Distribution of Interleukin-2 Receptor Bearing Lymphocytes in the Skin

A Comparative Study of Allergic and Irritant Contact Dermatitis, Tuberculin Reaction and Cutaneous T Cell Lymphoma

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The tissue distribution of T cells and interleukin-2 receptor bearing (Tac^+) lymphocytes was studied immunohistochemically in frozen sections of biopsies from inflamed skin. In skin lesions caused by irritant or immunologic stimuli, relatively few (less than 5%) of the total dermal infiltrating T cells were Tac^+, but in both types of conditions the Tac^+ cells showed a predeliction for the epidermis. In skin lesions from patients with cutaneous T cell lymphoma a relatively high proportion of dermal T lymphocytes were Tac^+. Tac^+ as well as Tac^- T lymphocytes may thus migrate to a tissue secondary to several different stimuli. The demonstration of a non-random distribution of Tac^+ lymphocytes in the non-malignant inflammatory conditions in the present study indicate that the epidermis may have a special role in the activation of T lymphocytes. *Key words: Tac^+ lymphocytes; Skin biopsies.* (Received November 18, 1985.)

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Migration of lymphocytes into sites of inflammation represents an early step in the inflammatory process irrespectiv of whether the inflammation is triggered by physicochemical or immunological factors (1–3). There is evidence that at least to a large extent extravasation of lymphocytes is unspecific (2). On the other hand, the extravasation cannot occur totally at random, as many inflammatory conditions for example in skin are even in their early phases characterized by the presence of many T lymphocytes but few if any B cells (3–6). Furthermore, the relative number of T lymphocytes that carry "helper" and "cytotoxic/suppressor" markers differ in between different types of inflammatory lesions (5–9). Unfortunately, however, knowledge of the lymphocyte traffic in inflammatory lesions is still incomplete and it is, for example, not known whether different types of T lymphocytes preferentially migrate to certain parts of inflamed tissues. A number of these problems are now amenable to analysis with the help of monoclonal antibodies towards functionally defined molecules on different subsets of lymphocytes.

In the present report we have studied one aspect of this problem by comparing the tissue distribution of interleukin-2 (IL-2) receptor positive (Tac^+) and IL-2 receptor negative (Tac^-) lymphocytes respectively in different inflammatory conditions in the skin.

MATERIALS AND METHODS

Biopsies

Seven patients with known contact dermatitis, 8 healthy, tuberculin-reactive individuals and 8 patients with cutaneous T cell lymphoma (CTCL; mycosis fungoides) with grade II or III skin lesions participated in the study (for further details see Tables I–II). The procedure for eliciting the allergic

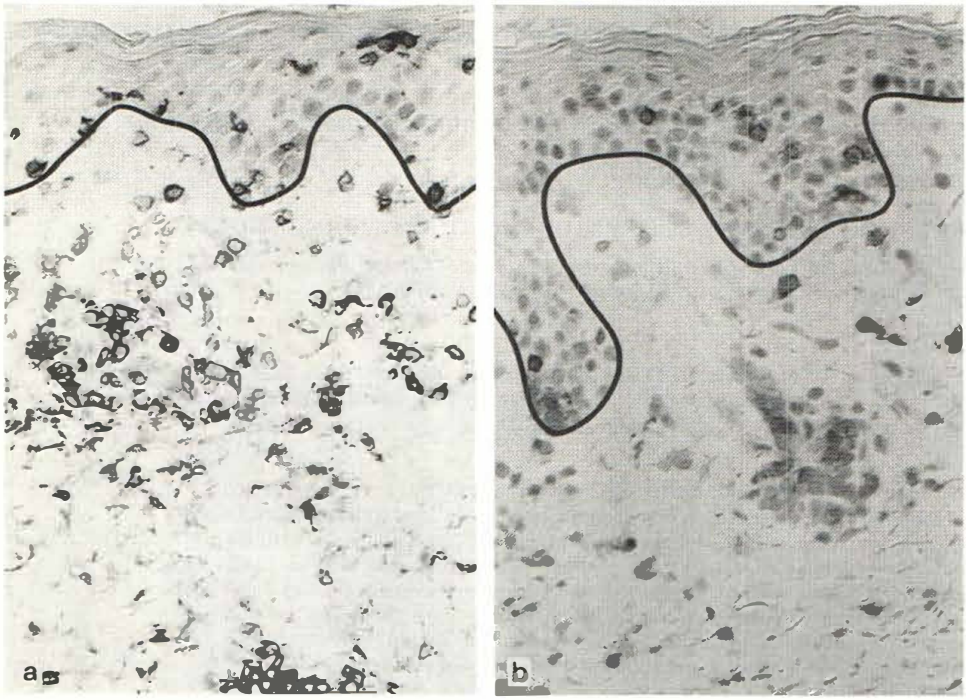

Fig. 1. Immunoperoxidase staining on serial frozen sections of a skin biopsy obtained at 72 hours after allergic provcation with 4×0.25% thiuram mixture. Stainings were in (*a*) with anti-Leu 1 and in (*b*) with anti-Tac antibodies. The sections were counterstained with haematoxylin. The solid lines indicate the epidermal basal membrane zone.

and irritant contact reactions by a patch test technique as well as the tuberculin reactions by intradermal injections of PPD (RT 23, State Serum Institute, Copenhagen, Denmark) have been described in detail elsewhere (3, 5). Three mm punch biopsies were obtained from the skin lesions, from normally appearing skin and from skin occluded by patch tests with only the vehicle (3, 5). The specimens were snap frozen in isopentane and kept at −70° before sectioning.

Immunohistochemical staining

Frozen sections, 4 μm thick, were investigated by immunohistochemistry using a conventional Avidin-Biotin peroxidase Complex (ABC) technique described in detail elsewhere (5, 10). Anti-IL-2 receptor (anti-Tac) mouse monoclonal antibodies were kindly given to us by Dr T. Waldmann, National Institute of Health, Bethesda, MD (11). Anti-Leu 1 antibodies that define a molecule mainly present on T cells (12) were obtained from Becton Dickinson (Sunnyvale, CA, USA). In control experiments staining was not observed when the primary antibodies were omitted or replaced by irrelevant antibodies.

Evalution of immunohistochemical staining

Serial sections were prepared and stained with anti-Tac and anti-Leu 1 antibodies. All positively stained cells present in the epidermis were counted and the ratios between the numbes of Tac^+ cells and Leu 1^+ cells were calculated and expressed as percentages. In the dermis, care was taken to enumerate positively stained cells in areas that were possible to define on serial sections. Due to the irregularity of this tissue and lack of delimiting structures an absolute quantitation of cells is, however, difficult and we have elected to express the data obtained from dermal infiltrates semiquantitatively. The tissues where the numbers of Tac^+ cells were less than 5% of those of the Leu 1^+ cells but where several Tac^+ cells are present are denoted +; tissues where the numbers of Tac^+ cells are between 5 and 20% of the numbers of Leu 1^+ cells are denoted as ++; higher proportions of Tac^+ cells are denoted as +++ (not seen in the present biopsies).

Table I. *Test subjects, skin challenge and results of immunoperoxidase staining*

Patients	Numbers of skin biopsies	Challenge	Skin biopsy taken after	% of Tac$^+$/Leu 1$^+$ Epidermis[a]	Cells in dermal infiltrates[b]
Healthy, tuberculin$^+$	3	PPD[c]	48 hours	27, 57, ND[d]	+
	2	PPD[c]	96 hours	36, 42	+
	3	PPD[c]	6–8 days	10, 19, 20	+
Contact allergic	2	Contact allergen[e]	24 hours	47, 60	+
	2	Contact allergen[e]	48 hours	28, 44	+
	4	Contact allergen[e]	72 hours	33, 45, 49, 51	+
	2	Irritant[f]	24 hours	0, 20	+
	2	Irritant[f]	48 hours	ND, ND	+
	4	Irritant[f]	72 hours	14, 33, 59, ND	+

[a] According to a quantitative evaluation of positively stained cells in epidermis within the actual sections.
[b] According to a semiquantitative evaluation where + means that the number of Tac-positive cells was less than 5% of cells stained with anti-Leu 1.
[c] 0.1 ml (2 TU) of PPD was injected intradermally in tuberculin-reactive healthy individuals (5).
[d] ND = not determined, due to bad morphology of parts of specimen.
[e] Epicutaneous patch testing was performed with 20% neomycin sulphate, 4×0.25% thiuram mixture, 1% tetramethyl-thiuram-disulfide or 1–5% nickel sulphate as earlier described (3).
[f] Epicutaneous patch testing was performed with 2 or 5% sodium lauryl sulphate as previously described (3).

RESULTS

Skin biopsies obtained from tuberculin reactions, allergic and irritant contact rections

Biopsies obtained at different times after injection of PPD intradermally in tuberculin-reactive individuals and after provocation with contact allergens or irritants were analyzed for the presence of Tac$^+$ and Leu 1$^+$ cells. These results are summarized in Table I.

In accordance with previously published data (3, 5), biopsies obtained at 6 hours after challenge (n=5) did not differ from controls (n=7): Anti Leu-1 reactive cells were found in low numbers scattered perivascularly in the dermis and occasionally in the epidermis. Tac$^+$ cells were not observed in the epidermis and were even less frequent than Leu 1$^+$ cells in the dermis.

After 6 hours Leu 1$^+$ T cells infiltrated the epidermis and perivascular cell infiltrates increased progressively. The cell infiltrates were usually larger in the tuberculin and in the allergic reactions than in the irritant reactions, but no differences as to tissue distribution of Leu 1$^+$ T cells was seen.

When analyses on presence of Tac$^+$ cells were performed on sections adjacent to those stained with anti-Leu 1 antibodies, the proportion of T lymphocytes in the epidermis, reactive with anti-Tac exceeded that observed in the dermal cell infiltrates independently of the type of challenge or time after challenge (see Table I and Figs. 1 and 2). The Tac$^+$ dermal cells were scattered and represented in all cases less than 5% of corresponding Leu 1$^+$ T cells, whereas the percentage of Tac$^+$ cells out of total Leu 1$^+$ cells in epidermis varied between 10 and 59% (see Table I). In biopsies taken at 6 and 8 days after PPD injection, a relatively lower number of Tac$^+$ cells was noted in the epidermis (see Table I); only few biopsies were, however, analyzed at this time period.

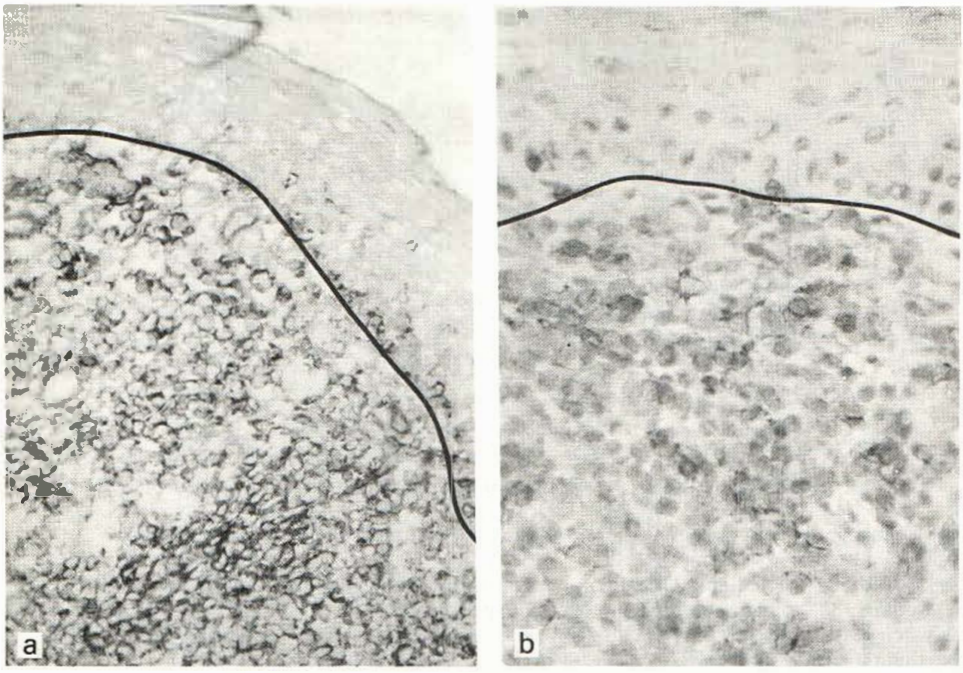

Fig. 2. Immunoperoxidase staining on serial frozen sections of an intraepidermal vesicle in a skin biopsy obtained at 72 hours after irritant provocation with 2% sodium lauryl sulphate. Stainings were in (*a*) with anti-Leu 1 and in (*b*) with anti-Tac antibodies. The sections were counterstained with haematoxylin.

Skin biopsies from patients with CTCL

Biopsies from 3 patients with stage II lesions of CTCL and biopsies from 5 patients with stage III lesions were investigated for the tissue distribution of Leu 1^+ and Tac^+ cells (see Table II). In all cases a significant infiltration of Leu 1^+ cells was seen in both the dermis and epidermis (Fig. 3*a*). In biopsies from stage III lesions 5 to 20% of dermal T cells were positive for the anti-Tac antibody in 4 out of 5 cases (see Table II). Furthermore, the Tac^+

Table II. *Results of immunoperoxidase staining in patients with CTCL*

Patients	Numbers	% Tac^+/Leu 1^+ cells in Epidermis[a]	Dermis[b]	Patterns of Tac^+ cells
CTCL stage II	3	5, 15, 20	+	Single, scattered
CTCL stage III	1	25	+	Single, scattered
CTCL stage III	4	3, 4, 25, ND[c]	++	Groups

[a] According to a quantitative evaluation of positively stained cells in epidermis within the actual sections.

[b] According to a semiquantitative evaluation where; + means that several Tac-positive cells were seen but that their total number were less than 5% of cells stained with anti-Leu 1; ++ means that the number of Tac^+ cells was between 5 and 20% of the number of Leu 1^+ cells.

[c] ND = not determined, due to bad morphology of parts of specimen.

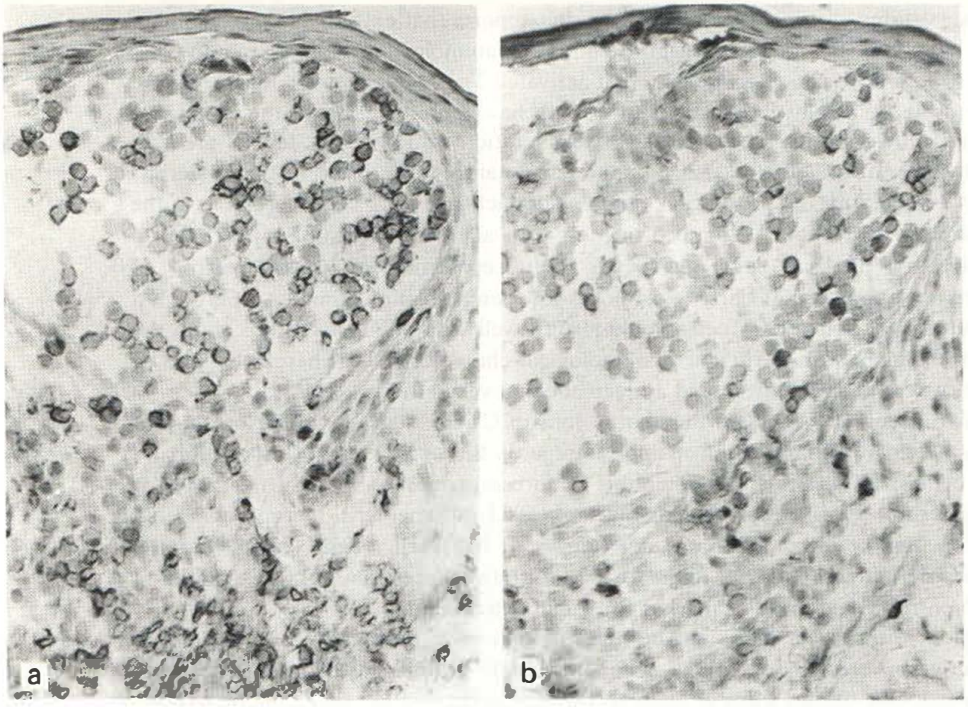

Fig. 3. Immunoperoxidase staining on serial frozen sections from skin lesions obtained from a patient with CTCL stage III. Stainings were in (*a*) with anti-Leu 1 and in (*b*) with anti-Tac antibodies. The secions were counterstained with haematoxylin. The solid lines indicate the epidermal basal membrane zone.

cells were characteristically arranged in groups (see Fig. 3*b*). In the epidermis, the percentage of Tac^+ T cells varied between 3 and 25% (see Table II) and no clear relationship to the stage of the disease was evident.

DISCUSSION

When T cells are activated, molecules such as the IL-2 receptor (Tac) and class II transplantation antigens that are not normally present on resting T cells are expressed (11, 13, 14). Analyses of class II and Tac expression on T lymphocytes have revealed the presence of in vivo activated T lymphocytes in different inflammatory conditions, most notably in synovial tissue from patients with active rheumatoid arthritis (15, 16). From analyses on cells eluted from rheumatoid synovial tissue it has also been demonstrated that IL-2 receptors and class II antigens are preferentially expressed on separate sets of T cells, and that the in vivo activated class II and Tac expressing cells differ in several respects from T cells activated in vitro (16). Consequently, a further understanding of the process that leads to activation of the immune system during inflammation in vivo would require further studies on the phenotypes of differently activated T cells *in situ* during various phases of inflammatory reactions.

In the present study we have found that Tac^+ cells occurring in the skin in the tuberculin reaction, in allergic and irritant contact dermatitis are proportionally more frequent in the epidermis, than in the dermis. Platt et al. (17) have recently described similar results in the tuberculin reaction.

These observations are in agreement with earlier reports that extravasation of lymphocytes occur largely unspecifically in response both to immunological and non-immunological stimuli (1, 2). Our results also indicate that activated Tac^+ lymphocytes do not migrate randomly into the tissues investigated, as these cells have a predeliction for the epidermis. Whether this feature is only due to a selective migration of Tac^+ cells to this area, or whether activation of previously Tac^- lymphocytes mainly takes place in the epidermis cannot be evaluated with the present technique. The fact that Tac^+ cells were seen in the epidermis already at 24 hours after provocation with allergen suggests however, that a selective migration of Tac^+ cells may occur to the epidermis; at least in vitro IL-2 receptors are not expressed as early as 24 hours after an activation stimulus (16). Such a migratory pattern for activated T cells would also fit well with previous notions about the epidermis as an organ with particular immunological characteristics, where activation of the immune system against introduced antigens occurs very efficiently (18).

The distribution of Tac^+ cells in the skin lesions of CTCL stage III differed from that seen in non-malignant inflammation mainly as regards the occurrence of proportionally more Tac^+ cells in the dermis. Whether this feature mirrors an abnormal migration or activation pattern of T cells in advanced stages of CTCL or whether it merely represents different intensities of inflammation is not known. It has earlier been shown that the majority of the dermal lymphocytes in the CTCL infiltrates express class II transplantation antigens, which represent another marker for T cell activation (19).

In conclusion, the present data indicate that interactions between activated T lymphocytes and antigen-presenting cells may occur differently in different microenvironments within one particular tissue. Careful analysis of local events within these microenvironments is thus essential for our understanding of the inflammatory processes in skin.

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