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IN SITU CHARACTERIZATION AND COUNTING OF MONONUCLEAR CELLS IN LESIONS OF DIFFERENT CLINICAL FORMS OF PSORIASIS

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Abstract. Mononuclear cells in the dermis were characterized and counted in cryostat sections of lesional skin from 34 patients with various clinical forms of psoriasis. T lymphocytes were detected with $F(ab')_2$ preparation of rabbit IgG against human T lymphocytes and acid α-naphthyl acetate esterase (ANAE) activity as histochemical marker. The ANAE method was also used for the detection of macrophages. Comparable results were obtained using both the anti-T lymphocyte serum method and the ANAE method. The highest proportion of ANAE-positive lymphocytes was found in sections from stationary lesions of psoriasis vulgaris (61.2%), and the lowest in lesions of erythroderma (38,5%). ANAE-positive macrophages constituted 51.3% of the mononuclear cells in lesions of erythroderma and 29.7% in stationary lesions of psoriasis vulgaris. Only a few B lymphocytes could be detected with goat antibodies against human Fab of Ig. Cells with receptors for the Fc part of IgG (FcR) were detected using soluble complexes of horseradish peroxidase (HRP)-anti-HRP. Most FcR-positive cells were found in lesions of pustular psoriasis (53.7%) and in erythroderma (45.5%), and fewest (18.3%) in stationary lesions of psoriasis vulgaris.

Key words: Psoriasis; Cryostat sections; T lymphocytes; B lymphocytes; Macrophages; Fcγ-receptors

The dermal mononuclear cell infiltrates in psoriatic lesions have recently been characterized using hemadsorption with various indicator cells to cryostat sections (3). The results obtained showed that T lymphocytes were the predominant mononuclear cells in psoriatic lesions, except in the clinically most active lesions where T lymphocytes and macrophages were present in approximately equal numbers.

The characterization of mononuclear cells in situ in tissue sections involves several problems concerning the specificity and sensitivity of the methods used. Therefore, it is important that different methods are used for the detection of various markers, to increase the reliability of the conclusions. In the present work, mononuclear cells in cryostat sections of lesional skin from patients with different clinical forms of psoriasis were examined using both immunological and histochemical methods. α-Naphthyl acetate esterase (ANAE) activity was used as marker for T lymphocytes and macrophages (15, 25), heteroantiserum for the demonstration of T lymphocytes (4, 22) and soluble immune complexes for the demonstration of cells with receptors for the Fc part of IgG (FcR) (4, 21). The aim was to obtain additional information about the quantitative and topographic distribution of the various mononuclear cells.

MATERIALS AND METHODS

Patients

Thirty-four patients with psoriasis were studied. Fourteen patients had fully developed, stationary psoriasis vulgaris; 12 patients had active psoriasis with exudative, peripherally spreading or guttate lesions; 6 had generalized exfoliative psoriasis (erythroderma), and 2 patients had generalized pustular psoriasis of the von Zumbusch type.

Tissues

Elliptical skin biopsies, approximately 10×15 mm in diameter, were taken from the periphery of the lesions. The lesions chosen for examination were not treated for at least one week prior to biopsy. The specimens were divided into two parts. One part was used for the demonstration of immunological markers. The other for the demonstration of ANAE activity, was fixed in buffered formol–sucrose (pH 6.8) at 4°C for 22 hours and kept in Holt's gum–sucrose at 4°C for another 22 hours (25). The specimens were quick-frozen in isopentane, pre-cooled with liquid nitrogen and sectioned (4–10 μm) on a cryostat.

Specimens of normal spleen and thymus were obtained from the Department of Surgery.

Sera

Anti-human T lymphocyte serum was raised by immunizing rabbits with human thymocytes (22).

Antiserum to horseadish peroxidase (HRP) (type IV, Sigma, St. Louis, Mo., USA) was also raised in rabbits (21).

Immunoglobulin preparations

Rabbit IgG antibodies were isolated by ion-exchange chromatography in DEAE–cellulose (Whatman DE-52) equilibrated with 0.015 M phosphate buffer, pH 7.6, followed by gel filtration on a Sephadex G-200 column.

$F(ab')_2$ fragments were prepared according to the methods described by Stewart et al. (31).

The $F(ab')_2$ preparation of IgG of the anti-human T lymphocyte serum was rendered specific as described elsewhere (22).

Fluorescein isothiocyanate (FITC)-conjugated swine antibodies to rabbit Ig were purchased from DAKO-immunoglobulins A/S, Copenhagen, Denmark (code no. F 2190, molar F/P ratio 2.3). FITC-conjugated $F(ab')_2$ of goat antibodies against human Fab of Ig was purchased from Nordic Immunological Laboratories, Tilburg, Holland (code no. 10-680, protein concentration 10 mg/ml).

The protein concentrations of the preparations were calculated assuming $E^{1\%}_{1\,cm}$ *280 nm* = 14.6. The preparations were filtered through a sterile 0.45 μm Millipore filter before use.

Immune complexes

Immune complexes were prepared by adding dilutions of the IgG or the preparations of $F(ab')_2$ fragments to HRP to equal amounts of four-fold dilutions of HRP from 1 mg/ml in phosphate-buffered saline, pH 7.2 (PBS). The mixtures were incubated for 2 hours at room temperature before use. In tests with tissue sections, complexes prepared at slight antigen excess were used (21).

Demonstration of T lymphocytes with anti-T lymphocyte serum

The procedure was as previously described (4). Briefly, cryostat sections were incubated at room temperature for 30 min with the IgG $F(ab')_2$ preparation (10 mg/ml) of anti-T lymphocyte serum diluted 1 in 4 in PBS, and then incubated for another 30 min with FITC-conjugated swine anti-rabbit Ig diluted 1 in 20 in PBS. The sections were mounted in PBS–glycerol and examined in a Leitz Orthoplan microscope with an Osram HBO-200 mercury lamp. The reactions were defined as 3+ when >70% of the mononuclear cells were stained, as 2+ when 30–70% were stained and as 1+ when <30% were stained.

Demonstration of ANAE activity

Cryostat sections of fixed tissue mounted on gelatin-coated slides were washed in distilled water for 20 min at room temperature and incubated for 2 hours in the following solution: 40 ml 0.067 M phoshate buffer of pH 5.0, 2.4 ml hexazotized pararosaniline (equal volumes of 4% Na nitrite in distilled water, and 1 g pararosaniline in 20 ml distilled water and 5 ml of concentrated HCl) and 10 mg α-naphthyl acetate (Sigma) in 0.4 ml acetone. The solution was adjusted to pH 5.8 with 2 N NaOH (25).

After washing for 20 min in distilled water, the preparations were counter-stained with 1% methylgreen in 0.1 M acetate buffer at pH 4.2 for 10–15 sec. The preparations were dehydrated in ethanol, cleared in xylene and mounted in Depex mounting medium (Difco laboratories, England).

The macrophages display a diffuse reaction product on the external surface of the plasma membrane (6), allowing them to be distinguished from lymphocytes (6, 25). A lymphocyte was considered as ANAE-positive when its cytoplasm contained a single or a few distinct spots of the reddish-brown reaction product (25). The proportions of macrophages and ANAE-positive and negative lymphocytes were estimated in each section by counting 200 mononuclear cells.

Demonstration of surface membrane bound Ig (SmIg)

Cryostat sections were washed at 4°C in PBS for 2 hours before incubation with FITC-conjugated $F(ab')_2$ of goat antibodies to human Fab of Ig diluted 1 : 16 in PBS. The sections were then washed for 30 min, mounted and examined as described above.

Demonstration of FcR using HRP-anti-HRP immune complexes

Cryostat sections were incubated with soluble immune complexes at room temperature for 45 min, and the peroxidase activity was revealed by incubating the sections in Graham & Karnovsky's solution as previously described (4). The sections were then stained with hematoxylin for 45 sec. Mast cells were demonstrated by staining some sections following the Giemsa method. Stained sections were mounted in Depex mounting medium before microscopical reading. The proportion of FcR-positive cells was assessed in each section by counting 200 cells.

Demonstration of FcR-positive cells and T lymphocytes with the same section

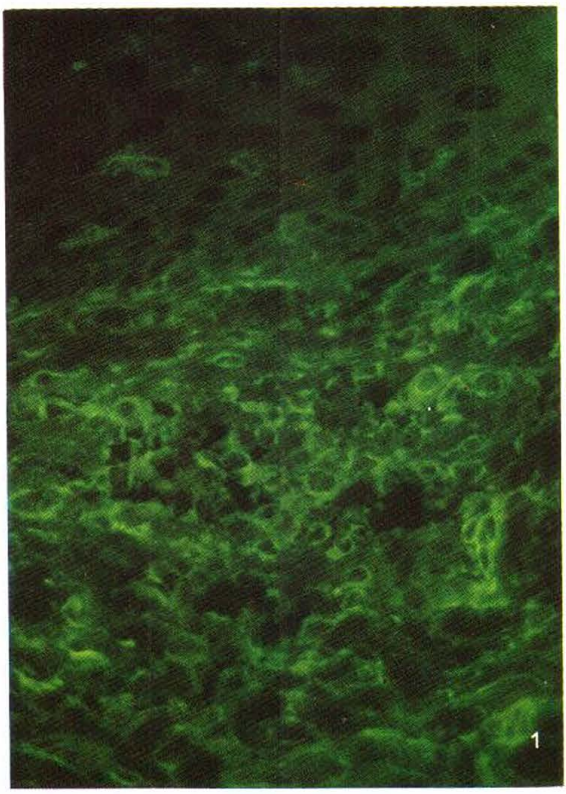
Sections of spleen were first incubated with soluble immune complexes of HRP-anti-HRP as previously described, stained for 10 min in Graham & Karnovsky's solution, and then incubated overnight at 4°C with a 1% solu-

Fig. 1. Section of an exudative psoriatic lesion incubated with $F(ab')_2$ preparation of anti-T lymphocyte serum and FITC-labelled swine anti-rabbit Ig. ×360.

Fig. 2. Section of a stationary lesion of psoriasis vulgaris stained for ANAE activity. Nearly all the lymphocytes contain one or two reddish-brown granules in the cytoplasm. The macrophages show reddish-brown staining of the whole cytoplasm. ×360.

Fig. 3. Section of an erythrodermic psoriatic lesion incubated with complexes of HRP-anti-HRP. Bound HRP revealed with diaminobenzidine is seen mainly along the cell membranes. Stained with hematoxylin. ×360.

Fig. 4. Section of normal spleen incubated with HRP-anti-HRP, stained with diaminobenzidine and then incubated with AET-treated E, fixed and stained with diaminobenzidine and hematoxylin. An arteriole is seen in the middle of the figure. The indicator cells adhere to the periarteriolar sheath in the white pulp (upper left part of figure). The cells in the white pulp show no membrane staining with HRP-anti-HRP. Only a few indicator cells adhere in the red pulp, where most cells show membrane staining with HRP-anti-HRP (lower right part of figure). ×225.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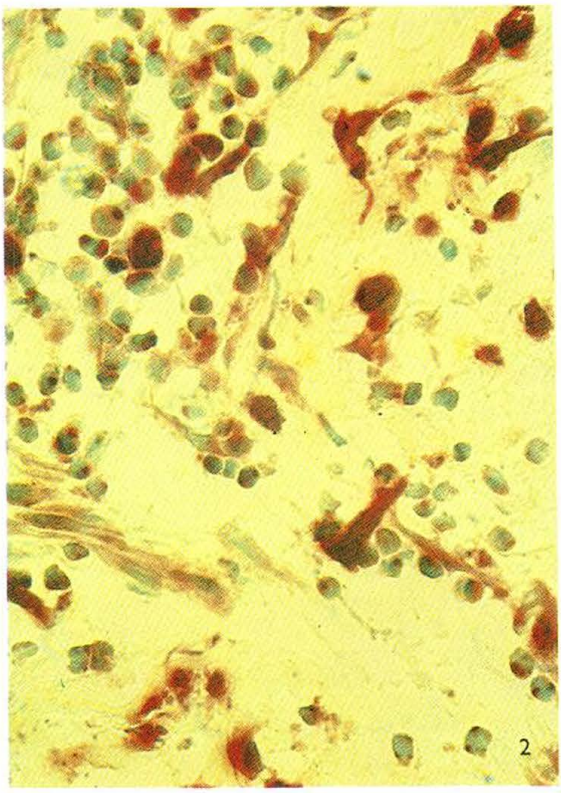
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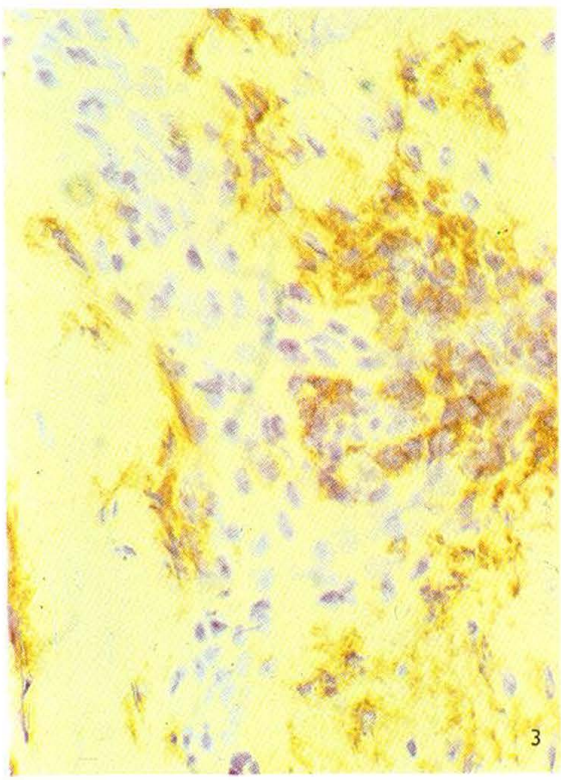
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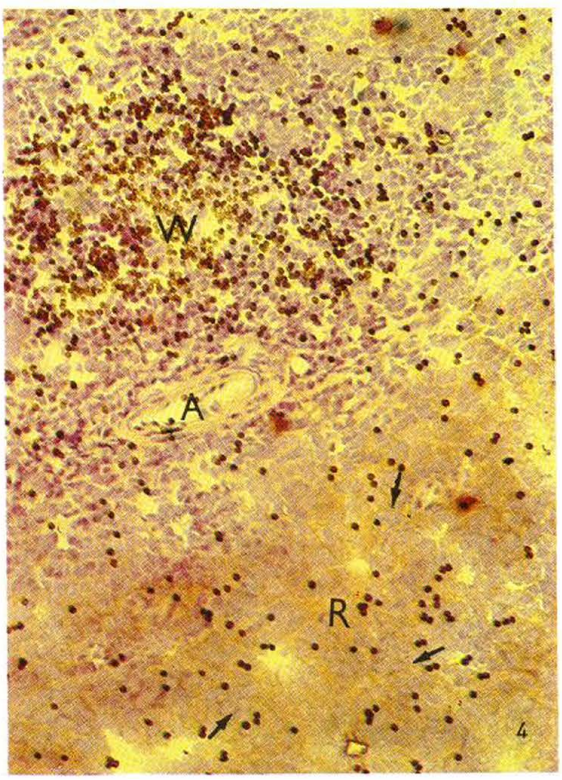
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Table 1. *Results obtained with sections of biopsy specimens from psoriatic lesions stained with immune complexes of HRP-anti-HRP, anti-T lymphocyte serum, or stained for ANAE activity*

Relative distribution of mononuclear cells

Patients	HRP-anti-HRP (%stained cells)	Anti-T serum[a]	ANAE (% stained cells)		
			Macrophages	T lymphocytes	Unstained
Psoriasis vulgaris					
T. N.	4	2+	26	64	10
O. H.	18	3+	29	62	9
I. Aa.	19	3+	31	62	7
Exudative psoriasis					
T. R.	25	2+	43	48	9
T. H.	29	2+	29	57	14
S. T.	30	2+	33	58	9
Erythroderma					
P. E.	34	2+	50	39	11
D. T.	50	2+	51	44	5
R. S.	52	2+	53	37	10
Psoriasis pustulosa					
E. N.	47	n.t.[b]	45	45	10
B. M.	56	2+	54	37	9

[a] Grading of reaction with anti-T lymphocyte serum. Percentage of cells stained: 3+ = >70%, 2+ = 30–70%, 1+ = <30%.
[b] n.t. = not tested

tion of sheep erythrocytes (E) treated with 2-aminoethylisothiouronium bromide hydrobromide (AET) using the closed chamber technique as described elsewhere (3, 22). The reactions were recorded while the preparations were unfixed and still cold, and also after fixation and staining with hematoxylin and eosin (3).

Controls

The IgG F(ab')₂ preparations of rabbit anti-human T lymphocyte serum stained thymocytes in sections of thymus and lymphocytes in the periarteriolar areas in spleen sections (11, 22). Sections incubated with PBS and FITC-labelled swine anti-rabbit Ig serum remained unstained. T lymphocytes in the periarteriolar areas in the white pulp also showed ANAE activity.

Complexes of HRP-anti-HRP were bound strongly to the red pulp areas of human spleen (4). Complexes prepared with F(ab')₂ fragments of IgG anti-HRP or HRP solution alone did not stain the sections. However, some cells revealed endogenous peroxidase activity when the sections were incubated with Graham & Karnovsky's solution alone.

Statistics

For statistical evaluation, differences between groups were tested by the Wilcoxon rank sum test.

RESULTS

The staining with F(ab')₂ preparation of anti-T lymphocyte serum was sharply delineated and localized to the cell membranes (Fig. 1). The highest proportion of stained cells (>70% of the mononuclear cells) was found in sections of lesional skin from patients with stationary psoriasis vulgaris, and the lowest (30–70%) in lesional skin from patients with erythroderma and pustular psoriasis (Table 1). A few cells in the epidermis were also stained with the anti-T lymphocyte serum (Fig. 1). Corresponding results were obtained using the ANAE method for demonstration of T lymphocytes (Tables I–II) (Fig. 2). The greatest proportion of ANAE-positive lymphocytes was detected in lesions of stationary psoriasis vulgaris (mean 61.2%, range: 49–68%) and the lowest proportion in erythroderma lesions (mean: 38.5%, range: 34–44%) and pustular psoriasis (mean: 41.0%, range: 37–45%). In each group of patients low mean values (9.1–13.4%) were registered for esterase-negative lymphoid cells. When only lymphoid cells were considered, T lymphocytes constituted 86.9% (mean value) in lesions of stationary psoriasis vulgaris, and 78.6% (mean value) in active psoriatic lesions. The difference is statistically significant ($p<0.005$). Most T lymphocytes showed one or two reddish-brown granules in the cytoplasm. In a few lymphocytes more granular staining was noted.

When using the ANAE method, the macrophages could easily be identified due to the reddish-brown

Table II. *Relative distribution of dermal mononuclear cells in psoriatic lesions characterized using immune complexes of HRP-anti-HRP and staining for ANAE activity*

		Cells demonstrated using (%)							
		HRP-anti-HRP		ANAE staining					
				Macrophages		T lymphocytes		Unstained	
Type of psoriasis	No. of patients	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Psoriasis vulgaris	14	18.3	4–33	29.7	26–36	61.2	49–68	9.1	6–16
Exudative psoriasis	12	34.4	21–46	37.1	29–44	49.5	40–58	13.4	9–21
Erythroderma	6	45.5	34–55	51.3	50–53	38.5	34–44	10.2	5–15
Psoriasis pustulosa	2	51.5	47–56	49.5	45–54	41.0	37–45	9.5	9–10

staining of the whole cytoplasm (Fig. 2). Many cells with a morphology similar to fibroblasts were also stained reddish-brown, and were excluded in the differential counts. There was a significant difference ($p<0.01$) between the proportion of ANAE-positive macrophages in lesions of stationary psoriasis vulgaris (mean: 29.7%, range: 26–36%) and exudative psoriasis (mean: 37.1%, range: 29–44%). A statistically significant difference ($p<0.01$) was also found between the proportion of ANAE-positive macrophages in lesions of exudative psoriasis and erythroderma (mean: 51.3%, range 50–53%) (Tables I–II). Sections of varying thickness (4–10 μm) were stained using the ANAE method. Thin skin sections, 4 μm, were found to be best suited for counting. When using the looser spleen tissue, 10 μm thick sections showed the best morphology.

Tests for demonstration of Smlg were hampered by some background staining of intercellularly located Ig. However, only a few cells showed rim-like fluorescent staining, indicating a small proportion of B lymphocytes.

The HRP-anti-HRP immune complexes bound to mononuclear cells in the dermal papillae and the upper part of dermis. The greatest proportions of stained cells were usually detected in the dermal papillae. The granular brown reaction product was localized mainly along the cell membrane (Fig. 3). In some sections there also seemed to be cytoplasmic staining. The cells stained with HRP-anti-HRP were mainly large mononuclear cells with abundant cytoplasm morphologically consistent with macrophages. Usually, both the topographic localization and the number of HRP-anti-HRP stained cells corresponded to results obtained when detecting ANAE-positive macrophages. However, in sections from some of the stationary psoriatic lesions there was a marked difference; fewer than 10% of the cells stained with HRP-anti-HRP, while ANAE-positive macrophages exceeded 25% (Table I). The highest percentage of FcR-positive cells was found in sections of lesional skin from patients with clinically active psoriasis (Tables I–II). There was a statistically significant difference ($p<0.01$) between the percentage of FcR-positive cells in lesions of stationary psoriasis vulgaris (mean: 18.3%, range: 4–33%) and of exudative psoriasis (mean: 34.4%, range: 21–48%). A significant difference ($p<0.05$) was found between the percentage of FcR-positive cells in lesions of exudative psoriasis and of erythroderma (mean: 45.5%, range: 34–55%). The colour of the reaction product varied; dark brown on most stained cells, but on some cells distinctly lighter brown.

Granular staining was noted on some cells with a morphology similar to fibroblasts. These cells were excluded when counting stained cells. In some sections, particularly those with many FcR-positive cells, there seemed to be extracellular staining, mainly located in the upper dermis. In order to demonstrate mast cells, some sections were counterstained with Giemsa. None of the mast cells showed any deposits of HRP-anti-HRP. The double staining experiments showed that the soluble immune complexes gave membrane staining of cells in the red pulp areas of spleen, while there was no staining of cells in the periarteriolar white pulp areas binding AET-treated E (Fig. 4).

DISCUSSION

The problem of identifying T lymphocytes *in situ* in tissue sections was approached by using an $F(ab')_2$

preparation of anti-T lymphocyte serum and also by staining for ANAE activity. Both methods showed that, except for the lesions of erythroderma and pustular psoriasis, T lymphocytes were the predominant mononuclear cell type in the psoriatic lesions. T lymphocytes were detected both in the dermal papillae and particularly in perivascular, sometimes follicle-like, lymphoid infiltrates in the reticular dermis. A few T lymphocytes present in the epidermis indicated exocytosis of these cells. The findings with regard both to the proportion and localization of T lymphocytes tally with data obtained previously using AET-treated E for the detection of T lymphocytes (3). Hovmark (14), examining the dermal infiltrates in lesional skin from 2 patients with long-standing psoriasis, demonstrated a majority of ANAE-positive lymphocytes. We found the ANAE method to be best suited for counting. The method is simple to perform. The stained sections can be examined in an ordinary light microscope, thus giving information on the quantitative and topographic distribution of the stained cells. The dotted ANAE staining is highly characteristic (15, 25), although not an absolutely specific marker for T lymphocytes (27). The majority of mature resting T lymphocytes express some ANAE activity (15), but activation of T lymphocytes in vitro may result in partial loss of ANAE activity (20, 35).

The thickness of the sections may affect the accuracy of the estimates obtained with the ANAE method. We found thin skin sections (4 μm), which gave the best morphological details, to be optimal for counting. Recently, Ranki et al. (28) have reported very little variation in the number of ANAE-positive lymphocytes when 4 μm versus 10 μm thick sections of lymph nodes were examined. Some investigators have reported that the ANAE staining pattern may allow differentiation between T lymphocytes with receptors for the Fc part of IgM (Tμ, helper T lymphocytes) (13) and IgG (Tγ, suppressor T lymphocytes (13, 20). According to these reports, Tμ lymphocytes are usually single or double spotted, while Tγ lymphocytes are more granular spotted. Thus, according to the staining pattern, the great majority of the ANAE-positive lymphocytes detected in the present study were consistent with Tμ lymphocytes.

The proportion of ANAE-negative lymphoid cells was higher than the proportion of B lymphocytes detected by the demonstration of SmIg (12) in the present study, or previously when characterizing B lymphocytes by receptors for the complement fragment C3d (3). Stingl et al. (32) demonstrated few B lymphocytes in sections of psoriatic skin when using a hemadsorption technique. T lymphocytes activated by antigenic and/or mitogenic factors in the skin may be present among the ANAE-negative lymphoid cells. Some ANAE-negative cells may also represent mast cells and plasma cells. Mast cells have previously been reported to be ANAE-negative (19). The results obtained when using Giemsa staining showed some mast cells in line with results obtained using other staining techniques (7, 10). Plasma cells, however, exhibiting moderate to weak non-specific esterase activity (19), could not be demonstrated in the dermis in psoriatic lesions by Braun-Falco & Schmoeckl (7) using hematoxylin and eosin staining.

The majority of the cells stained with HRP-anti-HRP seemed morphologically consistent with macrophages, previously shown to bear FcR (1). Other FcR-positive cells to be considered are B lymphocytes (34), subpopulations of T lymphocytes (24), K cells (8) and mast cells (30). However, as concluded above, B lymphocytes are probably present only in low numbers in psoriatic skin lesions. With the present methods it is not possible to outline FcR-positive T lymphocytes (Tγ) or to detect K cells that might be present in the tissue. However, the use of spleen sections in double-marking experiments showed that lymphocytes in the periarteriolar areas did not stain with HRP-anti-HRP. This may indicate that T lymphocytes (11) express FcR of low avidity which are difficult to detect in situ in tissue sections. In addition, the ANAE staining pattern of the lymphocytes indicated the presence of only a few Tγ lymphocytes. In the present study, FcR-positive mast cells were not demonstrated. This agrees with previous data, since Fc receptors only for IgE have been demonstrated on human mast cells (30). Accordingly, most of the cells stained with HRP-anti-HRP are probably macrophages. This conclusion is also supported by the results of ANAE staining of macrophages. The topographic distribution and the numbers of ANAE-positive macrophages and FcR-positive cells corresponded. However, in sections from some of the stationary psoriatic lesions there were markedly lower numbers of FcR-positive cells than ANAE-positive macrophages. In sections of specimens from these stationary psoriatic lesions the FcR-positive cells

were also more weakly stained, indicating a reduced biological activity or a blocking of FcR.

When interpreting results of the FcR test, anti-immunoglobulins (rheumatoid factors) previously reported to occur in psoriatic skin lesions (16) should be considered. However, current studies on the relationship between FcR and anti-immunoglobulins in psoriatic lesions indicate so far that the binding of both complexes of HRP-anti-HRP and antibody coated E to sections of psoriatic skin is mainly due to FcR (5).

Fibroblast-like cells were found to have FcR. These cells may actually represent fibroblasts, but also histiocytes at various levels of differentiation. FcR have been demonstrated on mouse fibroblasts (26), but to our knowledge not on human fibroblasts.

In some sections there seemed to be extracellular localization of FcR, as previously described in malignant tissue (33). In vivo extracellular shedding of FcR may be of functional significance. Recent data point to an important role of FcR in the induction and regulation of the immune response (18).

The dominance of T lymphocytes and macrophages in the dermal infiltrates suggest involvment of cell-mediated immunity in the development of psoriatic lesions. The varying proportions of macrophages depending on the clinical activity may reflect a common reaction pattern in the skin. However, a histiocytic reaction specific for psoriasis should also be considered. Several reports (2, 17, 23) have indicated an alteration of macrophage/monocyte function in psoriatic patients. A defective suppressor function in psoriasis has been indicated in other reports (9, 29). This may be related to the present demonstration of few lymphocytes showing the granular ANAE staining pattern, claimed to be typical for Tγ lymphocytes (15, 20). However, this possibility can only be clarified by further investigations.

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