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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.

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

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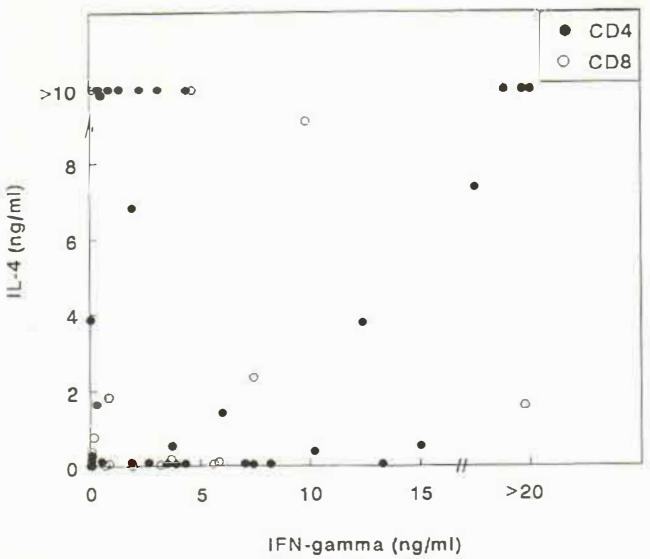

Fig. 1. IL-4 and IFN-γ secretion patterns of CD4⁺ (*closed circles*) or CD8⁺ (*open circles*) T-cell clones from psoriatic lesional skin. Secreted lymphokines were measured in supernatants of 24 h cultures of T-cell clones (10^6 cells/ml) stimulated with PHA and PMA and the values are expressed in ng/ml.

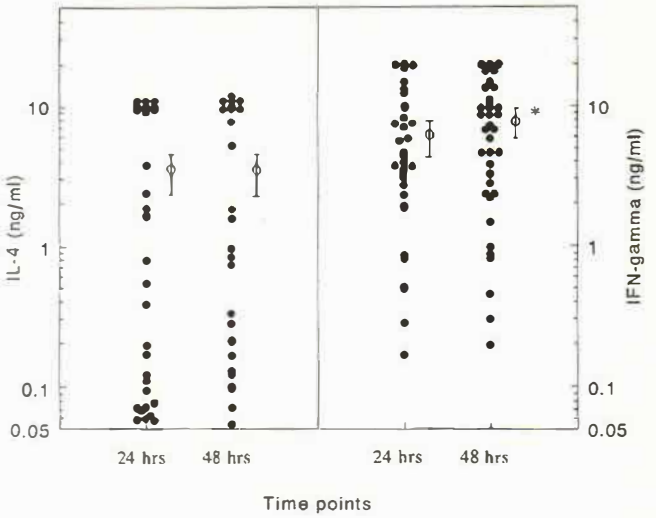

Fig. 2. TLC from 49 clones were stimulated by polyclonal activators (PHA + PMA). The conditioned media were harvested after 24 and 48 h of culture and IL-4 and IFN-γ activity was determined. Means ± SEM of the population are indicated. IFN-γ production is significantly higher on day 2 ($*p<0.01$).

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.

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 supernatants ($*p<0.05$).

γδ⁺ 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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REFERENCES

1. Wong RL, Winslow CM, Cooper KD. The mechanisms of action of cyclosporin A in the treatment of psoriasis. Immunol Today 1993; 14: 69–74.
2. Jegasothy BV, Ackerman CD, Todo S, Fung JJ, Abu-Elmagd K, Starzl TE. Tacrolimus (FK 506) – a new therapeutic agent for severe recalcitrant psoriasis. Arch Dermatol 1992; 128: 781–785.
3. Prinz J, Braun-Falco O, Meurer M, Daddona P, Reiter C, Rieber P, Riethmüller G. Chimaeric CD4 monoclonal antibody in treatment of generalized pustular psoriasis. Lancet 1991; 338: 320–321.
4. Mosmann TR, Coffman RL. Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; 7: 145–173.
5. Paliard X, De Waal-Malefijt R, IJssel H, Blanchard D, Chrétien I, Abrams JS, De Vries JE, Spits H. Simultaneous production of interleukin 2, interleukin 4 and interferon-gamma by activated human CD4⁺ and CD8⁺ T cell clones. J Immunol 1988; 141: 849–855.
6. Kapsenberg ML, Wierenga EA, Stiekema FEM, Tiggelman AMBC, Bos JD. Th1 lymphokine production profiles of nickel-specific CD4⁺ T-lymphocyte clones from nickel contact allergic and non-allergic individuals. J Invest Dermatol 1992; 98: 59–63.
7. Haanen JBAG, De Waal-Malefijt R, Res PCM, Kraakman EM, Ottenhof THM, De Vries RRP, Spits H. Selection of a human T helper type 1-like T cell subset by Mycobacteria. J Exp Med 1991; 174: 583–592.
8. Van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML. High frequency of IL-4 producing CD4⁺ allergen-specific T lymphocytes in atopic dermatitis lesional skin. J Invest Dermatol 1991; 97: 389–394.
9. Borst J, Van Dongen JJM, Bolhuis RLH, Peters PJ, Hafler DA, De Vries E, Van de Griend RJ. Distinct molecular forms of human T cell receptor γ/δ on viable cells by a monoclonal antibody. J Exp Med 1988; 167: 1625–1644.
10. Van der Pouw-Kraan TC, Van Kooten C, Rensink I, Aarden L. Interleukin (IL)-4 production by human T cells: differential regulations of IL-4 vs. IL-2 production. Eur J Immunol 1992; 22: 1237–1241.
11. Van der Meide PH, Dubbeld M, Schellekens H. Monoclonal antibodies to human immune interferon and their use in a sensitive solid-phase ELISA. J Immunol Methods 1985; 79: 293–305.
12. Gillis S, Ferm W, Ou W, Smith KA. T cell growth factor: parameters of production and a quantitative microassay for activity. J Immunol 1978; 120: 2027–2032.
13. Baadsgaard O, Tong P, Elder JT, et al. UM4D4⁺ (CDw60) T cells are compartmentalized into psoriatic skin and release lymphokines that induce a keratinocyte phenotype expressed in psoriatic lesions. J Invest Dermatol 1990; 95: 275–282.
14. Bos JD, Hagenaars C, Das PK, Krieg SR, Voom WJ, Kapsenberg ML. Predominance of "memory" T cells (CD4⁺, CDw29⁺) over "naive" T cells (CD4⁺, CD45R⁺) in both normal and diseased human skin. Arch Dermatol Res 1989; 281: 24–30.
15. Nikaein A, Morris L, Phillips C, Soliman M, Ordonez G, Silverman A, Stone MJ, Menter A. Characterization of T-cell clones generated from skin of patients with psoriasis. J Am Acad Dermatol 1993; 28: 551–557.
16. Kabelitz D, Pechhold K, Bender A, Wesselborg J, Wesch D, Friese K, Janssen O. Activation and activation-driven death of human γδ T cells. Immunol Rev 1991; 120: 71–88.
17. Baker BS, Bokth S, Powles A, Garioch JJ, Lewis H, Valdimarsson H, Fry L. Group A streptococcal antigen-specific T lymphocytes in guttate psoriatic lesions. Br J Dermatol 1993; 128: 493–499.
18. Gomi J, Shiohara T, Munakata T, et al. Interleukin 1 tumor necrosis factor-α, and interferon-γ in psoriasis. Arch Dermatol 1991; 127: 827–830.
19. Takematsu H, Tagami H. Interleukin-2, soluble interleukin-2 receptor and interferon-γ in the suction blister fluids from psoriatic skin. Arch Dermatol Res 1990; 282: 149–152.
20. Barker JNWN, Karabin GD, Stoof TJ, Sarma VJ, Dixit VM, Nickoloff BJ. Detection of interferon-gamma mRNA in psoriatic epidermis by polymerase chain reaction. J Dermatol Sci 1991; 2: 106–111.
21. Nickoloff BJ, Mitra RS, Elder JT, Fisher GJ, Voorhees JJ. Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factorα production in keratinocytes cultured from psoriatic lesions. Br J Dermatol 1989; 121: 164–174.
22. Cai JP, Falanga V, Taylor JR, Chin YH. Transforming growth factor-beta differentially regulates the adhesiveness of normal and psoriatic dermal microvascular endothelial cells for peripheral blood mononuclear cells. J Invest Dermatol 1992; 98: 405–409.
23. Wierenga EA, Snoek M, Jansen HM, Bos JD, Van Lier RH, Kapsenberg ML. Human atopen-specific types 1 and 2 T helper cell clones. J Immunol 1991; 147: 2942–2949.