

ACID α -NAPHTHYL ACETATE ESTERASE STAINING OF T LYMPHOCYTES IN HUMAN SKIN

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Abstract. T lymphocytes were stained in order to disclose α -naphthyl acetate esterase activity (ANAE staining) by adopting the method described by Mueller et al. In ANAE-staining of frozen sections from human lymph nodes, more than 90% of the lymphocytes in paracortical areas (T cell areas) were ANAE-positive, but in cortical follicles (B cell areas) less than 5% of the cells were positive. Lymphocytic infiltrations in various dermatoses (lichen ruber planus, psoriasis, SLE, atopic dermatitis, erythema multiforme, poikiloderma atrophicum vasculare, and Sézary syndrome) were investigated. A high percentage of ANAE-positive lymphoid cells (>80%) was found in most cases. One patient with chronic lymphatic leukaemia, however, had a smaller proportion of ANAE-positive lymphocytic cells in an erythema multiforme skin infiltrate. ANAE staining seems to be an easy method for the identification of T lymphocytes in skin sections. The results of this investigation support the hypothesis that T lymphocytes have an affinity to skin.

Key words: T lymphocytes; Staining enzymatic; Histology skin

Lymphocytes can be divided into two populations, having differing functions. The first group, the T lymphocytes, are essential for cell-mediated immune responses such as delayed hypersensitivity skin reactions and co-operate in humoral responses. The second group, the B lymphocytes, are precursors of antibody-forming cells, and are of importance in humoral immunity.

The nature and localization of lymphoid cells in dermal infiltrates are of importance in the pathogenesis of skin diseases. B lymphocytes can be identified in both suspensions and frozen tissue sections by the demonstration of immunoglobulins or C3 receptors on the surface of the cells. Viable T lymphocytes can be detected in suspensions with SRBC-rosette techniques, but most authors have

been unsuccessful in identifying T lymphocytes in tissue sections (2, 10, 11).

Mueller et al. (5) have described a method for the identification of T lymphocytes in frozen tissue sections of mouse lymphoid tissue and cell smears by using acid α -naphthyl acetate esterase activity. Positive, dot-like reaction products appeared in most of the lymphocytes located in the diffuse cortical ("thymus-dependent") areas of the lymph nodes. However, in the cortical follicles, which are composed predominantly of B lymphocytes, less than 7% of the lymphocytes were esterase-positive. Macrophages were intensely stained all over, and had no dots.

Ranki et al. have shown that the α -naphthyl acetate esterase staining (ANAE staining) of cell smears from mouse spleen and lymph node cells (7), and of human peripheral lymphocytes (8) gave reliable results. The ANAE marker-carrying cells were included in the population of lymphocytes forming rosettes with sheep red blood cells (T lymphocytes), but not in the surface-bound Ig-carrying lymphocyte population (B lymphocytes).

However, transformation of the lymphocytes to blast cells by *in vitro* stimulation with T- and B-cell mitogens showed that the ANAE marker was not a reliable criterion for the presence of T cells in the activated state. Thus the method could only be recommended for the testing of "resting" lymphocytes.

The purpose of this work was to use the ANAE staining method to identify T lymphocytes in frozen tissue sections from various dermatoses. Control staining was also performed on smears from human peripheral lymphocytes and frozen tissue sections from human lymph nodes.

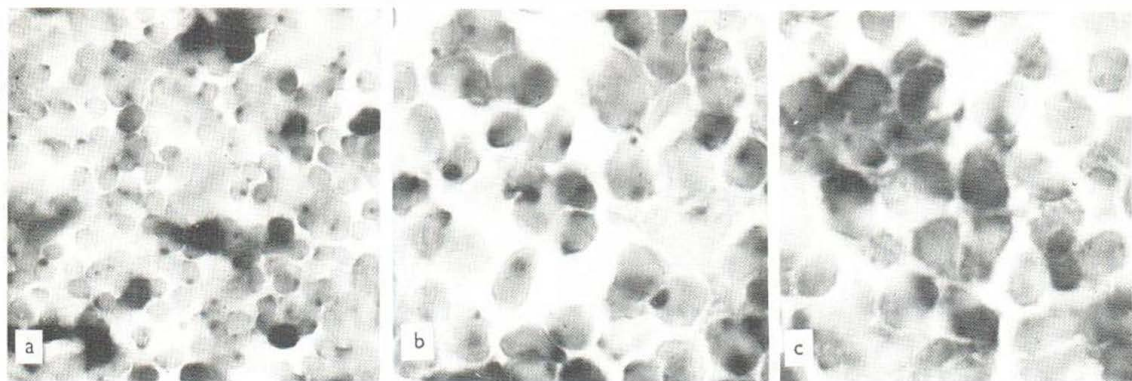


Fig. 1. Demonstration of ANAE activity in histological section of human lymph node. The majority of the lymphocytes in the paracortical areas (a, $\times 260$; b, $\times 650$) are ANAE-positive. In the cortical follicle (c, $\times 650$) most

of the cells are ANAE-negative. Larger cells (macrophages), which are stained intensely all over, are seen in a.

MATERIAL AND METHODS

Patients and controls

The patients were obtained from the Department of Dermatology, Södersjukhuset, Stockholm. Their diagnoses were verified by clinical and histological examinations.

Infiltrates where only mononuclear cells dominated were studied.

The patients investigated were: 2 with lichen ruber planus, 2 with longstanding psoriasis, 1 patient with poikiloderma atrophicum vasculare (probably mycosis fungoides, premycotic stage), 1 patient with Sézary

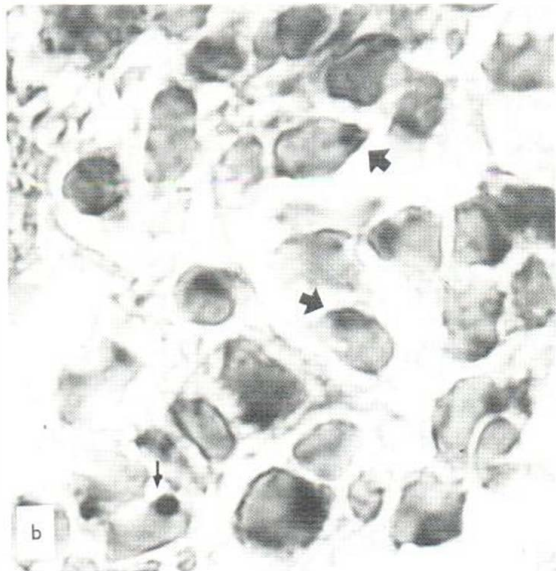
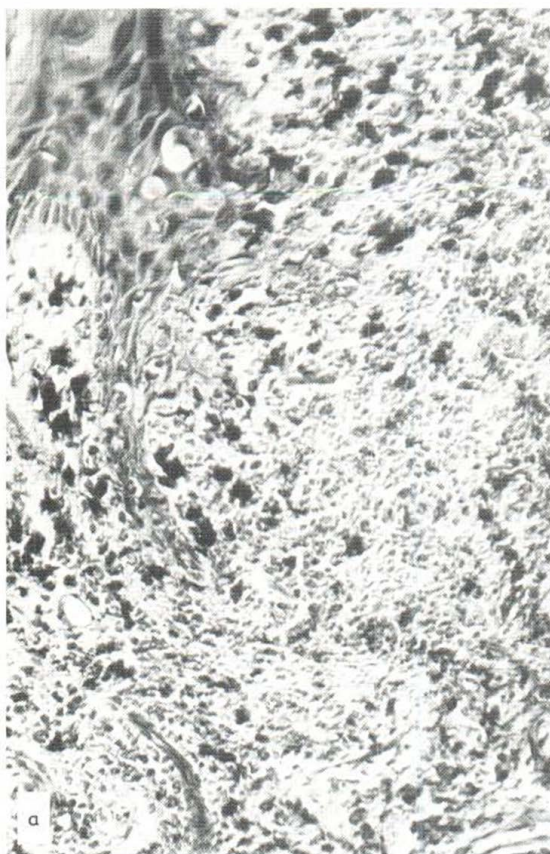


Fig. 2. Demonstration of ANAE activity in histological section of skin lesion in poikiloderma atrophicum vasculare (a, $\times 100$; b, $\times 1000$). Dense subepidermal infiltrate of small and medium sized lymphocytic cells, most of them ANAE-positive. The upper dermis also contains large cells (histiocytes), which are stained intensely all over.

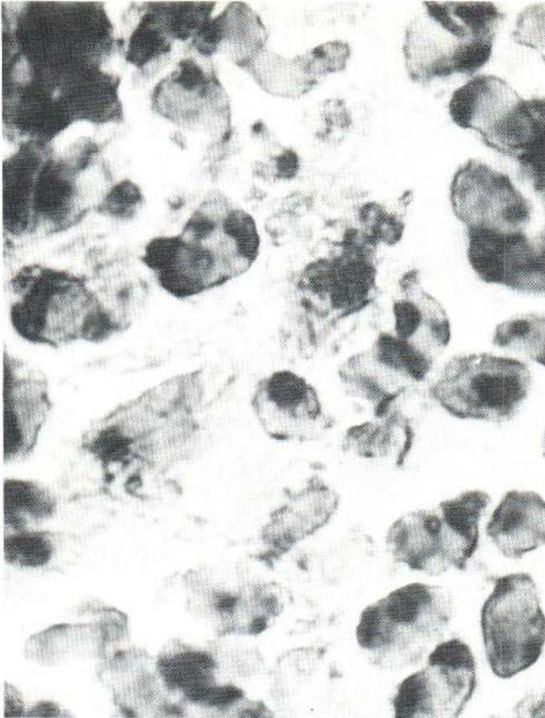


Fig. 3. ANAE-positive cells in skin lesion of psoriasis ($\times 1000$).

syndrome (the patient was treated with lekeran and prednisolon and had a total leukocyte count of $30\,000/\text{mm}^3$, of which 82% were lymphocytes), 1 patient with systemic lupus erythematosus (the patient was treated with prednisolon and was in remission), 4 with subacute atopic dermatitis, 1 patient with chronic eczema, 2 patients with erythema multiforme of whom one also had chron. lymphatic leukaemia. The latter patient had a total leukocyte count of $240\,000/\text{mm}^3$.

Smears of peripheral lymphocytes were investigated in

3 healthy controls, in the patient with Sézary syndrome and in the patient with chron. lymphatic leukaemia. Tissue sections from human mesenteric lymph nodes were also studied as a control.

Preparation and staining of tissue sections

The method used was essentially that described for ANAE staining of lymphoid organs by Mueller et al. (5). Human mesenteric lymph nodes and tissue from skin biopsies (3–4 mm cutaneous punch biopsies) were fixed in buffered formol-sucrose (pH 6.8) at 4°C for 22 h and kept in Holt's gum sucrose at 4°C for another 22 h. Frozen sections of 8–10 μm thickness were mounted onto slides which had been pretreated with formolgelatine. The preparations were allowed to dry at room temperature for 1 h. Two sections were stained for ANAE activity for 2 h and one section was conventionally stained with haematoxylin and eosin. Hexazotized pararosaniline was prepared as follows. Solution A: 1 g of pararosaniline (acridine-free) was dissolved in 20 ml distilled water and 5 ml concentrated HCl was added. After gentle warming, cooling, and filtration, the solution was stored in the dark at 4°C . Solution B: freshly prepared 4% aqueous solution of sodium nitrite. Equal parts of solutions A and B were mixed and shaken for a few seconds until the colour turned amber. Activity of ANAE was ascertained by incubation in a medium consisting of 40 ml of 0.067 M phosphate buffer, pH 5.0, 2.4 ml of hexazotized pararosaniline and 10 mg of α -naphthyl acetate in 0.4 ml acetone. The mixture was adjusted to pH 5.8 using 2 N NaOH. One of the two sections stained with α -naphthyl acetate was counterstained with 1% methylgreen in 0.1 M acetate buffer at pH 4.2 for 2 min and the other with 1% aqueous toluidine blue solution for 30 min. The preparations were dehydrated by passage through increasing concentrations of ethanol, cleared in xylene and mounted. In order to obtain a better quality of the cell morphology, separate biopsies, which were paraffin embedded instead of frozen before sectioning and haematoxylin and eosin staining, were also taken from most of the patients.

Smears from Ficoll-Isopaque separated human peripheral blood lymphocytes were allowed to dry at room

Table I. Results of ANAE staining in various dermatoses

Diagnosis	n	% small or medium sized mononuclear cells in HTX-eosin staining	ANAE staining	
			% infiltrating cells which stained intensely all over	% small or medium sized lymphocytic cells with dots (ANAE-positive cells)
Lichen ruber planus	2	>70	<20	>85
Psoriasis	2	>70	<30	>85
Atopic dermatitis	4	>70	<25	>80
Chronic eczema	1	>70	15	>90
SLE	1	20	75	>80
Erythema multiforme	1	>70	25	>80
Erythema multiforme+CLL	1	>70	25	<40
Poikiloderma atrophicans vasculare	1	>70	10	>85
Sézary syndrome	1	50	45	>90

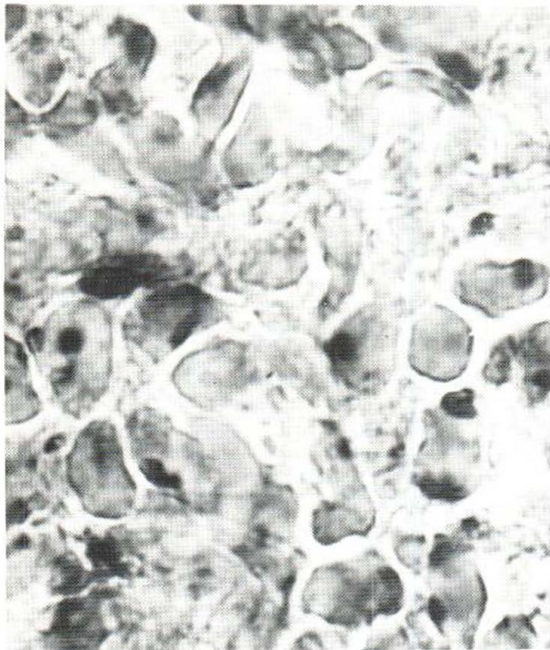


Fig. 4. ANAE-positive cells in skin lesion of lichen ruber planus ($\times 1000$).

temperature and fixed in cold Baker's formolcalcium, pH 6.7, at 4°C for 10 min and stained for ANAE for 21 h.

A lymphocytic cell was scored ANAE-positive if its cytoplasm contained a single or a few distinct spots (the reddish brown reaction product). At least 200 mononuclear cells from each staining were counted and classified in a light microscope ($\times 1000$).

RESULTS

Smears of peripheral human lymphocytes

About 60% of the cells from 3 healthy controls were ANAE-positive. The patient with the Sézary syndrome had about 50% ANAE-positive cells and the patient with chron. lymphatic leukaemia had about 1% ANAE-positive cells.

Human mesenteric lymph node

In paracortical areas, more than 90% of the lymphocytes were ANAE-positive (Fig. 1a, b) and in follicular areas less than 5% were positive and in the centre of the follicles almost no positive cells were seen (Fig. 1c). In both paracortical and follicular areas there were also larger cells (macrophages) which stained intensely all over the cell (Fig. 1) but in most areas they comprised less than 5% of the total cell population.

The results of skin biopsies are shown in Table I. Small and medium sized lymphocytic cells dominated in all patients investigated, with the exception of the patient with SLE, in whom larger cells were numerous, many of them identifiable as histiocytes in haematoxylin and eosin staining. In ANAE staining, most of the cells in the sparse infiltrate from the patient with SLE were stained intensely all over, and had no dots, but most of the few small or medium sized lymphoid cells were ANAE-positive.

The patient with Sézary syndrome had a sub-epidermal infiltrate which consisted of medium-sized lymphocytic cells with atypical cerebriform nucleus (about 50%) and bigger cells with large nucleoli (about 40%). More than 90% of the medium sized lymphocytes were ANAE-positive. The bigger cells with large nucleoli were stained intensely all over the cell in ANAE staining.

In the patients with lichen ruber planus, psoriasis, atopic dermatitis, chron. eczema, erythema multiforme, and poikiloderma atrophicum vasculare, small and medium sized lymphocytic cells were dominant in the infiltrates. Most of the lymphocytic cells (>80%) were ANAE-positive (Figs. 2-6) in all patients except for the patient with erythema multiforme who also had chron. lymphatic leukaemia. Here, <40% of the cells were ANAE-positive (Fig. 7). The percentages of ANAE-positive lymphocytic cells in infiltrates from

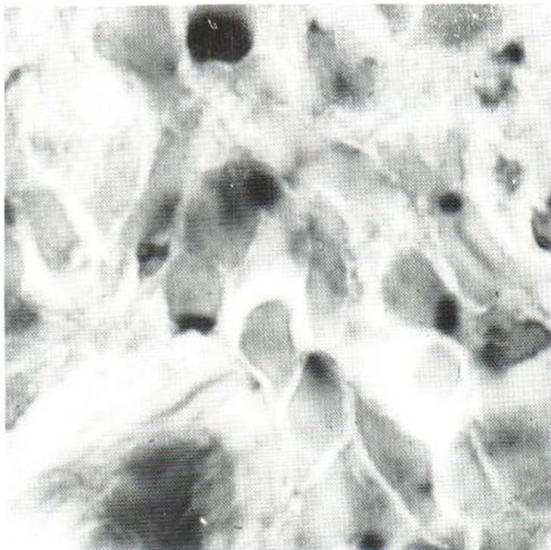


Fig. 5. ANAE-positive cells in skin lesion of atopic dermatitis ($\times 1000$).

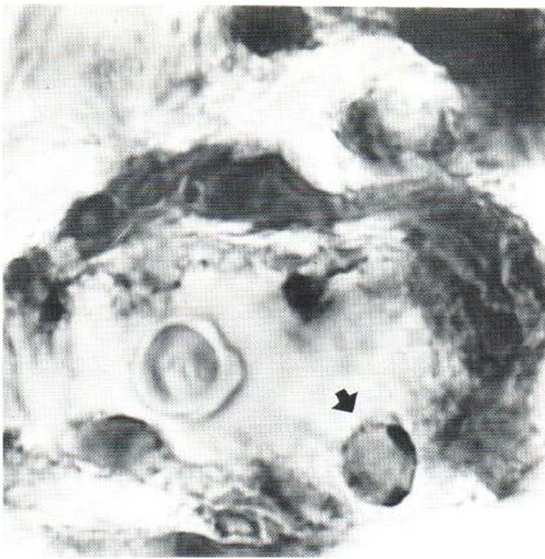


Fig. 6. ANAE-positive lymphocyte passing through the wall of a vessel ($\times 1000$).

the patients with lichen ruber planus, psoriasis, atopic dermatitis, and poikiloderma atrophicum vasculare were in many regions about the same as those found in the paracortical areas of the lymph nodes. Most of the cells which infiltrated the papillae were large cells, and many of them could be identified as histiocytes by haematoxylin-eosin staining. In ANAE staining, most of the large cells infiltrating the papillae were intensely stained all over the cell, but there were also a few small or medium sized lymphocytic cells, most of which were ANAE-positive.

DISCUSSION

Walker (11) has studied 11 patients with lichen ruber planus and found that the majority of the mononuclear cells in the infiltrates from skin or oral mucosa show neither the membrane receptor characteristics of B lymphocytes nor those of macrophages. He concluded by exclusion that the cells were probably T lymphocytes. Edelson et al. (2) and Tan et al. (10) have applied methods of tissue disaggregation in their studies on skin lesions seen in various dermatoses. Viable cells from skin infiltrates were liberated upon tissue disaggregation, and studied with immunological methods. Tan et al. found 66–86% T lymphocytes and less than 5% B lymphocytes in the skin infiltrates of 7 pa-

tients with mycosis fungoides or Sézary syndrome. In lesions from 5 patients with lichen ruber planus they found 67–84% T lymphocytes and less than 5% B lymphocytes. However, in 2 of 4 patients with non-Hodgkin lymphomas they found that B lymphocytes dominated (51–60%) the cellular content of skin infiltrates, and in those 2 patients the T lymphocytes accounted for 33–39% of the lymphocyte infiltrate. Also using the method of tissue disaggregation, Edelson et al. (2) found that a high percentage of the infiltrating cells in a Bowen's squamous cell carcinomatous lesion were T lymphocytes. However, it is often difficult with the tissue disaggregation method to obtain enough infiltrating cells to be able to quantitate T and B lymphocytes, and it is not possible to determine where in the skin lesion the different cell types are located, and whether the cells investigated are representative in general.

The ANAE-staining method is specific for T

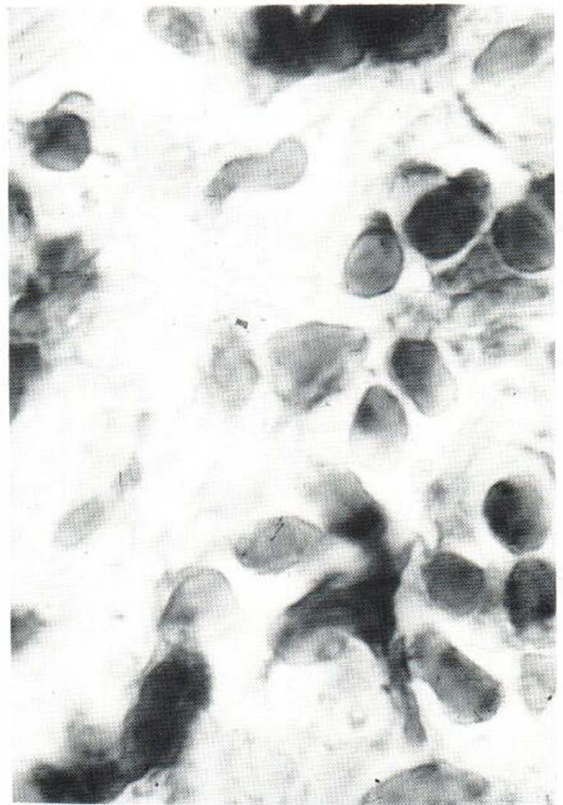


Fig. 7. Less than 40% of the lymphocytic cells were ANAE-positive in a skin lesion of erythema multiforme in a patient with chron. lymphatic leukaemia ($\times 1000$).

lymphocytes in frozen sections of mouse lymphoid tissue (5) and for "resting" human T lymphocytes in smears (8). In this study most of the lymphocytes in paracortical areas of human lymph nodes (T cell areas) were ANAE-positive and most of the cells in the cortical follicles (B cell areas) were ANAE-negative. Thus the ANAE staining method also seems to be specific for T lymphocytes in frozen sections of human tissue, although further investigations are necessary in order to determine whether false-negative or -positive ANAE staining can arise. In haematoxylin- and eosin-staining it is difficult to differentiate between lymphocytes and monocytes, and thus some of the small and medium sized lymphocytic cells in the mononuclear skin infiltrates could be monocytes. We could not conclude from this investigation how monocytes react to ANAE staining. The staining is easy to perform and the ANAE-positive cells are readily detected histologically by ordinary light microscopy.

The Sézary syndrome is known as "a T-cell erythrodermia" (1). In the skininfiltrate from the patient with Sézary syndrome about 50% of the cells were ANAE-positive medium sized lymphocytic cells. It could not be determined on the basis of this investigation whether the bigger cells, in which ANAE staining was intense and present throughout the cytoplasm, were of histiocytic origin, or were T lymphocytes and ANAE-negative, as they were abnormal (or stimulated) cells.

Chronic lymphatic leukaemias are known to be B-cell leukaemias in most cases (6). The patient with chron. lymphatic leukaemia had a low ANAE-positive cell count in the smears from peripheral blood lymphocytes. There was also a lower percentage of ANAE-positive cells in the erythema multiforme skin infiltrate from this patient than in any of the other lymphocytic skin infiltrates investigated.

Most of the lymphocytes in the skin infiltrates tested by means of ANAE staining were T lymphocytes. The results are consistent with those of Tan et al. (10), who found that T lymphocytes dominated in the skin infiltrates from patients with lichen ruber planus. They are also consistent with the results of Walker (11), who found that the majority of mononuclear cells in infiltrates from lichen ruber planus showed neither the membrane receptor characteristics of B lymphocytes nor those of macrophages.

The results of this investigation support the

hypothesis (3) that T lymphocytes have affinity to skin. The decreased number of T lymphocytes in peripheral blood, as reported in both atopic dermatitis (9) and psoriasis (4), may be explained as a translocation of T lymphocytes from blood to skin. Since many T lymphocytes seem to be localized in the skin in these conditions, the total number of T lymphocytes in the body need not necessarily be decreased in atopic dermatitis or psoriasis.

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