

IgE on Langerhans cells in the skin of patients with atopic dermatitis and birch allergy

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Epidermal cell suspensions from the skin of seven patients with atopic dermatitis (AD) and seven healthy non-atopic controls were investigated for the presence of surface HLA-DR and CD1 antigen, and IgE using indirect and double-staining immunofluorescence techniques. Fifty-seven percent of all CD1+ and 68% of all HLA-DR+ cells from the patients demonstrated IgE on their surface, indicating that Langerhans cells (Lc) in AD may be a heterogeneous population with regard to surface characteristics. No IgE+ cells were found in the epidermal cell suspensions from the healthy non-atopic controls. An attempt to demonstrate birch pollen antigen on the surface of Lc from the same patients all strongly allergic to birch pollen, using indirect immunofluorescence techniques, was unsuccessful, also after *in vitro* incubation of the Lc with high concentration of the birch antigen.

Key words: atopic dermatitis; birch allergy; IgE; Langerhans cells.

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CLINICAL ASPECTS

This study shows the presence of IgE on about two-thirds of epidermal Langerhans cells isolated from uninvolved skin of atopic dermatitis patients with birch allergy. The worsening of the dermatitis in these patients during the pollen season could be due to involvement of IgE+ Langerhans cells.

Recent studies indicate that both immediate and delayed type hypersensitivity reactions are involved in the pathogenesis of atopic dermatitis (AD) (8, 10, 15). The dermal mononuclear cell infiltrate consists mainly of T cells (1), in particular T helper cells, and relatively low numbers of suppressor/cytotoxic T cells (11, 15). Increased numbers of Langerhans cells (Lc) have been demonstrated in acute (7, 15), subacute (11), as well as chronic lichenified lesions (12). Atopic dermatitis patients may show positive reactions to inhaled allergens both in intradermal as well as in patch tests (5, 8, 10). Recently, Bruynzeel-Koomen et al. (4) demonstrated the presence of IgE molecules on epidermal Lc from patients with AD. Therefore, it has been sug-

gested that in patients with AD, epidermal Lc might bind allergens to IgE molecules on their surface, and thereby present these antigens to T cells in a delayed type hypersensitivity reaction.

Birch allergen is known to cause positive skin reactions of the immediate type in a large number of patients in Scandinavia. The present study performed in May/June with maximum exposure to birch pollen shows that epidermal Lc from patients with AD strongly allergic to birch pollen antigen have surface IgE, while non-atopic controls do not. An attempt to demonstrate birch allergen on the surface of Lc, also after *in vitro* incubation with birch allergen, was not successful.

MATERIAL AND METHODS

Patients

Seven patients with atopic dermatitis (3 females and 4 males, mean age 23 years, range 18–32 years) diagnosed according to the criteria of Rajka (9) were studied. They had a history of birch pollen allergy, a positive prick test, serum RAST class 2–4 to birch pollen and RIST >1000 units/ml. Most patients were allergic to other agents in the prick test series used, i.e. three patients reacted with most of the allergens, two reacted with house dust mite, timothy and cat and/or dog, and one reacted with house dust mite only. The patients received no systemic or local corticosteroid therapy for at least 2 weeks prior to the study. Seven healthy non-atopic volunteers (4 females and 3 males, mean age 31 years, range 27–42 years) with negative prick tests, negative RAST to birch allergen and normal total serum IgE level were used as controls.

Preparation of epidermal cell suspension

The epidermis was separated from the dermis at the basement membrane level *in vivo* using a suction blister device (6). The suction blisters were obtained from clinically uninvolved abdominal skin of the patients in the period of maximum exposure to birch allergen. The epidermal sheets were treated with trypsin/deoxyribonuclease as previously described (2). After washing, the cells were suspended in medium RPMI-1640 with L-glutamine (Gibco Bio-Cult Glasgow, Scotland) supplemented with penicillin, streptomycin and 20% pooled human serum. Viability demonstrated by trypan blue exclusion was usually >90%. No peroxidase positive monocytes/macrophages were detected in the epidermal cell suspensions.

Birch pollen antigen

A commercially available purified water-soluble reference birch antigen (Spectralgen, ref. BK 2/60) was kindly supplied by Nycomed

A/S, Oslo, Norway. This antigen has been previously characterized by Vik et al. (13).

Sera and antibodies

Lyophilised IgE-rich pooled serum (IgE-RPS) from 240 patients allergic to birch pollen with serum RAST class 4 to *Betula verrucosa* pollen, i.e. with a high concentration of anti-birch IgE, was purchased from Nycomed A/S, Oslo, Norway. Pooled serum from eight healthy non-atopic donors with negative prick tests and negative RAST to birch allergen was used as a control. A rabbit anti-birch pollen specific anti-serum was obtained from Nycomed A/S, Oslo, Norway.

The monoclonal antibodies selected for the detection of antigens present on the epidermal Lc included OKT6 (Ortho Pharmaceutical Corp. Raritan, N.Y.), and anti-HLA-DR (Becton-Dickinson, Sunnyvale, CA, USA) directed against a nonpolymorphic region of human HLA-DR antigen. Anti-human IgE antibody clone SRT-3 was obtained from Serotec, England, and tested for specificity before being used. Rabbit IgG antibodies against human IgE were produced, affinity-purified and tested to be isotype specific in ELISA. Fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG + IgM was obtained from Tago, Burlingame, CA, USA, FITC-conjugated swine anti-rabbit IgG was from Dako (Glostrup, Denmark) and FITC-conjugated sheep anti-rabbit IgG from Serotec, England. Tetramethylrhodamine isothiocyanate (TRITC) labelled swine anti-rabbit IgG was a kind gift from Prof. Brandtzæg, Laboratory for Immunochemistry and Immunopathology, Rikshospitalet, Oslo, Norway. Rabbit anti-rat antibodies IgG class were purchased from Nordic (Tilburg, The Netherlands).

Indirect immunofluorescence staining (IIF)

The epidermal cell suspensions were first incubated at +4°C for 30 min with the monoclonal antibodies, diluted in PBS containing 1% bovine serum albumin, pH 7.2, washed twice in supplemented RPMI-1640 and then incubated with

FITC-conjugated goat anti-mouse IgG + IgM. At the end the cell suspensions were washed twice and mounted with PBS-glycerol.

Double staining for detection of CD1 (OKT6) or HLA-DR antigens on IgE-positive Ec was performed by first incubating cells with the monoclonal antibodies OKT6 or anti-human IgE for 60 min at +4°C. The cells were afterwards washed twice with supplemented RPMI-1640 and then incubated with FITC-conjugated goat anti-mouse IgG + IgM antibody for 30 min. The cell suspensions labelled with OKT6 were later incubated with rabbit anti-human IgE, and the cells stained with monoclonal anti-human IgE were incubated with immunosorbent-purified rabbit anti-HLA-DR antibody. Finally, the cells were incubated with TRITC-labelled swine anti-rabbit IgG. The stained cells were enumerated using a Leitz fluorescent microscope. A minimum of 1000 cells were counted. As a control Ec were incubated with phosphate buffered saline (PBS) instead of the primary antibodies. As additional control Ec were first incubated with IgG rabbit anti-horse instead of rabbit anti-human IgE.

In some experiments IF staining with monoclonal anti-IgE was preceded by incubation of the cells with IgE-RPS (undiluted and diluted 1:20).

To study the possible binding of birch allergen on Lc, 10⁶ cells were incubated with either: 1) rabbit anti-birch specific IgG; 2) birch allergen followed by rabbit anti-birch IgG; or 3) IgE-RPS followed by incubations with birch allergen and anti-birch specific IgG. These incubations were performed as described by Yodoi & Ishizaka (14) with slight modification. The cells were incubated for 15 min at 37°C and then for 60 min at +4°C with occasional mixing and twice washing with supplemented RPMI-1640 in between. Both FITC-conjugated swine anti-rabbit and sheep anti-rabbit antisera were each added to one of duplicated Ec suspensions as control for binding specificity of the conjugates.

RESULTS

A mean of 1.4% of epidermal cells from AD patients strongly allergic to birch demonstrated surface IgE (Table 1). No staining for IgE was

found on Ec from healthy individuals. There was no staining of Ec when the primary antibody was omitted or replaced by rabbit anti-rat IgG.

Double immunofluorescence staining experiments showed that all IgE+ cells stained with OKT6 and/or anti-HLA-DR. A mean of 57% CD1+ and 68% of HLA-DR+ Ec from the atopic patients presented IgE on their surface (Table 2). Ec from seven patients with AD and seven non-atopic healthy individuals were incu-

Table 1
Percentage of epidermal cells stained with anti-IgE, OKT-6 and anti-HLA-DR monoclonal antibodies

Ec donors	IgE +	CD1 +	HLA-DR +
Atopic dermatitis			
KB	1.5	2.5	2.3
AA	1.6	2.3	2.3
GO	1.8	3.0	2.6
AK	0.9	2.6	1.3
PA	1.2	2.4	1.7
GB	1.5	2.5	2.2
MC	1.4	2.2	2.1
Mean±SEM	1.4±0.3	2.5±0.2*	2.1±0.4
Controls			
VS	0	2.7	2.1
PB	0	1.6	1.4
TK	0	1.9	1.9
TC	0	2.6	2.3
UL	0	1.4	1.1
KB	0	2.1	2.0
KV	0	1.9	1.1
Mean±SEM		2.0±0.4	1.7±0.5

* Statistical significance of the difference from normal controls (Student's t-test): $P < 0.05$.

Table 2
CD1 and HLA-DR antigens on IgE+ epidermal cells

Ec donors	IgE + /CD1 +	IgE + /HLA-DR +
Atopic dermatitis		
KB	60.0	65.2
AA	69.6	69.6
GO	60.0	69.2
AK	34.6	69.2
PA	50.0	70.6
GB	60.0	68.2
MG	63.6	66.7
Mean±SEM	56.8±10.5	68.4±1.7

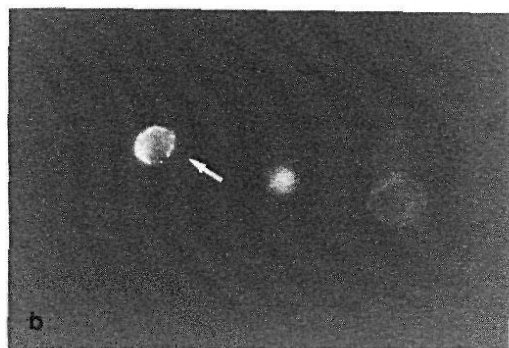
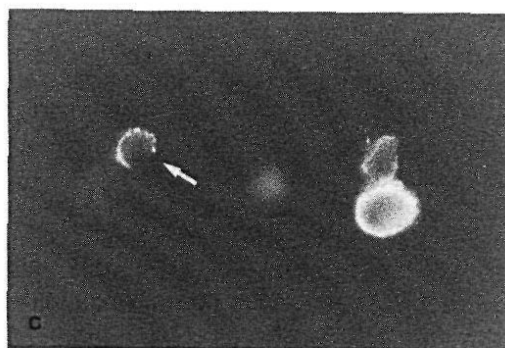
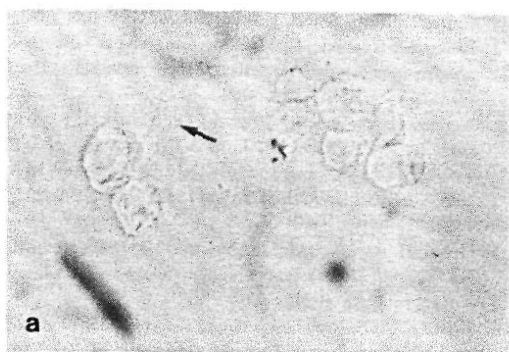


Fig. 1 a-c. Epidermal cells in suspension. Unstained (Fig. 1a). Double-stained with monoclonal anti-IgE + FITC-labelled goat anti-mouse Ig (Fig. 1b) and rabbit anti-HLA-DR-antibodies + TRITC-labelled swine anti-rabbit Ig (Fig. 1c). One cell is IgE + /HLA-DR + (arrows). $\times 450$.

bated with IgE-RPS from atopic patients allergic to birch. The number of IgE + Ec from AD patients before and after the incubation was the same. The IgE-RPS pre-incubated Ec from non-atopic healthy controls demonstrated no staining with monoclonal or polyclonal anti-IgE antibodies.

Birch allergen bound *in vivo* could not be demonstrated on epidermal Lc from any of the seven atopic patients examined. We were also unable to detect *in vitro* binding of birch allergen to Lc after incubation of Ec with birch allergen alone or with IgE-RPS followed by incubation with birch allergen. The same results were obtained whether swine anti-rabbit or sheep anti-rabbit antisera were used as FITC labelled antibodies.

A significantly higher percentage of CD1 + Ec was demonstrated in AD patients compared with the normal controls (mean $\pm$ SEM = 2.5 ± 0.2 versus 2.0 ± 0.4 ; $P < 0.05$, Student's t-test). In the patient group the percentage of CD1 + Ec was not significantly higher than that of HLA-DR + Ec (2.5 ± 0.2 versus 2.1 ± 0.4).

DISCUSSION

In the present study we have demonstrated IgE molecules on the surface of epidermal cells isolated from clinically uninvolved skin from the abdominal area of seven patients with atopic dermatitis. Double staining experiments demonstrated that all IgE + cells were either HLA-DR + and/or CD1 +, indicating that they were Lc. Fifty-seven percent of all CD1 + and 68% of HLA-DR + cells demonstrated IgE on their surface. Thus, it seems that Lc in normal-appearing skin from these patients are a heterogeneous population with regard to their affinity to IgE molecules. Our findings are in agreement with the findings of Bruynzeel-Koomen et al. (4) who recently reported that the majority of Lc from atopic patients were IgE +.

The percentage of IgE + Lc did not increase after incubation of the cells with IgE-RPS. This finding may indicate that IgE binding does not occur merely as a result of a high IgE concentration in the tissue. The possibility that trypsin and/or deoxyribonuclease treatment of the epidermal

cells affected the IgE presence on Lc cannot be excluded. Yodio & Ishizaka (14) found that treatment with 0.25% trypsin for 30 min abolished the expression of Fc ϵ R on lymphocytes, but it did not affect the transition of Fc γ R to Fc ϵ R after further incubation of the cells with IgE.

The positive patch test reactions to inhalant allergens demonstrated in patients with AD (5, 8, 10) may suggest an interaction between these allergens and Lc in the skin. In previous studies we have shown that Lc are the main antigen-presenting cells in epidermis (2). However, our attempt in the period of maximum exposure to demonstrate birch pollen on Lc from AD patients strongly allergic to this antigen was unsuccessful, also after incubation of the cells with the antigen *in vitro*. This may be due to lack of antigen bindings to the Lc surface or to concentrations so low as to be undetectable with the techniques used.

There was a significantly higher percentage of CD1 + Ec from uninvolved skin in AD patients compared with normal controls. To our knowledge, there are no previous data on the proportion of CD1 + and/or HLA-DR + Ec in uninvolved Ad skin. Sillevs Smitt et al. (11), examining skin sections from AD lesional skin, reported that the number of CD1 + cells exceeded the number of HLA-DR + dendritic cells.

The observation that about two-thirds of epidermal Langerhans cells from AD patients with birch allergy carry surface IgE may be of pathogenetic significance. The worsening of clinical status during the pollen season in these patients could be due to the involvement of IgE + Lc in a delayed type hypersensitivity reaction to birch allergen.

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