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Subpopulations of Mononuclear Cells in Microscopic Lesions of Psoriatic Patients. Selective Accumulation of Suppressor/Cytotoxic T Cells in Epidermis During the Evolution of the Lesion

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The age of microscopic lesions in psoriatic subjects was assessed from the stacking characteristics in the horny layer and related to type and density (cells/tissue volume) of mononuclear cells in the epidermis and the dermis determined by immunoperoxidase methods using monoclonal antibodies. Pan T cells (Lyt-2+, Lyt-3+, Leu-4+, OKT3+), T helper cells (Leu-3a+, OKT4+), T suppressor/cytotoxic cells (Leu-2a+, OKT8+), Ia+ cells and monocytes (OKM2+, BRL αmono+) were determined in epidermis and dermis. The psoriatic lesion was divided into regions underneath a parakeratotic and an orthohyperkeratotic/hypergranular portion of the horny layer and contrasted with perilesional and uninvolved psoriatic skin as well as with healthy skin. In the various regions and skin layers, the cell density was highest in parakeratosis and decreased toward normality with decreasing histologic abnormality. The relation between epidermal and dermal cell densities of the T-cell subsets was modified in the involved psoriatic skin with a selective preponderance of T suppressor/cytotoxic cells in the epidermis. The accumulation was present in the youngest lesion found (3 days) and cell densities were unchanged in older lesions. The finding suggests that the altered relationship in the subsets of T cells has an important role during the induction and progress of the psoriatic process in the skin.

The initial psoriatic lesion shows signs of epidermal inflammation and disturbance of keratinocyte maturation with increased cell proliferation [1]. The inflammatory reaction in the early lesions is variously described as mainly polymorphonuclear [2,3] or mononuclear [4,5]. It is clearly stated in such studies that the inflammatory infiltrate is a very early occurrence and presumably of importance during the initial phase of the psoriatic process. During the progress of the reaction, inflammatory cells always adhere to the most active part of the lesion containing T lymphocytes, macrophages, mast cells, and polymorphonuclear granulocytes [1,6–10]. In peripheral blood of psoriatics, abnormal proportions of subsets of lymphocytes are reported and the activity of T-cell subsets has also been altered [11–14].

In the present immunohistochemical study the skin infiltrate was analyzed with the aid of monoclonal antibodies defining functional T-lymphocyte subsets and other mononuclear cells. The hypothesis was advanced that a subset of cells might induce a proliferative and maturative disturbance of the keratinocytes present in psoriasis. In order to discriminate such effector cells, observations were analyzed per unit tissue volume (epidermis, reticular dermis) in various parts of the lesion. In order to gather material from very young lesions, microscopic psoriatic papules were selected. From earlier kinetic studies it is known that orthohyperkeratosis is a very early morphologic sign of alteration in the evolving psoriatic lesion [5]. The cellular infiltrate in the skin was therefore studied in regions within a lesion, in which, areas with orthokeratosis were differentiated from those with parakeratosis and compared with perilesional skin. Furthermore, based on the morphology of the horny layer, the age of the lesion was determined as detailed elsewhere [5]. Then, the age of the lesion could be included as a parameter to discriminate the proposed cells involved in the pathogenesis of the epidermal lesion.

MATERIALS AND METHODS

Nine untreated psoriatic patients in an active phase of their disease were selected. Seven patients supplied microscopic lesions, 2 patients submitted material from plaque lesions. One patient had 3 lesions investigated. In addition, material from 2 healthy subjects and from uninvolved skin in 2 psoriatics was included as control. The patients were 22–59 years of age. Five subjects were women. The actual phase of flare-up had been present during a period of up to 2 weeks before the patient was included in the study. Minute lesions were identified under a magnifying glass 3–10×. Punch biopsies were excised without anesthesia, frozen on solid carbon dioxide, and kept until further processing at −90°C. They were sectioned on a cryostat at 6 μm and each consecutive section was examined to collect all the material containing minute parafollicular lesions. Uninvolved skin obtained at least 5 cm outside lesions and involved (plaque) skin were included for comparison. The composition of the material is given in Table I, which shows the types of antibodies used to define the different mononuclear cells.

Immunohistochemical Stainings

The indirect immunoperoxidase or biotin-avidin-peroxidase staining methods were employed [15–17]. The monoclonal antibodies used were: Lyt-2 (10.2) 5 μg/ml, New England Nuclear (NEN) Dreieich, BDR; Lyt-3 (9.6) 10 μg/ml, NEN; Leu-4 (SK7), 5 μg/ml, Becton-Dickinson (BD) Oxnard, California; OKT3, 2 μg/ml, Ortho Diagnostic Systems, Raritan, New Jersey; Leu-3a (SK3), 6.7 μg/ml, BD; OKT4, 2 μg/ml, Ortho; Leu-2a (SK1), 2 μg/ml, BD; OKT8, 1 μg/ml, Ortho; Ia, 1:40 dilution of purchased material, Dr. I. S. Trowbridge, Scripps Clinic, La Jolla, California; OKM2, 1:20 dilution of purchased material, Ortho; BRL αmono (63D3), 10 μg/ml, Bethesda Research Laboratories, Gaithersburg, Maryland. OKT3, OKT4, and OKT8 were used with the biotin-avidin-peroxidase method (see below) since in our hands the immunoperoxidase method was less sensitive with the OKT antibodies.

Immunoperoxidase

The 6-μm cryostat sections were fixed in acetone at 4°C for 15 min, rinsed in 0.05 M Tris-buffered saline (0.14 M) pH 7.6 for 15 min, and

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Abbreviations:

PBS: 9.3 mM phosphate-buffered saline (0.14 M, Na^+:K^+ ratio = 38.1) pH 7.4

TABLE 1. *Material utilized for the study of mononuclear cells in the skin in 2 healthy and 9 psoriatic subjects and the monoclonal antibodies used*

Monoclonal antibody	Involved			Uninvolved	
	Parakeratosis	Orthokeratosis	Paralesional skin	Psoriasis	Normal
Lyt-2	6	4	2	1	1
Lyt-3	7	5	3	1	1
Leu-4	11 (2)	9 (2)	6 (2)	2 (1)	2 (1)
OKT3	3	2	2 (1)	0	0
Leu-3	11	9	6	2	2
OKT4	3	3 (1)	2 (2)	0	0
Leu-2	11	9	6	2	2
OKT8	2	3 (1)	2 (2)	0	0
Ia	8	6	4	1	1
OKM2	7	5	3	0	0
BRL αmono	2 (1)	2 (1)	1 (1)	1	1

Number of biopsies tested is indicated by given numbers for the reagents used. In some cases biopsies were examined with only one of the reagents used to assess a certain cell type. The number of such biopsies is indicated by numbers in parentheses for the particular reagent used.

incubated for 30 min with the appropriate monoclonal antibody. Incubations were done at room temperature. The concentrations given above were predetermined by serial dilutions in tests on normal tonsil or thymus sections. The antimonocyte monoclonal antibodies were tested similarly on mononuclear cell suspensions isolated on Ficoll-Hypaque.

After incubation the sections were washed for 10 min 3 times in Tris-buffered-saline and incubated 30 min with peroxidase-conjugated rabbit antimouse IgG (DAKO, Denmark) intensively absorbed by human Ig. After 3 washes in Tris-buffered-saline the sections were treated with the peroxidase substrate 3-amino-9-ethyl-carbazole (final concentration 0.88 mM) which was solubilized in 50 mM N,N-dimethylformamide dissolved in 0.1 M acetate buffer, pH 5.2. Immediately prior to use, the substrate solution was oxidized by 90 μM H_2O_2 and filtrated. After incubation for 30 min, the sections were rinsed in Tris-buffer-saline, counterstained with hematoxylin, and mounted in glycerin-gelatin. Control sections were prepared and run in parallel excluding the monoclonal antibody.

Biotin-Avidin-Peroxidase

After fixation in acetone for 5 min at 4°C the cryostat sections were rinsed at room temperature in 9.3 mM phosphate-buffered saline (0.14 M, Na^+:K^+ ratio = 38.1) pH 7.4 (PBS) for 10 min and oxidized by 0.9 mM H_2O_2 in water during incubation for 5 min and subsequently washed in the buffer for 10 min. Then, the sections were incubated with various OKT-monoclonal antibodies for 30 min. The appropriate concentrations were predetermined on additional skin sections after serial dilutions in PBS supplemented with 2% bovine serum albumin. After rinsing in PBS, the sections were exposed to affinity-purified biotinylated antimouse IgG (25 μg/ml, Vector Laboratories, Burlingame, California) for 30 min, again rinsed for 7 min 3 times in PBS and incubated with a complex of biotinylated peroxidase and avidin DH (17 μg/ml, Vector Laboratories) for 60 min, and again rinsed in PBS. Finally, the sections were incubated with the peroxidase substrate (3-amino-9-ethyl-carbazole) for 5 min and rinsed in PBS before mounting in glycerin-gelatin. Control sections were prepared and run in parallel excluding the monoclonal antibody or exchanging it with normal mouse serum or ascites fluid.

Histologic Analysis

The quantitative determinations of the mononuclear cell content in various portions of the skin is schematically illustrated in Fig 1. This enabled discrimination of the central parakeratotic part of epidermis and the underlying dermis and the paracentral orthohyperkeratotic and perilesional portions of epidermis and dermis. Within the borders all labeled mononuclear cells were counted. Two nearby cells were regarded as labeled only if the staining included other parts of their membranes than those which touched each other. The volume of tissue of the parts delineated was obtained from nominal thickness of the section and area determined by enlarging (219×) the microscopic image on a paper and the parts indicated cut out and the slip of paper weighed. Weights of the slip of paper varied mainly between 0.5–2 g (range, 44–9000 mg). Corrections for the tangent diameter of the cells according to stereologic techniques [18] were not done. The slides were read twice with an interval of at least 2 months by one of us (SQG). The error of the readings was estimated from the difference between the 2 examinations; it was 12%.

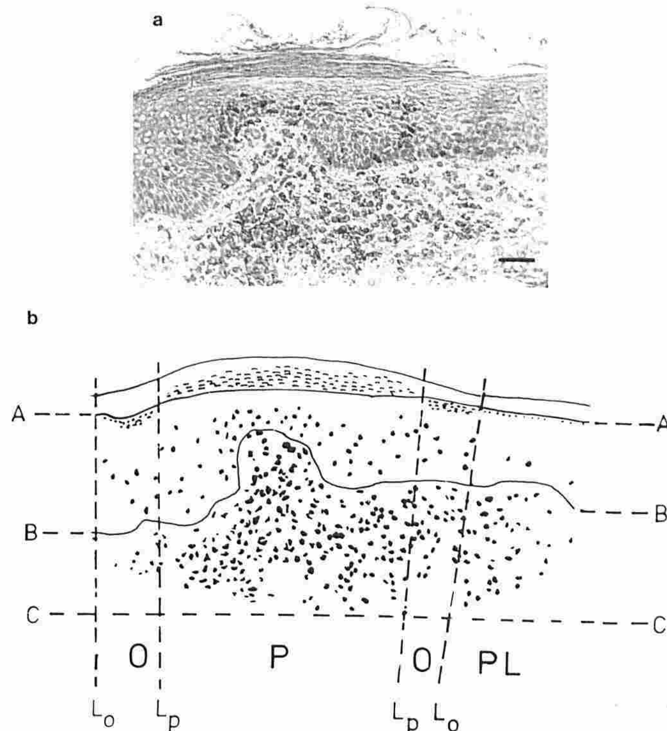

FIG 1. *a*, An initial psoriatic lesion with a central portion of parakeratosis stained with the peroxidase method for Lyt-2+ cells and counterstained with hematoxylin. *Bar* equals 50 μm. *b*, A schematic drawing of (*a*) indicating the various regions discerned. Lines L_p and L_o are drawn perpendicular to the borderline between the horny layer and stratum malpighii (*A-A*). The lines separate regions of parakeratosis (*P*) from orthohyperkeratosis (*O*) and from perilesional portion (*PL*). The line L_p is drawn through a point situated in the center between the most peripheral parakeratotic nuclei present in the horny layer and the margin of hypergranulosis present in the adjacent orthohyperkeratotic region. The line L_o is drawn at the opposite margin of the region with hypergranulosis. Epidermis is limited by the line *A-A* and the basal membrane (*B-B*). The line *C-C* is drawn parallel to *A-A* at the bottom of the dermal infiltrate.

Lyt-2 and -3, Leu-4 and OKT3 were found to react with number of cells per unit tissue volume approximately equal in size and their average was used to quantify pan T cells. T helper cells were identified by Leu-3a and OKT4, T suppressor/cytotoxic cells by Leu-2a and OKT8. Either OKM2 or BRL αmono were used to measure monocytes in separate cases. The results were combined since the various means did not differ in the subgroups.

Age of Lesions

The age of a lesion was assessed by the method of structure analysis [5]. This was accomplished on guide sections which were taken at regular intervals from the series of sections used for immunohistochemistry. Those cryostat sections were stained with fluorescein isothiocyanate and 4-4-diamidinodiphenylamine to reveal cell membranes and parakeratotic nuclei [5]. The section used contained a microscopic psoriatic lesion with a rim of normal horny layer on top of parakeratosis (Fig 2). The border between normal and abnormal corneocytes was used as a time mark to assess the age of the lesion. This border was traced from the center of the lesion into the perilesional part of the section. At this point all underlying corneocytes forming interdigitating sheets stacked without disorder on top of each other down to stratum granulosum were counted. An earlier finding indicated that 2 corneocyte layers were formed per day in uninvolved skin [19]. The age of the lesion for the period during which abnormal keratinization was present was calculated from these figures.

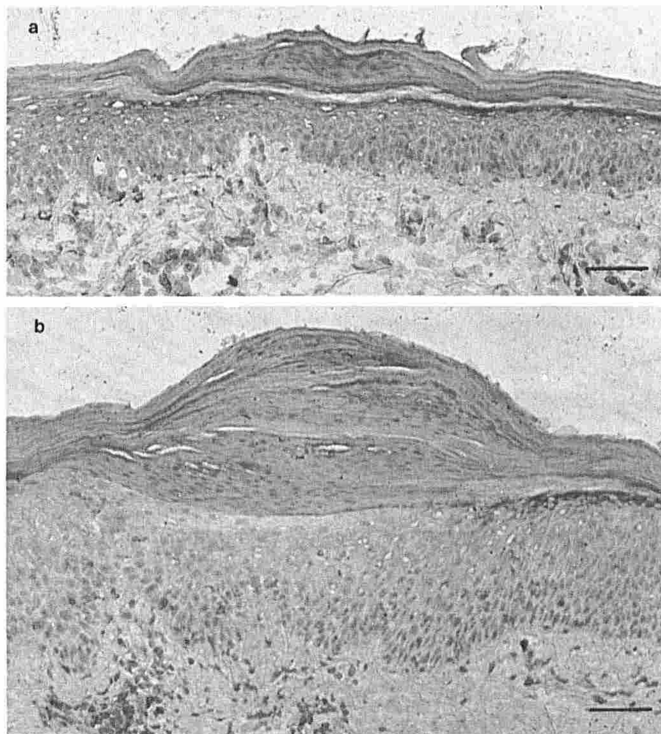

FIG 2. Psoriatic lesions varying in age. The horny layer was secured to reveal a top region of normal appearance lifted up to various degrees by the accumulation of parakeratotic corneocytes. Such layers were traced into the paralesional region which was used as a recorder of time assuming a steady state production and exfoliation of 2 layers per day [19]. *a*, Three and one-half-day-old lesion *b*, Eleven-day-old lesion. *Bars* equal to 50 μm.

Lesions were selected that showed parakeratosis just on top of stratum malpighii and with a normal horny layer in their most superficial part (Fig 2). The orthohyperkeratotic portion surrounding parakeratosis was labeled by the age of its parakeratotic center. Full-blown lesions of stable psoriasis were also examined with parakeratotic portions in various strata of the horny layer. These were older than 10 days but their real age was uncertain.

Statistical Methods

Individual number of cells was divided by volume of tissue to obtain density (cell/unit tissue volume). Cell densities ranged from over 400 to zero in the various groups. Proportionality between group means and their variances was found. A reason for this occurrence is that the data are exponentially distributed [20]. The presence of proportionality has to be alleviated before statistical tests can be done [20]. A logarithmic transformation of the original data incremented by unity rendered variances of the various groups homogenous. Systematic and random errors are not influenced by the transformation. Analysis of variance or covariance [20,21] was designated for this experimental study. The statistical model was $Y = \mu + I_i + C_j + L_k + R_l$ + interactions (between factors I, C, L, R) + error, since it was assumed that variation in cellular infiltrate was dependent on individuals (I), cell type (C), skin layers (L), and regions within and outside lesions (R). Covariation with age (A) was included when it was appropriate. The model used may discern a possible dependence between a special cell type and one or several of the other factors. In the analysis a significant interaction among the factors is then a necessary result to demonstrate their dependence.

RESULTS

General Findings

The histology of the lesions is exemplified in Figs 1–3. The cell densities in epidermis and dermis are summarized in Fig 4. In all cell groups revealed by the immunohistologic procedure the highest density was obtained in the portion of the lesion outlined by its parakeratotic horny layer, less in that outlined by orthohyperkeratosis and in that of perilesional skin, and the least in normal skin. Uninvolved psoriatic and healthy skin did not show variation. The cell density in the dermis was larger than that in the epidermis for pan T cells, helper T cells, and monocytes (for each $p < 0.001$). This was not shown for suppressor T cells and Ia-positive cells. Contrary to the other cell types, suppressor T cells varied differently in skin layers within the lesion than in skin outside the lesion (Fig 4). In order to test this statistically, helper T cells were chosen to represent the regular features of increasing cell densities toward the center of the lesion as a background control. The question is then asked whether suppressor T cells behave otherwise or not. This was submitted to an analysis of variance (model given above). A 3-factor interaction was found significant ($p < 0.01$). It was between the factors: cell types, skin layers, and regions (C×L×R). This means that within the lesion and in the epidermis, suppressor T cells do not follow the main pattern as given by helper T cells. On the contrary, the suppressor T cells allocate preferentially to the epidermis within the lesion. In the analysis of variance, significant variation was also present between layers and between the different regions ($p < 0.001$).

Age of the Lesion and Cell Density

Variation in cell densities by age was minor. The involved skin was compared with perilesional and noninvolved psoriatic skin under the assumption that the latter two expressed the base level equal to day 0 in the evolution of a lesion. Changes in cell densities of pan T and suppressor T cells are shown in Fig 5.

In the dermis of the orthohyperkeratotic region no significant variation by time from day 0 and throughout was present for pan T, helper T, and suppressor T cells, and the same was also true for helper T cells in the epidermis. Pan T and suppressor T cells were increased in the epidermis from day 3 and after without variation by age of the lesion.

In the parakeratotic region the densities of pan T, helper T, and suppressor T cells were increased compared to day 0. Both in the epidermis and in the dermis, changes were present at day 3 and did not significantly vary thereafter.

DISCUSSION

This study was designed to reveal a possible candidate among mononuclear cells which in the initial phase of the psoriatic process might act on the epidermis. An increase of invading lymphocytes in the epidermis was obtained. Among these, suppressor T cells were found to selectively favor the epidermis over the dermis both in the center as well as in the periphery of the lesion. This pattern was already present in the youngest lesions. Therefore, it is appropriate to assume that the preponderance of suppressor T cells may have a biologic role in the process of the disturbed keratinocyte function present in the psoriatic lesion.

Ia-positive cells were also increased within the lesion (Fig 4). These cells obviously parallel the number of infiltrating T cells, which probably indicates their activated state in accordance with other observations on Ia expression by T cells [22]. However, without double-staining experiments it cannot be excluded that other cells, i.e., monocytes or Langerhans cells, might contribute to the observed increase in Ia expression in the lesions.

Findings of a normal or increased ratio of helper/suppressor T cells in psoriatic blood [11–14] is compatible with the fact that suppressor T cells apparently are recruited into the skin (Fig 4). Recent communications by Bjerke and by Bos et al independently confirm our results for the full-blown psoriatic lesion [23,24]. At present, the stimulus for mononuclear cells to invade the epidermis in psoriasis is unknown. Clearly, further studies seem necessary to elucidate the role of T-cell function in psoriasis. Apparently, functions and/or factors including lymphokines [25] related to suppressor T cells may be involved

a

b

c

d

FIG 3. Initial psoriatic lesions stained with the peroxidase method to reveal mononuclear subsets and counterstained with hematoxylin. *a*, Leu-3a+ cells. *b*, Leu-2a+ cells. *c*, Ia+ cells. *d*, OKM2+ cells. *Bars* equal to 50 μm.

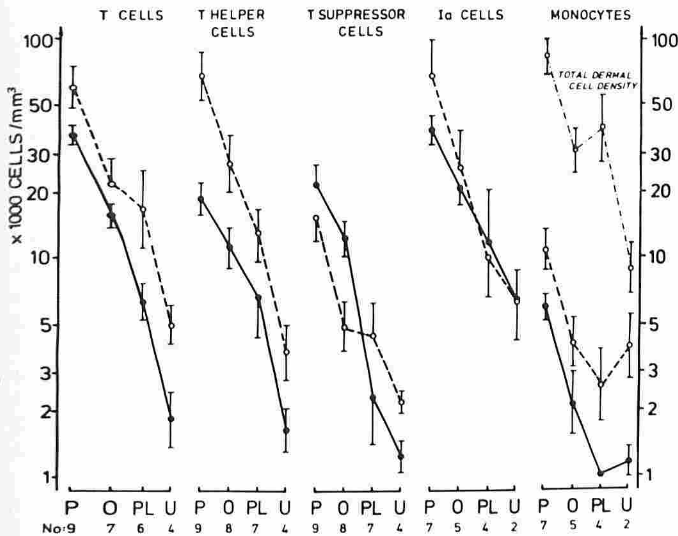

FIG 4. Mononuclear cell density in regions of the psoriatic lesion (see Fig 1*b*). Dermal infiltrate (O---O) and epidermal infiltrate (●——●) are given as means and SE taken from the logarithmic transform (ln(x + 1)) of actual values (x). Number of subjects is indicated at the bottom of the graph. Total dermal cells were measured in the same number of subjects as for T helper cells. Regions indicated: P = parakeratosis; and O = orthohyperkeratosis (within lesion), PL = perilesional regions, U = uninvolved psoriasis and healthy skin combined.

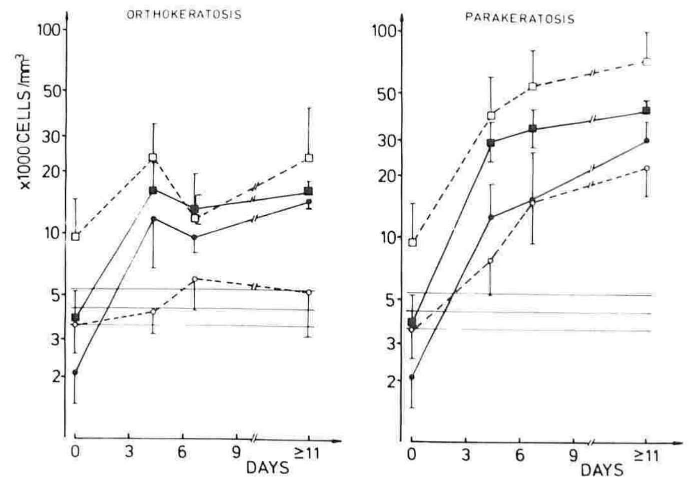

FIG 5. Pan T (*squares*) and suppressor T (*circles*) cells related to age (for its estimation, see text) of evolving psoriatic lesions. The cell densities in epidermis (*continuous lines*) and in dermis (*broken lines*) in the orthohyperkeratotic and parakeratotic region of the lesions given as means and SE. Day 0 values are combined from perilesional and uninvolved psoriatic skin. The dermal cell content of suppressor T cells (Leu-2a+, OKT8+) in the orthokeratotic region did not differ by time and its mean and SE are indicated by the *parallel lines* as reference. Age was grouped at 4.2 days (observed: 3, 4.5, and 5 days), 6.3 days (observed: 5.5, 6, 7.5 days), and ≥11 days (observed: 11, 14, and 14 days, and 2 lesions >14 days).

in the pathogenesis of the psoriatic lesion. At present, the nature of these functions and factors in vivo could, however, only be speculated upon.

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