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Reappraisal of in situ immunophenotypic analysis of psoriasis skin: interaction of activated HLA-DR$^+$ immunocompetent cells and endothelial cells is a major feature of psoriatic lesions

O. J. de Boer[1], C. M. van der Loos[2], F. Hamerlinck[1], J. D. Bos[1], P. K. Das[1,2]

[1] Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
[2] Department of Pathology, University of Amsterdam, Academisch Medisch Centrum, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

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Abstract. Psoriasis is an inflammatory skin disease of unknown aetiology. Many observations indicate that T cells play an important role in the pathogenesis of the disease. Upregulation of MHC class-II molecules on immunocompetent cells, endothelial cells and keratinocytes on lesional psoriatic skin has been regarded as a hallmark of the disease. However, there is some controversy in the literature regarding the cell types expressing class-II molecules and there is limited information about the presence of immune cells other than T cells and antigen presenting cells in the cellular infiltrates of psoriatic skin. We therefore reinvestigated the subject using immunocytochemical single and multiple staining techniques. In agreement with earlier reports, our studies showed that the cellular infiltrates in lesional skin consist largely of HLA-DR$^+$/IL-2R$^+$ T cells, HLA-DR$^+$/CD1a$^+$ Langerhans cells, and HLA-DR$^+$/CD68$^+$ macrophages. We found increased HLA-DR expression mostly on immunocompetent cells and endothelial cells, but no prominent HLA-DR expression on keratinocytes in lesional psoriatic skin. Upregulation of HLA-DR on endothelial cells and in mononuclear infiltrates was also evident in the non-lesional skin of psoriatic patients as compared with normal controls. B cells and natural killer cells were also found in the cellular infiltrates in lesional psoriatic skin. In spite of the presence of a large amount of activated T cells in the epidermis, we found that HLA-DR expression on keratinocytes was not a major feature of psoriatic skin.

Key words: Psoriasis – HLA-DR – Immunocompetent cells

Psoriasis is an inflammatory skin disease with hyperplasia of keratinocytes (KC), profound vascular changes and infiltrates of immunocompetent cells in the lesions [10, 34]. Studies of immunocompetent cells in skin biopsy specimens from lesional psoriatic skin have indicated the presence of a high proportion of interating HLA-DR$^+$/interleukin-2-receptor-positive (IL-2R$^+$) CD4 T cells [33] and HLA-DR$^+$/CD1a$^+$ Langerhans cells (LC) [6, 15]. Among other abnormalities in cellular infiltrates increased numbers of CD8$^+$ T cells [11], CD56$^+$ natural killer (NK) cells and F-XIIIa$^+$/HLA-DR$^+$/CD1a$^-$ dendrocytes have also been reported [30]. On the other hand, there are conflicting reports on the presence of B cells in psoriatic skin [7, 10, 13]. It is now widely believed that T lymphocytes together with other immunocompetent cells and their mediators cause increased epidermal proliferation [9, 15, 29, 40]. Furthermore, KC have been reported to be activated because at lesional sites they express higher levels of Fcγ receptors [25], β1 integrins [22], ICAM-1 [24], and (in some, but not all, reports) MHC class-II molecules (HLA-DR) [16, 36].

There remains controversy about the phenotypic composition of the cellular infiltrates in lesional and non-lesional psoriatic skin and about the expression of MHC class-II molecules on different cell types, particularly KC. The activity of the lesions and the presence of arthritis have been implicated as a contributory factor to the expression of HLA-DR by KC [17]. Furthermore, previous studies have been carried out using one kind of HLA-DR monoclonal antibody (MAb). In a previous study in our laboratory we have observed that varied expression of MHC class-II molecules is found in tissues by applying a panel of different commercially available MAbs to HLA-DR [35]. Consequently, we used a panel of HLA-DR antibodies to confirm the expression of HLA-DR staining on different cell types. Since it is also known that non-lesional skin of psoriasis patients shows many aberrant features [31, 32], the present investigation reevaluated the phenotypic characters of different cells in specimens from both lesional and non-lesional psoriatic skin as compared with normal human skin by single and multiple immunohistochemical staining methods.

Materials and methods

Patients

Seven patients (four male, three females; mean age 41.2 ± 11 years) with psoriasis vulgaris who had had no treatment for the previous

Correspondence to: P. K. Das

2 months were selected for the study, and gave informed consent to their participation in the study. Biopsies from five age-matched healthy individuals with no previous history of any skin abnormality (e.g. acne, contact dermatitis or infection) served as controls. Abdominal skin from three individuals undergoing plastic surgery was obtained as additional control material.

Specimens

Punch biopsies (4 mm) from the inside border of stable or slowly enlarging lesions on the trunk were obtained under lidocaine/adrenalin anaesthesia. Specimens of non-lesional skin from the same individuals with no signs of scaling, erythema or irritation and at least 5 cm away from any visible lesion were also included in the study. The biopsy specimens were immediately frozen in liquid nitrogen and stored at −80 °C. Serial cryostat sections (5 μm) were cut, fixed in cold acetone and stored at −20 °C until use.

Immunohistochemistry

Single immunohistochemical staining was performed using a three-stage indirect peroxidase technique as described previously [27]. In brief, the tissue sections were incubated with appropriate dilutions of the MAbs listed in Table 1. The sections were then incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulins (RAM) (Dakopatts, Glostrup, Denmark) followed by an additional incubation with HRP-conjugated swine anti-rabbit (SAR) immunoglobulins (Dakopatts). Before counterstaining with haematoxylin, HRP activity was detected with H_2O_2 as substrate and 3-amino-9-ethyl carbazole (AEC; Sigma, St. Louis, Mo., USA) as chromogen [18].

Double immunohistochemical staining was performed using various previously described protocols which depend on the type of combination of the primary MAbs used [27, 28]. For most of the double stainings, combinations of a fluorescein thiocyanate (FITC)-conjugated and an unlabelled MAb were used as the primary steps (protocol 1). First, the sections were incubated with the unlabelled MAb, followed by alkaline phosphatase (AP)-conjugated RAM (TAGO, Burlingame, Calif., USA). After a blocking step with normal mouse serum (Dakopatts), the sections were incubated with the second, FITC-labelled, antibody, followed by rabbit anti-FITC and HRP-SAR (both Dakopatts). Finally, first the AP activity was visualized using naphthol-AS-MX phosphate as substrate and fast blue BB (FBBB) as chromogen (both Sigma), and then HRP activity was revealed in a way similar to that for the single staining.

The second type of double staining consisted of a combination of a polyclonal rabbit and a monoclonal mouse antibody. According to this protocol, the sections were first incubated with a mixture of the polyclonal antiserum and the MAb. After washing with phosphate-buffered saline (PBS) the sections were incubated with a mixture of HRP-conjugated SAR immunoglobulins and AP-conjugated goat anti-mouse (GAM) immunoglobulins (TAGO). Finally, AP and HRP activities were detected in a way similar to that outlined above.

Triple staining

Triple staining was performed using one unlabelled, one biotinylated and a FITC-conjugated antibody [28]. In this protocol, the sections were first incubated with streptavidin followed by D-biotin, to avoid background staining. The sections were then incubated with the unlabelled MAb, followed by GAM-galactosidase (GAL; Southern Biotechnology Associated, Burmingham, Ala., USA). The sections were then incubated with normal mouse serum (NMS), followed by a mixture of the second, biotinylated, MAb and the third, FITC-conjugated, MAb. The next incubation step consisted of a mixture of avidin biotin complex (ABC)-HRP (Dakopatts) and rabbit

Table 1. MAbs uses in this study

MAb	Source	CD	Specificity	Reference	Dilution
OKT6	Ortho	CD1a	LC		1:50
Leu-4	B & D	CD3	Pan T		1:20
Leu-3a + 3b	B & D	CD4	Th/i		Undiluted
Leu-2a	B& D	CD8	Ts/c		1:20
Leu-7	B & D	–	NK		1:20
Leu-14	B & D	CD22	Pan-B		Undiluted
DK22	Dako	–	HLA-DR β-chain (DR, DP, DQw1; not DQw3)	[37]	50
L243	B & D	–	HLA-DR, α-chain (DR, DX)	[12, 23]	1:100
CR3/43	Dako	–	HLA-DR, β-chain (DR, DP, DO)	[42]	1:50
VICY1	Dako	–	HLA-DR		1:100
TAL. 1B5	Dako	–	HLA-DR, α-chain (HLA-DR)	[39]	1:10
Factor VIII	Dako	–	EC		1:200
PAL-E	Sanbio	–	EC		1:10
EN-4	Sanbio	–	EC		1:250
UEA-1	Dako	–	EC		1:100
βF1	TCS	–	αβ TCR		1:50
TCRδ1	TCS	–	γδ TCR, constant		1:50
δTCS1	TCS	–	γδ TCR, variable		1:50
IL-2R	Biotest	–	Interleukin-2 receptor		1:100
CK1	Dako	–	Human keratin		1:50

B & D, Becton and Dickinson, Mountain View, Calif.; Dako, Dakopatts, Glostrup, Denmark; Sanbio, Sanbio BV, Uden, The Netherlands; Ortho, Ortho Diagnostics, Raritan, NJ, USA; TCS, T cell Sciences, Cambridge Mass., USA; Biotest, Frankfurt/Main, Germany
CD, Cluster of differentiation; LC, Langerhans cells; EC, endothelial cells; TCR: T cell receptor

anti-FITC antiserum, followed by SAR-AP. Finally, respective enzyme activities were developed: first the β-galactosidase activity was detected using bromochloroindolyl galactopyranoside/ferri-ferrocyanide [8] (green), followed by AP activity (blue) and HRP activity (red) as described above.

At regular intervals during each staining with new samples of MAb and their combinations both positive and negative controls were always included.

Quantification

Single- or double-stained cells were counted in the epidermal and dermal compartments as previously described [11]. The differences between lesional and non-lesional psoriatic skin and normal healthy control skin were analysed using one-way analysis of variance and a two-sample *t*-test.

Results

The phenotypes of the interacting cellular compositions in different specimens were evaluated by both single- and double-staining methods and are summarized in Table 2.

Langerhans cells

An immunocytochemical single-staining pattern is illustrated in Fig. 1. The network pattern of $CD1a^+$ cells in the epidermis of normal skin appeared to be less frequent in lesional skin. The staining was observed mainly on the cell bodies, and to a lesser extent on the dendrites of $CD1a^+$ cells in the epidermis, suggesting shorter dendrites than in non-lesional specimens, and distinctly shorter dendrites than those found in normal skin. Besides the normal network pattern of $CD1a^+$ cells in the epidermis few of these cells were observed in the papillary and reticular dermis of normal skin. Some LC were found intraepithelially in sweat glands and hair follicles. In non-lesional skin the epidermal staining pattern of LC was similar to that found in normal skin, but increased numbers of $CD1a^+$ cells were found in the dermis, especially perivascularly located. Significantly increased numbers of $CD1a^+$ cells were found within clusters of perivascular infiltrates (Table 2).

T cells

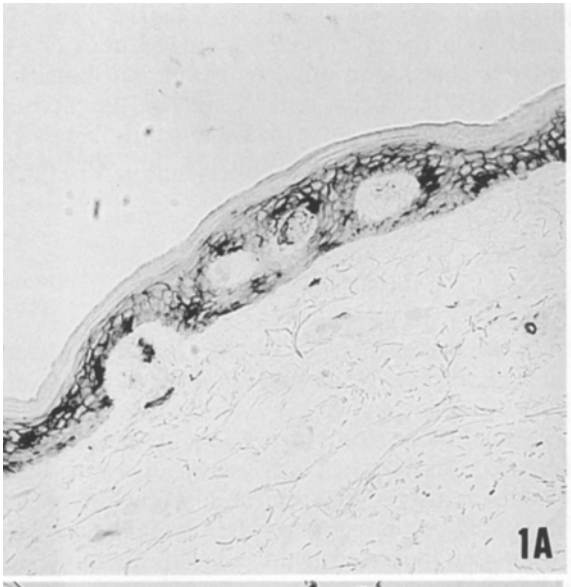
The results are summarized in Table 2. Few T cells were found in normal skin. When present, most of them were usually perivascular, and rarely found in the epidermis. The majority of these T cells were $CD4^+$. In non-lesional skin, increased numbers of T cells, especially of the CD4 subset, were found in the perivascular region of the reticular as well as the papillary dermis. Some of these T cells were seen in close attachment with the basement membrane of the papillary tip of the epidermis. In the same specimens both $CD4^+$ and $CD8^+$ T cells (although few) were also seen in the epidermis. In lesional specimens proportionately more $CD8^+$ intraepidermal cells were found. In the same specimens, significantly increased numbers of both T-cell subsets (proportionately more $Cd4^+$) were found clustered around the vessels in the dermis. By double staining, most of these T cells in involved psoriatic skin were found to be $IL\text{-}2R^+$. Interestingly, a large number of these $IL\text{-}2R^+$ T cells in lesional skin were found to be attached to the basement membrane zone in a necklace-like fashion and also in, or in close contact with, basal KC (as illustrated in Fig. 2). Increased numbers of $IL\text{-}2R^+$ T-cell subsets were found in lesional skin as compared with both non-lesional and normal skin specimens (Table 3).

Table 2. Cellular composition of normal, non-lesional and lesional psoriatic skin as evaluated by single and double immunostaining techniques

	Normal skin	Non-involved skin	Involved skin
$CD3^+$ T lymphocytes	Mostly TCR-$\alpha\beta^+$ (<10% TCR-$\gamma\delta$) 35 ± 9/mm	Mostly TCR-$\alpha\beta$ (<10% TCR-$\gamma\delta$) 65 ± 22/mm*	Mostly TCR-$\alpha\beta$ (<10% TCR-$\gamma\delta^+$) 260 ± 80/mm**
$CD4^+$ T lymphocytes	Epidermis: 0.6 ± 0.5/mm Dermis: 16 ± 5/mm	Epidermis: 1.2 ± 0.7/mm Dermis: 42 ± 17/mm*	Epidermis: 8 ± 5/mm** Dermis: 138 ± 40/mm**
$CD8^+$ T lymphocytes	Epidermis: 0.2 ± 0.2/mm Dermis: 5 ± 3/mm	Epidermis: 0.7 ± 0.4/mm Dermis: 14 ± 8/mm	Epidermis: 11 ± 4/mm** Dermis: 79 ± 40/mm**
$CD1a^+$ Langerhans cells[a]	Network pattern in epidermis; hardly any $CD1a^+$ cells in dermis; $HLA\text{-}DR^+$	Network attern, similar to normal skin, $HLA\text{-}DR^+$; increased numbers of cells in dermis	Loss of network pattern in epidermis; located in the more basal layers; many $CD1a^+$ cells in the dermis, especially
$CD68^+$ macrophages	Very few in dermis (counting not done)	Increased tendency (counting not done)	Increased in dermis and epidermis (counting not done)
$CD56^+$ NK cells	None	0.11 ± 0.27/mm	6.2 ± 2.1/mm**, mostly at epidermal/dermal junction; some scattered in dermis and epidermis
$CD19^+$ B lymphocytes	None	None	7.3 ± 2.3/mm**; all in dermis

[a] Because of their dendritic morphology accurate counting of this cell type not done (see also Table 3)

* Increased tendency: $P < 0.15$ (as compared with normal skin); ** Significantly increased: $P < 0.05$ (as compared with normal skin)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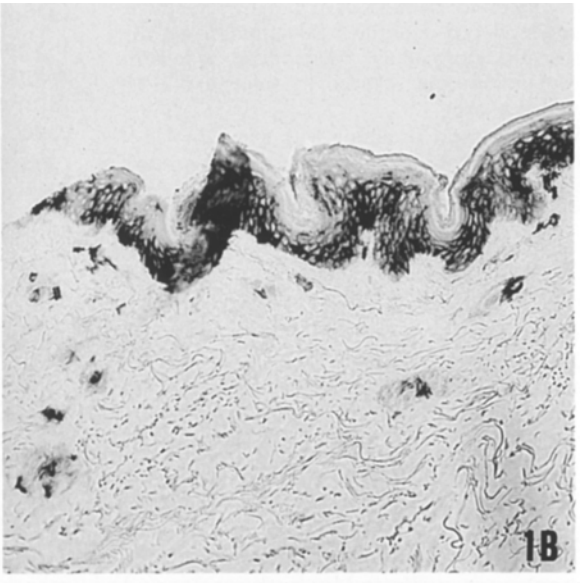
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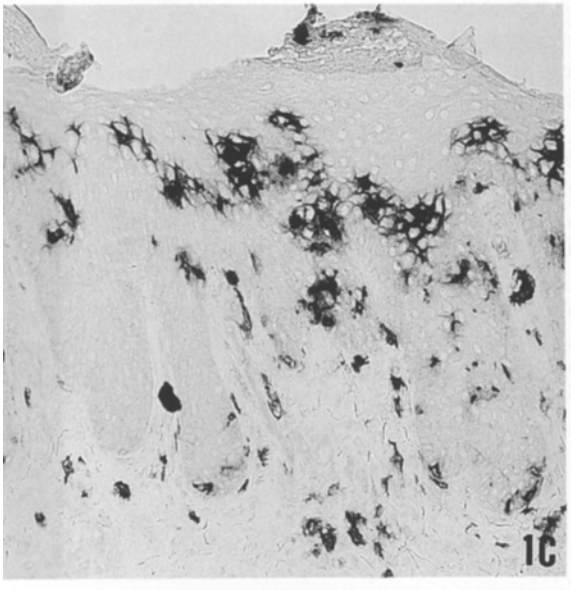
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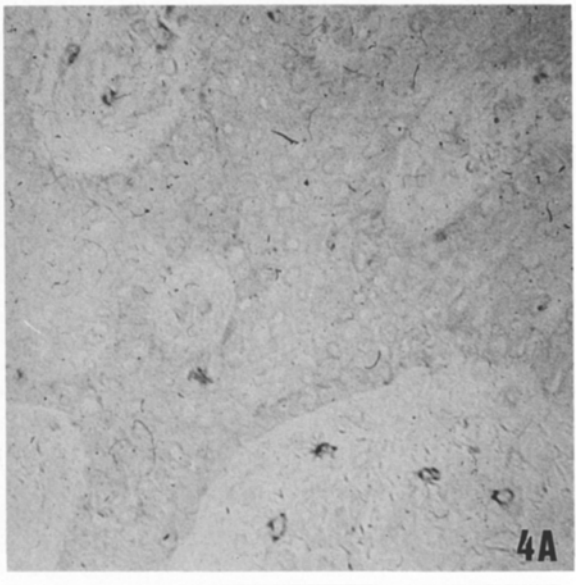
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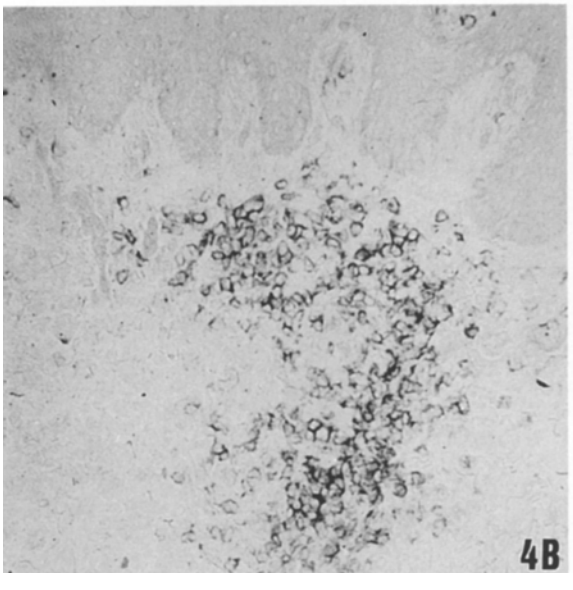
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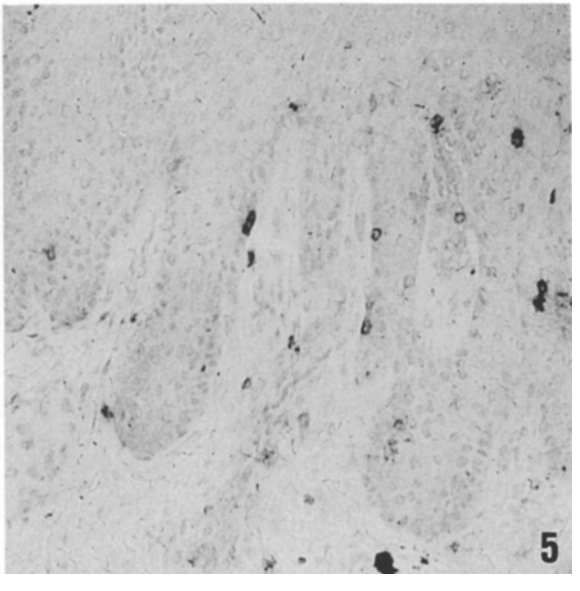
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Table 3. Expression of HLA-DR$^+$ and IL-2R$^+$ cellular components in normal, non-lesional and lesional skin

	Normal skin	Non-lesional skin	Lesional skin
HLA-DR$^+$/CD3$^+$	10%	40%	>80%
HLA-DR$^+$/CD1a$^+$	>80%	>80%	Approx 100%
HLA-DR$^+$/F-VIII	Most vessels ±	Most vessels +	Most vessels + + +
HLA-DR$^+$/CK1$^+$	None	None	Occasionally focal in basal layer
HLA-DR$^+$/CD19$^+$	ND	ND	Approx 100%
HLA-DR$^+$/CD68	20%	40%	Approx 100%
IL2-R$^+$/CD3$^+$	20–40%	20–40%	40–60%

±, weak staining; +, positive staining; + + +, strong staining

When the T cells in all the specimens were analysed at receptor level, it appeared that most expressed the $\alpha\beta$ T-cell receptor (TCR-$\alpha\beta$). Although the total number of $\gamma\delta^+$ T cells in psoriatic lesional skin was increased as compared with non-lesional and normal skin (Fig. 3), they were not found to be a prominent feature of any of the specimens.

B cells

B cells were occasionally seen in non-lesional and normal skin specimens as determined with MAbs against CD19 and CD22. Scattered B cells were seen in the upper dermis and, occasionally, in the epidermis of all lesional skin specimens (Fig. 4a). In two of the lesional specimens B cells were observed as clustered infiltrate in the reticular dermis (Fig. 4b). It should be noted that when similar stainings were performed on other inflammatory skin specimens, e.g. lesional specimens from contact dermatitis patients, no positively stained cells were found (data not shown). Although stainings with antibodies to surface immunoglobulin isotypes (IgM, IgG, IgA and IgD) were also performed, the results were unsatisfactory because of non-specific staining.

Natural killer cells

Leu-7$^+$ cells in normal and non-lesional skin were only found in a minority of skin specimens. Only nerve fibres showed cross-reactivity with the Leu-7 antibody in these specimens. On the other hand, in lesional sections Leu-7$^+$ cells were seen around the basement membrane in close proximity to the basal KC, and also a few in the epidermis. Increased numbers of NK cells were found scattered in the dermis, as well as in perivascular infiltrates in the lesional skin (Table 2, Fig. 5).

Endothelial cells and keratinocytes

We investigated possible changes in phenotype of endothelium using MAbs against Factor VIII, PAL-E and En-4, and lectin *Ulex europaeus* agglutinin (UEA-1). The only difference observed among the various specimens was an increased number of vessels in lesional skin. No difference in staining intensity between the various specimens was found with any of the antibodies used or with UEA-1. Interestingly, UEA-1 stained the uppermost KC (stratum granulosum) in normal skin and more layers of suprabasal KC in non-lesional skin (stratum spinosum), whereas in lesional skin progressively more layers of the stratum spinosum, reaching the basal layers, were stained with increasing intensities (Fig. 6).

Staining pattern of MHC class II

The various cell types expressing HLA-DR were evaluated by single staining of serial sections as well as by double and triple staining. Single staining was carried out using five different commercially available MAbs. Double and triple staining was performed with the CR3/43 antibody. The status of MHC class-II expression by different cells in both lesional and non-lesional specimens appeared to bear no relationship to disease activity. The single-staining patterns with all these MAbs in lesional skin specimens are illustrated in Fig. 7. The MAbs TAL. 1B5, CR3/43 and L243 all gave similar staining results. With DK22, however, the intensity of stained cells was significantly less than those identified by the other anti-HLA-DR MAbs. Similarly, VICY1 also showed a more limited staining pattern. In all sections fewer dermal mononuclear cells were positive, while the staining on endothelial cells (EC) was weaker.

In normal control specimens TAL. 1B5, CR3/43 and L243 with EC, and with stronger intensity on both cell bodies and dendrites of Epidermal LC. Mononuclear cells in these specimens stained intensely with these MAbs. In non-lesional skin these MAbs reacted with EC and LC with higher intensities than in normal skin. The mononuclear infiltrates, if present, were strongly positive with TAL. 1B5, CR3/43 and L243. Al-

Fig. 1A–C. Immunoperoxidase staining with MAb OKT6 on **A** normal, **B** non-lesional and **C** lesional psoriatic skin. Note the network staining pattern in normal skin and non-lesional skin (× 140)

Fig. 4A, B. Immunohistological demonstration of B cells in lesional skin sections. **A** Scattered CD22$^+$ B cells; **B** clustered CD22$^+$ B cells in the infiltrates (**A** × 225; **B** × 140)

Fig. 5. Leu-7 staining on lesional skin; note the scattered NK cells, some of them adhering to basal KC (× 140)

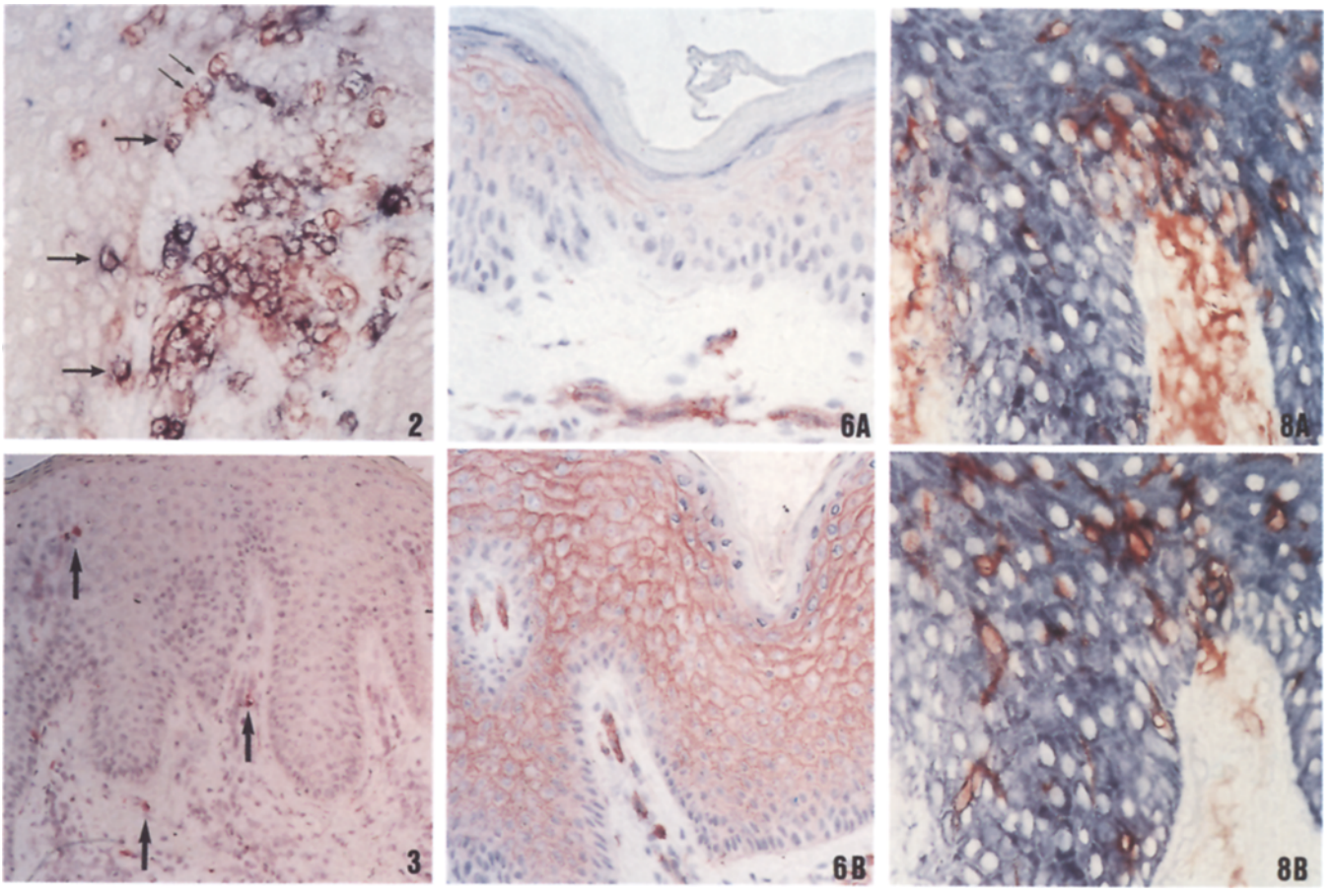

Fig. 2. Double staining with CD3 *(red)* and IL-2R *(blue)*. *Single arrows*, double-stained cells; *double small arrows*, necklace type of orientation of IL2R$^+$T cells ($\times$ 350)

Fig. 3. TCR-$\gamma\delta$ T cells in lesional section *(stained purple)*. Note only a few cells (some indicated by *arrows*) are positively stained ($\times$ 140)

Fig. 6A, B. A comparative staining pattern with lectin UEA-1. **A** Non-lesional and **B** lesional skin. Note that in lesional skin almost the whole epidermis is positively stained. In addition, blood vessels are also stained ($\times$ 225)

Fig. 8A, B. Double immunostaining with **A** keratin/HLA-DR *(blue/red)* **B** keratin/OKT6 *(blue/red)*. Note that double stained cells in the epidermis are mostly HLA-DR$^+$OKT6$^+$ cells ($\times$ 350)

Fig. 9. A representative example of a triple staining with OKT6 *(green)* Leu-4 *(blue)* and HLA-DR *(red)* to illustrate the cellular interactions between activated T cells and LC within the infiltrates. *Arrows* with *R*, *G*, and *B* indicate single-stained red, green or blue cells, respectively; *curved arrows* indicate clusters of interacting cells ($\times$ 225)

though intensely stained HLA-DR$^+$ EC, LC and mononuclear infiltrates were prominent features of lesional skin, we found hardly any HLA-DR$^+$ KC with any of the MAbs. Only in the basal layer of the hyperproliferative epidermis was a weak, fuzzy, but focal staining with these three MAbs seen. The staining on the basal layer as observed with TAL. 1B5, CR3/43 and L243 in lesional skin was not visible with DK22, and VICY1 also showed a more limited staining pattern. In all sections fewer dermal mononuclear cells were positive, while the staining on EC was weaker, and epidermal LC were stained more on the cell body.

In order to find out exactly which population of cell types in the skin specimens were HLA-DR$^+$, double stainings were performed with the CR3/43 MAb. After double staining with the combinations CK1/OKT6, CK1/HLA-DR, and HLA-DR/OKT6 we were able to confirm that almost all the HLA-DR$^+$ cells in the (suprabasal) epidermis of lesional specimens were not KC (Fig. 8).

Furthermore, almost all the interacting immunocompetent cells (CD3$^+$, CD1a$^+$ and CD68$^+$) in the perivascular region of lesional skin were HLA-DR$^+$, whereas in non-lesional skin we also found increased HLA-DR

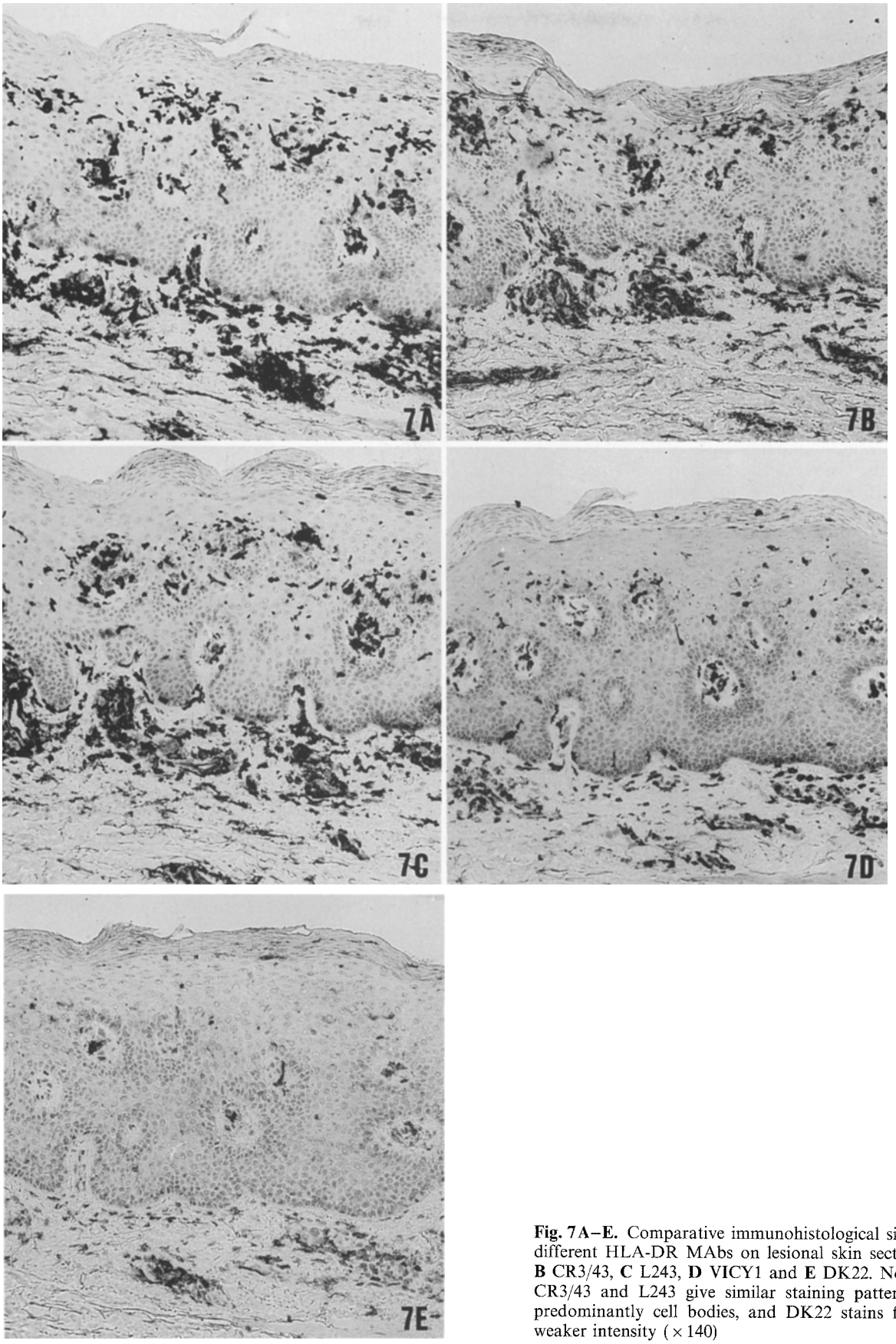

Fig. 7A–E. Comparative immunohistological single staining with different HLA-DR MAbs on lesional skin sections. **A** TAL.1B5, **B** CR3/43, **C** L243, **D** VICY1 and **E** DK22. Note that TAL.1B5, CR3/43 and L243 give similar staining patterns, VICY1 stains predominantly cell bodies, and DK22 stains fewer cells with a weaker intensity (× 140)

expression on T cells, macrophages, LC and EC in comparison with normal skin. Similarly, B cells in lesional skin were also HLA-DR$^+$ (data not shown).

In situ pattern of cellular interactions

Further triple stainings were performed to study the interacting pattern of different cell types in the infiltrates. These stainings were performed on sections from lesional, non-lesional and normal skin biopsy specimens. We performed triple stainings with the combinations FVIII/HLA-DR/CD3, FVIII/CD3/CD1a, FVIII/CD3/CD68, leu-7/CD3/CD1a, CD3/CD68/CD1a and CD1a/CD3/HLA-DR. A representative example of the last is illustrated in Fig. 9. This triple staining showed that HLA-DR$^+$ EC are surrounded by HLA-DR$^+$ T cells, HLA-DR$^+$ LC and HLA-DR$^+$ macrophages. In other triple stainings it was also shown that HLA-DR$^+$ macrophages are also present in the infiltrates (data not shown). Triple staining also showed the presence of some NK cells where T cells and antigen-presenting cells (APC) were found in lesional skin specimens. Furthermore, CD3/CD68/CD1a triple staining showed again that the interaction of these cells was mainly present as perivascular clusters (data not shown).

Discussion

It is well known that psoriatic lesions are characterized by epidermal hyperproliferation and localized perivascular infiltrates consisting of T cells, LC and macrophages [10, 34]. We have also found increased numbers of HLA-DR$^+$ T-cell subsets (CD4 and CD8), CD1a$^+$ LC and CD68$^+$ macrophages in lesional skin sections [10, 30]. Such increased infiltrates were also found in non-lesional skin specimens as compared with normal skin indicating that non-involved skin is also in a hyperimmune reactive state. Furthermore, we noted that the HLA-DR$^+$ IL$^-$2R$^+$ T-cell subsets (also in non-lesional skin) are in close contact with basal KC, suggesting that these interacting T cells recognize some epitopes of basal KC. As a result these T cells may secrete cytokines, leading basal KC to enter into an actively cycling state [5, 20]. Another explanation could be that T cells become activated regionally in an antigen-independent manner upon interaction with basal KC via specific adhesion receptors [2]. If this explanation is correct, it would be rational also to investigate phenotypic and functional characteristics of the T cells in non-lesional specimens; this is currently in progress in our laboratory. Whatever the exact pathway is for local T-cell activation, the end result is that T-cell-derived cytokines may influence KC proliferation [5, 20]. The majority of T cells in psoriasis and normal skin express $\alpha\beta$ T-cell receptors. Although the proportion of TCR-$\gamma\delta$ T cells in psoriasis as is increased, their numbers seem to be unaltered. The differential contribution by TCR-$\alpha\beta$ and TCR-$\gamma\delta$ T cells to the pathogenesis of psoriasis cannot, however, be determined from the results of the present study.

Interestingly, we oserved CD22$^+$CD19$^+$ B cells in all lesional skin specimens, which were mostly found scattered in the dermis in the region where T cells and APC were also present, but occasionally they were also found in the epidermis. The discrepancy between our findings and those reported in the literature regarding the presence of B cells could be due to differences in the methodology and reagents used. In previous studies, immunohistochemical analysis was performed using MAbs against the C3d receptor or antisera against surface immunoglobulins [10]. The function of these B cells in the pathogenesis of psoriasis remains unclear. We also found an increases presence of scattered NK cells in the dermis and in close contact with basal layer KC, which is compatible with some [30], but not all [10], earlier reports. Although NK cells contribute less to the inflammatory infiltrate than T cells, they may still play a role in the aetiology of the disease.

Besides the infiltrates of immunocompetent cells, EC and KC are known to undergo phenotypical changes [22, 24]. In this study, although increased numbers of immunoreactive EC were observed, no differences in staining intensity or pattern were observed among the skin sections studied. However, UEA-1 produced different staining patterns in the epidermal compartment between lesional, non-lesional and normal sections. The present findings on UEA-1 staining patterns are compatible with earlier reports of increased UEA-1 staining in both lesional and non-lesional skin [21]. EC from lesional, and to a lesser extent non-lesional, skin did show increased HLA-DR expression compared with normal skin. This indicates that EC in psoriatic skin are in an activated state, which facilitates the influx of various immunocompetent cells into both lesional and non-lesional sites. Indeed, an increased expression of various adhesion receptors and their ligands in both involved and non-involved psoriatic skin has been found (O. J. de Boer et al., submitted for publication).

Regarding the question as to the status of MHC class-II expression on various cell types in psoriatic skin, we found strong positivity on T cells, EC, LC and monocytes, but only occasionally and weakly on basal KC. Double stainings with HLA-DR confirmed that the positively stained suprabasal cells are not KC. Although there are differences in staining intensities by different MAbs to class II molecules, the present report showed that the expression of MHC class II on KC was not a major feature in psoriasis. It is well recognized that HLA-DR can be induced on most cell types, including KC after appropriate stimulation with cytokines, usually interferon-γ (IFN-γ) [1, 19]. IFN-γ, also known as immune interferon, is a cytokine which can be produced by NK cells and T cells, and virtually all cell types express receptors for this cytokine [41]. Reports concerning the presence of IFN-γ in psoriatic epidermis are contradictory. No IFN-γ could be detected in blister fluuid from lesional skin of psoriasis patients [38], and, using bioassays, no IFN-γ activity could be demonstrated in scale extracts from psoriasis patients [14]. In contrast, IFN-γ was found in scale extracts by immunoassays [14] and the presence of IFN-γ mRNA has been demonstrated in

epidermal sheets of psoriatic skin by the polymerase chain reaction [3]. Furthermore, Livden et al. demonstrated IFN-γ in the stratum basale of active plaques by immunohistochemical staining methods [26]. Interestingly, in our present study weak HLA-DR activity on epidermal cells occasionally appeared to be in the same area where IFN-γ was detected.

In conclusion, our studies indicate that interacting HLA-DR$^+$ cells ($\alpha\beta$ T cells, macrophages, LC and EC) but not HLA-DR$^+$ KC are a major feature of psoriatic skin. Furthermore, B cells and NK cells are present together with T cells and APC in lesions. Although the present thinking focuses mainly on T-cell mediated pathogenesis of the disease, the contribution of all immunocompetent cells present in skin infiltrates from psoriasis patients needs further investigation.

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