

Upregulation of CD40 and CD40 Ligand Expression in IgE-associated Cutaneous Diseases

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In order to better understand the immunological processes connected with IgE-associated cutaneous disease, we have examined the expression of CD40 and its ligand CD40L, required for the induction of IgE synthesis in B-cells, as well as of IgE and its receptors in various dermatoses (atopic dermatitis (AD), scabies, chronic recurrent urticaria) versus normal skin, and in one dermopathic lymph node versus normal lymphatic tissue by immunohistochemistry. Compared to normal skin, cells expressing IgE, FcεRI, FcεRII, CD40, CD40L and L26 were increased in the dermis, partly also in the epidermis, from patients with AD and scabies, but not in chronic urticaria. CD40 and CD40L were detected on numerous cells in lymphatic tissue from both normal donors and a patient with AD, whereas large numbers of IgE- and FcεRI-positive cells were only found in the dermopathic lymph node from the AD patient, in contrast to very few in normal lymphatic tissue. These results with selectively increased IgE/FcεRI and associated CD40/CD40L expression in the skin of AD and scabies suggest that cutaneous tissue, in addition to dermopathic lymphatic tissue, might contribute to IgE synthesis. **Key words:** atopic dermatitis; scabies; urticaria; IgE synthesis.

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Binding of allergens to specific IgE through FcεRI on mast cells and basophils results in degranulation and release of inflammatory mediators (1). The pathogenetic role of allergen-specific IgE has been well established for diseases like urticaria, allergic rhinoconjunctivitis and asthma (2). Recently, the detection of IgE-binding structures (FcεRI/RII) on dendritic cells in the skin has led to new concepts regarding the role of these structures in various cutaneous inflammatory disorders, particularly in atopic dermatitis (AD) (3–6). On the other hand, the mechanisms responsible for the association between certain dermatoses and markedly elevated serum IgE levels have not been completely elucidated; nor is it known whether induction of IgE synthesis in the skin itself may contribute to an increased IgE production in cutaneous diseases.

Induction of IgE synthesis in human B-cells requires two signals: the binding of the cytokines IL-4 or IL-13 to their respective receptors and the interaction of the CD40 molecule on B-cells with its natural ligand CD40L expressed on activated T-cells (7–9). Recently, it has been reported that in addition to T-cells, basophils and mast cells can express CD40L (10). Furthermore, CD40/CD40L interaction results in upregulation of adhesion and costimulatory molecules (11). This interaction has also been suggested to play a role in the induction of

inflammatory infiltrates through upregulation of adhesion molecules (12, 13).

In order to shed light on the relevance of these findings in cutaneous disease, we set out to evaluate the expression of CD40 and CD40L as well as of IgE and its receptors (FcεRI and RII) in normal skin and in various cutaneous diseases associated with an IgE-dependent pathological process. These findings were related to the presence of activated T- and B-cells and compared to findings in lymph nodes, in order to determine the relative contribution of skin versus lymph node tissue to IgE production in cutaneous disease.

MATERIAL AND METHODS

Patients and tissue samples

Skin biopsies were taken after informed consent from patients with moderate to severe chronic AD ($n=12$; mean of serum IgE levels = 4,865 kU/l, normal range < 150 kU/l), from lesional and non-lesional skin of patients with chronic recurrent urticaria ($n=8$; mean of serum IgE levels = 329 kU/l), and for diagnostic purposes from patients with scabies ($n=11$; mean of serum IgE levels of 8 of these patients = 361 kU/l; IgE not determined in 3 patients). Biopsy sites were the inner aspects of the upper arms in AD patients and mostly the trunk in urticaria and scabies patients, depending on the location of lesions. There was no preceding treatment with steroids. Normal skin samples were obtained from specimens of patients undergoing plastic surgery ($n=8$; serum IgE not determined). One dermopathic lymph node specimen from a patient with severe AD (serum IgE 5,360 kU/l) and normal lymphatic tissue ($n=5$; serum IgE not determined) were available from the tissue banks of the departments. All specimens were snap frozen in liquid nitrogen for immunohistochemistry.

Immunohistochemistry and antibodies

Cryostat sections were stained by using the APAAP technique, as previously described (14). The following antibodies were used: anti-IgE from DAKO (Denmark) at the dilution of 1:10,000; anti-FcεRI (29C6), a kind gift from J. Hakimi (Hoffmann La Roche, New Jersey), at the dilution of 1:7,500; anti-CD23 (Tü1), a gift from A. Ziegler (Virchow Klinikum, Berlin), undiluted; anti-CD40 (mAb 89) from Immunotech (France) and anti-CD40L (TRAP1) (15) at 1:25,000 and 1:10, respectively. Langerhans' cells were detected by using anti-CD1a (T6) from DAKO (Denmark), (1:100); mast cells with anti-tryptase (AA1) from A.F. Walls, Southampton (England), (1:10); activated T-cells with anti-CD30 (HRS-4) from Immunotech (France), (1:200); and B-cells with L26 from Dako (Denmark), (1:200). Double-labelling was attempted but abandoned because of loss of sensitivity of cell detection.

Assessment and statistical analysis

After the staining procedures, tissue sections were examined microscopically by two independent observers at 400× magnification. Nucleated, positively stained cells were counted in five different microscopic fields. Results are expressed as mean cell counts/high-power field (HPF). For some antibodies (anti-IgE, 29C6, Tü1, anti-CD40, anti-CD40L, HRS-4, L26) the pattern of expression in

the epidermis and dermis was determined separately. Comparative analysis between normal and diseased skin sections is based on the Mann-Whitney U test and the Spearman rank correlation test. Values ($p < 0.05$) are considered to be significant, values ($p < 0.01$) highly significant.

RESULTS

Increased presence of cells expressing IgE and the IgE receptors in atopic dermatitis and scabies

Fig. 1a, b shows the results of the expression of IgE and its receptors by using anti-IgE, anti-FcεRI and anti-FcεRII antibodies in skin biopsies (epidermis and dermis separately) from normal persons and from patients with AD, urticaria and scabies. Tissue sections from patients with AD and scabies showed a significant increase of IgE-expressing cells in the epidermis and dermis, compared to normal skin. The number of FcεRI-bearing cells was significantly increased in the epi-

dermis of lesional atopic skin and in the dermal compartment of tissue sections from patients with scabies. In contrast, staining of tissue sections from patients with chronic urticaria was unchanged for IgE in the epidermis and dermis, compared to controls, and cells expressing FcεRI in chronic urticaria sections were even significantly decreased. Cells expressing CD23, the low affinity IgE receptor (FcεRII), were rare in all sections studied. Nevertheless, a significant increase of CD23-bearing cells was detected in the epidermis and dermis of skin sections from patients with AD and in the dermis of scabies lesions. The number of cells expressing CD23 was not altered in chronic urticaria.

Increased numbers of CD40- and CD40L-expressing cells in lesional skin from patients with atopic dermatitis and scabies

Counts of cells expressing CD40 and its ligand in the same tissue from the patients with AD, urticaria and scabies are shown in Fig. 2. CD40- and CD40L-positive cells were barely detectable in the epidermis of normal skin, and only moderate numbers were observed in the dermis of these skin specimens. Tissue sections from patients with AD showed an increased presence of CD40- and CD40L-positive cells in both epidermis and dermis, compared to normal skin. A significantly augmented number of CD40- and CD40L-bearing cells was also determined in the dermal compartment of scabietic skin, but not in the epidermis. In contrast, no difference in the amount of CD40- and CD40L-positive cells, either in the epidermis or in the dermis, was observed in tissue sections from patients with chronic urticaria, compared to controls.

Since CD40L is mainly expressed on activated T-cells, but also on mast cells, we used anti-CD30 and anti-tryptase antibodies to determine activated T-cells and mast cells in

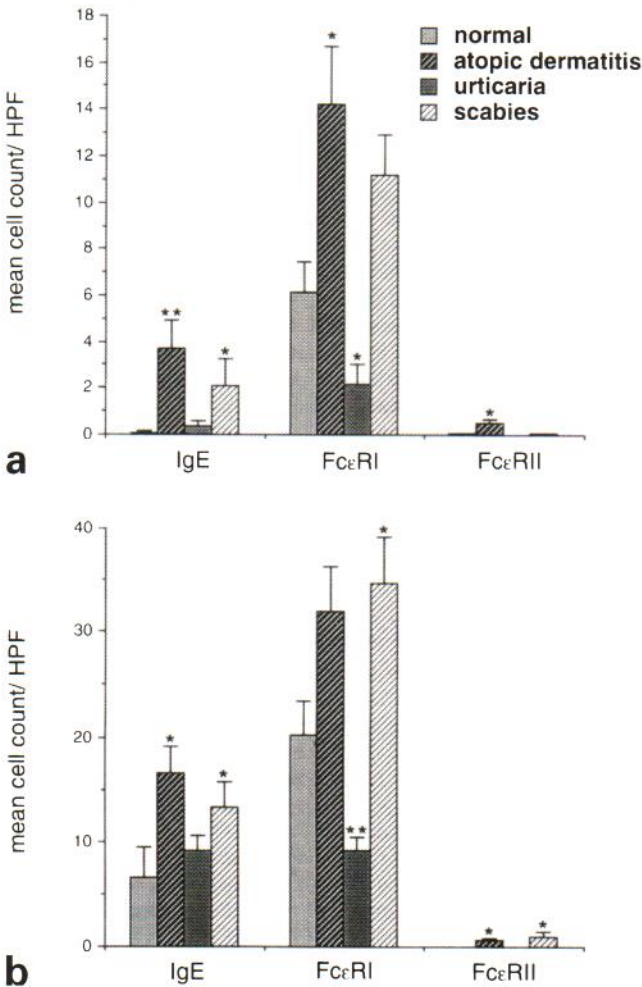


Fig. 1. Increase of cells expressing IgE and IgE receptors in AD and scabies lesions. (a) The expression of IgE, FcεRI and FcεRII (CD23) in the epidermis of skin sections from normal persons ($n=8$) and from patients with AD ($n=12$), urticaria ($n=12$) and scabies ($n=11$) was assessed by immunohistochemistry, using the APAAP method. Mean cell counts/HPF + SEM are shown. Significant changes compared to normal skin are indicated with an asterisk ($*=p < 0.05$, $**=p < 0.01$). (b) Same sections, antibodies and methods as in a, with evaluation of cells in the dermis.

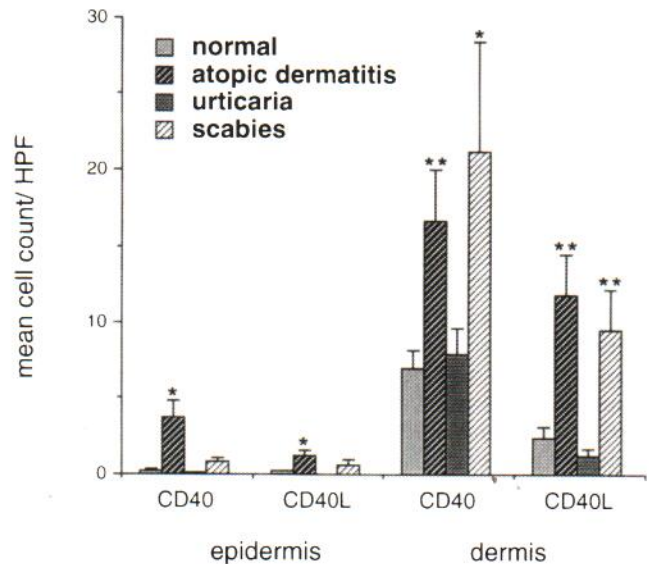


Fig. 2. Increase of cells expressing CD40 and CD40L in lesional skin from patients with AD and scabies. The expression of CD40 and CD40L in the epidermis and dermis of skin sections from normal persons ($n=8$) and from patients with AD ($n=12$), urticaria ($n=12$) and scabies ($n=11$) was assessed by immunohistochemistry, using the APAAP method. Mean cell counts/HPF + SEM are shown. Significant changes compared to normal skin are indicated with an asterisk ($*=p < 0.05$, $**=p < 0.01$).

normal skin and lesional skin from patients with AD, scabies and urticaria (Fig. 3). A highly significant ($p < 0.01$) increase of CD30-positive cells was detected in the dermis and a significant increase in the epidermis (not shown) of lesional atopic skin. In AD and scabies, numbers of CD30-positive cells in both epidermis and dermis correlated significantly with those of CD40L-positive cells. The mast cell counts were, however, not altered in skin biopsies from patients with AD and even decreased in scabietic skin, compared to normal skin.

Since B-cells are the source of all immunoglobulins, their numbers were examined as well, using the L26 antibody (Fig. 3). L26-positive B-cells were increased in the dermis but not in the epidermis of lesional atopic and scabietic versus control skin ($p < 0.05$), although overall numbers were low (1.44 and 2.37 versus 0.36 cells/high-power field).

Numerous IgE- and FcεRI- positive cells in lymph node tissue from a patient with atopic dermatitis

Next, we evaluated the amount of cells expressing IgE, the high affinity IgE receptor and CD40 and its ligand in lymphatic tissue from a patient with severe AD and from normal controls. Fig. 4a, b shows that IgE and FcεRI are expressed on a large number of cells in the dermopathic lymph node from the patient with severe AD. IgE-bearing cells were particularly accumulated within the lymph node follicles, while FcεRI-positive cells were restricted to the paracortical region. Very few IgE- and FcεRI-expressing cells were observed in lymphatic tissue from normal donors (Fig. 4c, d).

In the follicles, large numbers of cells expressed CD40 (not shown). Moderate numbers of CD40L-positive cells were found in the follicles, marginal and T zones of lymphatic tissue (Fig. 5a). For comparison, a skin section from a patient with

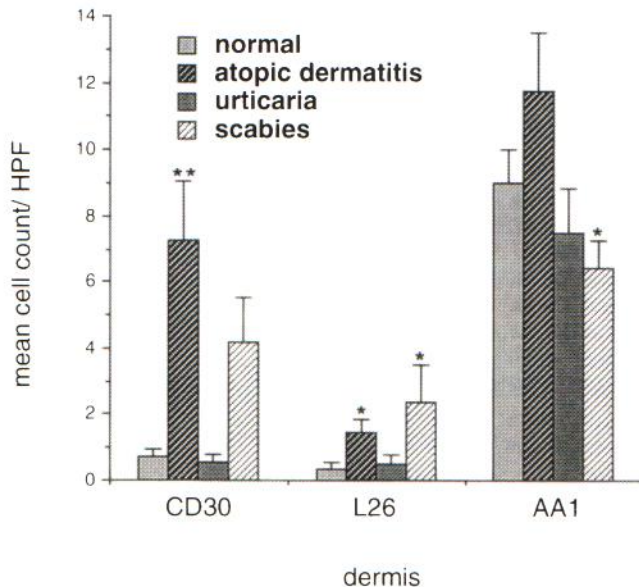


Fig. 3. Increase of CD30-positive cells in skin sections from patients with AD. The expression of activated T-cells (CD30), B-cells (L26) and mast cells (AA1) in the dermis of skin sections from normal persons ($n = 8$) and from patients with AD ($n = 12$), urticaria ($n = 12$) and scabies ($n = 11$) was assessed by immunohistochemistry, using the APAAP method. Mean cell counts/HPF + SEM are shown. Significant values are indicated with an asterisk (* = $p < 0.05$, ** = $p < 0.01$).

AD is shown with numerous CD40L-expressing cells in the dermal infiltrate (Fig. 5b). In contrast to findings in the skin (Fig. 2), we observed no quantitative differences between the counts of CD40- and CD40L-bearing cells in the lymph node from the AD patient, compared to normal lymphatic tissue (data not shown).

DISCUSSION

In the present study, we have shown increased numbers of cells expressing IgE, FcεRI, FcεRII, CD40 and CD40L in the epidermis and dermis from tissue sections of patients with AD and scabies. The data are in agreement with previous studies, which demonstrated increased IgE-binding and expression of FcεRI in the skin and particularly on Langerhans' and dermal dendritic cells in AD patients (4, 16, 17). Recent findings suggest that increased FcεRI expression may be of pathogenetic importance in the induction and maintenance of chronic IgE-associated inflammation as well as in sustained antigen presentation, which may occur in AD. It is thought that FcεRI binds specific IgE, and upon allergen exposure, the FcεRI-bearing cells are directly activated by allergen-mediated crosslinking of their Fcε-receptors. Subsequently, cytokines would be produced and adhesion molecules upregulated, thus supporting the chronic inflammatory process in the skin (18, 19).

In contrast to AD, the role of IgE-mediated mechanisms in scabies has not yet been well established. However, cross antigenicity between the scabies mite, *Sarcoptes scabiei*, and the house dust mite, *Dermatophagoides pteronyssinus*, has been described (20), and similar mechanisms as in AD are likely to be operative.

The role of CD23 in IgE dependent-disease is less well established. In contrast to the findings with FcεRI, we detected only low numbers of cells expressing CD23 in normal skin and in biopsies from patients with urticaria. Significantly increased CD23-positive cell counts were recorded in both epidermis and dermis of atopic skin lesions and only in the dermis in tissue sections from scabies, although absolute numbers were low. In agreement with our findings, Sakamoto et al. (21) have also reported an increased presence of CD23-positive lymphocytes in AD, although their numbers were higher. These quantitative differences may be due to the use of different antibodies, recognizing different epitopes. Using the Tü-1 antibody and the same technical procedure as in the present study, we have, however, previously reported large numbers of CD23-positive cells in cutaneous tissue, namely in a pseudolymphoma (22), indicating that CD23 expression is comparatively low in AD.

The present study provides for the first time data on the expression of CD40 and its natural ligand CD40L in normal and diseased skin. CD40 and CD40L are shown to be expressed at increased levels in lesional skin from patients with AD and scabies, but not urticaria. Since the interaction of the CD40 molecule on B-cells with CD40L on activated T-cells in the presence of the cytokines IL-4 or IL-13 is known to result in induction of IgE synthesis in human B-cells (8), the increased presence of CD40- and CD40L-bearing cells would agree with clinical observations reporting increased serum IgE in patients with AD (23). In addition, in atopic patients, genetic predisposition and other not yet identified factors favour the markedly augmented production of IgE (24). The presence of

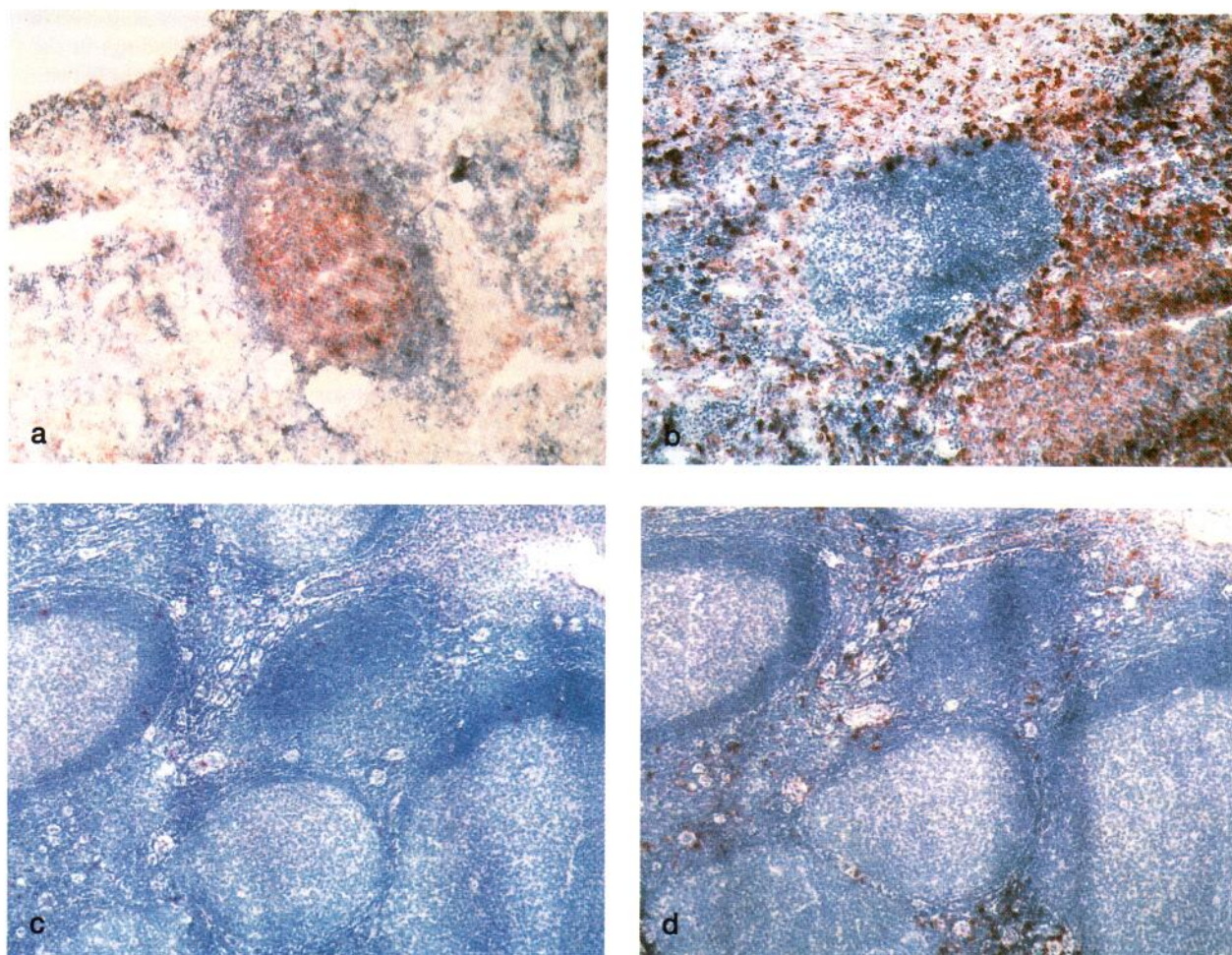


Fig. 4. (a) Staining with anti-IgE in a dermopathic lymph node from a patient with severe AD. Accumulation of IgE-positive cells within a lymph node follicle (APAAP technique, $\times 100$). (b) Numerous FceRI-expressing cells within the paracortical region of the same lymph node as in a (APAAP technique $\times 100$). (c, d) Comparatively few IgE- and FceRI-expressing cells in lymphatic tissue from normal donors (APAAP technique $\times 100$).

TH2-positive lymphocytes and mast cells as potentially IL-4- and IL-13-producing cells as well as the expression of CD40 and CD40L and the increased amount of B-cells (L26-positive) in AD would provide all elements currently known to allow local IgE production in the skin. It is unlikely that CD40L-positive mast cells make a major contribution to this process, since their numbers remained within the normal range and the increased expression of CD40L in skin biopsies from patients with AD and scabies correlated instead significantly with the expression of CD30, a marker for activated lymphoid cells. An additional contribution of CD40L on cutaneous mast cells in AD or scabies cannot, however, be entirely ruled out with the present data, since double-labelling could not be performed for reasons mentioned.

It should also be mentioned that the upregulation of CD40 in lesional skin of patients with AD, as noted here, may be due to cells other than B-cells, since dendritic, monocytic and endothelial cells have also been shown to express this molecule (12, 13, 25). The functional significance of CD40 on these different types of cells remains, however, to be determined. Certainly, the increased presence of dendritic cells in AD is well established (17) and may explain the marked upregulation of CD40 expression in the AD sections. On the other hand,

only few B-cells were detected in lesional dermis, which means that local IgE synthesis is not likely to occur on a major scale in the skin.

This concept is supported by the presence of numerous CD40- and CD40L-positive cells in lymphatic tissue from normal donors and a patient with AD. The accumulation of IgE-expressing cells in the lymph node follicles from a patient with AD reflects the high rate of IgE-producing plasma cells in this particular lymph node specimen.

Taken together, the local pathology in atopic and scabietic skin, including increased numbers of resident and infiltrating cells expressing IgE/FceRI and CD40/CD40L, with associated release of stimulatory cytokines, provides further insight into the understanding of local inflammation in the epidermis and dermis from patients with these diseases. In view of the marked expression of the same markers in lymphatic tissue, particularly in the dermopathic lymph node of an atopic patient, the relative contribution of nonlymphatic CD40/CD40L interactions to the induction of IgE synthesis within the skin needs to be further clarified.

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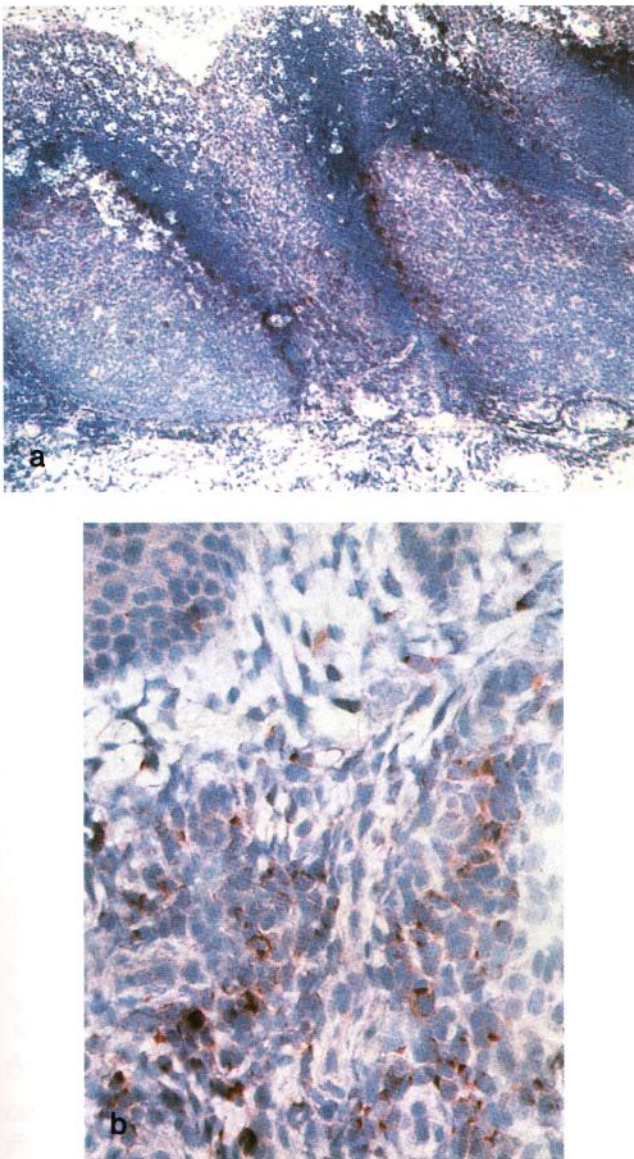


Fig. 5. (a) Moderate numbers of CD40L-positive cells in the follicles, marginal and T zones of lymphatic tissue from a normal donor (APAAP technique $\times 100$). (b) Lesional atopic skin with CD40L-expressing cells in the dermal infiltrate (APAAP technique $\times 400$).

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