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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-$\gamma\delta^+$ T cell lines to cytokine-stimulated and unstimulated endothelium is significantly increased as compared with that of $\alpha\beta$ T cell lines (a representative example of the adhesion of two TCR-$\alpha\beta$ T cell lines and two TCR-$\gamma\delta$T cell lines is illustrated in Table IV). This increased adhesion could not be blocked to TCR-$\alpha\beta$ 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-$\gamma\delta$ T cells to endothelium is surprising, because relatively few TCR-$\gamma\delta^+$ 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-$\gamma\delta$ 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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