Proceedings of the 4th European Symposium on Psoriasis

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Contents

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Aims of the Fourth European Symposium on Psoriasis

C. SCARPA (Trieste)

Inaugural Address, 17th September 1993

The chief reason why this series of Symposia thrives is the continuing interest in extrapolating from the wealth of pertinent information a tiny selection of real, true data concerning the pathogenesis and treatment of psoriasis. This implies making a clean sweep of the flood of those new data, which prove to be partial, inaccurate, unverified, or based on small groups of patients or concerning pathogenetic research performed *in vitro* only. This is the basic reason why these symposia need to be held. We must discuss in detail amongst ourselves the soundness of certain approaches which sometimes appear to be original, especially the biochemical studies, yet all too often prove groundless.

One can sometimes wonder why we should mishandle in every conceivable manner both psoriatic and non-psoriatic tissue under the pretext of detecting the minutest specific changes. This is like trying to tend a greenhouse full of beautiful flowers after a hurricane has devastated it! Is it not like that?

This meeting, if it is to take itself seriously, should outlaw anything that suggests hasty conclusions or highly tortuous and quibbling experimental 'proofs'! One should not work on living tissues in all manner of ways and then claim to have discovered true events in their molecular behaviour. For instance, it is true that DNA can withstand rather high artificial temperatures, but DNA represents the real basis of all living biomolecular scaffolding. This is not true of all the other fine tissue components, which deteriorate when subjected to any variation in their biological environment.

How can one think of or defend the squandering of huge resources in time and money in experimenting without consideration of the very demanding requirements of certain biomolecular chains which are vital for certain kinds of tissue? It is quite inconceivable. Moreover, how anyone can claim to have elicited real truths in this way is even less comprehensible.

If we wish to avoid falling completely into the power of the pseudoscientific press then we must dig in our heels and select only the truly scientific papers. We must plan the sort of research that takes into full account the vital metabolic needs of living tissues and therefore respects them. Something similar has been done in recent decades in attempts to cultivate *in vitro* certain tissues and attaining some degree of success in achieving their survival – at least acceptably if not of luxuriant growth.

Now let us turn our attention to *treatment*. The placebo effect is still prevalent in every field of human sickness, based on millennia of human medical treatment. Placebo is a factor of prime importance in all therapy, and especially of psoriasis which is also demonstrably susceptible to psychological influence. How on Earth can anyone think of conducting trials of a new antipsoriatic without first ascertaining its placebo effect? This should be absolutely mandatory.

As some cytokines (such as neuropeptides) and some immunomodulators (such as thymopentines) survive in the bloodstream for a few seconds only, how can anyone imagine that he can permanently quench this chronic and very peculiar inflammatory condition, namely psoriatic inflammation, by administering or applying anti-cytokines? Only thoroughly tested agents now in general use, such as the aromatic retinoids, PUVA, methotrexate, cyclosporin and calcipotriol, can be claimed to represent real progress. Other agents are for the present mere will-o'-the-wisps; they cannot be evaluated adequately in seriously conclusive terms. Nor do they appear to be effective in a consistent and safe way.

New, complete treatment trials are warranted only if preliminary trials elicit a successful response, as the PASI index diminishes by more than 75%. Full trials should then be sufficiently extensive, double-blind, random and placebo-controlled. They may then confirm our first, favourable impressions. Nothing else can be accepted on a seriously scientific level. If orthodox occidental medicine has achieved any progress, it is based on these very rules. But if these are ignored, then any alternative, anecdotal or old-fashioned remedy can equal or even surpass it. Committed dermatologists are invited to discuss this topic frankly, to take stock of relevant research and to point out new, clear approaches to advance our knowledge.

The vast network of already known and still to be discovered cytokines implies, in my opinion, that one cannot modulate any or some of these infinitesimal, labile fractions to achieve real and constant success in treating psoriasis. With cytokines, we have entered unwillingly into an inextricable maze which seems increasingly to entangle and constrict our understanding; it is becoming impenetrable. What we have succeeded in revealing in psoriatic skin at the biomolecular level are only secondary disturbances. Consequently we should in my opinion change direction and try to discern how we can work in a more constructive and rewarding manner. Candidly, this is the reality of the situation.

Some sessions of the Symposium will be devoted to already adopted, succesful ways of treating psoriasis: aromatid retinoids, PUVA, methotrexate, cyclosporin and calcipotriol. The point at issue here is to test therapeutic refinements or the selection of certain of these for sequential or combined therapeutic regimens. Compared with the huge number of therapeutic trials based on shaky foundations and therefore doomed to fail, the aforementioned agents, refined over several years by orthodox occidental medicine, will fairly achieve their greatest prominence at this Symposium.

I wish all delegates a pleasant stay in Trieste and fruitful deliberations.

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A Population Genetic Study of Psoriasis

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In the dermatological literature, simple models for the inheritance of psoriasis have been considered invalid (1,2). This is an important question in many respects. We have therefore found it necessary to analyse further the population genetics of psoriasis.

In Sweden, we have a patient organisation with 22,000 members that has been willing to let us use its membership register. At present we have data on psoriasis from more than 12,000. In about 300 individuals, both probands and relatives, the accuracy of reporting has been checked by an experienced dermatologist. There is underreporting of very mild psoriasis amounting to a few percent only. Data on psoriasis among the children of the probands have been requested for in 1,300 families. In population genetics studies of psoriasis, the late onset of the disease is a problem as many individuals with the psoriasis genotype will be classified as not having the disease. Of those who have psoriasis and are over the age of 50 years, only about half have developed the disease before the age of 25.

As we have a very large material, we have used information from psoriasis sufferers over the age of 55 years for this presentation in order to minimise the problem of late onset of the disease. We have used data on psoriasis among parents and siblings from 5,197 families and among children from 1,195 families.

The specific question we want to discuss is whether an autosomal dominant or an autosomal recessive mode of inheritance is compatible with population genetic data.

Psoriasis among the parents of the probands is given in Table I.

Psoriasis among the siblings of probands with both or one parent having psoriasis does not distinguish between the dominant and recessive modes of inheritance.

In nearly two-thirds of the probands, no parent had psoriasis. This would exclude a dominant inheritance if we did not assume a very low penetrance of the genotype. If we had a recessive mode of inheritance, the probability of the siblings' getting psoriasis would be 25%. The number of siblings affected is determined by a binomial distribution. However, we have no families where no one got the disease, as the probands always had psoriasis, by definition. We have thus a truncated binomial distribution with one term missing, viz. the one corresponding to no one having psoriasis. There are formulae for calculating the expected number of affected individuals in sibships of differing size under these circumstances (4).

Table II shows that the observed number of affected siblings closely corresponds to the expected number for a recessive inheritance.

A penetrance slightly over 90% is obtained. If we perform the same calculations for sibships where one parent or both parents had psoriasis, a slightly lower penetrance of the genotype is obtained. Psoriasis among siblings of probands is thus compatible with an autosomal recessive inheritance for psoriasis.

Calculations of this type are dependent on random mating. What is important is that mating is random with respect to the partner having or not having psoriasis. When collecting the data about psoriasis among children, we asked if the other parent of the children also had psoriasis. It turned out that 4.9% of the female probands had children together with a partner having psoriasis and the corresponding figure for male probands was 3.7%. This is what could be expected by chance in an adult population. We thus have no indications of a selection in this respect.

With regard to psoriasis among parents, it is dependent on the gene frequency, if we have an autosomal recessive mode of inheritance. This seems not to have been taken into account by earlier investigators (1,2) when they have discarded a recessive mode of inheritance for psoriasis. We can actually calculate the gene frequency from the data on psoriasis among parents.

The gene frequency of psoriasis turns out to be about 25%, giving 6% of the population being homozygotes and thus having the genotype for psoriasis. 38% will have one gene and cannot get the disease, while nearly 60% will carry no psoriasis gene.

We should point out that the data on psoriasis among the children of the probands are compatible with an autosomal recessive mode of inheritance with a gene frequency of 25%.

As in every other material, there is a certain risk of selection error. We have compared our data with those of Lomholt from the Faroe islands (1), however, and the correspondence is very good. Lomholt seems to have overlooked the consequences of a high gene frequency and discarded the possibility of a recessive inheritance incorrectly.

Table I. *Psoriasis among parents of probands*

Both parents had psoriasis	2%
One parent had psoriasis	34%
No parent had psoriasis	64%

Table II. *Number of siblings observed to have psoriasis in sibships of differing size when no parent has psoriasis, compared with the expected number (for p = 0.25)*

Size of sibships	Male probands		Female probands	
	Obs	Exp	Obs	Exp
2	492	504	563	571
3	423	454	526	547
4	289	325	343	366
5	199	234	211	247
Sum	1403	1517	1643	1731
Penetrance	92.5%		94.9%	

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What is the effect on the population genetic data of two clinically indistinguishable types of psoriasis vulgaris with different age at onset (3)? We have made a thorough investigation of this problem, which will be described in a separate paper. However, the risk of the children getting psoriasis when both parents have the disease is less, when we have two variants of the disease than when we have just one; when one parent has the disease the risk is about the same, and when no parent has the disease but both are heterozygotes, the risk is slightly higher than for one variant.

Even though an autosomal recessive inheritance is compatible with population genetic data, this does not prove that psoriasis is inherited in this way. However, this model is useful for genetic counselling.

ACKNOWLEDGEMENTS

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Characterization of Lesional Psoriatic Skin T Lymphocyte Clones

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T cells are considered to play a role in the pathomechanism of psoriasis. Therefore we investigated the cytokine production patterns of T cell clones that were randomly prepared from chronic plaque psoriasis lesions of 2 patients. 67% of the 49 T lymphocyte clones (TLC) expressed CD4 and 33% expressed CD8 (ratio 2:1), while γδ-TCR expression was absent. The production of IL-4, IFN-γ, IL-2 and IL6 was measured in supernatants of TLC following PHA plus PMA stimulation. Different groups of clones could be distinguished according to their IL-4/IFN-γ production ratio. In addition to Th0 cells (low IL-4/low IFN-γ), low IL-4/high IFN-γ producers as well as high IL-4/low IFN-γ producing clones were found, suggesting the presence of Th1– and Th2-like subsets. Upon stimulation, all TLC secreted low levels of IL-2 whereas a minority of the TLC secreted low levels of IL-6. These results may imply that T cells in psoriasis lesions do not show shifts towards either a Th1 or a Th2 cytokine production profile.

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A variety of observations such as the therapeutic efficacy of cyclosporin A (1), FK 506 (2) and CD4 antibodies (3) suggest a crucial role of $CD4^+$ helper lymphocytes in the pathomechanism of psoriasis. The accumulation of various cytokine-releasing T cell subsets in psoriatic (epi)dermis indicates that locally produced cytokines may regulate the inflammatory process and keratinocyte hyperplasia.

Helper T lymphocytes can be functionally distinguished according to their cytokine secretion patterns (4). Th1-like cells preferably produce IL-2 and IFN-γ, and Th2-like cells preferably secrete IL-4 and IL-5, although the majority of activated human T cells produce all these cytokines simultaneously (5). Certain inflammatory skin diseases are characterized by a predominance of T cells of one of the subgroups. Nickel-specific TLC from nickel contact-allergic individuals (6) or *Mycobacterium leprae*-specific TLC from leprosy patients (7) were found to be of Th1 type, while *Dermatophagoides pteronyssinus* (housedust mite = HDM) specific T cell clones from atopic, HDM-allergic individuals showed Th2-like patterns (8). Such findings prompted the search for similar functional T cell subsets that may be of pathogenetic importance in psoriasis.

For this purpose panels of randomly cloned T cells from lesional psoriatic skin biopsies of 2 patients were screened. Their cell surface phenotype was analysed for CD4, CD8, γδ-TCR expression, and their cytokine production by assaying the secretion of IL-4, IFN-γ, IL-2 and IL-6. The study demonstrates that significant levels of the Th1- and Th2-like cytokines are present in the supernatants of lesional skin-derived TLC, without a predominance of any T cell subset.

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MATERIALS AND METHODS

Biopsies and cloning procedure

Lesional skin biopsy specimens ($n = 2$) were obtained from the forearm of 2 male patients suffering from chronic plaque psoriasis. No local or systemic therapy had been given to either patient for 6 weeks before biopsy. 40 ml heparinized venous blood was taken as a source of autologous feeder cells (PBMCs). T cell clones were prepared according to Van der Heijden et al. (8).

Medium and cell culturing

During the cloning procedure, cells were cultured in Iscove's modified Dulbecco medium (IMDM) (GIBCO, Paisley, Scotland), supplemented with 10% pooled complement-inactivated normal human serum (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands), rIL-2 (20 U/ml) (Cetus Corp., Emeryville, Calif.) and gentamycin (80 μg/ml). When cells were stimulated for assaying cytokine production, human serum was replaced by 10% fetal calf serum (FCS) (HyClone Laboratories Inc., Logan, UT) and further supplemented with 35 μg/ml human transferrin (Behring-Werke, Magdeburg, FRG), 1.75 IE/ml human insulin (Actrapid, Novo Nordish A/S, Bagsvaerd, Denmark) and 3.5 μl/l β-mercaptoethanol (Merck, Munich, Germany). The Epstein-Barr virus transformed human B cell line JY was maintained in IMDM containing 5% FCS. All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Phenotyping of T cell clones

T cell clones were phenotyped by incubating on ice with anti-CD4 (OKT4) or anti-CD8 (OKT8) (Ortho Diagnostics Systems Ltd., High Wycombe, Bucks, England). The mAb anti TCR γδ-1 (9) was kindly provided by Dr. J. Borst (Dept. of Immunology, The Netherlands Cancer Institute, Amsterdam). FITC-conjugated $F(ab')_2$ fragments of rabbit anti-mouse IgG were purchased from Zymed (San Francisco, Calif.). The staining was quantified by flow cytometry (FACSscan, Becton-Dickinson).

Generation of clone supernatants

TLC cells (10^6/well) were stimulated with PHA (1 μg/ml) and PMA (5 ng/ml) (Sigma Chemical Co, St. Louis, Mo.) in Costar 24-well plates in 1 ml culture medium. Cell-free supernatants were collected after 24 and 48 h of culture and stored in aliquots at −20°C.

Cytokine measurements

The analysis of the IL-4 and IFN-γ contents of the TLC supernatants was performed with specific solid-phase sandwich ELISA systems, as described elsewhere (10, 11). The IL-2 activity in the supernatants was measured according to Gillis et al. (12). The IL-6 production in the cell-free supernatants was analysed using a commercially available ELISA kit (Central Laboratory, Netherlands Red Cross Blood Transfusion Service, M 1916).

Statistical analysis

Each group of cytokine measurements was expressed as a mean ± SEM. The difference between the means was analysed using Student's *t*-test.

RESULTS

Preparation of T cell clones from psoriatic skin

T cells were cloned directly from lesional skin of 2 patients

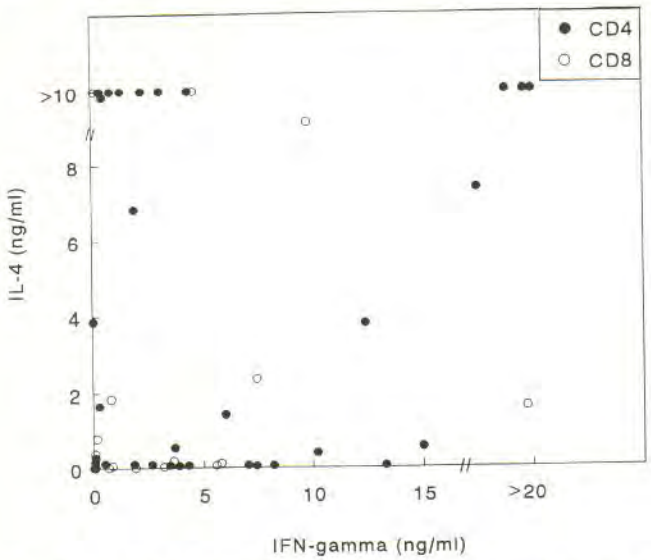

Fig. 1. IL-4 and IFN-γ secretion patterns of CD4⁺ (*closed circles*) or CD8⁺ (*open circles*) T-cell clones from psoriatic lesional skin. Secreted lymphokines were measured in supernatants of 24 h cultures of T-cell clones (10^6 cells/ml) stimulated with PHA and PMA and the values are expressed in ng/ml.

using a limiting dilution protocol with high efficiency. Of 37 TLC obtained from the first patient, 25 were phenotypically characterized as CD4⁺ (67.5%), and 12 as CD8⁺ (32.5%). Of TLC from the second patient, 8 out of 12 clones expressed CD4 (66.7%), while 4 expressed CD8. Thus, a consistent CD4/CD8 ratio (2:1) was observed for both patients. None of the TLC expressed the TCR-γδ.

Cytokine profile of TLC

T cell clones were stimulated with PHA and PMA and the supernatants were harvested after 24 and 48 h. IL-4, IFN-γ, IL-2 and IL-6 activity was determined. The clones demonstrated differential release of IL-4 and IFN-γ at 24 h after stimulation (Fig. 1). Seven CD4⁺ and 2 CD8⁺ clones secreted high levels of IL-4 but low levels of IFN-γ resembling Th2-type cells. Six clones (5 CD4 and 1 CD8) produced large amounts of IFN-γ and small amounts of IL-4 showing a Th1-like secretion profile. The majority of clones produced less than 100 pg/ml of these cytokines. However, we found 4 clones that showed a high production of both IL-4 and IFN-γ. The supernatants of 48 h cultures showed a quite similar distribution of IL-4 and IFN-γ production (data not shown). By calculating the means of IL-4 and IFN-γ production by the 49 clones (Fig. 2), no difference was found in the IL-4 production between day 1 and day 2, whereas the mean of IFN-γ levels was significantly higher in the 48 h supernatants ($p < 0.01$). Under the same conditions, small amounts of IL-2 (0.05–1.5 U/ml) were detected in the supernatants and only a few clones produced IL-6 (15–60 pg/ml), (Fig. 3). In both cases the level of cytokine production was significantly lower on day 2 ($p < 0.05$). The low IL-2 levels already after 24 h were probably due to consumption by the stimulated TLC.

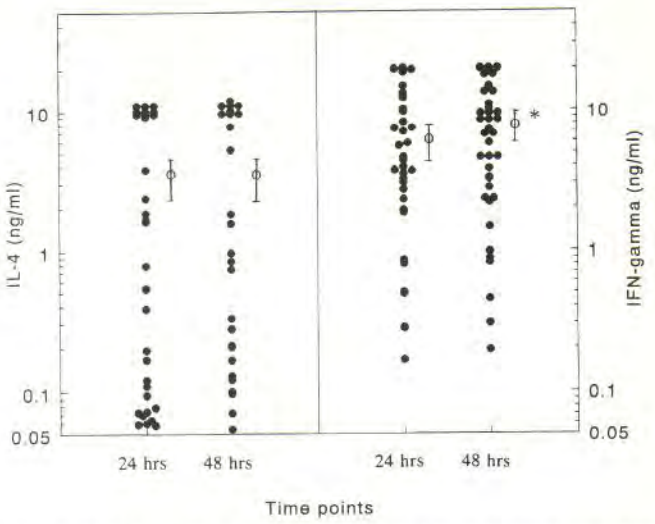

Fig. 2. TLC from 49 clones were stimulated by polyclonal activators (PHA + PMA). The conditioned media were harvested after 24 and 48 h of culture and IL-4 and IFN-γ activity was determined. Means ± SEM of the population are indicated. IFN-γ production is significantly higher on day 2 ($^*p < 0.01$).

DISCUSSION

To study the cytokine profile of psoriatic T cells, we expanded T cells from involved psoriatic skin. Immunophenotyping of psoriatic skin TLC of the 2 patients showed that the major cell type was the CD4⁺ helper T lymphocyte. The consistent CD4/CD8 ratio of TLC in this study is in accordance with the ratio previously observed by others in TLC panels (13) and with the ratio found *in situ* in lesional psoriatic dermis (14). None of the clones expressed γδ-TCR, thus supporting an earlier observation by Nikaein et al. who also did not find TCR-γδ⁺ cells (15). These findings do not entirely exclude that γδ-TCR⁺ cells are present in the initial culture. Kabelitz et al. reported that cloned

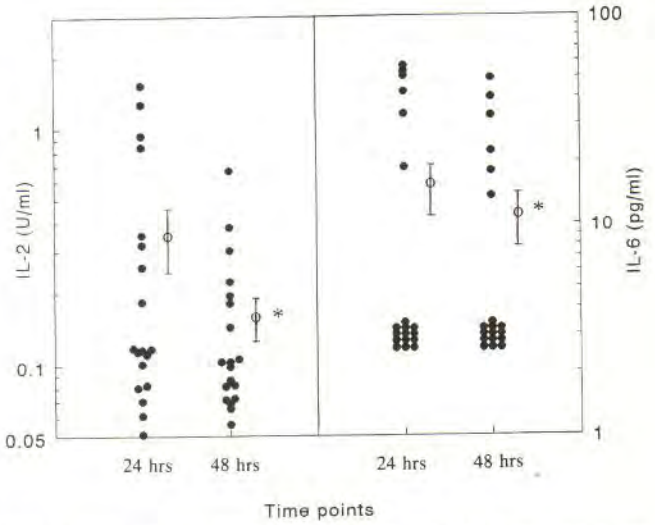

Fig. 3. IL-2 and IL-6 production by PHA + PMA stimulated T cell clones from patient 1 (with means ± SEM) of IL-2 and IL-6 activities. In the 48 h supernatants the levels of both cytokines were significantly lower than in the 24 h supematants ($^*p < 0.05$).

$\gamma\delta^+$ T cells underwent apoptosis when activated by anti-CD3 mAb (OKT 3) or PHA in the presence of IL-2 (16).

Results with the TLC suggest that according to the IL-4/IFN-γ ratio, different groups of TLC exist in lesional skin. Th1-like cells with high IFN-γ/low IL-4 values as well as Th2-like clones with high IL-4/low IFN-γ were found, whereas the majority of clones showed an intermediate profile (Th0 cells) (Fig. 1). A striking feature of these non-specifically stimulated clones was the presence of high IL-4 producer subsets. So far the only cytokines known to be produced by psoriatic skin-derived T cell clones were IFN-γ, IL-2, IL-10 and GM-CSF (17).

The IFN-γ levels were found to be elevated in serum (18) and suction blister fluid (19) of psoriatic patients, and its mRNA was present in epidermal sheets of lesional skin (20). Psoriatic keratinocytes are less sensitive to the growth inhibitory effect of IFN-γ than are those from normal skin (21). These observations emphasize the integral role of IFN-γ in psoriasis. IL-4 is produced by allergen-specific $CD4^+$ T lymphocytes in atopic dermatitis lesional skin (8). Cai et al. showed that IL-4 increased the adhesiveness of psoriatic dermal microvascular endothelial cells to peripheral blood mononuclear cells (22). Detection of IL-4 has not been reported before in psoriatic skin and its precise role in the inflammatory reaction in psoriasis is obscure.

Low levels of IL-2 were detected in our TLC supernatants compared with IL-2 production by atopic dermatitis clones (23). Similarly, Baker et al. (17) also found small amounts of IL-2 in TLC supernatants.

Only 6 out of 19 clones produced detectable levels of IL-6. All these clones were part of the high IL-4-producer subset.

Although we found clones with extreme Th1- or Th2-type cytokine profile, the variable IL-4/IFN-γ ratios of psoriatic TLC did not show a clear shift toward any helper T subset. Thus, in contrast to TLC obtained earlier from atopic dermatitis (8) and contact dermatitis (6) lesional skin, the psoriatic T cells cannot be clearly categorized on the basis of Th1- and Th2-associated cytokine production.

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ABSTRACT

The Cytokine Pattern in Psoriasis

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The pathomechanisms of various inflammatory disorders are revealed by distinct cytokine patterns. To elucidate the pathogenesis of psoriasis vulgaris (PV) we therefore have compared the profiles of cytokines expressed in affected skin with those of lesional psoriatic T cell lines (TCL) and clones (TCC) which had been activated via CD2 (2–3)/CD28 in the absence of monocytes, mRNA specific for TNF-α/β, TGF-α/β, IL-2/3/4/5/6/8, and GM-CSF, was determined by PCR and specific hybridisation. The PV-biopsies tested ($n = 4$) showed a combined transcription of IL-2/3/5/6/8, TNF-α/β, TGF-β and GM-CSF, but not of TGF-α, with a very faint signal for IL-4 in 2 samples. A similar cytokine pattern was found in 2 TCC, the supernatants of which enhanced keratinocyte proliferation in vitro, while 2 TCC suppressing keratinocyte growth as well 6 TCL showed also a strong signal for either or both IL-4 and TGF-α. No cytokine mRNA could be amplified from unaffected skin ($n = 2$). Collectively, psoriatic skin lesions express a complex cytokine profile uncharacteristic of a TH1- or TH2-response. This psoriatic pattern can fully be accomplished by psoriatic T lymphocytes with mitogenic effect on keratinocytes. The role of the psoriasis-associated cytokines for the clinical features of PV now needs to be determined. TGF-α as a cytokine mitogenic for keratinocytes, however, is appatently not involved in the increased epidermal turnover of psoriasis.

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ABSTRACT

A Subpopulation of T Lymphocytes Prepared from Psoriatic Skin Lesions Enhances Proliferation of Keratinocytes *in Vitro* via Secreted Products

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In order to substantiate the pathogenic role of T lymphocytes in the increased epidermal turnover of psoriasis vulgaris, 105 T cell clones (TCC) and 10 T cell lines (TCL) were prepared differentially from dermis and epidermis of biopsies taken from lesional psoriatic skin. Supernatants from 14 of 77 epidermal TCC, 7 of which were $CD8^+$ and from 8 of 28 dermal TCC, 5 of which were $CD8^+$, were found to enhance keratinocyte proliferation, with a more pronounced mitogenic activity of the dermal TCC. Another 9 epidermal and 3 dermal TCC had no effect, while supernatants from the remaining TCC as well as from the 5 epidermal and 5 dermal TCL inhibited keratinocyte growth to a varying degree. Both, mitogenic and suppressive capacities of T cell supernatants were largely abolished by an antiserum to interferon gamma (IFN-γ) whereas the effect of supernatants on keratinocyte growth was not altered in the presence of irradiated psoriatic T cells. Irrespective of their effect on keratinocyte proliferation, T cell supernatants promoted expression of MHC class II molecules and of ICAM-1 on the human epidermoid carcinoma cell line A431. Thus, a subpopulation of lesional psoriatic T lymphocytes is capable of enhancing keratinocyte proliferation in vitro via secreted products. This mitogenic capacity probably requires IFN-γ in conjunction with other cytokines which determine the ultimate effect of IFN-γ. Activation of these T cells in vivo could explain the keratinocyte alterations in psoriatic skin lesions.

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ABSTRACT

TNF-α Immunoreactivity and Bioactivity in Psoriasis

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Tumour Necrosis Factor-α (TNF-α) is a pleotropic cytokine which is thought to play an essential role as a mediator of inflammatory reactions. Involvement of TNF-α in the pathogenesis of psoriasis has been suggested previously, using immunological assays, but evidence for the presence of TNF-α bioactivity is lacking. We have therefore analysed aqueous extracts of stratum corneum (SC) from psoriatic lesions and uninvolved heels (from psoriatics and normals) using a TNF-α ELISA, a TNF cytotoxicity bioassay (WEHI.164 cl 13/2) and ELISAs for TNF receptors (p55 & p75). TNF-α ELISA measured higher immunoreactivity in lesional than in ininvolved SC. The immunoreactivity was found to be approximately twice as high as the bioactivity. This latter difference was expected to be due to the presence of TNF-soluble receptors (p55 & p75). ELISA for p55 showed higher immunoreactivity in lesional than uninvolved SC. p75 immunoreactivity was lower in lesional SC extracts and undetectable in uninvolved. TNF-α immunoreactivity was absent in psoriatic and normal sera, indicating a local effect. To determine whether the bioactivity, in lesional psoriatic SC, was due to the presence of TNF-α or TNF-β, lesional psoriatic SC extracts were incubated with an anti-TNF-α neutralising mAb prior to the bioassay. Results obtained, from all the six samples tested, showed complete neutralisation of the bioactivity. This confirmed that the bioactivity measured in the lesional SC extracts was due to the presence of TNF-α alone.

In conclusion, bioactive TNF-α is present in psoriatic lesions and may play a role in local cutaneous inflammatory events. It is probable that soluble TNF-α receptors are involved in the regulation of TNF-α activity in skin inflammation.

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Cellular Interactions and Adhesion Molecules in Psoriatic Skin

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T-cell activation probably plays the most important role in hyperproliferation of keratinocytes in psoriasis. We present here our results concerning the interacting immunocompetent cells and their phenotypic and functional characteristics in relation to psoriasis pathology. Immunohistochemical analysis of skin biopsies from psoriasis patients, did indeed show that hyperproliferation of keratinocytes is associated with increased vasculature and increased influx of MHC class II molecules expressing immunocompetent cells. Furthermore, in psoriasis, several adhesion molecules and other relevant activation markers were found to be upregulated even in the non-lesional psoriatic skin, indicating that psoriatic skin in general is in an activated state. This interpretation is further supported by the observation that the expression of several AR and other relevant activation markers when compared with those in non-lesional skin from contact dermatitis are increased in a significant manner in the non-lesional skin of psoriasis patients. We have then followed up our investigations by generating T-cell lines from lesional psoriatic skin and studied their adhesion patterns on cultured endothelial cells in order to get better insight into the migrationpattern of different T cell subsets in psoriasis pathology. Our results indicate that different T-cell subsets CD4$^+$, CD8$^+$ (both TCR-$\alpha\beta^+$) CD4$^-$/CD8$^+$ TCR-$\gamma\delta$+ and CD4$^-$CD8$^-$TCR-$\gamma\delta$ (Vδ1$^-$) T-cells can easily be generated from psoriatic patients. In a comparative kinetic study using unstimulated and stimulated cultured human umbilical vein endothelial cells, we observed that TCR-$\gamma\delta$ T cells showed different adhesion properties from that of TCR-$\alpha\beta^+$ T cell subsets. The overall results suggest that further studies on the cellular interactions (particularly concerning the expression and characteristics of the various adhesion receptors on different skin cells) together with the elaborate functional characteristics of T cells from psoriatic patients would help to elucidate the pathomechanism of psoriasis.

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Psoriasis is an idiopathic skin disease characterized by epidermal hyperproliferation and increased numbers of interacting activated lymphocytes and antigen-presenting cells (1). It is generally accepted that the presence of interacting T cells and APC followed by subsequent release of cytokines thereof are associated with the development of psoriatic lesions. There are only a few reports on the role of other immunocompetent cells in relation to this disease. Moreover, in spite of some occasional published data stating that psoriatic skin may constitutively be in an activated state (2), it is not clear whether non– lesional sites of psoriatic skin as compared with healthy skin from normal individuals and vis-à-vis that from other inflammatory dermatoses are prone to accumulate increased amounts of immunocompetent cells. The cellular influx and their subsequent activations, either in an antigen dependent or independent manner, are important in the chain of events leading to the perpetuation of the disease. The migration and activation of immunocompetent cells to and at the site of lesions are thought to be controlled by sets of adhesion receptors (AR) expressed on migrating cells and endothelial cells (3). These AR, in addition to MHC class II molecules and cytokine receptors, are regarded as the markers for activated states of these cells (4). In order to gain a better insight into the immunopathology of psoriasis, the essential steps for investigations are, firstly, an elaborate in situ phenotypic analysis of regionally accumulated interacting cells and their activation markers, including the adhesion molecules in both non-lesional (NL) and lesional (L) skin of psoriasis patients as compared with those from another hyperimmune reactive dermatosis and healthy individuals as controls.

Secondly, since it is believed that the role of T cell subsets is important in psoriasis pathology, it is essential to generate T cell lines from psoriatic skin and to study their adhesion and functional properties in relation to the disease.

In the present article we present a review of the results on (1) comparative in situ immunophenotypic analysis of interacting cells and their state of activation markers in L, NL skin from 7 psoriasis patients, the same from patch-tested DTH reactive skin from contact dermatitis patients and 7 healthy individuals; (2) characterization of adhesion kinetics of the in vitro generated subsets of T cells from psoriatic lesions. The detailed results of our studies have also been reported in separate manuscripts (5,6,7). Our results suggest that psoriatic skin is constitutively in an activated state, which facilitates the differential influx of different T cell subsets and other antigen-presenting cells into the skin. Moreover, we suggestthat cellular interactions and differential expression of adhesion molecules by different cell types emphasizes the fluctuating nature of the disease, e.g. exacerbation and remission.

MATERIALS AND METHODS

Immunohistochemistry

Biopsies from 6 patients with active psoriasis and 6 individuals with DTH reaction upon 72 h patch-tested contact dermatitis (CD) were removed from the inside border of lesions and also from random locations of non-involved area of the same groups of patients. Skin biopsies from 6 healthy volunteers were included as normal controls. Specimens were immediately frozen in liquid nitrogen and stored at −80°C until use. Immunohistochemical single and double stainings were performed on acetone-fixed cryostat sections by routinely performed immunohistochemical methods as described previously (8). MAbs used in this study were Leu4 (CD3), Leu7 (NK cells), Leu12 (CD19 Pan-8) and Leu14 (CD22 pan-8, all obtained from Becton & Dickinson, Mountain View, USA); anti- HLA-DR (CR3/43), Factor VIII, CD68 (EBM-11), CK1 (anti- keratin, all from Dakopatts, Glostrup, Denmark) OKT6 (Ortho Diagnostics, Raritan, N.J., USA), anti in-

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Table I. *Visual evaluation (arbitrary measurement) as determined by immunohistochemical single and double stainings, reflecting the gradient of intensity as well as percentage of cells present*

	Normal skin	NL*	L	NL	L
CD3	+	++	++++	+	++++
$CD3^+IL2R^+$	±	+	+++	±	++
$CD3^+HLA\text{-}DR^+$	±	++	++++	+	+++
CD1a	Normal network pattern ± in dermis	Normal	Loss of network pattern, more basal located	Normal network pattern	Loss of network pattern, +++ in dermis
$CD1a^+HLA\text{-}DR^+$	+++	+++	++++	+++	+++
CD68	+	++	+++	+	+++
$CD68^+HLA\text{-}DR^+$	+	+++	+++	+	+++
CD19	−	−	+	−	−
CD22	−	−	+	−	−
$CD22^+HLA\text{-}DR^+$	−	+	++++	−	−
NK cells	−	±	++	−	±
$CK1^+HLA\text{-}DR^+$	−	−	±	−	+++

*NL: non lesional skin; lesional skin.

terleukin 2 receptor (CD25, Biotest, Frankfurt, Germany); anti-LFA-1 (obtained from the American Tissue typing Center), anti-ICAM-1 (British Biotechnology, Abingdon, Berks, England), and HECA-452 specific for high endothelial venules (9) and cutaneous leukocyte associated antigen (10).

Immunohistochemical single and double stainings were performed using different staining protocols developed in our laboratory (8). Protocols differed according the combination of MAbs used for double and triple stainings (as described previously, ref 5 and 6). Percentages of interacting cells types as well as the activation markers expressed by them were evaluated light microscopically as described before (6).

Cell culture

T cell-lines from 6 psoriasis patients were generated from skin biopsies and peripheral blood as described previously (7) using 25 (Cetus) units r-IL-2, and either 10 μg/ml PHA (Wellcome, Dartford, Kent, England) or 10 μg/ml tuberculin PPD (Statens Serum Institut, Copenhagen, Denmark) as initial inducing mitogen or antigen. Initially irradiated autologous PBL were used as feeder cells until the point when T cells with homogeneous subsets phenotype were generated. Thereafter, irradiated autologous EBV-8 cells were used as feeder cells to expand the stable and homogeneous T cell lines of corresponding phenotypes. T cell lines from PBL were also generated using a similar culture protocol from 3 of these patients. T cell lines were analysed phenotypically by FACS analysis (7).

Human umbilical vein endothelial cells (HUVEC) were isolated according to the method of Jaffe et al (11), on 1% gelatin coated culture plastic in culture medium consisting of RPMI 1640, 10% fetal calf serum, 10% NHS, 2 mM glutamine and antibiotics.

Adhesion assays: 10.000 HUVEC were cultured for 3 days on 1% gelatin-coated microtitre plates, in presence or absence of 10 ng/ml TNF-α or 100 u/ml IFN-γ. Before the assay, HUVEC were incubated with appropriate dilutions of α-ICAM-1 MAb. Then 200,000 $Na_2{}^{51}CrO4$ labelled cultured T cells were added for 1 h. Plates were gently washed and percentage adhesion was calculated by (experimental release)/ (maximal release-spontaneous release).

RESULTS AND DISCUSSION

A T cell mediated immune response is considered to be the most important immunocompetent cell type in the pathogenesis of the psoriasis, and consequently investigators primarily focused their attention on the involvement of these cells and APC, e.g., LC and macrophages. However, other immunocompetent cells are also present in psoriasis lesions. Therefore, we investigated simultaneously the involvement of other cells in addition to T cells subsets present in lesional and non-skin specimens of psoriasis patients, using a panel of MAbs, and compared the results obtained with similar specimens from both CD patients and specimens from normal healthy individuals. In order to get a better insight into the pattern of in situ interaction between these regionally accumulated cells, we also investigated the expression of activation markers such as MHC class II molecules and IL2R. The results of both immunosingle and double stainings are summarized in Table I. We found increased amounts of T cells, Langerhans cells (LC) and macrophages in both lesional and non-lesional psoriatic, and that only in lesional CD skin. The percentages of these immunocompetent cells in non-lesional CD specimens were more or less similar to those found in normal controls. It is of particular interest to note that the percentages of MHC class II expressing immunocompetent cells and $IL\text{-}2R^+$ CD3 cells are also present in increasing amount in non-lesional psoriatic skin, as compared with those in CD non-lesional biopsies. Interestingly, in psoriasis, especially at the lesional sites, we found activated, $IL\text{-}2R^+$ T cells often in close contact with basal keratinocytes, suggesting that these cells may be involved in hyperplasia of keratinocytes. We also found the presence of B cells in all the lesional psoriasis, but not on any of the other (normal and CD) biopsies. This suggests that B cells might be important in certain stages of psoriasis. In contrast, only a few NK cells were also found to be present at the lesional psoriatic sites, often in close contact to basal keratinocytes. Only a few NK cells were found to be present in lesional but not any in non-lesional CD specimens. Although the numbers of B and NK cells in lesional psoriatic specimens are relatively small as compared with T cells, LC or macrophages, these cells should be regarded also to be important in both local immune responses and its regulation in relation to the disease.

Surprisingly, in spite of the presence of activated T cells in and around the epidermis, there was only very weak expression of HLA-DR in basal keratinocytes on psoriasis skin, whereas strong $HLA\text{-}DR^+$ areas of keratinocytes were encountered in CD lesional area. This suggests that the activation pattern of T cells

Table II. *Expression of adhesion receptors on different cell types the tested skin specimens*

	Psoriasis			Contact dermatitis	
	N[a]	NL	L	NL	L
CD1a⁺/HECA-452⁺	0[b]	60±24	92±6	5±3	15±8
CD3⁺/HECA-452⁺	40±8	85±8	83±9	50±26	53±13
CD68⁺/HECA-452⁺	8±4	11±7	10±5	9±10	10±4
CD1a⁺/LFA-1⁺	0	7±7	10±8	3±3	30±28
CD3⁺/LFA-1⁺	98±3	99±1	100	96±1	99±1
CD68⁺/LFA-1⁺	9±9	8±5	10±7	24±10	57±11
ICAM-1⁺/CK1⁺	–[c]	–	+	–	+++
ICAM-1⁺/CD3⁺	±	+	+++	±	+++

[a] N = normal skin; NL = non-lesional skin; L = lesional skin.
[b] mean numbers of double-stained cells (±SD).
[c] Visual evaluation (arbitrary measurement) as determined by immunohistochemical single and double stainings, reflecting the gradient of intensity as well as percentage of cells present.

and the locally produced cytokine profiles are different in these two diseases during the active phase. From the aforementioned results it is suggested that the psoriatic non-lesional skin is abnormal with respect to hyperimmune reactivity, whereby there is a constant tendency for local infiltration of activated immune cells. Adhesion receptors are important for leukocyte migration into skin, as well during cellular interactions in an ongoing immune response (3). Consequently, we also investigated the expression of these AR expressed by immunocompetent cells as well as keratinocytes, in normal, lesional and non-lesional psoriasis and CD specimens. The expression of "endothelium-related" AR is discussed in a separate paper (12). The results are summarized in Table II. It can be seen that increased numbers of HECA-452⁺ LC, HECA-452⁺ T cells and LFA-1⁺ LC are found in both lesional and non-lesional psoriatic specimens. Interestingly, in both lesional and non-lesional CD specimens, about half of the T cells were found to be HECA-452⁺, similar to that in normal skin. This is in contrast to psoriasis. In the latter case, percentages of HECA-452⁺ T cells were approximately 90% of both lesional and non-lesional specimens. Interestingly, we have also observed that the content of E-selectin (a possible ligand for HECA-452) positive vasculature in psoriatic non-lesional skin is increased, compared with normal and non-lesional CD skin (6,12). No such increase in any of the AR studied was observed in non-lesional specimens of CD. However, significantly increased expression of HECA-452 on LC, and LFA-1 on both macrophages and LC was found in lesional CD patients.These results indicate that the non-lesional skin of psoriasis patients is indeed constitutively in an activated state, to facilitate the accumulation of HECA-452⁺ cells. It appears therefore, that a cutaneous immune reaction against contact allergens leads to another type of immune response different from that in psoriasis which is reflected by the presence of high percentages of LFA-1⁺ LC and macrophages in CD, but not in psoriasis. These studies further point towards the fact that specific types of T cell migration and their activation upon interaction with other immunocompetent cells are associated with the pathology of psoriasis.

In an attempt to elucidate such an assumption, we isolated and cultured T cell lines from both lesional psoriasis biopsies and from the peripheral blood to study their phenotypes in regard to adhesion receptors and their adhesion kinetics, using both unstimulated and cytokine-stimulated HUVEC. The phenotypes of the cell lines obtained are summarized in Table III. Surprisingly, the majority of the T cell lines obtained were CD8⁺, and fewer cells lines were CD4⁺ in spite of the fact that in the lesional sites CD8⁺ cells form the minor population. In addition, relatively many cell lines were found to possess the γδ (Vδ1⁻) phenotype, even though these cells are relatively rare in lesional skin. In adhesion studies, as illustrated in Table IV, we did not find any differences in adhesion of CD4⁺ and CD8⁺ cells to HUVEC, nor did we observe differences in adhesion patterns between T cells

Table III. *Percentages of phenotypic similar in vitro generated T cell lines obtained from skin biopsies and peripheral blood from 6 different donors*

	Phenotype	%
Skin derived T cell lines ($n = 87$)	DC4⁺	14
	CD8⁺	43
	CD4⁻CD8⁻TCR-γδ	19
	CD4⁻CD8⁺TCR-γδ	1
	non homogenous	23
Blood derived T cell lines ($n = 6$)	CD4⁺	0
	CD8⁺	50
	CD4⁻CD8⁻TCR-γδ⁺	50

Table IV. *Percentage adhesion of two skin-derived TCR-αβ and TCR-γδ⁺ T cell lines to HUVEC*

	TCR-αβ (CD4⁺)	TCR-αβ (CD8⁺)	TCR-γδ	TCR-γδ
None	31±5	26±2	71±11	60±6
None + ICAM-1	23±5	23±1	60±15	52±6
IFN-γ	40±4	40±8	98±5	70±5
IFN-γ + ICAM-1	20±1	28±3	58±3	54±9
TNF-α	73±5	77±5	99±1	76±9
TNF-α + ICAM-1	50±2	52±7	63±5	ND

HUVEC were stimulated for 72 h with either 100 u/ml r-IFN-γ or 100 u/ml r-TNF-α (5 ng/ml).

isolated from the peripheral blood and from the skin. On the other hand, we found that the basal adhesion of TCR-γδ$^+$ T cell lines to cytokine-stimulated and unstimulated endothelium is significantly increased as compared with that of αβ T cell lines (a representative example of the adhesion of two TCR-αβ T cell lines and two TCR-γδT cell lines is illustrated in Table IV). This increased adhesion could not be blocked to TCR-αβ levels with anti-ICAM-1 antibodies, suggesting that this increased binding is due to other AR, possibly ICAM-2. Still, this increased adhesion of TCR-γδ T cells to endothelium is surprising, because relatively few TCR-γδ$^+$ T cells are present in human skin (normal and diseased) (5). It can be speculated that too-strong adhesion to endothelium inhibits the subsequent migration of these cells into the tissue.

In conclusion, our results indicate that the extravasation of these different immunocompetent cells into – and their subsequent activation at – lesional sites in psoriatic skin is associated with the differential upregulation of various adhesion molecules and activation markers on immunocompetent cells, as well as on other skin cell types, e.g., keratinocytes and endothelial cells. Furthermore, particularly TCR-γδ and CD8$^+$ cells, although constituting a smaller fraction of the total in situ T cell populations, might be of importance in regulating the hyperimmune reactivity associated with the hyperproliferation of keratinocytes. We hypothesize that the concerted interaction between the migrating immune competent cells and resident skin cell resulting in the keratinocyte hyperproliferation is dictated by the constitutively upregulated cell adhesion receptors in psoriasis.

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Soluble Intercellular Adhesion Molecule-1 and Procollagen III Peptide are Reliable Markers of Disease Severity in Psoriasis

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Levels of soluble intercellular adhesion molecule-1 (sICAM-1) and procollagen III peptide (PIIIP) were measured respectively by enzyme immunoassay (EIA) and radioimmunoassay (RIA) methods in sera from 14 patients affected with psoriasis. The same determinations were also performed on suction blister fluids (BFs) obtained from lesional and non-lesional skin. Fourteen normal subjects were used as controls. Significant correlations were found between the serum levels and psoriasis area and severity index (PASI), ($R = 0.62$ for sICAM-1 and $R = 0.73$ for PIIIp, respectively). Of the PASI components, infiltration and erythema represented the variables most closely related to PIIIP ($R = 0.85$; $R = 0.72$ respectively). Differently from PIIIP, whose levels were significantly lower in the sera than in skin BFs (serum: median value 1.05, range 0.7–2.3 vs. lesional skin fluid: 11.8, 4.8–30 U/ml), sICAM-1 molecules were found predominantly in the sera (serum: median 316, range 117–579 vs lesional skin fluid: median 70, range 31–252 ng/ml). These data cannot exclude that sICAM-1 molecules detected in suction BFs may derive from serum contamination. ***Key words: sICAM-1; PIIIP; psoriasis; blister fluids; serum; PASI.***

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Recent advances in our understanding the pathomechanisms of psoriasis are supported by the increasing knowledge of several soluble molecules synthesized and released by different cells(1). In this regard, we studied the sICAM-1 (soluble intercellular adhesion molecule-1) levels in involved (ISBFs) and uninvolved (USBFs) skin blister fluids (BFs) and sera of 14 patients affected with psoriasis. The procollagen III peptide (PIIIP) levels on the same samples were measured to study possible fibroblastic involvement in this disease. The clinical usefulness of the previous determination was evaluated correlating the results found with the commonly employed parameters of PASI score.

PATIENTS AND METHODS

Fourteen patients with active (non-arthropathic) psoriasis were studied (13 females and 1 male, median age 41, range 15–72 years) and 14 healthy volunteers (12 females and 2 males, median age 43, range 21–50). Twelve of the 14 patients were affected with plaque-type psoriasis, 1 with suberythrodermic psoriasis and 1 with pustular psoriasis. The median PASI of 13 patients was 11.4; range 3.0–40.5. None of the patients had ever received methotrexate or had liver dysfunction. The patients had received no treatment for at least 10 days before enrolment. Suction blisters were obtained both from lesional skin (ISBFs) (plaque) of 3–5 cm in diameter or edge of larger plaques) and from unaffected skin (UBSFs) (10–15 cm from the lesion) in all patients and from normal skin (NSBFs) in 5 out of 14 controls, by the Kiistala methods (2). Serum samples were obtained from all patients and controls studied. The methods and technical data are as follows:

Table I.

Test	Producer	Method	Sensitivity	Sample (µl)	Dilution
Serum					
sICAM	T Cell Sci.	ELISA	0.3 ng/ml	25	1/100
PIIIP	Behring	RIA	0.1 UPIIIP/ml	20	1/1
Fluid					
sICAM	T Cell Sci.	ELISA	0.3 ng/ml	25	1/100
PIIIP	Behring	ELISA	0.1 UPIIIP/ml	20	1/2

Statistical analysis

The results are expressed as median and range values. Accordingly, statistical comparisons were calculated by non-parametrical methods: Kruskal-Wallis or Rank Wilcoxon or Spearman Rank correlation tests were used as necessary.

RESULTS

The sICAM-1 and PIIIP levels in blister fluids (BFs) and sera from psoriatic patients and controls are shown in Fig. 1a, 1b. Serum levels of sICAM in 14 psoriatic patients (median 316 ng/ml; range 117–579), were statistically higher than in the controls (median 234 ng/ml; range 160–240; $p = 0.032$). With regard to the sera, in both patients and controls , lower sICAM-1 levels were detected in the USBFs (median 31.5 ng/ml; range 10–223; $p = 0.00002$, in the ISBFs (median 70 ng/ml; range 31–252; $p = 0.00001$) and in NSBFs (median 30 ng/ml; range 27–58; $p = 0.009$). The sICAM-1 concentrations in the ISBFs were significantly higher than in the USBFs ($p = 0.002$) or in NSBFs ($p = 0.005$). No statistical difference between the USBFs and the NBSFs sICAM-1 was found. Direct correlations were observed between the levels of sICAM-1 in sera and ISBFs ($r = 0.82$; $p = 0.000$.) as well as between ISBFs and USBFs ($r = 0.89$; $p = 0.00001$). A statistically significant correlation between serum sICAM-1 levels and the PASI score was also noted ($r = 0.62$; $p = 0.03$). PIIIP levels in the BFs and sera from psoriatic patients and controls are shown in Fig. 1b. PIIIP serum measured in the 14 psoriatic patients (median 1.05 U/ml range 0.7–2.6) were significantly lower than those of ISBFs (median 11.8 U/ml; range 4.8–30; $p = 0.000007$) or of USBFs (median 8.3 U/ml; range 1.7–30; $p = 0.00002$). The control sera also showed lower PIIIP levels (median 0.9 U/ml; range 0.6–1.0) when compated with NSBFs (median 8.6 U/ml; range 4.1–12.1; $p = 0.008$). In the ISBFs, a significant increase in the PIIIP levels was noted as compared with the USBFs ($p < 0.05$) and, although not statistically significant, to the NSBFs. Also in patients' sera, the PIIIP levels were significantly higher than in the control sera ($p = 0.035$). Direct correlations between the PIIIP serum levels

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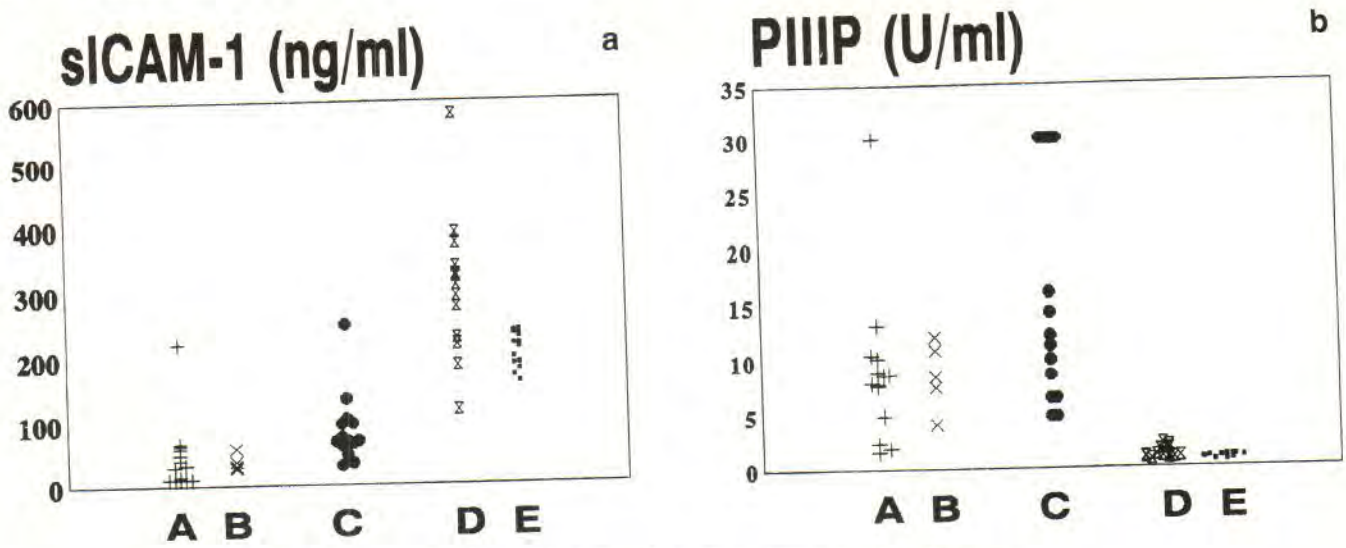

Fig. 1a, b. A = uninvolved skin; B = normal skin; C = involved skin; D = psoriasis serum; E = control serum.

and PASI score ($r = 0.73$; $p = 0.005$) and between the PIIIP and grade of erythema ($r = 0.72$; $p = 0.03$) and PIIIP and grade of infiltration ($r = 0.85$; $p = 0.0001$) were found.

DISCUSSION

ICAM-1 is a membrane glycoprotein (90–114 KDa MW) which plays a central role in several cell-cell interactions (3). Various cell types constitutively express ICAM-1 or may be induced to express it by cytokines (3). ICAM-1 may also be released into the fluids (4): in fact, the sICAM-1 serum levels are increased in many diseases characterized by an activation of phlogistic mechanisms (5). In our study, higher sICAM-1 levels, both in psoriatic and in control sera, were demonstrated, together with a direct correlation of sICAM-1 serum levels and PASI score. Similar data have been recently reported by Schopf R. E. et al. who did not study sICAM-1 levels in BFs (5). The significantly lower sICAM levels in ISBFs and in USBFs, as in NSBFs, is not surprising. In fact, sICAM-1, released in greater amounts at the level of the skin phlogistic focus, due to its high molecular weight (> 30 KDa) (6), can only partially penetrate into the BFs and is collected in the bloodstream.

PIIIP serum levels of psoriatic patients and controls were significantly lower than skin BF. However, higher serum PIIIP levels vis-à-vis control sera, were observed in the psoriatic patients. A significant direct correlation between PIIIP serum levels and PASI score was found. Other correlations between the PIIIP serum levels and the lesion grade of erythema or infiltration were observed. Serum PIIIP amounts can derive partly from interstitial skin. The higher PIIIP levels found in the ISBFs as compared with control BFs indicate a greater local fibroblastic activity, in partial agreement with previous reports (7). In ISBFs, higher PIIIP levels were found as compared with the USBFs ($p < 0.05$) and also vs. NSBFs ($p = NS$). However, no differences were observed between the USBFs and the NSBFs. These data suggest that serum sICAM-1 and PIIIP may be considered possible markers of disease severity in psoriatic patients. Further studies are warranted to verify in which cases they may be selectively better utilized.

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Differential Expression of ICAM-1, E-selectin and VCAM-1 by Endothelial Cells in Psoriasis and Contact Dermatitis

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Adhesion receptors on endothelial cells are considered to be important for cellular influx in tissue. In this regard, skin constitutes a specialised environment for migration of leukocytes during inflammation. Using immuno-enzymatic staining techniques, we compared the in situ expression of ICAM-1, E-selectin, and VCAM-1 on endothelial cells and inflammatory infiltrates in both lesional and non-lesional biopsied skin from two immuno-inflammatory diseases, viz. psoriasis and contact dermatitis. The results were compared with those in skin specimens obtained from normal healthy individuals free from any history of skin disease. Our results show that ICAM-1 and ELAM-1 are upregulated in psoriatic non-lesional and lesional skin. On the other hand, in non-lesional biopsy from contact dermatitis patients, all three AR molecules are sparsely present, similar to the situation in normal skin although they are overtly expressed in the lesional sites. Moreover, VCAM-1 was found to be significantly increased on endothelial cells in the lesional sites of contact dermatitis as compared with biopsied psoriatic specimens. Interestingly VCAM-1 was also found to be present on some T-cells and Langerhans cells in contact dermatitis alone. The present data suggest that in different inflammatory dermatitis, varying adhesion receptor-ligand interactions involving endothelial cells and leukocytes are involved, which may be due to the differing cytokine profiles of perivascularly located T-cells.

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The main constituents of inflammatory dermatoses are characterized by the presence of mutually interacting activated subsets of T cells and antigen-presenting cells (APC) at the lesional sites (1). Adhesion receptor (AR) expression on endothelial cells (EC) is regarded as important for cellular influx in tissue. In recent years, various AR on both EC and immunocompetent cells have been identified which are involved in adhesion and homing into various tissues and also causing their regional activation (2). The interactions between the accumulating cellular infiltrates cause the release of proinflammatory cytokines which then lead to tissue damage. Psoriasis is a disease which spontaneously exacerbate but also undergo remission. During our ongoing studies on cellular interaction in psoriasis pathology, we observed that the expression of various AR is upregulated in both non-lesional (NL) and lesional (L) skin of psoriasis patients, as compared with that in skin of normal healthy individuals (3–4). Our findings, together with those in the literature (5), show that the upregulation of AR in non-lesional psoriatic skin is due to an ongoing local hyperimmune reactivity as a constitutive phenomenon of the disease. However, in order to establish the validity of such a hypothesis, one essential prerequisite is to evaluate the status of AR in both NL and L skin from patients with other known inflammatory dermatoses, such as contact dermatitis, during the active phase of the disease.

The best characterized AR expressed by EC in relation to leukocyte migration and adhesion are ICAM-1, VCAM-1, and E-selectin (6). Consequently we examined the expression of these AR, particularly on EC and on immunocompetent cells, by immunohistochemical single and multiple staining methods.

MATERIALS AND METHODS

The details are similar to those described earlier (3–7). Patients: Groups of 6 patients, each with active psoriasis, and 72 h patch-tested contact dermatitis (CD) patients with DTH reaction, together with 6 healthy volunteers were included in this present study.

Skin biopsies were removed from the inside border of respective lesions and also from the non-involved area on a location at least 5 cm away from the involved area. Cryostat sections (5 μm) were cut, fixed in acetone and stored at −20°C until use.

Immunohistology and lightmicroscopic examination: Immunohist-

Table I. *Expression of ICAM-1, E-selectin and VCAM-1 by endothelial cells in cryostat sections from normal, lesional and non-lesional psor/asis and contact dermatitis skin biopsies*

	Normal skin	Psoriasis		Contact dermatitis	
		NL	L	NL	L
ICAM-1	+	+–>++	+++	+	+++
E-Selectin	±	+	+++	+	+++
VCAM-1	–	–	±	–	+++

-: no staining; ±: weak staining; +: positive staining; ++: strong staining; +++: very strong staining.

Table II. *Mean percentages of Langerhans cells and T cells expressing ICAM-1 and VCAM-1 in cryostat sections from normal, lesional and non-lesional psoriasis and contact dermatitis skil biopsies*

	N	Psoriasis		Contact dermatitis	
		NL	L	NL	L
CD1a/ICAM-1	0	10±8	38±30	5±8	70±30
CD1a/VCAM-1	0	0	0	0	35±12
CD3/ICAM-1	20±20	65±15	70±15	15±10	38±18
CD3/VCAM-1	0	0	0	0	8±8

N: normal human skin; NL: non-lesional skin; L: lesional skin.

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ological analysis by both single and multiple staining methods (using serial sections) were carried out described earlier before (3–7). MAbs used in this study were anti-ICAM-1 and VCAM-1 (both purchased from British Biotechnology, Abingdon, Berks, England), anti E-selectin (a gift from Dr D. Haskard, Rheumatology Unit, Postgraduate Medical School, London, England), Leu (4) (Becton & Dickinson, Mountain View, USA), OKT 6 (Ortho Diagnostics, Raritan, USA).

RESULTS AND DISCUSSION

The results of the immunostainings on AR expressed by endothelium are summarized in Table I. Endothelial cells in normal skin are ICAM-1 +, and increased expression of this adhesion receptor can be found in lesional psoriasis and CD skin biopsies. On the other hand, only the non-lesional psoriatic (and not CD) skin also shows increased expression of ICAM-1. E-selectin was found to be weakly expressed in normal as well as in non-lesional CD skin, but was increased in non-lesional psoriasis. This adhesion molecule was strongly expressed in lesional sites in both the diseases. On the other hand, VCAM-1 expression was negative in all normal and non-lesional skin specimens. However, strong expression of this AR was seen in lesional CD skin, while weak expression was evident in lesional psoriatic biopsies. These results show that EC in non-lesional psoriatic skin are in an activated state because of the higher expression of E-selectin and ICAM-1 as compared with non-involved CD and normal skin. The upregulation of these AR facilitates the recruitment of specific populations of T cell subsets.

The migration of lymphocytes into skin is thought to be a multistep mechanism, involving the initial adhesion, activation of leukocyte integrins, eventually leading to transendothelial migration (8). Regulation of lymphocyte migration into lesional psoriasis skin appears to be mediated mainly by ICAM-1 and LFA-1, and to a minor extent VCAM-1/VLA-4. In contrast, the migration of lymphocytes into CD skin involves both ICAM-1/LFA-1 and VCAM-1/VLA-4. In agreement with the present findings of low levels of VCAM-1 in psoriasis it can be reasoned that the critical time period for identifying the in situ identification of this molecule had already lapsed when the biopsy was taken. On the other hand, in CD lesions the biopsy was taken at the peak time of 72 h for *de novo* VCAM-1 expression on cytokine-stimulated endothelial cells.

Since both ICAM-1 and VCAM-1 also showed staining with mononuclear cells in the different skin specimens, double stainings with MAbs against LC and T cells were also performed. Double stainings of ICAM-1 and VCAM-1 against CD68 could not be performed since the MAbs were of the same isotype. In normal and CD lesional skin we found that a certain proportion of T cells were invariably ICAM-1+ (15–20%). However, the proportion of CD3 + ICAM-1+ cells was significantly increased in both non-lesional and lesional psoriasis skin biopsies. Quantities of ICAM-1+ CD3+ cells in CD lesional and non-lesional sites were less than those observed in psoriasis. We did not find any CD3+ or CD1a+ cells which were also VCAM-1 + in any of the normal or psoriasis skin specimens. Interestingly, VCAM-1+ Langerhans cells as well as occasionally T cells were encountered only at the site of DTH reaction. This indicates that VCAM-1+ Langerhans cells as well as T cells are important in interactions with antigen (contact allergen) specific T cell populations. Such an interpretation should be evaluated further by making in vitro studies.

In conclusion, our results show that the endothelium of non-lesional psoriatic skin is constitutively activated. Additionally, we hypothesize that, although psoriasis and CD are both hyperimmune skin disorders, leukocyte infiltration and migration into the lesional sites is regulated by varying adhesion pathways in different dermatoses.

ACKNOWLEDGEMENT

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Interleukin-1-beta, Interleukin-6, and Interferon-gamma in Suction Blister Fluids of Involved and Uninolved skin and in Sera of Psoriatic Patients

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Interleukin-1-beta (IL-1-beta), Interleukin-6 (IL-6) and Interferon-gamma (IFN-gamma) were measured by enzyme immunoassay (EIA) methods in blister fluids (BFs) obtained from both involved (ISBF) and non-involved skin (USBF) and in sera from 14 psoriastic patients. The same determinations were carried out in 14 sera and in 5 suction blister fluids from 14 normal subjects. IL-6 was always detectable in all skin fluids and in 3 psoriasis sera. IL-1-beta was measured only in 5 ISBFs and in 5 sera from the same patients. IFN-gamma was preasent in 11 ISBFs, in 5 USBFs and in 5 sera. The analysis of the levels found in the samples shows: 1) a local production of these cytokines, 2) the presence of detectable amounts of IL-6 and IFN-gamma in USBFs, and 3) a significant correlation between the IL-6 levels in the ISBFs and erythema score. ***Key words: psoriasis; blister fluids; IL-6; IL-1-beta and IFN-gamma.***

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Recent investigations emphasize the importance of the interaction of different cytokines in substaining the two main features of psoriatic lesions, viz. inflammation and kerationocyte hyperproliferation (1). In this context, we studied the presence in vivo of Interleukin-1-beta (IL-1-beta), Interleukin-6 (IL-6) and Interferon-gamma (IFN-gamma) in the sera and blister fluids (BFs) obtained from a group of 14 psoriatic patients.

PATIENTS AND METHODS

Fourteen patients with active psoriasis (13 females and 1 male, median age 41, range 15–72 years) and 14 healthy volunteers (12 females and 2 males, median age 43, range 21–50 years) were studied. Of the 14 psoriatic patients, 12 were affected with plaque-type psoriasis, 1 with suberythrodermic psoriasis and 1 with pustular psoriasis. The median Psoriasis Area and Severity Index (PASI) of 13 patients was 11.4; range 3.0–40.5. The patients were untreated for at least 10 days before enrolment. Suction blisters were obtained both from lesional skin (ISBFs) (plaque 3–5 cm in diameter or edge of larger plaques) or unaffected skin (USBFs) (10–15 cm from the lesion) in all patients and from the skin (NSBFs) in 5 og 14 controls, by the Kiistala method (2). Serum samples were obtained from all the patients and controls studied. Methods and technical data are listed in the Table I.

Statistical analysis

The results were expressed as medians and ranges. Accordingly, statistical comparisoons were calculated by non-parameterical methods: Kruskal-Wallis or χ^2 or Spearman Rank correlation tests were used, as necessary.

RESULTS

The IL-1-beta, IL-6, and IFN-gamma levels in BFs and sera from psoriatic patients and controls are presented in Fig. 1. IL-1-beta was detected in ISBFs in 5 out of 14 patients (median 121.5 pg/ml; range 70.4–190.9), and in the corresponding sera (median 15.4 pg/ml; range 7.5–45.5). IL-1-beta levels were consistently higher (8-fold) in the BFs than in the serum samples (Fig. 1a), with a significant direct mutual correlation ($r = 0.96$, $p = 0.006$). No IL-1-beta was detected in the USBFs or in the NSBFs. None of the normal sera had detectable amounts of IL-1-beta. IL-6 was consistently found in the ISBFs and USBFs as well as in BFs of healthy volunteers. As shown in Fig. 1b, the IL-6 levels in the ISBFs were significantly higher (median 1682.5 pg/ml; range 267–12168) than those in psoriatic USBFs (median 120.5 pg/ml; range 7.0–484.4; $p = 0.00002$) or BFs of normal skin (median 10 pg/ml range 7.0–11.0; $p = 0.005$). IL-6 levels in the USBFs were also significantly higher than those in NSBFs ($p = 0.002$). Only 3 of the 14 patients had detectable amounts of IL-6 in their sera (median 72.3 pg/ml range 4.3–230.0). None of the normal sera was positive. A direct correlation between the IL-6 levels in ISBFs and the erythema score was found ($r = 0.63$; $p = 0.01$). IFN-gamma was detected in 11 of the 14 ISBFs (median 0.04 IU/ml; range 0.02–2.94), while lower levels (median 0.03 IU/ml range 0.02–0.05) were found in 5 of the 14 USBFs. Furthermore, 5 of 14 patients had detectable IFN-gamma amounts (Fig. 1c) in their sera (median 0.03 IU/ml; range 0.02–0.05). Normal subjects invariably had undetectable IFN-gamma both in skin BFs and in their sera. The frequency of subjects positive for IFN-gamma in ISBFs differed from that of USBFs or normal skin controls (χ^2; $p = 0.02$; χ^2; $p < 0.05$ respectively). Only 3 of 14 patients had IFN-gamma simultaneously detectable both in the sera and in the ISBFs and USBFs. Patients with high IL-1-beta and IFN-gamma levels in ISBFs corresponded mainly to those with the highest IL-6 concentrations (not shown).

Table I

Test	Producer	Method	Sensitivity	Sample (µl)	DIL
Serum					
IL-1-beta	Medgenix	ELISA	3 pg/ml	100	1/1
IL-6	Medgenix	ELISA	3 pg/ml	200	1/1
IFN-gamma	Medgenix	ELISA	0.2 IU/ml	50	1/1
Fluid					
IL-1-beta	Medgenix	ELISA	15 pg/ml	20	1/5
IL-6	Medgenix	ELISA	15 pg/ml	20	1/5
IFN-gamma	Medgenix	ELISA	0.2 IU/ml	50	1/1

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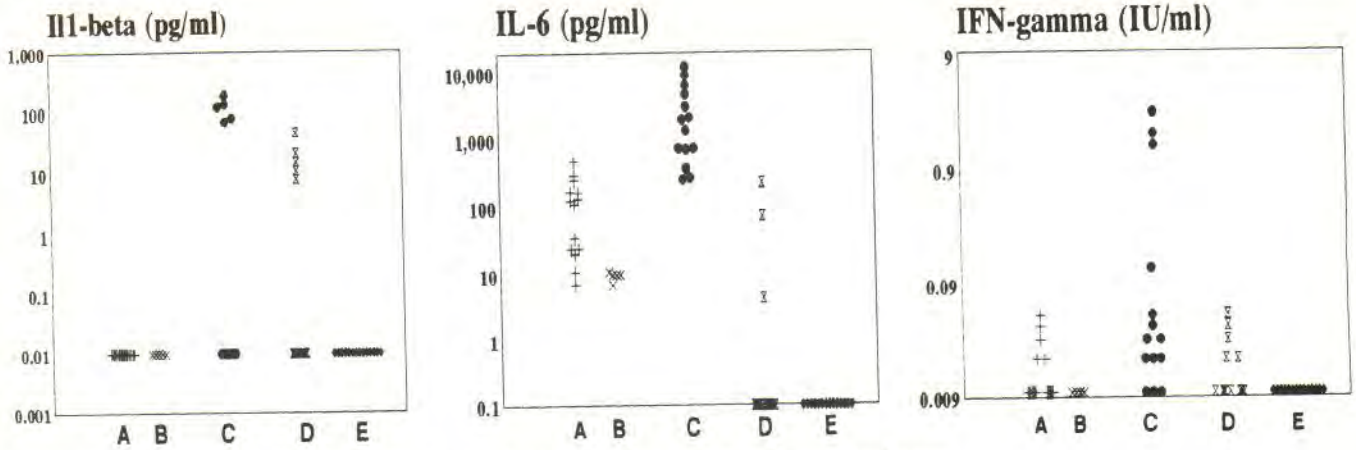

A = uninvolved skin; B = normal skin; C = involved skin; D = psoriasis serum; E = control serum.

DISCUSSION

The higher IL-1-beta levels in the BFs than in the sera confirm the local production of this cytokine at the lesional site. The direct correlation between BFs and serum levels of IL-1-beta may reflect its passage from the skin to the bloodstream. IL-1-beta is present in some ISBFs. Another study failed to detect IL-1-beta in ISBFs found overexpressed on the plasma membrane and in the intracellular compartment of epidermal cells from psoriatic-involved skin (3). This discrepancy may have been due to the different sensitivity of the assays used. At the present time, the role of IL-1-beta in the pathogenesis of psoriasis remains controversial even if an activation of lymphomonocytic cells (4, 5) has been indicated. IL-6 is a pleiotropic cytokine with a prominent pro-inflammatory activity; moreover, it has been shown to stimulate keratinocyte growth *'in vitro'* (6, 7). Interestingly, we could detect significant IL-6 levels in BFs derived either from involved and uninvolved psoriatic skin, or from normal skin. Significantly smaller IL-6 amounts were detected in psoriatic USBFs than in ISBFs, *al* though larger than in normal healthy skin. This finding could be important as it could be speculated that the pathogenetic mechanisms of psoriasis are activated, even to a lesser extent, also in apparently normal skin. Moreover, a direct correlation was found between the IL-6 levels in psoriatic ISBFs and the grade of erythema, suggesting a possible cause-effect phenomenon. Prens et al., using a different methodology, found no significant IL-6 levels in USBFs and in the normal skin (3). Differently from Grossman et al. (7), we detected IL-6 only in the sera of 3 of 14 patients. This disparity could also be due to the different methods employed, i.e. ELISA vs bioassay. The role of IFN-gamma in the pathogenesis of psoriasis is currently being investigated. This cytokine appears to possess many pro-inflammatory properties (8). Furthermore, IFN-gamma has been shown to act directly on keratinocytes to produce TGF-alpha (9). This latter can stimulate its own proliferation in an autocrine/paracrine manner (10). In our study, IFN-gamma could be detected in psoriatic ISBFs (78.5%), in USBFs (35%) and also in patients' sera (35%). In this regard, there are several disparities in the literature (11, 12). As reported for IL-6, some of the USBFs showed detectable IFN-gamma levels. The data provided herein confirm the presence of IL-1-beta, IL-6 and IFN-gamma concomitantly in lesions of a limited number of psoriatic patients. These individuals have the highest levels of IL-6, which seems to play a crucial role in the pathogenesis of psoriasis.

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Serum Levels of Interferons and TNF-α Are Not Correlated to Psoriasis Activity and Therapy

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Sera from 52 patients with psoriasis and 106 controls were tested for IFN-τ, IFN-α2 and TNF-α in ELISA and for total IFN activity using an infectivity inhibition micromethod. Psoriasis patients had lower serum levels of IFN-τ than had the controls: median 0.10 ng/ml vs. 0.16 ng/ml ($p = 0.01$). The highest median serum INF-τ levels were in patients with peripherally spreading psoriasis, 0.10 ng/ml, and acute guttate psoriasis, 0.09 ng/ml. Patients with stable plaque psoriasis had lower serum IFN-τ levels (median 0.0) than those with other forms of psoriasis, or blood donors. The serum levels of IFN-α2, total IFN activity and TNF-α did not differ between the psoriasis and control group. Treatment with cyclosporin, acitretin and the Goeckerman regimen increased the total IFN activity, but did not affect the levels of IFNs nor TNF-α. ***Key words: IFN-τ; IFN-α2; cyclosporin; acitretin; Goeckerman.***

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Psoriasis is a papulosquamous disease of unknown etiology. An infiltrate consisting mainly of activated CD4+ T lymphocytes and macrophages is regularly present in early and fully developed lesions (1, 2). Early in the disease process, T lymphocytes pass into the epidermis. The epidermal T lymphocytes are mainly of the CD8 phenotype and could react with autoantigens or foreign antigens (3). We have previously detected antiviral activity consistent with the presence of interferon (IFN) both in serum and in suction blister fluid from skin lesions in psoriasis patients (4). Both IFN-τ and IFN-α could be implicated. Another cytokine of interest in psoriasis is the tumour necrosis factor (TNF) which is synthesized by macrophages (5) and keratinocytes (6). TNF is cytotoxic for neoplastic cells and stimulates a variety of cells involved in immune responses.

The present study was undertaken to clarify whether the serum levels of IFNs and TNF-α are correlated to disease activity and therapy in psoriasis. We have examined IFN-τ, IFN-α2, total IFN activity and TNF-α in sera from patients with different clinical types of psoriasis, before and during treatment with cyclosporin, acitretin and Goeckerman regimen.

MATERIAL AND METHODS

The material consisted of 16 patients with stable, plaque-type psoriasis (A0) (age, mean 50.0 years, range 24–85), 22 patients with highly active psoriasis (A1) (age, mean 47.7 years, range 27–76) and 14 patients with acute guttate psoriasis (A2) (age, mean 31.3 years, range 18–49). None of the patients received local nor systemic anti-psoriatic therapy prior to the study. Eigth patients were treated with acitretin 0.5 mg/kg/day and 5 patients with cyclosporin 3–7 mg/kg/day. Nine patients received Goeckerman therapy (UVB radiation and 3% coal tar ointment) 3–6 times weekly. The serum samples were stored at -20°C until examination. Sera from 106 healthy blood donors (age, mean 36.6 years, range 19–65) were used as normal controls.

IFN-τ, IFN-α2 and TNF were measured using an ELISA method based on MoAbs developed at F.Hoffmann-La Roche Laboratories.

Total IFN activity was detected by an infectivity inhibition micromethod employing human embryonic lung fibroblast cells, in their fifth to fifteenth passage, and vesicular stomatitis virus as the challenge virus (7).

RESULTS

Psoriasis patients had lower serum levels of IFN-τ detected by ELISA than had the controls: median 0.10 ng/ml vs. 0.16 ng/ml ($p = 0.01$). The highest median serum IFN-τ levels were in patients with peripherally spreading psoriasis (A1), 0.10 ng/ml, and lowest in patients with stable, plaque psoriasis (A0), median 0.0 ng/ml. However, the differences between IFN-τ in the psoriasis groups were not statistically significant. On the other hand, there were significantly lower serum levels of IFN-τ in psoriasis patient groups A2 and A0 than in the healthy controls (Table I).

The serum IFN-τ levels did not change following therapy with cyclosporin, acitretin (Table II), or Goeckerman regimen (Table III).

The serum levels of IFN-α2 did not differ between the psoriasis (positive 2/33) and control groups (positive 7/34).

Nor was there any difference in serum levels of TNF-α between patients with psoriasis (positive 10/41) and controls (positive 7/40) (Table IV).

The serum levels of IFN-α2 and TNF-α did not change following therapy with the cyclosporin, acitretin and Goeckerman (Table IV).

Total IFN activity in serum increased following therapy with cyclosporin, acitretin and Goeckerman (Table IV). However, the number of sera tested was low and the increase was not statistically significant.

Table I. *Serum IFN-τ levels in patients with psoriasis*

Disease activity	No. of samples	IFN-τ (ng/ml)		
		Median	Mean±SD	Range
A2	14	0.09[a]	0.12±0.14	0–0.40
A1	22	0.10	0.44±0.75	0–2.90
A0	16	0.00[b]	0.05±0.09	0–0.30
Psoriasis total	52	0.10[c]	0.23±0.52	0–2.90
Controls	106	0.16	0.30±0.43	0–2.50

Statistically significant difference from controls at [a]$p = 0.01$, [b]$p = 0.0003$, [c]$p = 0.002$ using Mann-Whitney's test.

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Table II. *Acitretin therapy: Serum IFN-τ levels*

Weeks treated	Patients' initials							
	OL	HS	KH	GK	OV	RJ	AS	LB
Before	0.15	0	0	0.50	0	0	0.10	0
2	0.00	0	0	0.60	0		0.10	0
4	0.15	0	0	0.45	0	0	0.10	0
6	0.20	0	0	0.50	0	0	0.10	0
8	0.15	0	0	0.50		0	0	0
10	0.15	0	0	0.50				0
12	0.20	0	0					0
14	0.15	0	0	0.60				

Table III. *Goeckerman therapy: Serum IFN-τ levels*

Treatment no.	Patients' initials							
	DR	MN	AR	AT	MN	SS	HL	MK
Before	0.40	0.65	0.10	0	0	0	0.50	0
5	0.40	0.60	0.30	0	0	0	0.45	0
10	0.40	0.80	0.30	0	0	0	0.50	0
15	0.50	0.50	0.30	0	0	0	0.50	0
20	0.40	0.50	0.30		1.00	0.10		
25	0.50	0.50	0.30		0.10	0.10		
30			0.60		0	0.10		

DISCUSSION

In the present study the serum IFN-τ levels were higher in patients with the most active psoriasis than in patients with stable plaque psoriasis. However, the difference was not statistically significant. Taken together, the psoriasis patients had significantly lower serum IFN-τ levels than the healthy controls. There were no detectable differences in serum levels of IFN-α2 and TNF-α between the three psoriasis groups, nor compared with the controls.

IFN-τ produced by infiltrating activated T lymphocytes would induce KC in the psoriatic lesion to express HLA-DR antigens and intercellular adhesion molecule 1 (ICAM-1). TNF-α induces ICAM-1, but not HLA-DR. In psoriatic lesions the ICAM-1 expression (8) is more pronounced than the often weak HLA-DR expression (3,8). The reason might be that the infiltrating immune cells produce more TNF-α than IFN-τ. However, Takematsu et al. (9) reported the absence of TNF-α in suction blister fluids and stratum corneum from patients with psoriasis. On the other hand, there are data indicating production of IFN-τ in psoriatic lesions. IFN can be detected in suction blister fluid from psoriatic lesions (4) and in situ by staining with anti-IFN MoAbs (10). The normal TNF-α serum levels in patients with psoriasis contrast with the elevated levels we recently found in patients with systemic sclerosis (11).

Ultraviolet irradiation is a potent inducer of cytokine release from epidermal cells (12). The results were consistent with an increase in total IFN activity in serum in most patients following Goeckerman therapy, similar to what we have found earlier in sera and suction blister fluids (13). Diezel et al. (14) reported increased IFN activity after PUVA therapy. Measured by the infectivity inhibition method, we found an apparent increase in the anti-viral activity also after cyclosporin and acitretin treatment. There was no change in serum levels of IFN-τ, IFN-α2 and TNF-α measured by ELISA during therapy with Goeckerman regimen, acitretin, or cyclosporin. Konnikov et al. (15) reported elevated levels of plasma interleukin-1 (IL-1) in patients with psoriasis following UVB therapy for psoriasis, while Kowalzick et al. (16) found no change in serum levels of soluble IL-2 during PUVA therapy.

There are several possible explanations for the differing IFN results obtained with the infectivity inhibition method and the immunological assay. First, there may be other IFNs not detected by the ELISA: IFN-α subtypes, including acid-labile IFN-α and IFN-β. We have previously concluded that there are elevated levels of acid-labile IFN-α in sera from patients with psoriasis (17). Second, IFN-antibodies are inhibitors in the ELISA, but not in the virological IFN assay. Third, keratinocytes produce a variety of cytokines. Among these, IL-6 (IFN-β2) have a slight anti-viral activity such that we cannot exclude interference with the infectivity inhibition assay for IFN activity (18).

Table IV. *Effect of treatments for psoriasis on IFN and TNF. No. of positive sera*

No. pats treated	Treatment with		
	Goeckerman 9	Acitretin 8	Cyclosporin 5
Total IFN			
before	2	1	2
after	5	4	3
IFN-α2			
before	0	0	2
after	0	0	3
TNF-α			
before	1	1	2
after	1	1	2

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Neutrophil Responsiveness in Psoriasis

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During recent years, several studies have been focused on the involvement of immunological factors in the pathogenesis of psoriasis. In this regard, the accumulation of $CD4^+DR^+CD25^+$ lymphocytes into active psoriatic plaques appears to play a key role (1), even if recent data point out that also $CD8^+$ cells and $CD11c^+$ macrophages are implicated in the development of full-blown psoriatic lesions (2). At the same time, the psoriasis-related exaggerated release of several cytokines (CKs), such as interleukin 1 (IL-1), IL-6, IL-8, interferon τ (IFN-τ) and Tumour Necrosis Factor α (TNF-α), may either stimulate keratinocyte proliferation or trigger adhesion molecule expression on both endothelial and T cell surface, which is in turn responsible for T lymphocyte extravasation (3). The recent demonstration of an increased serum concentration of soluble intercellular adhesion molecule-1 in active psoriasis fully supports these findings (4).

On the other hand, the skin infiltration by polymorphonuclear cells (PMN), as detected in spongiform pustules of Kogoj and Munro's microabscesses, represents one of the salient histological features in active psoriasis (1). In the light of these results and on the basis of the elevated CK synthesis, a PMN activation has been suggested to occur at either skin or blood level. With regard to the latter point, our recent data clearly indicate that circulating PMN from active psoriatic subjects display a significant increase in their chemotactic responsiveness, superoxide anion (O_2^-) release, hydrogen peroxide (H_2O_2) generation, adherence property to either nylon fibers or fetal calf serum (FCS)-coated plates and lysosomal enzyme release (5).

Psoriatic scale-derived factors may account for the observed effects. Evidence has actually been provided for an enhanced production in psoriatic lesions of C5a anaphylatoxin, a peptide which stimulates PMN chemotaxis (6). Nevertheless, PMN enzyme activation might either upregulate the expression of surface chemotactic receptors or accelerate intracellular signal transduction (5). In this framework, a role for IL-8 cannot be ruled out, since an increase of surface IL-8 receptor density has recently been shown in PMN from psoriatic individuals (7). The augmented CK release may also be selectively involved in the triggering of PMN respiratory burst. TNF-α and IFN-τ are able to prime neutrophil suspensions for O_2^- generation by shortening the lag period following agonist stimulation (5). The resulting enhancement of oxidative metabolism may have an important in vivo counterpart, since reactive oxygen metabolites may exert noxious effects for host tissue by the induction of auto-oxidation processes (5).

The observation of an increased PMN adhesiveness to FCS-coated plastic substrates in active psoriasis implies the occurrence of a β_2 integrin-dependent mechanism. Proinflammatory CKs may, in fact, stimulate adhesion molecule expression on neutrophil membrane by favouring CD11b and CD11c translocation from intracellular stores to PMN surface (8). The pivotal role of these structures in such a phenomenon is also confirmed by the demonstration that monoclonal antibodies to β_2 integrins specifically inhibit PMN adherence to FCS-coated plastic plates (5). Finally, it should be stressed out that the augmented PMN adhesiveness gives rise to a further increase in respiratory burst, thus supporting a critical role for oxygen radicals in psoriasis-related skin damage (5).

In the light of these findings, the question arises whether peripheral blood PMN-mediated functional capacities and/or metabolic pathway positively correlate with disease activity. Our results clearly outline that PMN chemotactic and adhesiveness properties merely parallel the clinical course of psoriasis, since a strict relationship (85.7%) has been found between immunological and clinical data in a closed protocol (9).

As far as the mechanisms accounting for these effects are concerned, the possible influence of CK level on PMN-mediated responsiveness has to be pointed out. Elevated IFN-τ and TNF-α serum concentrations have been shown in untreated psoriatic patients, while cyclosporin-A or etretinate treatment down-modulates CK levels (10). Moreover, a transient IFN-τ increase usually precedes lesion relapses (10). Therefore, a drug-induced reduction of CK synthesis may account for the normalization of PMN chemotactic and adherence capacities. On the other hand, this is not the case for O_2^- generation, which is significantly enhanced in both active and inactive psoriatic individuals (9). A possible explanation for the unmodified response pattern might be represented by the elevated C5a serum levels in psoriatic-treated subjects, which is in turn responsible for the increased oxidative metabolism through complement pathway activation (9).

Taken together, these findings indicate that an activation status of circulating PMN occurs in active psoriasis and suggest the potential usefulness of PMN chemotactic and adhesiveness assays in the follow-up of the disease.

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The Hyperperfusion of the Psoriatic Plaque Correlates Histologically with Dilatation of Vessels

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We examined psoriatic lesions on the upper legs in 20 patients, using a two-dimensional Laser-Doppler-Scanner (Laser Doppler Perfusion Imager LDI, Lisca Development, Linköping/Sweden). The plaques were evaluated weekly during therapy with dithranol. Five plaques were reconstructed three-dimensionally before and after therapy (reconstruction program ANAT 3D, SIS, Münster, Germany). The psoriatic plaque was represented in the Laser Doppler Perfusion image as a sharply demarcated, hyperperfused area. The perfusion of the plaques dropped during therapy with dithranol to just slightly increased values, compared with normal skin (2.04 arbitary units AU, healthy skin 1.1 AU). Using three-dimensional reconstruction, we investigated the volume of dermal vessels and the density of papillae. When compared, the volume of papillary vessels was twice as large in psoriatic as in healthy skin. The number of the papillae per square millimetre, detected by three-dimensional reconstruction, was not reduced significantly during therapy. We think that the increased perfusion of the psoriatic plaque is due to the combination of morphological (dilatation of vessels), dynamic (increased blood flow) and optical effects (reduced scattering and increased sampling depth of the laser-beam in acanthotic tissue). ***Key words: psoriasis; perfusion; laser Doppler scanner; vascular alterations.***

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In psoriasis vulgaris we find specific alterations of vessels. Elongation and twisting of the papillary capillaries have been described using epiluminescence-microscopy (14). They are dilatated and seem to be, according to Barton et al. (2), increased in number.

Psoriatic lesions consistently show increased blood flow, described for example by MacDonald Hull et al. (15). We compared the perfusion-image of the Laser Doppler Scanner with the three-dimensional architecture of the capillaries to detect the histological correlate to the increased perfusion of the psoriatic plaque.

MATERIAL AND METHODS

We examined the psoriatic lesions on the upper legs of 20 patients, using a two-dimensional laser-Doppler-scanner (Laser Doppler Perfusion Imager LDI, Lisca Development, Linköping/Sweden). After removal of the scales with salicylic acid 5% over 3 days, the plaques were assessed weekly during therapy with dithranol. Five plaques were reconstructed three-dimensionally before and after therapy.

Laser Doppler scanner

The laser Doppler scanner works without direct skin contact, producing an image of the perfusion pattern of the skin. The laser beam scans the skin surface by means of a mirror system. A maximum area of 12 by 12 cm can be detected. The medium sampling depth of the instrument is 200 μm. According to the Doppler-effect, moving erythrocytes reflect the beam partly. The intensity of the Doppler signal corresponds to the product of the number per volume and the velocity of the erythrocytes. The Doppler signal is received by a photodetector and digitized. The colour-coded perfusion image consists of up to 4,096 measuring points. The intensity of the perfusion is expressed in arbitary units (AU), the value of the signal height ranges between 0 and 10 AU. In every area of the perfusion image, single point measurements as well as mean flux values and standard deviations can be calculated.

Three-dimensional reconstruction

Paraffin or Epon 811 embedded 7 μm resp. 1 μm sections were cut parallel to the skin surface. The structures of interest (papillary body and vessels of the papillary body) were fed into an IBM-compatible 386DX computer and reconstructed three-dimensionally by means of the reconstruction program ANAT 3D (8). Using this model we calculated the number and volume of the papillae as well as the volume of the vessels per papilla.

Epiluminescence-microscopy

Using epiluminescence-microscopy at magnification 400 (Scopeman 503, FORT-GmbH, Germany) we determined the number of capillaries per square-millimetre in the psoriatic lesion before and after the treatment period. The examinations were performed with the patient in prone position. For better visualization of the vessels we used immersion oil.

RESULTS

The psoriatic plaques were represented in the Laser Doppler Perfusion image as sharply demarcated, hyperperfused areas.

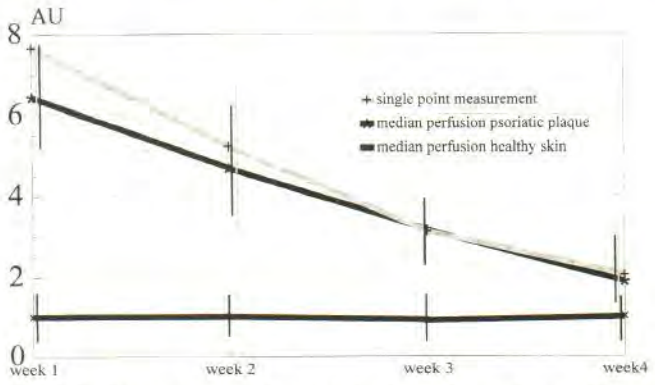

Fig. 1. Perfusion course of the psoriatic plaque during therapy. Single-point measurements and median perfusion of psoriatic and healthy skin of the upper leg.

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Fig. 2. Three-dimensional reconstruction of a psoriatic plaque. pink = papillae, yellow = hair follicle, green = irregularities of the dermo-epidermal junction.

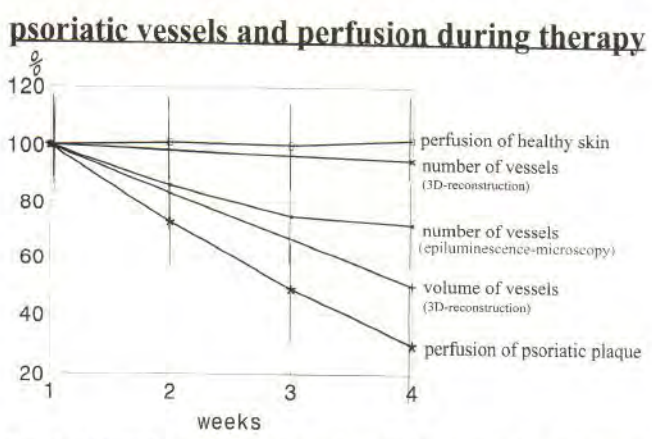

Fig. 3. Schematic image of the reduction in the density of vessels, vessel volume and skin perfusion of the psoriatic plaque (in percent). The reduction in the vessel volume per papilla parallels the decrease in the perfusion of the plaque.

The medium perfusion of the plaques before therapy was 7.66 AU ± 1.81 AU (surrounding healthy skin 1.0 AU ± 0.5 AU). The perfusion-image of the plaque showed marked inhomogeneities, the perfusion range of the untreated plaque was between 4 and 9 AU.

The perfusion of the plaques dropped during therapy with dithranol to just slightly increased values, compared with normal skin (2.04 AU, healthy skin 1.1 AU). In 3 of the 20 cases the decrease in the perfusion was not continuous. These 3 patients developed a dithranol dermatitis during the first week which was reflected in a short-term perfusion enhancement of 2.0 to 3.0 AU. Fig. 1 shows the median perfusion profile of the examined population during therapy.

The three-dimensional reconstruction (Fig. 2) shows the papillae of an active plaque before therapy, from the side. The papillae are displayed in pink, the irregularities of the dermo-epidermal junction in green. The papillae were elongated and the vessel volume of the papillae was enlarged. The mean papillary volume of the psoriatic skin was about 800 μm^3, one of the vessels being about 93.8 μm^3. This volume decreased during therapy to 669.9 μm^3 and 40.5 μm^3, respectively. This means that the vessel volume of the psoriatic papilla is about 10.5% in fully developed lesions and 5.7% after therapy. The number of papillae per square millimetre, determined by three-dimensional reconstruction, was not reduced significantly during therapy.

As opposed to the results of the three-dimensional reconstruction, epiluminescence microscopy showed a marked reduction in the number of papillae during therapy (30 to 21/mm^2). The capillaries of the psoriatic plaques were dilated and tortuous. The number of capillaries of the surrounding healthy skin remained constant.

Fig. 3 summarizes the results. The reduction in the perfusion of the psoriatic plaque correlates with the decrease in the vessel-volume per papilla. The number of capillaries, as shown by epiluminescence-microscopy, was reduced by as much as 72% during therapy. With three-dimensional reconstruction, however, no decrease could be detected concerning the density of papillae or capillaries.

DISCUSSION

The increased perfusion of the psoriatic plaque is due to a combination of morphological (dilatation of vessels), dynamic (increased blood flow) and optical effects (reduced scattering and increased sampling depth of the laser-beam in acanthotic tissue). The decreasing perfusion of the psoriatic plaque during therapy has been evaluated up to now only by means of one-dimensional Laser Doppler (6, 15). The extraordinary inhomogeneity in the perfusion of psoriatic skin however reduces the validity of single-point measurements such as Laser Doppler Fluxmetry. This is supported by our own comparison between single-point measurements and evalution of an area, represented by Laser Doppler Scanning (Fig. 1). Though healthy skin reveals inhomogeneities in perfusion as well (5, 16), the perfusion amplitude of the psoriatic skin is much higher.

According to Barton et al. (2) the blood-volume of the psoriatic capillary doubles or trebles. We were able to confirm this using three-dimensional reconstruction: the volume of the capillaries in the plaque was twice as large as in healthy skin. The increase in the number of psoriatic capillaries has been reported by several authors (2); their results are based on epiluminescence-microscopy or capillaroscopy. This increase seems to be due to functional rather than morphological reasons:

As epiluminescence-microscopy shows the functional density of vessels, only opened vessels can be identified, the large number of closed vessels in healthy skin remaining invisible (7). This means that by epiluminescence-microscopy, the diameters of the erythrocytes are determined by measuring the diameter of a vessel. Due to the dilatation up to twice or three times the volume in the psoriatic plaque, every capillary can be seen in the epiluminescence microscope (7). This relation between the number of the vessels and their volume has also been described for other diseases such as peripheral occlusive disease (10, 11). The number of the visible capillaries is reduced parallel with the arterial pressure (10).

The investigations of Bacharach-Buhles et al. (1) call for an unchanged course of the vessels in psoriasis, in comparison with healthy skin. Because of the acanthosis, the psoriatic vessels can

be seen more easily. As the first vascular plexus is located in the papillary body, the tortuous course and the elongation of the vessels revealed by epiluminescence-microcopy are caused by this visible first plexus of the skin.

In contrast to epiluminescence-microscopy, three-dimensional reconstruction is independent of functional parameters. Even very small capillaries are detected using serial sections. For that reason, in three-dimensional reconstruction, no additional capillaries or papillae can be seen in the psoriatic plaque (see Fig. 3). The results of this labour-intensive method are reliable, particularly due to its detailed illustration.

According to the results of Bacharach-Buhles et al. (1) it has to be discussed, whether the penetration depth of the Laser Doppler Scanner in psoriatic skin is increased and whether deeper vessels such as the deep cutanous and subcutaneous plexuses contribute more to the perfusion image. Contrary to Braverman and Sibley (4), Bacharach-Buhles et al. postulate an elongation of the rete pegs only, but not of the subepidermal vessels. The location of the former, in healthy skin subpapillary plexus in the dermis remains unchanged. With the elongation of the papillae during the psoriatic transformation, this first plexus is not displaced downwards but incorporated into the papillary body as an intrapapillary plexus. As the distance of this first plexus of the skin to the surface of the epidermis remains constant, only the relation between rete pegs and corium is changed in favour of the rete pegs. In the inhomogeneous corium, the laser beam is scattered more than in a homogeneous structure such as acanthosis. The laser beam is less scattered on its way to the deep plexus. This thesis is supported by the investigations of Utz (17), who described increased transmission of light having a wavelength in excess of 300 nm. The dependence of the median sampling depth from the tissue also has been described by Jakobsson and Nilsson (12) and differs by up to factor ten, according to the tissue. Not only are more vessels detected by the laser beam, but also the number and median velocity of the erythrocytes are higher in the vessels of the lower dermis than in the subpapillary network (12). The blood flow of the subcutis is increased even more. Using three-dimensional reconstruction, it has been proven that this increase is caused by subcutaneous shunt vessels (9). Apart from increased blood volume, the blood flow in the psoriatic lesion is increased as well (3).

To sum up: the density of neither vessels nor papillae is increased in the psoriatic lesion (7). Dilatation of vessels is the histological correlate to the increased perfusion in the psoriatic plaque.

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Sonographic and NMR Imaging Study of Sausage Digit

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Swelling of the fingers and/or toes, giving them a sausage-shaped appearance (7), is commonly called 'sausage digit' (SD) or even 'cocktail hot-dog digit' (3). Distal interphalangeal (DIP) and proximal interphalangeal (PIP) joints of the hands and feet, and metacarpophalangeal (MCP) or metatarsophalangeal (MTP) joints are usually involved and inflammation of associated tendon sheaths is present. Often other concomitant inflammatory signs are found (pain, inflammation and functional incapacity). SD consists of both intra-articular effusion of the involved joints and flexor tendon sheath inflammation (8, 9). More rarely, a periosteal reaction is present too.

SD is one of the clinical manifestations of asymmetric oligoarticular arthritis occurring in Psoriatic Arthritis (PA), which is the most common pattern of the disease, accounting for more than one-half of all patients (7). SD is seldom present also in Reiter's Syndrome (1, 2, 6). Much more rarely, and in the absence of trauma, a diffuse swollen digit can occur in osteomyelitis, cellulitis and gout, when it usually involves one single finger or toe, though sometimes two or three SD may be present simultaneously.

The presence of SD could be connected with major histocompatibility complex (MHC) and particularly with HLA-Bw38 and/or B27 (5).

In the international literature, there are few reports (4, 6) on the use of imaging methods in the study of SD – and they are restricted to low kilovolt radiography for the examination of generic soft tissue swelling.

In our study we examined SD by means of two imaging methods recently introduced into skeletal system pathology: Nuclear Magnetic Resonance (NMR) and Sonography (SN).

MATERIALS AND METHODS

The hands of 6 patients with SD and asymmetric oligoarticular pattern of PA, diagnosed using international criteria for spondiloarthropathies, were studied. They were 4 males and 2 females whose mean age was 33.2 years (range 23–54). The mean illness duration was 4.8 years (range 2–11). In 5 patients just one SD occurred, while one patient had three SD on a hand and two SD on a foot, simultaneously. All the patients were hospitalized at the Rheumatology Department of Rome University "La Sapienza".

No patient showed significant periosteal abnormalities of the hands, by standard radiographic imaging.

NMR was performed by means of an arthro-scanner instrument. SN was carried out by means of a 'real-time' instrument and a 7.5 MHz linear probe; when necessary, a gel spacer was interposed.

RESULTS

SD was present in 6 patients (17.1%) out of 35 subjects with an asymmetric oligoarticular pattern of PA who had been hospitalized in our Rheumatology Department.

In all patients the more significant finding of NMR was stretching of the tendon sheaths of the finger flexors. Three patients had also significant stretcing of the articular capsule of PIP and DIP joints. Those findings indicated the presence of effusion.

In the patient who had three fingers with a sausage shape, NMR imaging showed a stretching of the tendon sheaths of the finger flexors at the metacarpal level too (Fig. 1). At consecutive examinations made in the course of medical treatment which caused resolution of symptoms, we found gradual improvement of the sausage shape; morphological 'restitutio ad integrum' appeared in 4 patients with early onset therapy. Only in one patient who had PA at a very late stage did the tendon alterations persist.

In all cases, SN revealed the presence of a hypoechoic band surrounding the flexor tendons. Concomitantly, the soft tissues of the dorsal surface of the finger appeared hypoechoic, both because of the extensor tendon inflammation and also due to peritendinous and subcutaneous tissue edema. In all cases, SN confirmed the presence of effusion both of DIP joints (3 cases) and of PIP joints (6 cases), showing an unechoic area within the involved joints (Fig. 2).

DISCUSSION

NMR imaging has made it possible to study the soft tissues of the hand in SD. By means of this imaging method, stretching of tendons sheaths of the finger flexors was visualized. In some cases a common tendon sheath of the finger's flexors could be present at the metacarpal level and this anatomical anomaly may confirm the presence of multiple finger involvement and the appearance of multiple SD. Moreover, it is possible that SD is related to MCH, particularly to HLA-Bw38 and/or B27, which are often present when involvement of tendons is found in PA.

SN revealed the presence of effusion of interphalangeal joints and confirmed the involvement of tendons. Concomitant edema of soft tissues was also found.

SN and NMR imaging demonstrated their usefulness in the study of inflammatory changes of SD, and showed the relationship between imaging and pathological anatomy.

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Psoriatic Scales: An Ultrastructural Study

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Ultrastructural studies on psoriatic skin have revealed peculiar epidermal alterations (1). Above all, stratum corneum changes were considered of great importance in the pathogenesis and classification of psoriasis (2). Changes in the keratin pattern of corneum cells, reduction in the number of desmosomes and the presence of a large number of vacuoles in their cytoplasms are the most important ultrastructural findings reported (1). The aim of this study was to evaluate by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) the ultrastructure of psoriasis scales, in order to ascertain possible correlations between morphological data and pathogenetic hypotheses.

MATERIALS AND METHODS

To perform SEM and TEM analyses, we collected corneum scales from psoriatic patients on condition that they had stopped topical and/or systemic therapy for over a month. Specimens for SEM were obtained using (*a*) silicone replica technique, or (*b*) horny layer biopsy technique by means of cyanoacrylate adhesive stripping. Specimens for TEM were obtained using conventional technique. Ultrathin sectioning was carried out parallel to the major axis of the scales.

RESULTS

Our results are summarized in Table I. SEM observation of the stratum corneum surface revealed a smoothing out of the secondary texture lines. Besides this we noted desquamation of clusters of cells and corneum cells having a polyhedric shape. The surface of the great majority of squamous cells revealed a pronounced villous pattern (3) (Fig. 1). We also noted the presence of abnormal superficial keratinization.

TEM observation helped us to detect very tortuous cell to cell fittings. The keratin pattern appeared loosely aggreated. Dilatated and sinuous interlamellar spaces presented two types of interlamellar material: (*a*) finely granular, and (*b*) lamellar substructure. This material sometimes had a whorled appearance (Fig. 2). We observed many vacuoles in corneum cell cytoplasm and substructures resembling giant lamellar bodies (Fig. 3).

Table I. *Cellular changes*

	Normal stratum corneum	Psoriatic stratum corneum
SEM		
Surface texture	Presence of primary and secondary lines	Absence of secondary lines
Cellular desquamation	Single cells	Cell clusters
Villous protrusion	Regular	Pronounced
Parakeratosis	Absent	Presence of nuclear bulge
TEM		
Cellular surfaces	Linear	Wavy
Intercellular spaces	Regular	Dilated
Keratin pattern	Filamentous	Loosely aggregated
Vacuoles	Absent	Present

COMMENT

Psoriasis is and inflammatory skin disorder characterized by a pronounced hyperproliferation of keratinocytes. Hitherto, the aetiology of psoriasis has been considered obscure and it may be due to this that several pathogenetic hypotheses have been formulated to speculate on its origin (4–5). Stratum corneum in psoriasis presents several ultrastructural aspects evoking the proposed pathogenetic hypotheses. Smoothing of secondary lines and desquamation of clusters of cells could be considered, on the one hand, to be an effect of cellular infiltration into psoriatic plaques. On the other hand, it could be a symptom of increased cellular turnover. SEM evidence of a pronounced villosity of corneum cell surfaces correlates with tortous cell-cell fittings observed by TEM and this could partly explain the clusters of desquamating cells. Near the villi, we did not observe the "pore-like" structures described by Mishima (6) on the surface of psoriatic keratinocytes. We speculate that villi are sometimes substituted by a network of plasma membrane extraflexions – as if the villi could merge together to form the "pore-like structures".

To find abnormal keratinizing structures such as conic extraflexion on the cell surfaces at SEM and an abnormal keratin pattern at TEM calls attention to psoriasis as being an aberration of keratinization. The vacuoles in the cytoplasm of squamous cells have been regarded as lipid droplets (7). This finding could be correlated with the pathogenetic hypothesis that psoriasis is a disease characteristic of an altered interlamellar lipid metabo-

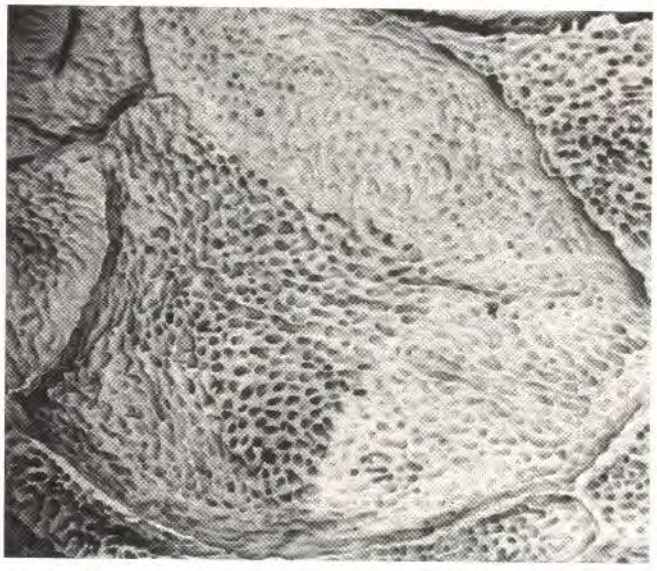

Fig. 1. Prominent cell surface villous pattern (×2100).

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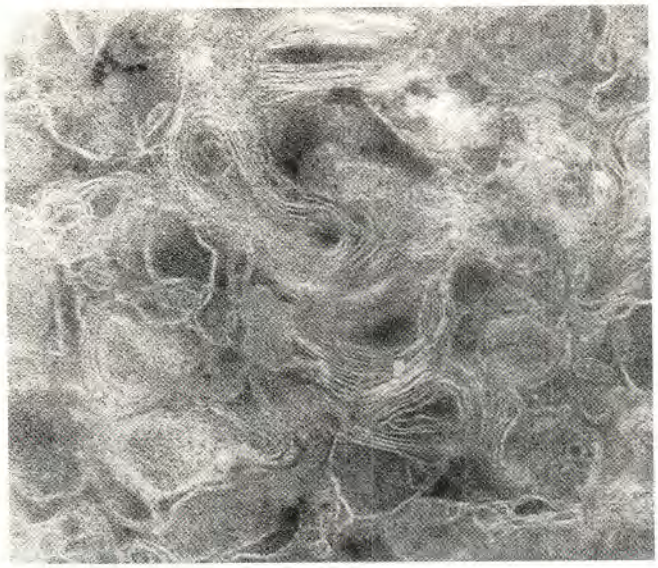

Fig. 2. Dilated interlamellar space filled with a material having a lamellar substructure (×52000).

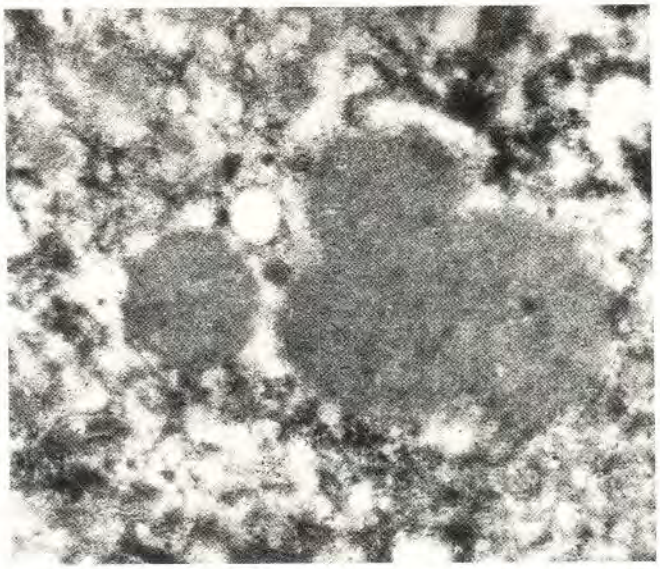

Fig. 3. Cytoplasmic organelles resembling abnormal and enlarged lamellar bodies (×21000).

lism (8). This alteration has its clinical evidence in a deranged water barrier function, as demonstrated by evaporimetric measurements on psoriatic skin. The numerous lipid vacuoles and the presence of a substructure reminiscent giant lamellar granules suggest that it is not the quantity but the quality of lipid composition that plays a role in the pathogenesis of psoriasis. We underline the observation of dilated intercellular spaces filled with finely granular material. This is a PAS-positive material interpreted as glycoproteins having cell-to-cell adhesive function. It is conceivable that this finding is linked to the pathogenetic view of an intercellular adhesion and alteration of membrane surface coats of epidermal cells in psoriasis (9).

In conclusion, our findings confirm that psoriasis has a complex pathogenesis, though morphological data suggest only a part of the pathogenetic hypotheses proposed to explain the formation of psoriatic lesions - but not others such as the hereditary, the biochemical and the immunological ones. Future studies should be oriented to establish the sequence of these pathogenetic mechanisms.

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ABSTRACT

Optical Properties of Psoriatic Epidermis

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We have performed in vitro measurements of diffuse reflectance (R) and total transmittance (T) coefficients (covered wavelengths from 240 to 400 nm) of the upper epidermis layers, obtained by means of a cyanoacrylate skin surface stripping technique. A commercially-available spectrophotometer (Cary 2415) with integrating spheres was used. The linear absorption and scattering coefficients of the samples were reconstructed from R and T measurements using 4-flux Kubelka-Munk theory. The optical properties of epidermis from patients in different stages of psoriasis vulgaris (PV, $n = 12$) and from normal individuals (N, $n = 15$) with no personal or family history of psoriasis were investigated. The results of in vitro measuring for R and T are given in the table.

λ, nm		260	280	300	320	340	360	380
R, %	N	5.5	4.7	5.6	7.5	8.2	9.0	10
	PV	9.8	10	10.2	13.5	14	14.2	15
T, %	N	38	35	46.6	58.4	62.1	67.4	74
	PV	27	24.5	54.8	81.5	78.3	80	80

The differences in optical properties of normal and psoriatic epidermis can be used to diagnose skin pathology and to develop new photo- and photochemotherapy techniques.

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Lipoprotein Peroxidation in Adult Psoriatic Patients

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Psoriasis, a chronic inflammatory skin disease characterized by an aberration of lipid metabolism, has been associated with an increased risk for atherosclerosis. Since oxidatively modified lipoproteins are involved in the pathogenesis of atherosclerosis, we investigated the lipid composition and in vitro induced peroxidation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) from psoriatic patients. 11 male adult psoriatics and 16 male age-matched healthy subjects were studied. Lipid peroxidation of VLDL and LDL was performed by incubation with $CuSO_4$ for 24 h at 37°C. The compositional analysis showed a significant increase in tryglycerides and phospholipids, both in VLDL ($p < 0.05$) and in LDL ($p < 0.001$) from psoriatic patients, compared with controls. Moreover a significant increase in total cholesterol (TC) ($p < 0.01$) and apoprotein (P) ($p < 0.05$) was found in LDL from psoriatics. The levels of thiobarbituric acid reactive substances (TBARS), as a measurement of lipid peroxidation, were significantly higher in Ox-VLDL and in Ox-LDL from psoriatics ($p < 0.01$) than the corresponding values in controls. Moreover, basal values of TBARS were significantly higher in VLDL and LDL from psoriatic patients than those from controls. In conclusion, the lipoprotein compositional changes associated with the modifications of TBARS before and after Cu^{2+} treatment of lipoproteins may suggest an increased risk for atherosclerosis in adult psoriatic patients. ***Key words: psoriasis; low density lipoprotein; very low density lipoprotein; lipid peroxidation; atherosclerosis.***

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The etiology of psoriasis, a chronic inflammatory skin disease characterized by an accelerated turnover of epidermal cells and an incomplete differentiation in lesional epidermis, is still unknown but genetic, metabolic and immunologic mechanisms have been suggested (1). At membrane level, modifications of phospholipid (PL) fatty acid composition with a significant increase in arachidonic acid (AA) in the plasma membrane of skin cells and erythrocytes of adult psoriatic patients (2) suggest an aberration in the lipid metabolism as a generalized phenomenon in psoriasis. The lipid compositional changes, as demonstrated in our previous studies, are associated with modifications of membrane fluidity in erythrocytes of adult and pediatric subjects (3, 4).

Changes in plasma lipid and lipoprotein composition in middle-aged male psoriatic patients (2) that include a tendency toward an increase in cholesterol and triacylglycerol, associated with very low density lipoproteins (VLDL) and a decrease in high density lipoprotein-cholesterol (HDL-C), also suggest that psoriasis may be linked to disorders of lipid metabolism (5). The modifications in plasma lipoprotein composition, more pronounced in patients with the severe form of the disease (5), may be related to the increased risk for atherosclerosis observed in adult psoriatic patients (6). In fact, many epidemiological studies have clearly demonstrated that the risk for coronary heart disease is positively correlated with low density lipoproteins (LDL) and plasma total cholesterol (TC) and negatively correlated with high density lipoproteins (7–8). Moreover, recent studies have demonstrated that also oxidatively modified lipoproteins play a key role in the pathogenesis of atherosclerosis (9–10).

To further investigate plasma lipoprotein alterations in psoriasis, we studied the chemical composition and the susceptibility to in vitro oxidation, triggered by copper ions, of VLDL and LDL isolated from adult psoriatic patients, compared with healthy adult subjects.

METHODS

Patients

We studied 11 male adult patients, affected by psoriasis. In all the patients the disease was moderate, stable, and of plaque type. Mean age was 58 ± 16 years. None of the patients had received any systemic or topical medication for at least 2 weeks preceding the study. The duration of the disease ranged from 5 to 30 years. None of the patients had a history of cardiovascular disease or familial hyperlipidemia, nor had a known diabetes mellitus and all had normal laboratory tests for liver and renal function.

The control group consisted of 16 healthy adult male subjects, of mean age similar to the psoriatic patients.

Isolation of VLDL and LDL and in vitro peroxidation

Human plasma VLDL and LDL were prepared by sequential ultracentrifugation (L70 Beckman Ultracentrifuge), according to Havel et al. (11) and they were dialysed against 150 mM NaCl before peroxidation treatment. The peroxidation of VLDL and LDL was performed as described previously (12).

500 µl of VLDL, with a protein concentration of 0.03 g/l, was incubated with 10 µM CuSO4 for 24 h at 37°C. LDL were incubated with copper ions (5 µM) for 24 h at 37°C.

The determination of peroxidation products was performed by evaluating thiobarbituric acid reactive substances (TBARS) as described by Yagy (13).

Chemical composition of lipoproteins

The levels of cholesterol (C), triacylglycerol (TG) and phospholipids (PL) in total plasma level and lipoprotein fractions were determined by enzymatic methods (14–16).

Protein concentration was determined as described by Lowry et al. using bovine serum albumin as standard (17).

Statistics

All the results are expressed as means ± SEM. Statistical differences between data from psoriatic patients and controls were determined according to Student's *t*-test.

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Table I. *Composition of VLDL and LDL from psoriatic patients and controls*

	VLDL	LDL
Cholesterol (mmol/l)		
controls (16)	0.6±0.5	4.1 ±2.54
patients (11)	1.5±1.2	9.67±3.41***
Phospholipids (mmol/l)		
controls (16)	0.4±0.3	1.3 ±0.83
patients (11)	1.1±0.7*	3.71±1.31***
Triacylglycerol (mmol/l)		
controls (16)	1.1±0.7	0.61±0.43
patients (11)	2.6±1.5*	1.30±0.69*
Proteins (g/l)		
controls (16)	0.2±0.2	1.54±1.01
patients (11)	0.3±0.3	2.97±0.97*

The values are expressed as means ± SEM; ***$p<0.01$, *$p<0.05$.

RESULTS

Lipoprotein composition

The contents in TG and PL were significantly increased in VLDL of psoriatic patients, compared with controls ($p<0.05$), while protein and cholesterol levels were not modified (Table I). Compositional changes have also been observed in LDL from psoriatic subjects, with a significant increase in TC and PL ($p<0.01$), TG ($p<0.05$) and P content ($p<0.05$) (Table I).

Lipoprotein peroxidation

The susceptibility of plasma VLDL and LDL derived from psoriatic subjects to in vitro oxidation with copper ions was compared with that of lipoproteins obtained from male adult controls. The levels of TBARS in VLDL and LDL from psoriatic patients were significantly higher compared with the corresponding values in lipoproteins from controls before chemical treatment (Table II).

In VLDL and LDL of controls, a significant increase in TBARS after incubation of lipoproteins with copper ions, demonstrates the efficacy of the peroxidation treatment (Fig. 1). A significant increase in TBARS in Ox-VLDL and Ox-LDL from psoriatic patients has also been shown, with respect to untreated VLDL and LDL (Fig. 1).

DISCUSSION

Psoriasis in middle-aged patients has been associated with alterations of plasma lipid levels (5). However, a detailed biochemical compositional study of plasma lipoproteins has not previously been carried out in psoriatic patients. In particular, the levels of PL and apoprotein that, at the lipoprotein surface, constitute the interphase between water and non-polar lipid core and which help to stabilize the characteristic structure of lipoprotein particles, has not been investigated previously. This interphase is directly involved in exchange processes between lipoproteins and cells (18).

The significant increase in TG content observed in the present study in VLDL and in LDL from psoriatics is in agreement with previous findings in adult and pediatric patients (5, 19). Moreover, a significant increase in PL has been observed in VLDL and in LDL from psoriatic subjects.

Table II. *Oxidative state of native VLDL and LDL in controls and patients*

Lipoprotein oxidation (nmol MDA/mg protein.

	VLDL	LDL
Controls	2.99±0.92	15.84±1.43*
Patients	9.87±3.00	35.22±4.74**

*$p<0.001$ and **$p<0.01$ vs controls.
Values are expressed as means ± SEM.

The regulation of lipid composition in lipoproteins involves complex mechanisms; crucial components are lipoprotein receptors in the liver and extrahepatic tissues that mediate the uptake and degradation of cholesterol-carrying lipoproteins (20). Changes in the transport of LDL receptors to the cell surface have been shown in psoriatic vis-à-vis normal skin (21). Moreover, a lowered LDL-receptor activity has been observed in cultured dermal fibroblasts isolated from the skin of psoriatic patients (22). The LDL receptor abnormalities, if confirmed in other psoriatic cells, could be involved in the changes in low density lipoprotein composition.

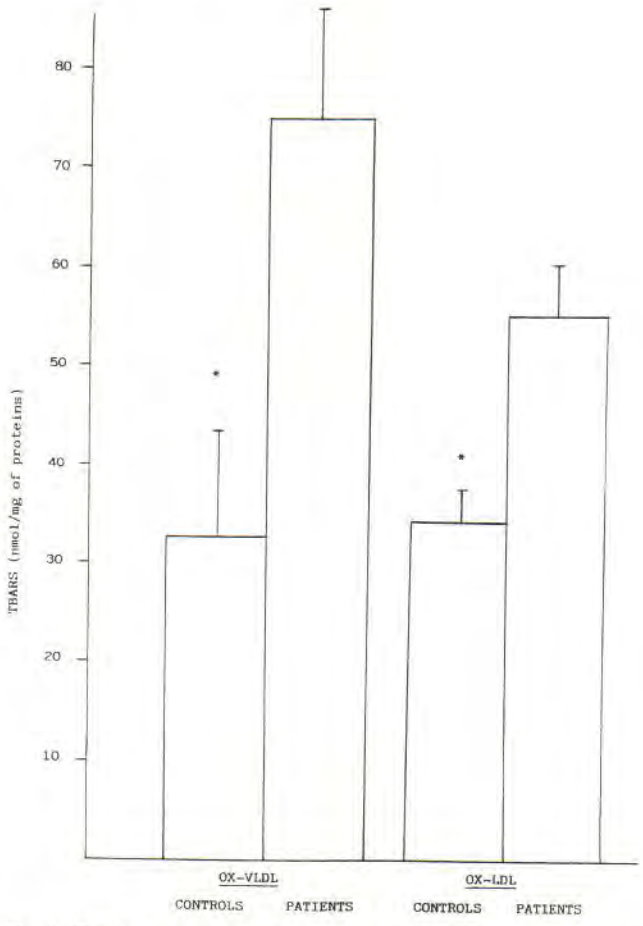

Fig. 1. TBARS content (nmol/mg of protein) in VLDL and LDL from controls and psoriatic subjects after incubation with copper ions ($p<0.01$).*

In lipoproteins from psoriatic patients the lipid compositional changes are associated with modifications of lipid peroxidation as demonstrated by the levels of TBARS, before and after Cu^{2+} treatment, that are significantly higher in VLDL and LDL of psoriatic subjects than in lipoproteins from controls. Lipoprotein oxidation is dependent on the availability of the lipid substrates; therefore the higher basal level mediated oxidation observed in VLDL and LDL can probably be ascribed to the increase in PL and TC in lipoproteins of patients compared with controls. In fact, Cu-triggered oxidation involves mainly PL and TC that are localized at the lipoprotein surface. In psoriasis, modifications in susceptibility to lipid peroxidation have also been demonstrated at membrane level, with an increased production of MDA following stimulation with thrombin in platelets of psoriatic adult patients (23). These alterations have been correlated to the changes in PL fatty acid composition, with an increase in AA that exposes the membrane to lipid peroxidation by free radicals (23).

In conclusion, our results demonstrate lipid compositional changes and a higher susceptibility to in vitro oxidation in VLDL and LDL of psoriatic patients vis-à-vis controls.

Some hypotheses can be advanced about the mechanism (s) that result in the compositional changes in lipoproteins in psoriasis.

As far as concerns the higher susceptibility to in vitro peroxidation of VLDL and LDL of psoriatics, since oxidatively modified lipoproteins play a key role in the pathogenesis of atherosclerosis, our results support an increased risk for atherosclerosis as previously suggested in adult psoriatic patients (6). This hypothesis is also supported by the significant increase in TG in lipoprotein of psoriatics. The role of triglycerides and of TG-rich-lipoproteins in atherogenesis and thrombogenesis has recently been affirmed (24–25).

The usefulness of fish oil supplementation, rich in polyunsaturated fatty acids (PUFA), in the treatment of psoriasis has been proposed previously (26). However, it must be emphasized that a large increase in PUFA in plasma lipoproteins increases the risk of lipid peroxidation (27). The higher susceptibility to in vitro oxidation demonstrated in the present study in lipoproteins of psoriatics and the modest clinical improvement in adult patients after fish oil treatment (28), do not justify a diet rich in PUFA in the treatment of psoriasis.

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Workshop on Calcipotriol

Chairman: J. D. Bos

J. Serup
O. Baadsgard
K. Kragballe
A. C. Chu
J. Berth-Jones
C. Scarpa

ABSTRACT

Calcipotriol Irritation: Mechanism, Diagnosis and Clinical Implication

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Irritant dermatitis of any degree occurs in 15–20% of psoriatics treated with calcipotriene (Calcipotriol INN, MC903) ointment. The onset is typically 2–4 weeks after treatment was started. There are two distinct clinical types, viz. lesional/perilesional and head and neck dermatitis. The dermatitis is both cases directly related to the drug in situ, topically applied to the lesions or transferred by the hand-to-face route with the facial region representing a locus of minor resistance.

Calcipotriene is not cytotoxic *per se* in clinically relevant formulations. The irritation is of the indirect or secondary type, in a way resembling irritation by retinoids. Occlusive patch testing of calcipotriene solution 50, 10, 2, 0.4 μg/ml on untreated controls shows many doubtful and 1+ reactions, while 2+ reactions are umcommon. 1,25-dihydroxy-vitamin D_3 in equimolar application shows the same frequency of irritant reactions. Application of calcipotriene 500 μg/ml results in no more frequent irritancy than 50 μg/ml, and the upper end of the dose irritation curve is not linear as it is with the primary irritant sodium lauryl sulphate (SLS). It is not known whether the irritant and the therapeutic effects of calcipotriene share a common mechanism.

Evaluation of positive patch test reactions to calcipotriene by means of bioengineering techniques shows increased blood flow but minor or no increase in transepidermal water loss (TEWL). The surface may be papular. Ultrasonography shows a subepidermal echolucent band and disturbed pilosebaceous units.

Irritancy to calcipotriene is to some extent vehicle dependent. The ointment vehicle containing propylene glycol 10% probably enhances shunt penetration via the pilosebaceous unit. In the scalp a gel and a cream formulation gave more frequent irritation than an isopropanol solution.

In patients exhibiting a severe dermatitis reaction during therapy, allergic sensitization is a possibility. Nevertheless, it is a clinical experience that calcipotriene is often tolerated again after an interval. Allergy patch testing should not be performed with the ointment, but with a buffered isopropanol solution and probably in 1% dilution.

The non-irritant threshold concentration is not yet known, but Leo studies to solve this problem are proceeding.

There is a high risk of false-positive reactions, particularly +?, and 1+, while 2+ reactions may be indicative of allergy, especially if reproducible after a period.

For the clinical dermatologist it is more simple and conclusive to perform a repeated open application test (ROAT) with the ointment (BID for 7 days, antecubital skin) and decide whether the patient should in the future avoid calcipotriene, or not.

It should be borne in mind that the sensitivity of individuals' skin to irritants is complex, with a number of endogenous and exogenous factors operating at any time, resulting in periods, of higher or lower susceptibility.

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ABSTRACT

Potential Mechanisms of Action of Vitamin D and Analogues in Psoriasis

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Psoriasis is an inflammatory skin disease characterized by increased numbers of proliferating keratinocytes, vascular expansion and leukocytic infiltration. These changes may be caused by a primary defect in keratinocyte growth and/or cytokine release, or as a phenomenon secondary to the release of cytokines from activated leukocytes.

The immune hypothesis, which has received increasing attention, is supported by associations with certain HLA types, the clinical efficacy of the immunosuppressant cyclosporin, and the ability of growth factors released from T cells from psoriatic lesions to directly stimulate keratinocyte growth.

Calcipotriol (Daivonex®) is a vitamin D analogue having demonstrated efficacy in psoriasis. In the circulation, vitamin D is bound with highest affinity by an alpha-2 globulin called the GC-globulin. It binds to the vitamin D receptor, a 50–60 kD intercellular protein related to the steroid, thyroid and retinoic acid receptor superfamily. The receptor complex is thought to interact with DNA sequences regulating synthesis of mRNA involved in cell growth and differentiation. The receptor is expressed by many cells involved in the pathogenesis of psoriasis, including keratinocytes, monocytes, macrophages and activated T cells.

In vitro, vitamin D and its analogues induce differentiation of resting monocytes, and demonstrate inhibitory effects on activated monocytes, causing impaired accessory cell function and decreased release of IL-1α, TNF α and IL-6. They also have a direct effect on T cell activation, resulting in decreased proliferation and release of cytokines including IL-2, interferon gamma, GM-CSF, TNF β and IL-6. Furthermore, in cultural human keratinocytes, vitamin D enhances transglutaminase activity and the formation of cornified envelopes. Simultaneously there was an inhibition of keratinocyte proliferation. *In vivo*, vitamin D and its analogues inhibit T cell mediated responses in animals, including allograft rejection and the development of autoimmune diseases. The effects of vitamin D in psoriasis may be twofold, since it demonstrates direct effects on both the immune system and on keratinocytes.

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ABSTRACT

Safety Aspects of Calcipotriol Treatment

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When excessive doses of vitamin D – including calcipotriol – are used, there is a risk of D hypervitaminosis. In cases of mild overdosage, the physiological control mechanisms cause compensatory hypercalciuria, whereas with true overdosage, hypercalcaemia develops.

Hypercalcaemia is a result of increased calcium absorption from the gut and resorption of bone. Symptoms which may arise include malaise, headache, drowsiness, constipation, polydipsia, polyuria, muscle weakness, fatique, irritability, nausea and vomiting. Chronic hypercalcaemia can result in urinary stones, soft tissue calcification in blood vessels, myocardium and cornea, nephrocalcinosis, and renal failure. Furthermore, hypercalciuria alone can cause urinary stones.

The extensive clinical research performed on topical calcipotriol has indicated a high degree of safety. Serum calcium was monitored during all the clinical studies performed on this compound, and none has shown a significant rise in the mean level, even in long-term use. Fewer data are available regarding urine calcium excretion during calcipotriol treatment.

Three studies have failed to demonstrate any increase in 24-hour calcium excretion in patients using up to 100 g of ointment weekly. Furthermore, no changes have been detected in other biochemical markers of calcium and vitamin D_3 metabolism.

As of today there are 4 cases of hypercalcaemia during calcipotriol treatment of psoriasis. These patients used, against the guidelines, between 150 g and 490 g of calcipotriol ointment per week. Symptoms of hypercalcaemia developed in only 1 of the 4 patients. In all cases the hypercalcaemia disappeared within a few days of discontinuing treatment. Three further cases of marginally increased serum calcium have been observed after use of between 70 g and 83 g of calcipotriol ointment per week.

Of the 7 patients with marginal or frank hypercalcaemia, 3 had renal impairment or previous urinary stones.

On current evidence, monitoring of serum or urine calcium does not appear necessary, provided less than 100 g of calcipotriol ointment is used weekly.

However, if quantities above this limit are required, it is essential to monitor the serum calcium closely.

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ABSTRACT

Calcipotriol in Combination Therapy

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Calcipotriol has proved to be an effective monotherapy for mild and moderate psoriasis. The purpose of this presentation is to demonstrate its application in combination therapy for patients with more severe forms of the disease.

In an open study of 20 patients, the efficacy of and tolerability to Calcipotriol ointment alone or with UVB radiation were compared.

After 8 weeks' treatment with Calcipotriol alone, 66% showed marked improvement and 17% showed clearance.

In the group treated with the combination of UVB with Calcipotriol ointment, 50% showed marked improvement and 39% showed clearance.

Although the study was too small to show stastistical significance between the two groups, the results were encouraging.

In a double-blind study, comparing Calcipotriol ointment with PUVA and vehicle control with PUVA, 104 patients were studied.

PUVA was given in stepwise increments up to a maximum dose of 8.5 joules/cm^2 for skin type II and 9 joules/cm^2 for skin type III, or until adverse events appeared or until a target treatment response was reached. A target of >90% reduction in PASI score was aimed for 71.7% of the Calcipotriol treated group and 55.6% placebo treated group achieved the target. In the Calcipotriol treated group there was a 26% reduction in total UVA dose (this did not reach significance). There was however, a statistically significant reduction of 37% in the cumulative UVA dose in the total Calcipotriol treated group analysed compared with the vehicle treated group. Combined treatment with PUVA and Calcipotriol is thus a highly effective and well tolerated treatment of extensive plaque psoriasis.

In a double-blind comparative study of Calcipotriol ointment with cyclosporin A and vehicle ointment with cyclosporin A in severe psoriasis (PASI $\geq$ 20), 69 patients were studied. Cyclosporin was given at a dosage of 2 mg/kg/day. The primary response criterion was clearance, or >90% reduction from baseline in PASI score in the 6-week treatment period. 53.3% Calcipotriol-treated compared with 12.5% of vehicle-treated patients achieved this primary target.

Calcipotriol thus appears to potentiate the effect of cyclosporin A in the treatment of psoriasis and allows better response rates which would only be achievable using cyclosporin A alone at higher doses and for longer periods of treatment.

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ABSTRACT

Comparison of Calcipotriol Ointment with Betamethasone 17-valerate Ointment and Dithranol and investigation of the Long-term Safety and Efficacy of Calcipotriol

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Calcipotriol ointment (50 μg/g) has been compared with betamethasone valerate (0.1%) and short contact dithranol treatment in large, multicentre, randomized studies on patients with chronic plaque psoriasis.

The first of these was a double-blind, right/left comparison of calcipotriol and betamethasone in 345 subjects. Each treatment was applied twice daily for 6 weeks. Response was assessed by investigators using the PASI, and by subjects on a five-point scale. There was a significant difference in PASI in favour of calcipotriol after 2 weeks, which persisted throughout the study. Subjects' grading of response also significantly favoured calcipotriol.

A second double-blind study, in which 405 subjects participated, compared the same treatments, using the same parameters, but a parallel-group format. Although there was a marked improvement in both groups, there was no significant difference in improvement of the PASI between the two treatments. However, subjects' grading of response again showed calcipotriol to be significantly more effective. Follow-up of subjects in both these studies showed little difference between treatments regarding the frequency, timing and severity of relapse. Further treatment with calcipotriol rapidly brought the disease back under control.

Calcipotriol has been compared with short-contact dithranol therapy in an open, parallel-group study. Two evenly matched groups of 239 subjects were treated for 8 weeks. Calcipotriol was applied twice daily and dithranol cream was applied daily for 30 min at the highest concentration tolerated. The improvement in PASI was significantly better with calcipotriol. Subjects graded both the response to treatment and cosmetic acceptability on five-point scales. The results differed significantly, in favour of calcipotriol, for both parameters.

Long-term use of calcipotriol has also been investigated. Continuing efficacy and safety have been demonstrated in large multi-centre trials performed both by dermatologists, in a study performed on 167 subjects, and by family practitioners, in a study with 211 subjects. Clinical improvement occurred most rapidly during the first 2 months of treatment and this was followed by further, but more gradual, improvement over the rest of the year.

Adverse events were carefully monitored throughout these studies.

Approximately 20% of subjects in the short-term trials experienced some irritation of the skin but this was rarely severe. Over the course of 12 months a larger proportion of subjects reported some degree of irration but very few discontinued treatment as a result. Although the treated areas were most commonly affected, the face was another common site for irritant reactions. There were no increase in the mean serum calcium in any study. Hypercalcaemia did not develop in any subject using the treatment as directed. One subject, who used 400 g of ointment over 10 days, developed asymptomatic hypercalcaemia which resolved promptly after stopping treatment.

These studies have shown calcipotriol to be more effective than betamethasone or short-contact dithranol. It remains effective in long term use as well as being safe and generally well tolerated.

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Calcipotriol: Clinical Trial versus Betamethasone Dipropionate + Salicylic Acid

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The four Italian dermatology centres involved in the clinical trial were: situated at the universities of Milan 2nd (Prof. A. Finzi), Milan 3rd (Prof. C. Crosti), Pisa (Prof. E. Mian) and Trieste (Prof. C. Scarpa).

Ointments were applied *bis in die* for 6 weeks without local occlusion. The two parallel groups of psoriatic patients (en plaque) were randomized within centres (20 + 20 per centre) and were treated as follows:

Group 1: calcipotriol, 50 mg/g.
Group 2: betamethasone dipropionate, 0.05‰ + salicylic acid, 3% (see Table I).

RESULTS AND COMMENT

Confidence intervals for the significant differences were obtained by *comparing certain proportions.* As they were rather small, their statistical significance was rather high, but the *p* (probability) obtained in all these comparisons of proportions was 0.7% for the healed group only (better for calcipotriol treatment after 10 weeks; values double underlined in the tables).

The overall impression gained from this trial was that after the first 2 weeks of treatment, the betamethasone +3% salicylic acid treated patients felt better and their clinical appearance was better, whereas in the long term, after conclusion of treatment and the observation interval, the calcipotriol-treated patients experienced a better overall outcome (statistically significant).

Table I. *Calcipotriol versus betamethasone + salicylic acid*

Centres involved	40 psoriatic patients per centre (20 vs 20)
C. Crosti	Dermatology clinic – Milano
A. Finzi	Dermatology clinic – Milano
E. Mian	Dermatology clinic – Pisa
C. Scarpa	Dermatology dept. – Trieste

Table II. *Calcipotriol versus betamethasone + salicylic acid 3%* b.i.d., 6 weeks, no occlusion. – Controlled, multicentre, parallel group, randomized within centres.

Psoriasis		Calcipotriol	Betamethasone + salicylic acid
No. of cases		80	80
Age, mean		50	50
Sex	Male	53	56
	Female	27	24

Table III. *Percentage of overall investigator's judgement* (n = 80 psoriatic patients enrolled).

	Cal 2°w	Bet 2°w	Cal 4°w	Bet 4°w	Cal 6°w	Bet 6°w	Cal 10°w	Bet 10°w
Excellent	5	0	18	14	39	27	42	19
Good	54	55	47	35	20	35	12	14
Moderate	32	38	14	35	20	20	9	19
Poor	6	7	17	14	16	12	20	18
Null	3	0	4	2	5	6	17	30

Table IV. *Percentage of patient's overall acceptance* (n = 80 psoriatic patients enrolled).

	Cal 2°w	Bet 2°w	Cal 4°w	Bet 4°w	Cal 6°w	Bet 6°w
Excellent	34	19	30	22	35	20
Good	45	64	52	57	49	56
Moderate	12	11	5	9	2	14
Poor	2	4	2	2	2	2
Null	4	0	5	1	1	5

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ABSTRACT

Strategy of Psoriasis Treatment: Alternate and Associate Therapies

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Despite many brilliant recent advances, a substantial effort remains necessary to improve the convenience, the efficacy and the safety of psoriasis tratments. Until to now we have no long-term safe monotherapy for psoriasis: PUVA can be given up to a cumulative dose of approximately 1500 Joules, Cyclosporin no more than 5 years in the best of cases, Methotrexate usually less than 7 years, Retinoids up to 12 years and more but for a few patients.

Thus in addition to a necessary research for new, more active and less toxic molecules, it seems of major interest to evaluate new strategies: 1) alternate treatments (for example one year Cyclosporin, one year Methotrexate) open the hope of obtaining with a succession of treatments, very efficient but with quite different side effects, a real improvement in the long-term tolerance; 2) another way, less comfortable for the patients, but also quite interesting, is the association between treatments to improve efficacy and to diminish the side effects. These associations could be between two systemic treatments with a different spectrum of toxicity, or between a systemic tratment at very low doses and a topical treatment used only to control the remaining lesions or a flare of the disease.

Finally its seems of key importance to develop therapeutic research, not only on a short-term basis with, as a major criterion, the clearing of skin lesions, but on a long-term basis in order to test the best strategy for maintenance treatment. The main parameters of evaluation should be not only the efficacy and the avoidance of relapse, but also the safety and the quality of life of the patients. These long-term studies are expensive and time consuming but offer the most important therapeutic information in a chronic disease such as psoriasis.

These two aspects of the strategy of psoriasis treatment will be discussed.

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Structural Alterations of Basal Keratinocytes and Capillary Loop in Psoriasis during Treatment with Topical Calcipotriol

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Recent research has demonstrated the activity of calcipotriol, effective as a potent inhibitor of cellular proliferation and known to increase differentiation in a number of cell lines in the topical treatment of psoriasis. Vit D3 receptors are expressed in keratinocytes and vascular endothelial cells. We studied the alterations in basal kertinocytes (stem cells or anchoring cells) and endothelial cell modification in 6 patients with psoriasis treated with calcipotriol ointment twice a day for 4 weeks. The samples were embedded in Epon resin for thin section and ultrathin section examination by electron microscopy. A normal pattern of distribution of the two different types of basal keratinocytes was observed before treatment. After treatment, only anchoring cells were detected. The alterations of endothelial cells in capillary loop disappeared after treatment, presenting normal aspects. Our morphological findings suggest that calcipotriol is therapeutically effective, due principally to an inhibition of cellular proliferation. ***Key words: calcipotriol; stem cells; serrated cells; non-serrated cells; psoriasis.***

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Calcipotriol (MC 903), a new synthetic analogue of Vitamin D, was recently introduced in the topical treatment of psoriasis. This drug has a high affinity for the vitamin D receptor expressed in the skin by keratinocytes, fibroblasts, endothelial cells, monocytes, activated T-lymphocytes and B-lymphocytes.

In vivo, calcipotriol induces a significant reduction in epidermal proliferation and increased maturation of keratinocytes, and it modifies the inflammatory infiltrate by decreasing the numbers of polymorphonuclear leukocytes and, later, T-lymphocytes. These effects are probably due to an interference with some immunological mediators (IL-1, IL-2, IL-6) as observed in vitro (1) (2). Many studies suggest that this is achieved, after binding of the specific cytosolic receptor, both through transportation to the nucleus and transcription of those genes involved in hormonal response as steroids do, and through a more immediate mechanism not mediated via the genoma, which directly increases intracellular free calcium (3).

In normal skin there are two cell populations of keratinocytes in the basal layer: (i) stem cells with non-serrated morphology, generally localized at the tips of the deep rete ridges, and (ii) anchoring cells with serrated morphology, distributed mainly along the sides of rete ridges (4) (5).

In psoriasis, epidermal hyperproliferation involves not only the stem cells of the basal layer, but also the suprabasal population named transient amplifying cells.

Epidermal hyperplasia cannot occur without vascular proliferation. In psoriasis, capillary loops of the dermal papillae are abnormally dilated and convoluted, and they display the characteristics of venous capillaries (6).

The aim of this study was to evaluate the effect of calcipotriol in psoriasis, by studying the morphology of the basal layer and the capillary loops.

MATERIAL AND METHODS

Biopsy specimens were taken from 6 patients with nummular psoriasis before and after treatment with calcipotriol ointment twice a day for 4 weeks.

The samples were fixed by immersion in 2% formaldehyde and 2.5% glutaraldehyde 0.1 M cacodylate buffer, pH 7.4, followed by 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, dehydrated in graded ethanol, passed through propylene oxide and embedded in Epon 812. Semithin sections were stained with methylene-blue and fuchsin for light micros-

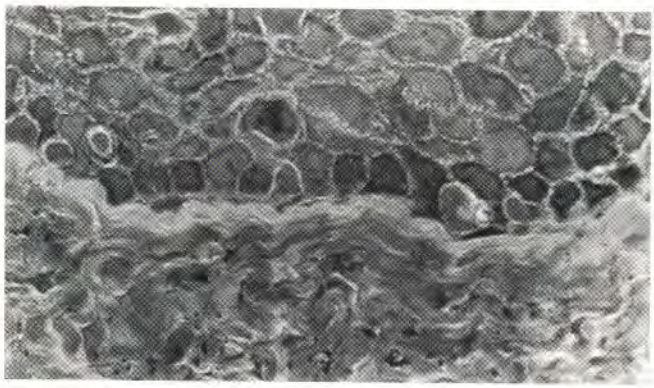

Fig. 1. Normal distribution of stem cells, localized at the tips of the deep rete ridges in the basal layer, before treatment. Semithin section, 2 μm, stained with methylene-blue and fuchsin (×1000).

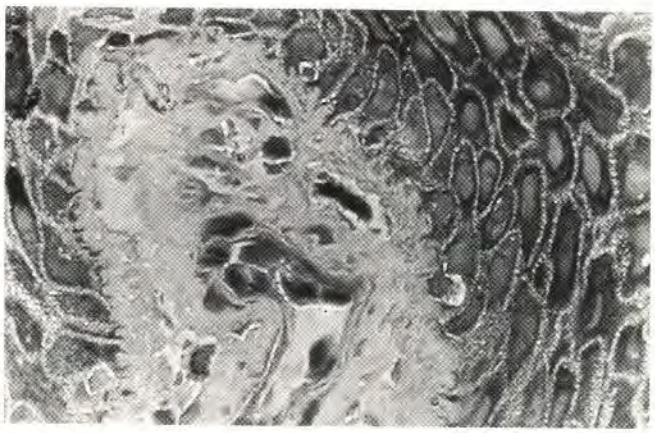

Fig. 2. Normal distribution of anchoring cells localized along the sides of rete ridges in the basal layer, before treatment, Semithin section, 2 μm, stained with methylene-blue and fuchsin (×1000).

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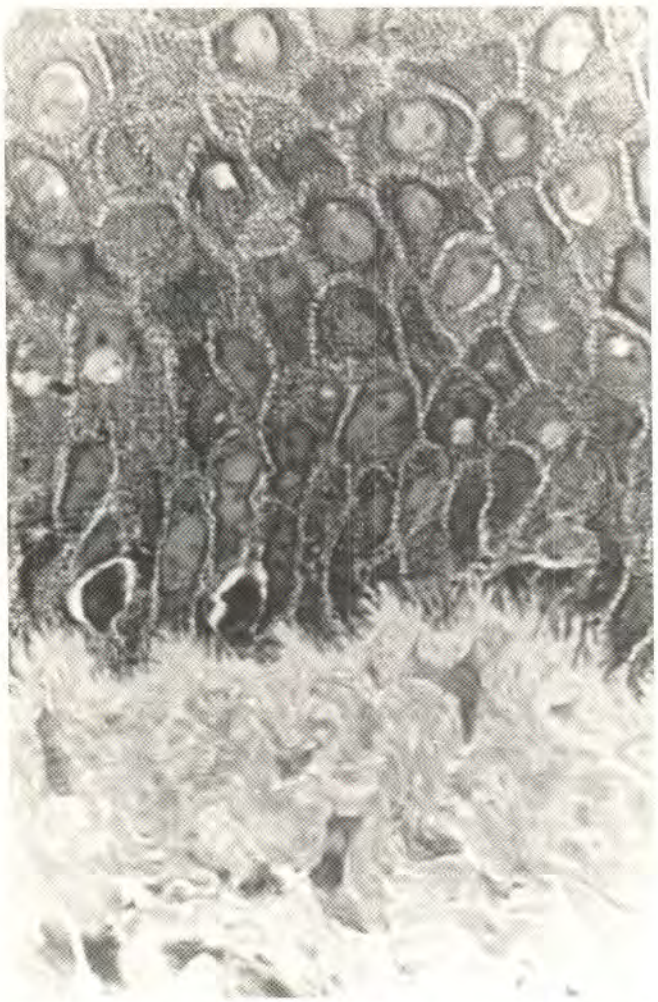

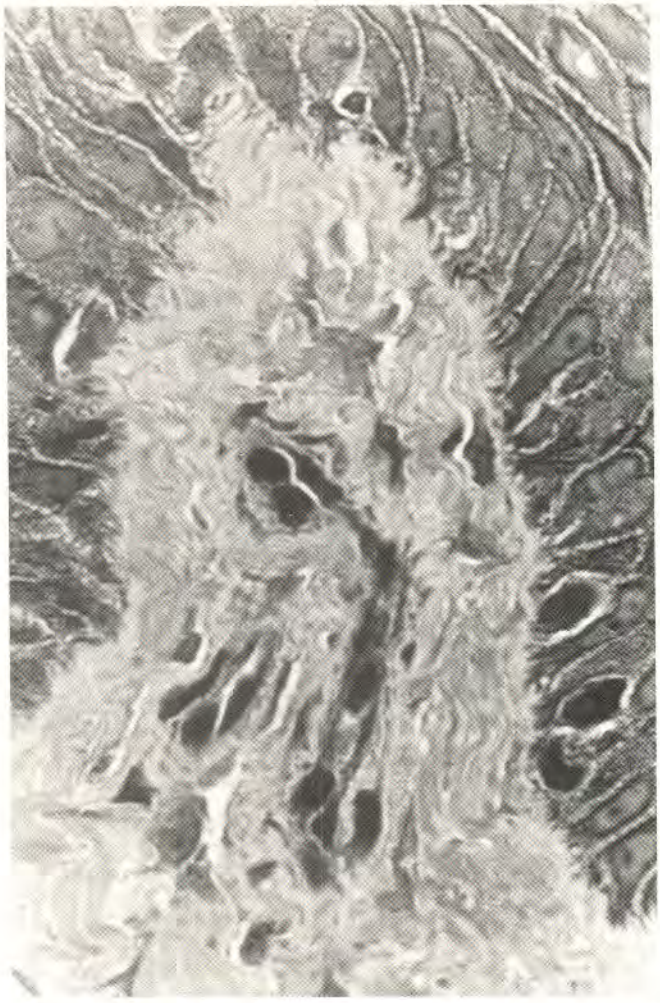

Figs 3, 4. After treatment with MC 903 the basal layer is composed only of serrated cells with amplified, highly convoluted dermal epidermal junction. Semithin section 2 μm, stained with methylene-blue and fuchsin (×1000).

copy. The samples were also cut in ultrathin sections, stained with uranyl acetate and bismuth tartrate and examinated in a Siemens Elmiskop 102 electron microscope at 80 kW.

RESULTS

Examination by light microscopy showed a normal distribution of stem cells (Fig. 1) and of anchoring cells (Fig. 2) in all samples before treatment. After treatment with MC 903, we found only serrated cells with amplified highly convoluted dermal epidermal junctions (Figs 3, 4). These data were supported by the electron microscopic observations (Figs 5, 6) which, after therapy, showed an elongation of cytoplasmic projections of serrated cells extending deeply into the papillary dermis (Fig. 6) and an absence of non-serrated cells.

Before treatment in all samples, the capillary loop appeared typically dilated and tortuous, with endothelial swelling and nuclei protruding into the lumen. Electronmiscroscopic observation showed that the intrapapillary portion of the capillary loop had a venular-type multilaminated basement membrane. Furthermore, the endothelial cells presented a well defined rough endoplasmic reticulum. After treatment, light microscopy demonstrated a normal capillary loop. Both endothelial swelling and capillary lumen were reduced. Electronmicroscopic study demonstrated a return to arterial capillary features with the basement membrane characterized by a single electron-dense band and many cytoplasmatic free ribosomes.

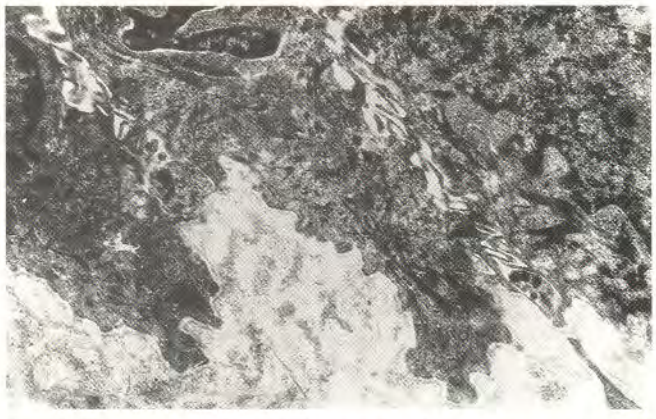

Fig. 5. Transmission electronmicrography showing the non-serrated basal keratinocytes with the regular dermal-epidermal junction with short villi-like projections (×7,500).

Fig. 6. Transmission electronmicrography showing the serrated basal keratinocytes with the elongation of cytoplasmic projections of serrated cells extending deeply into the papillary dermis after treatment (×7,500).

DISCUSSION

Our data suggest that this drug has a therapeutic effect, due principally to an inhibition of cellular proliferation, supported by the disappearance of keratinocytes with a non-serrated morphology in the basal layer. Previous studies (4, 5, 7) have correlated basal layer cell morphology to its function, evidencing a stem activity for non-serrated cells that gives rise to suprabasally located, highly proliferative transient amplifying cells. In psoriasis the hyperplasia is supported by these cells. The other basal layer cells presenting a serrated morphology are post-mitotic cells, presumably with an anchoring function. The basal layer is composed mainly of serrated cells, which indicates that calcipotriol is effective in inhibiting cell proliferation and inducing cell differentiation. The normalization of the capillary loop could be correlated with the reduction in skin thickness or directly with an antiproliferative action of calcipotriol.

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Acta Derm Venereol (Stockh) 1994; Suppl. 186: 52–54

Effect of Calcitriol on Growth, Differentiation, Chemokine mRNA Expression of Cultured Keratinocytes and on Keratinocyte-T Cell Binding

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Calcitriol has recently been shown to be effective against psoriasis. However, its mode of action is not exactly known. The present study focused on the influence of calcitriol on growth, differentiation, chemokine mRNA and ICAM-1 mRNA expression of keratinocytes (KC) and on the binding of T-cells to keratinocytes. In vitro studies showed that calcitriol has a strong anti-proliferative effect and induces terminal differentiation. γ-IP-10 and ICAM-1 mRNA were induced by γ-IFN, an induction not influenced by calcitriol. Moreover, the functional expression of ICAM-1 on the KC cell surface as measured by a cell adhesion assay, was not influenced either. IL-8 and huGRO mRNAs were constitutively produced in KC, as was demonstrated after incubation with cycloheximide. Up-regulation of both IL-8 and huGRO mRNA by IL-1α was also not affected by calcitriol. It is concluded that calcitriol has a strong antiproliferative activity and does not interfere with KC responsiveness to γ-IFN and IL-α induced chemokine expression or with the adhesion of T-cells to keratinocytes.

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Psoriasis is characterized by hyperproliferation and impaired differentiation of keratinocytes (KC) and the presence of activated T cells and neutrophils in the epidermis (1, 2). KC-leukocyte interactions and chemotactic factors are believed to play a crucial role in the pathogenesis of psoriasis (3, 4).

Recently a superfamily of chemokines with chemotactic properties has been described (5). γ-IP-10, IL-8 and huGRO, members of this superfamily, can be induced in vitro by pro-inflammatory cytokines (γ-IFN, IL-1 and TNF-α) and have been detected in psoriatic scales. Therefore they may be involved in the leukocyte infiltration (6, 7). Recent investigations demonstrated that calcitriol is effective in psoriasis. However, the way in which calcitriol exerts its beneficial effects is not exactly known. Several studies have demonstrated that it causes normalization of epidermal differentiation and suppresses leukocyte infiltration (8). In addition it was shown that calcitriol decreases IL-8 levels in KC (9); the effects on the other chemokines are currently unknown. Moreover, whether or not calcitriol interferes with the binding of T-cells to KC is unknown either.

The present study focuses on the effects of calcitriol on growth, differentiation and chemokine mRNA expression in cultured KC and the binding of T-cells to keratinocytes.

MATERIALS AND METHODS

Culturing conditions

Human foreskin keratinocytes (KC) were cultured in serum-free medium (keratinocyte-SFM, Gibco, Breda, The Netherlands) as described previously (3, 10). All studies were performed under low Ca^{++} (0.1 mM) culture conditions, except for the proliferation study which was done under high Ca^{++} (1.8 mM) conditions.

For proliferation studies, third passage KC (5×10^3 cells/cm^2) were grown for 48 h with or without calcitriol (10^{-7}, 10^{-8} M), kindly provided by Solvay Duphar, Weesp, The Netherlands. The cells were labelled with [^{3}H] thymidine (5 μCi/ml) for 4 h. Incorporation of [^{3}H] thymidine was expressed as the percentage of standard incorporation of controls (11). Experiments were performed in triplicate.

To measure differentiation of KC, the percentage involucrin- positive cells was determined (12). Therefore third passage KC (5×10^3 cells/cm^2) were grown for 96 h with or without calcitriol (2.5×10^{-7} M). After trypsinization, fixation and immunocytochemical staining with an antibody against involucrin (14), the cells were transferred to object slides and counted microscopically (12).

For RNA isolation, subconfluent cultures of KC (3–5 passages) were grown in the presence or absence of calcitriol (final concentration 10^{-7}, 10^{-8}, 10^{-9} M) with either TNF-α (1000 U/ml), IL-1α (1000 U/ml) or γ-IFN (300 U/ml), for the indicated periods of time (see legends to the figures). In some experiments, cycloheximide (CHX, 10 μg/ml) was added.

RNA isolation and Northern blot analysis

Total RNA was isolated from KC using the guanidinium-HCl method (3). RNA samples (10 μg) were fractionated on 1% agarose gels containing 2.2 M formaldehyde and transferred to Nylon Hybond N filters (Amersham, Slough, Bucks, England). The blots were hybridized with random ^{32}P labelled probes specific for huGRO, γ-IP-10, IL-8 and ICAM-1, while cyclophillin or β-actin were used as "housekeeping" genes.

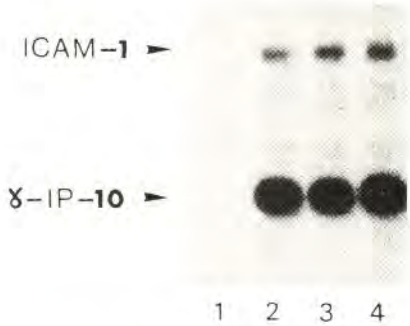

Fig. 1. Effect of calcitriol (D3) on the induction of ICAM-1 and γ-IP10 mRNA in KC. Northern blot analysis of RNA obtained from unstimulated KC (*lane 1*)and from KC stimulated for 3 h with γ-IFN alone (300 units/ml, *lane 2*)and γ-IFN (300 units/ml) in combination with D3 (10^{-7}M, *lane 3*; 10^{-8} M, *lane 4*). Total cellular RNA (10 μg/lane) was probed for ICAM-1 and γ-IP10.

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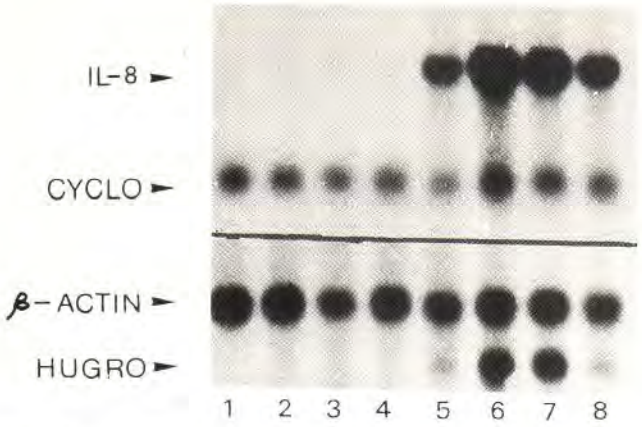

Fig. 2. Effect of Calcitriol (D3) on the induction of IL-8 and huGRO mRNA in KC. Composite figure of Northern blot analysis of RNA obtained from unstimulated KC (*lane 1*) and stimulated (3 h) KC. *Lane 2*: IL-1α (1000 units/ml); *lane 3*: IL-1α (1000 units/ml) and D3 (10^{-7} M); *lane 4*: IL-1α (1000 units/ml) + D3 (10^{-8} M); *lane 5*: cycloheximide (CHX, 5 μg/ml); *lane 6*: IL-1α (1000 units/ml) + CHX (5 μg/ml); *lane 7*: IL-1α + D3 (10^{-7} M) + CHX (5 μg/ml); *lane 8*: CHX (5 μg/ml) + D3 (10^{-7} M). Total cellular RNA (10 μg/lane) was probed for IL-8 and huGRO. Cyclophillin and β-actin were used as reference genes.

Cell adhesion assay

The adhesion of T-cells to the keratinocyte cell-line SVK-14 was measured in the enzyme-linked immuno-cell adhesion assay (10). Subconfluent cultures of SVK-14 cells were treated for 48 h with γ-IFN (300 U/ml) in the presence or absence of calcitriol (final concentration 10^{-7} M). Unstimulated or PMA stimulated (50 ng PMA/ml; 30 min.) normal human T-cells were added to KC and after 1 h of incubation the bound T-cell fraction was quantified spectrophotometrically.

RESULTS

Effect of calcitriol on proliferation and differentiation

[^{3}H]-thymidine incorporation in KC, reflecting cell proliferation, was reduced to 51.8% by 10^{-8} M calcitriol and to 26.8% by 10^{-7} M calcitriol as compared with untreated controls. The percentage involucrin-positive cells indicative of differentiation increased from 17% in control KC (without calcitriol) to 80% in KC grown in the presence of calcitriol.

Effect of Calcitriol on ICAM-1, γ-IP-10, IL-8 and huGRO mRNA expression

Cultured human KC did not produce detectable amounts of mRNAs coding for ICAM-1 and γ-IP-10 respectively (Fig. 1, lane 1). Even in the presence of CHX, there were no dectectable signals indicating that in growing KC, ICAM-1 and γ-IP-10 mRNAs are not constitutively produced (data not shown). KC can be induced to express ICAM-1 and γ-IP-10 mRNA with γ-IFN (Fig. 1, lane 2). Simultaneous addition of calcitriol and γ-IFN had no effect on the expression of ICAM-1 and γ-IP-10 mRNA (Fig. 1, lanes 3, 4). IL-8 and huGRO mRNAs are undetectable in normal KC (Fig. 2, lane 1), but accumulate in the presence of CHX (Fig. 2, lane 5), indicating that IL-8 and huGRO mRNA are constitutively expressed in growing keratinocytes. Addition of calcitriol had no effect on the constitutive synthesis of IL-8 and huGRO mRNAs (Fig. 2, lane 8).

IL-1α did not appear to induce IL-8 and huGRO mRNA to any detectable degree (Fig. 2, lane 2). However, in the presence of CHX, the IL-1α induced up-regulation of IL-8 and huGRO mRNA expression became very obvious (Fig. 2, lane 6). Addition of calcitriol had no effect on this IL-1α-induced IL-8/huGRO mRNA synthesis (Fig. 2, lanes 6, 7).

From a theoretical point of view one might argue that treatment with calcitriol for only 3 h (Figs 1 and 2) is insufficient to have any effect. That prompted us to choose an experimental approach for IL-8 and huGRO (Fig. 3) where KC were pretreated with calcitriol for 0, 5, 24 and 48 h prior to stimulation. From Fig. 3 it can be concluded that KC retain their capacity to produce IL-8 and huGRO mRNA following pretreatment with calcitriol, even after 48 h.

Although the strong growth-inhibiting properties of calcitriol are reflected by an overall down-regulation of mRNA synthesis, including the reference genes cyclophillin and beta-actin, (Fig. 3, lanes 13–16), the IL-8 to cyclophillin and the huGRO to β-actin ratios are not influenced by calcitriol, indicating that calcitriol does not selectively interfere with IL-8 and huGRO mRNA synthesis in KC.

Effect of calcitriol on KC-T-cell adhesion

Non-actived T-cells became minimally bound to unstimulated

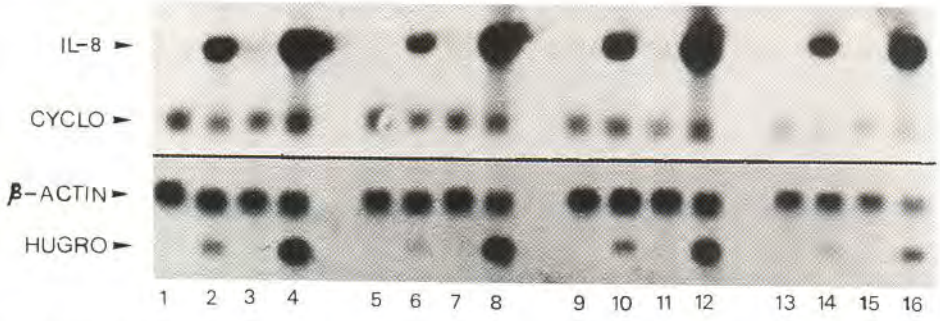

Fig. 3. Long-term effect of Calcitriol (D3) on the induction of IL-8 and huGRO mRNA in KC; a time course study KC were preincubated with D3 (10^{-7} M) for 0 h (*lanes 1–4*), 5 h (*lanes 5–8*), 24 h (*lanes 9–12* and 48 h (*lanes 13–16*) respectively. At the indicated time points following pre-treatment with D3 the KC were incubated for 3 h with: D3 (10^{-7} M), lanes 1, 5, 9, 13; CHX (5 μg/ml) + D3 (10^{-7} M), lanes 2, 6, 10, 14; IL-1α (1000 units/ml) + D3 (10^{-7} M), lanes 3, 7, 11, 15; and CHX + IL-1α + D3, lanes 4, 8, 12, 16. Total cellular RNA (10 μg/lane) was probed for IL-8, huGRO, cyclophilin and β-actin.

SVK-14 cells, in contrast to PMA-activated T-cells. After stimulation of SVK-14 cells by γ-IFN and a two-fold increase in binding of activated T-cells was found. Preincubation with anti-ICAM-1 inhibited the binding of T-cells to KC to 50% (10).

Addition of calcitriol simultaneously with γIFN activation of the SVK-14 cells had no effect on the proportion of adhering T-cells to this keratinocyte cell-line, as compared with calcitriol-untreated controls, which is consistent with our observation that ICAM-1 expression is not influenced by calcitriol.

DISCUSSION

In this study we investigated the effect of calcitriol on normal human KC with respect to growth, differentiation, chemokine mRNA production and T-cell adhesion in order to get an insight into the mode of action underlying its beneficial effects in psoriasis.

Calcitriol appeared to strongly inhibit proliferation and to induce differentiation, probably by increasing intracellular Ca^{++} levels (14). These findings are in agreement with data from the literature (11).

In psoriatic skin, increased amounts of the chemokines γ-IP-10, IL-8 and huGRO, which are chemotactic for T-cells and neutrophils, have been found (4, 7). Of these chemokines, γ-IP-10 and IL-8 proved normal after successful treatment with tar, UV, etretinate, or corticosteroids (4, 12). For calcitriol treatment, no data are available as yet. In this study we demonstrate that in appropriately stimulated KC, calcitriol does not selectively suppress gene transcription of these three chemokines. Though Larsen et al. (9) observed some suppression of IL-8 gene transcription in KC under the influence of calcitriol, we did not detect such an effect.

We also investigated the effect of calcitriol on γ-IFN-induced ICAM-1 expression in KC. Calcitriol did not seem to interfere with this stimulus-induced ICAM-1 mRNA expression at early time points (3 h of incubation). Moreover, calcitriol had no effect on adhesion of activated T-cells to KC monolayers, indicating that the functional expression of ICAM-1 on KC cell surface was not influenced by calcitriol. Matsumoto et al. (15) found a reduction in the number of high affinity EGF-receptors following incubation with calcitriol for 48 h. Moreover, c-myc expression was dramatically reduced already after 3 h of incubation with calcitriol, making c-myc a very early response gene to calcitriol. C-myc is known to play an important role in cell proliferation. The reduced number of high affinity EGF-receptors after 48 h as found by Matsumoto et al. (15) and the overall down-modulation of mRNA synthesis following treatment for 48 h observed by us (Fig. 3) is therefore probably more an indirect consequence of growth arrest induced by selective inhibition of c-myc expression, than a specific effect on the other genes.

In conclusion, in accordance with the literature, calcitriol arrests KC in their growth. This growth arrest is followed by differentiation. Calcitriol has no specific effect on stimulus-induced mRNA expression of chemokines and the adhesion molecule ICAM-1, nor on the binding of activated T-cells to keratinocytes.

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Treatment of Psoriasis Vulgaris with Topical Calcipotriol:

Is the clinical improvement of lesional skin related to a down-regulation of some cell adhesion molecules?

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Calcipotriol is demonstrably efficacious for the treatment of psoriasis by virtue of its effects on the skin's immune system and on epidermal growth. We performed this study to emphasize the difference in the expression of certain cell adhesion molecules (CAMs) (ICAM-1, ELAM-1, LFA-1, VLA-3, VLA-6) in lesional and perilesional skin of 10 patients with psoriasis, before and after treatment with topical Calcipotriol. We took two biopsies of lesional and perilesional skin from each patient before and after treatment and then performed an immunohistochemical study to observe the expression of these CAMs, utilizing monoclonal antibodies against these adhesion molecules. We noticed reduced levels of infiltrating cells along with the expression of ICAM-1, LFA-1, ELAM-1 and of CAMs VLA-3, VLA-6 in basal and suprabasal keratinocytes. On the basis of these data we hypothesize that, besides epidermal keratinocytes, another target for Calcipotriol may be the skin's own immune system, suggesting that Calcipotriol can modify T lymphocyte activity (IL-1 dependent) through a down-regulation of CAMs. ***Key words: vitamin D analogues; skin immune system; epidermal growth.***

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Calcipotriol is the structural analogue of calcitriol, bioactive form of Vitamin D_3. All clinical trials have demonstrated that both have good therapeutic efficacy against hyperproliferative diseases such as psoriasis (1). The efficacy of vitamin D analogues in psoriasis vulgaris was discovered in 1985 by Morimoto et al. They reported a case of significant amelioration of this dermatosis in a patient with psoriasis, treated with oral α-calcidol for a severe osteoporosis (2, 3).

Calcipotriol and calcitriol show similar receptor binding as found in different cells lineages: epidermal keratinocytes, dermal fibroblasts, endothelial cells and activated T lymphocytes (4, 5).

They both have similar effects on the immune system and on the epidermal growth-promoting terminal differentiation and inhibiting proliferation of keratinocytes (6, 7, 8).

The therapeutic index of vitamin D analogues orally administered, is low and a safe and effective dose has not yet been established; in fact, calcitriol implies a high risk of inducing the typical side-effects related to Vitamin D_3: hypercalciuria, hypercalcaemia and bone resorption.

Calcipotriol, on the contrary, as it has been developed for topical treatment of psoriasis, exerts only one-hundredth calcitriol's effect on calcium metabolism (9, 10, 11). Calcipotriol appears not to have a direct anti-inflammatory effect, but seems to modify epidermal growth and T-lymphocyte activity, so modulating the production and release of cytokines (12).

Cell adhesion molecules (CAMs) are cell surface receptors expressed on different cell lineages and involved in cell-cell and cell-matrix interactions in various physiological and pathological conditions. Some of these CAMs are involved in the interactions of lymphocytes with keratinocytes, endothelial cells and inter- and perivascular connective cells. Their expression is often up-regulated by cytokines (13, 14, 15).

These adhesion receptors mediate the trafficking and the homing of lymphocytes from circle to perivascular tissue sites of inflammation through 'transendothelial migration' (16).

MATERIAL AND METHODS

Ten informed outpatients (8 males, 2 females, 25–52 years old, mean 40.5) with symmetrical, plaque-type psoriasis were selected for the study. None of the patients had received antipsoriatic treatment for at least 5 weeks before the study. They all had normal serum levels of calcium. They were treated for 8 weeks with topical calcipotriol (50 μg/g twice daily). No other antipsoriatic treatment was permitted. Blood samples for standard laboratory examinations were repeated after the treatment.

All the patients underwent a biopsy on lesional and perilesional skin before and after treatment. Under local anesthesia, samples were taken with a cutaneous biopsy punch, 5 mm. The material was fixed in O.C.T. compound, i.e. an embedding medium that we used for freezing (−80°) our tissue specimens. Cryostat sections (7–8 μm) were studied with an immunohistochemical method (indirect immunoperoxidase). We utilized monoclonal antibodies directed against antigens CD3, CD4, CD8, CD1a, CD36, that respectively identify mature T lymphocytes, T helper/inducer lymphocytes, T cytotoxic/suppressor lymphocytes, antigen-presenting cells, perivascular dendritic cells, and monoclonal antibodies against cell adhesion molecules (CAMs) ICAM-1, ELAM-1, LFA-1 (which may be expressed respectively: ICAM-1 on endothelial cells, keratinocytes, antigen-presenting cells; ELAM-1 on endothelial cells, and LFA-1 on lymphocytes) which represent some of the most important adhesion molecules. We also utilized monoclonal antibodies directed against CAMs VLA-3 and VLA-6, that are expressed on basal and suprabasal keratinocytes.

For quantitative analysis, we used two sections (lesional and perilesional) per patient, before and after treatment, per antibody (CD3, CD4, CD8, CD36, CD1a, ICAM-1, LFA-1, ELAM-1, VLA-3, VLA-6). Expression of ELAM-1, ICAM-1, LFA-1,

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Table I. *Percentage of labelled cells in the dermis of psoriatic skin before and after tratment with topical calcipotriol*

BT = before treatment: AT = after treatment

Pats.	CD3		CD4		CD8		CD1a		CD36	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
1	35	12	40	16	15	13	30	15	18	14
2	20	5	38	18	13	11	35	18	21	15
3	30	0	34	20	10	10	30	23	25	12
4	30	10	36	12	15	14	33	12	16	14
5	22	8	30	15	16	15	27	16	22	20
6	36	5	35	17	18	16	28	10	27	23
7	18	8	32	21	12	12	21	19	19	16
8	28	12	28	10	14	13	34	22	23	15
9	34	10	25	12	12	10	37	20	25	16
10	35	6	35	15	10	9	30	18	20	12

VLA-3, VLA-6 was quantified *ad modum* Messadi et al. for ELAM-1: 0, no staining; 1, weak focal granular staining; 2, moderate staining; 3, strong staining; 4, very strong staining.

RESULTS

Topical application of calcipotriol caused a significant improvement (clinical remission of the lesions) with a decrease of 38 in the PASI score after 8 weeks of treatment ($p < 0.001$).

The urinary and blood parameters of calcium metabolism following treatment were within the normal range.

The immunohistochemical data of our study were as follows (for quantitative data, see Tables I and II):

Lesional skin before treatment

By light microscopy, two main immunohistochemical features were found:

1) Perivascular cellular infiltration.
We have demonstrated with the immunohistochemical method the presence of CD3+, CD4+, CD1a+ and CD36+ cells in the infiltrate.

2) Cell adhesion molecule expression.
We found expression of cell adhesion molecules in the perivascular dermis and in the epidermis of all the samples we studied. In particular we discerned an up-regulation of ICAM-1 (intercellular adhesion molecule-1) (see Fig. 1) by endothelial cells, antigen-presenting cells and keratinocytes and its counter-receptor LFA-1 (Leukocyte function antigen-1) situated on the T lymphocyte surface, whose expression could permit, after a linkage with ICAM-1 expressed on endothelial cells of 'high endothelial venules' (but not only in those cells), the transendothelial migration of T lymphocytes in the perivascular tissue. Finally we have observed a moderate expression of ELAM-1 on endothelial cells and of VLA-3 and VLA-6 on basal and suprabasal keratinocytes. On perilesional skin before treatment we have observed a scanty infiltrate and a slight expression of CAMs.

Table II. *Expression of cell adhesion molecules (ICAM-1, ELAM-1, LFA-1, VLA-3, VLA-6) in the epidermis and dermis of psoriatic skin, before and after an 8-week treatment with topical calcipotrol*

BT = before treatment: AT = after treatment, P = patients

CAMs	ICAM-1		ELAM-1		LFA-1		VLA-3		VLA-6	
Pats.	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
1	++++	+	+++	+	+	+	+	+	+++	+
2	+++	+	+	+	+	−	+++	+	+	+
3	+++	+	+	+	+	−	+	+	+	+
4	+++	+	+++	+	++++	+	+	+	+	+
5	+++	+	+	+	+++	+	+	+	+	+
6	+++	+	+++	+	+	−	+	+	+	+
7	+++	+	+	+	+++	+	+	+	+++	+
8	++++	+	+++	+	+++	+	+	+	+	+
9	+++	+	+	+	+++	+	+	+	+	+
10	++++	+	+++	+	++++	+	+	+	+	+

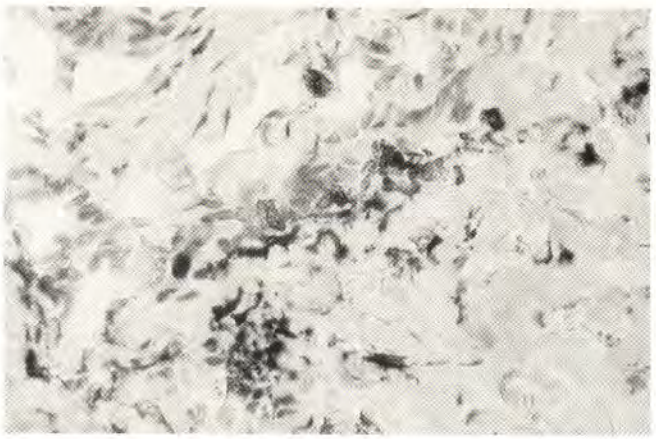

Fig. 1. Significant expression of ICAM-1 in the dermis of psoriatic skin before treatment. 40×.

Lesional skin after treatment

By light microscopy we have observed a significant reduction of CD3, CD4, CD1a and CD36 perivascular cellular infiltrate, a strong down-regulation of ICAM-1 (see Fig. 2), LFA-1, ELAM-1 and a moderate reduction of the expression of VLA-3

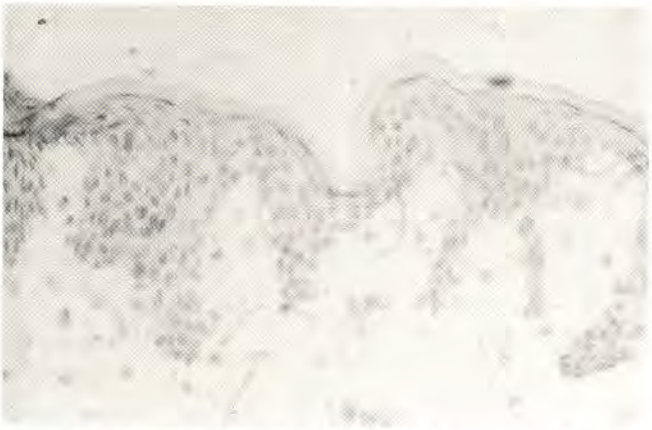

Fig. 2. Significant down-regulation of ICAM-1 expression after treatment. 25×.

and VLA-6 (see Table II). In particular we have discerned a very strong reduction of LFA-1 expression.

On perilesional skin, the infiltrate, present in moderate quantities before the treatment, was now completely absent. Some placebo-treated plaques in a patient with psoriasis have not improved.

DISCUSSION

All clinical trials have shown that topical calcipotriol is of significant therapeutic benefit in psoriasis vulgaris (17). Its mode of action has not been completely elucidated but it does not seem to have a direct anti-inflammatory action (12). Besides modulating epidermal growth-promoting terminal differentiation and inhibiting proliferation of keratinocytes, calcipotriol seems to have an immunoregulatory role that involves the skin immune system. In our data we have shown that VLA-3 and VLA-6 expression are not significantly modified by calcipotriol, while we have found a marked decrease in infiltrating cells and a down-regulation of ICAM-1, ELAM-1 and LFA-1 (18).

What is commonly accepted is the correlation between several weeks of treatment and a significant reduction of the perivascular cellular infiltrate on lesional and perilesional skin, represented mainly by activated T-lymphocytes (CD4+) and antigen-presenting cells (CD1a+) (19).

This reduction could be partly linked to the effect of this drug's inhibition of the production and release of cytokines. In fact calcipotriol reduces the release of various soluble factors (IL-2, IL-6, TNF) that normally are released by epidermal keratinocytes, monocytes and activated T-lymphocytes (20). Subsequently, this reduction could also be correlated to the effect of calcipotriol inhibiting the response of lymphocytes to IL-1 (21).

In this context, the role played by cell adhesion molecules is noteworthy. These adhesion receptors are known to mediate the trafficking and the homing of lymphocytes from the blood to the area site of inflammation, so representing the first step in the pathogenesis of many systemic and also cutaneous diseases.

On the basis of the results of our study, the reduction of cytokine release due to the therapy with topical calcipotriol might indicate/govern/regulate the down-regulation of the adhesion molecules ICAM-1, ELAM-1, LFA-1. In conclusion, calcipotriol may reduce the release of cytokines from different cell lineages, indicate/govern/regulate a down-regulation of CAMs that are known to mediate the passage of activated T-lymphocytes in the dermis and in the epidermis, thus producing a reduction of cellular infiltrate in psoriasis.

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Notes on History of Psoriasis

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To write, even briefly, of the history of this disease across the centuries becomes a difficult task because a complete treatise on the history of Dermatology has not yet been made. Let's look at the elements that are available in order to trace a historical profile of this clinical condition.

Clearly, the term psoriasis derives from "psora", meaning scale. Such term has been used in the course of time to indicate various diseases, characterized by the presence of cutaneous lesions of varying appearance, covered by scales. Already in the works of Hippocrates we can find this term, used to indicate a scaly rash localized on the face and genitals. Later, in Roman times, we can find mention of various dermatological conditions, but cannot trace a clinical picture referable to psoriasis as we intend it today. A precise idea of the status of medicine in this epoch can be found in the work of Aulo Cornelio Celso, "De re medica", written in the first century A.D. This work describes the various diseases known in that period. Of interest to us, in book 5, chapter 28, we can find "piaghe nate per corrompere alcune parti del corpo" (wounds born to corrupt some parts of the body), such as carboncello, foruncoli, ulcers, abscesses, fistole, pustole, rogna, impetigene, papule and vitiligo. In this treatise we can find, for the first time, mention of two conditions which can be referred to as psoriasis. In fact, when referring to the various types of impetigini (paragraph 17) he writes:"...l'altra specie è peggiore, quasi somigliante alle papule, ma più aspra e rossa, avente diverse figure; forma nella parte posteriore della cute delle squamette, il prurito é maggiore...ed a certi fissi tempi appare e si dilegua..." ("...the other type is worse, almost identical with papule, but harsher and red, having different shapes; forms on the exterior part of the skin small scales, the itch is greater...and appears and disappears at certain fixed times ..."). Another quote concerns vitiligini (paragraph 19) which are distinguished in alfo, melas and leuce; "dicesi alfo allorché ha color bianco... intanto che sembra come tante gocce sparse..." ("called alfo because of the white coloring...seeming like various drops...").

In later times, in the writings of Galeno, Ezio, Oribasio, Attuario, Paolo di Egina, we sometimes find the word psoriasis used to indicate various scaly rashes, of completely different nature; thus the recurrent terms "leuce, scabies, lepra, alphos, impetigene, lichen and elefantiasi", for example, were used to indicate chronic skin diseases very different one from the other, serious or not, characterized by localized conditions compromising the skin, in which large quantities of scales were also present.

In the epoch of Arab medicine, which reached its zenith between the VIII and XI centuries, various illustrious persons contributed with their work to the progress of medical knowledge of that period. Among these, we must remember Rhazes, Haly Abbas, author of "Liber regius", in which dermatology is especially discussed, Avicenna, Albucasis and Maimonide, as some of the most important. Their works also discuss diseases which are characteristic of the skin and the terms "morphea, albaras, serpedo" have been used to indicate, in particular, skin diseases characterized by spots, chapped skin and itching, covered by lamelle forforacee or rounded scales, similar to fish scales. The term "usagro" is also mentioned to define those chronic conditions of the skin in which a great quantity of such scales are present.

In order to clarify the terminology, we can today state that the condition that was the lepra of the ancients, that very serious deforming disease which had its maximum spread in medieval times, had various names such as: Zaraath, Lepra Arabum, Lepra Vulgaris, Elefantiasi dei Greci and in such conditions it was possible to distinguish also such clinical pictures as alphos, melas, leuke. But, the term "lepra" also included other chronic skin inflammations, not dangerous, covered by scales, that had nothing to do with the real leprosy. All of this only increased the confusion of terms and concepts.

In the Middle Ages, western medicine was mainly empirical, monastic, astrological and in part also magical. References to dermatological diseases can already be found among the Maestri of the ancient Scuola Salernitana, such as Costantion Africano; subsequently, various other authors studied these conditions, such as Henri de Mondeville, Yperman, Guy de Chauliac, Bernard de Gordon and John Arden, but in their work we do not find important contributions to the history of dermatology, since the literature in these centuries consists basically of collections or comments of ancient Greek and Arab texts.

During the Renaissance, the dermatology studies also had a positive influence from the renovative spirit present in the scholars of that period. Among the authors of that period, Girolamo Mercuriale (1530–1606) deserves particular mention: author of important works, he is also considered today the founder of modern dermatology since, in his treatise "De morbis cutaneis" he was the first to create a morphologial classification of these diseases. In the second chapter of this book, which covers skin diseases, itching, scabies, leprosy and lichens are lucidly described; concerning leprosy, he writes that it differs from scabies because the latter presents only small corpuscoli and dandruff, while the former is characterized by the presence of scales similar to those of fish.
However, confusion persists over descriptions of these various forms of disease.

During the 17th and 18th centuries medicine became experimental. The discovery of the microscope permitted the description of structures which were previosuly unknown; there was progress and important contribution to various fields of knowledge. Concerning dermatology, we must remember Jean Astruc (1684–1766), and Joseph Plenck (1735–1807) author of "Doctrina de morbis cutaneis" proposing for the first time a systemic classification of dermatological diseases in 14

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classes. But even with these authors, psoriasis does not seem to be defined; the use of the term "lepra" continues to indicate various diseases which leave the skin in a "disgusting" condition or diseases which were particularly resistant to treatment. Uncertainty persists between what was intended with leprosy, psora and psoriasis.

The Englishman Robert Willan (1757–1813) finally shed light on the problem. He was an author of basic works in this field, among which a 1799 treatise of great historical value being the first to have coloured illustrations of skin diseases. Willan distinguishes these conditions, classifying them from the point of view of objective examination, introduction a terminology which was to remain valid for a long time. The dermatoses are divided into seven orders; the second is of interest to us, characterized by scales, which includes: lepra, psoriasis, pitiriasi and ittiosi; he has the merit of being the first to indicate the clinical character of psoriasis and to have it termed by name and description as a separate disease, including all the squamous dermatoses of the skin, differing from leprosy. However, a few concepts still connected with the past persist in his writings: he felt, for example, that the diseases which he called lepra and psoriasis were the lepra and psora of the ancient Greek authors. Referring to the ancient writings of Paolo di Egina, stating that lepra formed circular spots on which appeared scales similar to those of fish and that the psora instead is more superficial and multi-formed, covered by a dandruff-like substance, he distinguished this squamous disease into two different morbid states defined as "lepra graecorum" the condition characterized by circular, ring-like spots, differentiating it from the other "psora leprosa" or better still, "psoriasis".

Notwithstanding this much discussed division of psoriasis into two different diseases, the terminology proposed by Willan was kept for a long time in dermatology; subsequently, the work of others such as Fuchs, Simon, Alibert and Hebra, just to name a few of the most important ones, recognized the indivisibility of these two clinical pictures and it was felt that the single term psoriasis or Lepra Willani should unite the various clinical aspects of the disease being discussed. Hebra, particularly, proved how the circular form held to be characteristic of leprosy could be present also in psoriasis, although these two forms where not considered to be different – nor should there have been subdivisions between the different types.

Therefore it was only around 1830–40 that the disease had its first precise definition.

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ABSTRACT

Epidermal Self-maintenance in Psoriasis

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Knowledge is accumulating on the bioregulatory factors of epidermal tissue, but we do not yet really understand how epidermal self-maintenance takes place. A kinematic model which helps elucidate both the mechanism of this self-maintenance and the formal pathogenesis of aberrations, are proposed in the present paper.

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Psoriasis and the Nervous System

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Both clinical and experimental evidence is accumulating on the role of the nervous system in the pathogenesis of psoriasis. Sporadic reports as well as extensive studies indicate that emotional stress can act as an exacerbating event in psoriasis. Moreover, that neurogenic mechanisms are operating in psoriasis is suggested by clinical, pharmacologic and experimental data. We have focused our investigations on the role of vasoactive intestinal peptide (VIP) and substance P (SP) in psoratic lesions using a variety of experimental approaches: 1) receptor autoradiography; 2) immunohistochemistry; 3) radio-imunoassay; 4) human keratinocytes cultures. Our results indicate that an imbalance of VIP and SP exists in psoriatic lesions, and that these neuropeptides exert different and specific effects on human keratinocytes. At present, however, the finding of psoriasis being exacerbated by psychological factors cannot be satisfactorily explained merely by alterations of neuropeptides in the skin. ***Key words: stress; vasoactive intestinal peptide; substance P.***

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Several observations have been reported in the literature concerning the correlation between psoriasis and the nervous system. Psychogenic or neurogenic mechanisms have alternatively been claimed to act as provoking or exacerbating factors, either in the onset or in the maintenance of psoriatic lesions.

Indeed, it is common experience in dermatologic practice to observe a stressful event being strictly associated with a psoriatic rash. In these cases, it is obviously tempting both to the clinician and to the patient to think of a causative linkage, with the emotional stress acting as a trigger. Although most reports in the literature are just anecdotal and the concept of "stress" as related to clinical conditions is still vague, some studies have shown with standardized and statistical methods a correlation between stress and psoriasis. Gaston et al. were able to demonstrate a statistically significant relationship between adverse life events and severity of psoriasis (1). In 132 psoriatic patients followed over a 3-year period, Seville found that a specific stress occurred within a month before the first psoriatic attack in 39% of the subjects (2). Interestingly the prognosis for these patients was better than for patients who could not recall such a stressful event. Moreover, classic psychologic therapeutic tools, such as the "insight" of the patients (3), hypnosis (4), biofeedback (5) and psychotherapy (6) have been successfully used in psoriasis. However, at present the pathomechanisms connecting psychologic factors with the appearance of the psoriatic lesions are completely unknown, and a direct relationship is still to be demonstrated.

As far as neurogenic mechanisms are concerned, clinical and experimental evidence points to a role of peripheral nerves and neural molecules in the pathogenesis of psoriasis. For example surgical denervation (7), dermabrasion (8), and skin injury (9) have been reported to induce local remission of psoriasis, possibly as a consequence of peripheral nerve damage. Neuropeptides (NP) have been considered the peripheral mediators of the neurogenic component underlying the pathogenesis of psoriasis (10). Indeed, these neural peptides are known to be antidromically released by sensory nerve fibres in the skin, where they are able to induce local inflammatory reactions (11). Several functions and cells, which are modulated by NP, such as vasodilation (12), mast cell activation (13), keratinocyte proliferation (14), play a basic role in the pathology of psoriasis (15, 16). Furthermore, several NP affect the function of immune cells involved in the pathogenesis of psoriasis, such as lymphocytes, neutrophils, and mast cells (17). The neurotoxin capsaicin, which depletes cutaneous sensory nerves of their neurotoxin content (18), is effective when topically applied to psoriatic lesions (19). Intravenous infusion of somatostatin, an inhibitor of the NP substance P (SP) (20), is beneficial in severe psoriasis (21). An accelerated turnover of neural elements (22), an increased innervation (23), and a greater number of SP-containing intra-epidermal nerve terminals (24) have all been reported in psoriatic lesions.

In recent years, our group has been evaluating the role of two important cutaneous NP, vasoactive intestinal peptide (VIP) and SP, in the pathomechanisms of psoriatic lesions, using a variety of experimental approaches. Using receptor autoradiography, we were able to detect SP receptors, on both normal and psoriatic skin (25). Specific SP-biding sites were found, both evenly distributed in the epidermis and also focally clustered in the dermis, in a location corresponding to the possible SP dermal target structures (microvessels, mast cells, keratinocytes). Quantitative computerized analysis revealed no significant differences in receptor density or receptor affinity between lesional and normal skin. Similarly, immunohistochemistry showed a comparable density and distribution pattern of SP– and VIP-positive fibres in psoriatic lesional, non-lesional and normal skin sections (26). It should be noted that SP and VIP appeared to be contained not only in cutaneous nerve fibres, but also in the cytoplasm of neutrophils in the infiltrate, thus suggesting another possible source of these NP in psoriatic lesions.

A third approach was to measure VIP and SP cutaneous levels in psoriatic lesional, non-lesional and normal skin. A radioimmunologic technique was employed on tissue homogenates. The local content of VIP was consistently increased in psoriatic lesions as compared with both non-lesional and normal skin. These data would be confirmed by the observation that larger amounts of capsaicin are needed to induce neurogenic inflammation in psoriatic vis-à-vis control skin (27).

Finally, since keratinocyte hyperproliferation is a feature of psoriasis, we evaluated the effects of VIP and SP on cultured normal human keratinocyte proliferation. We demonstrated that

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the VIP carboxy-terminal fragment was responsible for the dose-dependent growth-promoting effect on keratinocytes (26). By contrast, SP and SP fragments failed to stimulate keratinocyte proliferation. Furthermore, SP was shown to significantly block the VIP-stimulated proliferation.

The changes in SP and VIP skin content in psoriasis indicate participation of these NP and cutaneous nerves in the mechanisms underlying the production or the maintenance of psoriatic lesions. Their specific effects on keratinocyte proliferation, as well as their antagonistic action on the cells of the immune-inflammatory system seem to reflect a different role of SP and VIP in the pathogenesis of psoriatic lesions. In conclusion, two distinct series of evidence would seem to indicate a role for the nervous system in psoriasis. On the one hand, emotional stress has long been recognized as acting as a precipitating factor in psoriasis. On the other hand an increasing body of observations indicates that at least some NP intervene at a local level in the pathogenesis of psoriatic skin lesions. However, evidence of a direct connection between psychological stress and peripheral NP release is still lacking. Therefore, at present, these concepts should be regarded as two different components in the pathomechanism of psoriasis.

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Psoriasis, Stress and Psychiatry: Psychodynamic Characteristics of Stressors

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The aim of this investigation was to learn how a stressful event, often very mild, can determine a relapse of psoriasis. The research was carried out with clinical interviews and with the administration of Rorschach Psychoreactive, MMPI and H-T-P tests to 80 in-patients. Our data revealed a high prevalence of psychic disorders: 71.2% of patients showed symptoms which allowed a precise psychiatric diagnosis based on DSM-III-R criteria. 35% had personality disorders, 17,5% were moody, 12.5% were anxious and 6.25% had a schizophrenic trait. The analysis of the stressful events enabled us to determine the presence of a specific event in 88.7% of cases. For the majority of patients, the stressful event was felt as very mild: 67.6% of patients reported the existence of a low-impact stressful event according to the DSM-III-R classification. The average evaluation of the stressful event for all patients, based on a five-stage rating (ranging from 2 'light' to 6 'catastrophic') was 2.56. In conclusion, the analysis of the psychic conditions of in-patients showed that the importance in inducing an acute episode of psoriasis is the meaning of a stressful event as experienced by the patient, i.e. the questioning of his own identity, rather than the intensity of the aforementioned stressful event. In this case, the disease appears to be an attempt to express a defensive somatic response to a possible identity crisis. ***Key words: psoriasis; psychosomatic; stress; psychiatry.***

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The influence of psychological factors in the etiology and pathogenesis of psoriasis has been long recognized in the literature. As early as the 1950s, a clear correlation between stressors and psoriasis was established (Ingram, Susskind, Wittkover). This fact led to several studies which were aimed both at identifying the frequency of stressors present at the onset of the disease and at verifying the influence by the stressor on the clinical course of the already established disease.

As to the onset of the pathology, several authors have succeeded in establishing the presence of stressors preceding its first manifestation. Their percentages are remarkedly varied: 32–44% (5, 6); 90% (16); 39% (17); 80% (11).

Other authors have specifically investigated the exacerbation of the disease following stressful conditions and they have found positive correlations (3, 12); the disease worsens within a month after the stressor and, in two-thirds of cases, during the first 2 weeks after its occurrence (19).

Under standardized experimental conditions, Arnetz et al. (2) found significantly more intense responses to stress in patients with psoriasis, compared with the control group.

Several studies have been conducted also to verify the existence of recurrent specific personality traits in the psoriatic patients, but the results are, by and large, not very significant (13, 3, 4, 15).

As to the specifically pathogenetic aspects, i.e., how the cutaneous damage develops from the stressor, the studies by Farber et al. (7, 8, 9) have underlined the role played by the psychoneuroimmune mechanisms, with particular attention paid to the function of neuropeptides.

Finally, all of the data collected in literature (10) point to the fact that an effective therapeutic program against this disease clearly benefits both from psychotherapeutic efforts aimed at controlling the stress and from the promotion of healthy physical conditions.

Following these studies, we have decided to carry out an investigation to learn how the stressors find a way to discharge psychological discomfort into the body. Our aim, therefore, was to accomplish an accurate assessment of the patients' psychiatric conditions, as well as of the recurrence and intensity of the stressors at the onset of the disease. This could prove to be important, as the history of many patients suggested that their psychiatric disease preceded the dermatological one. First and foremost, we wanted to analyse in depth the psychodynamic experiences of the subjects, in order to understand how the psyche reacts to the stressor, thus causing this specific somatic response.

MATERIALS AND METHOD

The sample consisted of 80 in-patients, aged 19–59 (mean: 34.65 SD: 11.79), 38 females (47.5%) and 42 males (52.5%).

The intervention protocol applied to each patient was structured as follows:

* administration of a socio-demographic questionnaire;
* three consecutive psychiatric interviews;
* administration of psychodiagnostic tests: Rorschach, MMPI, House-Tree-Person Test.

These are all very well-known instruments, which can very accurately single out psychopathological signs and symptoms. They were administered and analyzed by psychologists who were unaware of the clinical diagnosis made by the psychiatrists. Only subsequently were the clinical and test data compared in order to make a definitive diagnosis. The detailed analysis of the results obtained through these tests will be reported in other works.

Both the psychiatric diagnosis and the assessment of the stressor intensity were based on the DSM-III-R diagnostic criteria.

RESULTS

The patients' symptoms evidenced in the psychiatric interviews and by the psychodiagnostic tests allowed of an accurate psychiatric diagnosis of 57 subjects (71.2%), based on the DSM-III-R

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Table I

Diagnosis		No.	%
Personality disorders		28	35
	Dependent	12	15
	NOS	12	15
	Histrionic	2	2.5
	Ossessive, compulsive	2	2.5
Mood disorders		14	17.5
	Dysthymia	8	10
	Major depression	2	2.5
	Cyclothymia	2	2.5
	Bipolar disorder, manic	1	1.25
	Bipolar disorder, mixed	1	1.25
Anxiety disorders		10	12.5
	Generalized	6	7.5
	Simple phobia	3	3.75
	NOS	1	1.25
Psychotic disorders		5	6.25
	Delusional (persec. type)	2	2.5
	Chronic schizophrenia, disorganized	1	1.25
	Schizof., residual type	1	1.25
	Schizoaffective disorder	1	1.25

diagnostic criteria. Table I shows the frequency of the various diagnoses.

Each patient's history allowed for the individuation of a triggering stressor in 71 cases (88.75%). Table II shows the patients' distribution according to the presence or absence of stressors as indicated in the DSM-III-R. Table III classifies patients on the basis of the stressor intensity, which was generally very low.

When the means of the stressors' intensity were compared for the two sexes, no significant difference emerged. The same result was obtained by comparing psychiatric and non-psychiatric patients (Tables IV, V).

DISCUSSION

The above results are, in a way, only a premise to our observations. The data relating to our patients' psychiatric disorders indicate the remarkable presence of personality disorders. This is a rather new result in the current literature, and it shows the existence of chronic disorders, as far as the patients' identity is concerned. Their onset is rather early, around adolescence or pre-adolescence.

Moreover, the analysis of the stressors indicates that their intensity is generally very low: our patients reported what to an outsider may appear as very mild stressors, such as quarreling with a sister-in-law or the possibility that one's brother might separate from his wife.

No statistical significance existed in the relationship between stressor intensity and patients' psychic health conditions. One could, therefore, suppose that equal intensity stressors might variously affect the patients according to their basic psychic conditions, i.e., that the subject's psychiatric pathology may render him/her more susceptible to stress. This is potentially the case in personality disorders, which generally precede the onset of psoriasis. This hypothesis, however, was not supported by solid data.

In any case, the presence of stressors was among the most comprehensive ever reported in the literature. This was probably due to the modalities used in data gathering. Indeed, we collected elements of psychologically significant episodes in the patients' history rather than verifying how aware the patients were of a more or less accidental connection between something that had upset them and the onset of the disease. Only later did we investigate the possibility of a temporal connection between these episodes and the onset of the dermopathic event. This probably allowed us to identify events that would have otherwise escaped the anamnesis.

In fact, our interviews have indicated that, in itself, the stressor intensity is not important: what counts is the meaning that said stressor takes on within the patients' way of 'viewing themselves' in the world, i.e., with respect to their psychological identity.

For these patients the stressor does indeed become so mea-

Table II

Code	Stressor	No. of pats	%
0.	Insufficient Info	5	6.25
1.	None	4	5
2.–6.	Present	71	88.75

Table III

Stressor intensity		No. of pats	%
2.	Low	48	67.6
3.	Moderate	10	14.1
4.	High	11	15.5
5.	Extreme	–	–
6.	Catastrophic	2	2.8
	Total	71	100
	Mean	2.56	
	SD	0.95	

Table IV

	Male	Females	Difference
Mean	2.703	2.412	0.291
SD	1.127	0.701	

$p = 0.411$

Table V

Psychiatric Pathology	Present	Absent	Difference
Mean	2.640	2.381	0.259
SD	1.051	0.584	

$p = 0.322$

ningful as to undermine their self-image. The phenomenon occurs in the most varied fashions, but in all cases the consequence is the same: the patient feels 'undermined' as a person, and thus the disease becomes a compensatory response capable of holding at bay the anxiety that the subject experiences when confronted with a crisis of identity.

All of our cases dealt with delicate subjects whose identity structure was rather defective and precariously balanced due to the most varied factors. For example, a particularly intense and symbiotic relationship with one's mother, the sense of family's unity, a stable and reassuring job, the school environment, one's bedroom walls, etc.

When the stressor endangers the protective factor to which the patient connects his/her identity, then the subject may face a crisis even though the stressor intensity is very low, because what really counts is the meaning that he/she attributes to it. In consequence, a family quarrel or a family member's potential marital separation can be experienced as an actual blow to the solidity of the group to which the patient is bound. The same can occur following changes in one's work environment (maybe just a change of room or of duties within the same organisation), in the school environment or a house move: clearly, subjects who are sufficiently sound at the psychological level can easily cope with events of this nature.

Such identity crises are not a rare event in psychopathology and they have no specific evolution pattern. In fact, in other types of patients they can develop into a variety of symptomatologic pictures: other psychosomatic diseases, hypochondriasis, psychic disorders of a different nature. We believe that this peculiar type of response in the psoriatic patients is determined by a widely recognized genetic predisposition.

Even the localization of the cutaneous lesions does not present characteristics that may somehow be correlated to specific problems. The disease seems rather to constitute a sort of generalized response: its para-hyperkeratosis apparently emphasizes and demarcates the boundaries of one's bodily and psychic self in a compensatory manner. Indeed, the disease allows these patients to shift their attentition from their inner to their outer world, thus providing the double benefit of defending them against the anxiety about internal disintegration and of clearly marking their body boundaries.

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Experience with Psoriasis in a Psychosomatic Dermatology Clinic

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We studied 179 psoriatic patients by semistructured colloquia and psychometric tests and determined their cutaneous psychoneurophysiological profiles by biofeedback methods. The Paykel scale for stressful events showed that 72% of psoriatics had experienced significant stressful events about one month before the appearance of the psoriasis. The Zung test for anxiety and depression showed a high level of anxiety in the psoriatic patients. 64% of the patients who were treated by BFBtraining had a decrease in their PASI index for severity and the extent of the disease and also fewer recurrences at the one-year follow-up. The results of the World Experience Inventory indicated difficulties related to body image and to relationships with others. Psoriasis influenced the sexuality of the patients. It is always difficult when one is afflicted by ill health to enjoy life and the general scores of SWL (Satisfaction with Life), were significantly lower than those of a control group. ***Key words:*** **psoriasis; stress; quality of life; alcohol consumption.**

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EXPERIENCE WITH PSORIASIS IN A PSYCHOSOMATIC DERMATOLOGY CLINIC

The observation that psoriasis often appears or recurs after trauma or stressful events has prompted investigations into the possibility that psychological factors may be a cause (or co-cause) of the disease (1–7). The relationship between pruritus and psoriasis is another aspect that indicates the importance of the bond between psychological factors and the manifestations of psoriasis (8, 9). In addition, the development of psoriasis easily causes anxiety and difficulties in social relationships (10, 11).

MATERIAL AND METHODS

We have approached psoriasis from the psychosomatic point of view at the Psychosomatic Dermatology Outpatient Clinic of the Institute of Dermatology in Milan. We studied 179 psoriatic patients by semistructured colloquia, psychometric tests (MMPI, Zung Anxiety and Depression, Paykel rating scale for stressful events) and we determined their cutaneous psychoneurophysiological profiles by biofeedback (BFB) methods.

RESULTS AND CONCLUSION

The Paykel scale for stressful events showed that 72% of psoriatics (category not requested and not checked) had experienced a significant stressful event about one month before the appearance of the psoriasis (12). The MMPI did not reveal any abnormalities in the personalities of our patients, while the Zung test for anxiety and depression showed a high level of anxiety in the psoriatic patients (12).

The cutaneous psychoneurological profiles showed statistically significant increases in muscle tension and cutaneous galvanic resistance under conditions of mental, emotional or physical stress (13). This we considered indicative of a possible link between the psoriasis and a corresponding psychoneurological

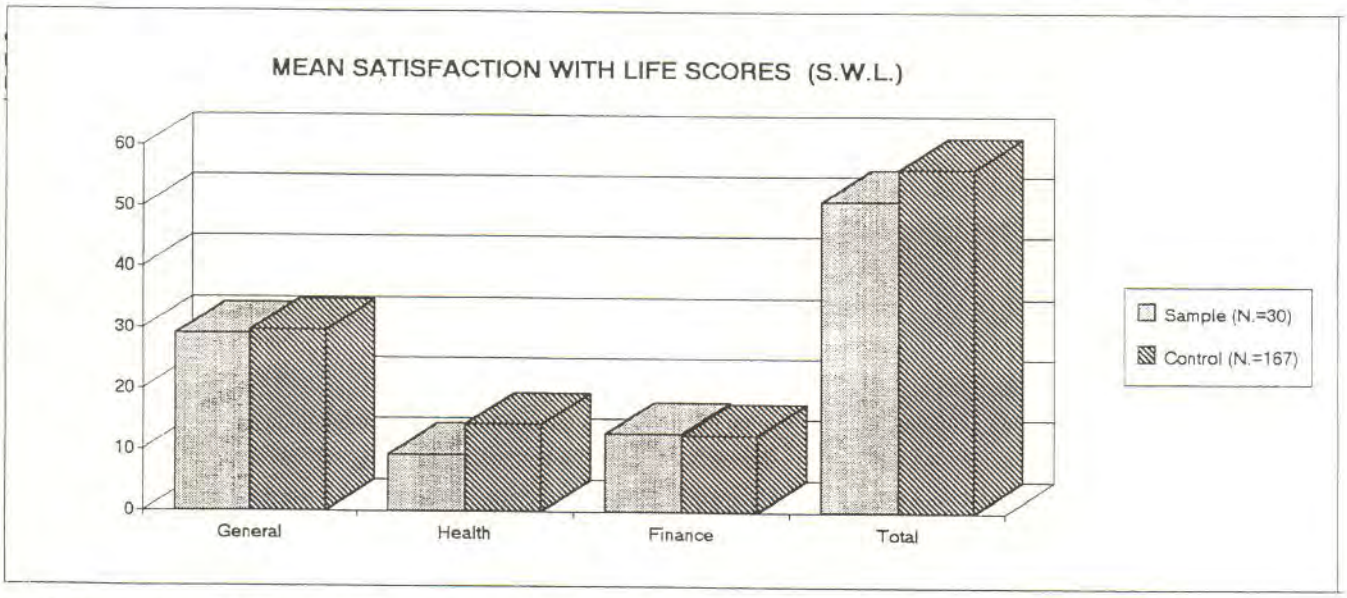

Fig. 1. The experience of Psoriasis in a Psychosomatic Dermatology Service.

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substrate. 64% of the patients who were treated by BFBtraining (EMG feedback) had a decrease in their PASI index (14) for severity and extent of the disease, in particular decreases in erythema, infiltration and desquamation. They also reported less extensive disease and fewer recurrences at the one-year follow-up (15).

Another aspect we investigated was the quality of life of psoriatic patients, which we studied in 30 psoriatic men.

The study design was transverse, with 10 subjects less than 33 years of age, 10 between 45 and 54 years and 10 between 63 and 72 years. We wished to see if the impact of psoriasis on the quality of life increases with increasing time. The instruments used to evaluate the quality of life were the WEI (World Experience Inventory), BSRI (Bem Sex Role Inventory) and SWL (Satisfaction with Life). The results of the WEI indicated difficulties related to body image and to relationships with others. The extent, the severity and the localization of the psoriatic lesions influenced and conditioned the sexuality of the patient, but without affecting the sexual identity. It is always difficult when one is affected by ill health to enjoy and be satisfied with life and the general scores were significantly lower than those of a control group. From the more generalized view of the clinical sessions, we concluded that there is no strict correlation between the severity of the disease and the psychological suffering. Each of the patients responds subjectively to the cyclicity of the disease, but from time to time assigns different significance to it. In an earlier study (16) we found a positive correlation between the duration of the disease and behaviour (e.g., alcohol consumption).

In conclusion, the results of this preliminary study emphasize the need for a more thorough investigation that will also take into account the changes in quality or life during the long-term treatment that is needed by psoriatic patients.

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Plasma Neuropeptide Levels in Psoriasis

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The immune system is important in the pathogenesis of psoriasis and emotional stress has precipitated psoriasis in many patients. Neuropeptides, alpha-Melanocyte stimulating hormone (alpha-MSH), beta-endorphin, met-enkephalin and substance P (SP) act as immunomodulators, and their secretion increases during periods of stress. To see whether these neuropeptides themselves might be related to psoriasis and/or to the aggressiveness of the disease, we evaluated the plasma neuropeptide levels in 13 patients with active psoriasis (patients with new lesions and/or pre-existing lesions that had become larger during the month before the study), in 11 patients with stable psoriasis and in 10 healthy controls. Plasma concentrations of neuropeptides were evaluated by RIA (immunoradiometric assay for beta-endorphin). Data were compared by the Student *t*-test for unpaired data. There were no significant differences between the plasma levels of any of the neuropeptides between active psoriatic patients and stable psoriatic patients, nor between the plasma levels of neuropeptides of psoriatic patients and those of control subjects. It seems unlikely that circulating neuropeptide levels are of primary importance in the manifestation of the psoriatic skin lesions. *Key words:* psoriasis; opioid peptides; substance P.

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The immune system is important in the pathogenesis of psoriasis (1) and emotional stress has precipitated psoriasis in many patients (2). Opioid peptides (i.e., alpha-Melanocyte Stimulating Hormone (alpha-MSH), beta-endorphin and met-enkephalin) are important regulators in the immune system, including T cell functions (3). Their release has also been shown to be affected by stress (4). Stress also influences the immune response and it has been suggested that opioid peptides might be mediators in stress-induced immunomodulation (5). Substance P (SP), a neuropeptide present in both central and peripheral nervous systems, is known to have some important physiological functions, including T cell immunomodulation (6). In addition, SP secretion is also influenced by stress (7). It has been suggested that the nervous system can influence the course of psoriasis and that neuropeptides, such as SP, may be of importance in the pathogenesis of psoriasis (2, 8). This hypothesis is supported by the observation that the treatment of psoriasis with topical capsaicin, which depletes primary-sensory nerves of neuropeptides, can improve psoriasis (9). Thus, opioid peptides and SP seem to have two important biologic characteristics in common: they act as immunomodulators and their secretions vary during periods of physical and/or psychological stress. For these reasons, the changing levels of opioids and neuropeptides might be correlated with the manifestations of psoriasis.

To see whether or not the levels of these peptides might be related to psoriasis itself and/or to the aggressiveness of the disease, we measured the plasma peptide levels in patients with active psoriasis, in patients with stable psoriasis and in healthy subjects.

PATIENTS AND METHODS

Twenty-four male patients (32 to 50 years) with extensive plaque-form psoriasis (mean PASI score 18) were studied. In 13 of the 24 patients, new lesions had developed and/or pre-existing lesions had enlarged during the month before the study. These patients were defined as having active psoriasis; the other 11 as stable. The control group consisted of 10 male healthy volunteers (30 to 46 years). Samples of peripheral blood were taken at 8 a.m. Plasma concentrations of neuropeptides were evaluated by RIA (Immunoradiometric assay (IRMA) for beta-endorphin) with ^{125}I-labelled specific rabbit antibodies and different types of bound-free separation. For met-enkaphalin, preliminary extraction of the sample on Sep-pack C18 silica columns (Waters Associated, Milford, Mass) was necessary. In the assays, the reagents

Table I. *Plasma concentration of opioid peptides and substance P in patients with psoriasis, compared with healthy subjects**

Peptide	active psoriasis (13 cases)	stable psoriasis (11 cases)	healthy subjects (10 cases)	*p*-values			
				Active P. vs. healthy subjects	Stable P. vs. healthy subjects	Active P. vs stable P.	Active plus stable P. vs. healthy subjects
Alpha-MSH	62.5+4.9	65.3+7.2	57.7+8.2	NS	NS	NS	NS
Beta-endorphin	26.0+1.3	23.2+1.0	24.3+1.6	NS	NS	NS	NS
Met-enkephalin	55.8+5.8	53.9+6.4	52.5+8.9	NS	NS	NS	NS
Substance P	32.8+4.7	39.4+4.2	38.4+3.3	NS	NS	NS	NS

* Plasma peptide levels are expressed in pg/ml (mean + SEM) NS, no significant difference (Student's *t*-test).
P psoriasis.

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used were from Immuno Nuclear Corporation, Stillwater, Minn, for alpha-MSH, met-enkaphalin and substance P; Allegra-IRMA from Nichols Institute Diagnostic, S Juan Capistrano, Cal., for beta-endorphin. The data were analysed by Student's *t*-test for unpaired data.

RESULTS

The results of neuropeptide plasma levels (mean + SEM) are given in Table I. There were no significant differences in the plasma levels of any neuropeptide between any two groups.

DISCUSSION

No previous study of plasma levels of opioid peptides in psoriasis has been made. Our data for SP accord with those of Eedy et al. (10), who found no differences in the plasma levels of SP between psoriatic patients and controls. In the present study the plasma levels of opioid peptides and of SP in psoriatic patients did not differ from those of normal subjects, and there was no correlation between the plasma levels of any neuropeptide and the aggressiveness of the disease. Since all of our patients had extensive psoriasis, it seems unlikely that circulating opioid peptides or SP are of primary importance in the manifestation of the psoriatic skin lesions.

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Psoriatic Arthritis: A Clinical, Radiological and Genetic Study of 58 Italian Patients

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It is well known that genetic heterogeneity and/or the complex interaction of several MCH-linked risk factors can explain the onset and the broad spectrum of Psoriatic Arthritis (PsA) from the clinical point of view. Fifty-eight patients with PsA (Moll and Wright criteria), 35 men and 23 women, mean age of 45, 14, were studied; all the patients were assessed by both clinical and radiological examination, with particular attention to the sacro-iliac joints. HLA typing of the patients confirmed the association between PsA and HLA-B39 ($p = 0.0008$) and Cw6 ($p = 0.0011$). In addition a significant increase in DQ2 antigen ($p = 0.004$) has been found. No correlation of any particular HLA antigen with clinical subsets (oligo-polyarticular peripheral PsA, axial PsA and axial with peripheral PsA) or erosive incidence of joint involvement – generally related to the duration of the disease – was found. ***Key words: psoriatic arthritis; HLA antigens; disease severity.***

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Increasing numbers of clinical, radiographic, serologic and epidemiological studies support the nosographic classification of psoriatic arthritis (PsA) as a true entity (1). Despite regarding Moll and Wright criteria as useful for diagnosis, a global definition of PsA may be "a syndrome in which psoriasis is associated with inflammatory arthritis, usually having a negative sheep cell agglutination test" (2, 3, 4).

The pathogenesis of PsA is still to be elucidated, but it is certain that genetic, environmental and immunologic factors are thought to be prominent in the development and perpetuation of the disease (5, 6). The opportunity to observe a clinical subset of rheumatoid arthritis-like PsA, in any case seronegative for rheumatoid factor, has stimulated the study of the genetic differences between PsA and rheumatoid arthritis (RA): psoriasis and PsA are associated with both Class I (B13, B17, B39, B27, Cw6) and Class II (DR4, DR7) HLA antigens, while RA is associated only with Class II antigens (DR4, DQ3) (1, 7).

While for other rheumatic diseases the role of the MHC in autoimmunity is hypothesized, the mechanism of the MHC association with PsA is still unknown (1). Contradictory is the association of PsA with DR and DQ antigens; DR4 in association with severity of the disease (8) and rheumatoid arthritis-like disease (8, 9); DR4 and DQ53 with earlier age of onset of arthritis (3). A9 and B5 antigens are associated with a higher radiological lesion (erosions) (3), A9 antigen with a later onset of psoriasis. Finally, there is a significant increase in A1, B38 in patients with asymmetric peripheral disease and B39 with symmetric peripheral disease (3). From the clinical and radiological points of view it has been noted that, in PsA, joint damage tends to occur early in the course of the disease (4); recent clinical studies suggest that PsA may be as destructive as RA (10, 11), but with the occurrence of both joint lysis and ankylosis in various joints in the same patient (1). Our aim is to contribute to the clinical and radiological physiognomy of PsA and to report the HLA antigens distribution in our patients and the possible association between clinical subgroups and HLA antigens.

MATERIALS AND METHODS

Fifty-eight italian outpatients with PsA were studied. The diagnosis was established according to the Moll and Wright criteria (2). All patients had cutaneous and/or nail psoriasis and underwent X-ray examination of hands and wrists, feet, spine and sacro-iliac joints. Modified ARA criteria of RA were used to document the radiological stage of each joint (4). Axial and sacro-iliac radiological involvement was evidenced

Table I.

	PsA (total)	Peripheral disease		Axial disease	
		Oligo-arthr.<5	Poly-arthr. ≥5	With periph. involv.	Without periph. involv.
No. of patients	58	29	23	4	2
Female/male	23/35	15/14	8/15	0/4	0/2
Mean age (years)	45.17	41.79	48.56	50.00	45.50
Mean age of onset PsA (years)	42.46	40.24	44.69	46.50	41.00
Mean duration of P. (months)	96.90	65.10	125.74	201.00	18.00
Mean duration of arthr. (months)	33.52	19.45	48.00	42.00	54.00
Family history of P. (%)	34.48	34.48	34.78	33.33	
Nail lesions	63.79%	16 pts	18 pts	2 pts	1 pt

Table II. *Radiological features of the 58 patients*

	PsA (total)	Peripheral disease		Axial disease	
		Oligo-arthr.	Poly-arthr.	With periph. involv.	Without periph. involv.
No. of patients	58	29	23	4	2
* X-ray stage:					
I		6/29	3/23	1/4	–
II		8/29	5/23	2/4	–
III		15/29	13/23	0/4	–
IV		–	2/23	1/4	–
Enthesitis	22.41%	3	8	2	–
Sacro-iliac joint inv.	43.10%	11	8	4	2

* Modified ARA criteria were used to document the radiological stage of each joint (radiographs of hands and feet only).

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Table III.

	Controls		Patients		p-value	RR
	n	%	n	%		
Locus B	(116)		(58)			
B13	7	6	6	10.34	ns	–
B17	14	12.1	12	20.69	ns	–
B27	7	6	5	8.6	ns	–
B38*	6	5.2	7	12.6	ns	–
B39*	1	0.8	8	13.79	$p=0.0008$	18.1
Locus C	(114)		(44)			
Cw6	20	17.5	19	43.18	$p=0.0011$	3.6
Locus DR	(116)		(56)			
DR2	32	27.6	8	14.28	$p=0.038$	0.4
DR3	12	10.3	12	21.43	$p=0.049$	2.2
DR7	33	28.4	21	37.50	ns	–
Locus DQ	(116)		(54)			
DQ2	36	31	29	53.7	$p=0.004$	3.2

* $n=114$

through the New York criteria and/or syndesmophyte presence. Clinical subsets of PsA were: oligo-polyarticular peripheral; axial; axial with peripheral joint involvement. HLA A, B and C antigens were typed by the standard NIH microlymphocytotoxicity technique (12); HLA DR and DQ antigens were typed by a prolonged cytotoxicity technique (13) on B lymphocyte purified preparation (14). Typing sera were selected from the 9th and 10th International Histocompatibility Workshop, and correlated sera were also used.

Data analysis was carried out using Fisher's exact test to compare the frequencies of HLA antigens. Relative risks (RR) were calculated using the Woolf method.

RESULTS

Clinical features of 58 patients with PsA are reported in Table I.

Radiologic features of our 58 patients are given in Table II.

The distribution of HLA antigens in our 58 patients of PsA, compared with normal controls, is shown in Table III.

DISCUSSION

Our data confirm the prevalence of psoriatic onset in the natural history of PsA and the importance of a family history of psoriatic disease. Psoriatic onychopathy was observed in 63.79% of total PsA, peripheral and/or axial not necessarily associated with distal interphalangeal articular involvement. From the radiological point of view, sacro-iliac involvement in our patients was 43.10%, with evidence of this location not only in the axial subgroup; enthesopathy was present in 22.41% of our patients. X-ray joint lesions, stages III and IV of modified ARA criteria, were present in 55.35% of our patients with an association with major duration of the disease (mean duration of 43.74 months in the patients with erosions versus a mean of 19.20 months in those without erosions). HLA typing of our patients confirms, according to other authors (1, 7), the increased association between PsA and HLA-B39 ($p=0.0008$) and Cw6 ($p=0.0011$) and for the II Class of antigens a significant increase in DQ2 antigen ($p=0.004$), the latter in partial contradiction of other reports (3). Despite the experience of other authors (1, 3), there was not significant statistical correlation between PsA and HLA-DR antigens either. The presence of HLA-B27 antigen was limited and not only associated with the 'axial' patients. No correlation of any particular HLA antigen with our clinical subgroups (oligo-polyarticular peripheral PsA, axial PsA and axial with peripheral PsA) was found, presumably due to the small number of patients in each subgroup.

We can conclude by underlining the importance of psoriatic familiarity and the presence of HLA-B39, Cw6 and DQ2 antigens, in order to define a 'risk' of disease, while the spreading of the radiologic lesions is particularly associated with the duration of PsA.

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Is HLA B27 a True Marker of Axial Involvement in Psoriatic Arthropathy?

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Sixty-six patients with psoriatic arthropathy were subdivided into nine groups on the basis of the presence of peripheral arthritis, axial disease whether or not fulfilling the New York criteria for ankylosing spon dylitis both associated and not associated with peripheral arthropathy and bilateral or monolateral sacro-iliitis. Only the group with axial disease (sacro-iliitis + spondylitis) without peripheral arthritis and not fulfilling the NY criteria showed a truly increased B27 prevalence. However, in this atypical group, only 2 patients had a true ankylosing pattern-like spondylitis. On the other hand, in the group with axial disease fulfilling the NY criteria, only one of 9 patients was B27+. We conclude that B27 is not a true marker of axial involvement in psoriatic arthropathy.

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On the basis of recent clinical, laboratory and radiological findings, psoriatic arthritis (PA) has been designated one of the "seronegative spondyloarthritides" with frequent axial involvement (1).

Though HLA B27 would be positive in 50%–70% of PA patients with an ankylosing spondylitis pattern when compared with 90% of those with idiopathic ankylosing spondylitis (2, 3), there is general agreement that an association between this histocompatibility antigen and psoriatic arthropathy does exist in the subset of patients having axial involvement (sacro-iliitis + spondylitis) indifferently with or without peripheral arthritis (4, 5, 6, 7, 8).

In the present paper, the prevalence of HLA B27 in 66 consecutive patients with PA, 26 of whom had a definite axial involvement, is reported.

PATIENTS AND METHODS

Sixty-six psoriatic patients with arthropathy, as defined by Wright and Moll (1), were studied. For the purpose of our study, patients were classified as having:

1) peripheral arthritis only
2) peripheral arthritis with or without axial disease, but not fulfilling the New York (NY) criteria for spondylitis
3) peripheral arthritis with axial disease
4) axial disease (sacro-iliitis or spondylitis, or both) with or without peripheral arthritis (and not necessarily fulfilling the NY criteria)
5) exclusively axial involvement, whether or not fulfilling the NY criteria
6) spondylitis fulfilling the NY criteria, with or without peripheral joint involvement
7) spondylitis only fulfilling NY criteria, but without peripheral arthritis
8) presence of bilateral sacro-iliitis
9) presence of monolateral sacro-iliitis

Radiologic sacro-iliitis was graded according to the NY criteria for the diagnosis of ankylosing spondylitis (9).

All patients had antero-posterior and latero-lateral radiographs of the spine, as well as a postero-anterior and oblique views of the pelvis. Patients with peripheral arthritis had radiographs of involved joints. A microlymphocytotoxicity technique test was used to type for HLA B27 antigen (10). The control population consisted of 340 blood donors from the same geographical area.

RESULTS

The general features of the patients studied are shown in Table I. Seventeen (25.8%) were B27+ (9 males; 8 females). An axial involvement was present in 25 patients (37.9%), of whom 7 (26.9%) were B27+, while this antigen was present in 8.8% of the control population. Table II gives the prevalence of HLA B27 in each group considered. In all groups, B27 was increased, but only in the patients with axial involvement and without peripheral arthritis did the percentage reach 60. This heterogeneous group consisted of 5 patients: 3 B27+ (only one fulfilling the NY criteria for spondylitis), 2 B27– (one NY criteria +). When we considered the group of 9 patients with axial involvement and fulfilling the NY criteria indifferently with or without peripheral arthritis, the prevalence of B27 was only 11.1% (one positive, 8 negative). Finally only one of the 2 patients fulfilling the NY criteria (group 7) was B27+.

DISCUSSION

Usually HLA B27 is considered to be a marker of spinal involvement (sacro-iliitis + spondylitis) in psoriatic arthropathy, whereas there is a normal prevalence in uncomplicated psoriasis (7, 10).

Table I. *Clinical features of 66 patients with psoriatic arthritis*

Sex:	Male	36	(54.5%)
	Female	30	(45.5%)
Age, yrs (mean ±/– SD)		51.7	±/– 12.6
Duration of psoriasis (mean ±/– SD)		13.0	±/– 13.4
Nail involvement		36	(54.5%)
Duration of arthritis, yrs (mean ±/– SD)		7.42	±/– 8.26
Peripheral arthritis		41	(62.1%)
Axial involvement		25	(37.9%)
Exclusive axial involvement		5	(7.6%)

Table II. *Prevalence of HLA B27 in 66 patients with psoriatic arthritis*

Group		B27	Total patients	%
1	Peripheral arthr. only	10	41	24.4
2	Per. arthr. ±ax NY crit.–	14	53	26.4
3	Per. arthr. +ax disease	14	61	22.9
4	Axial disease ± per. árthr.	7	25	28.0
5	Axial disease ± NY crit	3	5	60.0
6	Axial disease + NY crit ± per arth	1	9	11.1
7	Axial disease + NY crit	1	2	50.0
8	Sacro-iliitis, bilateral	3	16	18.7
9	Sacro-iliitis, monolateral	1	6	16.6

However, the percentage of B27 positivity in patients with psoriatic sacro-iliitis and/or spondylitis is lower than that reported in idiopathic ankylosing spondylitis (2, 4, 5, 6, 7, 12).

There is a general agreement that the prevalence of B27 in PA patients with pure peripheral arthropathy differs slightly from the normal population. In our series, B27 was present in 24.2% of all patients and in 24.4% of those in whom peripheral arthritis alone was considered. These results are not at variance from those reported by most authors (5, 13, 14, 15). In our experience however the prevalence of B27 in patients with axial involvement did not differ from the data recorded in a general group or in a group with peripheral arthritis alone. When we evaluated patients with spondylitis or sacro-iliitis, or both, with or without peripheral arthropathy (not necessarily fulfilling the NY criteria), or patients with spondylitis, fulfilling those criteria, and whether or not showing peripheral arthritis (9 pats: 8 B27–, 1 B27+), bilateral (16 pats: 13 B27–, 3 B27+) or monolateral (6 pats: 5 B27–, B27+) sacro-iliitis, the prevalence of B27 was invariably low (11.1–28%). Only when we took into account the patients with exclusively spinal disease did we find a prevalence of 60%. However, we must regard this group too small and heterogeneous to allow of statistical conclusions: 3 patients had syndesmophites only and did not fulfil the NY criteria for ankylosing spondylitis and cannot be classified as true ankylosing spondylitis pattern.

Our results agree with those reported by other Italian authors (11, 16, 17) and consequently we think that B27 is not a true marker of spondylitis in Italian psoriatic arthropathics.

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ABSTRACT

Psoriasis and Rheumatologic Manifestations in an Unselected Group of Dermatologic Patients

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The aim of this study was to ascertain the frequency of rheumatological manifestations associated with psoriasis in a group of patients from the Dermatology Clinic at Lucca Hospital, in view of the recently formulated classification of Seronegative Spondyloarthropathies and Psoriatic Arthritis (PA).

Much attention has been devoted to the presence of enthesopathy and dactylitis. We examined 90 consecutive (42 male + 48 female) patients, selected from a group of 150 between the ages of 23 and 77 years (median 52.5<) during the period of May 91 – to June 92.

A personal and familial history was taken from all patients. During the clinical examination we evaluated all peripheral joints, those entheses most frequently involved in SpA, thoracic expansion, cervical and lumbar mobility, and the extent of psoriasis, using the PASI index. They were studied for A, B, and C locus antigens, for inflammatory indexes, immunoglobulins, and pelvic radiographs.

The presence of sacro-iliitis was ascertained by staging pelvic X-rays, using a 'blind' observer. 25 patients (27.7%) were affected by PA; 19 of these had both spinal and peripheral involvement, with or without tendinitis, 4 had enthesopathies and dactylitis only and 2 had isolated enthesopathy.

All 25 patients fulfilled the Amor criteria for the diagnosis and classification of SpA.

The results of our study suggest that if patients with psoriasis are examined in the light of the whole clinical spectrum of SpA, the frequency of rheumatologic manifestations is much higher than the 5–10% found in other studies which evaluated only spinal and peripheral joint involvement.

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A Rare Enthesopathy in Psoriatic Oligoarthritis

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Peripheral enthesopathies have recently been attributed a crucial role in the definition of seronegative spondyloarthropathies. We report a case of psoriatic oligoarthritis in which a peripheral enthesopathy, occurring at the right olecranon, was the heralding sign of the disease. ***Key words: seronegative spondylarthropathies; psoriatic oligoarthritis; peripheral enthesopathies; olecranon spur.***

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Enthesopathies, bony proliferations around osseous erosions, are a prominent clinical aspect of seronegative spondylarthropathies (SSp) (1). Characteristically, the process occurs at sites of tendon and ligament insertion on bone, with either an axial or a peripheral pattern. Sometimes, as an isolated finding, they may be a presenting feature of disease (2–4).

We describe a case of psoriatic oligoarthritis in which an isolated enthesopathy, occurring at an uncommon site, represented the onset of articular involvement.

CASE REPORT

In January 1992 a 68-year-old office clerk with psoriasis was referred to our Department, complaining of pain of 3 years' duration at his right olecranon. Psoriasis had manifested itself in January 1990 at both elbows. Five months later he suffered arthritic involvement of the left knee.

On admission, physical examination revealed psoriasis vulgaris on the scalp, elbows, hands and legs. In addition, swelling and reduced joint mobility of the left knee were recorded. Relevant laboratory data showed elevated sedimentation rate (ESR 28 mm 1st h) and increased C-reactive protein (8 mg/dl; NV <1 mg/dl). Rheumatoid factor was negative. Bilateral sacro-iliitis (grade 2 on the left, grade 3 on the right) was noted on X-rays, and an increased radionuclide uptake was detected by technetium-99 scintiscaming. Increased uptake was present also on the left knee region, without radiological evidence. Coarse syndesmophytes were randomly seen along the thoraco-lumbar spine. A bony proliferation at the triceps tendon insertion was evident in a film of the right elbow, while minimal changes were present at the contralateral site (Fig. 1a, 1b.)

DISCUSSION

For several decades, axial enthesopathies, consisting of sacroiliitis and/or syndesmophytes, have been considered a useful hallmark for the classification of SSp. Recently, peripheral enthesopathies, have also been included among new sets of classification criteria, for both definite and unclassifiable spondyloarthritic patterns (5–6). We report a case of psoriatic oligoarthritis in which cutaneous and articular involvement had a simultaneous clinical onset.

Pain at the right elbow, recorded several years before the clinical appearance of both psoriasis and arthritis, could probably be related to the enthesopathic changes evidenced at the olecranon.

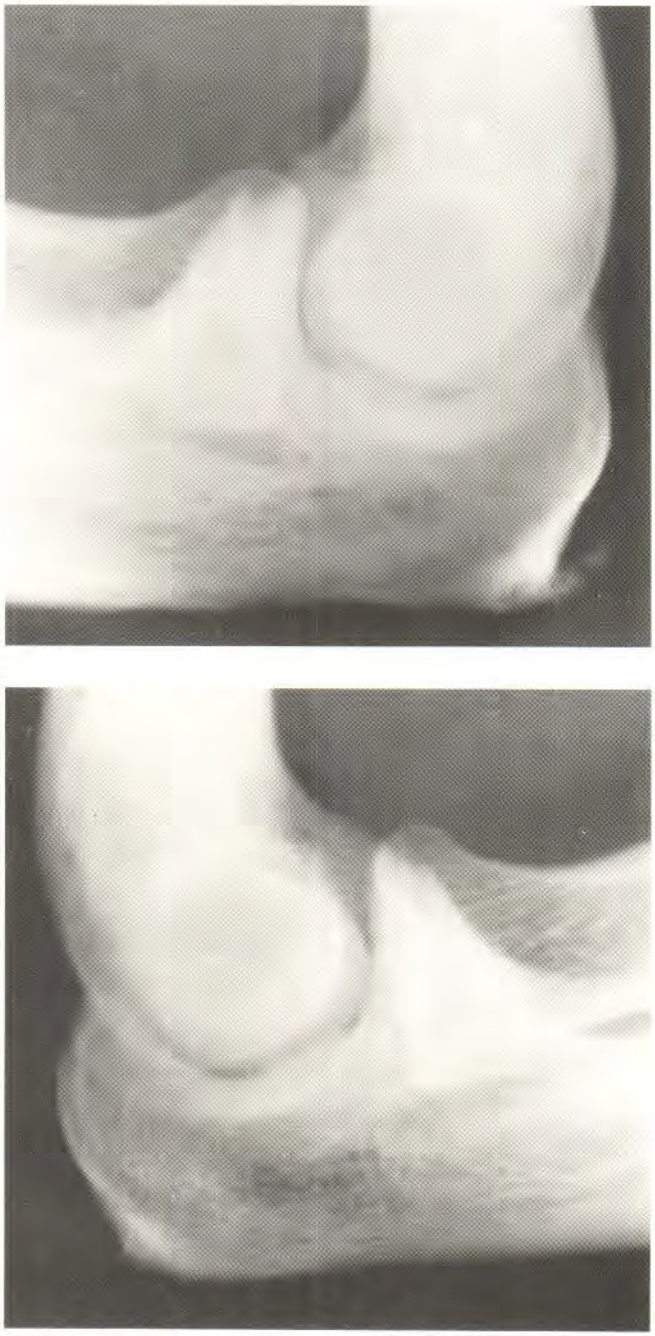

Fig. 1. (*a*) Right elbow: radiograph reveals an olecranon 'spur' at the site of triceps attachment to the ulna. (*b*) Left elbow: presence of only minimal changes.

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Table I. *Prevalence of symptomatic peripheral enthesopathies in 220 patients with psoriatic arthritis classified on the basis of different subsets (according to Moll and Wright)*

Subsets of arthritis	No. of cases	Symptomatic enthesopathies Affected patients	
		n	(%)
DIP arthritis (PA1)	13	1	(7.6)
Mutilans (PA2)	2	0	(0)
Polyarthritis (PA3)	50	6	(12.0)
Oligoarthritis (PA4)	21	3	(14.2)
Spondylitis (PA5)	67	21	(31.3)
Overlap: PA5–1	11	3	(27.2)
PA5–3	49	7	(14.2)
PA5–4	7	2	(28.5)
	220	42	(19.0)

(PA5–1, PA5–3, PA5–4 are mixed subgroups with the overlap of spondylitis and DIP, polyarthritic and oligoarthritic subset).

In SSp, inferior and posterior surfaces of the calcaneum are commonly involved sites concerned with enthesopathies (1). Bony proliferations at the triceps attachment have seldom been reported in osteoarthritis and diffuse idiopathic skeletal hyperostosis (7–8).

In our series, symptomatic peripheral enthesopathies were found in psoriatic arthritic patients in 19.0% of the cases (42 out of 220) (Table I). Among the different clinical patterns, spondylitis, with exclusive spinal involvement (PA5) or associated with peripheral arthritis (PA5-1, PA5–3, PA5–4), was the most commonly observed. Calcification in the calcaneal region occurred in 88.0% of the cases (Table II), while femoral trochanters and ischial tuberosities were involved in 28.5% and in 14.2% respectively. Proliferative changes of the triceps insertion seems a very rare feature, having been recorded only once in the 42 psoriatic arthritic patients with enthesopathy (2.3%).

In our case, olecranon enthesopathy has been the heralding sign of a psoriatic arthritis of later development, and, as suggested by Resnick, the term "olecranon spur" may describe this uncommon feature. On the other hand, this case reveals that a peripheral enthesopathy may represent an early finding of SSp. However, due to their low classification specificity when manifested in isolation, peripheral enthesopathies should stimulate a close follow-up of patients, in order to assign them to their proper diagnostic category.

Table II. *Percentage distribution of symptomatic enthesopathies at four different insertional sites in 42 patients with psoriatic arthritis*

Anatomical site	No. of cases	%
Calcaneal	37	88.0
Femoral	12	28.5
Ischial	6	14.2
Ulnar	1	2.3

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Peptide T: a New Treatment for Psoriasis?

A review of our experiences

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Peptide T (Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr) is a ligand of the CD4 receptor. It was discovered as part of the HIV envelope protein gp 120 (1). Synthetic Peptide T inhibits both the binding of HIV envelope to rat brain membranes and HIV infection of human T-cells (2). When used to treat an HIV-infected patient who has concomitant psoriasis, the skin disease disappeared. This prompted us to further treat a few HIV-negative psoriasis patients, with promising results (3, 4, 5, 6).

In the following we describe and discuss our further experience with this new treatment.

MATERIALS AND METHODS

Nine HIV-negative psoriasis patients with longstanding and recalcitrant psoriasis were included (2 females and 7 males). Their median age was 55 years (range 36–67). All the topical and systemic therapies were withheld 2 weeks prior to administration of Peptide T except for one patient (no. 6) who stopped etritinate and PUVA therapy one week before. The median dose of Peptide T/kilogram body weight was 26 μg (range 22–34). Only indifferent emollients were permitted during the entire period. Two milligrams of Peptide T were given intravenously in 500 ml saline for 28 consecutive days. All patients were hospitalized during the first week and thereafter treated as out-patients. The follow-up period lasted for another 3 months.

PASI score was employed (7) to evaluate the treatment effect. Biopsies from psoriasis lesions were taken before, weekly during the first 4 weeks and 4 weeks after discontinuing therapy. For the processing of the biopsies, see the appropriate papers (8, 9). The length of the epidermal rete lists was measured in the microscope from the tip of the rete lists, along the axial length to the junction between the epithelium and stratum comeum on haematoxylin eosin-stained histological sections.

RESULTS AND DISCUSSION

The clinical results are shown in Fig. 1. As can be seen the condition of all the patients except one had improved after 28

Fig. 1a

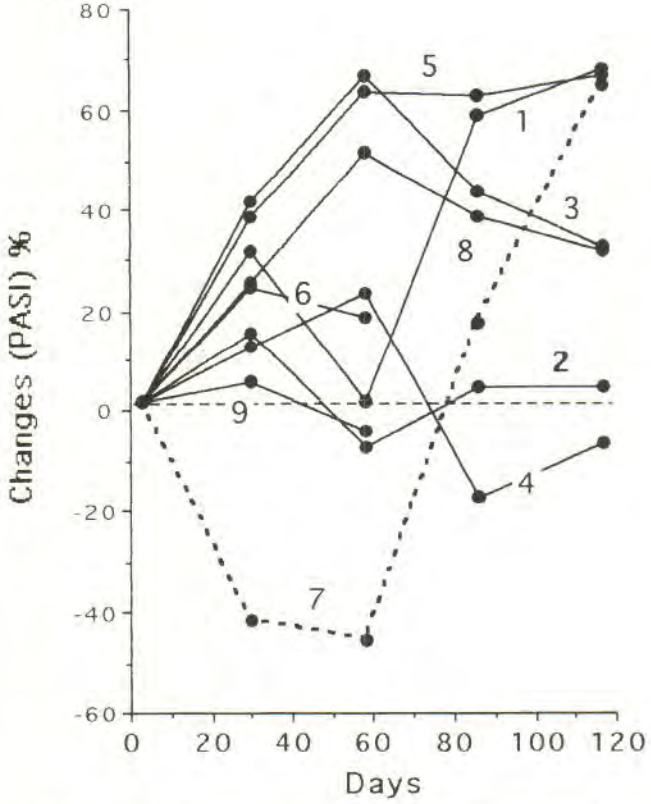

Fig. 1a. The clinical course is illustrated as a percentage of the pretreatment PASI score (zero level) during and after Peptide T treatment (2 mg/day from day 1 to 28). Increases denote improvements and decreases deteriorations. Figures on the graph stand for patient numbers. Patient no. 7 is denoted by a dashed line.

Fig. 1b

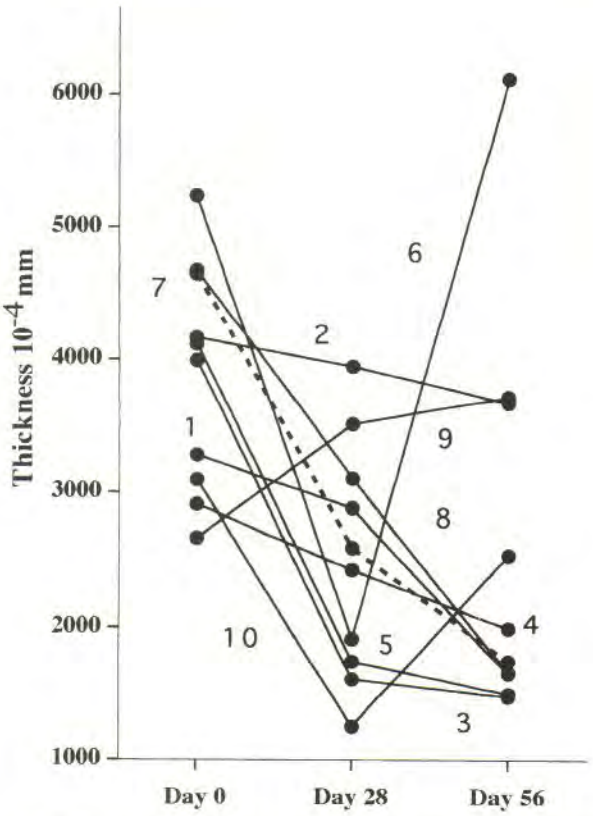

Fig. 1b. This graph shows the changes in the length of the epidermal rete lists (10^{-4} mm) for each patient during and after the Peptide T treatment. The figures correspond to those of Fig. 1. A 10th patient, shown in (*a*),is also included. Patient no. 7 is denoted by a dashed line.

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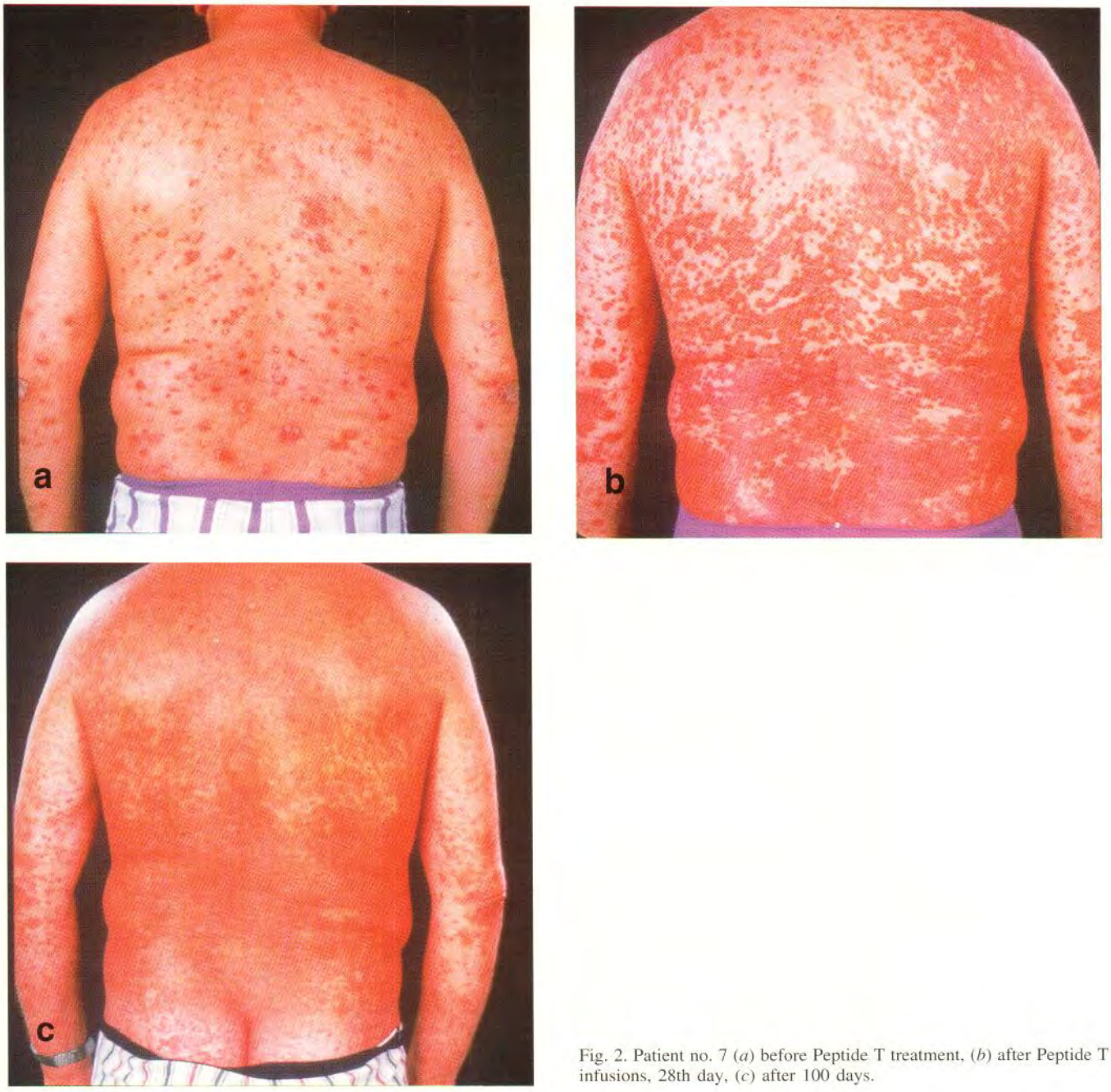
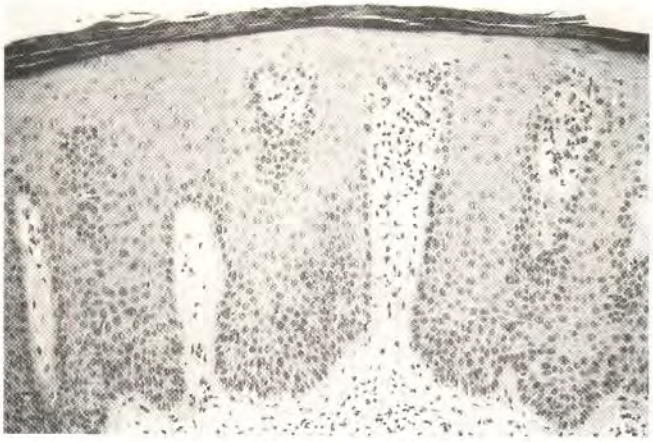
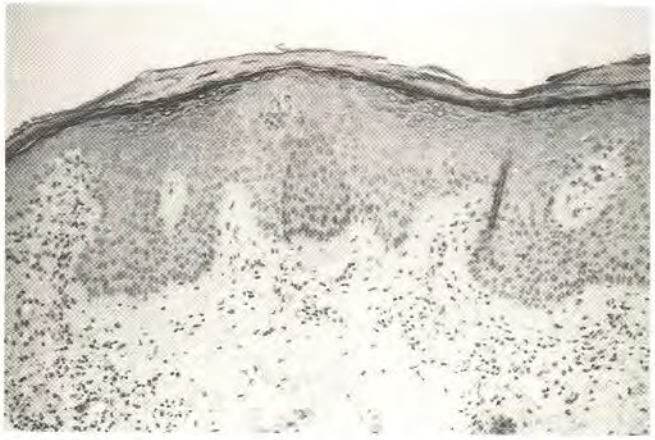


Fig. 2. Patient no. 7 (*a*) before Peptide T treatment, (*b*) after Peptide T infusions, 28th day, (*c*) after 100 days.

days, e.g. at the end of the time during which they were receiving Peptide T. The patients were followed for another 3-month period and an improvement of over 50% was noted in 5 patients. Interestingly, most of the conspicuous clinical improvements were seen after cessation of therapy. With regard to the histological improvement it can clearly be seen that epidermal thickness was reduced in all but one patient at the end of therapy (Fig. 1b) and increased thickness during the post-treatment period was observed in 2. We have previously presented data on other patients (4, 5, 6) showing continuous improvement throughout the observation period and we want to take this opportunity to describe one patient (no. 7) with an unusual pattern of response. Clinically he showed a severe deterioration at the beginning of the treatment which lasted till about day 60 (Fig. 2a, b). The deterioration measured by the PASI score was due to the increased area of involvement and the increased intensity of the redness. Then a pronounced clinical improvement was seen (Figs. 1a, 2a, b, c). During the worst period the affected areas were intensively red and glossy, with minimal scaling and induration. The recovery was seen during the follow-up period when no active treatment was given. With regard to the histological events it can be clearly seen that there was a continuous thinning of the epidermis (Figs. 1b, 3a, b, c). Farber et al. have also reported a positive clearance effect on psoriasis in a well designed controlled study (10). Thus we believe that Peptide T has a unique effect and that a placebo response is less likely, as has been suggested (11, 12).

The mechanisms of action of Peptide T are obscure. We have





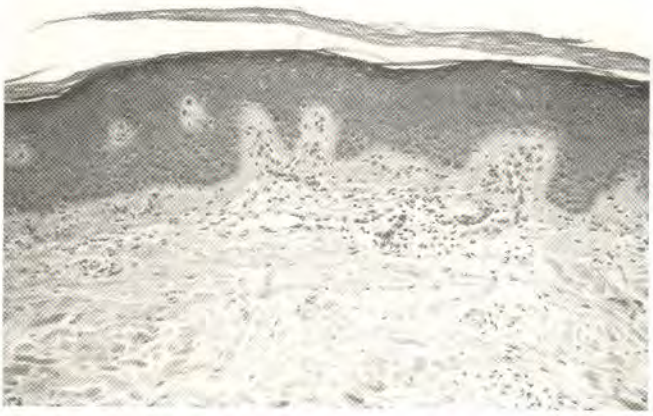


Fig. 3a. Pronounced psoriasiform acanthosis with agranulosis prior to Peptide T in patient no. 7.

Fig. 3b. During treatment, gradually decreasing epidermal thickness and normalization of the granular and corneal layers in patient no. 7 after 4 weeks.

Fig. 3c. Histological picture of lesional skin of patient no. 7 after 8 weeks.

analysed our serial biopsies from various aspects preferentially focused on the neuropeptides. One peptide, namely somatostatin, was found to be associated with dynamic changes in our biopsies. We have observed somatostatin-loaded dendritic cells in the dermis and a change in their numbers during therapy. In many instances the number is high initially or increases during the treatment and then decreases. We do not know exactly how to interpret our findings. Could it represent endogenously produced and released somatostatin, or are we observing migration of somatostatin-loaded dendritic cells? In view of the reported results from treatment with somatostatin, we believe that our findings are relevant (8) and that Peptide T may affect the synthesis of somatostatin. The true nature of these cells is at present unknown. Do they represent Langerhans cells or other cutaneous dendritic cells? With regard to epidermal Langerhans cells, they also show changes in number. At the beginning of therapy they are very few and during the course of treatment they increase in those who responded to Peptide T. We therefore believe that the Langerhans cells play an active role in the clearing of psoriasis. The possible relation and interaction between somatostatin, epidermal growth factor and vasoactive intestinal peptide has also been extensively discussed (9).

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Acta Derm Venereol (Stockh) 1994; Suppl. 186: 79–82

Fumaric Acid Esters (FAEs) Suppress CD 15- and ODP 4-positive Cells in Psoriasis

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A histological-immunohistological study was conducted to investigate the effect of systemically administered fumaric acid esters (FAEs) on epidermal thickness and composition of the inflammatory infiltrate in psoriatic plaques. The very first effect of systemic therapy with FAEs is the disappearance of CD 15-positive cells in and beneath the epidermis, accompanied by a significant reduction in T-helper cells beneath the epidermis, pointing to an immunosuppressive effect. This is followed after some delay by a reduction in acanthosis and hyperkeratosis. The reduction in infiltrating T-lymphocytes corresponds to that seen after systemic or intralesional therapy with cyclosporin. However, the normalization of the psoriatic plaques takes longer under the influence of FAEs than under cyclosporin.

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The clinical effects of fumaric acid esters (FAEs) on psoriasis were initially described in 1959 (1). Therapy with FAEs was developed further by the physician Schäfer (2, 3, 4). In the subsequent years FAEs were used effectively to treat psoriasis vulgaris at various clinical centres (5, 6, 7, 8). The effectiveness of FAEs was recently demonstrated again in a double-blind placebo-controlled study in 100 patients, carried out at five German hospitals (14). The toxic side effects referred to in some publications (9, 10, 11, 12, 13) were kept to a minimum by regular monitoring of laboratory parameters.

The mechanism of action of FAEs is not yet fully understood. We therefore conducted a histological-immunohistological study to examine the influence of systemically administered FAEs on the epidermis and the inflammatory infiltrate.

MATERIALS AND METHOD

In 33 patients suffering from severe psoriasis vulgaris (Table I) the histology of the psoriatic plaques was monitored during therapy with increasing dosages of FAEs. The drug consisted of a mixture of dimethylfumarate and monoethylhydrogenfumarate. It was available in two different enteric coated formulations: as a low-strength tablet containing 105 mg of ester mixture (30 mg dimethylfumarate/75 mg monoethylfumarate) and as a forte tablet containing 215 mg of ester mixture (120 mg dimethylfumarate/95 mg monoethylfumarate). The increase in dosage from week to week is shown in Fig. 1.

The biopsies were taken from comparable plaques on the trunk, measuring about 1 to 3 cm in diameter.

33 biopsies taken at the times shown in Table I, were fixed for 12 h in 5% formaldehyde, embedded in paraffin wax and used to assess the following histological criteria: parakeratosis, presence of stratum granulosum, acanthosis, spongiosis.

The intra-epidermal and dermal infiltrates were analysed immunohistologically. Table II shows the antibodies used and their optimal dilutions. The labelled sections (thickness 7 μm) were evaluated semiquantitatively (15, 16, 17) by counting the number of positive-labelled cells per total cell count in a defined area of 0.0324 mm^2 in each section. The infiltrate cells were counted at the intra-epidermal and subepidermal levels and in the mid-dermis in the way shown in Fig. 2, always at the site of densest infiltration. The percentages of positive-labelled cells per area were used to compare the composition of the infiltrate at the different times during treatment.

Statistical analysis

The proportions of stained cells were compared in the Wilcoxon-Mann-Whitney-U-test for independent samples. A significance level of $\alpha = 0.05$ was specified.

RESULTS

Histology

In the course of treatment with FAE the histological changes in psoriasis such as focal parakeratosis, hyperkeratosis, circumscribed spongiosis, mononuclear and granulocytic infiltration in the epidermal layer, focal loss of the stratum granulosum, and subepidermal mononuclear infiltration diminished. Two weeks after initiating treatment, no granulocytes were found at either the intra- or the subepidermal level.

In the sixth week of treatment, parakeratosis was seen in only a few cases. Stratum granulosum was still lacking in 20% of the tissue samples. Subepidermal mononuclear infiltration was present in all samples.

In the eighth week of treatment there was almost complete normalization of the parakeratosis, hyperkeratosis and the stratum granulosum. Focal spongiosis was present in 20% of the biopsies. The subepidermal mononuclear infiltration persisted in 80% of the cases.

Immunohistology

Before treatment, the intra- and subepidermal infiltrates consisted mainly of T-lymphocytes.

The intra-epidermal infiltrate consisted of 26.4% ODP 4-posi-

Table I. *Patient population, classified according to tissue embedding, sex and biopsy-sampling times (weeks 0, 2, 4, 6 and 8)*

	n	Female	Male	Age (in years)	Psoriasis since (in years)
Total	33	16	17	26–75	0.5–40
Week					
0	8	4	4	26–74	0.5–26
2	6	2	4	27–74	1.5–40
4	6	4	2	28–70	0.5–23
6	6	3	3	36–75	0.5–15
8	7	3	4	35–54	2.0–11

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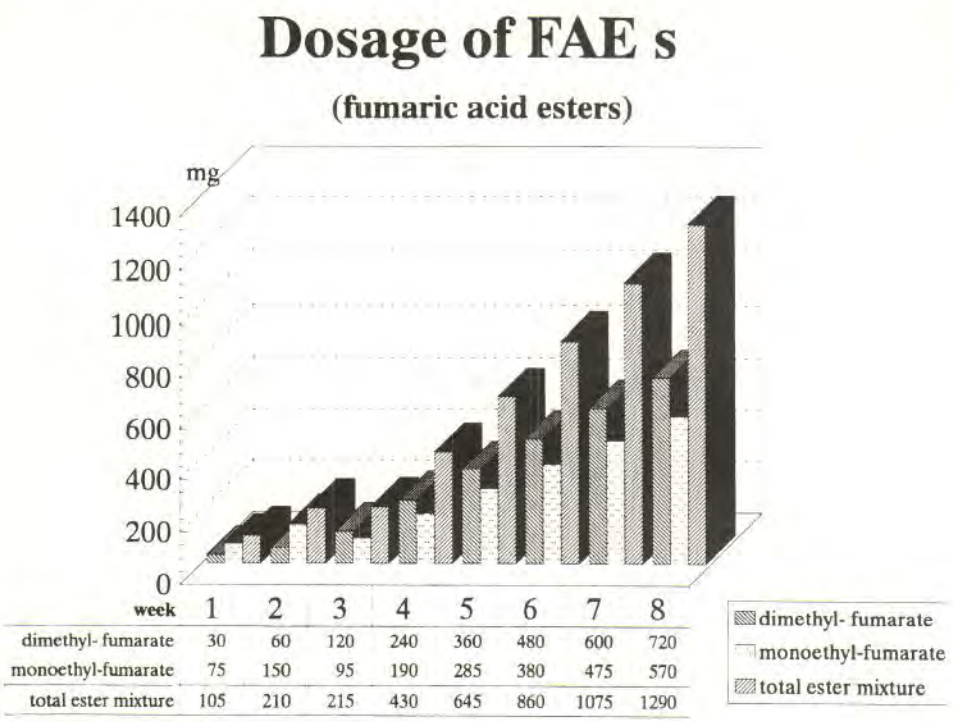


Fig. 1. Dosage schedule of fumaric acid esters from week 0 to week 8.

tive cells, 28.6% CD 45 RO-positive cells, 13% CD45R-positive cells, 23.4% CD 15-positive cells and 12.3% myeloperoxidase-positive cells. 13.3% of the cells stained positive with the HLA-DQ antigen. In the subepidermal infiltrate the total cell count consisted of 38.4% ODP 4-positive cells, 28.9% CD 45 RO-positive cells, 29.6% CD 45 R, 5.2% CD 15- and 1.6% myeloperoxidase-positive cells.

During therapy the intra-epidermal infiltrate diminished by 74.4% on the whole, with a significant decrease ($p = 0.05$) from week 0 to week 2 and a highly significant decrease ($p = 0.001$) from week 0 to week 8. Intra-epidermal ODP4-positive cells were reduced by 51.2%.

The subepidermal infiltrate decreased by 52.1% (Fig. 3), but due to the high standard deviation this decrease was not significant either between week 0 and week 2 or between week 0 and week 8. The number of ODP 4-positive cells at the subepidermal level decreased by 84.4% within the first eight weeks of treatment (Fig. 3). This reduction was even significant ($p < 0.05$) between week 0 and week 2.

CD 15-positive cells (granulocytes) which accounted before treatment for 23.4% of the intra-epidermal infiltrate, 5.2% at the immediately subepidermal level and 3.2% of the infiltrate deeper in the dermis, had disappeared completely at all localizations and in all sections by the second week (Fig. 3).

The proportion of CD 45 RO-positive cells showed a discrete increase from 28.6% to 33.0% at the intra-epidermal level and from 28.9% to 33.6% at the subepidermal level. Deeper in the dermis a reduction from 20.3% to 11.9% was observed. There was a slight increase in CD 45 R-positive cells during the treatment with fumaric acid esters. The percentages of HLA-DQ-positive, Ki 1-antigen-positive and myeloperoxidase-positive cells were largely unaffected by the treatment.

Table II. *List of antibodies, their specificity and optimal dilution, and suppliers*

Antibody	Identified cells	Optimal dilution	Supplier
ODP4	T-helper cells	1:50	Dako
CD45RO UCHL 1	activated T-cells	1:200	Dako
HLA-DQ	activated cells	1:10	Becton-Dickinson
CD15	mature granulocytes	1:50	Dako
CD45R	B-cells	1:100	Dako
Ki1	Hodgin cells	1:50	Dako
myeloperoxidase	granulocytes	1:200	Dako

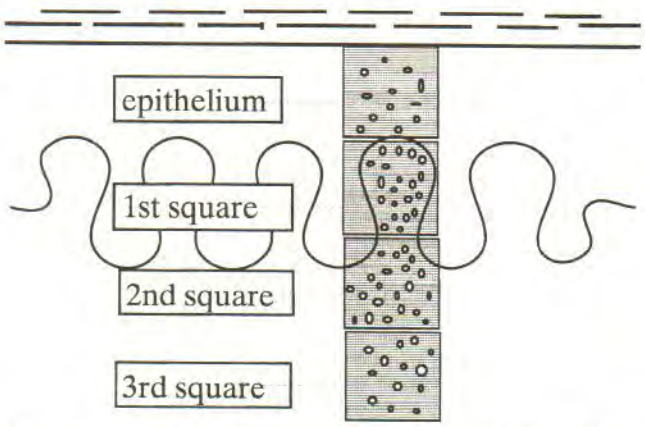
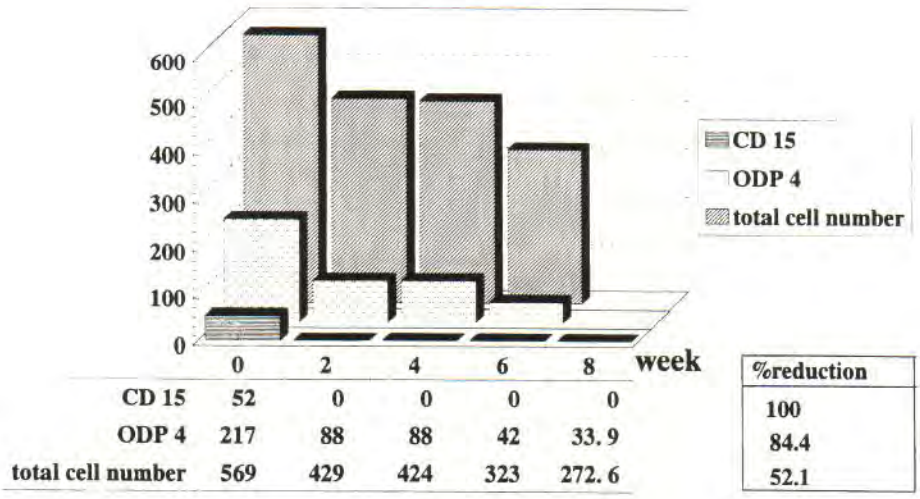


Fig. 2. System for the evaluation of labelled infiltrate cells in the epithelium and the subepidermal areas in squares measuring 0.90 μm × 0.90 μm.

Fig. 3. Number of CD 15- and ODP4-positive cells in comparison with the total cell number in an area 270 μm beneath the epidermis.



	0	2	4	6	8	week
CD 15	52	0	0	0	0	
ODP 4	217	88	88	42	33.9	
total cell number	569	429	424	323	272.6	

%reduction
100
84.4
52.1

DISCUSSION

During therapy with FAEs, psoriatic lesions heal during a period of 6–8 weeks (14). The very first effect of systemic therapy with FAEs is disappearance of the intra-epidermal infiltrate.

This is followed with some delay by a decrease in acanthosis and hyperkeratosis, while mononuclear infiltrates beneath the epidermis diminish slowly up to week 6–8.

Looking at the individual fractions of the infiltrate, the first and most striking changes are observed with respect to the granulocytes. They disappeared completely in the epidermis and the upper dermis only 2 weeks after starting therapy.

Parallel with the disappearance of the granulocytes the ODP 4-positive cells (T-helper cells) exhibit a significant reduction from week 0 to 2, while surprisingly the decrease in the total cell count is not significant at either time point.

The histological and immunohistological changes during therapy with fumaric acid esters point to an inhibitory effect on the granulocytes (CD 15-positive cells) in the first few days after starting treatment. There is also a disproportionately large reduction in the ODP 4-positive cells in comparison with the total cell count in the subepidermal infiltrate during the first 2 weeks. These results suggest an immunosuppressive effect of fumaric acid esters. Nieboer noted lymphopenia, especially a decrease in suppressor cells, and leukopenia during FAE treatment (6).

Hagedorn et al. (18) and Petres et al. (19) were able to demonstrate a dose-dependent reduction in the incorporation of [^{14}C]thymidine into the DNA of human lymphocytes. The authors discussed the influence of FAEs on the enzymes of nucleic acid synthesis, the citric acid cycle, or a defective synthesis of enzymes (19). Kuroda et al. reported that FAEs inhibit the growth of Ehrlich tumour cells in vivo (20), while mouse and chick embryo cells were resistant to fumaric acid (21).

The reduction in infiltrating T-lymphocytes corresponds to that produced by other systemic antipsoriatic drugs. A reduction in both T-helper cells (CD 4) and T-suppressor cells (CD 8) in psoriatic lesions is described during systemic or intralesional therapy with cyclosporin (22, 23). During intralesional therapy with cyclosporin, Baker et al. (24) found a significant reduction in CD 4-positive cells as well as in CD 8-positive cells in the epidermis within 12 days, while in the dermis only the reduction in CD 4-positive cells was significant. In the second week of treatment with FAEs, the T-helper cells in the tissue have decreased significantly. They then continue to fall, although no longer significantly, during the following weeks of treatment.

The reduction of granulocytes (CD 15) and T-helper cells (ODP4) during treatment with FAEs corresponds to the healing pattern found during intralesional or systemic treatment of psoriasis with cyclosporin (25).

There are, however, differences in the time course of healing of the psoriatic plaques. In contrast to therapy with cyclosporin, in our patients the reduction in the T-helper cells within the first 2 weeks did not correlate with the clinical healing of the lesion, which followed 4–6 weeks later, i.e. between the sixth and eighth week. The clinical healing of psoriasis under the influence of FAEs corresponds to the regression of epidermis hyperplasia and the extent of inflammatory subepidermal infiltrate.

FAEs induce exactly the same histological healing pattern as cyclosporin, although the time course differs considerably. Instead of hours or days, FAEs require weeks to achieve regression of a psoriatic lesion.

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Anthralin: How Does It Act and Are there More Favourable Derivatives?

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Anthralin is still the most effective and safest therapeutic agent for treatment of psoriasis. Our data may assist toward an understanding of its mode of action and introduce new derivatives, more antiproliferative and less toxic than anthralin *in vitro*. Anthralin exerts a direct effect on keratinocytes and leukocytes. In time-lapse studies it significantly prolonged the prophase of mitotic keratinocytes in subtoxic doses and suppressed the expression of keratin 6 mRNA in the immediately suprabasal layer of psoriatic epidermis in vivo. Anthralin inhibits the transformation of lymphocytes and the release of reactive oxygen species from activated leukocytes, *in vitro*. We provide evidence that these effects of anthralin are mediated by protein kinase C. Twelve new hydrophilic derivatives of anthralin, including a 1,8-dimethoxy compound, as well as C-2 and C-10 substituted anthrones were tested on human keratinocytes. The antiproliferative effect of those derivatives bearing lacton rings at a C-10, consisting of 4, 5, or 6 C atoms, exceeded that of anthralin and were equally or less cytotoxic than the parent drug. These compounds had no pro-drug character *in vitro*, since they did not metabolize via anthralin, as shown by HPLC. These data indicate that there may be anthralin derivatives with more favourable properties for topical therapy than anthralin itself. ***Key words: anthralin; analogues; protein kinase C; keratin; neutrophils.***

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PUTATIVE TARGETS OF ANTHRALIN (TABLE I)

Anthralin is an empirical agent whose mode of action is still unknown. It inhibits keratinocyte proliferation. Both the growth factor TGF-alpha and its receptor, the EGF receptor, are overexpressed in psoriatic lesions and are down-regulated by anthralin. By performing time-lapse studies on keratinocytes and titrating anthralin concentration to the lowest effective dose, we demonstrated a roughly sevenfold prolongation of the prophase, compared with untreated controls (1).

Treatment with anthralin also results in a normalization of keratin expression, i.e. increase in differentiation associated keratins K1, K2 and K10 and reduction of the proliferation associated keratins K6, K16, K17. In comparison with other antipsoriatic treatment modalities, anthralin shows the most rapid effect on keratins. Parallel to the reappearance of differentiation keratins, anthralin induces the reappearance of the differentiation marker filaggrin, which is, together with K10, essential for the restoration of the normal alpha keratin pattern in the horny layer. The reappearance of the differentiation keratins and filaggrin may be taken as indicators of clinical remission of psoriasis. On the other hand our findings give some evidence that K6 seems to be a more specific target for anthralin, since it is found elevated in uninvolved skin of psoriatic patients without changes in differentiation keratins and its mRNA reduction precedes by far that of K10 mRNA expression (2).

Another main target of anthralin appears to be T-lymphocyte activation, indicated for instance by the *inhibition* of DNFB-induced contact hypersensitivity, and of the mixed leukocyte/epithelial cell reaction, as well as the reduction of the urine neopterin level during treatment. This effect is evidently bidirectional, since anthralin directly inhibits T-lymphocytes, e.g. E-rosette formation and mitogen stimulation (3) and reduces T-cell activating cells, such as ATPase+ and CD1 (OKT6)+ Langerhans cells as well as Th1+ dendritic cells in mice.

The fourth main effect of anthralin is the inhibition of the chemotaxis of PMN and its intra-epidermal accumulation (4) as well as inhibition of the release of ROS from stimulated PMN. From the molecular point of view PKC is the most likely target for anthralin, since this enzyme is involved in controlling keratinocyte proliferation as well as ROS release from PMN (5).

PROSPECTS FOR IMPROVING THE BENEFIT/SIDE EFFECT RATIO (TABLE II)

The simplest way to improve the benefit/side effect ratio of anthralin is to restrict its application to the lesions only, e.g. by using stiff formulations or occlusive dressings. The rationale of short contact therapy is that anthralin penetrates faster through lesional than perilesional skin. Thus, short contact therapy may be improved by the additional use of penetration enhancers, such as urea, in order to better segregate the lesional antipsoriatic and the perilesional irritative effects. Similar penetration enhancing effects may be expected with microencapsu-

Table I. *Putative anthralin targets in psoriatic skin*

○	Keratinocyte proliferation EGF-receptors
○	Keratinocyte differentiation Keratin
○	T-lymphocyte activation mitogen stimulation, mixed epidermal lymphocyte reaction CD1 + cells, delayed hypersensitivity E-rosette formation
○	PMN chemotaxis and ROS release lipoxygenase
Other cells:	monocytes, mast cells, fibroblasts
Biochemically:	G-6-PDH, serine proteinases, thioredoxin reductase, mitochondrial respiration, PKC, polyamine biosynthesis, cyclic nucleotides

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Table II. *Possibilities (most experimental) to improve benefit/side effects ratio of anthralin treatment*

○	Applications:	short contact, low-dose, occlusion, gradual dose increase
○	Formulations:	plus urea, salicylic acid, tar, glucocorticoids vehicle: 70% o/w cream microencapsulated, liposomes
○	Combinations:	antioxidants, amines, glucocorticoids, lipoxygenase & cyclo-oxygenase inhibitors, serotonin antagonists, antihistamines, PAF antagonists, other anti-inflammatory drugs
○	New analogues:	10-acyl derivatives

lated or liposomal encapsulated anthralin. The problem with the liposomal encapsulated formulations is the instability of anthralin itself (6). To prevent the persistence of an anthralin reservoir in the horny layer, the use of easily washable ointments, anthralin-solvating baths, radical scavengers, or anthralin degenerating agents have been recommended at the end of medication. Moreover we showed that the addition of tar reduces anthralin irritation and leaves the antipsoriatic activity unchanged, provided that the ointment is freshly prepared, stored in a refrigerator, and used within the following 3 weeks (7).

Even though there is no clinical indication for tachyphylaxia it is obvious that the irritability of the perilesional skin abates as the anthralin therapy proceeds. There seems to be an adaptation of the skin to anthralin irritative activity. We found some evidence that danthrone, which may accumulate in the skin during treatment, inhibits NAD(P)H: quinone reductase activity, which is able to protect the cells from oxidative stress (8). Indeed, we also demonstrated in vitro an increasing tolerance of keratinocytes to anthralin after repeated exposures (9).

There are conflicting data concerning the advantage of combining anthralin with glucocorticoids. Bilateral comparison of anthralin treatment with and without one week of pretreatment with betamethasone dipropionate showed a faster response on the glucocorticoid-treated side after one week, but it took longer to achieve the same final result than on the side treated with anthralin alone (10). This is in agreement with a double-blind comparison of dithranol and a glucocoticosteroid-anthralin combination.

The most attractive attempts are those made by synthesizing new anthralin analogues. According to current knowledge, analogues obtained by substitution at the C10 position of anthralin seem to be the most promising: there is e.g. the 10 acylderivative, butanthrone, which is less irritative but also less effective than dithranol in clinical studies. Other 10-acylderivatives of anthralin have been developed, e.g. coupling products of anthralin with retinoids and salicylic acid, all of them inhibiting G-6-PDH. We tested 13 newly synthesized compounds by Wiegrebe on human keratinocytes and found that those derivatives bearing lactone rings at the C10 position had a favourable antiproliferative/cytotoxic ratio in vitro, which exceeded that of anthralin (11). Some of them were also more potent than anthralin in the inhibition of G-6-PDH and the 5-lipoxygenase pathway as indicated by a reduced production of 5-HETE and LTB4 by PMN. In conclusion, we feel that there is a realistic prospect of finding analogues of anthralin with a more favourable benefit/side effect ratio than anthralin itself.

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Tacalcitol in Psoriasis: A Video-Microscopy Study

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Video-microscopy is a video-imaging system which permits direct visualization of the skin surface and capillaries, by using a microscope attached to a camera, a video-recorder and a printer. This technique provides information on the morphology of capillaries in vivo and has been used both for research into normal skin microcirculation and as a clinical method to detect capillary changes in psoriasis and other skin diseases. The aim of this study was to evaluate the morphology of capillaries in psoriatic plaques before and after treatment with tacalcitol, a new topical vitamin D3 analogue. Clinical evaluation was made after 3 and 6 weeks of therapy. After 3 weeks a reduction in erythema and scaling was noted; and areas in which capillaries were less tortuous became evident. After 6 weeks, capillaries were less dilated and tortuous in the whole plaque and had lost the large and tortuous appearance of active psoriasis. ***Key words: tacalcitol; psoriasis; video-microscopy.***

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Tacalcitol is an active vitamin D3 derivative whose action of inducing cell division and of inhibiting cell proliferation is as good as or superior to that of calcipotriol (1α,25-(OH)2D3); it is assumed that this action is due to the binding with protein receptors specific to 1α,25-(OH)2D3 in the epidermal cells. However, since the effects of TC on the systemic calcium metabolism are less prominent, TC is deemed safer (1, 2, 3).

The aim of the present study was to demonstrate skin surface and capillary changes is psoriatic plaques during treatment with TC, using a video-imaging system.

Table I. *Quantitative VM evaluation of psoriatic plaques*

0, no response; 1, slight improvement; 2, moderate improvement; 3, marked improvement and 4, complete resolution

Patient no.	Sex/Age	Location	1st week			3rd week			6th week		
			E	S	TC	E	S	TC	E	S	TC
1	F/62	Elbow	4	4	4	2	3	4	0	1	4
2	F/33	Knee	3	4	4	2	3	3	1	2	3
3	M/33	Leg	2	2	4	2	2	4	3	2	3
4	M/59	Elbow	4	4	4	3	3	3	2	1	1
5	M/61	Leg	4	4	4	3	3	3	0	0	1

E = Erythema; S = scales; TC = tortuous capillaries.

MATERIAL AND METHODS

Five patients, from a multicentric double-blind study, who had chronic plaque psoriasis (3 males, 2 females) were studied (Table I). TC ointment 4 μg/g was applied once a day, without occlusion, to some plaques on their limbs. Treatment lasted 6 weeks. A 2-week wash-out period was carried out during which only white or 3% salicylic Vaseline could be applied. The following laboratory parameters were evaluated at the beginning of the study and after 3 and 6 weeks: serum calcium, albumin, creatining, phosphorus; alkaline phosphatase, lactate dehydrogenase, glutamic-pyruvic transaminase, blood cell count, thrombocytosis. Every week, serum albumin and calcium determinations were also performed. At the beginning of the study and after 3 and 6 weeks the psoriatic plaques were evaluated with video-microscope apparatus (VM) (4, 5) in order to study their evolution during the treatment.

The apparatus (Moritex Video Microscope System Scopeman, MS-504, Meisei Bldg., Japan) consists of a processing unit and a colour monitor (14"TTL CVS); light from the light source (a 100 W mercury vapour lamp) of the processing unit is guided with the optic fibre to the probe end. Objectives are equipped with non-contact lenses (×25, ×50) and with a contact lens (×200). A still video recorder and a colour printer may be attached. With this instrument we evaluated erythema, scales and capillary morphology. The effects of treatment were eval-

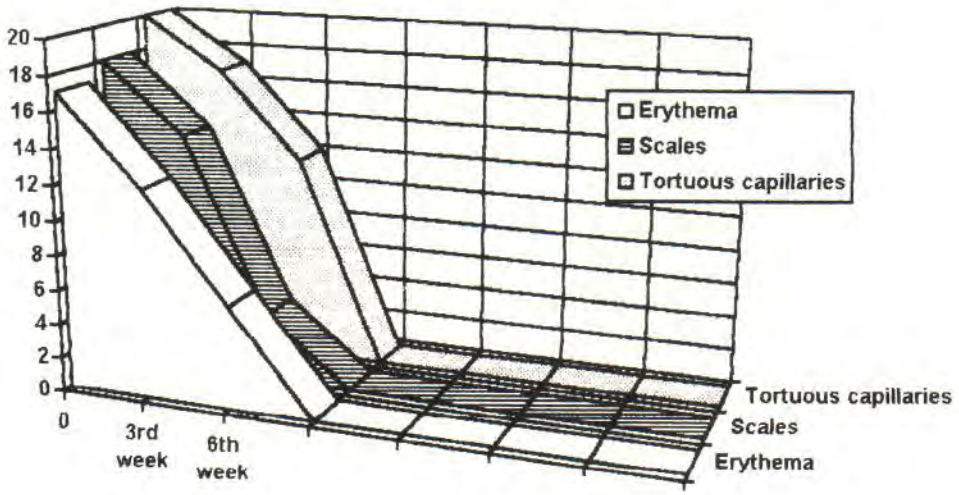

Fig. 1. Graphic representation of the behaviour of the parameters studied using VM during treatment with TC.

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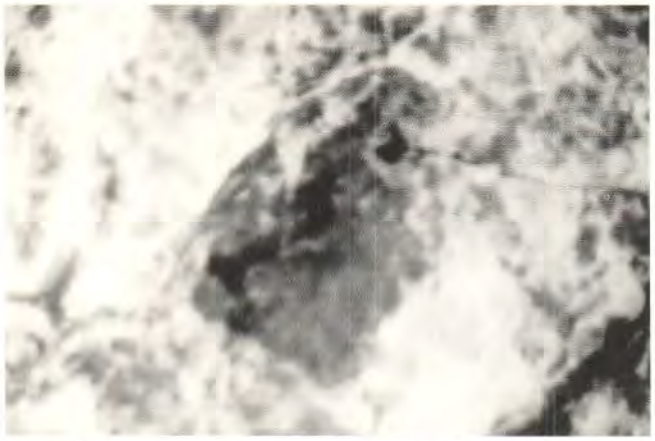

Fig. 2. Psoriatic plaque before the treatment (VM, ×50).

Fig. 4. Psoriatic plaque before treatment: tortuous capillaries are visible. This is a highly characteristic vascular pattern. VM, ×200).

uated by comparing video-microscope photographs taken at each examination and were scored as: 0, no response; 1, slight improvement; 2, moderate improvement; 3, marked improvement, and 4, complete resolution.

RESULTS

The results are reported in Table I and Fig. 1. Erythema was remarkably reduced after 6 weeks of therapy and had disappeared completely in 2 patients. Scales disappeared in one case and were considerably reduced in 2 others (Figs. 2–3). The grossly dilated and tortuous aspect of psoriatic capillaries also appeared to be reduced: a marked simplification of the coiling of the capillary ball occurred after 3 weeks in 2 cases. A prominent subpapillary plexus became clearly evident after 6 weeks in another case (Figs. 4–5).

DISCUSSION

The results of the present preliminary study demonstrate that VM is a good tecnique for the evaluation of psoriatic plaques during a specific treatment. It allowed us to study in vivo, with precision and high magnification, erythema, scales and capillary morphology. Once the technique has been mastered, patient examination requires little time and good photographs and slides are obtained. A video-recorder is very useful for providing a large archival database but the images are of poorer quality than those seen directly on the screen. The results of VM examinations seem to confirm the good therapeutic activity of TC. The drug was well tolerated by the patients. According to Kato et al. (2) the scale component improved more than erythema. In our opinion, the fact that variations in capillary morphology may be demonstrated during treatment is of interest; in fact the vascular pattern of active psoriatic plaque is highly characteristic. To the best of our knowledge a VM study has never been performed before in psoriatic plaques treated with TC. We consider VM to be a very useful technique with which to evaluate the results of topical and systemic therapies for psoriasis.

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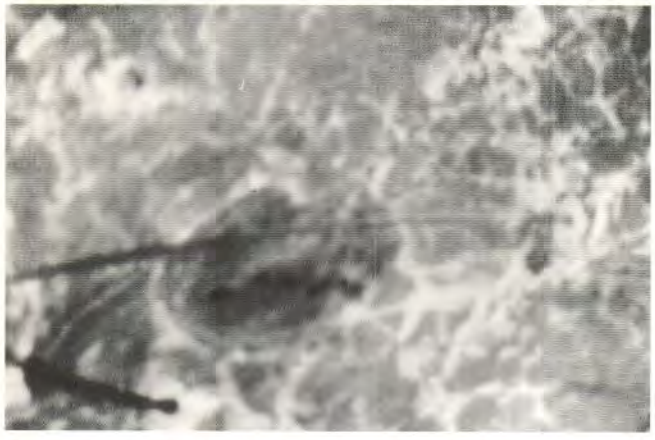

Fig. 3. Remarkable reduction in erythema and scaling after 6 weeks of therapy (VM, ×50).

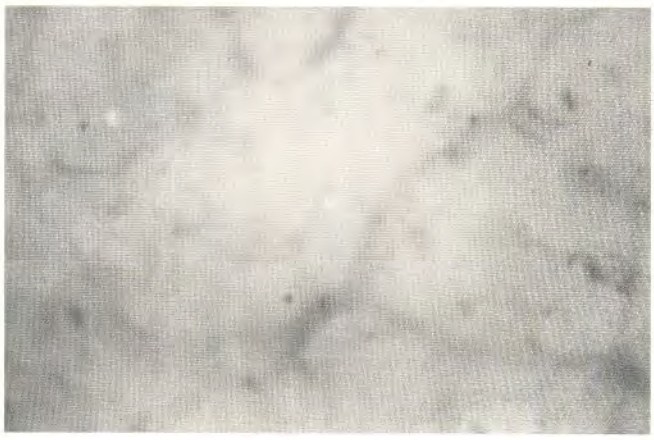

Fig. 5. Marked simplification of the coiling of the capillary ball after 6 weeks of therapy; the prominent subpapillary plexus is clearly visible, as in normal skin. (VM, ×200).

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ABSTRACT

Oral Ranitidine for Psoriasis Gave Promising Results in a Clinical Study

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Incidental reports have claimed clinical efficacy of long-term treatment with high doses of ranitidine in psoriasis. As ranitidine is well tolerated, compared with currently used systemic anti-psoriatic therapies, it is worth investigating its potential use in the treatment of psoriasis. In this open prospective multi-centre study, the efficacy of long-term administered ranitidine by means of generally accepted scores was evaluated under standardized conditions. Twenty patients with severe chronic plaque-type psoriasis were treated with oral ranitidine 300 mg twice daily. Patients were evaluated for efficacy monthly by means of the Psoriasis Area and Severity Index (PASI) during a period of 4 months. Patients with a PASI reduction of 40% or more were defined as responders. Initially, no distinct improvement in mean PASI was observed. After a 4-month treatment period, mean PASI was reduced from 15.9 to 7.6. A strong indication for regression of PASI was observed (Page test, $z = -14.17$, $p < 0.0001$). Responders had a mean PASI reduction of 67%. At the end of the evaluation period, a decreasing trend in PASI was still observed, implying that 4 months may be too short a period on which to assess the maximal efficacy of ranitidine in psoriasis. Neither clinical nor hematological side effects were observed during the study. The present results suggest such a beneficial effect of long-term systemic ranitidine that placebo-controlled confirmation should be performed as soon as possible.

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ABSTRACT

Assessment of the Effects of PUVA on Psoriatic Patient Skin by Computerized 20 MHz Sonography

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Sonography is a diagnostic technique which allows images to be obtained by means of ultrasound. B-scan technique was recently introduced in Dermatology, and has produced data on the dimensions and the depth of skin lesions. In this study, psoriasis was assessed by computerized sonography based on B-scan, but also incorporating the A-scan technique, in order to evaluate both the psoriatic lesion and perilesional skin before and after PUVA treatment. Dermascan C (Cortex Technology, Denmark) was used. Sixteen psoriatic patients (7 males, 9 females; mean age 42.14 ± 13.42) were examined sonographically on the elbows and lumbar region, both on psoriatic plaques and normal appearing skin. Sonography was performed on both sites before the treatment and after an average exposure of about 200 J/cm^2, usually corresponding to the resolution of the plaques. Before PUVA treatment, psoriatic plaques were characterized by a slight increase in the thickness of the epidermis and the dermis at both sites, as compared with uninvolved perilesional skin; the thickening was not statistically significant. After PUVA a significant reduction ($p < 0.02$) in the thickness of psoriatic epidermis was seen on the elbows. A non-significant reduction in thickness was detected after PUVA in dermis. In the lumbar region, psoriatic plaque showed a significant reduction in the thickness of both epidermis ($p < 0.001$) and dermis ($p < 0.005$) following PUVA treatment. A non-significant thickening of both the epidermis and dermis after PUVA was recorded on perilesional skin. The remarkable reduction in the thickness of the dermis after PUVA, seen mainly in the lumbar region, may be due to PUVA-induced dermal remodelling. Thickening of PUVA-exposed, normal-appearing skin may be interpreted as a consequence of a defence response to PUVA stimulus.

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Intermittent Cyclosporin A Treatment of Severe Plaque Psoriasis

Long-term follow-up of 26 patients

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The aim of this study was to evlauate the long-term effects of intermittent Cyclosporin A treatment of severe plaque psoriasis. For this purpose we considered the clinical records of 26 patients who had been intermittently treated with Cyclosporin A for 2 to 4 years. All 26 patients had severe plaque-type psoriasis (PASI score >18) that was unresponsive to conventional treatment. The initial Cyclosporin A dosage was 5 mg/kg/day in 8 cases and 3 mg/kg/day in 18 cases. In all patients, Cyclosporin A treatment was prolonged until complete or nearly complete remission of psoriasis (mean 2 months; range 1–4 months). All patients subsequently underwent a 2–4 months maintenance treatment with Cyclosporin A dosages that were gradually reduced until tapering off. In order to maintain clinical improvement after Cyclosporin A withdrawal, patients were treated with topical steroids, topical tar, emollients and UVA exposure and/or eliotherapy. Cyclosporin A treatment (2.5–3 mg/kg/day) was reintroduced only when clinical relapses reached a PASI score of 12 or more. Duration and dosages of Cyclosporin A cycles were always adapted for the purpose of obtaining an improvement acceptable to the patient (PASI <8) rather than total clearance of psoriasis. So far, the 26 patients have undergone 3–5 cycles of therapy with low doses of Cyclosporin A. None of these 26 patients interrupted Cyclosporin A treatment because of side effects. In conclusion, in our experience cyclic CyA treatment is effective for the long-term treatment of psoriatic patients.

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The efficacy of oral Cyclosporin A (CyA) in inducing remission of severe psoriasis has been widely demonstrated in a number of open and blind studies (1–9). However, optimal modalities of long-term management of psoriatic patients using CyA, as well as the side-effect profile of prolonged or intermittent administration of the drug, are still under discussion. The aim of this study was to evaluate the long-term effects of CyA cyclic treatment in severe plaque psoriasis. For this purpose we scrutinized the clinical records of 26 patients who had been periodically treated with CyA over a period of 2 to 4 years. These 26 patients formed part of an original cohort of patients who entered two multicentre Italian studies regarding the safety and efficacy of CyA in severe plaque psoriasis (8, 9).

PATIENTS AND METHODS

All 26 patients (11 females and 15 males) aged 22–65 years (mean 47.92 years) had severe plaque-type psoriasis (defined by a PASI score >18) which had been unresponsive to conventional treatment. They were otherwise in good health. Previous treatments included 1–3 courses of methotrexate (8 patients), etretinate (5 patients), PUVA therapy (7 patients) and both methotrexate and etretinate (7 patients). Patients had interrupted all systemic therapies at least 4 weeks before entering the study. None were taking drugs known to interact with CyA. Women of childbearing age were using effective contraception during CyA treatment.

The initial CyA dosage was 5 mg/kg/day in 8 patients and 3 mg/kg/day in 18 patients. In all patients, initial CyA dose was prolonged until complete or nearly complete remission of psoriasis (mean 2.53 months; range 1–3 months). All patients subsequently underwent 2 to 4 months of maintenance treatment with CyA dosages that were gradually reduced until tapering off. In order to maintain clinical improvement after CyA suspension, the patients were treated with topical therapies: steroids, tar, emollients and UVA exposure and/or eliotherapy. Since June 1992, 2 patients have been utilizing topical calcipotriol.

CyA treatment (2.5–3 mg/kg/day) was reintroduced only when clinical relapses reached a PASI score of 12 or more. Duration and dosages of CyA were always adapted to the purpose of obtaining an improvement acceptable to the patient (PASI <8), rather than total clearance of psoriasis.

Clinical and laboratory monitoring

During CyA therapy, patients were examined every 1 to 4 weeks, depending on drug dosages. A complete laboratory monitoring, including serum CyA levels, was performed at each visit. Twelve-hour whole-blood levels of CyA were measured using a radio-immunoassay (RIA) with a specific monoclonal antibody. Target range was 50–275 ng/ml and dosages were adjusted if levels were outside these values. Glomerular renal function was checked at pretherapy, and at the end of each cycle of CyA therapy. In patients who exhibited a rise of >30% in their baseline creatinine levels, the dose of CyA was reduced by 0.5–1 mg/kg/day and creatinine levels were monitored every 2 weeks.

During the CyA-free period, patients were examined every 1–2 months according to their clinical state and response to adjuvant treatments.

RESULTS

Initial CyA treatment produced a reduction of more than 85% in PASI score in 15 patients and a reduction of 75% in 11 patients. Psoriasis relapsed gradually in all patients 2 to 4 months after interruption of CyA (mean 3.19). PASI scores of relapses were always below baseline PASI. In 12 patients, adjuvant therapies permitted maintenance of a PASI score <12 for a period ranging from 3 to 4 months. Four of them did not require CyA reintroduction for 5 to 6 months.

Nine patients, on the other hand, required reintroduction of CyA, 2 to 3 months after interruption, to control their psoriasis despite of regular use of adjuvant therapies.

In all our patients, an acceptable control of psoriasis was obtained by using 2–5 cycles of CyA treatment in 24 months. During the 1st cycle of CyA reintroduction, 4 of the 8 patients who had started CyA therapy with 5 mg/kg/day required more than 3 mg/kg/day in order to control their psoriasis (3.5 to 4 mg/kg/day). However, dosages of 3 mg/kg/day were always sufficient in successive CyA cycling.

Side effects

None of the 26 patients interrupted CyA treatment because of

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side effects. Four of the 26 patients developed hypertension, 5 transient renal impairment and 5 both. Hypertension occurred during the first 2–4 weeks of treatment in 12 patients. In 5 patients, hypertension was only transient and controlled by reducing the CyA dosage.

Seven of the 12 patients developed mean blood pressure values (160/95 Hg) that required treatment with hypotensive agents. In all these patients, blood pressure returned to normal values when CyA therapy was stopped. In 4 of these 7 patients, hypertension reoccurred with every cycle of CyA. In 6 patients, after a slight increase in serum creatinine within normal range during 1–2 months of treatment, no significant change was subsequently detected.

A transient rise of more than 30% in serum creatinine level occurred in 14 patients, including 5 patients treated with CyA 5 mg/kg/day and 9 patients treated with CyA 3 mg/kg/day. The rises occurred within the first 3 months of treatment in all the 5 patients taking 5 mg/kg/day. Three of the 9 patients treated with 3 mg/kg/day showed a rise in serum creatinine after 4 months of therapy, whereas the remaining 6 patients developed a transient rise of creatinine after the third cycle of treatment. In all cases, serum creatinine value returned to pretreatment level within 1–2 months of CyA reduction.

Renal dysfunction was seen only 5 out of 12 patients with hypertension. No correlations were apparently detected between of creatinine increase above baseline, or incidence of hypertension and previous systemic antipsoriatic treatment.

Minor side effects noted during our study included headache (48%) and paresthesias (34%).

DISCUSSION

Our study indicates that intermittent treatment with low dosages of CyA was effective in maintaining an acceptable control of psoriasis in all our patients. During the follow-up period, however, the frequency and duration of cycles varied considerably in one and the same patient. Furthermore, a considerable variation in the minimal CyA dosage capable of maintaining adequate disease control was observed among the different patients. CyA dosages of 3 mg/kg/day were, however, effective in controlling psoriasis recurrences in most of our cases. This indicates that modalities and dosages of CyA treatment in the long-term management of psoriatic patients are strongly related to the clinical response of the individual patient.

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Short-term Treatment with Cyclosporin in Severe Psoriasis: Four Years of Experience

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The effectiveness of cyclosporin (CsA) in the treatment of severe psoriasis is now well recognized (1, 2). Since psoriasis is a chronic recurrent disease, we used a discontinuous treatment instead of a long-term treatment in order to minimize the side effects of CsA. The preliminary results of a 4-year experience with short-term treatment with Csa in psoriasis are reported.

PATIENTS AND METHODS

In an open study 142 consecutive patients (94 M, 58 F) aged 11–80 years (mean 42.84 ± 15.3) affected by severe psoriasis underwent one or more short-term treatment cycles with Csa. The duration of the disease ranged between 1.5–55 years (mean 10.3 ± 8.2).

The patients had previously been treated with retinoids, PUVA, systemic corticosteroids, or methotrexate. We have differentiated the patients depending on the various clinical variants of the disease: 121 patients with extensive chronic plaque psoriasis (ECPP), 11 with guttate eruptive psoriasis (GP), 5 with chronic palmo-plantar pustular psoriasis (CPPP), 1 patient with generalized pustular psoriasis (GPP), and 4 patients with sub-erythrodermic psoriasis (sE).

The exclusion criteria were as follows: psoriatic arthritis, renal and liver disease, pregnancy, neoplastic diseases, arterial hypertension, infections and diseases requiring the use of nephrotoxic drugs.

The efficacy of Csa treatment was assessed using PAI index (Psoriasis Area Involvement). The extent of the rush was estimated by the rule of nines. Clinical evaluation was performed every 4 weeks during the treatment: physical examination, resting blood pressure, heart rate, hematochemical tests, Csa blood levels. In the post-treatment period (6 months) each patient was examined every 1–2 months. Hematochemical tests were performed 1 month after stopping therapy.

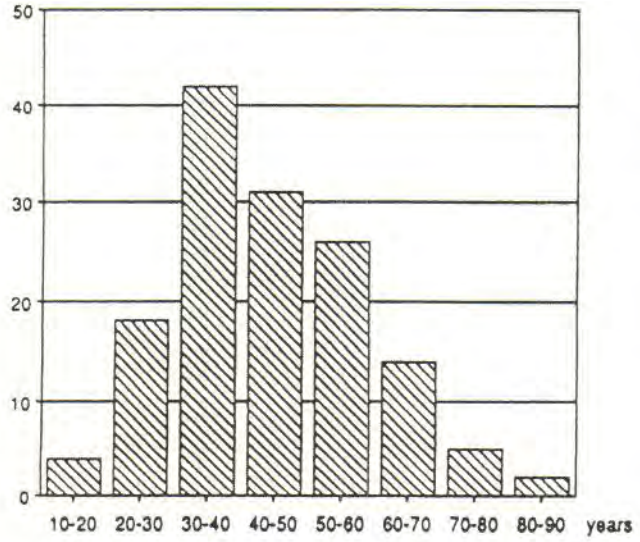

Fig 1. Distribution of the patients according to age.

SCHEDULE OF TREATMENT

The initial CsA daily dose was 5 mg/kg in 4 patients (suberythrodermic patients) and 4–3 mg/kg in 138 patients. The daily Csa dose was gradually reduced to 3 mg/kg according to clinical response. The average rate dose was 3.7 mg/kg/day. The mean duration of a single treatment cycle was 7.5 ± 2 weeks (range 4–12). Remission was defined as reduction by >75% of the baseline PAI score and resolution of at least 2 points out of 0–3 scale score of desquamation, erythema and infiltration; significant improvement was defined as reduction of >50% in the PAI score.

The only adjuvant therapy allowed during CsA treatment was white petrolatum. During a disease-free interval, patients were allowed to use topical steroids, topical tar or eliotherapy in order to control new starting lesions. A new CsA treatment period was started when the patients showed a recurrence of the disease. Relapse was defined, according to the Italian Multicenter Study Group(2), as clinical worsening characterized by an increase in the baseline PAI index of more than 50% of original baseline value.

RESULTS

In ECPP, after the first cyle of CsA therapy, clinical remission was observed in 93 patients, considerable improvement in 25 patients, while 3 patients did not respond because of poor compliance. The mean time of clearing was 7 ± 2 weeks, range 3–8. The mean duration of remission was 12 ± 4 weeks.

After stopping therapy, we found a relapse rate of about 70% during a follow-up of 6 months. Neverthless, the relapse rate was similar after consecutive CsA treatment cycles.

In the GP, after the first CsA cycle, clinical remission was

Table I. *Results after 1st cycle of CsA therapy*

	ECPP	GP	CPPP	GPP	sE
Remission (>75)	93/121	11/11	4/5	1/1	3/4
Improvement (>50<75)	25/121	0/11	1/5	0/1	1/4
Failure	3/121	0/11	0/5	0/1	0/4
Time to clearing					
Mean (weeks)	7±2	6±2	7±1	4	8
Range	3–8	3–6	4–8	4	7–9

Table II. *Relapse rate of consecutive CSA treatment in chronic extensive plaque psoriasis*

Cycle no.	Patients evaluable *n*	Relapse rate (%)	Follow-up (months)
1	58	76	6
2	19	71	6
3	11	67	6

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observed in 11/11 patients, the time to clearing was 6 ± 2 weeks (range 3–6). Nine of the 11 patients are still in remission (the follow-up 8 months). Only 2 patients required a seond cycle of treatment (the follow-up being 6 months).

In CPPP, clinical remission was achieved in 4/5 patients and considerable improvement in 1/5. The mean time of clearing was 7 ± 1, range 3–8 weeks. One patient healed sufficiently and did not require further treatment, the disease was easily controlled by local therapy (the follow-up being 6 months). The other 4 patients underwent three consecutive CsA treatments in a period of 2 years. The only patient affected by GPP healed after 4 weeks of treatment. In the 4 month follow-up he noticed a few lesions easily controlled by local therapy.

Out of the 4 patients affected by sE psoriasis, 3 presented remission, while 1 showed considerable improvement after the first CsA treatment. One patient dropped out after the first CsA cycle; 3 patients are still taking CsA.

The time to clearing and the duration of remission was found to be almost identical in the different clinical variants of the disease after consecutive cycles of treatment, except that the time to clearing was longer for the sE patients.

Concerning side effects, the most frequent were found to be mild subjective symptoms such as abdominal pains, nausea, meteorism, paresthesias, asthenia, cephalea, myalgia. We also observed mild arterial hypertension in 4 patients, and hypertriglyceridemia in 6 patients. None of these patients discontinued their treatment because of the side effects.

In 15 patients, after consecutive Csa cycles, a slight increase in serum creatinine level up to 30% of the baseline value, was found. None of the relapsing patients presented any signs of disease rebound after repeated short-term CsA treatment cycles.

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Short-term "Cyclosporin A" Therapy for Psoriatic Arthritis

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The usefulness of low-dose cyclosporin A (CsA) in psoriatic skin lesions is well known (4) and during the treatment an improvement of the sometimes associated arthritis has been noted (6).

There are few published studies on psoriatic arthritis (PA) patients treated with CsA, showing variable experiences both on effectiveness and safety (5, 8, 9).

We investigated the efficacy and safety of low-dose CsA therapy in a 6-month open study on 13 patients with PA to assess the main clinical and laboratory changes.

MATERIALS AND METHODS

13 patients affected by PA, according to Moll and Wright criteria (7), 9 men and 4 women aged 30 to 62 (mean age 45), were recruited for the study. All of them had active polyarthritis with a mean duration of 9 years (range 1–32 yrs). All patients had already been treated unsuccessfully with other disease-modifying antirheumatic drugs, but had not taken these drugs for at least 3 months prior to starting CsA therapy. They all were heated by non-steroidal anti-inflammatory drugs (NSAIDs), while 4 of them were also taking low-dose steroids (6-methylprednisolone 4 mg/daily). Patients were excluded for any of the following reasons: abnormal hepatic and renal function, recent major surgery, hypertension, concomitant uncontrolled infections, history or presence of malignancy, pregnancy.

The starting oral dose of CsA was 3 mg/kg daily administered twice a day for 6 months. If the articular improvement was then unsatisfactory, increments of 1 mg/kg daily were permitted at monthly intervals to a maximum dose of 5 mg/kg. A reduction of the dose by 1 mg/kg daily was made if the serum creatinine concentrations increased by more than 50% over the baseline value, or for any other significant side effects.

Each patient was examined by the same rheumatologists at entry, and then monthly throughout the trial. The rheumatological evaluation included number of painful joints; Ritchie articular index; patient's pain using a 10 cm visual analogue scale, and duration of morning stiffness (min). Blood pressure was measured at each visit.

The following laboratory investigations were carried out at entry and at each visit: complete blood cell count; Westergren erythrocyte sedimentation rate (ESR); C-reactive protein (CRP), serum urea nitrogen; creatinine; uric acid; electrolytes; albumin; transaminases; alkaline phosphatase; total bilirubin, and urine analysis. Creatinine clearance was measured before therapy began and again every other month. At entry, all the patients lacked rheumatoid factor and antinuclear antibodies.

Statistical analysis differences between paired data were analysed using the Wilcoxon signed rank test. The significance of correlations was determined using the Spearman rank correlation test. *P*-values less than 0.05 were considered significant.

RESULTS

Table I shows the main clinical data of the 13 patients on entry. Ten patients (77%) completed the 6-month study period, while 3 of them (23%) interrupted CsA treatment, owing to gastrointestinal problem in one case, uncontrolled elevation of blood pressure in another and poor compliance in the third.

Table II shows the changes in main clinical and laboratory parameters.

At the end of the trial, all the patients showed an improvement in their arthritis and in one case a complete remission of articular disease was achieved. Statistical analysis showed a significant improvement in the number of painful joints, Ritchie index, and subjective pain. Regarding laboratory investigations, reductions in ESR and CRP were detected in the study period, but a significant laboratory change occurred only in serum creatinine levels, although it was not over 50% from baseline value and did not require any reduction.

Steroid therapy was discontinued in all cases but one, while all the patients were encouraged to reduce their intake of NSAIDs during CsA treatment.

DISCUSSION

Our short-term study of CsA in PA patients showed that the number of painful joints, Ritchie index and patient's assessment of pain improved significantly during the treatment. However, no significant decrease in ESR was found, as already reported by other authors (3, 11, 12). It is questionable whether ESR can be considered a reliable variable for considering CsA a remittive

Table I. *Demographic and clinical features of the study patients (N = 13).*

Sex (M/F)	9/4
Mean age (yrs)	45±12
(range)	(30–62)
Mean age of onset of arthritis (yrs)	36±10
(range)	(23–57)
Mean duration of arthritis (yrs)	9±9
(range)	(1–32)
Steroid treatment (no. of pts)	4

Table II. *Outcome at baseline (T_0) and after 6-months (T_6) of CsA therapy in 10 PA patients.*

	T_0 M±SEM	T_6 M±SEM	*p*
Painful joints (no.)	9.9±1.2	4.9±1.4	0.001
Ritchie index	18.8±4.7	6.8±2.4	0.005
Pain analogue scale (cm)	5.2±0.6	3.4±0.6	0.01
Morning stiffness (min)	27±30	9±6	n.s.
ESR (mm/1 hr)	41.8±9.7	27.1±5.3	n.s.
CRP (mg/l)	19.2±6	10.8±4.9	n.s.
Serum creatinine (mg/dl)	0.9±0.1	1.1±0.1	0.05
6-methylprednisolone (mg/daily)	1.6±0.5	0.2±0.2	0.05

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agent, since these studies report that other acute-phase reactive proteins decrease during CsA treatment.

In our group of patients a significant rise in serum creatinine was found at the end of the 6-month study, although the values were still within the normal range and no other renal functional parameters revealed any significant modification. Hypertension occurred in one patient which led to withdrawal within the first month of therapy, despite the concomitant antihypertensive therapy.

However, the side effects, occurring only in 16% of cases, were mild and similar to those reported by others (3). The major problem with CsA is undoubtedly nephrotoxicity, even if it is conceivable that the duration of the treatments as well as the drug dosage are contributory factors for the renal damage (2, 10). The risk of interaction with other potentially nephrotoxic agents such as NSAIDs has been pointed out (1). We underline the significantly successful reduction of steroid consumption achieved quite soon in our trial. In fact, in all cases the reduction of 6-methylprednisolone dose was made within the first 3 months of CsA therapy.

In this study, low-dose CsA proved to be highly effective in the improvement of articular involvement in active PA, but in our opinion, the criterion of an aimed selection of the subjects to be treated must be followed, in order to avoid the most common side effects. Furthermore, larger double-blind studies and longer treatments will also be required to make a better assessment of the efficacy and tolerability of CsA in PA.

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Psoriasis and Cyclosporin: Immunohistochemical Aspects of the Basement Membrane

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We have demonstrated both in vivo and in vitro that Cyclosporin (CsA) treatment during psoriasis induced a regression of typical keratinocyte alterations and normalization of the basement membrane (BM). It is also known that the structure of BM implies cohesion between the networks formed by laminin and type IV collagen and that these components positively influence the cytomorphosis processes of keratinocytes. According to these results, we have evaluated, by immunohistochemical study, the behaviour of laminin and type IV collagen on psoriatic skin prior to the therapy and at the end of pharmacological treatment with CsA. This study was carried out on biopsies of involved skin taken from 12 patients with severe psoriasis and with PASI between 50 and 70. Our results can be summed up as follows:

Untreated psoriasis: **absence of laminin within BM; modest staining in basal keratinocytes; intense staining in suprabasal keratinocytes; discontinuous staining of Type IV collagen in the BM.**

After treatment: **evident and continuous staining of laminin and Type IV collagen within the BM.**

The obtained results confirm the positive effect of immunomodulation determined by CsA in the regulation of the functional activity of cells implicated in BM component production. In conclusion, the authors discuss the pathogenesis of the disease.

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It is known that the basement membrane (BM) of the epidermis influences a series of fundamental biological functions of keratinocytes such as growth, differentiation, polarization, adhesion, migration and can constitute a selective filtration barrier against biologically active substances which may be of importance in the pathogenesis of hyperproliferative diseases of the skin (1–5).

Our recent ultrastructural researches (6–9) have confirmed that in psoriasis, a typical hyperproliferative disease, evident anomalies of the BM constitution are always present, and have shown that these regress, together with keratinocytic alterations, following treatment with Cyclosporin (CsA).

Following these researches and with the aim of evaluating the behaviour of the main components of BM during the thriving phase of the illness, and after the treatment with CsA, we undertook a study of immunolocalization with direct monoclonal antibodies against laminin and collagen of IV type on bioptic fragments of the involved skin from psoriatic patients before and after a therapeutic cycle with CsA.

MATERIALS AND METHODS

We carried out cutaneous biopsies in the involved skin with punch biopsies "Stiefel" (3 mm ∅) on 12 patients affected by serious and diffuse psoriasis (PASI: 50–70) before and after a therapeutic cycle with diminishing doses of cyclosporin (3 mg/kg/per day during the first month; 2 mg/kg/per day during the second month; 1 mg/kg/per day during the third month). The bioptic fragments after repeated washings

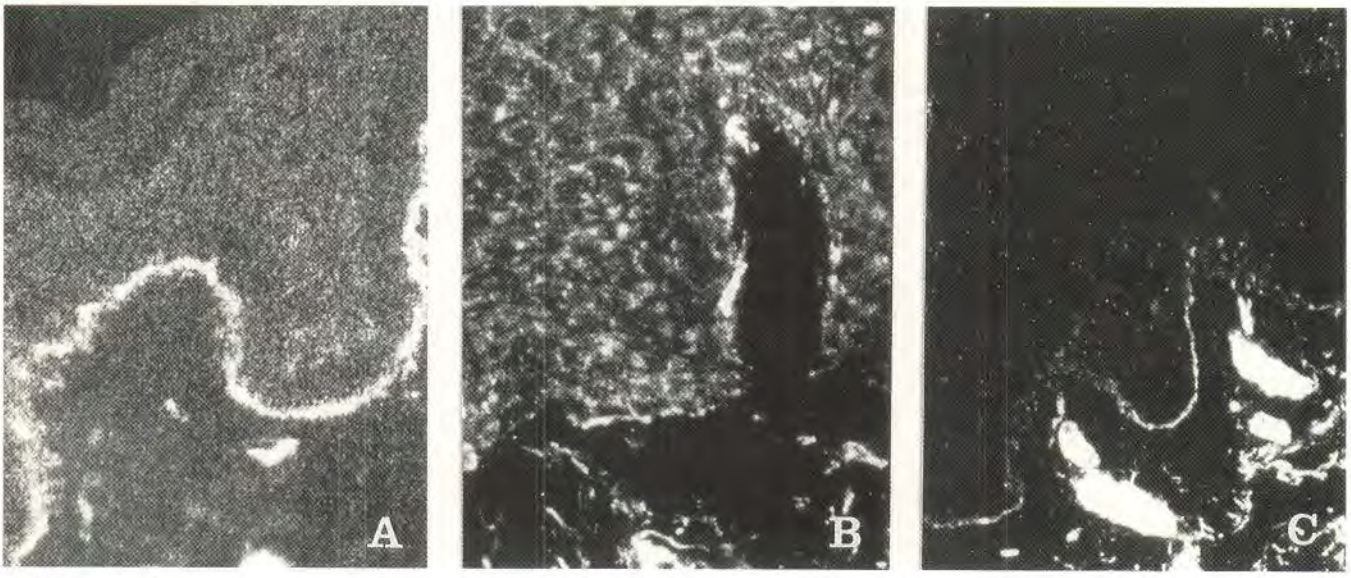

Fig. 1. Immunofluorescence staining of laminin in normal (A), involved psoriatic skin before (B) and after CsA (C). A: 400×; B: 400×; C: 200×.

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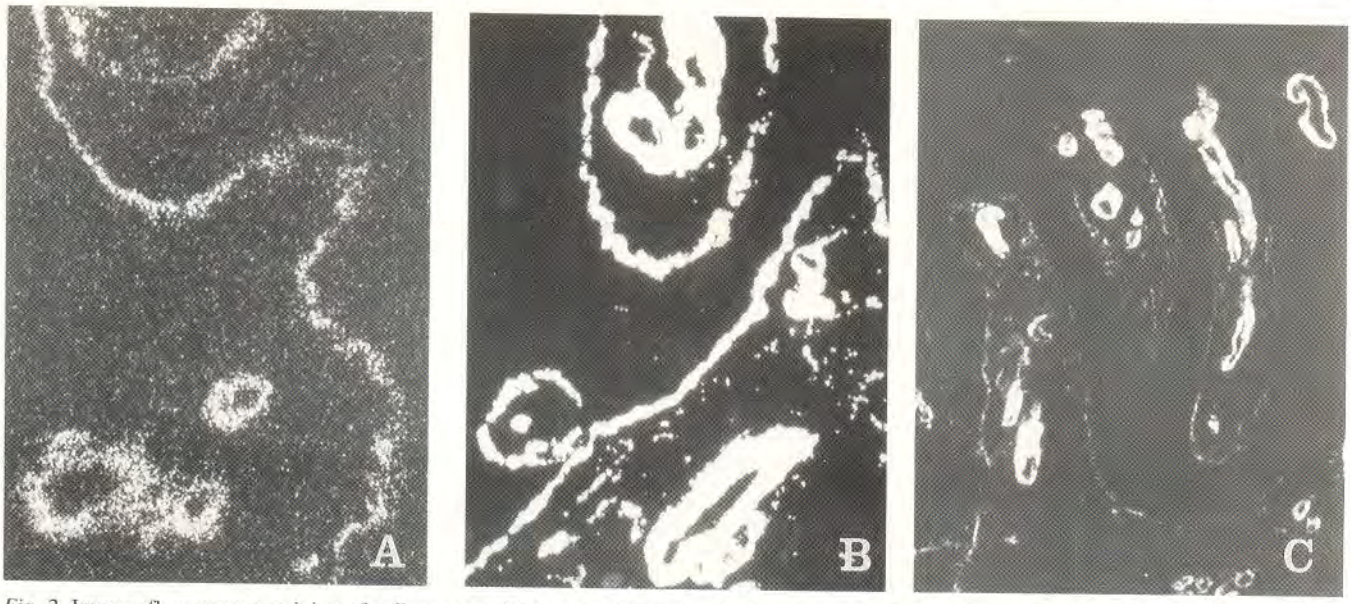

Fig. 2. Immunofluorescence staining of collagen type IV in normal (A), involved psoriatic skin before (B) and after CsA (C). A: 400×; B: 400×; C: 200×.

in phosphate 0.2 M at pH 7.4 and salt phosphate buffer, PBS were infiltrated with saccharose 2.3 M in PBS and then frozen in liquid nitrogen. Sections of about 14 μm, cut with a cryotome were gathered on gelatinized slides and tested with primary anti-laminin and anti-collagen, type IV (Sigma) at a dilution of 1:250; as a secondary antibody we used an Ig-biotinated anti-mouse in sheep at a dilution of 1:500; as fluorochrome, the Red-Streptavidin at a dilution of 1:100. The observations were carried out with a confocal microscope with laser scanning LSM 310 of Carl Zeiss.

RESULTS

Distribution of the laminin in normal and psoriatic skin. Frozen sections of normal skin stained by immunofluorescence with mouse antiserum against laminin showed a linear and continuous staining at the level of the BM and in the walls of dermic vessels (Fig. 1A). By contrast, in psoriatic skin, the staining of the BM appears completely absent in some and in others present in short segments, while it is possible to discern a positivity of the reaction in the walls of the dermic vessels and inside superbasal keratinocytes (Fig. 1B). After the treatment with CsA the laminin staining is distributed in an almost uniform way along all the BM with sporadic and short interruptions. The positivity at the keratinocytic level is no longer appreciable, while the walls of the vessels can still be discerned (Fig. 1C).

Distribution of the collagen of IV type in normal and psoriatic skin. In normal skin, staining is discerned as a continuous line at the level of the BM and in the walls of the dermic vessels (Fig. 2A). In psoriatic skin, on the other hand, staining appears less intense and clear-cut interruptions of the continuity – especially at the apex of the dermic papillae – are sometimes to be observed. The staining of the walls of the vessels is very intense and they appear tortuous and stretched (Fig. 2B). After the treatment with CsA the distribution of the staining for collagen of type IV appears almost similar to the normal skin, even if sporadic breaks are present (Fig. 2C).

DISCUSSION

From the examination of the reports it is seen that the known structural anomalies in the BM of psoriatic epidermis are in correlation with evident alterations of the molecular composition of the same; among these alterations, particular importance is attributable to the anomalous localization of the reaction of the laminin which is almost completely absent in the context of the BM, while it is positive inside the suprabasal keratinocytes.

This condition permits us to hypothesize a functional anomaly of the keratinocytes such that on the one hand they keep this glycoprotein inside the cellular body instead of releasing it, as in normal circumstances, into the extracellular matrix to consent the assemblage in the BM context; on the other hand, they are hindered in their development according to the normal scheme of epidermal differentiation.

The lack of laminin in the BM constitutes a decisively favourable condition for the manifesting of hyperproliferative phenomena which characterize psoriasis. In fact it is known that the network formed by laminin (10), in the context of the "lamina lucide", constitutes the main system of adhesion, mediated by integrins (11–12) of the basal keratinocytes to BM. The connection that is established between this transmembrane protein and the laminin fulfils the assumption why the signals that govern adhesion, migration, proliferation, polarization, cellular differentiation, biological functions these highly altered in psoriasis, have their origin.

On the other hand, the laminin, besides being connected with the basal keratinocytes, is closely connected, on the opposite side, with the components of the 'lamina densa', such as type IV collagen and heparan sulfate proteoglycan, and the normal function of the selective barrier of BM depends on the aggregation of this molecular complex (13–14). The absence of laminin, altering the normal assemblage of BM, surely facilitates the passage of the biologically active substance which, as is known, can be important in inducing keratinocytic hyperproliferation (5).

Furthermore, we also recently observed (15) a lack of laminin in the BM context in the uninvolved skin of psoriatic patients. This situation even upholds the hypothesis of an anomaly of the keratinocytes, similarly genetic, regarding the production and the liberation of laminin; therefore, the consequent lack and the alteration of the BM permeability, might explain the ease of the onset of the pathological picture in response to the variety of stimuli in subjects that bear such an anomaly.

After pharmacological treatment, the arrangement of the BM seems actually to normalize the positivity of the reaction to the laminin which is distributed in an almost homogeneous manner in the BM context; appears evident in addition to maintaining the continuity of the reaction to type IV collagen. All this confirms, at the molecular level, a morphologic normalization of the BM already observed by us by TEM (6–7) under the same experimental conditions and permits us to hypothesize a normalizing action of CsA on keratinocytic functionality. This activity of CsA, as is known (16), leads back to its capacity, of hindering lymphocyte T from producing those cytokines which, having keratinocytes has their target, alter the normal functionality and probably manifest an existing genetic anomaly.

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Workshop on Cyclosporin A

Chairman: P. C. M. van de Kerkhof

M. H. Schreier
P. Amerio
J. D. Bos
L. Fry
A. F. Finzi

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Immunophilins and Immunosuppression by Cyclosporins and Macrolide Structures

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The immunosuppressive drug Cyclosporin A has long been used in the prevention and treatment of transplant rejection and a variety of autoimmune diseases, before a basic understanding of its mode of action emerged. Its powerful selective immunosuppressive effect rests on the inhibition of lymphokine gene transcription. In the absence of IL-2, clonal proliferation of antigen-activated T cells cannot occur and therefore the immune system is reversibly inhibited.

A breakthrough in our understanding of the molecular mechanism involved in Cyclosporin-mediated immunosuppression came from the discovery of a highly specific binding protein for Cyclosporins, called Cyclophilin. Since then four different Cyclophilins have been characterized and cloned: Cyclophilins A, B, C and D, which occur in virtually all cell types and are highly conserved in evolution. They are peptidyl-prolyl isomerases (rotamases) which are inhibited by Cyclosporin-binding.

Studies on the molecular mode of action of Cyclosporin were strongly stimulated when the structurally unrelated macrolide FK 506 was found to have effects strikingly similar to those of Cyclosporin A. Like Cyclosporin, FK 506 inhibits T cell proliferation by preventing IL-2 gene-transcription, binds to a family of specific binding proteins called FKBPs, which are also peptidyl-prolyl isomerases (rotamases) and are inhibited by ligand binding.

The lack of correlation between immunophilin binding and immunosuppressive effects of a large variety of Cyclosporins and different macrolides showed unambiguously that binding of Cyclosporins or macrolides to their respective binding proteins (immunophilins) is required, but is not sufficient for immunosuppression. The immunophilin-ligand complexes (Cyclosporin–Cyclophilin or FK 506 FKBP) bind to the calmodulin-dependent serine/threonine phosphatase, calcineurin, thereby inhibiting its phosphatase activity for phospho-peptide substrates.

The molecular targets of the serine/threonine phosphatase calcineurin are most likely cytosolic components of the transcription complex which has to be assembled or activated to make possible coordinated expression of the early T cell activation genes, particularly the IL-2 gene.

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Cyclical Immunotherapy in Patients with Psoriasis

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Psoriasis, a chronic and unpredictable dermatosis, is a constant therapeutical challenge to dermatologists. However, new knowledge in immunodermatology has stimulated interest in and encourage the use of new molecules, especially cyclosporin A (CyA). Thanks to certain characteristics, this molecule is capable of modulating and blocking the intense network of cytochine that seems to cause this dermatosis. The first clinical experiments have demonstrated that low dosages (3–5/kg die) can achieve rapid and effective remissions. The Italian experience gathered from numerous centres has been assessed to better understand and manage the use of CyA, especially as regards to tolerance and reliability. There is proven remission in 77% of the cases of plaque psoriasis. The duration of remission, as well as the paucity of side effects, has brought to the concept of cyclical therapy with CyA. The advantages of this mode of therapy are: the possibility of determining the most effective dosage; quantification of the dermatosis-free period; opportunity to personalize treatment and decide its duration; early intervention, should the dermatosis recur; exclusion of side effects and better control over those remaining. ***Key words: therapeutical pattern; cyclosporin A; side effects.***

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Cyclosporin A and psoriasis

A number of studies have demonstrated a distinct immunological activity in the etiopathogenesis of psoriasis. Great importance is attached to the associated actions between SALT (Skin Associated Lymphoid Tissue) and the keratinocytes (1–2). These associated actions cause the release of some cytokines from keratinocytes. Fig. 1 outlines graphically what probably happens in psoriasis. It is believed that an initial and not well identified 'noxa' (auto/hetero antigens, trauma, stress, infections, neuropeptides) can stimulate the cells of the APC system that cause an increase of the IL-1. This interleukin stimulates the T lymphocytes to produce other cytokines (in particular, IL-2, IFN-γ) (3) and various growth factors that stimulate the hyperproliferation of keratinocytes. The activated keratinocyte (ICAM-1+, HLA-DR+) produces other cytokines, necrosis factors and inflammation mediators (LTB4) that support the immuno-mediated inflammation.

The outcome of these various activities is psoriasis. Knowledge of this process has allowed us to establish new therapeutical possibilities, in particular through the use of Cyclosporin A (CyA). This particular drug is capable of reducing the activity of the APC cells, thus blocking the secretion of IL-1 (Fig. 1, violet ellipse) (4). In this way, it also blocks the activation of the homing T lymphocyte and of the SALT, thus blocking the production of IL-2 and the cascade of events that follow (Fig. 1, violet ellipse) (3): clonal expansion of the T lymphocytes, endothelial proliferation and hyperproliferation of the keratinocytes (3, 5, 6, 7). The direct action on the keratinocytes is not evident with the low dosage (3–5 mg/kg/die) used in clinical studies (8). However, the hyperproliferation of the keratinocytes is reduced by the inhibitory action on the APC cells and the T lymphocytes (Fig. 1). Briefly, we perceive a direct action that affects the immunological cell-mediated activity responsible for the persistence of the disease. This mechanism of action is quicker and more efficient than that of the standard therapies. It also is more tolerable and easier to manage (9–10). The aim of multicentre Italian studies was to improve the evaluation of the efficiency, tolerability and safety of low-dosage CsA therapy in Erythrodermic Psoriasis and Plaque Psoriasis, by means of an open, multicentre, uncontrolled study lasting 6–12 months. The

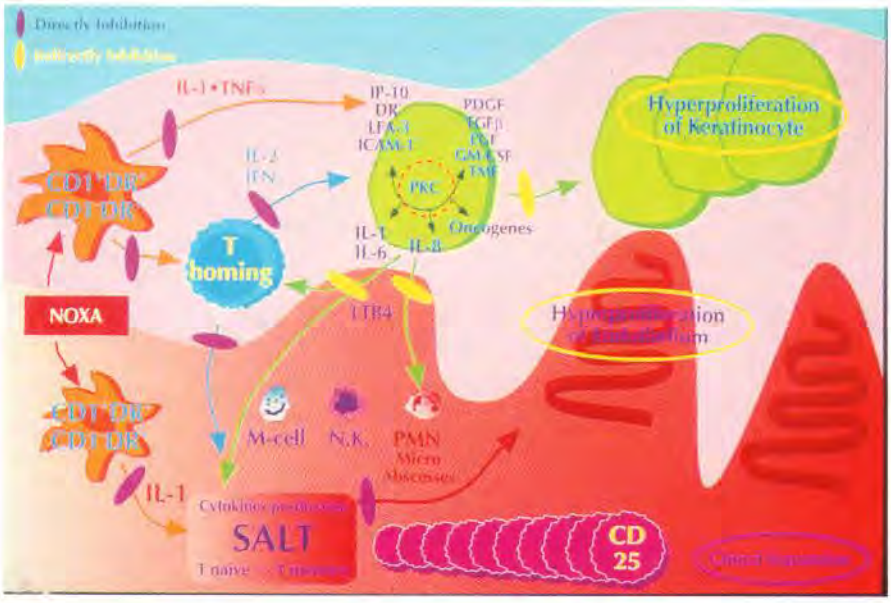

Fig. 1. How Cyclosporin works. For details, see main text.

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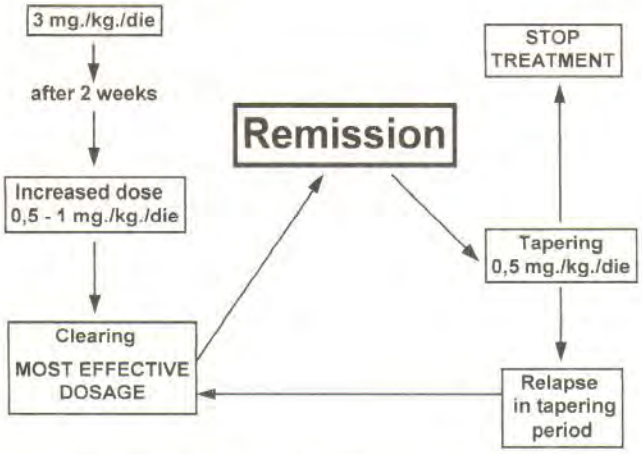

Fig. 2. Schematic illustration of therapy for psoriasis.

therapeutical scheme used was a dose of 3–5 mg/kg/die for the plaque form and 5 mg/kg/die for the erythrodermic form. At remission, a gradual reduction of the drug was practised (0.5 mg/kg/die, every 2 weeks) until suspension.

RESULTS

Plaque psoriasis

314 patients were treated and 293 patients were evaluated. Remission (defined as a reduction in the spreading of the lesions by at least 75%) was present in 225 patients (76.8%). Of these, 73% achieved remission after 2 months (median), on a dosage of CsA of 2.5–3.5 mg/kg/die. On a dosage >3.5 mg or <2.5, remission occurred 3 and 4 months, respectively, after commencing the study.

At remission, 133 pts. who were checked after 8 months, did not manifest relapse. Relapse (defined as a worsening of the clinical aspects of the disease involving more than half of the cutaneous surface) was present in 92/225 patients: 24 patients relapsed at a median time of 6 months, and the remainder 4 months (median) after remission. Side effects were present in 109 of the 293 patients studied (37%) but only 76/293 (26%) were caused by CsA and only 9 pts. (3%) had to suspend therapy because of drug-related side effects. The more frequent side effects were altered renal function, hypertension and an altered lipid profile. All patients could be treated either by reducing the dosage or its suspension (11).

Erythrodermic psoriasis

Remission (total disappearance of erythema and desquamation) was present in 22/33 pts. (67%) at a median time of 2 months in the 12 pts. who took 4–5 mg/kg/die and at a median of 4 months in the 10 pts. who took doses less than 4 mg/kg/die. Relapse occurred in 4 of the 22 patients in remission. Side effects, which were not very serious, were present in 15/33 pts. (45%) of which 8 were caused by the therapy. These side effects disappeared following appropriate reduction of the dosage, and in 6 cases after suspending the drug altogether (12).

DISCUSSION

From the information obtained from the multicentre studies and our own personal experiences, we can see the characteristics of therapy with CsA: *a*) rapid action; *b*) lengthy remission; *c*) a small proportion of side effects (caused by a long and, especially, continuous therapy). In view of this information, it is appropriate to use a cyclical therapy (Fig. 2). This method of therapy offers many advantages which, all things considered, permits a better control of the disease in every single patient, despite its instability. The advantages of cyclical therapy with CsA are as follows. Identifying the most effective dosage: the gradual reducing is the first step towards obtaining optimization of the therapeutic dose. Confronting its therapeutical validity with the dosages of the preceding therapy is the second step. Quantifying the period free from disease: this information is found by confronting the various cycles of therapy instituted, but its identification permits the personalizing and adjusting the duration of therapy: a therapy based on the administration of CsA during the winter months can be established and, at remission, lengthening it with the help of heliotherapy (at the seaside or in the mountains), where possible. Early control of relapse: in fact, in the early stages of this disease, topical therapy with steroids and/or keratolytics can be used. Reducing and better control of the side effects: damage caused by CsA can be manifested by altered renal and liver functions and hypertension. These side effects are all reversible and can be prevented or eliminated (even during therapy) by appropriate reduction of the dosage (reduce by 1 mg/kg/die). In the case of altered renal functions, the use of diuretics is not advised, as it is better to use ionic exchange resins. To conclude, the careful, individual, rational and especically cyclical use of CsA affords good results with only occasional and always reversible side effects.

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ABSTRACT

Cyclosporin and Alternative Cyclical Immunosuppressants in Psoriasis

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With the introduction of cyclosporin as the parent molecule of a whole new class of immunosuppressants, a fundamental change in the management of a series of dermatological disorders has ensued. Not only psoriasis, but also atopic dermatitis, pyoderma gangrenosum, morbus Behçet, actinic reticuloid and lichen ruber planus have been found to be responding. Cyclosporin is a cyclic peptide that, together with cyclic lactones, forms a new class of cyclic immunosuppressants. Their precise mechanism of action is as yet not defined, and may differ from molecule to molecule. It seems evident that they interact with cytokine production regulation at the genetic level and intercept T-cell production of IL-2, IFN-τ, IL-4 and IL-5.

The molecular weight of cyclic immunosuppressants is generally well over 500 D, which makes their possible use as topical agents highly unlikely, perhaps with the exception of application on non-cornified mucous membranes. Cyclosporin is registered for severe psoriasis in many countries and is tolerated reasonably well by a subgroup of patients. Dosage usually starts at 3 mg/kg/day, and it is advised to use cyclosporin as part of rotational therapy in psoriasis patients. Side effects of major concern are nephrotoxicity, development of hypertension, and possible facilitation of malignancies, especially squamous cell carcinoma, in patients who have received high dosages of photo-(chemo)therapy. In view of the benefit/risk ratio, that is now well established in psoriasis, alternative therapies with other cyclic immunosuppressants are under development.

IMM-125 is a cyclosporin-derivative with a certain anti-psoriatic effect, but the number of patients studied thus far is limited. FK-506 (tacrolimus) is a cyclic lactone that has proved efficacious when given systemically to patients with psoriasis and with pyoderma gangrenosum. Again, its risk/benefit ratio remains to be precisely defined. Alternative cyclic immunosuppressants such as desoxy-spergualin, tetranactin, didemnin-B and others have as yet not been investigated in dermatology.

ABSTRACT

Long-term Cyclosporin Therapy for Psoriasis

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Eighty-four patients (mean age 43; range 18–76; M:F 18:35) with severe chronic plaque psoriasis have been treated with CyA for 1–72 months (mean 18.5 months). The mean reduction in PASI score for the group was 69%. The mean maintenance dose of CyA was 3.3 (range 2–5) mg/kg/day. Continuous treatment with CyA was required for 35 (42%) patients in order to control psoriasis, whereas 32 (38%) patients were managed with intermittent courses. CyA was ineffective in 5 patients, defined a failure to control psoriasis on CyA 5 mg/kg/day. CyA was withdrawn in 13 cases because of: renal impairment ($n=5$); relative contra-indication with high-dose NSAID for arthritis ($n=2$); severe nausea ($n=1$); death ($n=1$); noncompliance and infrequent attendance ($n=4$). Three patients have developed malignancies. Hypertension (systolic BP >160 mmHg or diastolic BP >90 mmHg) developed in 27 (32%) patients with the onset at between 0.5 and 62 months (mean 11.5 months). Hypertension was reversible when CyA was discontinued. Glomerular filtration rate estimation fell 16% from a mean (SD) value of 110 (23.9) ml/min prior to commencing CyA to 94 (29) ml/min after treatment. 24 (28.9%) patients had >25% fall in GFR with the mean time of onset 22 months (range 3–65 months). The GFR improved in all patient when CyA was stopped for one month, but the GFR did not return to its pretreatment value in 12 of the 24 patients. A correlation between the fall in GFR and length of treatment with CyA was seen ($r=0.41$; P, 0.05). 17 (20%) of the patients had a rise in their serum creatinine rate >30% of the baseline value, and 12 (14%) had a rise >50%. A correlation between duration of treatment with CyA and rise in serum creatinine was demonstrated ($r=0.49$; P, 0.05). 8 patients who have been treated with CyA underwent renal biopsy after an average of 60 months (range 48–66). 6 of the 8 biopsies showed some evidence of CyA nephrotoxicity, but only in 2 were the changes serious enough to warrant discontinuing CyA.

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The Modern Approach: New Combined Treatments for Psoriasis

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The clever physician, especially if he is also an investigator, tries, whenever possible, to treat his patients with only one active drug, so that he can obtain exact information about the effectiveness of the drug and about any side effects that it may produce.

For psoriasis, a chronic, recurrent and often recalcitrant disease, attempts have been made to combine more than one drug in the hope of improving the effectiveness of the treatment. We have demonstrated that since psoriasis has precise self-maintaining mechanisms, recurrences are significantly more frequent when the disease has been only alleviated, instead of when the clinical symptomatology has completely disappeared (Table I).

There are many valid and non-valid reasons for which several treatments are often combined. Sometimes the drugs in the combination act at different sites, and therefore the combination may be more effective. Sometimes drugs with differing toxicity are combined to reduce the risk, or one can combine low doses of toxic drugs to reduce the toxicity. Sometimes high doses of toxic drugs are given in the hope of curing the disease within a short period of time. The ultimate and not really rational reason for combining several treatments is a very recalcitrant case which is treated 'shotgun', in the hope of finding an effective cure. Combining several drugs can result in cancellation of their individual effects (antagonism, which must absolutely be avoided) or else a simple summation of their effects, but ideally will have synergistic effects, which is to say that the effectiveness of each drug is greater than the same dose given alone. Summation may make it possible to shorten the period of treatment without increasing any side effects. Synergism, instead, increases the therapeutic effectiveness and decreases the side effects at the same time, which is the definition of the term 'better therapeutic ratio'. Drugs may interact directly (chemically or physically) or through reciprocal alterations of gastrointestinal absorption or of protein binding, or else of metabolism or renal excretion. Finally, they may interact at receptor sites. Receptor site interactions may be competitive or may be chemical alterations of the receptor or biological changes at different sites in the same system, or else work through different systems with similar biological effects.

Psoriasis can be treated locally or systemically and I will speak for the most part about systemic treatments. The currently available systemic treatments for psoriasis that have proved effective are photochemotherapy, retinoids, methotrexate and other cytostatic drugs such as hydroxyurea, cyclosporin and perhaps some fumaric acid esters, but the effectiveness of the latter has not been definitely demonstrated and we do not know the mechanism of their action.

I will now summarize rapidly the mechanisms of action of the drugs listed above, with the aim of deciding whether or not it might be possible to combine them.

Retinoids, especially etretinate, which is by far the most widely used drug of this class for psoriasis, act by regulating the differentiation of keratinocytes, inhibiting the chemotactic activity of neutrophilic granulocytes and reducing the adhesion of the cells and their anchorage-independent growth. Their major targets are the keratinocytes and the neutrophilic granulocytes.

Photochemotherapy acts by inhibiting the activities of the keratinocytes, the Langerhans cells, the T-lymphocytes and the neutrophilic granulocytes. The same cells are the targets for methotrexate.

Cyclosporin blocks the production of IL-1, IL-2, TNF, GM-CSF and gamma-IFN, decreases the activity of the Langerhans cells, inhibits phospholipase A2 and – only in vitro and at doses much higher than the therapeutic ones – decreases keratinocyte mitoses. The target cells for cyclosporin are, therefore, the T-lymphocytes, the monocytes and the Langerhans cells.

When designing a combined treatment, the first thing to keep in mind is to avoid summing of the same side effects of each drug. PUVA treatment, methotrexate and cyclosporin are all cancerogenic drugs and therefore must not be combined. The retinoids have been shown to have antineoplastic activity and might therefore be combined with each of the drugs listed – except methotrexate, which has hepatotoxic effects, as have the retinoids.

In Table II, I have summarized all the possible useful combinations of drugs for treatment of psoriasis, both systemically and topically, based on what I have listed theoretically above, on my personal experience and on the data in the literature. PUVA therapy can be combined locally with calcipotriol and systemically with retinoids. This combination (Re-PUVA) has been known for years and often reported in the literature to be one of the most effective treatments for psoriasis, with fewer side effects.

Table I. *Relapses of psoriasis after remission in 147 pts (median follow-up: 15 months)*

	Cleared 75–99%	Cleared 100%	
Relapses	68 (47%:	28 (27%)	χ^2: $p < 0.001$
No Relapses	76 (53%)	75 (73%)	

Table II. *Drug associations for psoriasis*

PUVA	MTX	CsA	RETINOIDS
Retinoids	Calcipotriol	Retinoids	PUVA
Calcipotriol	Dithranol	Calcipotriol	CsA
		Dithranol	Calcipotriol

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Methotrexate can be combined, essentially, only with such topical drugs as calcipotriol and dithranol, excluding the tars because of their potential oncogenic activity. Although the combination of methotrexate and retinoids should be effective theoretically, it should absolutely be avoided because of the high probability of hepatotoxicity, also reported in the literature.

Cyclosporin can be combined with non-oncogenic topical drugs such as calcipotriol and dithranol and, very likely, might be useful in alternating cycles with retinoids.

Finally, retinoids can be combined with PUVA therapy, calcipotriol and, apparently with cyclosporin.

The combination of cyclosporin with etretinate has different targets and thus should produce a truly synergistic therapeutic effect as well as reducing the possibility of side effects, especially the widely-feared oncogenic effects of cyclosporin. The long half-life of etretinate may also prolong the interval free of psoriasis after cure as I have seen in a large case list.

Recent studies by Weber and Back have also demonstrated that there is no metabolic interaction in the liver between cyclosporin and etretinate. Probably the two drugs are metabolized by different isoenzymes of cytochrome P-450 and, therefore, their use either in combination or alternatively does not increase their toxicity.

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Frequency of Psoriatic Arthritis in General Population and Among the Psoriatics in Department of Dermatology

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During the period 1989–90, the prevalence of psoriatic arthritis was studied in 10,500 randomly selected subjects in Osijek Region, while articular changes were studied in 553 psoriatic patients hospitalized at the Dept of Dermatovenereology, Zagreb University Hospital. For the former, prevalence rates of 0.97% for psoriasis vulgaris and 0.095% for psoriatic arthritis were found. Among those with psoriasis, 9.8% had psoriatic arthritis. Among the hospitalized patients, the corresponding rate was 7.23%.

INTRODUCTION

According the the definition by Moll & Wright (1), psoriatic arthritis (PA) is associated with inflammatory arthritis and psoriasis, giving negative results when testing serologically for rheumatoid factor. The prevalence of PA in the general population has not been studied exhaustively, but according to data already published, PA prevalence ranges between 0.02% and 0.1% (2). Among psoriatics, PA prevalence ranges between 0.5% and 39% (3, 4), though most the consensus of opinion is 7% (2, 5). It is conceivable that the differences in prevalence estimates are a consequence of applying different diagnostic criteria.

The objectives of our study were therefore 1) to establish the prevalence of PA in the general population, 2) to establish the prevalence of PA in a population of psoriatic patients, and 3) to establish the characteristics of PA in our patients.

SUBJECTS AND METHODS

Two groups of subjects were enrolled in the investigation. A random sample numbering 10,500 inhabitants of Osijek Region were studied to determine the prevalence of PA in the general population. The PA prevalence in psoriatics was studied in a sample of 553 patients hospitalized for psoriasis vulgaris (PV). In addition to an examination by a dermatologist, all patients with PV and PA were investigated by questionnaire in a genetic study of psoriasis. The diagnosis of PA was established by a rheumatologist.

In all patients with PA, particular attention was paid to anamnestic data on psoriasis and psoriatic arthritis in the family; time of onset of psoriatic changes affecting skin and joints, and spreading of psoriasis to the nails. In 35 of the altogether 40 patients, HLA was classified according to type. The affected joints in all the PA patients were radiographed and the X-rays were interpreted by a radiologist.

RESULTS

The systematic examination of the 10,500 randomly selected subjects from Osijek Region revealed 10 with a verified diagnosis of PA. Hence the prevalence of PA in the general population of that region is 0.095%, ranging between 0.04% and 0.15%, the probability being 95%.

In the group of patients with PV, 40 (7.23%) were diagnosed as having psoratic arthritis. In most of the patients (82.5%), psoriatic skin changes preceded changes in the joints. In 17.5% of the PA cases there were anamnestic indications of psoriasis in the patients' families.

16.6% of the PA patients were positive for HLA-B 27, whereas 66.6% and 16.6% respectively were positive for HLA-B 17 and HLA-B 39.

Clinical classification of the PA patients was made according the criteria of Moll & Wright (Table I). The clinical picture of the skin changes was worse in patients with PA than in psoriatic patients generally. The most frequent deviations from usual laboratory findings were the accelerated ESR (65%) and disproteinemia (47%). 25% of PA patients had an elevated uric acid value, while 35% had elevated IgA. All patients had a negative ANF finding. 85% of PA patients had affected nails, compared with 34% of the psoriasis group generally. There was a preponderance of males (85%) among the patients.

DISCUSSION

The prevalence of psoriatic arthritis (PA) in a random sample of the general population of Osijek Region was found to be 0.095%. Although PA prevalence has been insufficiently studied, rates reported in the meagre literature (2) range between 0.02% and 0.1%. The prevalence of psoriasis generally in the above sample was 0.97%, while the PA rate in this group was 9.8%, which tallies with figures published by other authors (3, 4).

In our group of 553 hospitalized psoriatic patients, 40 were diagnosed as suffering from PA (7.23%). This finding tallies with Leczinski's observation (4) of 6.8%, and with the results published by Sigler et al. (6) who investigated the prevalence of PA in a sample of hospitalized psoriatics, their finding being

Table I. *Clinical picture in 40 patients with psoriatric arthritis (PA)*

Clinical picture	Number of patients	%
Symmetrical polyarthritis	4	10
Asymmetrical oligo/polyarthritis	25	62.5
"Classic" PA	5	12.5
Psoriatic spondylitis	6	15
Arthritis mutilans	0	0
Total	40	100

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some 5–7%. Other PA prevalence data are: 39% according to Leonard et al. (7) and 34.4% according to Oriente et al. (8) and Scarpa R. et al. (9). As only the more serious psoriasis cases are hospitalized, however, such samples are inadequate for epidemiological studies.

There was a male preponderance (85%) among our subjects. Moll & Wright (1) and Leonard et al. (5) report such similar sex ratios as well, while Baker (2) conversely had a preponderance of female PA patients. There was a positive family anamnesis in 17.5% of our cases, whereas Baker reported (2) that only 4.4% of his patients had a positive family history of PA. The lag between onset of psoriasis and onset of arthritis averaged 10.25 years in our material (range 6 months – 40 years), which also tallies with the findings of Leonard et al. (7) and Mollin et al. (10).

Knowledge of the clinical and radiological characteristics of psoriatic arthritis is crucial where psoriasis is either not manifest at all, or the changes are visible only on the nails. As PA inhibits the ability to work and can even lead to complete disability, the diagnosis and treatment of the disease requires constant collaboration between specialists in dermatology and rheumatology.

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Prevalence and Clinical Features of Juvenile Psoriatic Arthritis in 425 Psoriatic Patients

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The aim of this study was to define the prevalence and clinical features of Juvenile Psoriatic Arthritis (JPsA). According to the definition by Ansell and Bywaters, we identified the population at risk of JPsA in 425 patients with psoriasis with onset occurring before the age of 31. Among these, 85 were younger than 16 years. Five patients with JPsA were found (prevalence 1.0%). All had a family history of psoriasis and onset of skin disease in the age range 10 to 20 years. Arthritis preceded psoriasis in two cases, while in the remainder the converse occurred. The interval between the onset of cutaneous and articular involvement never exceeded 8 years. Previous studies reported the low frequency of JPsA among juvenile arthritides. Our data appear to underline the rarity of the arthritic form. ***Key words: psoriatic arthritis, seronegative spondyloarthropathies.***

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First described by Ansell and Bywaters in 1962, Juvenile Psoriatic Arthritis (JPsA) is considered to be an inflammatory arthritis associated with psoriasis, either preceding the onset of arthritis proper, or occurring within the subsequent 15 years, and persisting for at least 6 weeks and beginning before the age of 16 years (1). Usually, rheumatoid factor is not present in the serum. In 1982 a survey was started in Naples with the aim of studying the prevalence and clinical features of the arthritis associated with psoriasis (2). 759 psoriatic patients have so far been considered and, of these, 85 children have been observed. In this paper, the prevalence and clinical features of JPsA cases are detailed and the dermatological characteristics and relationships between psoriasis and arthritis are also discussed.

PATIENTS AND METHODS

Eight-five children with confirmed psoriasis (59 F and 26 M, mean age 12.47, range 3–16) and 340 patients with onset of the skin disease between 16 and 31 years of age, were admitted due to cutaneous involvement to the Dermatological Department of Health Unit no. 42 of the Region of Campania. On the basis of the definition set out above, all subjects were regarded as a population at risk of JPsA (Table I). A complete dermatological and rheumatological assessment was carried out.

Dermatological assessment was based on scrutiny of familial and personal medical records, concerning age at onset of and localization and extent of skin involvement.

Rheumatological evaluation included the detection of peripheral and/or axial involvement, the assessment of its activity and age at onset.

Diagnosis of peripheral chronic arthritis was based on the presence, for at least 6 weeks, of joint swelling, or at least two of the following clinical features: morning stiffness, limited joint motion, pain accompanying joint movement, or joint margin tenderness in the medical history and/or at clinical examination. Radiological examination was also performed in adult men with a positive medical history. The localization of arthritis was also recorded.

The presence of axial involvement was determined by the detection of inflammatory back pain (IBP) (3) plus at least one of the following features, or by two of them alone: (*a*) limitation of lumbar spine motion in anterior and lateral flexion and extension; (*b*) limitation of chest expansion to 2.5 cm (1 inch) measured at the fourth intercostal space; (*c*) enthesopathies; (*d*) sacro-iliitis revealed by radiography in adult men with IBP lasting from pediatric age, or by tenderness evidenced by at least two of the following clinical techniques in all patients: 1) direct pressure over the sacro-iliac joints; 2) mutual impaction of the iliac bones; 3) hyperextension of one hip with the other in full flexion.

The arthritic activity was graded according to the severity of pain, duration of early morning stiffness and number of affected joints.

Serum rheumatoid factor was searched for by Latex test (4) and by a haemoagglutination slide test (5).

RESULTS

Only 4 of the 85 psoriatic children exhibited arthritis and 1 of the remaining 340 patients observed revealed articular involvement before the age of 16 (prevalence of JPsA: 1.0%). All 5 patients had active psoriasis.

Patient 1

A 16-year-old girl whose onset of psoriasis was at the age of 12. Skin disease involved scalp, arms, legs and back. Family history of psoriasis was positive (2 relatives).

Arthritis followed psoriasis after 3 years, involving MCP, PIP and DIP of both hands, with insidious onset and moderate activity.

Patient 2

A 10-year-old girl had psoriasis 2 months before the time of observation, with a diffuse localization. Her sister also had psoriasis.

Mild arthritis had preceded psoriasis by 4 years, with an insidious presentation in bilateral MCP and PIP and in both knees.

Patient 3

A 13-year-old girl had psoriasis at the age of 11, involving both

Table I. *Characteristics of the population at risk of JPsA*

Sex	Number	age (years)	
		Mean	Range
Female	240	27.60	4–68
Male	185	30.78	3–76
Total	425	28.98	3–76

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Table II. *Involved joints in the 5 patients with JPsA*

Pat. no.	MCP	PIP	DIP	SI	Knee	Ankle
1	++	++	++			
2	++	++			++	
3	++			+		
4				++		
5					+	+

elbows and knees, and then the legs and back. She also had onycopathy. One relative was also affected by psoriasis.

Two months after the onset of psoriasis, the patient suffered inflammatory back pain and peripheral arthritis localized in the knees and MCP of both hands, with moderate activity. At the time of our observation she had unilateral sacro-iliitis.

Patient 4

A 21-year-old woman developed psoriasis 7 months before our examination, localized on the scalp, face, upper arms, elbows, hands and feet. She also had severe onycopathy, but no family history of psoriasis.

Eight years before the onset of skin involvement, the patient had had inflammatory back pain. Bilateral sacro-iliitis with mild activity was evident at the time of our clinical examination.

Patient 5

A 12-year-old girl whose psoriasis appeared at the age of 8 years. Skin involvement was localized on the scalp, arms and legs. One relative of the patient was affected by psoriasis.

Two years after the onset of psoriasis a mild arthritis involved left knee and ankle.

DISCUSSION

JPsA was first described by Ansell and Bywaters in 1962 as an inflammatory arthritis associated with psoriasis either preceding the onset of arthritis or occurring within the subsequent 15 years, persisting for at least 6 weeks, beginning before the age of 16 years and usually with an absence of rheumatoid factor in the serum (1).

The aim of our study was to define the prevalence and clinical features of JPsA among psoriatic patients, within the scope of an epidemiological survey started in Naples in 1982 (2).

On the basis of the definition of JPsA quoted above, we identified a population at risk of JPsA comprising 425 patients with psoriasis onset occurring before the age of 31 years. Of these, 85 were less younger than 16 years old. Five patients with JPsA were identified (prevalence 1.0%).

Four patients had a family history positive for psoriasis and all had the onset of skin disease in the age range 10 to 20 years.

Arthritis preceded psoriasis in 2 cases, while in the remainder the inverse occurred. The interval between the onset of cutaneous and articular involvement never exceeded 8 years.

Localization of joint involvement is shown in Table II.

Previous studies reported a low frequency of JPsA among Juvenile arthritides (6–10). Our results, although not comparable to similar data, underline the rarity of this arthritic form. Nevertheless, JPsA needs a new diagnostic approach based on a forewarning detection of psoriasis and a definition of a typical pattern of arthritis.

At first, based on the high frequency of nail changes, dactylitis and a positive family history of psoriasis in children without typical psoriatic rash, a new diagnostic set allowing a forewarning detection of JPsA has been proposed (11).

Moreover, in the presence of joint manifestations alone, typical articular patterns of JPsA have been described (12).

Among our patients, although representing a small sample, all with definite psoriasis, 2 showed onycopathy, but, as previously observed (13), tenosynovitis and dactylitis was not found.

However, JPsA can at present be diagnosed only by the simultaneous detection of arthritis and definite psoriasis. This problem can only be solved by follow-up studies, lasting long enough to match probable JPsA with the definition criteria of Bywaters and Ansell.

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Calcium Metabolism and Psoriatic Arthropathy

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Calcium plays a fundamental role (1) in psoriasis through its influence on the rate of mitosis and cell differentiation. These effects are mediated by its role in arachidonic-acid metabolism (which leads to the production of leukotrienes, prostaglandins, etc.), the cAMP/cGMP ratio, the formation of desmosomes and factors effecting cellular cohesion (2). The involvement of calcium in psoriasic disesase is also reflected in the differenctial distribution of this element within the lesional and non-lesional skin of psoriasis patients and healthy skin from non-psoriatic subjects (3).

Psoriatic arthropathy is a condition characterized by phenomena of erosion, reabsorption, destruction and regeneration of the bone tissue. These phenomena would lead us to expect to see changes in blood and urinary levels of Ca++, but this aspect of psoriasis has, thus far, received little attention.

Purpose of the study

Our objective was to evaluate calcium metabolism in psoriasis patients as a possible index to the presence of arthropathic changes. A marker of this sort might eliminate the need for radio-scintigraphic studies, which are not without risk, but are currently the primary means of diagnosing psoriatic arthropathy.

MATERIALS AND METHODS

Fifty-two subjects with various types of psoriasis (with the exception of pustular pustular forms) participated in this study (tab. I); anamnesis was positive for arthropathy in 4 cases. A panel of bone studies, which has been described in our previous publications (4, 5, 6), was performed in 42/52 subjects; the other 10 were excluded from these studies because they were under the age of 15 or because they might have unknowingly been in the early stages of pregnancy.

This panel included plain X-ray, microradioscopy, computerized tomography, and radionuclide scan).

Calcium metabolism was evaluated in each subject on the basis of serum and urinary calcium levels, serum levels of ionized calcium, albumin, phosphorus, alkaline phosphatase, creatinine, glucose, uric acid, total cholesterol, triglycerides, RCP and RA tests.

RESULTS

The findings from the radio-scintigraphic studies (performed in 42/52 subjects) confirmed our previous observations of the almost constant presence of bone involvement in patients with psoriasis (4, 5, 6) (Table II). In most reports, the incidence of arthropathy in psoriasis patients has been estimated at around 20%, but these figures are generally based exclusively on the results of one type of bone study. Early arthropathic involvement can only be detected with a panel of sensitive and sophisticated imaging studies based on various techniques, such as the one used in this study.

In all of the subjects we examined, at least one of the imaging studies revealed signs of arthropathy ranging from minimal changes, such as increased bone-cell turnover, geodes and/or marginal erosions of the interphalangeal joints, to those classical alterations associated with disabling forms of ankylosing arthropathy.

Laboratory studies (Table III) confirmed the frequency of lipid metabolism disorders in psoriasis patients that has been noted by a number of authors. Serum glucose levels were within normal limits in all 52 patients, and normal blood and urinary Ca++ levels were found in 51 of the 52, including many with severe arthropathy, extensive cutaneous disease and/or associated metabolic disorders. A mildly elevated ionized calcium level was noted in one elderly patient with frank, long-standing arthropathy, but this elevation could have been due to his general condition rather than his arthropathy.

DISCUSSION

The reliability of our findings of normal calcium metabolism in these psoriasis patients seems to be confirmed by the concordance among serum and urinary calcium levels, and the levels of ionized calcium (therefore free calcium) and albumin, which normally binds over half of all circulating calcium.

The fact that all of the patients we studied, regardless of the

Table I. *Patients.*

	n	Age range	Average age	Mean of duration of the disease
Females	15	24–68 y	49 y	11.7
Males	37	11–77 y	50 y	12.5 y
Total	52			

Table II. *Instrumental investigation*

Skeleton radiography:	specific lesions in 21 cases
Skeleton scintigraphy:	specific lesions in 25 cases

Table III. *Laboratory investigations in 52 patients*

Hypercholesteraemia	12
Hyperglyceridaemia	11
Hyperuricaemia	17
Hyperglycaemia	0
Albuminaemia	normal
Creatinaemia	normal
Alkaline phosphatase	normal
Phosphataemia	3> 1<
Calcaemia	normal
Calciuria	normal
Ionized calcium	1>

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degree of cutaneous and bone involvement or the presence of metabolic disorders, presented serum and urinary Ca++ levels that were within normal limits, suggesting that there must be regulatory systems at the cutaneous and osseous levels that modulate release and sequestration of calcium ions.

Within the skeletal system, such regulation might be effected by bone-growth factors and bone-derived growth factors which have been found to play important roles in bone metabolism in human and animal studies (7, 8, 9).

Unfortunately, there have been no studies on the activities of these factors in humans that could confirm or disprove this hypothesis.

CONCLUSIONS

Our findings indicate that blood and urinary levels of Ca++ offer little useful information on the degree of cutaneous or skeletal involvement in psoriasis patients. Further study will be necessary to identify the metabolic pathways at the bone and skin levels that regulate the intracellular/extracellular gradient of this ion, which is fundamental to the development of psoriasis.

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The Nails in Psoriatic Arthritis

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Nail involvement is often present in patients suffering from psoriatic arthritis. Up to the present, no data concerning the rate of onychopathy associated with psoriatic arthropathy have been reported. 52 patients with psoriatic arthropathy have been studied with particular attention to the possible presence of and the typical features of onychopathy. Nail changes were noted in 86.5% of patients affected by arthropathic psoriasis. The commonest toenail alteration was subungual hyperkeratosis, while the most frequent fingernail change was pitting. ***Key words: onychopathy; arthropathy; psoriasis.***

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Nail involvement in psoriasis vulgaris is reported in some 10–55% of patients, while psoriatic arthropathy changes are present in about 85% of cases. The possible relationship between psoriatic arthropathy and onychopathy regarding morphology, localization, and time of occurrence of the ungual alterations is still unknown. In this study we evaluated nail alterations in a group of patients affected by arthropathic psoriasis.

PATIENTS AND METHODS

During the period May 1990–August 1993 we studied 52 patients admitted to our Institute and affected by arthritic psoriasis. All these patients (28 men and 24 women; mean age: 48 years) with a clinical (Ritchie Index), biohumoral and instrumental diagnosis (bone total body scanning; radiography) of arthropathic psoriasis have been studied in order to evaluate the presence of associated onychopathy. All of them also presented skin lesions due to psoriasis (PASI >1). Typology, localization of the lesions and time of occurrence of nail alterations have been evaluated.

RESULTS

Nail changes were noted in 45 out of 52 patients (86.5%) affected by arthropathic psoriasis and were observed in all of the 16 patients affected by severe arthopathy (Ritchie Index: >9). We noted the following lesions: subungual hyperkeratosis (hands: 35%; feet: 61%), pitting (hands: 47%), onycholysis (hands: 35%; feet: 36%), severe nail plate surface abnormalities (hands: 25%; feet: 27%), salmon patches (hands: 22%; feet: 14%) and splinter hemorrhages (hands: 1%).

In 69% of the patients, ungual changes concerned both hands and feet, while in 19% only fingernails were involved and in 12% only toenails. As regards the time of occurrence, onychopathy had occurred prior to arthropathy in 52% of the cases, later in 38% and at the same time in 10%.

DISCUSSION

The Present study has confirmed the high frequency of nail alterations in patients affected by arthropathic psoriasis (86.5%). In agreement with both Baker (83%) and Eastmond (84.8%), this result underlines the difference of these data from the incidence of onycopathy associated with scalp psoriasis which is lower, ranging from 10% to 55% (1–6).

In our cases we observed both matrix and bed onycopathy; the commonest toenail alteration was the subungual hyperkeratosis often associated with distal onycholysis while, according to Baker et al. (1), the most frequent fingernail change was pitting. In the majority of our cases there was an involvement of both hands and feet, while no particular relationship between site of onychopathy and localization of arthopathy has been noted.

The occurrence of alterations has been evaluated anamnestically: in 52% of the cases, patients reported onychopathy prior to arthropathy, in 38% arthritis had occurred prior to onychopathy and in 10% the two features had started simultaneously. We emphasize the correlation between severity of the arthropathy and nail involvement; in fact in all the patients with a Ritchie Index higher than nine, some nail involvement was present. We have not noted any particular relationship between the various features of psoriatic arthritis and onychopathy. Moreover we stress that onychopathy associated with arthropathy could be of use in the diagnosis of psoriasis when cutaneous manifestations are not present.

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Methotrexate in Psoriatic Polyarthritis

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Methotrexate (MTX) is widely accepted as an effective treatment for psoriasis and chronic polyarthrides. We report data from 54 psoriatic polyarthritis patients (354 encounters) treated with MTX. 65% were males, mean age 47.9 years, mean disease duration 9.8 years for arthritis, 14.2 years for psoriasis. The MTX dose was 10–12.5 mg/week. 32 patients are still on MTX after a mean treatment time of 1.6 years. 22 patients discontinued MTX treatment: 11 felt it to be ineffictive, in 6 patients there were side effects, 3 patients obtained remission, while 2 patients underwent surgery. Efficacy was good on the whole: number of swollen and tender joints, global disease activity score, ESR, and CRP all underwent a significant reduction. The intake of symptomatic drugs was reduced in 40%. Psoriasis as assessed with the Psoriasis Activity and Severity Index showed a significant improvement. Our data confirm that MTX is of value in most psoriatic polyarthritis patients (60%). In our experience, this drug, gives the maximum efficacy within 6 months of therapy.

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The association between psoriasis and arthritis has been studied and described by many authors, but not until 1973 did Moll and Wright identify the partiular characteristics of articular involvement in psoriatic arthritis (PA).

Even if from a rheumatological point of view PA is less disabling than rheumathoid arthritis, it has recently been confirmed from studies (1) that one-fifth of PA patients had very severe diseases. We therefore need drugs capable of treating PA without exacerbating psoriasis, as some NSAIDs do. We decided to form a combined task force of rheumatologists and dermatologists to study this therapeutic problem and particularly the effects of Methotrexate (MTX).

MTX was first used to treat PA in 1951 (2), with good results. Since then, rheumatologists have treated PA with long-term (1–10 years), low doses of MTX, whereas dermatologists have used the drug for short periods only. The most feared side effect, hepatotoxicity, is a matter of discussion and analysis. Some think that MTX-induced cirrhosis cannot be aggressive in the absence of any clear correlation between MTX dose and duration of administration (3). Moreover the hepatic damage is reversible with the suspension of the treatment (4). Other authors state that a liver biopsy should be made after a cumulative dosage of 1,500 mg or after a period of 2 years of therapy (5). However, it is known that patients with hepatic or renal alterations, drinkers of alcohol or diabetics, are more predisposed to hepatic MTX-induced damage.

MATERIAL AND METHODS

We have studied 54 PA patients, 65% male, with mean age of 47.9 years. All the patients are affected by polyarthritis (more than 10 joints involved) and psoriasis. The mean psoriasis duration is 14.2 years, while that of arthritis is 9.8 years. The mean Psoriasis Area and Severity Index (P.A.S.I.) at the first encounter is 6.8. Every patient was initially assessed with examination, laboratory testing and X-ray of the involved or suspected joints. These tests and clinical evaluation were repeated at regular intervals. MTX was given intramuscularly at a dose of 10 mg/week (7.5–15 mg/week).

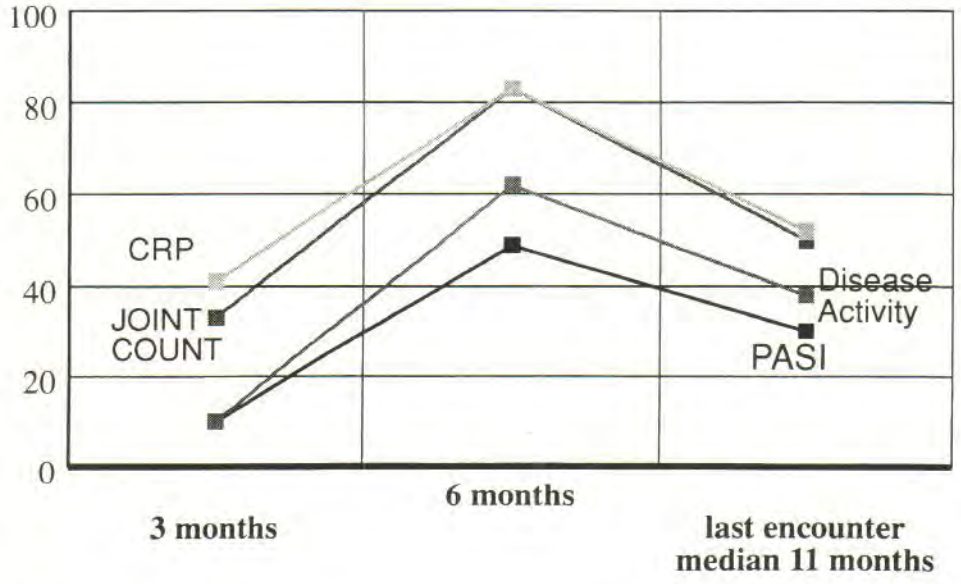

Fig. 1. Efficacy over time. Percentage of patients improved by at least 70% for each parameter.

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RESULTS

The mean period of treatment was 1.6 years (4 months – 3 years) for the 32 patients (59%) still taking MTX, and 1 year (3 months – 3 years) for the 22 patients (41%) who discontinued the drug. Interruption of the treatment was in 11 patients (50%) for inefficacy, in 6 patients (27%) for side effects, in 3 patients (14%) for remission and in 2 patients (9%) for surgery. To study the efficacy of MTX, we have considered four parameters: P.A.S.I., joint count, CRP, global disease activity score (Fig. 1).

Psoriatic skin symptoms generally sustained stable amelioration during treatment, judging by the P.A.S.I. However, complete resolution of the rash was seen in only 3 patients, in contrast to the 90% reported by other authors (5). Moreover, we did not observe any relevant change in psoriatic nail involvement, even after years of therapy. Fig. 1 shows the proportion of patients who improved by at least 70% for each parameter. We can see a significant amelioration for all the parameters after 6 months of treatment. During this time a good proportion of the patients were able to reduce or discontinue their symptomatic drug intake. The side effects noted were frequently moderate (nausea), but 6 patients withdrew MTX, due to severe stomatitis (2 patients), marked liver function test abnormalities (LFT) (2 patients), and longstanding skin infection (2 patients). The withdrawal was decided because side effects were still relevant despite a gradual reduction of MTX dose. A moderate increase in aminotransferase values was observed in the 11 other patients (23.4%).

DISCUSSION

Our experiences confirm that MTX is a valuable therapeutic agent for psoriatic arthritis, probably the most effective and tolerable among the so-called 'disease-modifying' agents. About 40% of our patients responded poorly to MTX and the side effects were dyspepsia, stomatitis, moderate LFT abnormalities; the resulting risk/benefit ratio still seems to be questionable. Probably this is also due to the variable and unpredictable course of psoriatic arthritis. Intramuscular MTX does not appear to be preferable to oral MTX in terms of efficacy and tolerability, nevertheless in our patients this route of administration seems to contribute to a higher patient compliance.

We conclude that MTX, in our experience, provides an overall 60% satisfactory response (with a 20% total remission). Moreover, MTX gives the maximum efficacy within 6 months of therapy: thereafter there is no further reduction in disease activity and the degree of improvement appears to diminish. All this suggests, in our opinion, a different approach to MTX therapy for both rheumatologists and dermatologists: if the best clinical results are achieved in 6 months, we should choose a cyclical 6-month schedule for MTX therapy in PA.

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Methotrexate and Cyclosporin Combined Therapy in Severe Psoriatic Arthritis. A pilot study

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Methotrexate (MTX) and cyclosporyn-A (Cy-A) are two effective agents in the treatment of psoriatic arthritis (PA), though some patients are resistant to these drugs when they are administered separately.

There is increasing interest, however, in combining two or more disease-modifying antirheumatic drugs (DMARD).

Clinical studies have evaluated several combinations of DMARD and have shown improved efficacy – but typically at the expense of increased toxicity, – compared with single agents. The combination of MTX and Cy-A has been used with encouraging results as prophylactic therapy for graft-versus-host disease (GVHD) in bone marrow transplant recipients (1, 2) and for suppression of collagen-induced arthritis in rats (3).

We present here the results of an open study on the efficacy and safety of MTX and Cy-A given in association, in patients with severe psoriatic arthritis.

MATERIALS AND METHODS

Eight patients (4 male, 4 female; average age 53 years) were entered into the study. All patients were in the 3rd functional class. The disease pattern was peripheral, with axial involvement in 6 cases, asymmetric peripheral involvement in 2. All were unresponsive to antimalarials, gold sodium thiomalate and 5 patients also to MTX or Cy-A when given separately. The above-mentioned drugs were discontinued at least 6 months before the study commenced. Non-steroidal anti-inflammatory drugs were allowed during the study. The dosages of MTX Cy-A were 10–15 mg/week and 3–5 mg/kg/die, respectively. Each patient was examined before starting treatment and monthly thereafter. The following clinical variables were evaluated at each visit:

- overall joint tenderness, expressed as Ritchie articular index modified for distal interphalangeal joints;
- duration of morning stiffness;
- patient's assessment of pain graded on a visual analogue scale.

The following tests were carried out at each visit: complete blood and differential count, erythrocyte sedimentation rate, C-reactive protein, serum creatinine and urine analysis. Serum potassium and sodium, uric acid, albumin, total bilirubin, liver transaminase, alkaline phosphatase were also measured.

RESULTS

General characteristics of the patients studied are shown in Table I.

All patients showed a rapid and significant improvement after the first month of therapy. All patients but one showed clinical improvement after 6 months of therapy (Fig. 1). In fact, one of them discontinued the drugs during the fourth month of treatment due to a mild increase in blood creatinine, which returned spontaneously to normal levels after 2 weeks.

Two patients suffered from a recurrence of the disease process after 21 and 19 months respectively; in one of them, reversible arterial hypertension also ensued. The remaining 5 patients are showing a marked and stable improvement after 32, 24, 19, 13 and 9 months of therapy, respectively. All but one patient showed a significant decrease in erythrocyte sedimentation rate and c-reactive protein level.

DISCUSSION

MTX is a folate antagonist which binds to dihydrofolate reductase. It inhibits purine synthesis and interferes with DNA and protein synthesis. Moreover, MTX has a strong anti-inflammatory and immunomodulatory activity by inhibiting interleukin 1 (IL-1). Cy-A is an immunosuppressive agent; it inhibits IL-2, IL-3 and interferon γ, with subsequent inhibition of T helper/inducer cell proliferation.

The association of low dose MTX + Cy-A (10–15 mg/week and 3–5 mg/kg/die respectively) was rapidly and persistently effective in our cases of severe PA. A close correlation was found between improvement in clinical and laboratory assessment. The therapeutic benefit of this drug combination appears to be additive or synergistic, since the drugs were ineffectual as single agents. Unwanted side effects were all mild and reversible.

In conclusion, this pilot study indicates that the combination MTX + Cy-A may be successfully employed in the management

Table I. *General characteristics of 8 patients with severe psoriatic arthritis*

Patient	Sex	Age	Disease duration (years)	Disease pattern
B.M.	F	57	18	P
C.S.	M	59	23	A + P
D.P.R.	M	54	1	A + P
F.M.S.	F	64	10	A + P
S.S.	F	45	22	A + P
R.G.	M	46	6	A + P
B.M.	M	52	8	A + P
C.C.	F	49	5	P

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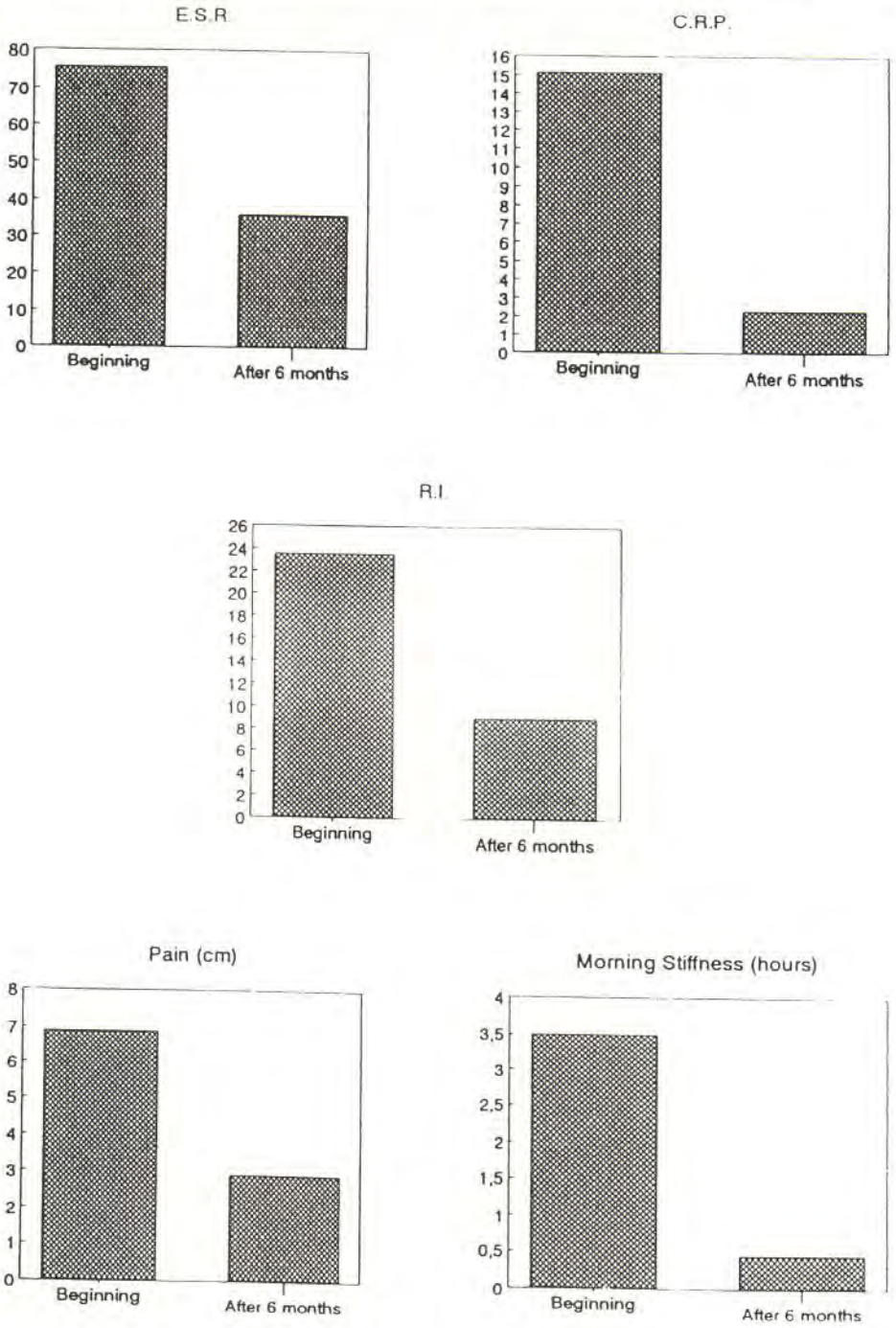

Fig. 1. Mean values of clinical data for 7 patients with psoriatic arthritis before and after 6 months of combined therapy with MTX and Cy-A.

of severe psoriatic arthritis. Further studies will be necessary to define the clinical impact of MTX + Cy-A on larger series of psoriatic arthritis and to assess the role of this association as a first line therapy in problematic cases.

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Scintigraphic Assessment of Disease Activity in Psoriatic Arthritis with 99m-Tc-labelled Non-specific Polyclonal Human Immunoglobulin G

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Technetium-99m-labelled, non-specific, polyclonal, human immunoglobulin G (99mTc-hIG) is a radiopharmauceutical agent recently proposed for the scintigraphic assessment and measurement of synovitis (1, 2). The aim of this work was to evaluate the usefulness of this tracer to quantify synovial inflammation in psoriatic arthritis.

MATERIALS AND METHODS

Twenty patients affected by psoriatic arthritis (Moll and Wright criteria) and suffering from clinically active synovitis were studied. The numbers and sites of the involved joints had been assessed previously by clinical examination on the basis of the presence of pain and/or swelling. All the patients underwent scintigraphy of several groups of joints with 99mTc-hIG. 4 and 24 h after i.v. administration of the radiopharmaceutical agent, static assessment of the joints had been performed with a large-field-of-view digital gamma camera at the real time of 5 and 15 min, respectively. None of the patients had been taking anti-inflammatory therapy for at least 7 days previously. Radiological examination had been carried out earlier on all of the joints. The scintigraphic images were assessed separately as positive or negative by two specialists who were unaware of each patient's clinical status. Foot and carpal joints were considered as a whole because of the difficulty of distinguishing the individual articular segments. Only the joints deemed positive by both specialists were finally accepted as really being positive.

RESULTS AND DISCUSSION

The joints included in this study numbered 283. The results of the comparison between clinical examination and scintigraphic studies are shown in Table I.

100% (16/16) of the clinically positive joints had a positive hIG scan and 86% of the clinically negative joints turned out to be negative on the hIG scan. Therefore, whereas 38 clinically negative joints had a positive hIG scan, none of the clinically positive joints was found negative by hiG scanning.

One explanation for these findings could be that hiG scans produce some 'false positive' results; however, this hypothesis admits the possibility of a different phlogistic process at the same articular site. This is rather improbable in such a large number of joints. On the contrary, it is conceivable that clinical examination cannot detect very early lesions and that the disparity between scintigraphic and clinical results could be the measure of the reliability of the scintigraphy. It has been noted that in psoriatic arthritis, damage tends to occur early in the course of the disease (3), thus the possibility of a scintigraphic assessment of the early lesion could be of great interest.

The comparison with X-ray studies shows that the frequency of hIG scan-positive joints corresponding to clinically negative examination is about the same both in patients with negative X-ray findings (28/38) and in those with minimal and early radiological signs of joint involvement (10/38).

Table I. *Scintigraphic results*

		+	scan	−	*n*
Clinical	+	16		0	16
Examination	−	38		229	267
	n	54		229	283

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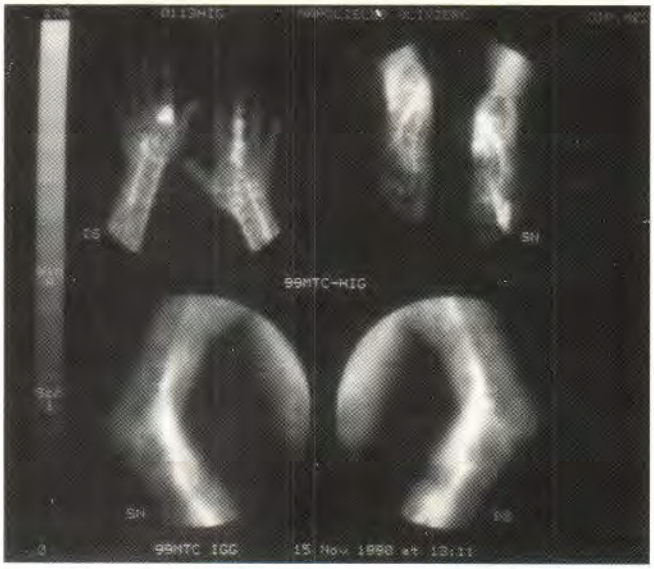

Fig. 1. Patients affected by psoriatic arthritis. 99mTc-hIG scan shows involvement of 2nd metacarpophalangeal of the right hand, 3rd metacarpophalangeal and 3rd proximal interphalangeal of the left hand, left forefoot. All of these joints were positive on clinical examination. Left and right knee scan were quite positive, whereas clinical examination of these joints was negative.

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Psoriasis and Lyme Arthritis

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Psoriasis is a distinctive skin disease that may sometimes be triggered by different agents, such as streptococci and staphylococci, and this may also occur in psoriatic arthropathy. (1). Moreover, psoriasiform lesions may be the first clinical manifestations of HIV infection (2).

Interesting relationships also exist between the course of psoriasis and infections: frequently, streptococcal infections can aggravate the disease (3), and HIV infection may exacerbate the psoriatic condition, including the joint manifestations, in individuals with pre-existent psoriasis (2).

Reactive arthritides are a group of joint diseases characterized by the fact that arthritis follows infections in other organs, caused by various bacteria (Streptococcus, Yersinia, Chlamydia, Salmonella, Shigella), and that they are frequently linked to the genetic make-up of the patient (4). The clinical manifestations are often similar to that of HLA-B27 related spondyloarthropathies, in particular Reiter's syndrome.

Lyme borreliosis (LB) is a multisystem disease, caused by the spirochaete *B. burgdorferi*, characterized by involvement of the skin, joints, nervous system, and heart (5). Some facts support the possibility that, at least in some cases, the joint manifestations caused by *B. burgdorferi* could be a reactive arthritis.

We studied the relationship between *B. burgdorferi* infection, Lyme arthritis, and psoriasis in a group of patients with suspected LB.

Patients and methods

The study population consisted of 1,321 patients examined at the Rheumatological Centre of the University of Genoa because of signs and symptoms possibly related to LB. Patients, all living in an area endemic for LB, were referred to us by rheumatologists, dermatologists, neurologists, and by general practitioners.

A complete rheumatological examination was performed, and patients were diagnosed as having LB according to a set of criteria (6): living in an area endemic for LB or previous tick bite; presence of serum antibodies to B. burgdorferi; at least two of the signs and symptoms compatible with LB (erythema migrans, systemic, neurological, cardiac, articular).

Antibodies to *B. burgdorferi* were measured by both indirect immunofluorescence (IFA) and enzyme-linked immunosorbent assay (ELISA), with previous absorption with *Treponema phagedenis* to avoid cross-reactions with *Treponema pallidum*.

Antibodies vs *B. burgdorferi*:	Positive *n* (%)	Negative *n* (%)
Psoriasis (*n* 32)	11(34)	21(66)
Psoriatic arthropathy (*n* 16)	2(12)	14(88)
All psoriatics (*n* 48)	13(27)	35(73)
Non-psoriatics (*n* 1273)	320(25)	953(75)

RESULTS

Forty-eight (3.6%) patients out of 1,321 were affected by psoriasis. Eight (16.6%) of these 48 had LB, and 2 of them fulfilled the criteria for both LB and psoriatic arthropathy, male-female ratio was 5/3 and mean age was 44 years. Among the 40 (83.4%) non-LB psoriatic patients, 26 (65%) had psoriasis only, and 14 (35%) psoriatic arthropathy. Male-female ratio was 25/15 and mean age was 47 years in this group.

One-hundred and eighty-seven (14.7%) out of 1,273 non-psoriatic patients had LB, male-female ratio was 66/121, and mean age was 46 years. The remaining 1,086 patients were affected by neither psoriasis nor LB.

LB was diagnosed in 195 (14.8%) out of 1,321 subjects examined. The prevalence of psoriasis in patients with LB was 8/195 (4.1%), and 40/1126 (3.6%) in non-LB patients. The presence of antibodies to *B. burgdorferi* in psoriatic and non-psoriatic patients is shown in the table.

There was no difference in the sero-prevalence of antibodies to *B. burgdorferi* between the psoriatic and the non-psoriatic patients.

DISCUSSION

Both psoriasis and psoriati arthropathy can be triggered by bacterial and viral infections, and recently HIV infection has been related to the appearance and the exacerbation of psoriasis and psoriatic arthropathy.

Infectious agents are also responsible for the appearance of reactive arthritides, and in some cases the arthritis of LB seems to follow the clinical pattern of reactive arthritis. This suggest that the spirochaete *B. burgdorferi* may act as a trigger for joint inflammation.

Thus it is conceivable that *B. burgdorferi* could act as a trigger in psoriasis and/or in psoriatic arthropathy.

Our results do not confirm this hypothesis, as we did not find any difference in the prevalence of LB in psoriatic and non-psoriatic patients. Nor was any difference found in the prevalence of psoriasis between patients with and without LB.

Moreover, the prevalence of antibodies to *B. burgdorferi* did not differ between patients with and without psoriasis. However, the presence of 2 patients who fulfilled the diagnostic criteria for both LB and psoriatic arthropathy raises the possibility that some relationship with *B. burgdorferi* infection could exist, at least in patients with psoriatic arthropathy.

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Workshop on Retinoids

Chairman: L. Juhlin

W. Lambert
C. Bodemer
P. C. M. van de Kerkhof
A. Giannetti
J. Lauharanta

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Pharmacokinetics of Acitretin

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Acitretin, the metabolite of etretinate, is eliminated far more rapidly from the human body than is etretinate. It has therefore been suggested that only a short period of contraception would be required following the completion of long-term therapy. However, recent studies have demonstrated the presence of etretinate in the plasma of acitretin-treated patients. In this paper, we review the results of studies at our centre in view of the recently discovered metabolic pathways for acitretin. Re-esterification of acitretin to etretinate, however, results in a loss of the metabolic advantages of acitretin. Because of this new knowledge, the recommended contraception period after acitretin therapy has been lengthened to 2 years.

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Etretinate and acitretin have similar antipsoriatic activity (1). However, since both compounds are teratogenic a contraception period following completion of treatment with these molecules is obligatory (2). Because of its high lipophilic properties, etretinate is stored in the adipose tissue and, after discontinuation of a treatment, the drug is released very slowly from this compartment, necessitating the mandatory contraception during the ensuing 2 years (3, 4).

Acitretin, the main metabolite of etretinate, does not accumulate in any particular tissue and is eliminated far more rapidly than etretinate. Consequently, a period of contraception lasting only 2 months is deemed sufficient after acitretin treatment (5, 6). In consequence, large numbers of patients previously treated with etretinate have been placed on acitretin therapy (7). Results of the analysis of blood samples from these patients indicated that acitretin did not drastically prolong the elimination of the etretinate that was stored in the adipose tissue. The very slow elimination of etretinate in one of the subjects was attributed to his obesity.

However, some years later and contrary to all expectations, a peak co-eluting with etretinate was detected in high performance liquid chromatographic traces of plasma samples from acitretin-treated patients.

Additional spectroscopic analysis (UV, MS) unequivocally identified this peak as etretinate. Based on results of animal studies at Hoffmann-La Roche (Basle, Switzerland and Nutley, NJ, USA), it is now known that alcohol can play an important role in the synthesis of etretinate from acitretin (8, 9).

At our centre, 20 patients (17 males, 3 females; age range 20–65 years) were treated for at least 12 weeks with acitretin (30 mg/day, orally). Blood samples were taken on starting the therapy, during the treatment, and also after completion of the therapy. The samples were analysed by high performance liquid chromatography for acitretin, 13-*cis*-acitretin, as well as for etretinate (10). The method consists of purification of the injected extract on a precolumn followed by column-switching and gradient elution of a reversed phase column. A representative chromatogram is shown in Fig. 1. In none of the patients was etretinate present in the plasma when starting the acitretin therapy. However, in 10 of the 20 patients the extra etretinate peak was found after only a few days of therapy with acitretin. Moreover, in the same patients, acitretin and 13-*cis*-acitretin persisted in the plasma much longer than expected after the discontinuation of acitretin treatment. In 6 of the etretinate-positive patients, the levels of etretinate were ≤5 ng/ml. Two patients had levels around 10 ng/ml, while in the remaining 2 patients, levels up to 60 or even 100 ng/ml were found. The samples positive for etretinate on the column-switching HPLC system (10) were reanalysed on an adsorption system based on the isocratic elution of a 15 × 0.46 cm I.D. Chromspher Silica column with *n*-hexane-methylsalicylate-acetic acid (200:18:0.3, by vol) at a flow rate of 0.85 ml/min. The effluent was monitored at 360 nm. The results obtained on both HPLC systems were in close mutual correlation and again positively identified 'the peak' as etretinate, as in both systems it co-chromatographed with an etretinate standard.

In a more recent study the pharmacokinetic behaviour and the distribution of acitretin were evaluated in its target tissue itself, i.e. the epidermis, in healthy volunteers. 13-*Cis*- and *trans*-

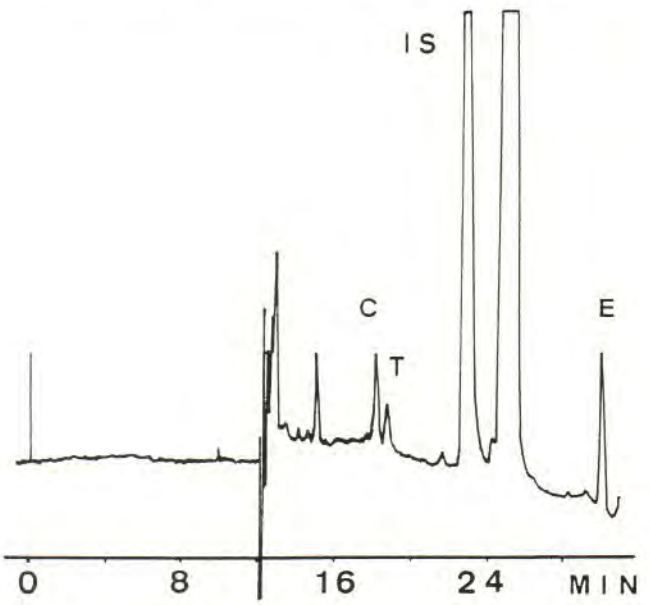

Fig. 1. Representative HPLC chromatogram on the column-switching system. Peak identification, C: 13-*cis*-acitretin; T: acitretin; IS: internal standard (13-*cis*-retinoic acid); E: etretinate. Levels were 8.1, 4.6 and 12.9 ng/ml for 13-*cis*-acitretin, acitretin, and etretinate, respectively.

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acitretin were measured by HPLC in plasma, blister fluid and epidermal samples. After multiple dosing (50 mg orally for 13 days) no accumulation of *trans*-acitretin was found in plasma or blister fluid. However, AUC values for *trans*-acitretin in the blister roof tended to be higher after multiple dosing (11). In an extension of this study to 12 psoriatic patients and to longer periods of treatment with acitretin (2 months to 3.5 years, 25 mg acitretin/day) plasma, skin biopsy material and subcutaneous fat were analysed. Trough levels of acitretin were consistently at the limit of quantification in both adipose tissue and skin, while the peak level was reached within 5 h of the intake of acitretin, suggesting a rapid penetration of the drug into skin and adipose tissue and indicating that neither skin nor adipose tissue functions as a storage compartment for acitretin. Here too, etretinate formation was demonstrated in the plasma of 2 patients who later admitted to being regular beer drinkers. In the same 2 patients, the etretinate concentrations in adipose tissue exceeded 1 μg/g wet weight, thus confirming the storage of etretinate in this tissue and illustrating again this unusual metabolic pathway of acitretin (12).

These results, together with data from other centres, have serious implications for acitretin management of patients. The recommended 2-year contraception period following etretinate therapy has now been extended to acitretin and leads to the loss of many of the earlier-described advantages of acitretin, compared with etretinate.

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ABSTRACT

Acitretin in Children

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Acitretin treatment offers the same clinical benefits to children as etretinate, namely effective, convenient oral therapy for severe dermatological conditions in which hyperkeratinization is the main feature. Its important advantage is the shorter period of potential side effects following completion of treatment. However, the possibility of bone disturbances means that their use in children should be restricted to severe forms resistant to the usual treatments. The best indications in children are severe forms of psoriasis, especially erythrodermic psoriasis and pustular psoriasis. Remission or marked improvement can be obtained in severe congenital disorders of keratinization: non-bullous ichthyosiform erythrodermia, bullous ichthyosiform erythrodermia and recessive x-linked ichthyosis. There are other anecdotal reports on foetal Harlequin Papillon Lefevre Syndrome... The spectrum of adverse events is typical of hypervitaminosis A and similar to that reported with the use of other retinoids. With regard to the very marked clinical benefit, acitretin can be prescribed for children, provided certain rules are observed: do not exceed an initial dose of 1 mg per kg per day and quickly reduce to the minimal effective dose, establish a protocol for detection of musculoskeletal complications with very careful surveillance of the child's growth parameters. The incidence of bone disturbances in acitretin treatment in children and the frequency of radiological surveillance is not yet well established. We have set up a protocol in which patients systematically undergo radiological work-up before and once a year during treatment; bone density is assessed by means of computerized tomography. In addition, every 6 months we measure phosphorus and calcium concentrations in blood and urine, together with those of vitamin D metabolism, osteocalcin and PTH. 14 children treated with acitretin have been studied so far. Interestingly we found anomalies in pretherapy values in 6 of the cases studied: 3 had a deficiency, and 3 had abnormally high 1–25 OH D values. So far, no changes in these parameters have been found during treatment. No radiological abnormalities were found during therapy, except in 1 patient who had evidence of demineralization before treatment. The absence of anomalies is no doubt related to the low doses of acitretin used: never exceeded 1 mg/kg/day.

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ABSTRACT

Acitretin – Clinical Effiacy and Side Effects

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Retinoids constitute important agents in the treatment of psoriasis and monogenic disorders of keratinization. Clinical efficay is impressive, whereas the side effects are acceptable and can be controlled as long as patients are supervised according to the guidelines.

A moderate to marked improvement is recorded in 70% of patients with chronic plaque psoriasis, when treated with acitretin (35–60 mg/day). In the case of a combined treatment with Acitretin plus PUVA or UVB, the improvement rate is enhanced substantially. In pustular and erythodermic variants, acitretin monotherapy is highly effective, the optimal dosages being 60 and 10–25 mg/day.

Various side effects during acitretin treatment may occur. Mucocutaneous side effects are the well known witness a sufficient bioavailability of acitretin. Hyperlipidaemia and increases in transaminases are common, but less frequent. Toxic hepatitis and formation of hyperostoses are recorded sporadically. Restrictive use of acitretin is indicated in females, in view of the teratogenicity of the drug.

Risk benefit evaluation of acitretin demonstrated that the drug is indicated in severe manifestations of psoriasis, especially in the erythrodermic and pustular variants. The choice between acitretin, methotrexate and cyclosporin should be made on an individual basis, reconciling the manifestation and the relative and absolute contra-indications.

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ABSTRACT

Acitretin: The Italian Experience

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Acitretin is a synthetic vitamin A analogue of the aromatic retinoid generation. The main acid derivative and metabolite of etretinate, the drug has been developed for the treatment of psoriasis and other diseases. Studies on the efficacy of Acitretin, compared with etretinate, carried out on over 700 psoriatic patients, showed a substantial improvement in around 70% of both groups of patients. 50 mg/day was the optimal dose. Combination therapy with PUVA or UVB was found to be of clinical value. Side effects were similar to those observed with etretinate. Acitretin is more rapidly eliminated from the body than is etretinate and it appears not to accumulate in human adipose tissue. Therefore its teratogenic potential can be limited to a shorter period following treatment withdrawal. However, etretinate traces have been detected in the plasma of some patients treated with acitretin.

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ABSTRACT

Acitretin and PUVA in Psoriasis

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Acitretin causes a rapid initial desquamation and thinning of psoriatic lesions. Occasionally, lesions may spread. Subsequent improvement is rather slow and complete clearing is seldom achieved if monotherapy alone is used. However, if acitretin is combined with PUVA therapy or other phototherapies, efficacy is improved and complete clearing is achieved in most patients, even in widespread severe cases.

In combination therapy, the initial acitretin dose is 40–50 mg/day. After 1–2 weeks' pretreatment with acitretin alone, simultaneous PUVA therapy is started and given 3 times weekly using normal or slightly reduced doses. The combination is continued until clearing, which is usually achieved in 6–8 weeks. Various modifications of this combination can be used, depending on the type and severity of the disease as well as on the therapeutic response.

The combination treatment is more effective than either treatment alone and the cumulative UVA dose needed can be reduced by 25–40%, depending on the mode of combination. This may reduce the risks associated with long-term PUVA therapy.

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ABSTRACT

Quantitation of Soluble Interleukin-2 Receptor (CD25) and Soluble CD27 in Serum from Psoriasis Patients during Cyclosporine A Treatment

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Psoriasis is a T-cell-mediated dermatosis which can be treated successfully with cyclosporin A (CsA). To date, no valuable parameter is available to evaluate disease activity or in-vivo T-cell activation in psoriasis patients. We therefore studied the serum levels of the soluble (sol) T-cell activation antigen CD25 (interleukin-2 receptor) and sol CD27. Sixteen psoriasis patients were treated for a period of 16 weeks with an optimal CsA dose (3–5 mg/kg/day). The mean PASI before treatment was 16.2, vs. 3.6 after CsA treatment. Serum samples were taken every 4 weeks. Serum sol CD25 levels (normal 268–620 U/ml) were elevated in 12/16 untreated patients and returned to normal following CsA treatment. The mean serum sol CD25 level before treatment was 784, vs. 603 U/ml (normal) within 4 weeks and 480 U/ml after 16 weeks of CsA treatment. The mean sol CD27 level (normal 112–217 U/ml) in all untreated patients was not increased (210 U/ml). However, 6/10 patients with severe psoriasis (i.e. PASI ≥16) showed elevated sol CD27 levels, which in 3 patients returned to normal values after CsA treatment. These findings indicate that sol CD25 – and to a lesser extent sol CD27 – can be used to monitor immunosuppressive treatment with CsA in psoriasis patients.

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ABSTRACT

T Cell Receptor Vβ Gene Expression in Psoriasis Vulgaris

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The identification of activated T cells in psoriatic skin lesions indicates that these cells are critical to the pathogenesis of psoriasis vulgaris. Using the polymerase chain reaction and Cβ-specific hybridization, we have analysed whether infiltrating lymphocytes – in comparison with peripheral blood – are characterized by a restricted set of T cell receptor Vβ genes. When we compared the relative expression of 20 Vβ gene families we found an overexpression of Vβ2, −6, −7, and −13.1 in the majority of biopsies and in paired blood lymphocytes that have been tested so far. Of these, Vβ6 was most prominent in skin biopsies, as compared with the degree of expression of the other Vβ genes. Although Vβ6 was also prominently expressed in blood T cells, it was usually paired with other overexpressed Vβ families.

Our preliminary results show a consistent pattern of highly expressed T cell receptor Vβ gene families in lesional skin and blood lymphocytes of psoriatic patients. The dominant overexpression of Vβ6 in psoriatic skin might be due to a specific local stimulation of particular T cell clones. Whether skin autoantigens or microbial products are the inducers of this stimulation remains to be determined.

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ABSTRACT

Enhanced Serological Reactivity of Psoriatic Patients with Keratinocyte Proteins Is Partly due to Crossreactivities with Streptococcal Antigens

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Serological cross-reactivities between streptococci and keratinocytes have been suggested to contribute to the pathogenesis of psoriasis vulgaris. Therefore we have analysed by immunoblotting the reactivity of sera from psoriatic patients (PP) and healthy individuals, using lysates of human keratinocytes (KC), EBV-transformed B-cell lines (BCL) and lysates of group A streptococci (ST), strains M1 and M12. When compared with the control group, sera from PP reacted more strongly with lysates of KC and ST. Here, a broad range of proteins of varying molecular size were detected. No additional reactivities could be induced by heat shock treatment (45°C, 2 h) of KC. Following preincubation of patient sera with sonicated ST, the intensity of several bands was decreased in the KC- and ST-, but not in BCL-lanes. Thus, cross-reactivities with ST antigens apparently contribute to the enhanced serological reactivity of PP with KC proteins. Although autoantibodies are most likely not involved in the generation of inflammatory psoriatic skin lesions, their presence suggests an enhanced cellular immune response to ST which, on the level of T lymphocytes, could very well be involved in the pathogenesis of psoriasis vulgaris. This work was supported by SFB 217 and Wilhelm-Sander-Stiftung, grant 92.032.1.

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Interlamellar Lipid Differences between Normal and Psoriatic Stratum Corneum

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Intercellular lipids of the stratum corneum are involved in permeability barrier integrity and function. In psoriasis, desquamation and permeability barrier homeostasis are modified; these observations are consistent with an alteration in stratum corneum lipid production. Therefore, in the present study, we determined and compared the total content of the three main intercellular lipids in psoriatic scales and normal human stratum corneum. Our results showed that the relative free fatty acid content decreased remarkably (46%) in psoriatic scales, compared with normal human stratum corneum. This decrease may reflect a general state of emergency of keratinocytes, in which free fatty acids can be employed. ***Key words: pemeability barrier; linoleic acid; abnormal desquamation.***

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The functional structure of stratum corneum is appropriately explained by the 'two compartment model' devised by Elias (1). According to this hypothesis, corneocytes are stacked together by intercellular lipids to form membranous bilayers. Both the cellular and the lipid components are essential to maintain such a protective and permeable barrier.

Free fatty acids (FFA), cholesterol (CHOL) and ceramides (CER), the three main species of intercellular lipids, are implicated in horny layer homeostasis. In psoriasis, the increase in keratinocyte proliferation and the incomplete process of differentiation lead to the formation of a highly deranged horny layer and, as a consequence, permeability functions are impaired, causing an increase in water flux and absorption (2). These observations are consistent with an alteration in stratum corneum lipid production. Up to now, few data are available on psoriatic skin lipids: together with the abnormal formation of the protein envelope (3), an elevated level of bound linoleic acid, associated with alterations in the covalently-bound ceramides have been described by Wertz et al. (4). In a previous work (5) we observed that in psoriatic scales the relative total content of ceramides, the main sphingolipid class present in the stratum corneum, is the same as in normal human stratum corneum.

These data are largely insufficient to justify the impairment in psoriatic barrier homeostasis. Therefore, in the present study, we determined the total content of the three main intercellular lipids of stratum corneum, FFA, CHOL and CER, in psoriatic scale[3], and compared the results with normal human stratum corneum.

MATERIAL AND METHODS

Abdomen skin sample of 6 normal subjects of both sexes were obtained from plastic surgery and processed according to Elias (6). Psoriatic scale samples were obtained from lesional areas of 10 psoriatic subjects, all males. EDTA and trypsin-obtained normal stratum corneum sheets (6) and psoriatic scales were minced and lipids were extracted by the Bligh – Dyer method (7). All separations were carried out using thin-layer plates (10 × 20 HPTLC, Merck), based on a previous report (8), with slight modifications. Pure lipid standards: free cholesterol, cholesteryl oleate, palmitic acid tripalmitin, 1-eicosene, Type III and Type IV ceramide, purchased from Sigma (St. Louis, Mo.) were applied (10 μg each) in parallel for identification and calibration purposes. After elution, the plates were air-dried, sprayed and charred on a 180°C hotplate (9). Quantification of different lipid classes (ceramides, cholesterol and free fatty acids) was done by densitometry, using a Camag TLC Densitometer equipped with a computerised image analyser (10).

RESULTS

The results, expressed in μmoles/mg total lipids of each lipid fraction, are shown in Fig. 1. The mole ratio among FFA/CHOL/CER in normal human stratum corneum was 4.1/1.3/1; in psoriatic scales, 2.2/1.3/1.

The main result was the remarkable decrease (46%) in FFA ratio vs. other lipid fractions of the psoriatic scales vs. normal stratum corneum.

DISCUSSION

Many earlier studies on the stratum corneum lipids indicated their role in maintaining permeability barrier homeostasis (11, 12) and in regulating lamellar stacking and desquamation (13, 14). However, studies on lipids of normal human stratum corneum are few and not particularly comparable because of the different extraction methods and site of harvesting. The FFA/CHOL/CER ratio of normal stratum corneum may be considered in the range of literature data shown in Table I.

Recently, Elias and co-workers, using lipid synthesis inhibitor

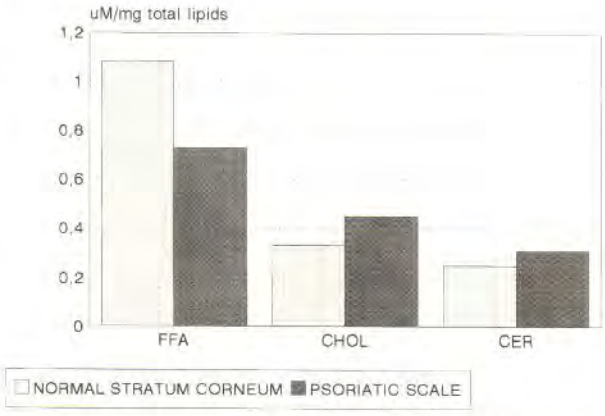

Fig. 1. Content of FFA, CHOL and CER in normal stratum corneum and psoriatic scales.

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Table I. *Literature review and new data on quantifiation of interlamellar lipid species in normal human stratum corneum and psoriatic scales*

Reference	Origin	FFA/CHOL/CER
Elias P.M. J. Lipid Res. (1983) 24, 131–140	Abdomen	2.9/1.5/1
Melnik B. et al. J. Invest. Dermatol. (1989) 92, 231–234	Plantar	1.5/1.6/1
Melnik et al. Arch. Dermatol. Res. (1990) 282, 549–551	Plantar Lumbar Nail	1.5/1.6/1 4.8/0.6/1 8.5/3.1/1
Melnik et al. J. Invest. Dermatol. (1991) 96, 959–962	Lumbar	10/0.8/1
Our data 1993	Abdomen NSC Abdomen PS	4.1/1.3/1 2.2/1.3/1

Data are expressed as a molar ratio, FFA/CHOL/CER.

of each species, demonstrated the importance of each single class of lipids for barrier homeostasis (15). If the stratum corneum lipids are synthesized in a functionally determined ratio, in psoriatic scales, in which both permeability and desquamation are altered, this ratio should be modified, too.

Knowledge of psoriatic scale lipids is scanty. Wertz and co-workers investigated the normal human stratum corneum covalently bound lipids and demonstrated a similar total concentration in psoriatic scales, but a different proportion of the individual lipid (4).

We observed, in a previous work on stratum corneum ceramides, that the relative content of this class of total lipids was the same in psoriatic scales as in normal human stratum corneum. However, the species isolated from psoriatic scales displayed a different pattern of ceramide distribution, particularly a decrease in ceramide 1, a complex species in which an additional ester linkage with linoleic acid is present (5).

In the present study, in order to extend our knowledge in this field, we investigated the content of the three main classes of interlamellar lipids in psoriatic scales, compared with normal stratum corneum. Our results showed that relative FFA content decreased remarkably (46%) in psoriatic scales, compared with normal human stratum corneum.

The decrease in free fatty acids in psoriatic scales may reflect a general state of emergency of keratinocytes in the disease, in fact the rapid turnover of these cells may also involves energy-consuming processes, in which free fatty acids can be directly or indirectly employed.

The previous evidence of an accumulation of covalently bound linoleic acid in psoriatic scales (4) appears to agree closely with our results. Moreover, preliminary data from our laboratory seem to indicate a tendency to increase triglycerides and sterol-esters.

In conclusion, a shift from the 'free' to the 'bound' form of fatty acids (as in covalently bound lipids, triglycerides and sterol-esters) appears to be a distinctive biochemical feature of psoriasis. Further investigation of free fatty acid species, absent or depleted in psoriasis, will help to shed light on the biochemical effect present in psoriasis at this level.

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The Pseudo-elongation of Capillaries in Psoriatic Plaques

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The intrapapillary vessels in the psoriatic plaque are described as elongated, twisted and multiplied. However there is neither proliferation nor necrosis of vessels in growing and dissolving psoriatic plaques. In 120 patients suffering from psoriasis vulgaris, computer-supported image analysis and in 8 patients additionally 3D reconstructions were made to investigate the regression process of the intrapapillary capillaries in the active and resolving psoriatic plaque. In acanthotic epidermis with a thickness of >400 μm the first subpapillary horizontally oriented plexus is included in the papilla due to the down-growing of the epidermal rete pegs. In the evaluation of the computer-supported image analysis there is only little variation in the levels of the different vascular plexuses within the dermis, while the epidermis is decreasing from >600 μm to >100 μm. In the 3D reconstruction of the transition of a psoriatic lesion into adjacent non-involved skin it could be proved that, apart from the epidermal alterations, there is virtually no difference in the arrangement of the vessels between the psoriatic lesion and the adjacent non-involved skin. In psoriasis the vessels do not proliferate, they rest as resident structures and are embraced by the down-growing rete pegs.

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So far, the function of the vascular system in the pathogenesis of psoriasis has remained speculative. The intrapapillary vessels in the psoriatic plaque seem elongated, twisted and multiplied (1, 2, 3, 4). This elongation and increased density of the capillaries could only be explained assuming blood vessel growth (2, 3, 5, 6). However, there are factors contradicting angiogenesis in psoriasis: first of all, no increase in endothelial cell proliferation in psoriatic lesions could be demonstrated; secondly, no decay products accumulate in the regression phase of a psoriatic plaque (6).

There is thus a discrepancy between the histological aspect of elongated multipled capillaries in the papillary region of a psoriatic lesion and the lack of proliferative processes of the vascular endothelia.

The subepidermal capillaries have to be viewed at their connection with the deeper situated vessels of the dermis. The dermal vessels are classified into several segments: from ascending arterioles the terminal arterioles, develop by arborizing within a superficial, horizontal plexus and from these arise the intrapapillary capillary loops. The venous limbs of the capillary venules evolve into postcapillary venules which form the venous network of the superficial vascular plexus. This plexus is connected to the subcutaneous venous system by the descending collecting venule (2, 7, 8, 9).

Using computer-supported three-dimensional reconstruction (10) and image analysis (11) we investigated the regression process of the intrapapillary capillaries in the active and resolving psoriatic plaque.

MATERIALS AND METHODS

Patients and biopsies

Punch biopsies were obtained from fully developed and resolving lesions of 120 patients with psoriasis vulgaris, in 10 cases additionally from adjacent, clinically normal skin. Table 1 shows the patient material and gives information about the biopsy sites.

Biopsies were fixed in formalin 5% for 12 h and embedded in paraffin wax. From 7 patients part of the tissue was also fixed in glutaraldehyde 3.5% and embedded in Epon 811.

Computer-supported image analytic evaluation (program analySIS®)

All 120 paraffin-embedded specimens were assessed by means of image analysis. From each biopsy at least 10 serial sections were obtained. Measurements were performed at 2 different locations on the 7 μm thick paraffin sections, 6 distances were measured each.

In this study we evaluated the following parameters (Fig. 1).

Parameters measured

epmax = epidermal thickness from the bottom of the rete pegs to the top of the granular layer
epmin = epidermal thickness from the top of the papillae to the top of the granular layer
pap = distance between basement membrane beneath epmin and the loop of the intrapapillary capillary
pl 1 = distance between the basement membrane below epmax and the first subpapillary, horizontally oriented plexus
pl 2 = distance between the basement membrane below epmax and the second subpapillary, horizontally oriented vascular plexus
pl 3 = distance between the basement membrane below epmax and the third horizontally oriented vascular plexus in the deep dermis

Table I. *Patients and biopsy sites assessed by means of computer-supported image analytic evaluation, program analySIS®*

Sex	*n*	forearm	upper arm	lower leg	thigh	back	abdomen	head	hand	armpit
Males	74	32	16	9	2	15	2	3	1	2
Females	46	16	10	7	1	12	2	1	1	0

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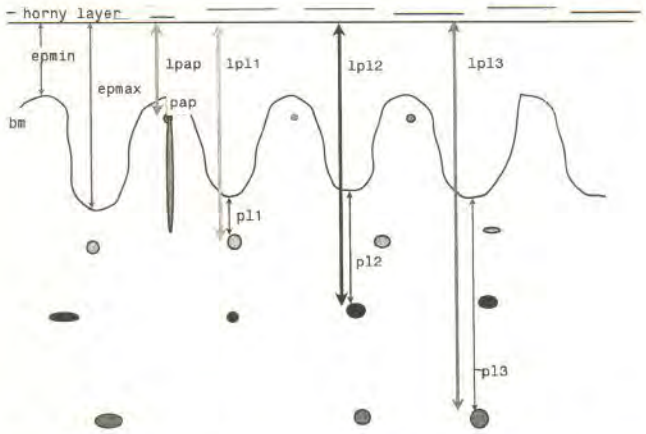

Fig. 1. Measured and calculated parameters evaluated by image analysis.

Calculated parameters

lpl 1 = epmax + pl 1 = level of pl 1 in the dermis (distance of pl 1 to skin surface)
lpl 2 = epmax + pl 2 = level of pl 2 in the dermis (distance of pl 2 to skin surface)
lpl 3 = epmax + pl 3 = level of pl 3 in the dermis (distance of pl 3 to skin surface)
lpap = epmin + pap = level of pap in the dermis (distance of the intrapapillary capillary loop to skin surface).

Specimens were evaluated at magnification of ×40 and ×250 under a Laboval (Zeiss) light microscope equipped with a CD-camera connected to an IP-8/AT matrox frame grabber board within an IBM-compatible 486 PC/AT. We used the image analysis program analySIS®, Soft-Imaging Software GmbH, Münster, Germany. The above-mentioned parameters (e.g. epmax, epmin, etc) were assessed by manual outlining of the structures of interest (using the mouse) on the digitalized microscopic image displayed on the monitor. Calibration of the video input channel in analySIS® allowed us to measure all distances in their actual length in micrometres.

To determine the exact location of the horizontally running vascular plexuses, the vessels were followed up over the whole length of the specimens; frequently, additional controls in adjacent serial sections were necessary (7).

In each specimen, too different measurements of each distance were obtained.

The measured values were further processed and displayed in graphs using the program Excel and MS-Windows®.

All measured values were sorted with regard to eprnax in descending order. Groups were formed with epmax ≥ 600 μm (group 1), ≥ 500 μm (group 2), ≥ 400 μm (group 3), ≥ 300 μm (group 4), ≥ 200 μm (group 5) and ≥ 100 μm (group 6).

For every group the mean value and standard deviation (δ) of all parameters were determined.

The mean values of the parameters of each group are presented in line diagrams.

Table II. *Patients sorted with regard to epmax*

group 1 = epmax ≥ 600 μm
group 2 = epmax ≥ 500 μm
group 3 = epmax ≥ 400 μm
group 4 = epmax ≥ 300 μm
group 5 = epmax ≥ 200 μm
group 6 = epmax ≥ 100 μm

Group	epmax	n	m	f
1	> = 600	4	2	2
2	> = 500	5	3	2
3	> = 400	18	12	6
4	> = 300	28	16	12
5	> = 200	34	23	11
6	> = 100	31	18	13

Computer-supported 3-dimensional reconstruction

Using the program 3D SIS, we 3-dimensionally reconstructed in 8 patients the vessels in the fully developed and resolving psoriatic plaques by evaluation of serial sections. Apart from the vessels, the stratum corneum and the basement membrane were also demonstrated.

In 1 patient we reconstructed the subepidermal vessels and the epidermis at the border of a psoriatic plaque, using serially cut 7 μm paraffin sections (transition of lesional skin into adjacent, clinically normal skin).

Results

Image analytic evaluation

In the fully developed psoriatic plaques the mean values of epmax range from 646 μm (δ = 27, 22) (group 1) to 437 μm (δ = 20) (group 3). During healing, epmax decreases to 150 μm (δ 28). Epmin shows, independent of epmax, a constant value of about 70 μm (Fig. 2). Table II gives information about biopsy-sites in the different groups.

Lpl 1, lpl 2 and lpl 3 are reduced with decreasing epmax (Fig. 3). During healing of the psoriatic plaque pl 1, pl 2 and pl 3 change levels in the dermis:

When epmax is higher than 500 μm, lpl 1 is on average 800 μm, which means that the first subpapillary, horizontally ori-

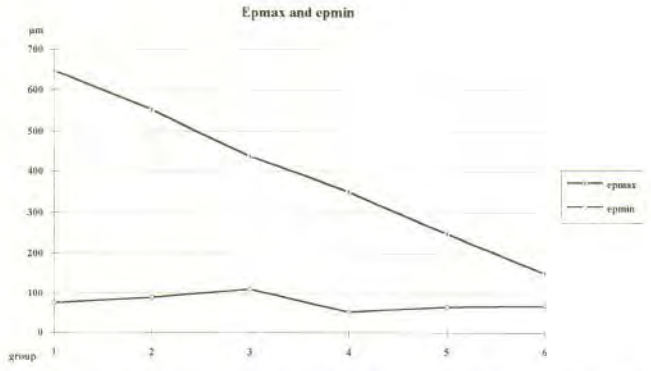

Fig. 2. Values of epmax and epmin in the different groups of patients (Table II).

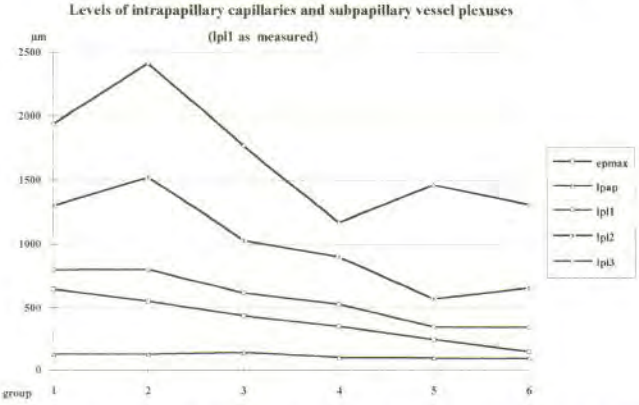

Fig. 3. Values of lpap, epmax, lpl 1, lpl 2, and lpl 3 (as measured), in the different groups (Table II).

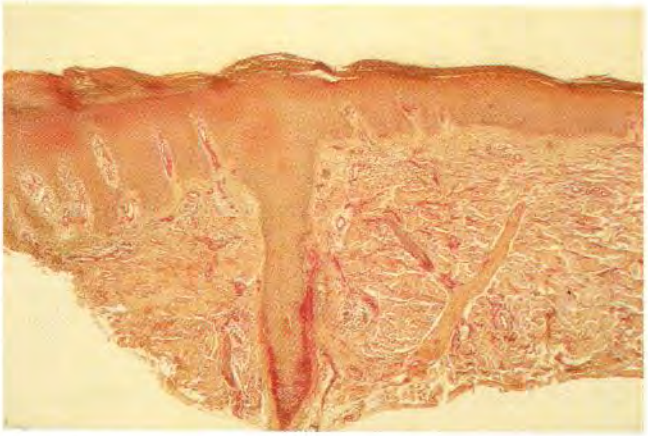

Fig. 4. Paraffin section showing the transition of a psoriatic plaque (*left*) into adjacent, clinically normal skin (*right*). lpl 1 and lpl 2 cf. Fig. 1.

ented plexus lies 800 μm below the skin surface. When epmax is less than 400 μm, at approximately this distance (900 μm from the skin surface) the second subpapillary plexus is located.

In the line-diagram in Fig. 3, the depth of the dermis is represented on the *y*-axis in 500 μm steps. Epmax, lpap, lpl 1, lpl 2 and lpl 3 are displayed as lines. From group 1 to group 6, lpap decreases slightly.

It is obvious that in groups 1, 2 and 3 there are no subpapillary vessels with a distance shorter than 500 μm to the skin surface. In groups 5 and 6, where the values of epmax drop below 300 μm, lpl 1 adopts values of less than 500 μm. Parallel lpl 2 reaches the values that lpl 1 occupied at an epmax higher than 400 μm, and lpl 3 those previously occupied by lpl 2.

Fig. 4 shows the transition of a psoriatic plaque into adjacent, clinically normal skin. Lpl 1 and lpl 2, as measured by image analysis, are displayed as lines.

Comparison of the two sides reveals that the vessels of the first subpapillary, horizontally oriented plexus on the right (healthy skin) lie inside the dermal papilla in the psoriatic lesion, with an epmax of 500 μm on the left.

Therefore this – in normal skin extrapapillary vessel is regarded as belonging to the ascending capillary of the dermal papilla in psoriatic skin. We can say that with increasing epmax

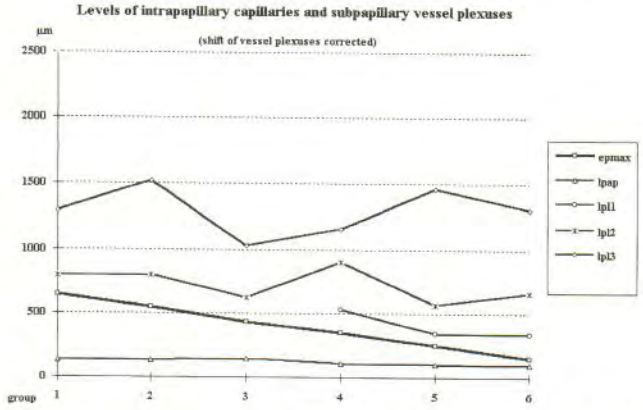

Fig. 5. Values of epmax, lpap, lpl 1, lpl 2 and lpl 3 with corrected shift of the different plexus levels. The values of lpl 1 are attributed to lpl 2 at epmax ≥ 400 μm; that of lpl 2 to lpl 3.

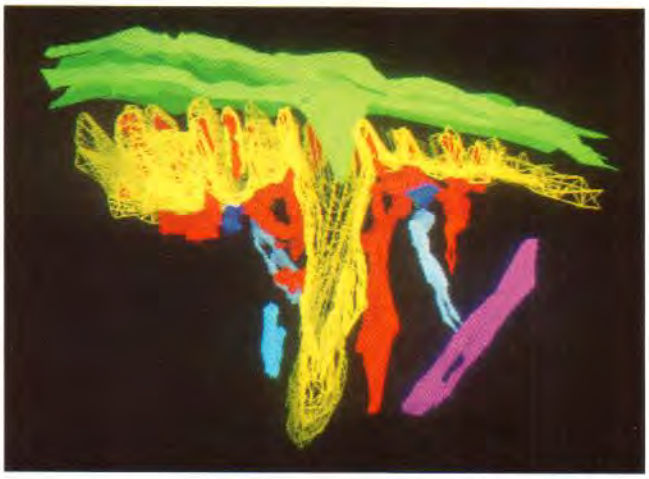

Fig. 6. 3D reconstruction of the transition of a psoriatic lesion (*left*) into adjacent, non involved skin (*right*).
green: horny layer
yellow: basement membrane
red: vessels
violet: smooth muscle.

there is an elongation of the intra-papillary capillaries due to the inclusion of the first subpapillary plexus in the dermal papillae.

As by definition, we call the distance to the uppermost recognizable subpapillary plexus pl 1, we erroneously consider in lesional skin (Fig. 4, left side) the plexus as first subpapillary plexus which corresponds to the second subpapillary vascular plexus in normal skin (Fig. 4, right side, margin of the lesion). When in psoriatic skin epmax increases above lpl 1 in non-involved skin, the designations of pl 1 and pl 2 differ from the correct plexus names by one number.

After correction of this shift we realize that lpl 1 is missing above an eprnax ≥ 400 μm (Fig. 5). The values of lpl 1 are attributed to lpl 2 at epmax ≥ 400 μm and the ones of lpl 2 to lpl 3 respectively. Fig. 5 shows that after this correction there is little variation in the levels of vascular plexuses within the dermis.

Computer-supported 3 dimensional reconstruction

The results obtained by 3-dimensional reconstruction of serial sections confirm that the upper horizontal vascular plexus dis-

Fig. 7. The same 3D reconstruction as in Fig. 6, omitting the epidermal basement membrane.

appears due to inclusion in the dermal papillae by the acanthotic epidermis of the psoriatic plaque.

Fig. 6 shows the 3-dimensional architecture of the transition of a psoriatic lesion into adjacent, non-involved skin (the same situation is presented 2-dimensionally in Fig. 4). In the non-involved skin on the right, the horizontal cross-connections of the subpapillary plexus are located underneath the epidermis; in the psoriatic plaque on the left, they disappear in the basement membrane, displayed in yellow. The same situation, omitting the basement membrane is displayed in Fig. 7: neglecting the epidermal component there is virtually no difference in the arrangement of the vessels. Only the increasing diameters of the vascular lumina, corresponding to the dilatation of capillaries, indicate the location of the psoriatic lesion.

DISCUSSION

The subepidermal, intrapapillary capillaries in the psoriatic plaque are only seemingly elongated. This elongation is not – as previously assumed – due to a proliferation of vessels by angiogenesis (3, 5, 6), but has its reason in the inclusion of the horizontal venous plexus into the dermal papilla, normally located below the rete pegs.

The downgrowing epidermal rete pegs embrace the existing vascular structures and integrate the originally subpapillary vessels of the superficial plexus into the thus elongated papillae. In the psoriatic plaque therefore not only the intrapapillary capillaries but also the first and in extensive acanthosis even the second horizontally running vascular plexus lie within the dermal papillae. In the resolving plaque, once the epidermal thickness is reduced below 400 μm, the horizontal plexuses are again located beneath the papillae.

Braverman and Yen (5) classify the capillary loops of normal forearm skin into two segments: an intrapapillary and an extrapapillary portion. An imaginary line drawn between the deepest points of adjacent rete pegs defines these two zones. While the intrapapillary part shows the ultrastructural characteristics of an arterial capillary, after leaving the papilla, it abruptly displays venous characteristics, showing bridged fenestrations and a multilayered basement membrane.

In the psoriatic plaque, however, Braverman et al. (2, 5, 12) found that the intrapapillary capillary loops were predominantly venous capillaries, leading them to the conclusion that there is increased proliferation of the venous part of the capillaries.

Our reconstructive and histometric results indicate, however, that the intrapapillary venous capillaries described by Braverman and Yen are actually vessels of the subepidermal vascular plexus (postcapillary venules).

When the acanthosis (epmax) increases above 400 μm, the first subpapillary, horizontally oriented vascular plexus is located higher than the deepest point of the subepidermal basement membrane zone. Therefore we erroneously interpreted the vascular layer which in healthy skin is called the second vascular plexus as the first subpapillary vascular plexus in psoriatic lesions (see Figs. 3 and 4). The 3-dimensional reconstruction (see Fig. 6) confirms that the upper horizontal vascular plexus disappears in the papillary region. When we look at the microvasculature disregarding the dermo-epidermal junction (Fig. 7), there are almost no differences between involved and normal skin, i.e. a real elongation of the papillary capillaries did not take place. As the upper vascular plexus, thus integrated into the elongated papillary region, consists mainly of postcapillary venules (7, 9), the proportion of the arterial and venous limbs of the intrapapillary vessels is shifted towards the venous part.

Interestingly, in another paper Braverman states that "following three weeks of Goeckerman therapy, the morphology of psoriatic capillary loops changed from venous capillaries to arterial capillaries which are found in the papillae of normal skin" (12). Five years later together with Sibley (6) he found that this 'transformation' of venous in arterial limbs of capillaries correlates with the labelling index of the basal keratinocytes. He concluded that epidermal hyperplasia requires vascular proliferation. According to Braverman, epidermal hyperplasia causes an increase in papillary volume and height and with increasing papillary height the intrapapillary capillary loops elongate (2).

Our histometric investigations confirm the correlation between epidermal hyperplasia and papillary height. The acanthosis in the psoriatic plaque is caused exclusively by hyperplasia of the rete pegs, there is no increased thickness of the epidermal part overlaying the papillae. As epmin is relatively constant (Fig. 2), the papillary height, being the difference of epmax and epmin, is directly related to epmax. The length of the intrapapillary capillaries thus depends on epmax as well. When epmax is reduced below 400 μm, accompanied by a corresponding decrease in papillary height, the first subpapillary vascular plexus appears (Fig. 4).

The integration of the previously subpapillary vascular plexus into the papillary region also explains the low labelling index of the endothelia of psoriatic lesions after incubation with tritiated thymidine (6). As there is no proliferation of vessels in the psoriatic plaques, there is also no need for them to decompose in resolving lesions. Indeed, no necrotic endothelia could be demonstrated during healing (6): "We never observed necrosis of endothelial cells in the intrapapillary loops or a spotty return to normal within these loops".

In this connection the observations of Ryan by means of epiluminescence microscopy are interesting (13), describing the association of hyperplastic or hypertrophic papillary vessels and epidermal hypertrophy. He states that in atrophic epidermis the intrapapillary vessels appear atrophic as well, in normal skin they are normal and in epidermal hyperplasia, coiling occurs. In clinically psoriasiform appearing skin lesions, he invariably found coiled capillaries; on the other hand the psoriasiform aspect could never be seen when coiled capillaries were lacking (14). Whether there is altered density of capillaries in psoriatic plaques is not yet clear.

While some authors favour an increased capillary density (4), others speak of convolute formation. Based on observations by epiluminescence, Schlosser and Pullmann (4) reported in initial poriasis compared with normal skin an actual increase in capillary density caused by angiogenesis. Comparing initial with fully developed psoriatic lesions, however, the authors felt that the further increase in capillaries was due to convolute formation of the capillaries, though they could not definitely exclude angiogenesis.

Barton et al. (1) compared capillaries microscopically in 20

psoriasis patients and 10 healthy control subjects. They found a successive increase in the number of capillary profiles from normal skin to non-involved skin at the margin between psoriatic plaques and lesional psoriatic skin. The authors concluded that there is increased vascular material as well as dilatation of vessels in psoriatic skin. It is not clear, however, whether they evaluated the complete biopsies or a defined area of the dermis.

Our findings inevitably lead to the question which has been discussed for decades: whether psoriasis starts from the epidermis or from the vasculature. What hyperplasia is concerned we can answer this question: the vessels do not proliferate, at least not the same extent as the epidermis. What the role of the endothelial cells is concerned with, regarding their activation and their immunologic part in psoriasis (15, 16), no conclusion can be drawn from our histological and histometric results or from the 3-dimensional reconstructions.

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Cutaneous Microcirculation in Psoriasis

A videocapillaroscopic morphofunctional study

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The capillaroscopic picture of psoriasis has been widely described in literature (1–8). Clinical alterations in the microcirculation of psoriatic plaques have been attributed prognostic significance (9), although the exact nature of the capillary damage remains uncertain (10). The latest capillaroscopic investigations of microcirculation functioning have shown that there is increased blood flow in the capillaries of psoriatic plaques and uninvolved skin, compared with normal skin (11). Ultrastructural examination revealed that the capillary wall takes on venous characteristics beyond the limits of the norm and endothelial volume is greatly increased in psoriatic lesions. Significant differences were also found between values for control subjects and those of both involved and uninvolved psoriatic skin for luminal volume (7–9, 12). Therefore, in this type of dermatitis, microcirculatory damage is characterized by vasodilatation with elements of dystrophy, an incrase in the number of capillaries per square millimetre and increased blood flow in the already dilated capillary loops.

The aim of the present study was to ascertain and evaluate, by means of computerized video-capillaroscopy, a non-invasive method we described at the 6th World Conference on Medical Informatics, Medinfo 89, Singapore, 1989 (13): the possible morphofunctional alterations induced by sensitized stasis test (modified Lunedei's test) in the cutaneous capillaries of psoriatic patients.

A group of 20 patients suffering from plaque-form psoriasis without onychopathy entered in the study (8 men and 12 women, mean age 49 years and PASI rating of more of 18). For control purposes, we utilized a group of 5 non-psoriatic subjects (3 men and 2 women, mean age 45 years). The two groups of subjects underwent a sensitized stasis test which consists in enforcing for 3 min, by a sphygmomanometer applied on an arm, a pressure of 5 Hgmm over the systolic pressure (SP) if this is less than 120 Hgmm, or a pressure of 10 Hgmm higher than SP in the case of SP greater than 120 Hgmm. Following these 3 min of complete cessation of blood flow, we made a reduced compression, for 5 min in any case, of 10 Hgmm in relation to SP. For every patient, capillaroscopic observations were made of a psoriatic plaque on a forearm, of the apparently healthy perilesional skin and of the nail fold of the fingers, before the test, at the end of the highest pressure occlusion, at the end of lowest pressure occlusion and 10, 20 and 30 min after removing the occlusion. Each subject stayed for 30 min in the laboratory before the sensitized stasis test and the capillaroscopic observation began. The study area was covered with oil of cedar wood to clarify the microscopic field.

At the end of the test, the control group presented very few haemorrhages and a moderate dilation of microvessels which disappeared after 20 min.

The uninvolved skin of psoriatic patients has shown an increase in the calibre of capillaries and some haemorrhages beaker-like observable after more 30 min.

The microvessels of uninvolved nail fold presented a slight dilatation with very few heamorrhages.

On the psoriatic plaque, we observed the greatest increase in vasodilatation with tortuosities, volutes and numerous haemorrhages. At the end of the compression, the visible capillaries were at least four times more numerous than before the test, and the colour of the field was darker, demonstrating a sufference of the subpapillary plexus. Afterwards the darkness of the field and the number of capillaries decreased; 30 min after the test the saturation of field colour was quite similar to baseline. Moreover, the number of pervious capillaries increased in calibre and with many beaker-like haemorrhages, was reduced by 50% in comparison with the capillaroscopic pattern at the end of stasis.

We maintain that the morphofunctional damage to the cutaneous microcirculation observed in this research could be an expression of endothelial alterations to the capillaries in psoriasis, not only in involved but also in apparently healthy skin.

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Psoriasis and Endothelins

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Background: Psoriasis is characterized by an abnormal proliferation and increased turnover of keratinocytes, the presence of acute and chronic inflammatory cells and microangiopathic changes. Endothelins are a family of peptides which have been investigated especially for their effects on the cardiovascular system. Recent studies have demonstrated their involvement also in human skin.

Aim of the study: We evaluated the Endothelin-1 and 2 plasma levels in psoriatic patients, as endothelin-1 can be produced in vitro by keratinocytes and can stimulate the proliferation of fibroblasts as well as modify the skin microcirculation dynamics.

Patients and methods: We studied 30 patients: 10 affected with psoriasis (PASI from 5 to 10), 10 affected with cardiovascular diseases and 10 healthy controls. The Endothelin-1 and 2 plasma levels were evaluated by radio-immunoassay procedure.

Results: A significant increase in Endothelin-1 and 2 plasma levels was observed in the psoriatic patients, in comparison with the controls.

Conclusions: Our data seem to suggest a possible relationship between psoriasis and increased plasma level of endothelin-1 and 2, though the possible role played in the pathogenesis of psoriasis needs further studies. ***Key words: psoriasis, endothelins.***

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The endothelins (ETs) are a family of recently discovered 21 aminoacid peptides (1). In Man, ETs occur in the isoform ET-1, ET-2 and ET-3, after a proteolytic processing of the specific preproendothelin (1). Yohn et al. (2) have recently found that human keratinocytes are able to synthesize and secrete ET-1 in vitro. Macrophages (3), monocytes (3) and astrocytes (4) can also secrete ET-1. Many human cancer cell lines, including those from human colon and liver, can produce ETs (5). ET-1 is the most powerful vasconstrictor substance known.

These properties suggest that ET plasma levels could vary in psoriasis, a pathology characterized by abnormal proliferation and increased turnover of keratinocytes, the presence of acute and chronic inflammatory cells and microangiophatic changes. In the present study we evaluated ET-1 and 2 plasma levels in 10 psoriatic patients and in two control groups.

PATIENTS, MATERIALS AND METHODS

Ten patients (5 males and 5 females, range: 38–77 years mean age: 60.9 years) affected with psoriasis (PASI from 5 to 10) were compared with two control groups: 10 patients affected with cardiovascular diseases (hypertension or ischaemic cardiopathy) and 10 healthy subjects comparable for age and sex.

A blood sample was taken from each of them, between 7.30 and 9.30 a.m., from the antecubital vein without blood stasis, after the subject had rested supine for 10 min.

The blood samples, collected with a 19 G neddle and mixed in Vacutainer tubes with 7.5 mM ETDA (13:1), were immediately placed in melting ice. The samples were centrifuged within 1 hour at 2,000 *g* for 10 min in a refrigerated centrifuge (4°C). Plasma aliquots were frozen at −70°C until assay.

An extraction procedure of ETs from plasma was performed before analysis. After thawing, 1 ml plasma was acidified using 0.25 ml 2 M HCl and centrifuged at 10,000 *g* for 5 min at room temperature.

1 ml of surnatant was charged on an Amersham C2 column (500 mg) (Amersham, Bucks, England) previously conditioned by washing with 2 ml methanol followed by 2 ml water. The column was rinsed with 5 ml water + 0.1% trifluoroacetic acid. The eluate was collected after washing the column with 2 ml 80% acetonitrile in water + 0.1% trifluoroacetic acid in a polypropylene tube. All the washing procedures were performed with a VACELUT vacuum system (Analitichem International) maintaining the flow rate below 5 ml/min. The eluate was dried under vacuum and reconstitued in 250 µl of assay buffer for R.I.A. ET-1 and 2 (high sensitivity) assay system with magnetic separator (Amersham).

STATISTICAL METHODS

The Wilcoxon test was employed to compare the three groups.

RESULTS

Psoriatic patients showed a significant increase in ET-1 and 2 plasma concentration (6.4 + 1.9 pg/ml) in comparison with both the patients with cardiovascular diseases and the normal subjects ($p = 0.0274$ v. cardiovascular patients; $p = 0.0357$ v. normal subjects) (Fig. 1). The two control groups did not show any significant difference when compared with each other (4.8 + 0.8 pg/ml v. 4.7 + 1.3 pg/ml, $p = 0.8336$).

DISCUSSION

A marked vascular and lymphatic dilatation and increased blood flow (twice the normal) appeared at the site of the psoriatic lesions (6, 7). The capillary loops of the lesional dermal papillae were dilated and tortuous (8). These microcirculation changes were present before psoriatic skin lesions appeared (7, 9).

Endothelins (ETs) were isolated from supernatant of cultured porcine aortic endothelial cells by Yanagisawa et al. (10) in 1988. They act via cell surface receptors, whose binding is rapid, specific and saturable (11).

Hitherto, ETs are the most powerful vasoconstrictor substances known. ET-1 induces strong and long-lasting constrictor effects in microvessels. It also causes bronchoconstriction (12), inhibits renin release from juxtaglomerular cells (13, 14), modulates autonomic transmission (15) and exerts positive inotropic and chronotropic effects on the myocardium (16, 17, 18, 19). By contrast, the activities of ET-2 and ET-3 have not hitherto been well defined.

ETs are mitogenic for vascular smooth muscle cells (20), fibroblasts (21) and renal mesangial cells (13, 22). Specific

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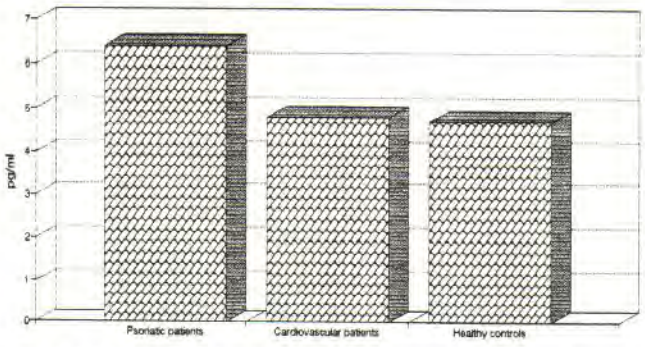

Fig. 1. ET-1,2 plasma levels.

high-affinity receptors for ET-1 have been identified in melanocytes and in fact ET-1 stimulates melanocyte proliferation and tyrosinase activity (2).

5 pmol/l of ET-1 has been determined in human plasma (23), thus suggesting that there is a basic physiological secretion. Numerous agents and several mechanical stimuli enhance *de novo* synthesis of ET-1. Many investigators have reported that thrombin, transforming growth factor β (TGβ), angiotensin II, ephineprine, arginine vasopressin, bradykinin, interleukin 1, calcium ionophore A 23187, inomycin, phorbol esters, and hypoxia all increase the expression of the preproendothelin gene or the ET-1 release (1, 10, 24).

ET-1 is clearly implicated in Raynaud's phenomenon; in this pathology an increase in the plasma ET-1 levels is observed and the cold challenge of an arm leads to a pronounced ipsilateral increase in plasma ET-1 (25).

In this paper we have described a significant increase in plasma levels of ET-1 and 2 in psoriatic patients, compared with two control groups. In our opinion these data are quite unexpected in view of the known vasoconstrictor properties of ETs and could perhaps be considered an epiphenomenon of the alterations observed in psoriasis. Moreover, we recall that some authors emphasize that ETs have a dose-related action on vessel resistance: little vasodilator effect at low doses and marked vasoconstriction at higher doses (19).

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Fcγ-Receptors in Skin and Serum from Patients with Psoriasis, Before and After Therapy

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IgG-Fc receptors (FcR) are present on most immune competent cells. We have examined FcR in skin lesions from 8 patients with stationary plaque psoriasis and 12 patients with highly active psoriasis using MoAbs against FcR and binding of soluble immune complexes. FcR in serum were measured in ELISA. The patients were treated with cyclosporin ($n = 5$), acitretin ($n = 7$) and Goeckerman regimen ($n = 8$). As controls served 8 skin biopsies and 22 sera from healthy individuals. Highly active psoriatic lesions showed strongest activity for FcRI, II and III and immune complex binding. The FcR+ mononuclear cells were located perivascularly and along the dermo-epidermal junction. The FcR activity decreased in correlation to the improvement following therapy. Epidermal Langerhans cells (LC) were positive for FcRII and immune complex binding. FcR activity on LC decreased during therapy. Keratinocytes expressed FcRI and III, irrespective of disease activity and therapy. FcR levels were lower in sera from psoriatics than in controls, median 0.15 vs. 0.27 ($p < 0.01$), and not correlated to disease activity. In 4 patients the FcR levels increased during therapy. The reduced levels of FcR in psoriatic sera might be due to consumption in the skin or anti-FcR autoantibodies. ***Key words: IgG-FcR; cyclosporin; acitretin; Goeckerman.***

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IgG-Fc receptors (FcR) are present on most immune competent cells, being involved in antibody dependent cell-mediated cytotoxicity, in the release of cytokines, and in phagocytosis (1). Soluble FcR is a mediator of immunomodulation (2). There are at least 3 types of FcR, characterized using monoclonal antibodies (MoAbs). FcRI (CD16) is a strong receptor. FcRII (CD32) and III (CD64) are weak receptors (1). Dermal histiocytic cells express strong FcR activity (3). Epidermal Langerhans cells (LC) express weak FcR activity (FcRII)(4, 5). Keratinoctes (KC) have weak FcR activity (4), but react with both anti-FcRI and anti-FcRIII MoAbs (5).

In psoriatic lesions there is increased FcR activity, particularly on histiocytes (6) and in stratum corneum, but also on KC (7).

The aims of the present study were to:

1) characterize FcR in situ in psoriatic lesions,
2) examine soluble FcR in serum from patients with psoriasis,
3) examine the effect of different treatment modalities on the FcR.

MATERIALS AND METHODS

Skin biopsies and serum samples from 8 patients with stationary plaque psoriasis and 12 patients with highly active psoriasis were studied. The patients were treated with cyclosporin ($n = 5$), acitretin ($n = 7$) and Goeckerman regimen ($n = 8$). As controls served 8 skin biopsies and 22 sera from healthy individuals.

Cryostat sections were stained with:

1) anti-FcR MoAbs: 32.2 (anti-FcRI), IV3 (anti-FcRII), Leu11b (anti-FcRIII) (5) and B1D6 (against a weak unclassified FcR) (8).
2) soluble immune complexes of horseradish peroxidase (HRP) and rabbit IgG antibodies to HRP to detect functional FcR activity (6).

Sera were examined for soluble FcR in ELISA with the MoAb B1D6.

RESULTS

Dermis

The FcR+ mononuclear cells were mainly located in the perivascular infiltrates, usually strongest in the dermal papillae, and in some lesions particularly strong along the dermo-epidermal junction. Highly active psoriatic lesions showed strongest activity for immune complex binding as well as reactivity with FcRI, II and III MoAbs. The number of FcR+ cells decreased in correlation to the improvement following therapy.

Epidermis

LC-bound immune complexes and were positive for FcRII. KC

Table I. *Serum levels of FcR (O.D.) in patients with psoriasis, before and after treatment*

Type of psoriasis	No. of patients	FcR (O.D.)			
		Before treatment		After treatment	
		Median	Range	Median	Range
Stable	8	0.175	0.141–0.955	0.147	0.133–0.950
Highly active	13	0.146	0.119–0.392	0.212	0.121–0.669
All patients	21	0.154	0.119–0.955	0.166	0.121–0.950
Controls	22	0.267	0.110–2.319		

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Table II. *Effect of psoriasis therapy on serum levels of FcR (O.D.)*

	Patients treated with					
	Cyclosporin		Acitretin		Goeckerman	
	(n = 5) Mean	Range	(n = 7) Mean	Range	(n = 9) Mean	Range
Before therapy	0.254	0.127–0.456	0.199	0.129–0.392	0.300	0.119–0.955
After therapy	0.320	0.130–0.493	0.281	0.130–0.669	0.305	0.121–0.950

expressed FcRI and III, strongest in highly active lesions. The FcR activity on both LC and KC decreased during therapy.

Sera

The FcR levels detected by B1D6 were lower in sera from patients with psoriasis than in controls, median 0.154 vs. 0.267 ($p < 0.01$), and were lower in highly active than in stable psoriasis, median 0.175 vs. 0.146 (Table I). In patients with highly active psoriasis, the median FcR levels increased during therapy, but after remission were still lower than in controls (Table I). This FcR increase occurred in patients treated with cyclosporine and acitretin (Table II) for highly active psoriasis.

DISCUSSION

The results showed that in psoriatic skin lesions there is an influx of FcR+ cells as well as increased FcR activity on KC and LC. In addition, the FcR activity is considerably increased in stratum corneum of psoriatic skin. The strongest FcR activity was detected in lesional skin from highly active psoriasis. The majority of the FcR+ mononuclear cells are histiocytes (6), which are important in the induction and control of immune responses. The large proportion of FcR+ histiocytes in the clinically most active psoriatic lesions sustains the concept of a local immune reaction early in the disease process (6, 9).

The reduced levels of FcR in psoriatic sera might be due to consumption in the skin or to anti-FcR autoantibodies (10). In systemic lupus erythematosus too, a reduced level of serum FcR has been found (11). During therapy, the FcR levels increased, but did not reach the levels in normal controls indicating an immunological defect not corrected by effective anti-psoriatic therapy.

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Evaluation of Serine α1-antitrypsin and Polymorphonuclear Leukocyte Elastase Contents and Their Immunogenetic Correlation in Psoriasis

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The purpose of our study was to quantify the serum content of α1-antitrypsin (α1-AT) and polymorphonuclear leukocyte elastase (PMN-E) in of 21 patients affected by active and stationary psoriasis, and 12 normal controls. HLA typing was also performed to identify a correlation among HLA antigens, age at onset of psoriasis and biochemical results. α1-AT levels were within the normal range in all patients, even in those with active, extensive, inherited and juvenile psoriasis, and in the controls. These data allow us to exclude, in our patients, the presence of rare or defective phenotypes, frequently associated with reduced serine levels of α1-AT. The PMN-E serine content was greatly increased in 3 patients, increased in 2, and slightly modified in 6 cases. All patients with the highest PMN-E levels reported a positive family history and absence of pulmonary, hepatic and atopic diseases. An increased psoriatic inheritance has been observed in the CW6-positive subjects (7/20), comparing B13 and DR6 antigen frequency. No correlation among HLA antigens, age at onset, clinical phase, or biochemical results could be established. ***Key words: psoriasis; α1-antitrypsin; elastase; HLA***

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Since proteolytic processes are prominent features in psoriasis, sera of 21 non-pustular and non-arthropathic psoriatic patients, genetically investigated for HLA antigens, were examined for α1-antitrypsin (α1-AT) and peripheral blood polymorphonuclear leukocyte elastase (PMN-E) contents.

It is well known that neutral serine proteinases such as PMN-E and their inhibitors can contribute to the pathogenesis of psoriasis because of their role as inflammatory agents causing damage to matrix components.

α1-AT, the most important serum inhibitor of PMN-E, protects the skin and pulmonar and hepatic tissues from the increased enzymatic activity of PMN-E. A decrease in serum concentration of neutral serine proteinase inhibitors, especially during flare and in patients with early onset of psoriasis, has been described (1), whereas increased elastase activity in neutrophils has been found in patients with active plaque lesions (2). These data may indicate that a protease-antiprotease imbalance could be related to the proteolytic tissue degeneration as the basal keratinocyte herniations observed in psoriatic skin (5).

The purpose of our study was to quantify the α1-AT and PMN-E serum contents in patients with active and stationary psoriasis and in normal controls. Human lymphocyte antigen (HLA) typing was also performed to identify a correlation among HLA antigens, age at onset of psoriasis and biochemical results, as proposed by several authors (4, 6).

PATIENTS AND METHODS

A total of 21 non-pustular and non-arthropathic psoriatic patients with active or stationary plaque lesions, randomly selected, and 12 normal healthy controls, have been studied for α1-AT and PMN-E serum concentrations. Pulmonar, hepatic, infective and joint diseases were absent in both groups.

In the performance of α1-AT test, a quantitative determination of antigen-antibody reaction was evaluated by rate nephelometry (Beckman Immunochemistry System, Calif., USA).

PMN-Elastase IMAC assay (Merck Diagnostica, Darmstadt, Germany) for elastase determination was employed.

Tissue typing for human histocompatibility antigens was applied in all patients studied.

RESULTS

α1-AT serum content was within the normal range for our control group (90–295 mg/dl) in all patients tested (Table I). PMN-E serum level was increased in patients nos. 18, 19, 20

Table I. *Total psoriatic patients: age of onset, clinical phases, α1-AT, PMN-E serum contents*

Patient	Age at onset	Clinical phase	α1-AT (mg/dl)	PMN-E (μg/l)
1. CA, f.	20 yrs	Active	141	43
2. NE, f.	25 yrs	Stationary	208	40
3. RM, m.	5 yrs	Stationary	151	59
4. DA, m.	31 yrs	Active	162	35
5. SA, m.	7 mo	Active	145	72
6. ML, m.	30 yrs	Stationary	163	60
7. TM, m.	4 yrs	Active	144	58
8. FR, f.	10 yrs	Stationary	175	124
9. VA, f.	30 yrs	Stationary	152	40
10. OE, f.	50 yrs	Stationary	160	50
11. BR, m.	22 yrs	Stationary	126	52
12. TN, f.	13 yrs	Active	145	41
13. RC, f.	30 yrs	Active	168	53
14. PA, m.	12 yrs	Stationary	128	50
15. PG, f.	20 yrs	Stationary	169	37
16. DG, m.	5 yrs	Stationary	129	50
17. BP, m.	54 yrs	Active	164	47
18. ZG, m.	40 yrs	Active	202	1967
19. DB, f.	30 yrs	Stationary	166	1504
20. MG, m.	3 yrs	Active	145	1596
21. GE, m.	40 yrs	Active	191	377

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Table II. *HLA antigens, age of onset, family history of psoriasis*

Patient	HLA antigens	Age at onset	Family history
1. CA	A2 A30 B13 B49 CW6	20 yrs	Negative
2. NE	A1 A26 B7 B37 CW6 DR2 DR5 DQ1 DQ3	25 yrs	Mother brother
3. RN	A24 A29 B17 B44 CW5 DR2 DR6 DQ1	5 yrs	Grandmother
4. DA	A1 A33 B14 B57 CW6 DR1 DR7	31 yrs	Mother
5. SA	A1 A2 B18 B35 CW4	7 mo	Mother
6. ML	A24 A30 B13 B44 CW6 DR1 DR2 DQ1	30 yrs	Grandmother
7. TM	A3 A23 B7 B44 CW4 DR2 DR5	4 yrs	Negative
8. FR	A24 A30 B7 B44 CW2 DR1 DR4 DQ1 DQ3	10 yrs	Mother
9. VA	A2 A24 B7 B18 CW2 DR4 DR8 DQ3	30 yrs	Negative
10. OE	A9 A30 B13 B18	50 yrs	Negative
11. BR	A13 A30 DR3	22 yrs	Negative
12. TN	A9 A30 B13 CW6	13 yrs	Sister
13. RC	A2 B13 CW4 DR6	30 yrs	Negative
14. PA	A24 A31 B51 B53 CW4 DR5 DR6	12 yrs	Negative
15. PG	A29 B44 CW5 DR2	20 yrs	Negative
16. DG	A3 A24 B18 CW6 CW7 DR1 DR5	5 yrs	Negative
17. BP	A2 B13 CW4 DR5	54 yrs	Negative
18. ZG	A2 B13 CW4 DR6	40 yrs	Brother
19. DB	A1 B7 CW5 DR1	30 yrs	Daughter
20. MG	A24 B44 CW6 DR2	3 yrs	Mother
21. GE	not done	40 yrs	Negative

Table III. *PMN-E level, inheritance, clinical phase*

Patient	PMN-E	Inheritance	Clinical phase
18, 19, 20	++++	Positive	Active: nos. 18, 20 Stationary: no. 19
8, 21	++	Positive: no. 8 Negative: no. 21	Active: no. 21 Stationary: no. 8
3, 5, 6, 7, 11, 13	+	Positive: nos. 3, 5, 6 Negative: nos. 7, 11, 13	Active: nos. 5, 7, 13 Stationary: nos. 3, 6, 11

(++++), 8, 21 (++), 3, 5, 6, 7, 11, 13 (+), as shown in Table I vis-à-vis the normal control value (2–42 μg/l).

All these serine data have been correlated to the age at onset (early onset = 0–20 yrs) and the different clinical phases (active – stationary) of the disease (Table I).

HLA antigen typing results, correlated to the age at onset and the family-history of psoriasis, are reported in Table II.

DISCUSSION

α1-AT levels were within the normal range in all patients, even in those with active, extensive, inherited or juvenile psoriasis, and in controls.

These data allow us to exclude the presence of the rare or defective phenotypes with very low levels of this enzyme, described in patients affected by inherited psoriasis, especially with early onset and during flare-up of the disease (1, 3).

PMN-E content was greatly increased in 3 patients (nos. 18, 19, 20), elevated in 2 cases (nos. 8, 21), and slightly modified in 6 patients (nos. 3, 5, 6, 7, 11, 13). All patients with high PMN-E levels reported a positive family history for psoriasis, but absence of pulmonary, hepatic and atopic diseases (Table III). These data need to be further investigated. We underline the results in cases 19 and 20, mother and daughter, respectively, in whom the biochemical values were similar, normal α1-AT content and very high PMN-E level, but the age at onset and the clinical phase of the disease were different.

We may suggest a genetic influence leading to an elevated PMN-E level despite different clinical features. All the patients investigated reported absence of either atopic dermatitis or contact dermatitis.

Regarding the HLA psoriatic antigens, CW6 was found in one-third of psoriatic patients (7/20), 4/10 cases with early onset and 3/10 cases with late onset of the disease. B13 was present in 7/20 patients, mostly with late onset of psoriasis; DR6 was revealed in 4/20 cases.

An increased positive family history in CW6 positive patients (5/7) can be highlighted by comparing B13 (3/7) with DR6 (2/4) antigen frequency, considered as genetic markers of inherited psoriasis. No correlation between HLA antigens and biochemical results could be established.

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Prevalence of Alexithymic Characteristics in Psoriatic Patients

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Although many skin diseases have a more or less objective etiological pathogenesis, none can leave out of consideration a neuropsychologically reactive conditions. This circumstance adds a pathogenetic component which, if not exclusive, is certainly remarkable and should not be underestimated for a more precise diagnostic identification of the disease and especially as regards prophylaxis and integrated therapy. Among psychosomatic patients, including those suffering from dermatological conditions, many authors have drawn attention to the remarkable preponderance of personalities having a restricted cognitive-affective style, deficient verbal expression of the emotions, mental faculities deficient in the abstract processes, and deficient introspective abilities, i.e. alexithymic personalities. In addition to having any of these features, alexithymic patients find it difficult to acknowledge their innermost feelings and therefore cannot employ them as 'signals' of emotional stress; their imaginative process is impaired and their verbalization and thoughts are fundamentally concrete and practical. In the present study we evaluated the prevalence of alexithymic characteristics in a sample of subjects diagnosed as suffering from psoriasis.

METHOD

*Patients (n = 32) were assessed using a measurement instrument specific for Alexithymia, viz. the Toronto Alexithymia Scale (TAS), and an unspecific instrument, the Rorschach test. Total and TAS factor scores were compared with those of 120 healthy controls matched with the experimental group by sex, age, and educational level (Table I).

*TAS is a 26-item self-report scale regarded as reliable and valid. Cut-of = or >74. TAS has a 4-factorstructure:
F1 = difficulty identifying and distinguishing between feelings and bodily sensations
F2 = difficulty in communicating feelings
F3 = inhibited daydreaming
F4 = externally oriented thinking

*Rorschach is a projective test. Its variables indicating alexithymia are:
1. Low response productivity
2. Low human movement percepts
3. Restricted affective response
4. Poorly adapted affect
5. Perceptual stereotype
6. Deficient ideational and affective assets.

RESULTS

*(See Table II): Our patients have more alexithymic characteristics than controls, but without statistically significant difference. Borderline scores result in higher percentages in controls than in patients. We can think that the difference between alexithymic and non-alexithymic subjects is sharper in our patients than in controls.

*(See Table III): Comparison between all samples and controls regarding total and TAS factor mean scores shows an alexithymic level higher in patients than in healthy subjects, considering only the total TAS and factor 1. This makes us think that our patients have secondary alexithymic characteristics, i.e. are reactive against affectively negative emotions with the aim of protecting themselves from those.

*(See Table IV): In agreement with Taylor, we found no statistically significant differences concerning sex and low educational

Table I. *Sociodemographic characteristics of patients and controls*

	Patients (n=32)	Controls (n=120)	t-test
Age			
Mean	37.0	34.16	p=0.137*
(SD)	(12.27)	(8.7)	
Range	20–55	18–50	
Sex			
M	12	60	p=0.554**
F	20	60	p=0.603**
Educational level			
E/H (low)	10	60	p=0.312**
S/U (high)	22	60	p=0.402**

*t-test. **χ^2.

Table II. *T.A.S. scores*

	Patients (n=32)	Controls (n=120)	t-test
Range	40–86	26–91	
Mean	60.94	54.50	p<0.05*
(SD)	(12.21)	(12.80)	
TAS=>74	15.6%	9.1%	p<0.001**
TAS <74e>63 (Borderarea)	25.0%	34.1%	p<0.01**

*t-test. **χ^2.

Table III. *Total and factors T.A.S. mean scores*

	Patients (n=32)	Controls (n=120)	t-test
TAS	60.94	54.50	p<0.05
(SD)	(12.21)	(12.80)	
F1	26.12	21.90	p<0.05
(SD)	(9.75)	(10.05)	
F2	18.00	16.20	n.s.
(SD)	(5.96)	(9.75)	
F3	13.19	12.90	n.s.
(SD)	(6.28)	(7.84)	
F4	11.25	11.00	n.s.
(SD)	(3.49)	(5.98)	

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Table IV. *Mean T.A.S. scores for sex and educational level*

	Patients	*n*	Controls	*n*	*t*-test
TAS M (SD)	61.42 (9.64)	12	54.2 (12.9)	60	n.s.
TAS F (SD)	60.65 (13.75)	20	54.8 (12.8)	60	n.s.
TAS E/M (SD)	61.40 (8.88)	10	56.0 (14.4)	60	n.s.
TAS S/U (SD)	60.73 (13.64)	22	52.9 (10.9)	60	$p<0.01$

level, but patients with a high educational level seem to be more alexithymic than the others.

*As regards the Rorschach test, all patients have a fantasy and affectivity lower than that of the controls, restricted affective responses, poorly adapted affect, and a perceptual stereotype higher than our healthy subjects, according to literature data.

CONCLUSIONS

Many authors think that alexithymia can vary along a continuum, so that all subjects, in particular situations, regress to a less symbolic communicational style. This is conceivable in our psoriatic patients with a probable secondary alexithymia. We believe that an integrated therapy could be useful for them, especially with the aim of helping alexithymic psoriatics to recognize their inner feelings and to employ them as 'signals' of emotional stress.

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ABSTRACT

The Hand in Psoriasis

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Among the many pathological conditions of the human hand, psoriasis is the most serious. A personal history of the psoriatic patient, of his hand, of evolving symptoms and severity, is outlined.

One can even express a reasonable prognostic evaluation regarding this condition, notwithstanding the limits set by this capricious morbid entity.

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Psoriasis of the Palms and Soles is Frequently Associated with Oropharyngeal *Candida albicans*

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Patients seen at our psoriasis clinic are studied for the possible presence of microbial factors that might be activating the disease. We have previously reported associations between certain clinical variations in the appearance of psoriasis and specific microbial findings (1).

Here we report 14 patients in whom palmar/plantar psoriasis was associated with the recovery of *Candida albicans* on culture from their throat and/or dental plate (Table I).

TREATMENT

Patients were treated with oral nystatin, fluconazole, or ketoconazole. Nine patients were evaluable following adequate treatment. Of these, 7 were cleared or substantially improved.

DISCUSSION

Baker (2) and Crutcher et al. (3) have previously reported the usefulness of oral nystatin in the treatment of psoriasis, presum-

Table I

Case no.	Age	Sex	Duration	Clinical description	*C. albicans* culture	Dental plate present	Treatment	Response
1	40	M	17 y	Red palms	+ Throat	–	Fluconazole	No response
2	63	F	20 y	Thick scale soles & palms, moderate erythema	+ Throat & dental plate	+	Nystatin	Improved
3	37	F	5 y	Scaly, red palms & soles	+ Throat & dental plate	+ (5 years)	Fluconazole	Pending
4	64	F	8 m	Thick palms & soles	+ Throat	–	Nystatin	Pending
5	39	F	5 y	Red, thick scale on palm	–	+	Ketoconazole	Cleared
6	49	M	–	Red, cracked fissured palms	–	–	Fluconazole, nystatin	Lost to follow-up
7	69	F	10 m	Red palms	+ Throat & dental plate	+	Nystatin	No help
8	43	M	6 m	Scaly palms	–	–	Nystatin, ketoconazole	Nystatin no help; ketoconazole improved
9	30	F	6 m	Very red, scaly palms & soles; pustules on feet	+ Throat	–	Nystatin Ketoconazole Fluconazole	Ketoconazole improved fluconazole cleared
10	37	F	2 y	Pink, scaly palms & soles	–	–	Nystatin yogurt	Improving
11	58	F	15 y	Red, scaly palms & soles	–	–	Nystatin	Lost to follow-up
12	56	F	1 y	Red, scaly palms & soles diabetes	+ Throat & dental plate	+	Ketoconazole, then fluconazole	Ketoconazole improved; fluconazole improved further
13	69	F	10 y	Red palms	+ Throat & dental plate	+	Nystatin	Cleared
14	57	F	6 m	Red palms	+ Throat & dental plate	+	Nystatin	Pending

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ably by virtue of its effects on Candida residing in the gastrointestinal tract.

Wachoviak (4) found Candida more prevalent in stools of psoriasis patients than in controls. Hanel et al. (5) found an increase in phospholipase A activity of *Candida albicans* strains isolated from the intestines of patients with psoriasis. Treatment with methotrexate made mouse intestine more vulnerable to candidal adherence (6).

Duvic et al. (7) reported the appearance of a psoriasis-like picture on the palms and soles in 6 of 20 patients who were being treated with intravenous glucan (an aqueous extract of yeast cell wall) in an attempt to stimulate their reticulo-endothelial system.

Patients with dental plates were advised to purchase an ultrasonic cleaning device for their dentures (Tatung Corp of America, Marietta, Ga). The use of such a device has been shown to reduce the numbers of recoverable yeasts from dental plates (8).

CONCLUSION

Psoriasis of the palms and soles is frequently associated with oropharyngeal candidal carriage.

Management of these patients can be successfully achieved with the use of oral antifungal drugs and attention to candidal carriage on their dentures.

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Association of Etretinate and Fish Oil in Psoriasis Therapy. Inhibition of Hypertriglyceridemia Resulting from Retinoid Therapy after Fish Oil Supplementation

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We studied the main papers concerning treatment with fish oil (EPA and DHA) of patients with psoriasis vulgaris, psoriatic arthritis and pustular psoriasis. In our investigation, 25 patients with psoriasis vulgaris evidenced a statistically significant increase in triglyceride serum levels, compared with controls. 10 of these patients underwent therapy with etretinate 0.75–1.0 mg/kg daily for 2 months followed by 2–3 months of etretinate 0.35–0.50 mg/kg daily associated with fish oil 1.5 g (EPA and DHA) daily. According with several authors, fish oil is able not only to produce good clinical results, but also to minimize the side effects of retinoid therapy, especially hypertriglyceridemia.

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INTRODUCTION

The essential fatty acids (EFA) are polyunsaturated acids with 18 or more carbon atoms and with the first double-link in position 3 or 6. The human organism cannot synthesize them, being unable to form double-links in position 3 or 6, which is why these substances must be introduced with the diet.

The omega-3 family is derived from the alfa-linolenic acid, after are synthesized eicosapenta-enoic acid (EPA) and docoso-hexa-enoic acid (DHA).

The omega-6 family derives from linoleic acid, after are synthesized gamma-linolenic acid (GLA), diomo-gamma-linolenic acid (DGLA) and arachidonic acid. The EPA therapeutic mechanism consists in promoting the formation of metabolites (monohydrossilic acids) with anti-inflammatory action which act as powerful inhibitors in vitro of LTB 4 which, on the contrary, produces a remarkable inflammatory effect (1).

Kragballe (2) found in psoriasic skin a mass of eicosanoids with an inflammatory action, particularly 12-idrossi-eicosatetra-enoic acid (12-HETE) and LTB 4. By contrast, some EPA derivatives (PGE 3 and LTB 5) have an anti-inflammatory and competitive action towards the inflammatory eicosanoids (PGE 2 and LTB4).

These factors justify the therapeutic use of EPA in certain dermatologic diseases. This study deals with psoriasis, in particular, where the best results could be observed with EPA omega-3 series (fish oil). Interest in EPA developed after discovering that the eskimo population, whose diet is based mainly on fish and therefore rich in EPA omega-3 series, have an incidence of psoriasis equivalent to 1/20 in comparison with that found in the population of industrialized countries.

Since 1985, several authors have tested fish oil treatment on psoriatic patients, with doses varying between 1.8 and 9 g/daily for 2–3 months, obtaining encouraging clinical results (3–7).

Lassus treated 46 patients with psoriasis vulgaris and 34 patients with arthropathic psoriasis, using EPA and DHA, obtaining improvement of skin lesions and also reduction of articular pains (8).

Kettler (9) obtained positive clinical results in patients with psoriasis vulgaris as well as in a case of pustular psoriasis. By contrast, Bjorneboe (10) found no clinical improvement in 30 psoriatic patients treated with 1.8 g of EPA daily for 2 months.

Dewsbury (11) and Escobar (12) found clinical improvement in psoriatic patients treated topically with fish oil.

The association of EPA omega-3 series with omega-6 series also gave very good results: therapy with GLA and DGLA leads to the formation of PGE 1 as an anti-inflammatory compound (4).

Gupta (13) moreover obtained an appreciable improvement in clinical parameters in psoriatic patients, when combining EPA with UVB exposure.

However, the most interesting association for psoriasis treatment seems to be the one between fish oil (EPA and DHA) and etretinate. This association not only produces a twofold therapeutic effect, but it also reduces the hyperlipidemia due to etretinate, particularly hypertriglyceridemia (14, 15) and in a lower degree the total hypercholesterolemia (14).

The aim of this work was to verify these last reports and to study the possibility of reducing etretinate dosage if used in association with fish oil, in order to obtain good therapeutic results with the slightest side effects.

MATERIALS AND METHODS

We studied 25 psoriatic patients, 13 women and 12 men, 20 with psoriasis vulgaris, 2 with psoriatic arthritis, 2 with palmopustular psoriasis and 1 with pustular psoriasis; 5 of these patients were affected by diabetes and 4 were affected by thyroid diseases. 30 subjects were tested as controls. The range of age was 20–62 years. Blood samples were tested for: triglycerides (TG), total cholesterol (TC), HDL cholesterol and also total cholesterol/HDL cholesterol fraction.

In 10 out of 25 patients, 5 women and 5 men, exams were determined three times: before therapy, after therapy with retinoids and after therapy with retinoids associated with fish oil.

Serum triglycerides (TG) and total cholesterol (TC) were measured by automated enzymatic methods. HDL cholesterol was measured with the above procedures after isolation by polyanion precipitation of other lipoprotein classes with heparin/magnesium/chloride.

In these patients we also considered certain clinical features, especially erythema and desquamation. We denoted no clinical change with 0 and progressive improvement with +, ++, +++, indicating with ++++ the complete remission of psoriatic lesions. Good results were considered ++ or more, corresponding to a reduction by at least 50% of psoriatic lesions. Our 10 patients were submitted to therapy with etret-

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Table I. *Mean values of TG, TC, HDL and TC/HDL in controls and psoriatic patients*

	TG	TC	HDL	TC/HDL
Controls (n=30)	131.5±71.4*	219.0±39.1	59.1±15.5	3.7
Psoriasic patients (n=25)	193.1±80.2**	230.0±40.2	52.2±16.1	4.3

*p <0.001 **v.s

Table II. *Mean values of TG, TC, HDL and TC/HDL in psoriatic patients before and after therapy*

Psoriatic patients (n=10)	TG	TC	HDL	TC/HDL
Before therapy	190.0±80.4+	228.2±41.3	53.1±17.2	4.4
After retinoids	218.5±86.2++	240.5±46.2	55.2±15.1	4.4
After retinoids + Fish Oil	169.0±75.1+++	235.4±48.2	61.7±16.5	3.8

+p <0.05 ++vs and + +++vs.
++p <0.001 +++vs.

inate 0.75–1.0 mg/kg daily and fish oil 1.5 g daily (0.9 EPA and 0.6 DHA).

RESULTS

Clinical improvement with remarkable reduction of erythema and desquamation was observed in 5 patients after 2 months (retinoids) and in 3 patients after 4–5 months (retinoids associated with fish oil).

In the other 2 patients there were no clinical results. Alterations of triglycerides (TG), total cholesterol (TC), HDL cholesterol and total cholesterol/HDL cholesterol fraction are shown in Tables 1 and 2.

DISCUSSION

Dietary supplementation with fish oil rich in eicosapenta-enoic (EPA) and docosohexa-enoic (DHA) may alleviate psoriasis (3–7).

In involved plaques of psoriasis there is a marked increase in eicosanoids (leukotriene B4 and 12-HETE) related to the 5-lipoxygenase pathway: this increase is associated with inflammation and cell proliferation (1, 2). By contrast, some EPA and DHA derivatives (PGE3, LTB5, 15-HETE), have an anti-inflammatory action: this action is competitive toward the inflammatory eicosanoids (PGE2, LTB4, 12-HETE) (16).

Several authors drew attention to the importance of EFA omega-3 series in the treatment of psoriasis vulgaris, but there were good results also in arthropathic psoriasis (8) and in pustular psoriasis (9). Only Bjorneboe did not find clinical improvement of psoriatic patients treated with fish oil (10).

Other authors found improvement in psoriasis following topical treatment with fish oil (EPA) (11, 12) alone or associated with GLA (omega-6 series): this therapy leads to the synthesis of another anti-inflammatory compound as PGE 1 (4). In recent years there have been only two reports on the treatment of psoriasis with etretinate and fish oil combined (14, 15): the EFA supplementation results in decreased hypertriglyceridemia (14, 15) and hypercholesterolemia following etretinate therapy (14).

Psoriatic patients sometimes evidenced high levels of total cholesterol and triglycerides in serum, even before etretinate therapy: in our 25 psoriatic subjects, versus 30 healthy controls, we found a high level of TG. Our study showed a significant improvement in clinical features (remarkable reduction of erythema and desquamation of psoriasis in 8 of 10 patients) after treatment with retinoids and fish oil. There is a significant difference in TG serum levels between psoriatic patients before therapy (190.0±80.4 mg/dl) and after therapy with retinoids (218.5±86.2 mg/dl) compared with those treated with retinoids and fish oil together (169.0±75.1 mg/dl).

It is very important to consider that supplementation with EPA and DHA can reduce the dosage of etretinate (0.75–1.0 mg/kg daily for 2 months, followed for 2–3 months by etretinate 0.35–0.50 mg/kg daily) and minimize the side effects (hypertriglyceridemia) of retinoids (17). The treatment with fish oil (0.9 g of EPA, 0.6 g of DHA daily for 2–3 months) increases the clinical improvement in psoriasis vulgaris after etretinate therapy.

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Characteristics and Possible Significance of the Answers to Rorschach from Patients Suffering from Psoriasis

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The authors have tried to ascertain the statistical significance of the differences between the answers to H. Rorschach Psychodiagnostic tests given to 80 male and female adult patients, suffering from psoriasis and by 'normal' patients. The absolute frequences and the averages of the results concerning 55 items obtained through the administration of Rorschach to patients with psoriasis were compared with the results of the test made to the general population. The analysis of the data and of the correlations has confirmed the hypothesis that the pathology of psoriasis, is (seriously) damaging, especially as regards the inhibitions, the cerebral potential, the emotional balance and the social relationships of the patient and furthermore, it can be related, ecologically, to problems concerning the identification-individualization process of the patient. This hypothesis has taken into account the high emotional value, in particular as regards the image of oneself, self-acceptance and self evaluation as well as social acknowledgement, and the communicative value given to the skin. ***Key words: psoriasis; psychosomatic; Rorschach test.***

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1. INTRODUCTION

1.1. The significance that specific psychosocial factors may have in the etiology, development and treatment of psoriasis is presently universally accepted (cfr. Arnetz et al., 1985; Farber, Lanigan & Rein, 1990; Farber & Nall, 1993; Goldsmith, Fischer & Wacks, 1969; McEnvoy & Roeningk, 1990).

1.2. The accurate identification of these factors would indicate the presence in said patients of specific personality and/or conduct characteristics, which could play a predisposing, precipitating and/or reinforcement role with regard to the psoriatic pathology (cfr. Baldaro et al., 1989; Baughman & Sobel, 1977; Cabras, Pastorino & Calabresi, 1980; Engels, 1982; Farber & Lanigan, 1991).

1.3. Several psychodiagnostic tools can be used to detect such hypothetical factors. Some 'personality questionnaires' (such as the M.M.P.I., the IPAT and the 16PF) and some 'projective tests' (such as the House-Tree-Person Test, the Rorschach Test, etc.) seem to be particularly suitable to this end (cfr. Buck & Hamer, 1969; Fabricci, 1987; Hammer, 1977; Kanno, 1981; Mendez Garcia & Garcia Besteiro, 1986).

1.4. To specifically analyse the presence of possible personality and/or conduct common characteristics, which might hypothetically be significant elements in the etiology of psoriasis, we have administered a series of psychodiagnostic tools (cfr. Decaminada et al., 1992) to a group of 80 psoriatic patients (cfr. Tables I, II, III and IV) hospitalized in the IRCCS IDI in Rome.

1.5. Hereinafter we report only the results obtained until now through the administration and evaluation of the Rorschach test.

1.6. Though it carefully considers all the data gathered (summarized in a total of XVII Tables), here the discussion of the results refers exclusively to Table V (XVII): due to evident space limitations, we are not in a position to present now all the Tables relative to the data processing per each indicator (they will soon be published in *Chronica Dermatologica*).

2. THE SAMPLE

Eighty psoriatic patients (40 males and 40 females, aged 19–59, hospitalized in the IRCCS IDI) were considered in the study.

3. METHODOLOGY

The sample is homogeneous from the point of view of pathology and present environmental situation.

The Rorschach test and the other psychodiagnostic tools were always administered by the same psychologist under adequate conditions (based on the physical environment, and, what is more important, on the subjects' willingness to collaborate). They were checked and assessed by the same specialist, who has mainly used the fundamental criteria for response tabulation and result analysis suggested by Aronow & Reznikoff (1976), De Cato et al. (1990), Exner (1976), Foglio Bonda (1986) and Piotrowsky (1957).

The cases in which the administration conditions did not sufficiently guarantee the test reliability were not considered, as well as the cases when the test had been administered to the subject over the previous 12 months, or when, for any number of reasons, the patient stated and proved that he/she already knew the tables and the possible responses to them.

4. DISCUSSION

4.1. The first evident and remarkably important element is represented by the high "significance" of the data obtained from the experimental sample compared with those typical of the adult "general population"; indeed, only 7 out of the 53 "indicators" considered (7.50%) show a "non-significant difference" reference data.

4.2. The second equally clear aspect is the "generalized decrease" in the quantitative production (cfr. total N● of responses, N● of r/ G, Dd, Dbi and Ddbi, movement r/ of any type, H r/ and O r/) and, especially, in the qualitative production (in particular, note the decrease in % of F+ and FQE+; the increase in chromatic and achromatic colour r/ and in the shading r/ where F component is secondary or non- existent; the decrease in the

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Table I. *Subjects, by sex and age*

Age (years)	Males	Females	Total
19	–	2	2
20–29	12	12	24
30–39	14	10	24
40–49	4	10	14
50–59	10	6	16
Total	40	40	80
Mean age	38.0	34.2	36.1
S.D.	13.01	11.84	12.42

Table II. *Subjects, by schooling*

Schooling	Males	Females	Total
Illiterate	–	–	0
Elem. school	4	8	12
Lower educ.	14	16	30
High school	22	14	36
Univ. degree	–	2	2
Total	40	40	80

Table III. *Subjects, by status*

Status	Males	Females	Total
Single	12	9	21
Married	21	25	46
Divorced	5	1	6
Widow/er	2	5	7
Total	40	40	80

Table IV. *Subjects, by occupation*

Occupation	Males	Females	Total
Unemployed	2	4	6
Pensioners	–	–	–
Students	4	2	6
Housewives	–	8	8
Labourers	10	4	14
Shop-owners	6	6	12
Craftsmen	2	2	4
Employees	12	12	24
Civil servant	2	–	2
Managers and Professionals	2	2	4
Total	40	40	80

number of non-H and non-A contents; the decrease in the % of O+ r/ and the increase in O–; and the remarkable increase in "special phenomena", which are indicators of conflict, defensiveness, anxiety, insecurity, immaturity, impulsiveness and problems related to the "reality testing").

4.3. An immediate conclusion that can apparently be drawn from these two observations is that in these subjects we have detected a "specific and global inhibition" of their potentialities (intellectual, affective, impulsive, socio-relational and relative to their occupation). When we use the term "inhibition", and not "inability" or "delay", it is because several data exclude this hypothesis with sufficient certainty (cfr., in particular, the % of G and D; the presence – though reduced – of "elaborated" answers: 137 "elaborated" G – 22.9% of the total G, and 47 "elaborated" D – 0.05% of the total D; the fact that F+% and, especially, FQE+% are within normal values; the average of at least 1 M r/ per each subject – 86 r/M = 1.07%, and of 1.5 MA r/ per subject – 124 r/MA = 1.55%; an adequate number of B and a B% within normal values).

4.4. In the group of psoriatic patients we seem to be able to observe a marked tendency to promote and maintain a superficial type of thinking, which eludes reflection and in-depth examination of things, and to clearly opt for the "operational thinking", which is directly aimed at the solution of concrete problems (between these problems, top-priority for the patient is that of his/her pathology: characteristics, consequences on one's personal, relational and social images; work problems; possibilities and ways to recover, etc.). This tendency to "operational thinking" can be inferred, in particular, from the interviews, but also from the symbolism of some test responses; from the significant increase in the number of primary G, of D% and A%; from the decrease in the elaborated answers, in Dd locations and in Dbi% + Ddbi%, in the movement responses, in the "conflict" content and, in "particular answers"; and from the marked elevation of "special phenomena" which indicate defensiveness, insecurity, immaturity and problems related to maintaining and efficiently using the "reality testing".

4.5. Equally evident are the strong "inhibition" of affective-emotional reactions, especially of those which can be voluntarily and consciously controlled (cfr. the increase in the number of F% and of "special phen." indicating "defensiveness"; and the significant decrease in FC and FE responses and of "special phen." related to affection); correlated with the "affection's inhibition", we find also in the patients of our group a scant willingness to open up and establish socio-affective contacts (note chiefly the significant decrease in the number and % of H and in the number of human movement – M – responses).

4.6. All subjects show a significant elevation of the "special phenomena" which indicate conflict, defensiveness, anxiety, insecurity, immaturity, impulsiveness and problems related to the "reality testing". All of these elements (plus the type of apprehension modality, the number and the % of F responses, the indicators of affective hypercontrol, the type and symbolism of the contents, particularly those wich are r/O+ or r/O–) suggest the prevailing and characteristic presence in these patients of sickness in the "personality disorders" area.

The specific "personality disorders" often identified in the group are those of "avoidant", "dependent" and, especially, "mixed" or "atypical" disorders.

Table V. *Comparison between the samples most significant data and the general population normal means; significant level of difference between the two samples expressed by Student's t-test*

Variable	Sample mean	Gen. pop. mean	S.D.	*t*	Sign. level
Responses	19.65	27.50	3.56	–16.67756	$p<0.0001$
Location					
G	7.47	7.00	1.39	3.034663	$p<0.005$
G %	38.15	25.00	4.25	27.67463	$p<0.0001$
D	11.67	16.50	2.41	–17.92566	$p<0.0001$
D %	69.17	60.00	4.69	17.48805	$p<0.0001$
Dd	0.42	2.00	0.49	–31.40434	$p<0.0001$
Dd %	2.02	7.50	2.42	–20.25397	$p<0.0001$
Dbi + Ddbi	0.52	1.00	0.49	–8.586501	$p<0.0001$
Dbi % + Ddbi %	2.40	4.00	2.36	–6.063914	$p<0.0001$
Determinants					
N* F	13.20	15.00	2.87	–5.609649	$p<0.0001$
F %	66.85	55.00	7.55	13.97913	$p<0.0001$
F+ %	80.67	85.00	15.99	–2.422059	N.S.
FQE+ %	75.52	80.00	13.26	–3.021898	$p<0.005$
M	1.07	4.00	1.38	–18.85375	$p<0.0001$
MA	1.55	2.50	1.35	–6.247837	$p<0.0001$
m ogg.	0.32	1.00	0.64	–9.357085	$p<0.0001$
FC (chromatic)	1.37	4.00	1.25	–18.66939	$p<0.0001$
CF	2.27	1.50	1.44	4.749717	$p<0.0001$
C	0.55	0.00	0.83	5.926928	$p<0.0001$
Color chr. score	3.78	3.35	2.25	1.749102	N.S.
FC' (achromatic)	0.45	0.50	0.77	–0.580797	N.S.
C'F	0.32	0.00	0.68	4.209069	$p<0.0001$
C'	0.10	0.00	0.30	2.981424	$p<0.005$
Color achr. score	0.70	0.25	0.91	4.422992	$p<0.0001$
FE (shading)	1.07	1.50	1.05	–3.628337	$p<0.005$
EF	1.05	0.00	1.20	7.826238	$p<0.0001$
E	0.05	0.00	0.21	2.032789	N.S.
Shading score	1.66	1.75	1.28	6.358819	$p<0.0001$
FT (texture)	0.40	0.50	0.53	–1.656347	N.S.
TF	0.05	0.00	0.22	2.032789	N.S.
T	0.00	0.00			
Texture score	0.25	0.25	0.35	0.00	N.S.
Content categories					
N* H	1.50	4.00	1.07	–20.89783	$p<0.0001$
H %	7.15	15.00	4.63	–15.16469	$p<0.0001$
N* A	14.87	9.50	3.42	14.04408	$p<0.0001$
A %	75.90	35.00	11.30	32.37352	$p<0.0001$
Conflicting cont.	3.67	6.50	4.35	–5.818.918	$p<0.0001$
Filling-up cont.	5.32	7.50	3.32	–3.873046	$p<0.0001$
Special cont.	0.57	1.50	0.83	–10.0219	$p<0.0001$
Frequency					
N* B	5.40	6.50	1.26	–7.808491	$p<0.0001$
B %	27.70	22.50	5.19	8.960	$p<0.0001$
Neiger index	4.45	6.50	1.80	–10.18653	$p<0.0001$
N* O+	2.17	9.50	1.59	–42.02661	$p<0.0001$
O+ %	10.50	30.00	6.70	–26.03184	$p<0.0001$
N* O–	1.77	0.00	1.06	14.79567	$p<0.0001$
Special scorings					
Conflict	4.08	1.50	2.26	10.21072	$p<0.0001$
Devensiveness	7.32	3.00	2.59	14.91863	$p<0.0001$
Affect	2.87	5.50	2.53	–9.297801	$p<0.0001$
Anxiety	4.52	2.50	2.63	6.869745	$p<0.0001$
Insecurity	5.60	2.00	2.56	12.57788	$p<0.0001$
Immaturity	2.60	0.50	2.75	6.830171	$p<0.0001$
Impulsiveness	3.05	1.50	2.85	4.864429	$p<0.0001$
Problems with "Reality testing"	3.33	0.50	2.81	9.007932	$p<0.0001$

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ABSTRACTS

Cyclosporin A in the Treatment of Psoriasis

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The aim of present study was to evaluate the efficacy of cyclosporin A therapy in patients with severe plaque-type psoriasis. For this purpose, 10 patients; 9 men and 1 woman, aged between 27 and 61 years, 9 with severe plaque type and 1 erythrodermic type psoriasis were entered into this study between November 1991 and July 1992. Cyclosporin A was given for 12 weeks. Patients were first treated with cyclosporin A, 2.5 mg/kg/day; in case of inadequate response the dosage was increased to a maximum of 5 mg/kg/day. Clinical efficacy was evaluated by the psoriasis Area and Severity index (PASI) score. At the end of the third month of the treatment, 7 patients (70%) showed a marked improvement, with $\geq 75\%$ reduction of PASI. Maintenance therapy in these cases has still been continued. During the treatment, the most common observed adverse side effects were headache (30%), pruritus (30%) and conjunctivitis (20%). In conclusion, oral cyclosporin A is recommended as an alternative medication to obtain remission in patients with chronic severe psoriasis in whom no contra-indications had been identified.

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Photofibrosis: A Further Histopathological Change Induced by PUVA Therapy via the Mast Cell in Guttate Psoriasis

Preliminary report

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Twenty-five psoriatic patients were studied histologically before and after PUVA therapy in order to delineate the relationship between dermal mast cells, psoriasis healing process and collagen changes. A number of mast cells were found in the psoriatic lesion both before PUVA and also after PUVA therapy in 22 of the 25 patients. Fibrosis of the papillary dermis and upper reticular dermis was found in 3 cases. Increased collagen deposition and increased numbers of fibroblasts were accompanied by verticalization of ectatic and elongated blood vessels, with an overall pattern of relatively recent scarring. Mast cells were no longer detectable in the fibrosis area. We cannot exclude the possibility that PUVA therapy exerts a further stimulus on mast cell histamine and heparin degranulation in this type of psoriasis, thus leading to dermal fibrosis and blood vessel neogenesis.
Key words: PUVA therapy; dermal changes; mast cell.

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INTRODUCTION

Histopathological changes induced by PUVA therapy in the dermis are characterized by an increased deposition of mucopolysaccharides in the papillary dermis, associated with decreased and fragmented dermal elastic fibres (1). Most of these changes are reversible after PUVA therapy is discontinued (2), although marked ultrastructural changes in the elastic fibres are still found 15 months after PUVA discontinuation (3). Disappearance of the elastic tissue from the papillary dermis and replacement with homogeneous and sclerotic material have been reported in one case after PUVA (4). Collagen is usually reported as not being affected by PUVA therapy. In addition to lymphocytes, the presence of considerable numbers of mast cells characterizes the inflammatory cell infiltrate of psoriasis (5). Mast-cell released mediators may stimulate both endothelial cells (6) and epidermal proliferation (5). The role of the mast cell in wound healing (7) and its relationship with dermal collagen deposition have been demonstrated (8).The aim of this work was to examine the relationship between dermal mast cells, PUVA-induced healing of psoriatic plaques, and possible collagen tissue changes.

MATERIALS AND METHODS

Twenty-five psoriatic patients (17 males, 8 females; mean age 40.3, range 20–72 years) were seen consecutively at the Department of Dermatology of the University of Pavia. 16 had previously been given PUVA therapy (energy dose range 109–4831 J/cm^2; mean energy dose 1247.3 ± 1689.5 J/cm^2). Skin biopsies were performed under local anaesthesia before PUVA therapy on the buttocks, both on psoriatic plaques and on perilesional skin. When no psoriatic lesion was present on the buttocks, biopsies were taken either from the elbow or from the extensor surface of the forearm. A second biopsy was made on the same area after the clearing of the pre-existing plaque, but naturally avoiding the site of the first biopsy. The 9 patients undergoing PUVA therapy for the first time received a mean exposure of 110.75 ± 35.96 J/cm^2 (range 50–160 J/cm^2). All the patients considered had avoided any local or systemic treatment for at least one month before PUVA therapy. They did not suffer from itching and had no self-induced excoriations. Haematoxylin and eosin, Toluidine Blue and Orcein-Giemsa stains were used.

RESULTS

A large number of mast cells were found in the psoriatic lesion in 22 of the 25 patients before PUVA therapy, and this number was not significantly decreased by PUVA. In 90% of the specimens, after regression of the psoriatic lesion, some degree of homogenization of the papillary dermis and a decrease in or fragmentation of the elastic fibres were seen. In 14 of the 25 cases (56%), disoriented and increased, coarse eosinophilic collagen fibres were found in the reticular dermis, with a decreased number of fibroblasts, thus featuring a variable degree of dermal sclerosis. Fibrosis of the upper and reticular dermis was found in 3 cases (Fig. 1). In these cases, epidermal atrophy was accompanied by an increased number of fibroblasts, increased collagen deposition and an increased number of neoformed, vertically oriented blood vessels (Fig. 2). The overall histopathological

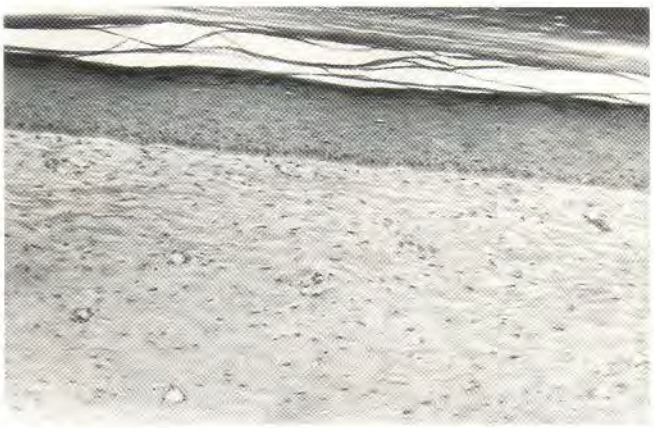

Fig. 1. Fibrosis of upper dermis, with increased numbers of fibroblasts, new collagen deposition and neoformed capillaries. H&E, ×160.

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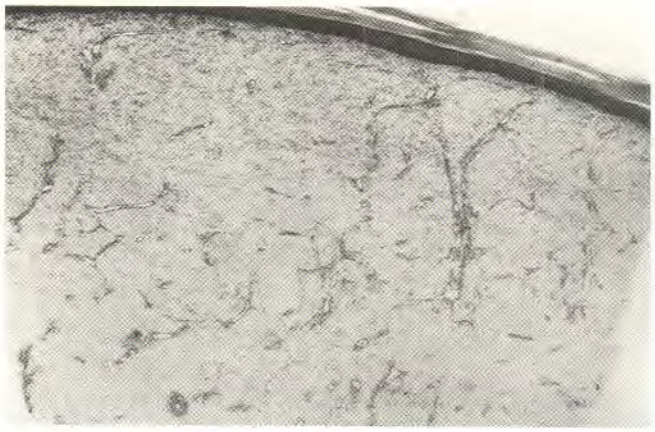

Fig. 2. Fibrosis of dermis, with atrophy of epidermis and compact hyperkeratosis. The overall pattern is that of a scar, with prominent, vertically oriented ectatic blood vessels. Orcein-Giemsa stain, ×40.

pattern was that of a relatively recent scar. In the 3 fibrosis cases, mast cells were no longer detectable in the dermis (Fig. 3).

DISCUSSION

The effects of PUVA therapy on mast cells are controversial. In fact, according to some authors (9, 10), degenerative changes are seen in mast cells of urticaria pigmentosa after PUVA. Some other authors, however, failed to demonstrate any changes in the number and structure of mast cells after PUVA (11). In our case series, the persistence of mast cells suggests that they escape the effects of PUVA therapy, even when the plaque has fully cleared. Persistence of mast cells during clearing of guttate psoriatic lesions after topical steroid therapy and after bath-PUVA therapy have been reported by Töyry et al. (12). In our series too, systemic PUVA therapy did not affect dermal mast cells, but this did occur in the 3 cases in which fibrosis was associated. We could not correlate the fibrosis finding either with clinical phototype, site, age, or with the energy dose given to these 3 patients, but rather with the type of psoriasis, which in all 3 cases was relatively recent and of the guttate and/or small nummular type. An early and constant mast cell degranulation characterizes eruptive guttate psoriasis (13). The type of psoriasis (i.e. guttate psoriasis) may thus be regarded as a main condition in leading to fibrosis after PUVA-induced healing. However, mast cells alone are unlikely to be the only factor in the induction of fibrosis, when it is recalled that some pathological conditions, such as mastocytoma and urticaria pigmentosa, may heal spontaneously without scarring fibrosis. The possibility cannot be ruled out that PUVAtherapy itself is the cause of the fibrotic response through the mast cells in guttate psoriasis (photofibrosis): nor can the possibility be excluded that the histological negativity of the mast cells in the fibrotic area may be attributable to mast cell degranulation and hence to its non-identification with the stains used. Granules may be released by mast cell cytoplasmic pseudopodia and actively taken both by fibroblasts and endothelial cells. This passage of granules (transgranulation) has been demonstrated both in vivo and in vitro (14). The transgranulation from cell to cell of heparin granules stimulates the migration of capillary endothelial cells (15), while the passage of histamine granules activates fibroblasts (16). Both these events are crucial steps in the fibrosing and/or scarring process. Our findings, albeit preliminary, suggest that in guttate psoriasis, a peculiar condition exists in which PUVA can induce a fibrosing response through increased mast cell activation.

Fig. 3. Fibrosis of upper dermis after PUVA-induced healing of a psoriatic plaque. Absence of elastic tissue, inflammatory cell infiltrate and mast cell are noticeable. Orcein-Giemsa stain, ×160.

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PUVA-treated Psoriatic Skin as a Model for Cutaneous Wrinkling Assessed by Skin Replicas

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Psoriatic patients may offer a useful model for PUVA-induced skin wrinkling. This study deals with the changes induced by PUVA therapy on the cutaneous microrelief of psoriatic patients assessed by surface replicas. A non-exposed body area (buttocks) was considered. The microrelief was evaluated by means of replicas analysed by an automatic image analyser. Three groups of patients were considered: 1) 10 psoriatic patients who had been undergoing PUVA treatment for the first time and who had received a total PUVA dose of 200±20 J/cm²; 2) 16 psoriatic patients in long-term PUVA treatment (>1000 J/cm²); 3) 13 psoriatic controls whose buttocks had never been affected by psoriasis nor exposed to sunlight or PUVA. The results showed that the number and the entity of the cutaneous crests and furrows had been increased by PUVA therapy. In particular the skin pattern analysis showed significant statistical differences between the second and the third group, while no changes were evident between the first and third group (ANOVA and Tukey test for multiple comparisons). In conclusion, our findings indicate that long-term PUVA therapy causes marked changes in the cutaneous microrelief, that this phenomenon can be measured non-invasively and that the changes observed are dependent on the PUVA-dose energies received.
Key words: microrelief; skin replicas; psoriasis; PUVA therapy.

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INTRODUCTION

PUVA therapy, which is characterized by the association of psoralens and exposure to UVA radiation (365 nm), is an effective and well-tolerated therapy for psoriasis (1). This treatment does not always produce lasting remissions and therefore long-term, repetitive treatments are required. One of the adverse effects of prolonged use of PUVA is described in the form of premature cutaneous ageing resembling photoageing (2–5). In particular, one of the clinical stigmata of prolonged PUVA therapy is the alteration of the cutaneous microrelief (6).

The aim of this work was to assess, non-invasively, changes in skin microtopography caused by short and long-term PUVA therapy, in psoriatic patients, using the buttocks, a skin area not usually exposed to sunlight.

MATERIALS AND METHODS

Three groups of patients were considered:

Group 1: 10 psoriatic patients (male, average age 40 years) who were undergoing PUVA treatment for the first time and had received a total PUVA dose of 200±20 J/cm².

Group 2: 16 psoriatic patients (male, average age 45 years) who had been undergoing PUVA therapy for 5 years and had received a total PUVA dose of 1000 J/cm².

Group 3: 13 control psoriatic patients matched for age and sex who had never been treated with photochemotherapy.

The measurements were carried out on the right buttock, a skin area never exposed to sunlight. Furthermore, no psoriatic plaques were present at the site of the measurement. The study was carried out using negative replicas of the skin surface (7, 8). These replicas consist of a rubber monomer of "Siflo Flexico" silicone which, when mixed with some drops of a catalyst, is applied to the surface to be tested. A Hewlett Packard adhesive disc is placed on the skin surface, as a support for the resin. As the skin surface pattern is anisotropic in most body areas it is advisable to make a mark on the replica indicating the direction of the body, e.g. a notch or a tag. The Hewlett Packard disc is supplied with a tag indicating the direction of the measurement; in our case the tag was pointed in the direction of the head.

All the replicas were assessed using a computerized image analyser, Philips NMS 8280 Software videographics MSX-DOS. The replica was placed under a macro-Philips 12 NC videocamera and laterally illuminated by a halogen lamp (50 Watts) at an angle of 26° and placed opposite to the video scan direction. The replica was always placed under the videocamera with the tag parallel to the light and the primary furrows vertical to the monitor that they appeared on. The data were processed using a Philips NMS 8280 computer with a software program evaluating the intensity of the greys in relation to the length of the shadows projected by the crests; the computer gives an average reading,

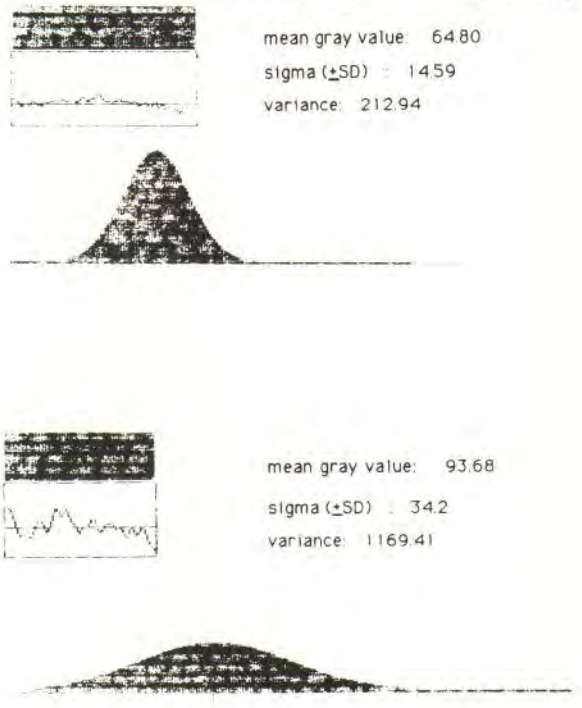

Fig. 1. Graphic elaboration of the image analysis made by the software program. The graph represents the analysis of the skin replica; the horizontal line expresses the mean grey level *M*; the crests and furrows correspond to the standard deviation of the mean grey level (sigma). *A*) Elaboration relating to a control's skin replica. *B*) Elaboration relating to a patient's skin replica (PUVA dose > 1000 J/cm²).

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M, which represents the mean grey level. Furthermore, the software program also calculates sigma σ, corresponding to the standard deviation of the mean grey level line (Fig. 1). The data collected were analysed using standard statistical methods (ANOVA and Tukey test for multiple comparisons).

RESULTS

No significant differences between Group 1 (PUVA dose 200±20 J/cm^2) and Group 3 (control group) were found as regards either the *M* level or the sigma σ level. On the other hand the comparison of measurements between Group 2 (PUVA dose ≥1000 J/cm^2) and Group 3 (control group) revealed significant differences as regards both mean grey level *M* and sigma σ level (± SD) (Table 1). ANOVA analysis is significant at a level of $P = 0.001$ for mean grey levels and at $P = 0.03$ for sigma levels respectively. ($P = 0.05$ Tukey test for multiple comparisons).

Table I. *Mean grey M and sigma σ levels (±SD) in psoriatic patients during photochemotherapy and controls*

	Mean grey level *M* (±SD)	Sigma σ (±SD)
Control	87.5±5.2*	15.0±3.6°
180 J	92.2±4.3	17.7±5.5
1000 J	95.9±6.2*	21.2±77°

ANOVA analysis is significant at a level of $p = 0.001$ for mean grey *M* levels and at $p = 0.03$ for sigma σ levels respectively. Controls and patients treated with doses higher than 1000 J/cm^2 are significantly different (*°, $p = 0.05$ Tukey test for multiple comparisons).

DISCUSSION

Wrinkles are the most commonplace of all the signs of cutaneous aging (9). Structural changes in the skin occurring with ageing and after sun-exposure alter the skin's surface clinical appearance. The cutaneous microrelief reflects the condition of the epidermis and the dermis, their thickness, the presence of papillae and the amount of elastic and collagen tissue; it reflects the anisotropy of the epidermis and the dermis (10, 11).

Many studies have demonstrated that changes in each of these structures alter the cutaneous microrelief, as shown in intrinsic ageing and photoageing (8, 12, 13, 14).

PUVA therapy offers a useful model when studying the effects of UVA irradiation on the skin in a controlled manner. This treatment, using long-wave ultraviolet light plus psoralens, causes wrinkling, teleangectases and changes in the skin markings (5, 6, 15). Many studies have described numerous changes in PUVA patients' skin histologically, histochemically and ultrastructurally (16).

In our work we studied the changes in cutaneous microrelief of PUVA-treated patients using a non-invasive method. (7, 8).

Several conclusions may be drawn from the data produced in this study:

1) PUVA therapy does not induce changes in the surface skin pattern at doses of 200±20 J/cm^2 (average value considered: 180 J/cm^2);

2) significant differences in the surface patterns are seen after cumulative doses of PUVA of 1000 J/cm^2 or more. These changes are significant both for the *M* values, that is for the number of wrinkles present (which had increased) and for the sigma σ level which increases with the increase in the depth of the wrinkles;

3) in practice, prolonged photochemotherapy causes changes to the skin surface: not only does the depth of the wrinkles increase, but new crests and depressions are formed, as can be seen from the morphometric analysis. There is, therefore, a huge increase in skin surface which is difficult to interpret as anything else but an attempt by the skin to deaden and dispel the energy of the photonic impact, spreading it out over a greater surface area;

4) the importance of the damage is also dose-related and proportional to the amount of energy received even though in this study the difference between 200 and 1000 J/cm^2 is too great to establish the critical PUVA level which is required to cause significant surface changes. Studies are being carried out by us to identify this critical point.

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Ocular Side Effects of PUVA-treated Patients Refusing Eye Sun Protection

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We have investigated short- and long-term ocular side effects of psoralen plus UVA (PUVA) therapy in 82 patients who refused to wear UVA blocking sunglasses after the treatments. They had received 321.7 ± 328.8 J/cm² of UVA in 148.8 ± 113.9 exposures over 2–4 years. Results were compared with findings obtained in 749 patients who shielded their eyes. They received 402.6 ± 302.2 J/cm² of UVA in 167.8 ± 136.9 treatments over 2–6 years. 20 patients refusing eye sun protection developed conjunctival hyperemia and 21 patients decreased lacrimation. Among patients who adequately protected the eyes, we observed 5 cases of conjunctival hyperemia and 1 case of decreased lacrimation. Lens opacities did not develop in any patient. Adequate eye sun-protection is thus needed to avoid acute toxicity of cornea and conjunctiva but lens opacities do not appear to be a side effect of long-term PUVA-therapy. ***Key words: cataract; psoralen; photochemotherapy.***

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The risk of short- and long-term ocular side effects of oral psoralen plus UVA (PUVA) therapy is still debated (1–8). We have evaluated the risks run by patients who refuse to wear the recommended ocular sun-protection.

MATERIALS AND METHODS

In a retrospective questionnaire, 749 (360 men; 389 women) psoriatic patients (mean age 43.7 yrs; range 17–75 yrs) claimed to have adequately shielded their eyes following the ingestion of psoralens. They underwent 2–5 therapy cycles over 2–6 years and received 402.6 ± 302.2 J/cm² of UVA split into 167.8 ± 136.9 treatments. 82 (43 men, 39 women) patients (mean age 42.3 yrs, range 22–74 yrs) refused the recommended UVA-opaque spectacles. They had received 321.7 ± 328.8 J/cm² of UVA split into 26 therapy cycles over 2–6 years. The cumulative number of exposures was 148.8 ± 113.9. Patients were examined by trained ophthalmologists before each therapy cycle and then at 1–2 and 6–12 month intervals. They underwent measurements of visual acuity and lacrimal secretion by Schirmer's test and slit lamp examination of the conjunctiva, cornea and anterior chamber. Lens, vitreous body and fundus oculi were examined through dilated pupil. In addition, patients were continuously monitored for acute ocular side effects throughout the treatment period. Data were analysed by χ^2 test. The level of significance was set to $p < 0.05$.

RESULTS

Among the 82 patients without adequate eye protection after PUVA treatments, conjunctival hyperemia developed in 21 (25.6%) and decreased lacrimation in 20 (24.4%). Among 749 patients who had shielded their eyes, 5 (0.7%) had conjunctival hyperemia and 1 (0.1%) decreased lacrimation. The comparisons of the incidences in the two groups were statistically significant ($p < 0.05$). No patient showed an impairment of visual acuity or developed lens opacities or lesions of the vitreous body and fundus. 24 patients refusing ocular sun-protection were affected by lens opacities prior of the treatment, but they did not get worse.

DISCUSSION

Patients undergoing PUVA therapy should be required to wear UVA blocking sunglasses for 12 h when exposed to sunlight after the oral intake of psoralens in order to prevent acute toxicity of cornea and conjunctiva. However, photochemotherapy does not appear to represent a risk factor for the induction of cataracts both in patients wearing adequate ocular sun-protection (1) or, in the present study, in patients refusing it.

Cataract formation was not previously found in 12 patients after 2–12 years of therapy for vitiligo with low doses (10 mg t.i.d.) of psoralen and natural sunlight, despite the fact that eye protection was not recommended (4). In contrast, rare reports have described presumptive PUVA-induced cataracts, almost all in patients with a history of inadequate ocular protection (2, 3) and these discrepancies have not so far been explained.

Concern about the risk of cataract formation was aroused by findings of experimental studies (5–8). Permanent lens opacities were observed in animals after over-treatment with PUVA (5, 6): psoralens were delivered in excess of the amounts usually administered to human beings and the cumulative UVA irradiation was much greater than natural environmental UVA content at an intermediate latitude (5, 6). Furthermore, the extent and reversibility of the damage appeared to vary with the dose and among animal species (6). Studies in humans and other animals have reported that environmental UVA can induce psoralen photoadducts with tryptophan, lens proteins and DNA, resulting in photoproducts that may remain permanently in the lens (3, 7, 8). However, there is no conclusive evidence that they could represent the primary chemical mechanism of the cataractogenic damage.

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Tacalcitol Ointment for Psoriasis

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Tacalcitol (1 α, 24 (R)-$(OH)_2$V.D_3), a synthetic analog of active vitamin D_3, is a new antipsoriatic agent (1). Although this compound is applicable topically, transdermal absorption of this vitamin D_3 derivative may cause hypercalcemia. For clinical use, from the standpoints of safety and efficacy, transdermal absorption of this agent from topical preparations should be limited, though the antipsoriatic effect should be satisfactory. To elucidate these points, we studied pharmacokinetics, clinical efficacy and safety of the ointment containing 2 μg/g of tacalcitol.

METHODS

1. Transdermal absorption of tacalcitol from ointment

(1) Serum tacalcitol concentration after topical application

[^{3}H]-tacalcitol ointment was prepared by incorporating the tacalcitol into a white petrolatum-based ointment vehicle to obtain a final concentration of 2 μg/g. 50 mg of the ointment ([^{3}H]-tacalcitol: 100 ng equivalent) was applied on shaved back skin (9 cm^2) of 4 hairless rats. They were immobilized for 24 h and blood was drawn from the orbital sinus periodically to determine radioactivity.

(2) Urinary and fecal excretion of tacalcitol from ointment

The [^{3}H]-tacalcitol ointment was applied to 5 hairless rats in the same way as described above. After 24 h the applied area was washed with KimwipeTR and the rats were placed in metabolism cages for 6 days. Urine and feces were collected to determine cumulative excretion of radioactivities.

(3) Tacalcitol permeability through human skin

The [^{3}H]-tacalcitol ointment was applied on human epidermis mounted on a diffusion cell (Fig. 1). The epidermis had been isolated from surgically excised abdominal skin by thermal treatment at 60°C for 30 min. The cell was equipped with a water jacket and kept at 37°C. Permeation through the epidermis to receptor fluid, whose composition was Hanks' balanced salt solution mixed with 30% of fetal bovine serum, was measured by determining radioactivity in the fluid. The permeation rate through human epidermis was compared with that of hairless rat epidermis.

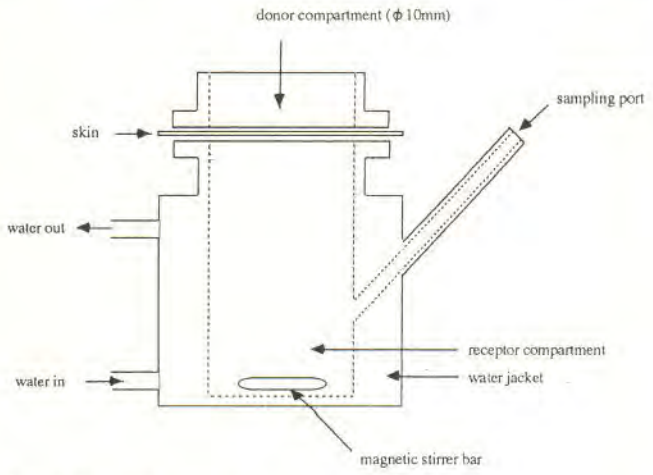

Fig. 1. The diffusion cell.

2. Clinical efficacy and safety

Psoriatic lesions of two symmetrical skin sites in 12 patients each were selected and a placebo-controlled double-blind right/left comparison was performed. One side was treated with ointment containing 2 μg/g of tacalcitol and the other with blank vehicle twice daily for 4 weeks. During the course of the study, weekly scoring for scaling, erythema and thickness of the psoriatic lesions was done using the following gradings: 0, none; 1, slight; 2, mild; 3, moderate; 4, severe. The investigator's global assessment was based on a comparison with the clinical condition at week 0 and used the following gradings: 1, marked improvement; 2, fair improvement; 3, slight improvement; 4, no change; 5, worse. A check was made for local side effects every day. Cumulative doses of tacalcitol were recorded and serum Ca levels were determined before and after the study.

RESULTS AND DISCUSSION

1. Transdermal absorption of tacalcitol from ointment

In *in vivo* studies in hairless rats, serum concentration profile suggested non-negligible transdermal absorption (Fig. 2) and recovery in urine and feces accounted for about 25% of the dose (Fig. 3). Ohta et al. also reported that about 30% of the radioactivity was recovered in urine and feces after a single topical application of a tacalcitol ointment in Wistar rats (2). Thus it was evident that tacalcitol in ointment was rather permeable through rat skin. However, in vitro study showed that the per-

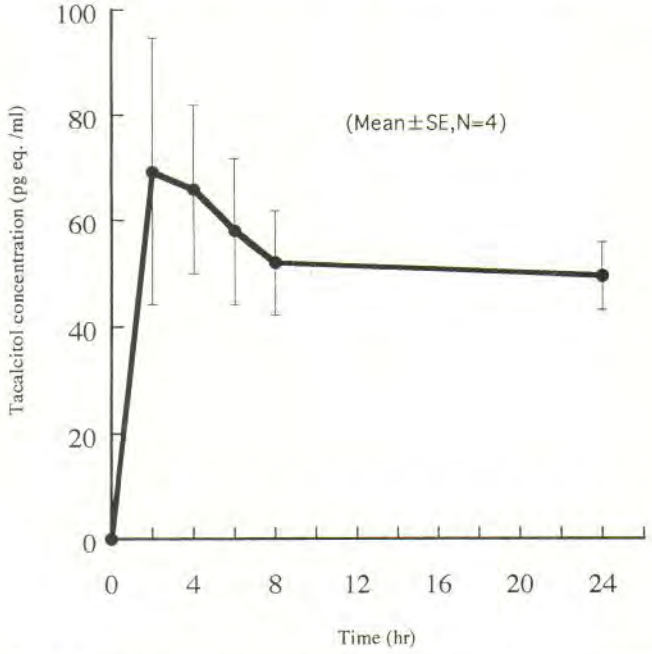

Fig. 2. Serum tacalcitol concentration after topical application of [^{3}H]-tacalcitol ointment on back of hairless rat.

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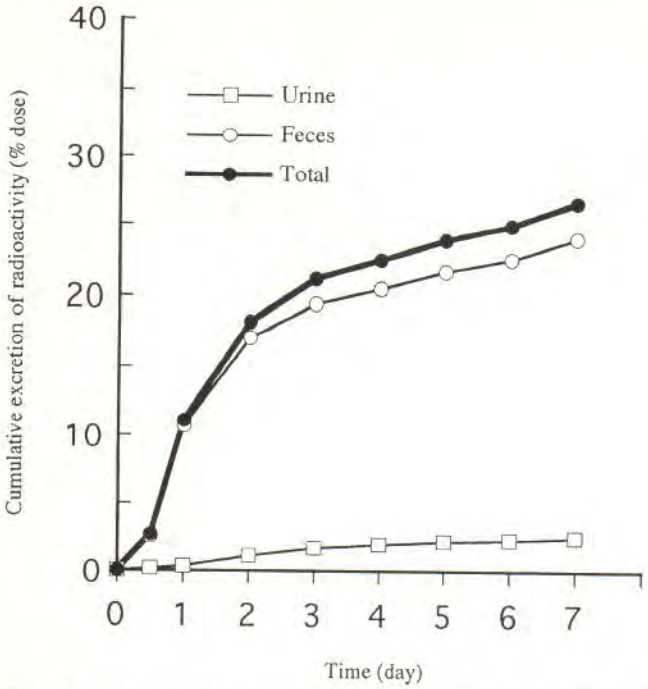

Fig. 3. Cumulative urinary and fecal excretion of radioactivity after topical application of [^{3}H]-tacalcitol ointment on back of hairless rat. The ointment was applied for 24 h.

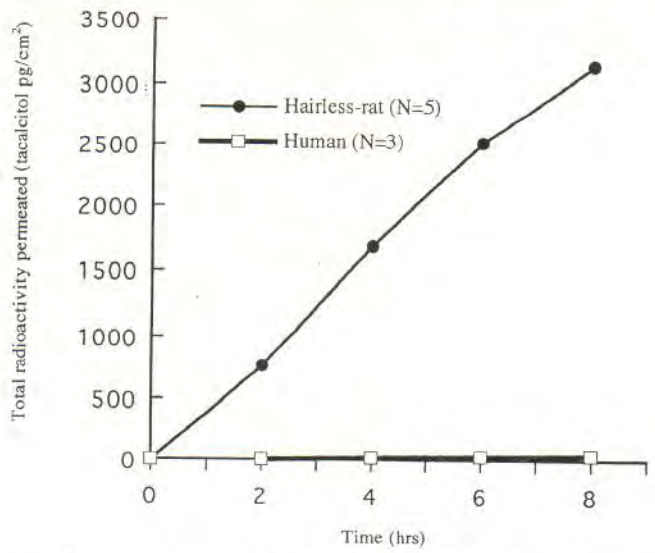

Fig. 4. In vitro permeability of [^{3}H]-tacalcitol in ointment through epidermis of different species. 3.9 mg of the ointment was applied.

meation rate through human epidermis was about one-fiftieth of that through hairless rat epidermis (Fig. 4). Therefore it was expected that tacalcitol might not be practically absorbed transdermally and pharmacological effects would be limited to the local skin site, when the ointment was applied to human skin.

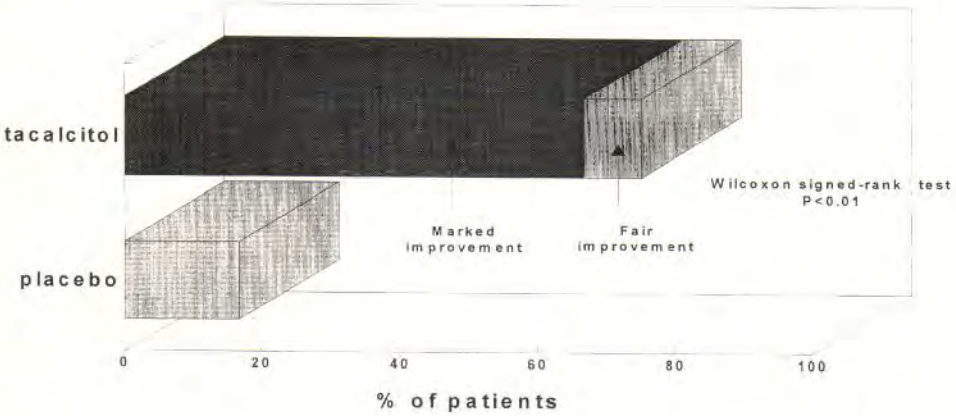

Fig. 5. Overall assessment of clinical efficacy.

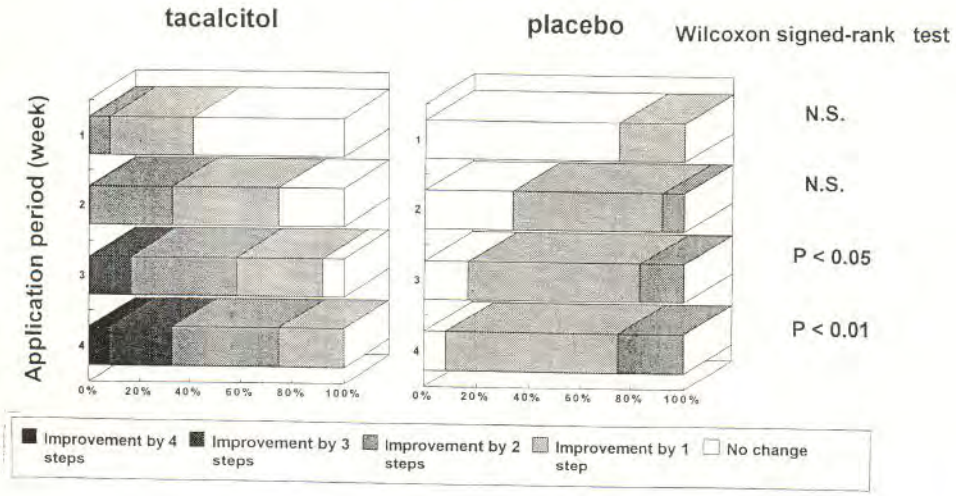

Fig. 6. Weekly improvement of erythema.

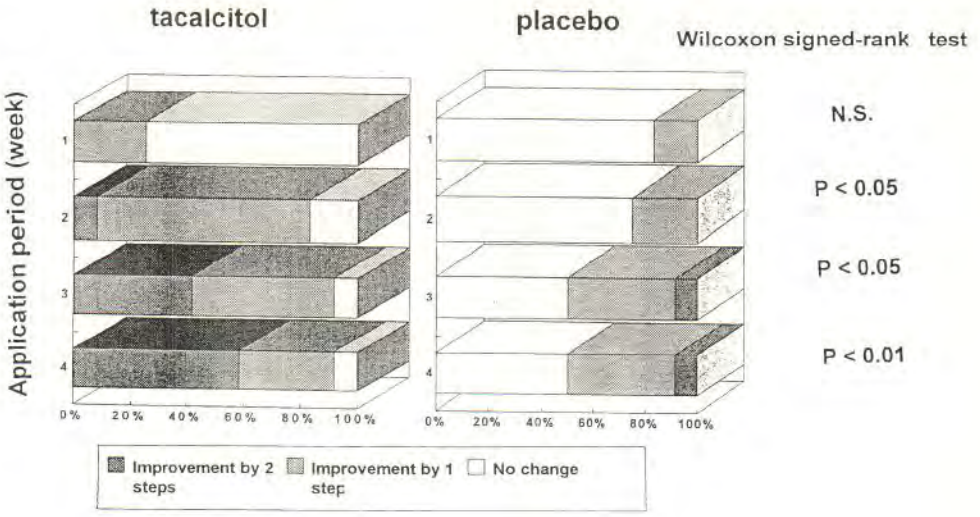

Fig. 7. Weekly improvement of scaling.

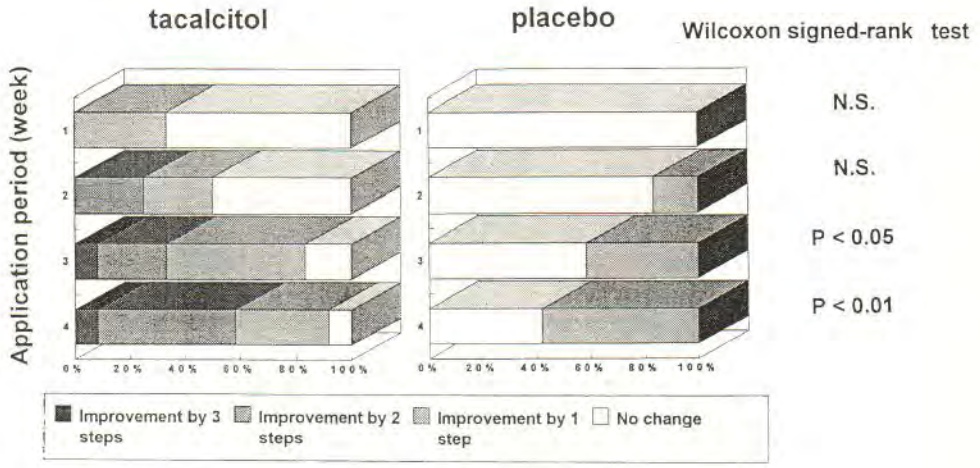

Fig. 8. Weekly improvement of thickness.

2. Clinical efficacy and safety

After 4 weeks of the treatment, the rate of improvement (better than fair) with tacalcitol ointment (75.0%) was significantly higher than that with placebo ointment (16.7%) (Fig. 5). The rate of marked improvement with tacalcitol ointment was 66.7%, while no case showed this rank of improvement with placebo ointment. The superiority of tacalcitol vis-à-vis placebo ointment was evident for scaling even at 2 weeks after starting the treatment and 3 weeks after for erythema and thickness (Figs. 6, 7 and 8).Cumulative doses of tacalcitol were 220 to 640 μg (average 340 μg). None showed any local adverse effects or elevation of serum Ca level in the clinical study.

From the viewpoint of safety and efficacy, we consider that tacalcitol ointment is a sophisticated antipsoriatic agent. However, its tolerability in long-term treatment should be further studied to define the safety of tacalcitol ointment in the treatment of psoriasis.

SUMMARY

Transdermal absorption of tacalcitol from the ointment containing 2 μg/g was studied using hairless rat and human skin. In the animal experiments, a non-negligible amount of tacalcitol was absorbed transdermally, whereas in the case of human skin, this compound was hardly absorbed at all. A placebo-controlled double-blind right/left comparison confirmed that this ointment is effective and safe for the treatment of psoriasis.

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Topical Calcipotriol (MC 903) for Psoriasis: A Clinical Study

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Calcipotriol, a non-calcemic vitamin D3 analogue, inhibits the proliferation and is necessary for final differentiation of keratinocytes. The aim of the present study was to determine the efficacy and tolerability of calcipotriol ointment in patients treated for 6 weeks. Twenty patients with chronic plaque-type psoriasis were treated twice daily with calcipotriol ointment 50 ng/g. After 6 weeks' treatment there was a marked and statistically significant decrease in the PASI score values for 17 patients, no improvement was seen in 1 patient and local adverse events occurred in 2. Hypercalcemia or other laboratory abnormalities did not develop in any patient. ***Key words: vitamin D3; psoriasis; calcipotriol.***

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Psoriasis is a very common skin disorder with increased epidermal cell proliferation and incomplete terminal differentiation of the keratinocytes.

Recent studies have shown that 1,25(OH)2-D3, the hormonally active form of Vitamin D3, inhibits the proliferation and is necessary for final differentiation of keratinocytes (1, 2). The systemic and topical administration of 1,25(OH)2-D3 may however cause a high frequency of side effects on calcium/phosphorus metabolism (hypercalcemia and/or hypercalciuria) (3, 4).

Calcipotriol (MC 903) is a Vitamin D3 analogue which is at best 1/100th as active as 1,25(OH)2-D3 in causing hypercalcemia and hypercalciuria, even though it seems to act through the same mechanism and has the same clinical efficacy (5–8).

The aim of the present clinical study was to evaluate the efficacy and tolerability of topical Calcipotriol in patients with chronic plaque-type psoriasis.

PATIENTS AND METHODS

Twenty patients (13M/7F) with psoriasis vulgaris, mean age 52 years (range 20–67) were studied. The mean duration of the disease was 10 years (range 1–26 years). All the patients had slight or moderate chronic plaque-type psoriasis with PASI (Psoriasis Area and Severity Index) scores between 5.1 and 17.1 (mean 9.53). Exclusion criteria were: pustular and erythrodermic psoriasis or a rapidly worsening type; patients who had been treated with systemic, intralesional or ultraviolet irradiation therapy in the last 2 months or with topical therapy except bland emollients in the last 2 weeks before therapy; pregnancy or lactation; patients with basal serum hypercalcemia or abnormalities of liver and kidney function. No patients was taking calcium and/or Vitamin D tablets.

After a wash-out period of 2 weeks, the patients were treated twice daily for 6 weeks with topical calcipotriol ointment (50 μg/g) (Formenti, Milan, Italy). The psoriasis was evaluated by the same physician at weeks 0, 2, 4, 6, 10 and routine blood analyses (plus calcemia, phosphoremia) and evaluations of the PASI scores were done at each visit. Overall evaluation of efficicacy and tolerability of the medication was made at each visit by both the physician and patients as: 3 = very good, 2 = good, 1 = moderate, 0 = poor, −1 = very poor.

At the end of the treatment the condition of the patients was evaluated after a follow-up period of 4 weeks.

RESULTS

18 of 20 patients (90%) completed the study. 2 patients interrupted the treatment, one because of aggravation of the skin lesions and another because of increased erythema and development of vescicles where the drug was applied.

Treatment with Calcipotriol ointment resulted in a marked and statistically significant ($p < 0.0005$) decrease in the PASI score (Fig. 1) (mean PASI score 9.53 at TO to 2.23 at T6) in 17/18 patients. No improvement of the skin lesions was seen in 1 patient after 6 weeks.

During the therapy, progressive reduction of desquamation was noted, followed by decreased erythema and infiltration (Fig. 2). After 4 weeks of follow-up (T10), PASI score values were

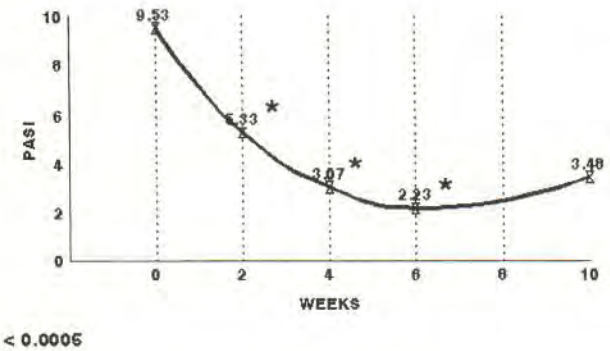

Fig. 1. Calcipotriol (MC903) for Psoriasis. PASI.

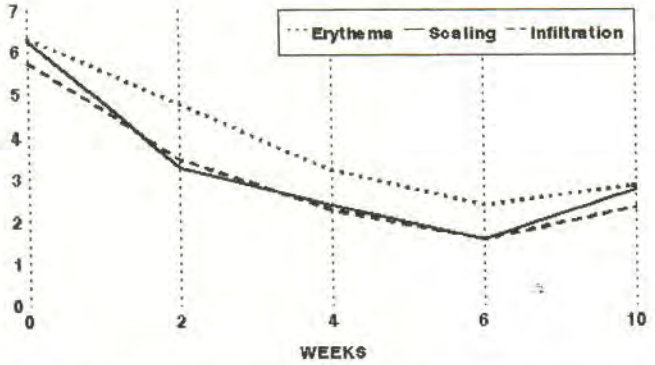

Fig. 2. Calcipotriol (MC903) for Psoriasis. Erythema, Scalling, Infiltration.

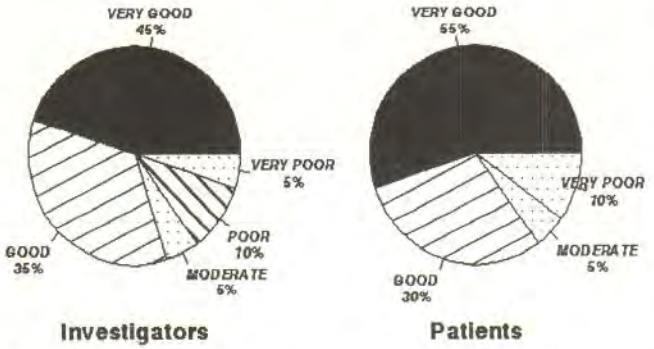

Fig. 3. Calcipotriol (MC903) for Psoriasis. Efficacy and Tolerability Evaluation.

slightly higher (T10=3.48) than at T6 (2.23). Although there was a slight increase in the PASI scores during the follow-up period, the decrease at T10 from T0 was still markedly and statistically significant.

The investigator's assessment of efficacy and tolerability was: very good for 9 cases (45%), good for 7 (35%), moderate in 1 case (5%), poor in 2 cases (10%) and very poor in 1 case (5%). The patient's assessment was: very good in 11 cases (55%), good in 6 (30%), moderate in 1 case (5%), but very poor in 2 cases (10%) (Fig. 3).

No patient had significant changes in routine blood analyses, calcemia or phosphoremia.

DISCUSSION

Our data confirm the efficacy of topical calcipotriol treatment for chronic plaque-type psoriasis. Positive results were obtained in 85% of the patients, unsatisfactory results in only 15%. One patient did not respond to the treatment and 2 developed local side effects (erythema and aggravation of the dermatosis).

The assessment of efficacy and tolerability by the investigator was: positive in 85% of the cases, while the patients' assessments were: positive in 90% of the cases.

No patient showed any systemic side effects.

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Topical Calcipotriol for Psoriasis – An immunohistologic study

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The aim of the present study was to investigate the distribution of Langerhans cells and T cells in the lesions and also the phenotypic expression of markers of activation on lesional T cells and keratinocytes, before and after 2 weeks of topical treatment of 7 psoriatic patients with calcipotriol. Before treatment, the infiltrate was composed mainly of T cells and there was decreased expression of CD1 on the intra-epidermal Langerhans cells. ICAM-1 and EGF receptor were present throughout the epidermis, but keratinocytes expressing Transferrin receptor were detected only in the basal layer. After 14 days of calcipotriol therapy, there were significantly fewer CD4T cells in the dermis and an increased number of intra-epidermal CD1 + Langerhans cells. ICAM-1 expression on lesional keratinocytes was reduced in all patients, but the expression of EGF receptor was decreased in 3 patients only, and Transferrin receptor expression on keratinocytes had not changed. All these changes were concurrent with moderate clinical improvement of the lesions. The results suggest that in the early stages of the clinical response to calcipotriol there is an immunomodulating effect of the drug associated with variable decreases in keratinocyte expression of markers of activation.

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The clinical antipsoriatic efficacy of Calcipotriol (C) is well established (1, 2), but its mode of action is not. C has antiproliferative effects and induces differentiation in cultured keratinocytes (Ks) (3, 4). Immunomodulating effects of C have also been demonstrated. Like calcitriol (the natural bioactive form of Vitamin D3), C inhibits the thymocyte proliferative response to IL1 (5) and it is conceivable that C has analogous inhibitory effects on T Lymphocyte (TL) proliferation, on IL2 production, on Transferrin-receptor (TFr) expression.

The aim of the present immunohistologic study was to determine the distribution of lesional TL and Langerhans cells (LCs) and the phenotypic expression of markers of activation on lesional TL and Ks, before and during topical C treatment, to obtain additional informations pertinent to the mechanism of action of C in Psoriasis.

PATIENTS AND METHODS

Seven psoriatic patients (36 to 50 years) were treated with C ointment (50 μg/g) (Formenti, Milan, Italy) twice daily for 6 weeks. The clinical severity of psoriasis was assessed by PASI scores, evaluated before and every 2 weeks during therapy, and analysed by Student's *t*-test.

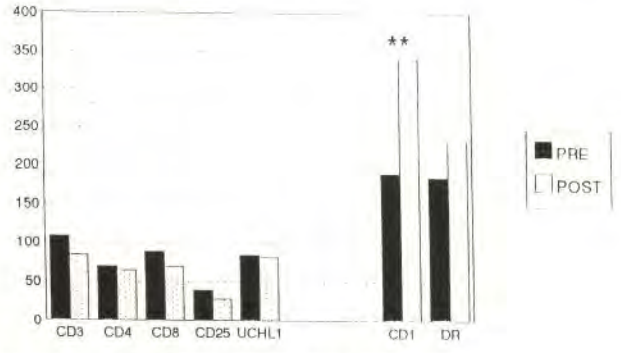
Punch biopsies, taken from patients before and after 2 weeks of treatment were embedded in OCT compound, snap frozen and serial cryostat sections prepared. A panel of monoclonal antibodies (MoAbs) was used to detect TL (Leu4), T cell subpopulations (Leu3a and Leu2a), memory TL (UCHL1) and LCs (OKT6 and HLA-DR). MoAbs against HLA-DR, TFr and IL2r (TAC) were markers of activated TL. To assess metabolic activation of Ks, MoAbs detecting Epidermal Growth Factor-receptor (EGFr), TFr and ICAM-1 were used.

A standard immunohistochemical technique, using APAAP (6), was applied to pathologic and normal skin samples. The APAAP processed slides were viewed with an ocular square grid and the absolute numbers of positive staining cells for each MoAb were counted. Twenty adjacent grid fields of epidermal sections, representing a surface area of 1.68 mm^2, were examined. Positive cells in papillary and reticular dermis were counted as described for epidermis. K expression of markers of activation was evaluated in 4 normal controls and psoriatic patients. The intensity of immunostaining (absent, faint, moderate, strong) and the extension of the epidermal distribution were assessed.

RESULTS

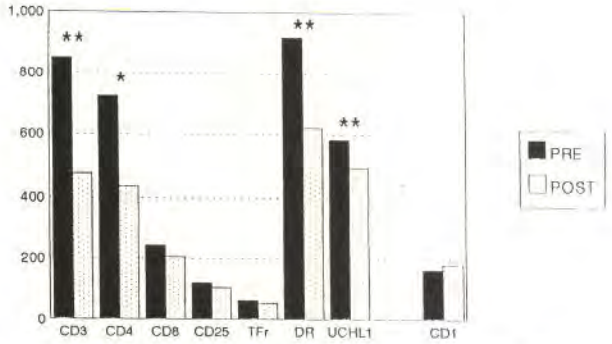
C improved psoriasis in all patients after 2 weeks of therapy, as confirmed by a significant decrease in the PASI score (mean values, 9.8 ± 4.0 before vs. 4.6 ± 1.6 after therapy, $p < 0.05$).

The pretreatment immunohistologic study, showed a cellular infiltrate consisting mainly of primed/memory TL (CD3+, UCHL1+), with a prevalence of CD4T cells in the dermis.



**, p< 0.01 PRE, before C therapy POST, after C therapy

Fig. 1. Total number of positively staining cells in 1.68 mm^2 of continuous psoriatic epidermis (mean values).



*, p< 0.02 **,p<0.01 PRE, before C therapy POST, after C therapy

Fig. 2. Total number of positively staining cells in 1.68 mm^2 of continuous psoriatic dermis (mean values).

Infiltrating TL expressed markers of activation. There was strong positivity for HLA-DR in dermal TL, and a small number of TL were positive for IL2r and for TFr (Figs 1 and 2). Intra-epidermal CD1 + LCs were fewer and irregularly distributed. After treatment, there was a decrease in dermal memory and CD3TL: Only the dermal CD4TL were decreased, with no changes in the CD8T cell subset. There were no changes in the numbers of IL2r + cells and TFr + cells, but there were fewer dermal HLA-DR + cells. In the epidermis we observed normalization of the distribution of LCs, associated with increases in CD1 + LCs (Figs 1 and 2).

Before treatment, ICAM-1 and EGFr expression in the Ks of the lesional epidermis was moderate in the basal layer, and in the upper squamous layers was faint. Immunoreactivity for TFr was similar to that observed in normal skin, being limited to basal layer. After C treatment, the expression of ICAM-1 was reduced in intensity and extent in all patients, whereas decreased expression of EGRr on Ks was observed in only 3 patients. No significant changes were observed in the expression of TFr on Ks.

DISCUSSION

The results of the present study indicate that in the early stages of the clinical response to C there is an immunomodulating effect of the drug on cutaneous immunocytes, associated with variable decrease in K expression of markers of activation.

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Psoriasis Vulgaris in 50 MHz B-scan Ultrasound – Characteristic Features of Stratum Corneum, Epidermis and Dermis*

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One hundred and forty fully developed, non-treated plaques of psoriasis vulgaris from the arms and legs of 22 patients were examined using 50 MHz B-scan ultrasound and compared with the images from adjacent, clinically normal skin. To visualize the dermis, high pre-amplification (digitization range 200 mV) was used, determined according to A-scan images. For evaluation of epidermal phenomena, low pre-amplification (digitization range 380 mV) was chosen in order to avoid overmodulation of the skin entry echo. In 10 patients, sonographic images were compared with histological sections from the exact same planes at the same magnification. At low pre-amplification, the skin entry echo is displayed as a markedly widened, frequently interrupted band composed of spots varying in thickness, height and echo density. Within these spots, several lamellae can be observed, represented as fine, echo-rich lines stacked one upon another. These phenomena correspond histologically to focal hyperparakeratosis, scaling and cracking of the stratum corneum. Due to the low amplification of the echo-signal the dermis is not visible. High pre-amplification allows evaluation of dermal changes. Below the entry echo there is an echopoor band (EPB) corresponding to the sum of acanthosis and infiltrate in the upper dermis. Underneath the EPB the dermis is represented as a zone with scattered internal echoes which are less intense than in normal skin. Dorsal shadows are typically present. They are artifacts emanating from epidermal regions with marked hyperkeratosis and disappear when the sonographic characteristics of the epidermis are changed, for instance by application of ointments prior to sonographic examination. Up to a thickness of the EPB of 800 μm, the total dermal thickness remains constant, not differing from the one in normal skin. With further increase in EPB-thickness, the total dermal thickness increases proportionally. Evaluation of psoriatic plaques with 50 MHz ultrasound at different pre-amplification levels of the echo signal allows to exactly visualize stratum corneum changes, to determine the thickness of acanthosis plus infiltrate and to quantify the thickness and echo-density of the dermis. ***Key words: 50 MHz-sonography; psoriasis vulgaris; follow-up; stratum corneum.***

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Ultrasound has been proposed as a non-invasive method to quantify the activity of psoriatic plaques and to study the effects of different treatment regimens (8). So far, B-scan sonography up to 25 MHz was used (1, 5, 8); evaluations focused on the so-called echopoor band which histologically correlates to the sum of acanthosis and infiltrate in the upper dermis. By raising the centre frequency and bandwidth of the ultrasound transducer, higher resolution can be obtained. It is thus possible to assess characteristics of the stratum corneum and the epidermis as well. We studied the epidermal and dermal sonographic features and their histological correlate in chronic psoriasis plaques using 50 MHz ultrasound.

PATIENTS AND METHODS

22 patients (13 men, 9 women, age range 25–55 years) with a more than 10-year history of chronic psoriasis vulgaris were studied. In each patient, several non-treated psoriatic plaques on the arms and legs were examined. All lesions were fully developed, showing the following criteria of psoriasis: silvery scales, marked infiltration and erythema. A total of 140 sonographic images were obtained. Moreover, in each patient, healthy skin adjacent to each plaque was assessed sonographically.

To correlate the ultrasound image with histology, an excision was performed in 10 patients as follows: a 10 mm long line was drawn on the lesion with a waterproof pen to define the plane of the B-scan. After sonography, the area was anesthetized. In a first step, the skin was cut

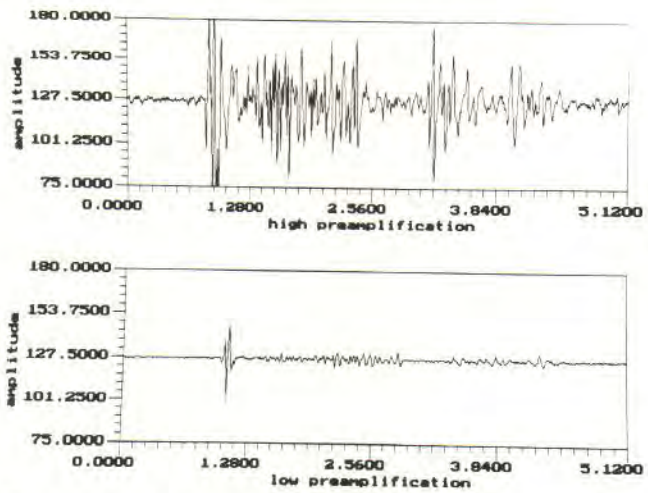

Fig. 1. A-scan of normal skin at high (upper curve) and low (lower curve) pre-amplification. Upper curve: First peak = entry echo, following peaks = dermis, following oscillations with low amplitude = subcutis, last peaks with higher amplitude = trabeculae and muscle fascia.

*This publication contains essential parts of the dissertation of Thorsten Auer.

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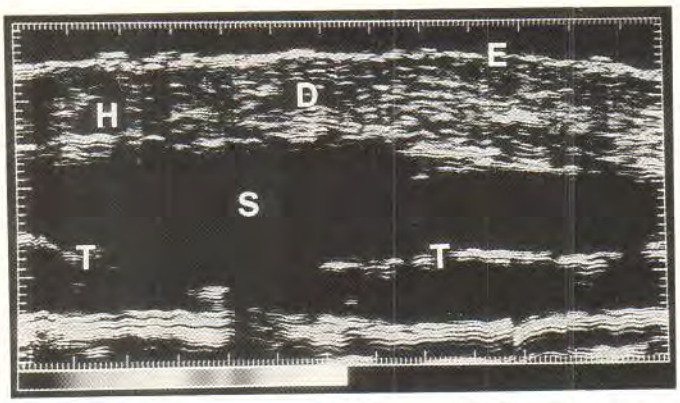

Fig. 2. B-scan of normal skin at high pre-amplification. The distance between the smaller scaling-marks at the border of the ultrasound images is 0.1 mm, between the larger ones, 1 mm. E = skin entry echo, D = dermis, S = subcutis, H = hair follicle, T = connective tissue trabeculae.

along this line down to the subcutis. Then a spindle-shaped excision was performed with this cut in the centre. The two halves of the tissue spindle were separated and their central cutting planes were placed on cardboard. This prevents warping of the tissue during fixation in 10% formalin. Biopsies were processed for light microscopy and stained with H&E.

Sonography was performed using a 50 MHz experimental system. We used a point-focused ultrasound transducer on polymer base (PVDF, polyvinylendifluoride) with a centre frequency of 40 MHz and a bandwidth of 30 MHz. Within the focal area, the transducer has an axial resolution of 37.5 μm and a lateral resolution of 125 μm (6). The inverted plexiglass pyramid socket in the lower part of the applicator has a slit, which is pressed lightly on the skin surface. Water is used as coupling medium between the transducer and the skin.

A-scan oscillations were registered using different echo-signal pre-amplifications prior to digitization of the high-frequency signal (Fig. 1).

High pre-amplification (digitization range 200 mV) was chosen in order to visualize the dermis (Fig. 1, upper curve): after a short time lapse (coupling water path) a large echo signal oscillation is seen (skin entry echo). When its amplitude exceeds a certain height, its peaks are cut off during analog-to-digital conversion. Cutting off of the signal is accompanied by an audible sound. The entry echo is followed by multiple irregular oscillations at lower amplitude (dermis). There is an abrupt transition of these oscillations to very low amplitudes, originating from the fatty tissue of the subcutis. Connective tissue trabeculae and fasciae are represented as echoes with high amplitude.

Low pre-amplification (digitization range 380 mV) was used to evaluate epidermal ultrasound characteristics: the oscillation curve has a markedly lower amplitude and the skin entry echo is located within the digitization range; hardly any signals follow the entry echo.

B-scans were registered with these pre-amplifications; the transducer was moved laterally in the applicator on the skin. Details of the technical setup have been described elsewhere (6). The original A-scans and their demodulated counterparts provide information about the distortion due to cutting off of the signal within the quantitization range of the analog-to-digital converter.

In 34 lesions, the thickness of the echopoor band and the thickness between the entry echo and the border dermis/subcutis were measured using an image analysis program (AnalySIS, SIS, Münster, Germany). Clinically normal skin adjacent to the psoriatic plaque served as a control; there the thickness between the entry echo and the dermis/subcutis interface was determined as well.

RESULTS

Healthy skin

High pre-amplification (Fig. 2):

In the B-scan image there is a rather linear, regular skin entry echo with a fine echo-lucent line beneath. Below this line, a wide zone with multiple, scattered echo-reflexes is seen. Often from this zone, several obliquely oriented, partly interrupted, linear, echo-rich stripes project into a wide, echo-lucent region.

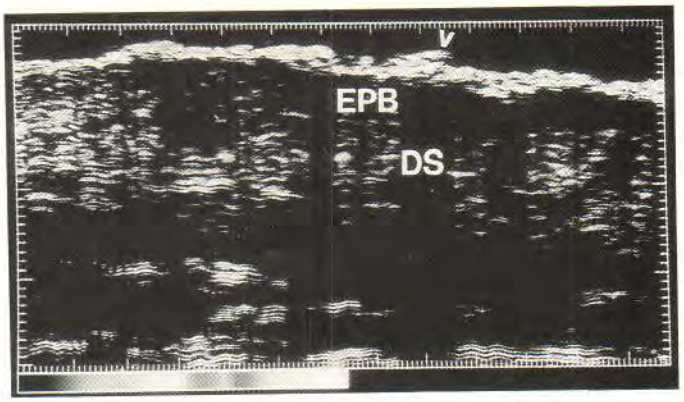

Fig. 3. B-scan of a psoriatic plaque at high pre-amplification. EPB = echopoor band, DS = dorsal shadow. Arrow head = scale.

Low pre-amplification:

The skin entry echo is represented as a white, homogeneous line, which is less wide than at high pre-amplification; no reflexes are visible beneath the entry echo.

Psoriatic plaques

All lesions show similar sonographic characteristics.

High pre-amplification (Fig. 3):

In the B-scan images, we see an irregularly thickened skin entry echo.

Beneath, an echo-poor band (EPB) of variable thickness separates the skin entry echo from a zone with numerous scattered echoes. The reflexes within this zone are less intense than in healthy skin. In regions with marked irregularity and thickening

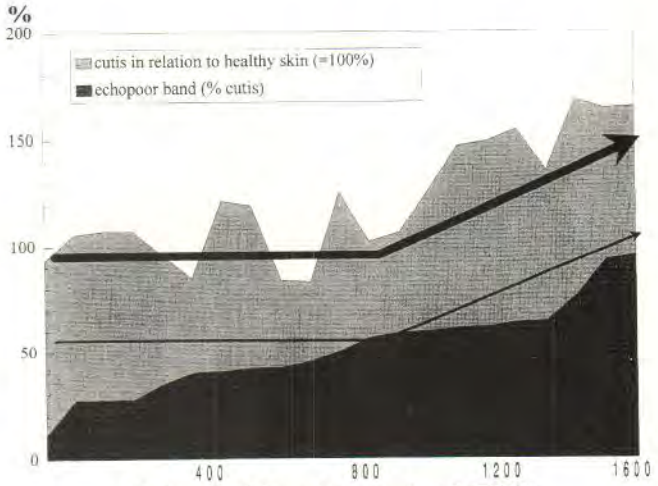

Fig. 4. Thickness of the echopoor band and the cutis (= distance between entry-echo and dermis/subcutis interface). Thickness values (y-axis) are expressed as percentage differences to the values obtained from normal skin adjacent to the plaques (= 100%).

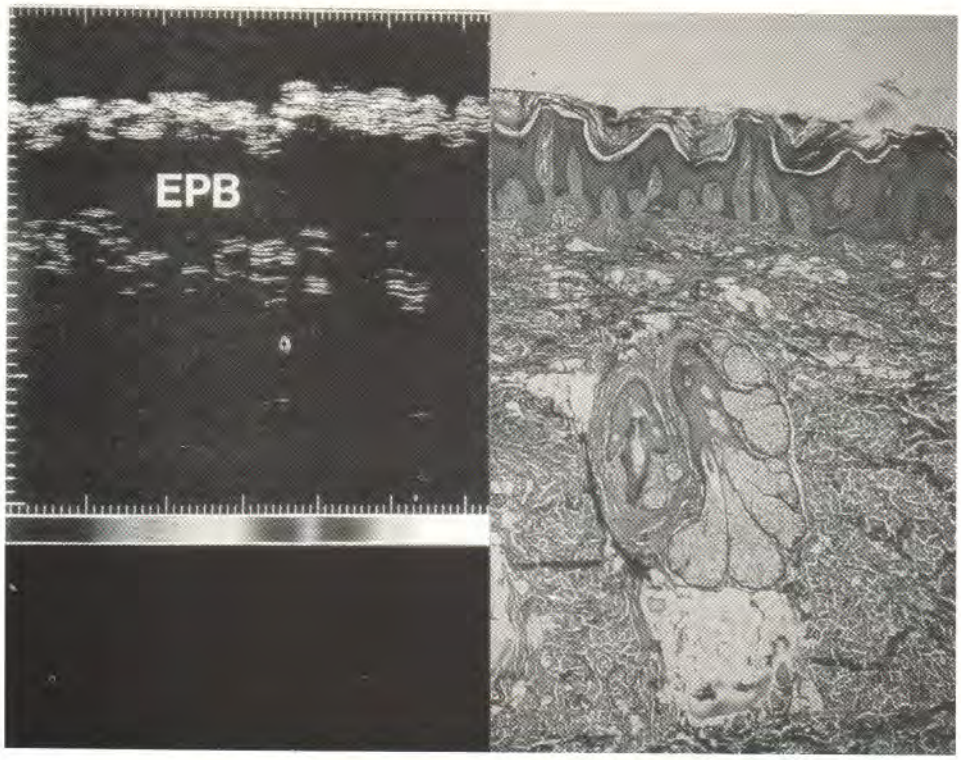

Fig. 5.
(*a*): B-scan of a psoriatic plaque at low pre-amplification. EPB = echopoor band.
(*b*): Histological section at same magnification.

of the entry echo, dorsal shadows (echo-free stripes oriented in the direction of the ultrasound beam) are observed.

Comparison with the histological sections at exactly the same magnification demonstrates that the skin entry echo corresponds to the irregularly thickened stratum corneum. The EPB corresponds to the sum of the acanthotic epidermis and the inflammatory infiltrate in the upper dermis. The dermis underneath the infiltrate is represented as the zone of scattered reflexes in the sonographic image.

The subcutaneous fat is visualized as an echo-lucent region.

Fig. 4 shows the results of the thickness measurements. Thickness values (*y*-axis) are expressed as percent differences vs. the values obtained from normal skin adjacent to the plaques (100%). With a thickness-increase of the EPB (*x*-axis) up to 800 µm, the total cutis-thickness (distance between entry-echo and dermis/subcutis interface) remains constant and does not differ from normal skin. With a further increase in EPB-width (from 800 to 1300 µm) there is a corresponding linear increase in the total cutis thickness as well. In other words, the EPB first expands at the cost of the cutis, until reaching 50% of the total cutis thickness. From that point on, both EPB and cutis increase proportionately in thickness.

Low pre-amplification (Fig. 5):
The skin entry echo differs significantly from the one in normal skin: it is a markedly widened, frequently interrupted band composed of spots varying in thickness, height and echo density. Within these spots several lamellae (up to 5) can be observed. These are represented as fine, echo-rich lines stacked one upon another. Generally, the uppermost of these lamellae shows the most intense reflex (see Fig. 5*a*).

Comparison with the histology (Fig. 5*b*) shows the pathological changes in the psoriatic plaques responsible for these ultrasound characteristics: there is focal hyperparakeratosis with irregular thickening of the stratum corneum, scaling and cracking of the stratum corneum and the upper epidermis.

DISCUSSION

Combining ultrasound at high resolution (50 MHz) with low pre-amplification of the echo-signal, we were able to visualize the specific characteristics of the stratum corneum in psoriasis vulgaris. The entry echo is widened and fragmented and shows a lamellar structure with spots of varying echo-density. The upper border of the entry echo is irregular and shows level jumps.

Visualization of these changes is possible only when the pre-amplification of the echo-signal is reduced so that the oscillation peak in the A-scan curve is located within the digitization range of the transient recorder. This avoids cutting off the oscillation peaks and subsequent distortion during demodulation. At this low pre-amplification however, the dermis is no longer visible. During normal sonographic skin examination, the pre-amplification is usually chosen to particularly judge the dermis (1, 5, 7). This leads to an overmodulation of the entry echo; the B-scan image shows a wide, echo-rich band (4) from which information about structural details cannot be obtained. Reduction of the overmodulated amplification parallels a narrowing of the entry echo. When the entry echo no longer narrows and only fades, the optimal preamplification for its evaluation is reached.

To observe the sonographic characteristics of the horny layer in normal skin, we previously examined the palms and soles at low pre-amplification (3, 4). The orthohyperkeratotic stratum corneum is represented as an echopoor structure, bordered by echo-rich lines at the water/stratum corneum interface (entry echo) and stratum corneum/stratum Malpighii interface. In

healthy skin of other body regions the horny layer is too thin to be resolved into sonographic images at 50 MHz, as confirmed by our study. As opposed to normal skin the hyperparakeratotic stratum corneum of psoriasis vulgaris is echorich, no matter how low the pre-amplification chosen. We believe that this is due to air trapped in the scales of the upper stratum corneum and to the higher reflectivity of the parakeratotic and thus inhomogeneous horny material. The irregular, scaly surface of the lesions leads to jumps of the entry echo. Hyperparakeratosis is represented as widening, fragmentation and lamellar structure of the entry echo and spots of varying echo-density. In fully developed psoriatic plaques, the width of the entry echo therefore represents the thickness of the stratum corneum.

Underneath the entry echo, psoriatic plaques exhibit an echopoor band. This corresponds histologically to the acanthosis and infiltrate in the upper dermis. This band is by no means specific for psoriasis, but can be demonstrated in all skin diseases which are accompanied by acanthosis and/or infiltrate in the upper dermis (3–5, 7). In psoriasis, it corresponds to the clinically palpable papule as assessed in the PASI index. Sonometry of the thickness of this band has been used to quantify the activity of psoriatic lesions (5, 7).

In chronic psoriatic plaques the dermis below the entry echo shows decreased echo-density compared with normal skin. Numerous echo shadows are visible. This is due to intense signal absorption and reflection in the hyperkeratotic stratum corneum, as discussed above. Shadows and decreased echogenicity are therefore artifacts emanating from the horny layer. Topical application of ointments for 30 minutes causes swelling and moisturization of the stratum corneum and removes air inclusion in the scales. This significantly reduces the shadows (3) and the decreased echogenicity of the dermis is no longer obvious.

Our measurements showed that the total cutis thickness remains constant as long as the thickness of the echopoor band does not exceed 50% of the total cutis thickness, respectively 800 μm. The echopoor band thus expands at the cost of the cutis. Bacharach-Buhles et al. (2) analysed the architecture of the elongated rete pegs and their spatial relation to the dermal vascular plexuses in psoriasis by means of image analysis of serial histological sections. They could demonstrate that the vascular architecture remains unchanged while the epidermal rete pegs grow down and include the vessels of the first horizontal dermal vascular plexus into the dermal papillae. This is entirely consistent with our finding that the total cutis thickness remains constant.

Further acanthosis, however, leads to an exophytic growth and thus to a thickening of the total cutis thickness, as demonstrated histologically (2). Sonographically, this finding is supported by the fact that further widening of the echopoor band results in a linearly proportional increase in cutis thickness.

Using 50 MHz sonography, stratum corneum, epidermal and dermal characteristics of psoriatic plaques can be quantified in vivo. This method is particularly valuable for follow-up studies evaluating different therapeutic regimens.

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Acta Derm Venereol (Stockh) 1994; Suppl. 186: 177

ABSTRACT

Instrumental Evaluation of Psoriatic Lesions Treated with Tacalcitol

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Eighteen patients affected by psoriasis were studied. Treatment was carried out on symmetrical lesions with tacalcitol ointment or with placebo, applied once a day for 6 weeks. Instrumental evaluations were performed at the beginning of the treatment, and subsequently once a fortnight, by colour measurements (Minolta Chroma Meter), hydration measurements (Evaporimeter EP1, Servo Med and Corneometer CM 820, Courage + Khazaka) and by 20 MHz B-scanning (Dermascan C, Cortex Technology). The echographic images were processed by a program, enabling a numerical representation of the pictorial data, based on the attribution of fictional values to the echoes' amplitudes, the possibility of selecting amplitude bands of interest, the binary transformation of the image, and the calculation of the extension of areas reflecting within the same amplitude range. Amplitude bands of interest were a 201–255 and a 150–255 interval assessing the entry echo, and a 0–10 and a 0–30 interval highlighting the hypo-echogenic parts of the dermis. Echographic measurements showed a progressive reduction of skin thickness, a decrease in the superficial hyper-echogenic band corresponding to epidermis and a thinning of the echolucent part of the dermis at tacalcitol-treated sites. Modifications of the skin at placebo-treated lesions were less marked according to clinical evaluation and to colour and hydration determinations. The echographic approach proved to be an effective way to evaluate the response to treatment by psoriatic skin, at the same time making possible skin thickness measurements, evaluation of the echographic equivalent of epidermis thickness, and assessment of the hypo-echogenic part of the dermis, corresponding to the inflammatory component.

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ABSTRACT

Topical Calcipotriol vs Clobetasol: Instrumental findings

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A new important advance in the treatment of psoriasis has been achieved by the development of vitamin D analogues for topical use. In this study, topical calcipotriol has been compared with clobetasol in the treatment of psoriasis. The efficacy of the treatments has been evaluated both visually (PASI index) and instrumentally (transepidermal water loss and blood flow measurements). Treatment was given for 3 weeks, twice a day to 12 psoriatic males with plaques on their limbs. Both treatments induced a significant improvement of skin lesions as monitored by PASI score. Treatment with clobetasol resulted in a quicker recovery of the skin barrier than with calcipotriol. No differences were found between the two products concerning blood flow recordings.

Conclusion: after 3 weeks of treatment the efficacy of calcipotriol is comparable to clobetasol, thus supporting the use of vitamin D analogues for the topical long-term treatment of psoriasis.

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ABSTRACT

Clinical Features of Psoriasis in Drug Abusers

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A total of 623 drug abusers have been screened for dermatologic diseases. Skin diseases were detected in 28% of these drug abusers; this was almost 4 times more than in the reference group. Psoriasis was found 10 times more frequently in these patients than in the controls.

Clinical features of psoriasis have been examined in 53 drug abusers. The incidence rate of complicated (exudative) forms of psoriasis, associated with manifest subjective sensations, was higher in this patient population than generally in psoriasis. The psoriasis in drug abusers tends to be much more active and resistant to most forms of therapy. Laboratory studies have revealed a number of disorders contributing to the more severe course of psoriasis in drug abusers than in the reference group: (1) decreased natural killer-cell activity, (2) decreased production of lymphokines which enhance natural killer cell activity (i.e. interferon gamma), and (3) an increased proportion of T cells with a suppressor phenotype.

These factors may exacerbate the course of psoriasis in drug abusers.

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ABSTRACT

Immunotherapy of Psoriasis with Anti-CD4 Monoclonal Antibodies

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Psoriasis is a chronic inflammatory skin disease in the pathogenesis of which activated T cells in the skin are thought to play an important role. CD25+ CD4+ T cells are the main inflammatory cell type found in active lesional skin. Cyclosporin is an effective treatment for psoriasis and anti-CD3 MoAb and peptide T have been occasionally reported to improve the psoriatic skin lesions.

In a recent study we treated 3 patients with severe and/or recalcitrant psoriasis by infusing anti-VD4 MoAb (0.2 to 0.8 mg/kg/day for 8 consecutive days). Clinical improvement, as assessed by a decrease in the PASI, started between day 4 and day 8, culminating around day 21. Relapse was observed in all 3 patients between day 30 and day 60. Anti-CD4 MoAb-mediated clinical improvement was not associated with a decrease in the number of CD4+ T cells in the blood and in the skin and was achieved using concentrations of MoAb which were only one-hundredth of that needed to saturate all CD4 molecules on T cells. Indirect evidence suggests that the mode of action of anti-CD4 MoAb in psoriasis is by down-regulation of CD4+ T cell activation in the skin, leading to the reduced production of inflammatory cytokines. This study suggests that anti-CD4 MoAb can be effective in clearing psoriatic lesions without deleterious immunosuppressive effects.

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POSTER AWARDS
(to the value of 1 million ITL each)

The Jury: Lennart Juhlin
Lionel Fry
Aldo Finzi

The Winners

No. 5, P. D. Pigatto et al. [Methotrexate in psoriatic polyarthritis].

No. 13, M. Bacharach-Buhles et al. [Are the capillaries in psoriasis really elongated?]

No. 16, J. R. Bjerke et al. [Fc receptors in skin and serum from patients with psoriasis before and after therapy]

No. 31, S. Seidenari et al. [Instrumental evaluation of psoriatic lesions treated with Tacalcitol]

CLOSING REMARKS (C. Scarpa)

At the conclusion of these 3 days of hard work (I could say 3 years), I wonder how best to sum up my feelings.

In 6 months' time, after having digested the Proceedings of this meeting, each of us will have a better idea of the results of the whole event. I suppose that until then, only skin deep impressions will have been gathered!

Concerning pathogenesis, we can now perhaps ask ourselves if the profusion of data has led to confusion or whether a new beacon is shining in the distance which could guide us all in the right direction.

Cyclical or combination therapy, using new drugs and strongly individualizing them, appears from now on to be a basic goal we should aim at. We can no longer avoid doing so, in my opinion, and it will be increasingly used by dermatologists. This is the future we can easily foresee.

Finally, I declare here and now my intention to resign from this creation, this Symposium I mean, and I hand over the reins to my old friend, Professor Aldo Finzi, of Milan. He will shoulder the burden next time, in 5 years, and will plan the meeting in Milan. I could find no better successor and it gives me great satisfaction to entrust the quinquennial European Symposium to him.

Here in Trieste we are proud to have hosted four of these meetings.

I would like now to thank Dr Franco Kokelj, who so brilliantly performed his task as General Secretary of the Symposium, and also my staff. My thanks go as well to all moderators, all presenters, all delegates and all participants.

The Fourth European Symposium on Psoriasis is herewith declared concluded.

Thank you all once again.

Pergolizzi S
Persia S
Pietravalle M
Pigatto PD
Pileri M
Pincelli C
Pollenghi MH
Polignano A
Polito R
Prinz JC
Puddu P
Van Der Raay MH
Rabbiosi G
Rantuccio F
Ranza R
Rebora A
Riccieri V
De Rie MA
Romani I
Romano E
Rosenberg JEW
Rovetta G
Ruju GP
Sacerdoti G
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