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Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis

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SUMMARY

The presence of Fc receptors for IgE on epidermal Langerhans cells (LC) from patients with atopic dermatitis (AD) was demonstrated by three different types of experiments. Firstly, cell-bound IgE on LC was removed by acid elution and restored by highly purified human myeloma IgE (IgE_κ). Secondly, after pepsin digestion of cell-bound IgE the number of LC staining with anti-human light chain (κ, λ) antibodies significantly decreased in contrast to the number of LC staining with anti-human ε heavy chain antibody. Thirdly, LC formed rosettes with sheep red blood cells (SRBC) coated with IgE_κ. Epidermal LC from normal non-atopic controls, did not form rosettes with SRBC-IgE. The SRBC-IgE rosette formation could be inhibited by preincubation with IgE_κ and BB10 (MoAb directed against the Fc receptor for IgE on human eosinophils, platelets and macrophages), but also with human IgG, whereas the SRBC-IgG rosette formation could be inhibited neither by IgE_κ nor by BB10. Both the SRBC-IgE and the SRBC-IgG rosette formation could be inhibited by OKT6 (anti-CD1) antibody. The results of inhibition studies with OKT6 antibody on the reconstitution of IgE on epidermal LC after acid elution suggest an associated expression of the CD1 antigen and the Fc receptor for IgE.

Keywords Langerhans cell atopic dermatitis $Fc_\varepsilon R$ IgE CD1 antigen CD1 molecule

INTRODUCTION

Epidermal Langerhans cells (LC) are important in the induction of delayed hypersensitivity responses to exogenous and possibly endogenous antigens. Glycoproteins present on cell membranes of LC include CD 1 (Murphy, Bhan & Sato, 1981), M241 (Bronstein, Murphy & Knowles, 1984), and HLA-class I (Bronstein *et al.*, 1983) and II antigens (Murphy, Bahn & Sato 1981). The CD1 antigen is also found on the surface of T cells undergoing maturation in the cortex of the thymus and on T cell leukaemia cell lines (Terhorst, Van Agthoven & LeClair 1980). The CD1 antigen on LC cannot be distinguished chemically from the CD1 antigen found on thymocytes, being a 49,000 D glycoprotein, associated with a smaller (12,000) β_2 microglobulin unit (Van Agthoven & Terhorst, 1982). The functional significance of the CD1 antigen is still unknown. There has been speculation that CD1 belongs to a family of related class 1 MHC antigens (Van de Rijn *et al.*, 1983). Recently, it was reported that the CD1 antigen can be internalized by receptor mediated endocytosis (Hanau *et al.*, 1987).

Besides the above-mentioned cell membrane antigens, LC express Fc receptors for IgG and for C3 (Stingl, Wolff-Schreiner & Pichler, 1977). In previous work (Bruynzeel-Koomen *et al.*, 1986), we demonstrated the presence of IgE molecules on epidermal LC from patients with atopic dermatitis (AD). Epidermal lying anti-IgE positive cells were present in skin biopsies from both clinically involved and clinically normal looking skin from patients with AD with high serum IgE levels. Immunogold electron microscopy studies on LC enriched epidermal cell suspensions from these patients showed that the anti-IgE positive cells were LC. The present study was initiated to demonstrate and further characterize Fc receptors for IgE on epidermal LC, using a rosette assay with fixed sheep erythrocytes (SRBC) coated with purified human myeloma IgE (IgE_κ). This rosette formation could be inhibited by a MoAb directed against the Fc receptor for IgE on human eosinophils, platelets and macrophages (BB10) (Capron *et al.*, 1986a) suggesting that the same type of Fc receptor for IgE might be involved on epidermal LC ($Fc_\varepsilon R_2$) (Capron *et al.*, 1986b). Furthermore, inhibition studies of the rosette assay were performed with antibodies directed against glycoproteins present on epidermal

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LC. These studies suggest an associated expression of the CD1 antigen and the Fc receptor for IgE on epidermal LC from patients with AD.

MATERIALS AND METHODS

Reagents

Minimal essential medium (MEM) and RPMI 1640, supplemented with 10% foetal calf serum (FCS), penicillin (100 IU/ml), streptomycin (100 μg/ml) glutamine (2 mM) and amphotericin (2·5 μg/ml) were obtained from GIBCO (Grand Island, NY, USA). Highly purified human myeloma IgE ((B.L.) κ light chain (IgE_κ)) and anti-$Fc_\varepsilon R_2$ antibody (named BB10) (IgM) with a specificity to $Fc_\varepsilon R_2$ on human eosinophils, platelets and macrophages were kindly provided by Dr. M. Capron and prepared as described (Capron *et al.*, 1986a). Highly purified human IgG was prepared by chromatography on a DEAE cellulose column. Immunosorbent purified goat anti-human Ig heavy chain (ε) or light chain (κ, λ) antibodies were obtained from TAGO (Burlingham, Ca, USA). Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human Ig heavy chain (ε, γ, μ, δ, α) or light chain (κ, λ) antibodies were obtained from Dakopatts (Copenhagen, Denmark). Tetramethylrhodamine isothiocyanate (TRITC)-conjugated swine anti-human heavy chain (ε) antibody was purchased from Nordic Immunological Laboratories (Tilburg, The Netherlands), and TRITC-conjugated rabbit anti-human light chain antibody from Dakopatts. OKT6 (anti-CD1) (IgG_1) and OKT6-FITC conjugated antibodies, used as a marker for LC (Harrist *et al.*, 1983), were obtained from Ortho Pharmaceutical (Raritan, NJ, USA), anti-human HLA-DR antibody (IgG_2) was obtained from Becton-Dickinson Monoclonal Center (Mountain View, Ca, USA), and trypsin from BHD Chemicals (Poole, UK). We obtained DNA-ase 1 from Boehringer (Mannheim, FRG), Ficoll-Hypaque from Pharmacia (Uppsala, Sweden) and pepsin from Sigma Chemical Company (St. Louis, MO, USA).

Patients

Twelve patients with AD were selected for this study. The AD diagnosis was made according to the criteria of Hanifin and Rajka (1980); the following four basic features were present: a chronic or chronically relapsing dermatitis, flexural lichenification, pruritis and a personal or family history of atopy (asthma, rhinitis, AD). All patients had extensive skin lesions involving the face, neck, upper part of the back and the greater part of arms and legs. Anti-IgE positive (indirect immunoperoxidase technique) cells were present in the epidermis of skin biopsies from clinically involved (erythematous and lichenified) and in clinically normal looking (dry or slightly lichenified) skin. None had received local corticosteroid therapy for at least two weeks before sampling. Their total serum IgE level was measured using the paper-radio-immunosorbent test (PRIST, Pharmacia, Uppsala, Sweden) and varied from 1,200 to 5,000 kU/L (1 kU = 2·42 ng IgE). The control group consisted of four healthy, non-atopic volunteers. All patients and controls gave their informed consent.

Preparation of LC enriched epidermal cells

Single cell suspensions were prepared from shave biopsies, obtained from clinically normal looking skin (dry or slightly lichenified skin) of patients with AD and from the flexor side of the fore-arm of normal non-atopics. The skin slices were washed in phosphate-buffered saline (PBS) and treated for 60–120 min in an EDTA-containing medium (Juhlin & Shelly 1979). The epidermis was detached from the dermis with a fine forceps and was soaked with 0·3% trypsin in PBS for 30 min at 37°C. The trypsinized epidermis was covered with RPMI medium and was gently stroked with a fine forceps. Subsequently, the cell pellet was resuspended in 1–3 ml of 0·025% deoxyribonuclease 1 in PBS for 1–3 min at 37°C, followed by repeated washing with RPMI medium. LC enrichment was obtained by centrifugation over Ficoll-Hypaque. Dispersed skin cells (4×10^6 cells/ml) in RPMI were layered on the gradient and centrifuged (30 min, 400 *g* at room temperature (RT)). The cells at the interphase were washed and resuspended in RPMI medium. The cell suspension contained 15–30% OKT6 positive cells. Viability after centrifugation, estimated by trypan blue exclusion, was 90–95%. The variable number of cells at the interphase ($2–6 \times 10^5$) limited the number of experiments that could be performed with each epidermal cell preparation.

Table 1. Demonstration of FcR for IgE on LC-enriched epidermal cells from AD patients

	% positive cells*				
	Acid elution†			Pepsin treatment‡	
IF reagent	before	after	$+IgE_\kappa$	before	after
OKT6	19·3 ± 2·6	19·3 ± 2·6	19·6 ± 3·1	19·0 ± 1·6	18·0 ± 2·5
anti-ε	18·3 ± 4·2	0	19·5 ± 2·7	18·0 ± 2·5	18·0 ± 1·4
anti-κ	17·9 ± 3·9	0	19·3 ± 2·6	16·2 ± 3·8	4·2 ± 3·6§
anti-λ	16·9 + 3·5	0	0	17·2 ± 3·4	7·0 ± 3·4§

* Immunofluorescence analysis of LC-enriched epidermal cells from AD patients ($n = 3–6$). The results are expressed as the percentage of fluorescent cells, mean ± s.d.

† Acid elution followed by incubation with IgE_κ.

‡ Pepsin digestion of Ig bound to LC.

§ Comparison with FITC-conjugated anti-κ and anti-λ staining before pepsin treatment, $P < 0·02$.

Immunofluorescence technique

LC enriched epidermal cells (5×10^4 cells) were incubated with FITC-conjugated OKT6 (1:80 in PBS with 1% HSA, 45 min at 4°C), anti-ε, anti-κ or anti-λ antibodies (1:20 in PBS with 1% HSA, 30 min at 37°C). After washing with PBS the number of cells positive with FITC-conjugated antibodies was determined by fluorescence microscopy. At least 500 cells were counted on each slide for each test.

Treatment at acid pH

To remove cell-bound Ig the LC enriched epidermal cells were treated with glycine-HCl buffer (0·05 M, pH 2·5) for 1 min at 4°C (Ishizaka & Ishizaka 1974), immediately followed by washing with RPMI twice. The viability of the cells after glycine-HCl treatment was 75–85%. In some experiments the cells (5×10^4 for each test) were subsequently incubated with IgE_κ (100 μg/ml/10^6 cells, 60 min at RT).

Pepsin cleavage of cell-bound Ig

To achieve fragmentation of cell-bound IgE, the LC enriched epidermal cells were treated with 0·003% pepsin in acetate buffer (pH 5·0) for 10 min at 37°C. Subsequently the cells were washed with RPMI and incubated with FITC-conjugated anti-ε, anti-κ or anti-λ antibodies. The viability of the cells after pepsin treatment was 80–90%.

Coating of sheep red blood cells (SRBC)

SRBC were treated according to the method of Spiegelberg and Melewicz (1980) by successive incubation with trypsin, pyruvic aldehyde and formaldehyde. The fixed SRBC were coated with IgEκ, IgG, OKT6 or goat anti-ε antibodies (400 μg/5 × 10^8 cells/ml) as described by Capron *et al.* (1981). Non-coated SRBC or BSA-coated SRBC were used as negative controls.

Rosette-assay

For this test 50 μl LC enriched epidermal cells (10^6/ml) were mixed with 25 μl coated SRBC (3 × 10^6/ml) and 25 μl MEM containing 4% BSA. After centrifuging this mixture for 5 min at 225 *g* at 4°C, the sediment was resuspended and the number of rosettes was determined by counting 400 cells in a haemocytometer. Only cells binding three or more erythrocytes were considered positive.

Statistical analysis

Statistical analysis was performed by using Student's *t*-test. For paired observations, the paired *t*-test was used. *P* values of ≥0·05 were considered significant.

RESULTS

Presence of cell-bound IgE

The presence of cell-bound IgE on LC enriched epidermal cells from patients with AD was demonstrated by immunofluorescence with FITC-conjugated anti-ε antibodies (Table 1). Before any treatment of the cells, a similar proportion of the epidermal cells stained with FITC-conjugated OKT6 antibody. Immunofluorescence with other fluorescent anti-human Ig heavy chain (γ, δ, μ and α) antibodies was negative, but immunofluorescence with anti-human Ig light chain antibodies (κ, λ) was positive. After acid elution immunofluorescence with FITC-conjugated anti-ε and anti-κ or anti-λ antibodies was negative. However, if these LC were further incubated with IgE_κ the same levels of positively stained LC were present with FITC-conjugated anti-ε and anti-κ antibodies (Table 1). The anti-ε staining on LC enriched epidermal cells from normal non-atopic controls was negative (0·0 ± 0·0%), even after incubation with IgE_κ.

Treatment of the LC enriched epidermal cells with pepsin resulted in a significantly ($P < 0·02$) decreased number of cells positive with FITC-conjugated anti-κ or anti-λ antibodies. However, the number of cells positive for FITC-conjugated anti-ε antibody was in the same range as before pepsin treatment (Table 1).

Rosette-assay with SRBC-IgE

Freshly isolated epidermal cells from patients with AD formed rosettes with SRBC-IgE. With non-coated or BSA-coated SRBC no rosettes were formed. After overnight incubation in RPMI medium of the epidermal cells at 4°C (viability 80–90%), the percentage of cells forming rosettes with SRBC-IgE did not differ significantly ($P > 0·05$) from the percentage of cells forming rosettes with SRBC-OKT6 (Table 2). The rosette formation with SRBC-IgE could be significantly ($P < 0·02$) inhibited by preincubating the LC enriched epidermal cells with IgE_κ (100 μg/ml/10^6 cells, 60 min at RT), with BB10 antibody (1 mg/ml/5 × 10^6 cells, 60 min at 4°C), but also with human IgG (500 μg/ml/10^6 cells, 60 min at RT). However, the rosette formation with SRBC-IgG could not be inhibited by preincubation with IgE_κ or BB10 antibody (Table 2). Mouse IgM MoAb with a specificity for *Echinococcus multilocularis* (Capron *et al.* 1986a) was used as a negative control. LC enriched epidermal cells from normal non-atopic controls, freshly isolated or after overnight incubation in RPMI medium or RPMI medium supplemented with IgE_κ (100 μg/ml/10^6 cells at 4°C or 37°C), formed rosettes only occasionally with SRBC-IgE (in two out of four normals, only one SRBC-IgE rosette was observed).

Table 2. Inhibition studies on FcR binding of LC-enriched epidermal cells from AD patients (using SRBC-IgE and SRBC-IgG)

	% rosette forming cells (mean ± s.d.)			
	SRBC-IgE		SRBC-IgG	
Preincubation	day 1*	day 2†	day 1*	day 2†
—	6·9 ± 1·9	17·8 ± 3·4	23·7 ± 2·9	15·0 ± 4·0
IgE_κ	2·0 ± 0·5‡	2·5 ± 1·3‡	21·7 ± 1·3§	
BB10		5·0 ± 2·2*	23·0 ± 2·5§	
IgG		9·3 ± 4·8*	3·7 ± 0·5*	
OKT6	0	3·7 ± 1·3	9·6 ± 0·5*	

* Freshly isolated epidermal cells.

† Epidermal cells after overnight incubation at 4°C ($n = 3–6$).

The percentage rosette forming cells with SRBC-OKT6 is 22·2 ± 4·9. Paired Student's *t*-test performed comparing values to those in line 1. ‡$P < 0·02$. §Not significant.

To identify the epidermal cells forming rosettes with SRBC-IgE or SRBC-IgG, the LC enriched epidermal cells were preincubated with OKT6-FITC antibody. This revealed the presence of OKT6-FITC positive cells forming rosettes with SRBC-IgE and SRBC-IgG. OKT6-FITC negative rosettes with SRBC-IgE and SRBC-IgG were not observed, suggesting that only epidermal LC (and indeterminate cells) were forming rosettes with SRBC-IgE and SRBC-IgG. However, after preincubation of the LC enriched epidermal cells with OKT6-FITC the rosette formation with SRBC-IgE or SRBC-IgG was significantly decreased ($P < 0·02$). The rosette formation with SRBC-IgG of LC enriched epidermal cell suspensions from normal controls was decreased also after preincubation with OKT6-FITC antibody (results not shown).

Associated expression of the CD1 antigen and the Fc-receptor for IgE

To further investigate the inhibition of the rosette formation with SRBC-IgE by OKT6 antibody, the LC enriched epidermal cells were incubated with OKT6, washed twice with RPMI medium and subsequently incubated with FITC-conjugated anti-ε or anti-κ antibodies. The percentage of cells staining with

Table 3. Inhibition studies, before and after acid elution, on the demonstration of cell-bound IgE on LC-enriched epidermal cells from AD patients

	% positive cells†					
	before‡		after acid elution§			
IF reagent	—	OKT6*	—*	IgE_κ	OKT6+I-gE_κ*	BB10+IgE_κ*
OKT6	19·3±2·6	nd	19·3±2·6	19·6±3·1	nd	18·3±1·2
anti-ε	18·3±4·2	4·3±2·9**	0	19·5±2·7	7·8±1·2††	11·0±0·8††
anti-κ	17·9±3·9	17·8±3·0	0	19·3±2·6	18·3±1·2	10·7±1·2††

* Pre-incubation treatment.

† Immunofluorescence analysis of LC enriched epidermal cells from AD patients ($n=3$–6). The results are expressed as the percentage of fluorescent cells, mean±s.d.

‡ Inhibition by OKT6 antibody before acid elution. ** Comparison with the FITC-conjugated anti-ε staining without preincubation with OKT6 antibody, $P<0{\cdot}02$. nd not done.

§ Inhibition of the rebinding of IgE_κ on glycine buffer treated LC enriched epidermal cells after preincubation with OKT6 or BB10 antibodies. †† Comparison with the percentage of cells staining with anti-ε or anti-κ antibodies after acid elution, followed by incubation with IgE_κ, but without preincubation with OKT6 or BB10 antibodies, $P<0{\cdot}02$.

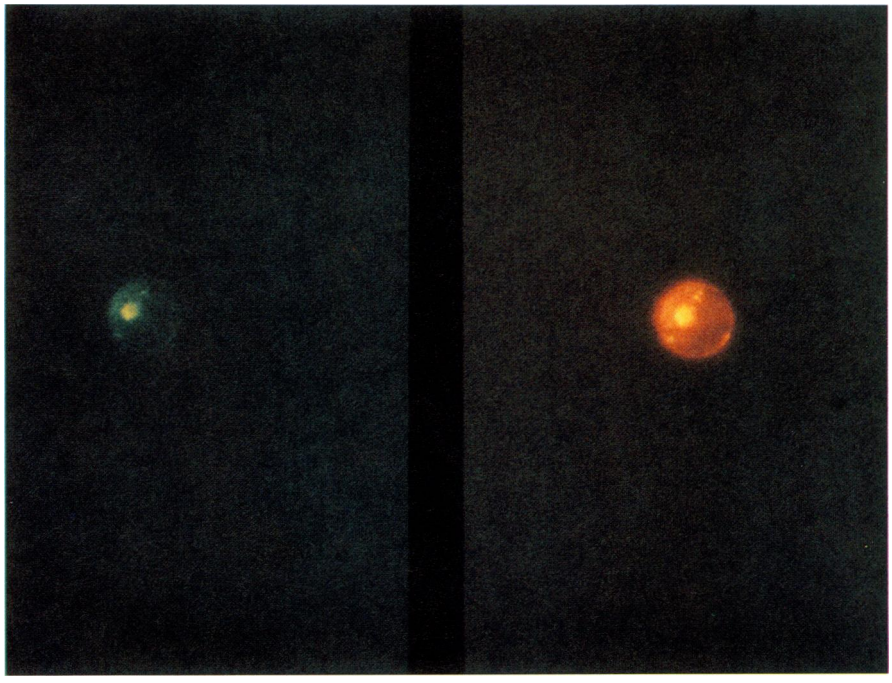

Fig. 1. Epidermal Langerhans cell showing caps with TRITC-conjugated anti-ε and OKT6-FITC antibodies on identical parts of the cell.

FITC-conjugated anti-ε antibody was significantly decreased ($P<0{\cdot}02$), but the percentage of cells staining with FITC-conjugated anti-κ antibody remained in the same range as that without OKT6 preincubation (Table 3). Non-immune mouse serum was used as a negative control. If the LC enriched epidermal cells were preincubated with anti-ε antibody, followed by incubation with OKT6-FITC antibody, the percentage of OKT6-FITC positive cells was in the same range as without preincubation with anti-ε antibody. If the epidermal cells were co-incubated, with either OKT6-FITC and anti-ε antibodies or with OKT6 and FITC-conjugated anti-ε antibodies no decrease in the number, respectively, of OKT6-FITC positive and FITC-conjugated anti-ε positive cells was observed (results not shown).

These experiments were repeated with glycine-HCl buffer-treated, LC-enriched epidermal cells. The results show that the binding of IgE could be restored, as was demonstrated by immunofluorescence with FITC-conjugated anti-ε and anti-

antibodies (Table 3). If the glycine-HCl-treated, LC-enriched epidermal cells were preincubated with OKT6 antibody before incubation with IgE_κ, the percentage of FITC-conjugated anti-ε positive cells was significantly decreased ($P < 0{\cdot}02$). However, the percentage of FITC-conjugated anti-κ positive cells was still in the same range as before preincubation with OKT6 (Table 3). Substitution of OKT6 by BB10 antibody, followed by incubation with IgE_κ, significantly ($P < 0{\cdot}02$) decreased the percentage of cells staining with FITC-conjugated anti-ε and anti-κ antibodies (Table 3). Preincubation with anti-HLA-DR antibody (1:300 in PBS with 1% HSA, 45 min at 4°C), followed by incubation with IgE_κ, did not influence the FITC-conjugated anti-ε and anti-κ antibody staining (results not shown). The same inhibition experiments with anti-CD23 antibody were also negative. A cross-reaction between BB10 and the CD1 antigen was excluded by blocking experiments with BB10 on human thymocytes. The OKT6-FITC staining of human thymocytes remained unchanged after preincubating the thymocytes with BB10. Furthermore, preincubation of the glycine-HCl-treated, LC-enriched epidermal cells with BB10 did not influence the number of OKT6-FITC positive cells. Finally, a cross-reaction between OKT6 and IgE antibodies was excluded by incubating SRBC-IgE with OKT6-FITC. No fluorescence was observed.

Associated expression of the Fc receptor for IgE and the CD1 antigen was studied in capping experiments. LC-enriched epidermal cell suspensions from patients with AD were incubated with TRITC-conjugated anti-ε antibody, followed by washing and incubation with OKT6-FITC antibody. To allow capping, the cells were incubated for 4 h at 37°C. After this procedure, caps with the TRITC-conjugated anti-ε and the OKT6-FITC antibodies were present on completely overlapping parts of the cells (Fig. 1). Incubation of the cells with TRITC-conjugated anti-ε, and OKT6-FITC antibodies in PBS, containing 0·5% azide, did not result in capping. If the cells incubated with TRITC-conjugated anti-ε antibody were allowed to cap for 4 h at 37°C and were then subsequently incubated with OKT6—FITC under non-capping conditions (0·5% azide, 45 min at 4°C), again caps with both antibodies were found on completely overlapping parts of the cells.

DISCUSSION

Previous studies (Bruynzeel-Koomen *et al.*, 1986) demonstrated the presence of IgE bearing cells in the epidermis of patients with AD. Furthermore, immunogold electron microscopy studies revealed that these cells were LC. The results of the present study show that epidermal LC from patients with AD bind IgE molecules by their Fc-fragment and strongly suggest the presence of an $Fc_\varepsilon R$ on LC from these patients. This could be demonstrated by the rebinding of IgE_κ after acid elution of cell-bound IgE, the enzymatic splitting of cell-bound IgE by pepsin and the rosette assay with SRBC-IgE. After overnight incubation the number of cells forming rosettes with SRBC-IgE was in the same range as the number of cells forming rosettes with SRBC-OKT6. Although IgE molecules in supernatants were not quantified, the increase in the percentage of cells forming rosettes with SRBC-IgE may be explained by shedding of IgE molecules from occupied Fc receptors. The rosette assay with SRBC-IgE was negative on epidermal cells from normal non-atopic controls. This suggests that normal non-atopic controls do not possess an Fc receptor for IgE. This discrepancy between LC from AD patients and those from normal non-atopic controls may be explained as follows. The density of the $Fc_\varepsilon R$ on LC depends on the total serum IgE level. In normals with low serum IgE levels the density of the $Fc_\varepsilon R$ on LC is too low to be demonstrated by the above described methods. The $FC_\varepsilon R$ is present on a limited number of epidermal LC from normal individuals. This has been described for the $Fc_\varepsilon R$ on T lymphocytes, monocytes (Spiegelberg & Melewicz, 1980) and eosinophils (Capron *et al.* 1981) from normal non-atopic controls. At this moment the limitation of the total number of LC which can be obtained from one individual interferes with extensive binding studies on these cells to clarify both points.

The specificity of the rosette formation with SRBC-IgE was demonstrated by the inhibition experiments with IgE_κ and BB10. Since anti-CD23 antibody (directed against the $Fc_\varepsilon R$ on B lymphocytes) did not inhibit the rosette formation with SRBC-IgE, the $Fc_\varepsilon R$ on LC differs structurally from the $Fc_\varepsilon R$ on human B lymphocytes. The results of the inhibition experiments with BB10 strongly suggest that the Fc receptor for IgE on LC is of the same type as on eosinophils, platelets and macrophages ($Fc_\varepsilon R_2$) (Capron *et al.*, 1986b).

The inhibition of the SRBC-IgE rosette formation by IgG may be explained either by steric hindrance of IgG, bound to an excess of Fc receptors for IgG, or by a cross-reaction between the Fc receptors for IgE and IgG. So far, inhibition of IgE binding by IgG antibodies has not been described in human $Fc_\varepsilon R$ bearing cells. Human basophils (Ishizaka *et al.*, 1979), eosinophils (Capron *et al.*, 1981) and monocytes (Melewicz & Spiegelberg, 1980) have separate Fc receptors for IgG and IgE. If only one type of Fc receptor is involved in LC, one would expect inhibition of the SRBC-IgG rosette formation by IgE_κ or BB10, but this is not observed in our studies with LC. Therefore, structural differences must be present between the IgE and IgG binding sites on epidermal LC. In rat basophil leukaemia cells two different types of Fc receptors are present, one with high affinity for IgE and one with affinity for IgE and IgG (Kepron, Conrad & Froese, 1982). The selective inhibition of the SRBC-IgE rosette formation by IgG but not of the SRBC-IgG rosette formation by IgE_κ, suggests that in the case of epidermal LC from patients with AD, two types of Fc receptors are involved, one with affinity for IgG and one with affinity for IgG and for IgE.

The inhibition of the rosette formation with SRBC-IgE and SRBC-IgG by OKT6 antibody may be explained either by Fc receptor blockade or by steric hindrance. The Fc receptor for IgG on epidermal LC has a low affinity for IgG since the presence of cell-bound IgG *in vivo* has never been reported. Therefore, the inhibition of the rosette formation by OKT6 antibody was investigated for the IgE-Fc receptor binding. The results of the OKT6 inhibition experiments on the restoration of the IgE-binding on LC-enriched epidermal cells after acid elution preclude the possibility of receptor-blockade and strongly suggest steric hindrance.

Since cross-reactivity between OKT6 and the Fc-fragment of the IgE-molecule was excluded, the negative FITC-conjugated anti-ε antibody staining after preincubation with OKT6 may be explained by masking of the ε-chain by OKT6. Because of the consistent observation of this inhibition phenomenon, an association of the CD1 antigen and the Fc receptor for IgE is highly probable. This was substantiated further by the capping experiments with TRITC-conjugated anti-ε and OKT6-FITC

antibodies. The results of the OKT6 inhibition experiments on the rosette formation with SRBC-IgG may suggest a common mechanism for expression of the CD1 antigen and the Fc receptor on LC in general.

An associated expression of the CD1 antigen with other cell membrane components has not been described yet. On the other hand, the Fc receptor for IgE on RBL and normal rat mast cells seems to be in close proximity with a 30,000 D glycoprotein (Kulczycki, 1981). Furthermore, an associated expression of the Fc receptor for IgE on human platelets with the glycoprotein IIb and IIIa complex (Ameisen *et al.*, 1986) and on human B lymphocytes with HLA-DR antigens (Bonnefoy *et al.*, 1988) was recently described. Whether these glycoproteins form a functional association with the Fc receptor for IgE has not been investigated.

In conclusion, we demonstrated the presence of an Fc receptor for IgE on human epidermal LC from patients with AD. This $Fc_\varepsilon R$ binds IgE *in vivo*, is trypsin resistant, reacts with BB10 antibody and probably cross-reacts with IgG. Furthermore, our results strongly suggest an associated expression of the Fc receptor for IgE with the CD1 antigen. Altogether, this $Fc_\varepsilon R$ on LC from AD patients seems to possess some unique properties which makes it differ from the $Fc_\varepsilon R$ on other human cell types. Further characterization of its properties, its functional role, the significance of the association with the CD1 antigen and its presence in patients with elevated serum IgE levels without atopy or atopy without AD, will be the subject of future investigations.

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