Immunology 1990 **69** 335–341

Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE

G. C. MUDDE,* F. C. VAN REIJSEN,* G. J. BOLAND, G. C. DE GAST, P. L. B. BRUIJNZEEL*‡ & C. A. F. M. BRUIJNZEEL-KOOMEN*† *Departments of Immunohaematology, †Dermatology and ‡Pulmonary Disease, University Hospital Utrecht, Utrecht, The Netherlands*

Accepted for publication 2 November 1989

SUMMARY

To investigate the role of IgE-bearing Langerhans' cells (LC) from atopic dermatitis (AD) patients in antigen presentation, IgE$^+$LC and non-IgE bearing LC (IgE$^-$LC) from AD patients were investigated for their antigen-presenting capacity and compared to antigen-presenting cells (APC) from peripheral blood. The T-cell response to *Candida albicans*, using IgE$^+$LC from AD patients as APC, was in the same range as with IgE$^-$LC. Also, the T-cell response to *Candida* with autologous APC from peripheral blood did not significantly differ between these groups. In contrast to this finding, the T-cell response to house dust allergen (HDA) was dependent on the type of APC used. If non-T cells from peripheral blood were used as APC, both AD patients and controls responded to HDA. However, when LC were used as APC, a T-cell response to HDA was only observed in the presence of IgE$^+$LC. IgE$^-$LC from AD patients or LC from normal controls were unable to present HDA. Preincubation of IgE$^+$LC with anti-IgE or anti-kappa/lambda antibodies inhibited HDA-induced T-cell proliferation, whereas the response to *Candida* was not affected. These *in vitro* results, which demonstrate the necessity of cell-bound IgE on LC for the presentation of aero-allergens, strongly correlate with the *in vivo* presence of a positive delayed patch reaction to the same antigens. When the LC of a patient appeared to be IgE$^-$, the *in vitro* proliferative response as well as, in most cases, the *in vivo* patch test reaction to the same antigens was negative. In conclusion, these experiments demonstrate that there are at least two different mechanisms by which LC capture antigens for antigen presentation. In one of them cell-bound IgE, as can be demonstrated on LC from AD patients, plays a crucial role. The binding and presentation of HDA by APC from peripheral blood can take place independently of cell-bound IgE. *Candida* can be presented by both types of APC in the absence of IgE

INTRODUCTION

Peripheral blood (PB) mononuclear cells from atopic patients show a lympho-proliferative response when challenged *in vitro* with environmental allergens such as house dust (Romagnani *et al.*, 1973; Yoo, Kuo & Heath, 1977), house dust mite (Romagnani *et al.*, 1973; Elliston, Heise & Huntley, 1982; Black & Marsh, 1980; Hiratami *et al.*, 1981; Lanzavecchia *et al.*,1983; O'Hehir *et al.*, 1987; Rawle, Mitchell & Platts-Mills, 1984; Cavaillon *et al.*, 1988) and grass pollen (Buckley *et al.*,1977; Geha *et al.*, 1975; Matthews, Pan & Weisberg, 1977; Phillips *et al.*, 1987). In this respect no distinction can be made between patients with allergic asthma, rhinitis or atopic dermatitis (AD) (Black & Marsh 1980; Lanzavecchia *et al.*, 1983; Rawle *et al.*, 1984). It has been shown that in atopics these circulating sensitized T cells may act as helper cells for antibody production by B cells (Lanzavecchia *et al.*, 1983; Ricci *et al.*,1985; Ishizaka, 1984; Zimmerman *et al.*, 1986). Whether these T cells play a role in the pathogenesis of the allergic reaction mechanism and are responsible for the symptomatology of atopic diseases like asthma, rhinitis or AD is still obscure. In AD evidence is accumulating that T lymphocytes may be involved in the pathogenesis of the skin lesions; the dermal cellular infiltrate consists of activated CD4$^+$ T lymphocytes, with APC such as Langerhans' cells (LC), interdigitating reticulum cells and macrophages (Sillevis Smitt *et al.*, 1986; Leung *et al.*, 1983; Zachary, Allen & MacDonald, 1985). Furthermore, patients with AD may show eczematous patch test reactions to aero-allergens, which are not present in normal controls. It was

Abbreviations: AET, 2-aminoethyl isothiouroniumbromide hydrobromide; APC, antigen-presenting cell(s); BSA, bovine serum albumin; HDA, house dust allergen; IgE$^+$LC, Langerhans' cells with membrane-bound IgE; IgE$^-$LC, Langerhans' cells without membrane-bound IgE; PB, peripheral blood; PBS, phosphate-balanced saline.

*Present address: Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, 7270 Davos, Switzerland.

Correspondence: Dr G. C. Mudde, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, 7270 Davos, Switzerland.

hypothesized that among others allergen-specific T cells are involved in the reaction mechanism behind these allergic patch test reactions (Bruijnzeel-Koomen *et al.*, 1988b; Reitamo *et al.*, 1986). Since normal non-atopics also have allergen-specific T cells in the circulation (Romagnani *et al.*, 1973; Cavaillon *et al.*, 1988; Buckley *et al.*, 1977; Matthews *et al.*, 1977), the question arises why normal non-atopics do not show delayed patch test reactions to allergens. This may be due to differences in APC in the skin between AD patients and normal controls. The most important APC in the skin is the LC. A striking difference between LC from normal and AD patients is that epidermal LC from AD patients bear Fc receptor-bound IgE molecules (Bruijnzeel-Koomen *et al.*, 1986, 1988a), probably depending on the stage of disease. The present study was initiated to investigate the antigen-presenting function of IgE^+LC from AD patients and to compare this with the antigen-presenting function of IgE^-LC and APC from PB. Therefore, lympocyte proliferation tests to HDA and *Candida* were performed with T cells from PB using either autologous epidermal LC or autologous non-T cells from PB as APC. The results of this study show that the presence of IgE molecules on epidermal LC is necessary to present HDA to T cells.

MATERIALS AND METHODS

Reagents

Culture medium consisted of RPMI-1640 (Gibco, Grand Island, NY, supplemented with 10% pooled human AB serum, penicillin (100 IU/ml), streptomycin (100 U/ml), glutamine (2 mM) and amphotericin (2·5 U/ml). FITC-conjugated OKT6 (anti-CD1) antibody and OKM1 antibody were obtained from Ortho Pharmaceuticals (Raritan, NJ), TRITC-conjugated anti-human IgE heavy chain (anti-IgE) from Nordic Immunological Laboratories (Tilburg, The Netherlands), rabbit anti-human kappa, lambda light chain (a-κ/λ) and rabbit anti-human epsilon heavy chain (a-IgE) antibodies from Dako (Copenhagen, Denmark), and anti-human HLA-DR (a-HLA-DR) antibodies from Becton-Dickinson (Mountain View, CA). Trypsin was purchased from BDH Chemicals (Poole, Dorset, U.K.), deoxyribonuclease-1 (DNAse-1) from Boehringer (Mannheim, FRG), Ficoll–Hypaque from Pharmacia (Uppsala, Sweden), 2-aminoethyl isothiouroniumbromide hydrobromide (AET) from Sigma (St Louis, MO), bovine serum albumin (BSA) from Janssen Pharmaceuticals (Goirle, The Netherlands), concanavalin A (Con A) and *Candida albicans* from Precipital (Haarlem, The Netherlands) and house dust allergen (HDA) from Haarlems Allergenen Laboratorium (HAL; Haarlem, The Netherlands). HDA was dialysed overnight in Hanks' balanced salt solution before use; no mitogenic activity, as a result of bacterial endotoxins (Berrens & Herings, 1984; Siraganian *et al.*, 1979) in concentrations up to 200 μg/ml, was found.

Patients and controls

All 20 AD patients and five normal controls selected for this study gave their informed consent. Patients (both with IgE^+LC and IgE^-LC) and controls were age and sex matched. The diagnosis of AD was made according the criteria of Hanifin and Rajka (Hanifin & Rajka, 1980). The AD patients were in clinical remission, using topical emollients, but no corticosteroids. Skin tests with HDA were performed by means of intracutaneous administration at a concentration of 0·5% (w/v) and read after 20 min. Skin tests with HDA were also performed by means of epicutaneous administration (patch test) at a concentration of 50% (w/v) as described before (Bruijnzeel-Koomen *et al.*, 1988b). In short, the upper part of the stratum corneum from normal looking skin of the back is removed by tape stripping; after this patch tests with the allergen are performed and the reaction is read after 24 and 48 hr. The total serum IgE level was measured using the paper-radioimmunosorbent test (PRIST; Pharmacia) and allergen-specific serum IgE was measured using the radio-allergosorbent test (RAST; Pharmacia). Total serum IgE values were expressed as kU/l; the RAST as a percentage of the total amount of added labelled anti-human IgE which was bound to the allergen-coated disc. These values were corrected for the blank binding of labelled anti-human IgE to the disc.

Langerhans' cell-enriched epidermal cell suspensions

Epidermal cell suspensions were prepared from shave biopsies from clinically normal looking skin (dry or slightly lichenified) from patients with AD or normal non-atopic controls. The skin slices were incubated in phosphate-balanced saline (PBS) containing 0·3% trypsin for 30 min at 37°. An epidermal cell suspension was obtained by gently stroking epidermis and dermis with a fine forceps. The epidermal cells were resuspended in 2 ml 0·025% DNAse 1 in PBS for 2 min at 37°, followed by repeated washing in RPMI-1640 supplemented with 10% fetal calf serum. LC enrichment was obtained by centrifugation of the epidermal cells over Ficoll–Hypaque gradient (30 min, 400 ***g*** at room temperature). The LC-enriched epidermal cell suspension consisted of $2\text{–}5 \times 10^6$ cells, of which 3–30% were $CD1^+$ cells. The viability of the LC-enriched epidermal cell suspension, estimated by trypan blue exclusion, varied between 80% and 90%. No significant differences in cell number, purity or viability were found in LC-enriched cell suspensions between controls and both patient groups. To detect the presence of cell-bound IgE the LC-enriched epidermal cell suspensions were incubated with TRITC-conjugated anti-IgE antibodies (1:5, 30 min, at 37° in PBS with 1% BSA). In eight out of 20 patients with AD, no IgE^+LC were observed (all $CD1^+$ were IgE^-), in the other 12 at least 50% of the $CD1^+$ cells were also IgE^+. The absolute number of LC strongly hampered the variety and number of experiments which could be performed with the cells of one donor.

Preparation of T cells from peripheral blood

Heparinized PB from AD patients and normal controls was diluted 1:1 with RPMI-1640. The diluted blood was layered on Ficoll–Hypaque and centrifuged (20 min, 1000 ***g***, room temperature). The cells at the interphase were counted, followed by a T/non-T separation. For this purpose the mononuclear cells were mixed with sheep red blood cells (SRBC), treated with AET. After density centrifugation of Ficoll–Hypaque (20 min, 1000 ***g***, room temperature) the T cell-SRBC rosettes were lysed with ammonium chloride. The interphase contained the non-T mononuclear cells (containing about 60% monocytes) (Mudde, Van Dam & de Gast, 1986). This fraction was used as APC from PB.

Lymphocyte proliferation test

The experiments were performed in 96-well round-bottomed culture plates. LC-enriched epidermal cells were used as APC in

numbers corresonding to 10^3 LC per well; higher numbers of LC did not improve the net responses to HDA or *Candida*, whereas lower numbers were suboptimal. As control APC, 10^5 non-T cells per well derived from PB were used. Autologous T cells were used as responder cells (10^5 per well). Con A was used at a concentration of 2·5 μg/ml and left in the culture medium during the complete period of stimulation. Preincubations (1 hr, room temperature) with LC-enriched epidermal cells and non-T cells were performed with HDA (50 μg/ml), *Candida* (50 μg/ml) or RPMI. Subsequently the cells were washed in culture medium, centrifuged (10 min, 200 *g*, room temperature) and co-cultured with autologous T cells for 5 days at 37°. At the fifth day the cells were pulsed with 1 μCi [^{3}H]thymidine per well. After 8–18 hr, the cells were harvested and [^{3}H]thymidine incorporation was measured with a liquid scintillation counter (Searle, Isocap/300). Responses with a net result smaller than 3000 c.p.m. were considered negative. The net response was calculated as the mean c.p.m. of the stimulated wells (e.g. HDA) minus the mean c.p.m. of the unstimulated wells (background). In the inhibition studies an extra preincubation step (1 hr, room temperature), with a-κ/λ (1/1000) or a-IgE (1/100), was introduced before incubation of the APC with antigen. In experiment numbers 3 and 7, optimal inhibition with a-κ/λ antibodies was obtained when the antibodies were used in a 1/100 dilution instead of 1/1000. The studies with a-HLA-DR antibodies were not performed as preincubation experiments but the a-HLA-DR antibodies (1/200) were added to the wells immediately before the T-cell suspensions. Addition of a-IgE or a-κ/λ antibodies after preincubation with antigens did not influence the response (data not shown). All experiments were performed in triplicate. T-cell responses are expressed as mean ± SD. The percentage inhibition was calculated as follows:

$$\frac{\text{c.p.m. Exp. a} - \text{c.p.m. Exp. a} + \text{antibodies}}{\text{c.p.m. Exp. a}} \times 100.$$

Statistical analysis

Statistical analysis was performed using the *t*-test for paired observations, and the Wilcoxon test was used for non-parametric distributions. $P \leq 0{\cdot}05$ (*t*-test) and $a \leq 0{\cdot}05$ (Wilcoxon test) values were considered significant.

RESULTS

Background stimulation of T lymphocytes

Non-T cells from peripheral blood (Fig. 1a). The results of the background stimulation of T cells in the presence of autologous non-T cells from PB, but in the absence of mitogens or antigens, are shown in Fig. 1a. No significant difference was present between AD patients and normals or between AD patients with IgE$^+$LC or with IgE$^-$LC.

LC (Fig. 1b). Figure 1b shows that the background response of T cells in the presence of autologous LC from AD patients is increased compared to normals ($P \leq 0{\cdot}05$). The difference between IgE$^+$LC and IgE$^-$LC from AD patients was not significant. The background response of T cells was significantly ($P \leq 0{\cdot}0025$) inhibited, when a-HLA-DR antibodies were added to the culture medium (data not shown).

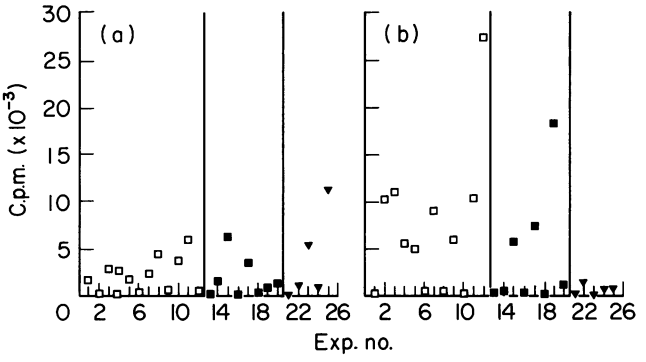

Figure 1. Background T-cell response. (a). Background T-cell response expressed in c.p.m. in the presence of non-T cells from PB. There was no significant difference between AD patients with IgE$^+$LC (open squares; Exp. nos 1–12) and IgE-LC (closed squares, Exp. nos 13–20) or between AD patients (open and closed squares; Exp. nos 1–20) and normals (triangles, Exp. nos 21–25). (b) Background T cell response (in c.p.m.) in the presence of LC. The background in AD patients was significantly increased compared to normals ($a \leq 0{\cdot}05$). There was no significant difference between the background response in the presence of IgE$^+$LC (Exp. nos 1–12) compared to IgE$^-$LC (Exp. nos 13–20). The background response in the presence of IgE$^+$LC from AD patients was significantly increased compared to the background T-cell response in the presence of non-T cells of these patients ($P \leq 0{\cdot}01$). No significant difference was present in the background T-cell response in the presence of IgE$^-$LC of AD patients or non-T cells of these patients. The experiment numbers mentioned correspond with the experiment numbers in Figs 2–6 and patient numbers in Table 1.

Lymphocyte proliferation to *Candida*

Non-T cells from peripheral blood (Fig. 2a). There was no significant difference in the T-cell response to *Candida* presented by non-T cells from AD patients (with IgE$^+$LC or with IgE$^-$LC), or normal controls. The T-cell response to *Candida* in

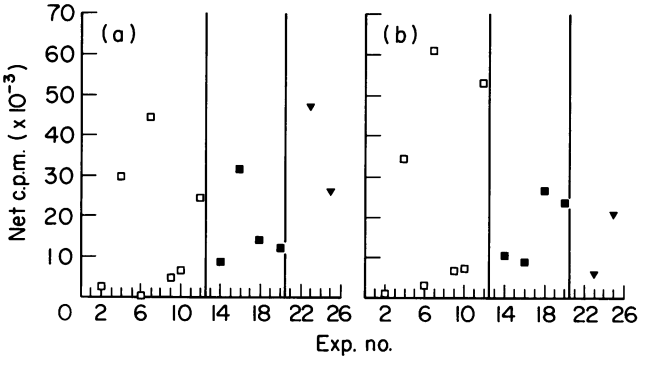

Figure 2. T-cell response to *Candida*. (a) T-cell response to *Candida* using non-T cells as APC. The results are given as net c.p.m. (c.p.m. T-cell response to *Candida* minus background c.p.m.). There was no significant difference between AD patients with IgE$^+$LC and IgE$^-$LC.
(b) T-cell response to *Candida* using LC as APC. The results are given as net c.p.m. (c.p.m. T-cell response to *Candida* minus background c.p.m.). There was no significant difference between AD patients with IgE$^+$LC and AD patients with IgE$^-$LC. Symbols as for Fig. 1.

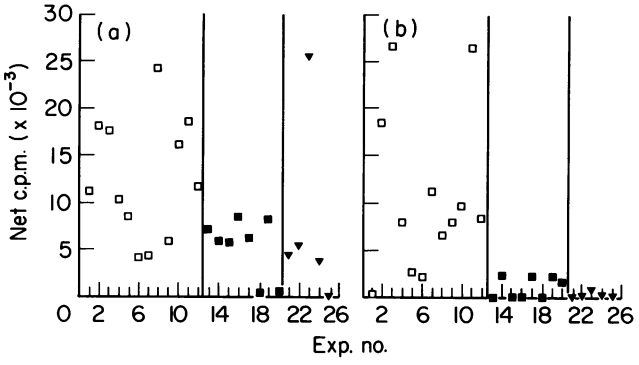

Figure 3. T-cell response to HDA. (a) T-cell response to HDA using non-T cells as APC. The results are expressed as net c.p.m. (c.p.m. T-cell response to HDA minus background c.p.m.). The T-cell response to HDA in the presence of non-T cells from AD patients with IgE$^+$LC (Exp. nos 1–12) was significantly increased compared to AD patients with IgE$^-$LC (Exp. nos 13–20) ($a \leq 0{\cdot}01$). There was no significant difference of the T-cell response to HDA between AD patients with IgE$^-$LC and normal controls. (b) T-cell response to HDA using LC as APC. The results are expressed as net c.p.m. (c.p.m. T-cell response to HDA minus background c.p.m.). There was a significant difference between AD patients with IgE$^+$LC and IgE$^-$LC ($a \leq 0{\cdot}005$) and also between IgE$^+$LC and normal controls ($a \leq 0{\cdot}005$). There was no significant difference between AD patients with IgE$^-$LC and normal controls. Symbols as for Fig. 1.

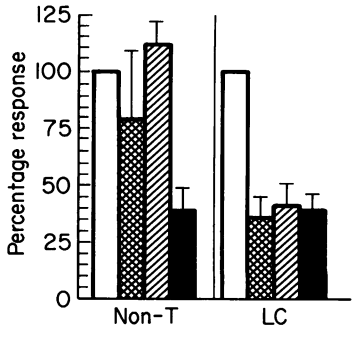

Figure 4. Effect of a-IgE, a-κ/λ and a-HLA-DR on net HDA response. The effect of preincubation with a-IgE, a-κ/λ and a-HLA-DR antibodies on the T-cell response to HDA presented by IgE$^+$LC or non-T cells is shown as a percentage of the normal HDA response set at 100% (open bars). Preincubation with a-IgE (crossed bar), a-κ/λ (striped bar) and a-HLA-DR (closed bar) inhibited the response to HDA presented by IgE$^+$LC significantly ($P < 0{\cdot}0125$, $P \leq 0{\cdot}0125$ and $P \leq 0{\cdot}025$, respectively). The response to HDA presented by non-T cells was not significantly influenced by a-IgE, but a-κ/λ had a slightly stimulating effect on the response ($p \leq 0{\cdot}05$). Anti-HLA-DR inhibited the HDA response induced by non-T cells ($P \leq 0{\cdot}0005$).

the presence of IgE$^+$LC as APC did not significantly differ from the T-cell response in the presence of IgE$^-$LC (Fig. 2b). Keratinocytes could not present *Candida* in the absence of LC (data not shown). There was no significant difference in the T-cell response to *Candida* between non-T cells and LC as APC in the same donor.

Lymphocyte proliferation to HDA

Non-T cells from peripheral blood (Fig. 3a). The results of the T-cell response to HDA using non-T cells from PB as APC, are given in Fig. 3a. T cells from AD patients with IgE$^+$LC or IgE$^-$LC or from controls significantly responded to HDA using non-T cells as APC ($P \leq 0{\cdot}0005$, $P \leq 0{\cdot}0005$, and $P \leq 0{\cdot}05$, respectively). The T-cell response to HDA in the presence of non-T cells from AD patients with IgE$^+$LC was significantly increased compared to AD patients with IgE$^-$LC ($a \leq 0{\cdot}01$). There was no significant difference between AD patients with IgE$^-$LC and controls.

LC (Fig. 3b). The results of the T-cell responses to HDA, using epidermal LC as APC, are given in Fig. 3b. IgE$^-$LC from normal controls or AD patients could not induce a significant T-cell response to HDA. A significant T-cell response to HDA was only observed when IgE$^+$LC from AD patients were used as APC ($P \leq 0{\cdot}0025$). The magnitude of the net T-cell response induced by IgE$^+$LC was not significantly different from the one induced by non-T cells of the same donor (Fig. 3a). HDA (at a concentration up to 200 μg/ml) did not induce a T-cell response in the absence of non-T cells or IgE$^+$LC. No antigen-presenting capacity of purified keratinocytes, from AD patients with IgE$^+$ LC or IgE$^-$ LC, in the absence of LC was detected.

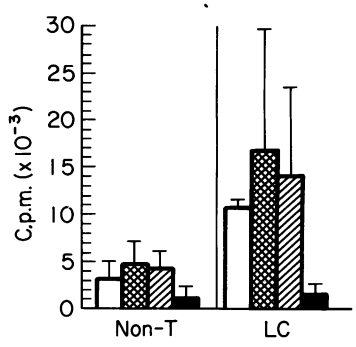

Figure 5. Effect of a-IgE, a-κ/λ and a-HLA-DR on the background response. The effects of preincubation with a-IgE (crossed bar), a-κ/λ (striped bar) and a-HLA-DR antibodies (closed bar) on the background T-cell response to IgE$^+$LC or non-T cells are shown as cp.m. $\pm$ SD together with the normal background response (open bar). Both a-IgE and a-κ/λ had a stimulatory effect on the background to IgE$^+$LC ($P \leq 0{\cdot}0125$ and $P \leq 0{\cdot}001$, respectively), these two antibodies did not have a significant effect on the background to non-T cells. Preincubation with a-HLA-DR inhibited the background responses to both non-T cells ($P \leq 0{\cdot}0025$) and IgE$^+$LC ($P \leq 0{\cdot}025$).

Effect of a-IgE on the response to HDA

Non-T cells from peripheral blood. When non-T cells from patients with IgE$^+$LC were used as APC (Fig. 4), in three (Exp. nos 3, 4, 12) out of six patients the net HDA response was inhibited by preincubation with a-IgE, whereas in two (Exp. nos 8 and 11) patients a-IgE increased the response to HDA (data not shown). The overall response was not significantly inhibited or enhanced by a-IgE (Fig. 4) and no significant effect on the background response was observed (Fig. 5).

LC. T-cell proliferation to HDA—using LC as APC—was significantly inhibited by a-IgE ($P \leq 0{\cdot}0125$) (Fig. 4). There was

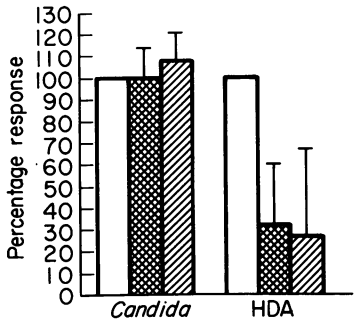

Figure 6. Effect of a-IgE and a-κ/λ on *Candida* response. The effect of preincubation with a-IgE (crossed bar), and a-κ/λ antibodies (striped bar) on the T-cell response to *Candida*, presented by IgE$^+$LC is shown as a percentage of the normal *Candida* response (open bar) set at 100%. Only the HDA response is significantly influenced by preincubation with the antibodies ($P \leq 0{\cdot}0125$).

a minimum inhibition of 42% (Exp. no. 8); the mean of the inhibitory effects was 64% ± 9. The background T-cell response increased significantly ($P \leq 0{\cdot}025$) when a-IgE was added in the absence of antigen (Fig. 5). The mean background increase amounted to 56% ± 21.

Table 1. Relation between *in vivo* patch tests and *in vitro* stimulation with HDA of patients with AD

Patient no.	IgE on LC	PRIST kU/L	RAST HDA %	IC 20 min	PT 24–48 hr	LPT non-T	LPT LC
1	+	12,000	ND	+	ND	+	−
2	+	21,000	23	+	+−	+	+
3	+	16,000	26	+	++	+	+
4	+	11,000	17	+	++	+	+
5	+	2550	11	+	++	+	−
6	+	6800	12	+	−−	+	−
7	+	3600	9	+	++	+	+
8	+	9250	19	+	ND	+	+
9	+	15,000	26	+	++	+	+
10	+	860	6	+	++	+	+
11	+	5300	9	+	++	+	+
12	+	12,750	21	+	++	+	+
13	−	1200	5	+	−−	+	−
14	−	2100	10	+	ND	+	−
15	−	875	8	+	++	+	−
16	−	1100	8	−	−−	+	−
17	−	3700	11	+	ND	+	−
18	−	2900	12	+	−−	−	−
19	−	800	6	+	++	+	−
20	−	16	0	−	−−	−	−

The patient numbers correspond with the experiment numbers in Figs 1–6

ND, not done; IC, intracutaneous test with HDA read after 20 min; PT, patch test with HDA read after 24 and 48 hr; LPT non-T, lympho-proliferation test in the presence of non-T from peripheral blood as APC; LPT-LC, lympho-proliferation test in the presence of LC as APC. The LPT-non-T and LPT-LC were considered negative when the net c.p.m. of the LPT was lower than 3000 c.p.m. (see Fig. 3a, b).

Effect of a-κ/λ on the response to HDA

Non-T cells from peripheral blood. When non-T cells were used as APC, in four (Exp. nos 4, 7, 8, 11) out of six patients a (small) stimulatory effect on the HDA response was observed, when a-κ/λ was added (data not shown). The mean stimulation (12% ± 10) was significant ($P \leq 0{\cdot}05$) (Fig. 4). Like a-IgE, a-κ/λ did not affect the background HDA response to unstimulated non-T cells (Fig. 5).

LC. When LC were used as APC, the T-cell response to HDA could be significantly ($P \leq 0{\cdot}0125$) inhibited by a-κ/λ (Fig. 4).In all six patients the proliferation to HDA was inhibited. The mean inhibition of the HDA response was (59% ± 10). In these experiments, like in the a-IgE inhibition experiments, the mean background increased significantly ($P \leq 0{\cdot}001$) when a-κ/λ was added (Fig. 5). The mean increase was 51% ± 12.

Effect of a-HLA-DR on the response to HDA

Non-T cells from peripheral blood. When non-T cells were used as APC, a-HLA-DR inhibited the lymphocyte response to HDA (Fig. 4) as well as the RPMI background (Fig. 5) significantly (respectively $P \leq 0{\cdot}0005$ and $P \leq 0{\cdot}0025$). The mean inhibitions amounted to 71% ± 10 and 68% ± 5, respectively.

LC. When LC were used as APC, the lymphocyte response to HDA (Fig. 4) and the background responses (Fig. 5) were also decreased significantly (both $P \leq 0{\cdot}025$). The inhibitions were 61% ± 7 and 62% ± 17, respectively.

Effect of a-IgE and a-κ/λ on the response to *Candida*

In three patients (Exp. nos 4, 7, 12) the a-IgE- and a-κ/λ-induced inhibition of the lymphocyte response to HDA and *Candida* were compared in the presence of LC as APC. The results are shown in Fig. 6. There was no significant inhibition of the T-cell proliferation induced by *Candida*. The T-cell response to HDA was significantly inhibited.

Relation between *in vitro* and *in vivo* findings with HDA

The results concerning the total serum IgE level, the specific serum IgE level for HDA, the intracutaneous and epicutaneous tests with HDA and the lympho-proliferative tests with HDA are given in Table 1. The total serum IgE level in AD patients with IgE$^+$LC was 9659 ± 1762 kU/l (mean ± SEM). All AD patients with IgE$^+$LC had elevated serum IgE levels, a positive RAST for HDA and immediate type skin reactions to this allergen. In nine out of 12 patients with IgE$^+$LC, positive lympho-proliferative responses in the presence of LC (LPT-LC) to HDA were present. In all AD patients with a positive LPT-LC to HDA, delayed patch test reactions to HDA could be observed. On the other hand two patients with IgE$^-$LC showed a positive delayed patch test reaction. The serum IgE level in AD patients with IgE$^-$LC was significantly lower: 1586 ± 432 kU/l (mean ± SEM) ($P \leq 0{\cdot}0005$). Seven out of eight AD patients with IgE$^-$LC showed a positive RAST and six showed immediate type skin reactions to HDA. The LPT-LC was negative in all patients with IgE$^-$LC. Patch test reactions to HDA were present in two out of six tested patients with IgE$^-$LC (patient numbers 15 and 19), in contrast to nine out of 10 tested patients with IgE$^+$LC.

DISCUSSION

In this study we present results from antigen-specific T-cell stimulations, using APC from PB and skin. The number of LC obtained after each isolation was, as expected, dependent on the size of the shave biopsy, and greatly influenced the variety and number of incubations that could be performed with the cells of one skin donor. In spite of this difficulty we were able to compare the antigen-presenting function of APC from PB and skin from AD patients and normal controls.

We demonstrate here that there is a difference in antigen presenting capacity of LC showing cell-bound IgE (AD patients) and LC without IgE (normal controls). AD patients without IgE^+LC reacted like normal controls. The presence of IgE on LC correlates with the clinical activity of the disease (Bruijnzeel-Koomen *et al.*, 1986). LC from AD patients in clinical remission may become IgE^-. In contrast to other reports (Kapp *et al.*, 1987; McGeady & Buckley, 1975), the responses of T cells from AD patients in the presence of APC from PB were of the same magnitude as the T-cell responses from normal donors. In our study T cells of all groups responded to HDA, *Candida* and Con A (data not shown) presented by non-T cells. Patients with IgE^+LC showed a stronger proliferative response to HDA in the presence of non-T cells than patients with IgE^-LC and normal controls ($P < 0{\cdot}01$). This may be due to the activation state and/or a higher frequency of HDA-specific T cells in blood from patients with IgE^+LC. However, we did not find differences in the magnitude of the T-cell responses when within the same patient (with IgE^+LC) the antigen was presented by LC or non-T cells. Therefore, neither the frequency nor the activation state of the T-cell population can account for the non-responsiveness of T cells to antigen presented by IgE^-LC. The proliferative response of T cells from normal controls to HDA (presented by non-T cells), however, confirms that non-atopics may also have been sensitized to HDA *in vivo* (Romagnani *et al.*, 1973; Yoo *et al.*, 1977). This has also been reported for house dust mite (Cavaillon *et al.*, 1988) and pollen allergen (Buckley *et al.*, 1977); Matthews *et al.*, 1977). The difference between AD patients and normal controls was found to be the capacity of LC to present aeroallergens *in vitro*. In the presence of epidermal LC as APC, we found that only T cells from AD patients with IgE^+LC proliferated to HDA. Patients with IgE^-LC on a certain occasion and with IgE^+LC a month later would respond likewise (data not shown). This indicates that the presentation of HDA by LC is dependent on the presence of cell-bound IgE. Cell-bound IgE is not just a marker for *in vivo*-activated LC. This was demonstrated by the inhibition studies with a-IgE and a-κ/λ antibodies. T-cell proliferation induced by IgE^+LC was selectively inhibited for HDA but not for *Candida*. Furthermore, the *Candida* response induced with IgE^+LC or IgE^-LC was not different, and the background response in the presence of both IgE^+LC and IgE^-LC was increased compared to that of LC from normal controls. We hypothesize that this increased background response, which can be completely blocked by a-HLA-DR antibodies, is most likely due to the presence of antigens on these LC as a result from *in vivo* stimulation. However, the increased background responses do not influence the capacity to respond to HDA or *Candida*. Since it was found that the net *Candida* response is not inhibited by the preincubation with a-IgE or a-κ/λ antibodies, inhibition of the HDA response by these antibodies cannot be explained by negative signals via the FcR and steric hindrance. In fact, in several patients, preincubation with a-IgE or a-κ/λ was found to stimulate T-cell proliferation. The stimulatory effect of the antibody preincubations seems to be contradictory to the inhibition of the LC-mediated T-cell response to HDA. However, this is not the case: only responses which may be mediated by IgE are inhibited (HDA/LC), whereas IgE-independent responses such as the background/LC and *Candida*/LC (net response *Candida*/LC not influenced) as well as HDA/non-T may be increased. In the case of non-T cell-mediated T-cell responses to HDA, there appears to be a competition between inhibition with a-IgE (three out of six cases) and stimulation with a-IgE (two out of six) cases. The same holds for a-κ/λ, which inhibits in two and stimulates in four out of six cases. These results indicate that even non-T cells from peripheral blood may use cell-bound IgE for antigen uptake. However, this also implies that, at least in part, these cells should possess cell-bound IgE. Indeed we found that a substantial proportion of PB non-T cells from AD patients, with IgE^+LC, bear IgE on their surface. In double-labelling experiments all these IgE^+ cells appeared to be CD11 positive, which indicates that in AD patients with elevated serum IgE levels, IgE^+ monocytes exist. This confirms recent results from Ferreri, Zeiger & Spiegelberg (1988) who found IgE on monocytes from patients with AD.

At least two explanations may be given for the inhibition of the HDA response by a-IgE and a-κ/λ antibodies. Firstly, the antibodies may change the conformation of the antigen-binding site of the IgE molecules on the surface of the LC, resulting in a lower binding efficiency of HDA. Secondly, a-IgE and a-κ/λ may cause receptor-mediated endocytosis (RME), removing the IgE molecules from the LC membrane, and thereby preventing HDA binding. In both models, the IgE molecules serve as antigen receptors, very much as was suggested for antigen-specific B cells (Lanzavecchia, 1985). Like IgE^+LC these B cells are very potent and efficient APC.

In conclusion, the *in vitro* observations presented here may offer an explanation for the reaction mechanism behind the delayed patch test reactions to HDA (and other aero-allergens) in AD patients. The dependence of this T-cell response on LC-bound IgE emphasizes the important or even crucial role of LC-bound IgE and elucidates why the delayed patch test reactions to aero-allergens are specific for patients with AD.

REFERENCES

BERRENS L. & HERINGS J. (1984) Bacterial endotoxins in allergic extracts. *Arbeiten aus dem Paul Ehrlich Institut, dem George Speyer Haus und dem Ferdinand Blum Institut.* **79,** 269.

BLACK P. & MARSH D. (1980) Correlation between lymphocyte responses and immediate hypersensitivity to purified allergens. *J. Allergy clin. Immunol.* **66,** 394.

BRUIJNZEEL-KOOMEN C., VAN DEN DONK E.M.M., BRUIJNZEEL P.L.B., CAPRON M., DE GAST G.C. & MUDDE G.C. (1988a) Associated expression of CD1 antigen and the Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin. exp. Immunol.* **74,** 137.

BRUIJNZEEL-KOOMEN C., VAN WICHEN D.F., SPRY C., VENGE P. & BRUIJNZEEL P. (1988b) Active participitation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br. J. Dermatol* **118,** 229.

BRUIJNZEEL-KOOMEN C., VAN WICHEN D.F., TOONSTRA J., BERRENS L. & BRUIJNZEEL P.L.B. (1986) The presence of IgE molecules on

epidermal Langerhans cells from patients with atopic dermatitis. *Arch. Dermatol Res.* **278,** 199.

BUCKLEY R., SEYMOUR F., SANAL O., OWNBY D. & BECKER G. (1977) Lymphocyte responses to purified ragweed allergens *in vitro*. *J. Allergy clin. Immunol.* **59,** 70.

CAVAILLON J., FITTING C., GUINNEPAIN M., RASSEMONT R. & DAVID B. (1988) Lymphocyte proliferative responses to the purified *Dermatophagoides farinae* major allergen (Der F1) in untreated and hyposensitized atopic patients. *Allergy*, **43,** 146.

ELLISTON W., HEISE, E. & HUNTLEY C. (1982) Cell-mediated hypersensibility to mite antigens in atopic dermatitis. *Arch. Dermatol.* **118,** 26.

FERRERI N.R., ZEIGER R.S. & SPIEGELBERG H.L. (1988) IgE, IgA and IgE induced release of leukotriene C4 by monocytes isolated from patients with atopic dermatitis. *J. Allergy clin. Immunol.* **82,** 556.

GEHA R., COLTEN H., SCHNEEBERGER E & MERLER E. (1975) Cooperation between human thymus-derived and bone marrow-derived lymphocytes in the antibody response to ragweed antigen E *in vitro*. *J. clin. Invest* **56,** 386.

HANIFIN J. & RAJKA G. (1980) Diagnotic features of atopic dermatitis. *Acta Derm. Venereol.* **92,** 44.

HIRATAMI M., MUTO K., OSHIDA Y., ITO S. & KASEI M. (1981) Lymphocyte responsiveness to Dermatophagoides farinae extract in mite sensitive patients: effect of immunotherapy on cellular proliferative response and specific immunoglobulin E antibody (RAST score). *J. Allergy clin. Immunol.* **68,** 205.

ISHIZAKA K. (1984) Regulation of IgE synthesis. *Ann. Rev. Immunol.* **2,** 159.

KAPP A., GILLITZER R., KIRCHNER H. & SCHOPF E. (1987) Production of interferon and lymphoproliferative response in whole blood cultures derived from patients with atopic dermatitis. *Arch. Dermatol. Res.* **279,** S55.

LANZAVECCHIA A. (1985) Antigen-specific interactions between T and B cells. *Nature (Lond.)* **314,** 537.

LANZAVECCHIA A., SANTINI P., MAGGI E., DEL PRETE G., FALAGIANI P., ROMAGNANI S. & FERRARINI M. (1983) *In vitro* selective expansions of allergen specific T-cells from atopic individuals. *Clin. exp. Immunol.* **52,** 21.

LEUNG D., BHAN A., SCHNEEBERGER E. & GEHA R. (1983) Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J. Allergy clin. Immunol.* **71,** 47.

MCGEADY S. & BUCKLEY R. (1975) Depression of cell mediated immunity in atopic eczema. *J. Allergy clin. Immunol.* **56,** 393.

MATTHEWS K., PAN P. & WEISBERG S. (1977) Lymphocyte transformation by pollen extracts and purified protein derivate (PPD) in leukocyte cultures of normal human subjects. *Cell Immunol.* **32,** 120.

MUDDE G.C., VAN DAM D. & DE GAST G.C. (1986) Human peripheral blood B cell subpopulations: Surface IgD+ cells respond to pokeweek mitogen with plasma cell differentiation. *Clin. exp. Immunol.* **64,** 150.

O'HEHIR R., YOUNG D., KAY A. & LAMB J. (1987) Cloned human T lymphocytes reactive with Dermatophagoides farinae (house dust mite): a comparison of T and B cell antigen recognition. *Immunology*, **62,** 635.

PHILLIPS L., CHAMBERS C., UNDERDOWN B. & ZIMMERMAN B. (1987) Lymphocyte proliferation to antigen E: demonstration of the restriction of antigen E specific T-cells to ragweed-allergic donors. *J. Allergy clin. Immunol.* **79,** 933.

RAWLE F., MITCHELL E. & PLATTS-MILLS T. (1984) T-cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus* antigen P1: comparison of the patients with asthma, atopic dermatitis and perennial rhinitis. *J. Immunol.* **133,** 195.

REITAMO S., VISA K., KÄHONEN K., STUBB S. & SALO O. (1986) Eczematous reactions in atopic dermatitis patients caused by epicutaneous testing with inhalant allergens. *Br. J. Dermatol.* **114,** 303.

RICCI M., DELPRETE G., MAGGI E., LANZAVECCHIA A., SALA P. & ROMAGNANI S. (1985) *In vitro* synthesis of human IgE: reappraisal of a 5 year study. *Int. Archs. Allergy Appl. Immunol.* **77,** 32.

ROMAGNANI S., BILIOTTI G., PASSALEVA A. & RICCI M. (1973) Mites and house dust allergy. III. *In vitro* lymphocyte transformation and precipitating antibodies to house dust and mite (*Dermatophagoides pteronyssinus*) extract in atopic and non-atopic individuals. *Clinical Allergy*, **3,** 51.

SILLEVIS SMITT J., BOS J., HULSEBOSCH H. & KRIEG SA. (1986) *In situ* immunophenotyping of antigen presenting cells and T cell subsets in atopic dermatitis. *Clin. exp. Dermatol.* **11,** 159.

SIRAGANIAN R., BAER H., HOCHSTEIN H. & MAY J. (1979) Allergenic and biologic activity of commercial preparations of house dust extract. *J. Allergy Clin. Immunol.* **64,** 526.

YOO T., KUO C. & HEATH C. (1977) A study of dust induced lymphocyte blastogenesis. *Acta Allergol.* **32,** 227.

ZACHARY C., ALLEN M. & MACDONALD D. (1985) In situ quantification of T-lymphocyte subsets and Langerhans cells in the inflamatory infiltrate in atopic eczema. *Br. J. Dermatol.* **112,** 149.

ZIMMERMAN B., UNDERDOWN B., ELLIS J. & JAMES D. (1986) Cloned helper T cells for IgE: characterization of T cells cloned from atopic donors with a high serum IgE level. *J. Allergy clin. Immunol.* **77,** 70.