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New Aspects in the Pathogenesis of Atopic Dermatitis

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Atopic dermatitis (AD) has a complex pathogenesis. Many factors may be involved in the circulation and in the skin. In the circulation the most important changes are an abnormal T-lymphocyte function (1) and an increased releasability of basophils (2), which are possibly due to an increased intracellular c-AMP phosphodiesterase activity (3), an increased serum IgE level with a specificity to a wide variety of allergens (4) and blood eosinophilia (5).

In the skin of patients with AD there exists an infiltration of activated T-lymphocytes (with an increased T4/T8 ratio) (6) and antigen presenting cells (CD1+ and RFD1+) (7, 8), lying in the upper part of the dermis and around blood vessels. Although intact eosinophils are only occasionally observed, they may play a role in the inflammatory mechanism since abundant depositions of extracellularly lying eosinophil derived proteins (major basic protein) have been reported in AD skin (9). The increased serum IgE level is reflected by an increased binding of IgE molecules to mast cells (10) and also by binding of IgE molecules to dendritic cells (CD1+) in the epidermis and dermis (11, 12, 13, 14, 15) (Fig. 1). The latter phenomenon is present in clinically involved and, to a lower degree, also in clinically "normal" looking skin from AD patients with elevated serum IgE levels. Immunoelectron microscopy studies on epidermal cell suspensions from AD patients revealed that IgE molecules were present on CD1+ cls containing Birbeck granules and, therefore, being Langerhans cells (LC) (11). Occasionally IgE+/CD1+ cells without Birbeck granules (indeterminate cells) were also observed.

The epidermal anti-IgE staining in AD patients disappears after 2 weeks of local corticosteroid (triamcinolon acetonide) treatment, whereas the epidermal CD1 staining is still present (personal observation).

Since the tissue IgE level is proportional to the serum IgE level it may be expected that the presence of IgE on epidermal LC is not specific for AD and may be observed in other skin diseases with elevated serum IgE levels. Indeed, the clinically involved skin of patients with mycosis fungoides and psoriasis with elevated serum IgE levels may also show a dendritic anti-IgE staining (personal observations).

Further studies on LC enriched epidermal cell suspensions from AD patients revealed that IgE is bound to LC by a Fc-receptor. This FcεR on LC is trypsin resistant, has affinity for IgG, binds with BB10, a monoclonal antibody directed against the FcεR on eosinophils, platelets and macrophages, does not bind to anti-CD23 antibodies directed against the FcεR on B-lymphocytes, and is associated with the CD1 antigen (16). The significance of this latter association is not yet clear.

The question arises whether IgE molecules, present on epidermal LC, have biological significance. *Are IgE molecules on epidermal Langerhans cells specific for and do they bind allergens?*

Patch test reactions to allergens

Environmental allergens (airborne or acroallergens) may reach the skin via the circulation after inhalation or via direct contact with the skin. It is still obscure if eczematous skin lesions in AD patients can be induced after inhalation of allergens (17, 18). Evidence has been presented that aeroallergens can penetrate the skin after direct epidermal contact and induce eczematous skin lesions in AD patients (19, 20). The penetration through the epidermis of molecules with a large molecular weight (compared to classical contact allergens) may be explained by an epidermal barrier dysfunction, which has been described in clinically involved and clinically normal looking AD skin (21, 22, 23). Several groups (20, 24, 25, 26, 27) have reported the presence of delayed type patch test reactions (positive after 24–48 h) to aeroallergens in patients with AD. These patch test reactions may be observed after epicutaneous application of aeroallergens on slightly abraded (24), stripped (25, 27) or even intact skin (26). Furthermore, these delayed patch test reactions with aeroallergens seem to be specific for AD patients, since they cannot be observed in non-atopic normals or atopics without AD.

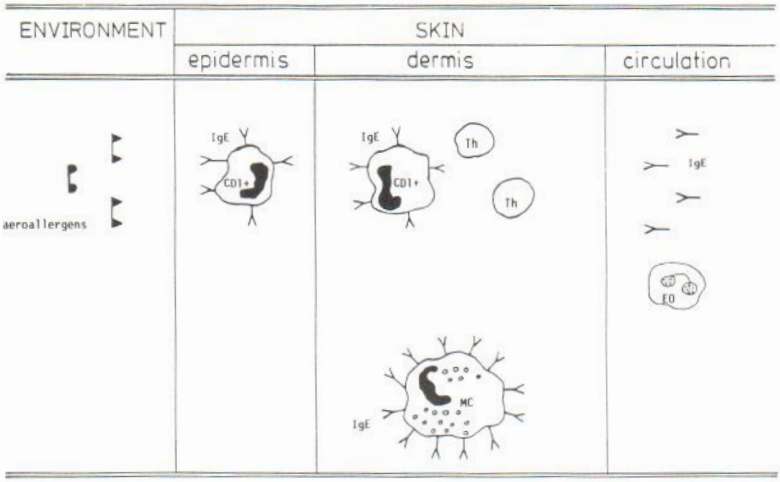

Fig. 1. Inflammatory cells in AD skin. EO = eosinophil; MC = Mast cell; Th = CD4+ cell (T helper/inducer cell); CD1+ = Langerhans cell/indeterminate cell.

Clinically and histopathologically the patch test with aeroallergens induces an eczematous response with spongiosis in the epidermis and a cellular infiltrate in the dermis, mainly consisting of activated T-lymphocytes, IgE+/CD1+ cells and eosinophils (20, 24, 25, 26, 27). The observations made on eosinophils in the patch test reaction to aeroallergens will be discussed later. An influx of basophils was also reported (24), but could not be confirmed in later studies (20, 25, 26, 27). Neutrophils were not present (27).

In conclusion, the patch test with aeroallergens induces an eczematous response, which is specific for AD patients and which has clinical and histopathological similarities with clinically involved AD skin. Therefore, the patch test reaction with aeroallergens forms an attractive working model to investigate the reaction mechanism by which aeroallergens play a role in the pathogenesis of AD. The most important cell types, which are involved in the delayed in time patch test reaction to aeroallergens are IgE+/CD1+ cells, T-lymphocytes and eosinophils.

In vitro lymphocyte response to aeroallergens

Atopic patients have circulating aeroallergen specific T-lymphocytes (28–36). Therefore, we performed lymphocyte stimulation tests with house dust allergen, using epidermal LC as antigen presenting cells to investigate if LC from AD patients can present aeroallergens to T lymphocytes. LC enriched epidermal cell suspensions were prepared from clinically non-involved skin from AD patients and autologous T lymphocytes were obtained from peripheral blood. Commercially available crude house dust extract was used as allergen. T-lymphocytes from AD patients and normal non-atopic controls proliferate to house dust allergen if monocytes from peripheral blood are used as antigen presenting cells. This is in agreement with other reports (28, 35). However, if LC are used as antigen presenting cells, only T-lymphocytes from AD patients show a proliferative response (Table I). Furthermore, the T-lymphocyte proliferative response to house dust allergens, using epidermal LC as

Table I. *Net cpm of T cell proliferation on house dust antigen (50 µg/ml)*

cpm = counts per minute, AD = atopic dermatitis, LC = Langerhans cells, MNC = non T cells from peripheral blood, sIgE on LC = the presence of cell-bound IgE on LC

AD patients	10^3Lc 10^5T cells	sIgE on LC	MNC $(2 \cdot 10^5)$
1	1 452	+	9 593
2	8 034	+	5 106
3	6 205	+	16 142
4	2 140	+	5 242
5	643	+	10 458
6	2 448	+	6 926
7	18 630	+	14 607
8	27 312	+	15 318
9	26 403	+	17 534
10	28	–	10 869
11	–207	–	12 561
12	68	–	472
Non-atopic controls			
1	–140	–	6 386
2	206	–	4 365
3	208	–	6 146

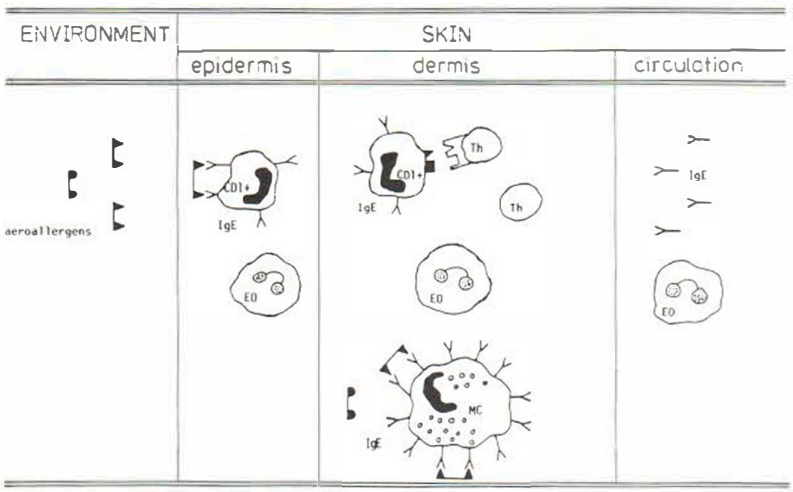

Fig. 2. Inflammatory cells in AD skin during a patch test reaction with aeroallergens.

antigen presenting cells, is restricted to AD patients with IgE-bearing LC. The T-lymphocyte response can be inhibited by anti-HLA-DR antibodies. T-lymphocytes do not react with house dust in the absence of antigen presenting cells. Keratinocytes are not able to induce a T-lymphocyte response to house dust allergen.

These preliminary results suggest that, in contrast to IgE- LC, IgE+ LC from AD patients may be immunologically active and induce a T-lymphocytic response to allergens. However, direct evidence that allergens indeed bind to IgE molecules on LC before presentation to T lymphocytes, is still missing. Furthermore, it is unknown which fraction of the allergen extract is involved in IgE binding and which fraction is recognized by T-lymphocytes. Therefore, studies with more purified allergen fractions are needed.

The possible role of IgE-bearing Langerhans cells

Sofar, arguments have been put forward which suggest that IgE+ LC may play a role in the reaction mechanism behind the patch test reaction to aeroallergens. Aeroallergens may bind to allergen-specific IgE on LC, which present the allergen to T-lymphocytes, inducing an eczematous response (Fig. 2). This mechanism may also be involved in the pathogenesis of the eczematous skin in AD. However, allergen-specific T-lymphocytes, present in AD skin may not only be involved in the eczematous response, but also in the regulation of the IgE production by B-lymphocytes. Two arguments favour this possibility. The first one comes from a recent study of Carswell et al. (37). They reported that in children with AD, with or without asthma, the level of serum IgE antibodies with a specificity to mite body allergen was significantly more elevated than in children with only asthma, whereas the level of serum IgE antibodies with a specificity to the faecal mite allergens was not significantly different. Since the mite body is 12 times greater in size than the faecal particles, these allergens are not likely to be inhaled and it was hypothesized that sensitization to allergens from the mite body occur via penetration of the (eczematous) skin.

A second argument comes from recent work of Hauser et al. (38). They reported that in mice epidermal LC were capable of inducing antigen-specific T-lymphocytes of the TH-2 subtype, which were able to produce Interleukin-4 (IL-4). IL-4 induces the IgE synthesis by B lymphocytes (39). Furthermore, IL-4 induces the expression of a low affinity FC-receptor for IgE not only on B cells (40), but also on monocytes (40). Furthermore, IL-4 induces the differentiation of monocytes into dendritic cells, increases class II MHC expression of monocytes and inhibits the secretion of IL-1 by monocytes (41). If we translate this to AD skin, the following pathway is possible after epidermal contact of aeroallergens with AD skin (Fig. 3).

Aeroallergens are capable of penetrating the skin, bind to IgE on LC and induce a T-lymphocytic response. These T-lymphocytes may be involved in the induction of the eczematous response. However, some T-lymphocytes may belong to the TH-2 subtype, which produces IL-4. IL-4 induces IgE-production by B-lymphocytes in afferent lymph nodes. Furthermore, IL-4 may be involved in the induction of a FcεR on monocytes, which further differentiate into

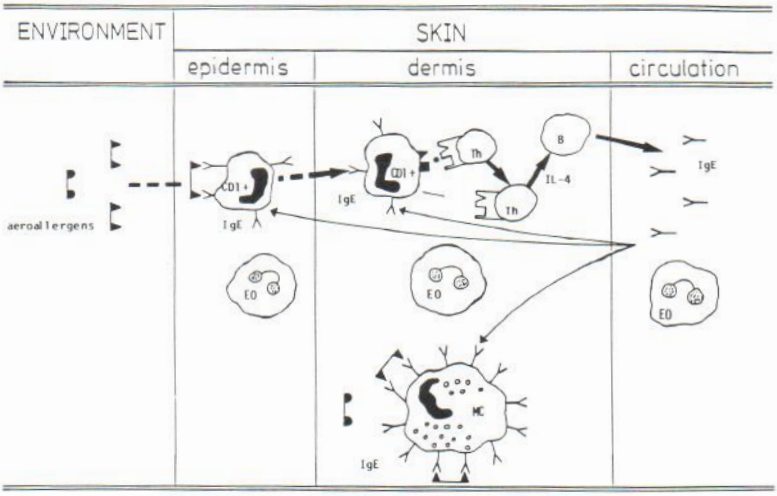

Fig. 3. Induction of IL-4 production after epicutaneous contact with aeroallergens.

dendritic cells, or directly on dendritic cells and LC in the skin, thereby amplifying the response to allergens. In vitro, IL-4 producing T-helper cells are increasingly present after repeated exposure to antigen, suggesting that IL-4 producing cells may play a particular important role in the immune response to antigen that persists or that is encountered repeatedly (42). Contact with environmental allergens like aeroallergens is indeed frequent or even continuous.

IL-4 induces the expression of a low affinity IgE receptor. The IgE receptor on LC seems to have a comparatively high affinity for IgE, since IgE is easily bound in vivo. Therefore, some factors must be involved to increase its affinity for IgE. Good candidates are local inflammatory mediators like platelet-activating-factor, leukotriene B4 and histamine, which have been reported to increase the affinity of the FcεR on eosinophils (43).

The possible role of eosinophils

6–24 h after patch testing with aeroallergens eosinophils are infiltrating the dermis and at 24 h they also appear in the epidermis. At 24 h they are occasionally lying close to IgE+ LC. The eosinophils, lying in the dermis at 6–24 h after patch testing, are in an activated stage since they stain with EG2 antibodies (recognizing eosinophil cationic protein (ECP) from activated eosinophils and also bind to the secreted forms of ECP and eosinophil protein-X (EPX)). However, the eosinophils lying in the epidermis 24 h after patch testing are EG2 negative (27).

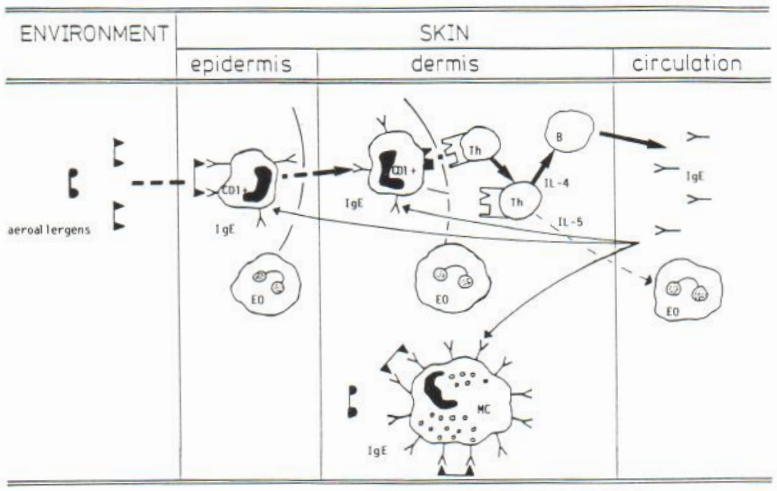

Fig. 4. The role of eosinophils during a patch test reaction to aeroallergens.

If we compare a patch test reaction to aeroallergens with a patch test reaction to conventional contact allergens (thiuram) in the same AD patient, the presence of eosinophils in the epidermis was observed in the 24 h patch test reaction to aeroallergens but not in the thiuram patch test reaction. Therefore, the presence of eosinophils in the epidermis seems to be specific for the patch test reaction to aeroallergens.

The presence of eosinophils may relate the patch test reaction to aeroallergens to the late phase allergic reaction, which may occur after intracutaneous administration of the aeroallergen and is IgE and mast cell dependent. However, a late phase allergic reaction in the skin does not show eczematous changes and is characterized by an infiltration of neutrophils (44, 45). Neutrophils are not observed in the patch test reaction to aeroallergens. This suggests that in the patch test reaction to aeroallergens a reaction mechanism is involved which differs from a classical contact allergic reaction and also from a late phase allergic reaction in the skin.

The TH-2 lymphocyte subtype, which can produce IL-4 is also capable of producing IL-5 (46). Since IL-5 is known as eosinophil colony stimulating factor, this may explain why many AD patients have peripheral blood eosinophilia. In patch test reactions eosinophils are lying in the dermis in mononuclear cell infiltrates and in the epidermis close to LC. Recently, it was reported (47) that eosinophil derived eosinophil cationic protein is capable of inhibiting a T-lymphocyte proliferative response. Therefore, we speculate that eosinophils in patch test reactions to allergens form a defending mechanism of the body to block or inhibit the LC-T cell-B cell amplification pathway (Fig. 4).

In conclusion, these results favour a role for aeroallergens, LC, T-lymphocytes and eosinophils in the pathogenesis of AD. These allergens may after contact with the skin via binding to IgE positive LC induce a cascade of events which may be responsible for the induction of an eczematous response but also for the induction or regulation of the IgE production by B-lymphocytes.

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