

Serum-IgE-Facilitated Allergen Presentation in Atopic Disease

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ABSTRACT. The occurrence of facilitated Ag presentation (FAP) was investigated in atopic disease using an in vitro model in which the ability of CD23-expressing EBV-B cells was tested to present *Der p* II, a major allergen of housedust mite *Dermatophagoides pteronyssinus*, to cells of autologous *Der p* II-specific CD4⁺ T lymphocyte clones. Purified *Der p* II protein was immune complexed with Ig by preincubation in sera from atopic patients containing *Der p* II-specific IgE. Incubation of EBV-B cells with these complexes before using the cells as APC results in proliferation of the *Der p* II-specific T cells at a 1000-fold lower *Der p* II concentration than required for T cell activation by presentation of uncomplexed *Der p* II. FAP is not evident using sera from allergic and nonallergic control donors not containing *Der p* II-specific IgE. FAP is mediated by IgE and not by IgG, because it can be blocked completely by preincubating the serum from atopics with polyclonal anti-IgE and not with polyclonal anti-IgG. After pulsing EBV-B with precomplexed *Der p* II at 4°C, FAP is as strong as after incubation at 37°C, suggesting that FAP is the result of facilitated trapping of IgE-complexed allergen. CD23 was involved in facilitated trapping of the complexes, because FAP is blocked by preincubation of EBV-B with anti-CD23. In contrast to FAP of *Der p* II, which was mediated by all *Der p* II-specific sera tested, only one of four sera containing cat allergen-specific IgE was appropriate to mediate FAP to cat allergen-specific T cell clones. Inasmuch as FAP enables functional presentation of allergen in vivo at doses as low as several nanograms, FAP may contribute to the continuing of chronic allergic reactions in response to minute doses of aeroallergens, especially housedust mite allergens, in the environment. *Journal of Immunology*, 1993, 150: 3643.

Atopic disease is associated with the occurrence of allergen-specific CD4⁺ T cells of the Th2 cell subset (1, 2). In contrast to Th1 cells, activated human Th2 cells secrete large quantities of IL-4 without substantial IFN- γ and, as a consequence, they can induce IgE synthesis by B cells (1). The predominant Th2 type response, therefore, may explain the elevated levels of allergen-specific serum IgE as commonly observed in

atopic disease. In addition, supernatants of activated Th2 cells strongly enhance expression of the low affinity IgE-receptor (Fc ϵ RII/CD23) on the cell surface of APC in vitro (3), explained predominantly as another effect of the Th2-derived cytokine IL-4 (4, 5). Indeed, the frequency of CD23-expressing cells is increased in PBMC fractions from atopic patients (6, 7). Moreover, CD23-expressing cells are also present in lesional skin of atopic dermatitis patients (7, 8).

Recently, Ag-specific IgE-mediated FAP² was described,

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² Abbreviations used in this paper: FAP, facilitated Ag presentation; Dp, *Dermatophagoides pteronyssinus*; Der p II, major allergen protein of *Dermatophagoides pteronyssinus*; Fd, *Felis domesticus*; IMDM, Iscove's modified Dulbecco's medium; PPD, purified protein derivative; RAST, radioallergosorbent test; TLC, T lymphocyte clone.

implicating more efficient trapping of IgE-complexed Ag by CD23-expressing APC (9, 10). EBV-B cells, which express CD23 constitutively, could activate Ag-specific T cells at a 100-fold lower Ag concentration in the presence of Ag complexed to IgE than in the presence of uncomplexed Ag. The elevated levels of allergen-specific serum IgE and the elevated numbers of CD23-expressing cells in atopic patients suggest the possible occurrence of a similar FAP mechanism for the presentation of allergen to allergen-specific CD4⁺ T cells in atopic disease.

In the present study, we have investigated the occurrence of IgE-mediated FAP in an in vitro model by incubating EBV-B cells from an atopic patient that constitutively express CD23 in various conditions with complexes formed between *Der p* II and Ig in serum of atopic *Der p* II-allergic patients or Ig in serum of nonallergic control subjects. The effects of these preincubations were analyzed by measuring the proliferative response and the cytokine production levels of *Der p* II-specific TLC from the same atopic patient.

Materials and Methods

Ag and antibodies

Der p II was used unpurified as protein extract of Dp, a kind gift of HAL Allergener Laboratorium (Haarlem, The Netherlands) containing 0.32 mg/ml *Der p* II. Synthetic peptide 116–129 of *Der p* II was kindly provided by Dr. W. van't Hof (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands) (11). Cat dander extract (*Felis domesticus*; Fd extract) was also a gift from HAL B. V. PPD was from Statens Serum Institute (Denmark). Anti-CD23 mAb MHM6 was obtained from Dakopatts (Glostrup, Denmark). Affinity-purified goat anti-human IgG and anti-human IgE were from Tago (Burlingame, CA). Anti-CD40 mAb G28–5 was a kind gift of Dr. J. Ledbetter (Oncogene, Seattle, WA). Anti-HLA-DR mAb B8.11.2 was a kind gift of Dr. F. Koning (Department of Immunohematology and Bloodbank, University Hospital, Leiden, The Netherlands).

T cell clones and APC

Type 2 TLC JBA2, derived from PBMC of an atopic patient, recognizes an epitope in the 116–129 amino acid sequence of *Der p* II in association with HLA-DR1, expresses CD3 and CD4, and secretes IL-4 and IL-5 and almost no IL-2 and IFN- γ after Ag-specific activation (J. van Neerven, unpublished observations). PBA1, a Th0 type clone, derived from a nonatopic donor, recognizes peptide 20–33 of *Der p* II. PBB1 and PBC1 are Th2 type clones that recognize different as yet undefined proteins in cat dander extract. Neither clone reacts to the major cat allergen *Fel d* I and cat albumin. TLC N3Ag is specific for a protein of mycobacterium in PPD. This TLC was a kind gift of Dr. T. Ottenhoff (Department of Immunohematology and Bloodbank, University Hospital, Leiden, The Netherlands).

As APC, autologous or HLA-DR matched EBV-B cells were used. CD23 expressed by EBV-B cells varied between 60 and 80%.

Preparation of APC for FAP

Serum of atopic patients containing high concentrations of IgE was incubated for 1 h at 37°C with *Der p* II in serial dilutions of 4 μ g to 0.4 ng/ml or with Fd extract in serial dilutions of 100 μ g to 1 ng protein/ml. Then EBV-B cells were added at a concentration of 3×10^5 /ml allergen-complexed serum and incubated for 1 h at 4°C or at 37°C. After incubation, the cells were washed three times in HBSS with 2% bovine calf serum (HyClone, Logan, UT), irradiated (3000 rad), and washed again. In blocking experiments with polyclonal anti-IgG or anti-IgE, the sera were incubated with these antisera for 1 h at 37°C before adding *Der p* II. In blocking experiments with anti-CD23, EBV-B cells were incubated for 1 h at 37°C with this antibody before adding the cells to the sera.

T cell proliferation assays

In the standard T cell proliferation assay, 4×10^4 TLC cells were cultured in 200 μ l culture medium with 1×10^4 irradiated autologous EBV-B in the presence of 4 μ g to 0.4 ng/ml *Der p* II or 100 μ g to 1 ng protein/ml Fd extract. FAP was studied with TLC cells and EBV-B cells that were preincubated with Ig-complexed allergen. Cells were cultured for 40 h in 96-well flat bottomed culture plates (Costar, Cambridge, MA), with the last 16 h in the presence of 0.3 μ Ci/well of [³H]TdR (Radiochemical Centre, Amersham, United Kingdom). Incorporation of [³H]TdR was determined by liquid scintillation spectroscopy and expressed as mean cpm and SD of triplicate cultures. If not indicated, SD was always lower than 15% of the average.

Sera and RAST

Sera were obtained from healthy volunteers and from atopic patients suffering from atopic dermatitis or allergic asthma. To assess the antibody content to common allergens, as Dp, cat, and grasspollen allergens, a RAST was performed. RAST assays for the presence of *Der p* II-specific IgE were expressed semiquantitatively as the percentage IgE binding of the total serum IgE, using anti-*Der p* II mAb. The assays were performed by Dr. S. Stapel (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands).

Culture medium and cell culturing

TLC cells were cultured and experiments were performed in IMDM (GIBCO, Paisley, United Kingdom), supplemented with 10% C-inactivated normal human serum (Central Laboratory Blood Transfusion Service), containing no detectable levels of IgE. EBV-transformed B cell lines were

maintained in IMDM supplemented with 5% bovine calf serum (HyClone). All cultures were performed at 37°C in a humidified atmosphere containing 5% CO₂.

Fixation of APC

Fixation of the APC was performed in 0.006% glutaraldehyde in HBSS for 30 s at 4°C. Fixation was stopped by 0.2 M glycine in HBSS for 10 min. After this procedure, the cells were washed three times and incubated for 30 min in IMDM with 10% human serum at 37°C. Then APC were irradiated (3000 rad), washed, and added to the TLC cells.

IL-4 and IFN- γ assays

Estimation of IL-4 and IFN- γ contents of the TLC supernatants was performed with specific solid-phase sandwich ELISA, as described elsewhere (12, 13). As references, rIL-4 and natural IFN- γ were used. The sensitivity of both assays was 100 pg/ml.

Cytofluorimetric analysis

Cells were labeled on ice for 30 min with the primary antibody MHM6, or polyclonal rabbit anti-human IgE in HBSS containing 2% FCS and 0.1% sodium azide. Subsequently cells were washed three times and incubated with the second antibody rabbit anti-mouse-FITC conjugate or goat anti-rabbit Ig-FITC conjugate. Cells were washed three times again and fluorescence was analyzed on a FAC-Scan (Becton-Dickinson). For each sample, 5000 cells were analyzed.

Results

FAP

The occurrence of FAP in atopic disease was studied in an in vitro model using *Der p* II-specific CD4⁺ TLC JBA2 from severe atopic dermatitis patient 1 and autologous CD23-expressing EBV-B cells as APC. In the standard proliferation assay in the presence of untreated EBV-B cells, JBA2 showed half maximal *Der p* II-specific proliferation after addition of a few μ g/ml of *Der p* II (Fig. 1). IgE-facilitated allergen presentation was investigated by preincubation of the EBV-B cells with *Der p* II that was precomplexed with IgE by mixing serially diluted *Der p* II protein for 1 h at 37°C with serum obtained from patient 1, which contained a considerable quantity of *Der p* II-specific IgE (32% binding of total IgE in RAST). EBV-B cells were incubated for 1 h at 37°C with these mixtures and subsequently thoroughly washed. This precomplexing procedure in atopic serum could bring the *Der p* II concentration required for half maximal proliferation of JBA2 cells down to values as low as several ng/ml (Fig. 1). FAP does not depend on properties of atopic APC, because sim-

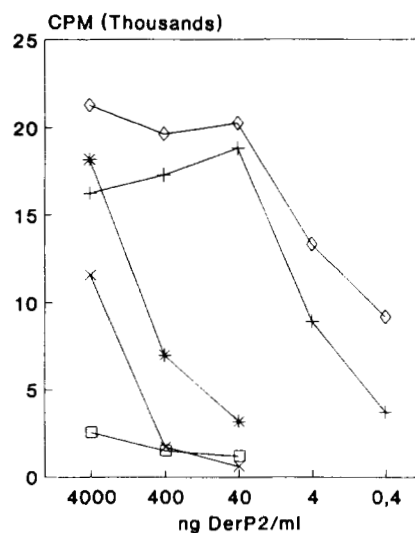


FIGURE 1. FAP after preincubating *Der p* II with atopic serum and EBV-B. Atopic and control serum were incubated with *Der p* II in serial dilution for 1 h at 37°C. After 1 h, EBV-B cells were added and incubated for 1 h at 4°C or 37°C. After washing, EBV-B cells were added to TLC cells. Atopic serum, 4°C (+), 37°C (◇); control serum, 4°C (□), 37°C (*), without preincubation (×).

ilar results were obtained with class II MHC-matched CD23-expressing EBV-B cells from a nonallergic control subject (data not shown).

The results indicate that atopic serum is able to increase the efficiency of allergen presentation, thereby reducing the minimally required allergen concentration for T cell activation at least a 1000-fold. Activation of the JBA2 cells at low doses of allergen (40 ng/ml) requiring the FAP mechanism resulted in the same cytokine secretion pattern as found at the allergen doses not requiring FAP (4 μ g/ml). At both allergen concentrations, the JBA2 cells secreted high levels of IL-4 and no IFN- γ (Table I).

FAP is dependent on specific IgE

FAP is strictly dependent on the presence of allergen-specific IgE in serum. In control experiments, in which *Der p* II protein was incubated in serum obtained from nonallergic control subjects (Figs. 1 and 2) or from atopic donors (Fig. 2), containing undetectable amounts of *Der p* II-specific IgE, FAP to *Der p* II-specific TLC JBA2 is not observed. FAP was found with sera from three different atopic patients containing *Der p* II-specific IgE (Fig. 2). The serum dilution that still induced half maximal proliferation of JBA2 cells could vary considerably from patient to patient and was not clearly related to the anti-*Der p* II IgE RAST values. With sera from two of the three patients, half maximal proliferation could be obtained at dilutions of as high as 20 times (Fig. 2).

FAP of *Der p* II could also be observed with other *Der p* II-specific TLC including TLC PBA1 prepared from a

Table I
Cytokine secretion by TLC JBA2 upon unfacilitated and IgE-facilitated recognition of Der p II presented by autologous EBV-B cells

Der p II Dose (ng/ml)	IL-4 (ng/ml)	IFN- γ (pg/ml)
4000 (unfacilitated ^a)	3.1	<200
40 (unfacilitated)	0.4	<200
4000 (facilitated by IgE ^b)	3.5	<200
40 (facilitated by IgE)	2.6	<200

^a T cells and EBV-B cells were cultured in the presence of the indicated concentrations of Der p II.

^b T cells were cultured in the presence of autologous EBV-B cells pulsed with complexes prepared by incubation of the indicated concentrations of Der p II with anti-Der p II IgE containing autologous serum.

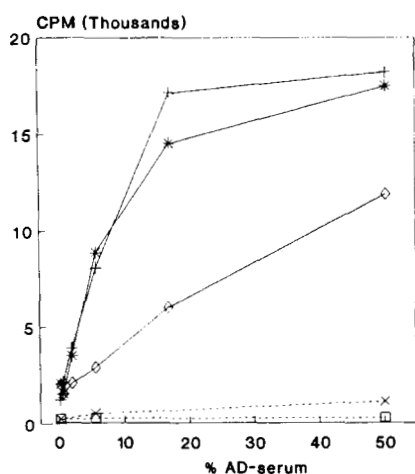


FIGURE 2. FAP effects after preincubating EBV-B cells in allergen-incubated sera obtained from atopics (solid lines) and a control (dotted line) in a serial dilution. Serum was preincubated with 400 ng Der p II/ml. Anti-Der p II-IgE RAST values were for patient 1, 32% (◇); patient 2, 39% (+); patient 3, 31% (*); patient 4, 0% (×); and normal donor, 0% (□).

nonatopic donor. TLC PBA1 is reactive to peptide sequence 20–33 and secretes both IL-4 and IFN- γ . In this case, FAP could be demonstrated with various Der p II-specific IgE-containing sera (Fig. 3), but not with the autologous serum that did not contain Der p II-specific IgE. This finding stresses that FAP strictly depends on the presence of allergen-specific IgE in atopic sera.

The crucial role of atopic serum IgE in FAP was further demonstrated in blocking experiments using polyclonal antisera against human IgE or IgG molecules. Blocking of complex formation by preincubation of serum of the atopic patient with anti-IgE, before incubation with Der p II, eliminated FAP in a dose-dependent fashion, whereas preincubation with anti-IgG did not show significant inhibition (Table II).

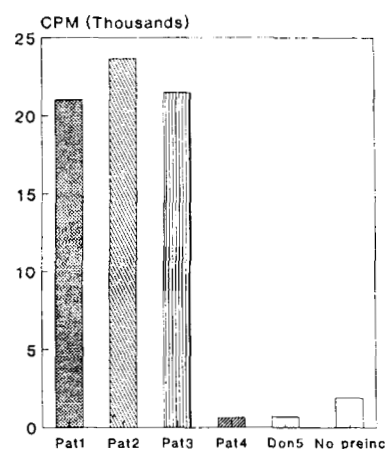


FIGURE 3. FAP to Der p II-specific TLC PBA1 from a non-atopic donor. EBV-B cells were preincubated with autologous and various atopic sera precomplexed with 400 ng Der p II/ml (hatched bars). The anti-Der p II-IgE RAST values of patients 1 to 4 are the same as in Figure 2. The RAST value of the autologous control donor (Don 5) was 0%. Open bar, proliferation without preincubation and Der p II added into the well.

Table II
Effects of anti-IgG and anti-IgE on FAP

Blocking Antisera ^a	Proliferative Response of JBA2 (cpm $\times 10^{-3}$)
No antibody	47.6
Anti-IgE ^a (μ g/ml)	
3	43.1
15	22.4
75	0.3
Anti-IgG ^a (μ g/ml)	
2	46.9
10	46.5
50	43.9

^a Atopic serum was preincubated with polyclonal affinity-purified anti-IgE or anti-IgG before complexing with Der p II allergen. EBV-B cells were incubated for 1 h with Der p II-complexed serum and cultured with JBA2 cells for 40 h, after which proliferation of the JBA2 cells was determined.

FAP of cat protein and FAP of nonallergic protein PPD

FAP is not restricted to house dust mite allergen, because FAP to cat protein-specific TLC could also be demonstrated (Fig. 4). Using the same protocols, EBV-B cells were preincubated with cat allergen-specific IgE-containing sera precomplexed with a varying concentration of cat allergen and subsequently cocultured with cat allergen-specific TLC PBA1. Notably, in contrast to FAP of Der p II with the Der p II-containing sera, only one of four cat allergen-containing sera was able to mediate FAP to cat allergen-specific TLC.

FAP could not be demonstrated in a model using a TLC specific for a protein in PPD. With either autologous serum or various sera from atopic patients, including the serum of

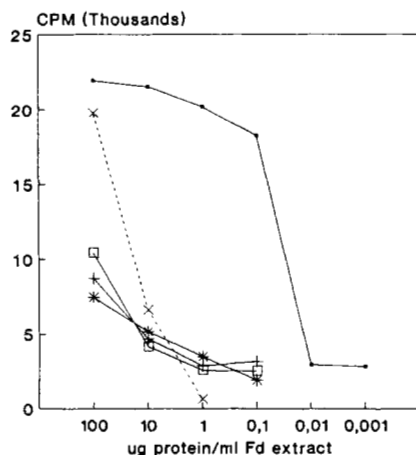


FIGURE 4. FAP to cat protein-specific TLC PBC1 from an atopic patient hypersensitive for cat allergen. EBV-B cells were preincubated with atopic sera precomplexed with cat protein at the indicated dilution and subsequently cocultured with TLC PBC1 (solid lines) or EBV-B cells were not preincubated and the cat protein added to the final culture instead (dotted line). RAST values for cat dander extract were patient 1 (·), 4+; patient 2 (+), 4+; patient 3 (*), 4+; patient 4 (□), 5+. Autologous serum from patient 4.

patient 1 that mediated FAP of *Der p* II and cat protein, FAP to PPD-specific TLC remained undetectable (data not shown). This finding stresses that FAP is not mediated by a nonspecific factor in the sera-mediating FAP of common allergens.

Role of complex formation in FAP

Sequential incubation of the EBV-B cells first in medium with atopic serum followed by incubation with *Der p* II did not result in FAP (Fig. 5), suggesting that FAP is dependent on complex formation between *Der p* II and *Der p* II-specific IgE before binding to the EBV-B cells. This implication is in line with the notion that CD23 has a higher binding affinity for complexed IgE compared to uncomplexed IgE (14). The requirement for complex formation was further evident from experiments using the synthetic *Der p* II peptide 116–129, which contains the relevant amino acid sequence recognized in association with HLA-DR1 by JBA2 cells. Peptide 116–129 is not bound by serum IgE of atopic patients (11). Incubation of EBV-B cells with atopic serum IgE that was allowed to complex with 0.4 μ g/ml *Der p* II or peptide both resulted in T cell proliferation (Fig. 6). Proliferation to peptide-containing serum may be explained by direct binding of uncomplexed peptide to HLA-DR molecules, because the peptide also induced proliferation in the T cells after preincubation with peptide in control serum. At 40 ng/ml, a dose that requires FAP, however, only native *Der p* II could induce T cells to proliferate, in agreement with the notion that the peptide 116–129 is unable to form complexes with serum IgE.

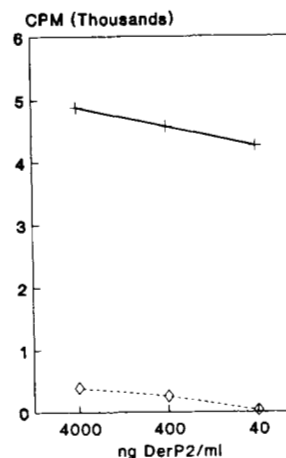


FIGURE 5. No FAP effect after preincubation in atopic serum without allergen. EBV-B cells were incubated sequentially in atopic serum for 1 h at 4°C, washed, and incubated in medium with allergen in a serial dilution for another 1 h at 4°C. After washing, EBV-B cells were added to TLC cells (dotted line). As control, EBV-B cells were incubated in allergen-incubated serum for FAP effects (solid line).

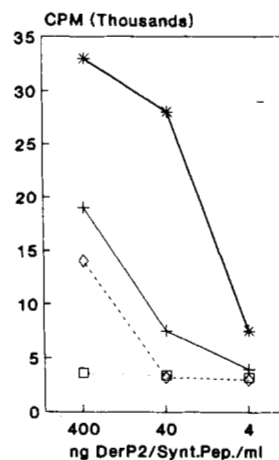


FIGURE 6. FAP after preincubating *Der p* II or synthetic peptide 116–129 with atopic or control serum for 1 h at 37°C. After 1 h, EBV-B cells were added and incubated for 1 h at 37°C. Atopic serum preincubated with *Der p* II (*) or with synthetic peptide (+). Control serum preincubated with *Der p* II (□) or with synthetic peptide (◇).

FAP results from facilitated IgE-mediated binding of allergen to FcεRII/CD23

FAP was still evident if EBV-B cells were preincubated for 1 h at 4°C with the same preparations of *Der p* II precomplexed in atopic serum, instead of at 37°C (Fig. 1). Because in these conditions EBV-B cells will bind but not internalize and process Ag or Ig-complexed Ag, this finding indicates that the increased efficiency of allergen presentation results from more efficient trapping of Ag, as was suggested before in mouse and human model experiments (9, 10), rather than from increased efficiencies in allergen processing or allergen re-exposure. Facilitated binding of allergen was further

Table III
Effect of anti-CD23 on FAP by CD23-expressing EBV-B cells

Blocking Antisera/Body	Proliferative Response of JBA2 (cpm $\times 10^{-3}$)
No antibody	14.9
Anti-CD23 ^a (μ g/ml)	
0.08	15.6
0.4	14.3
2	7.8
10	3.0
50	0
Anti-CD40 ^a (μ g/ml)	
0.08	14.3
0.4	11.4
2	10.2
10	12.0
50	9.7

^a EBV-B cells were preincubated with monoclonal anti-CD23 (MHM6) or anti-CD40 before adding precomplexed atopic serum.

implicated by the observation that, after pulsing with precomplexed allergen, EBV-B cells express membrane-bound IgE as determined by cytofluorimetry (data not shown).

To investigate whether indeed FAP follows from binding of IgE-*Der p* II complexes to Fc ϵ R/CD23 on the cell surface of the EBV-B cells, neutralizing antibodies to CD23 were added before and during incubation of EBV-B cells with IgE-precomplexed *Der p* II. Preincubation of EBV-B cells with anti-CD23 mAb MHM6, before incubation with the IgE-*Der p* II complexes, resulted in a dose-dependent inhibition of IgE-mediated *Der p* II presentation (Table III), whereas preincubation with the isotype-matched Anti-CD40 mAb G28-5, which is irrelevant for FAP, did not inhibit significantly.

FAP requires Ag processing

Presentation of exogenous protein Ag requires degradation of Ag in the lysosomal compartment of the APC into small peptides that subsequently are re-exposed in association with class II MHC molecules on the cell surface of the APC. TLC JBA2 requires Ag degradation, because the cells of this clone recognize an epitope within amino acid sequence 116–129 of the *Der p* II molecule. To investigate whether facilitated presentation of *Der p* II bound as IgE complex to CD23 also involves intracellular routing of Ag processing, IgE-*Der p* II-preincubated EBV-B cells were fixed mildly with glutaraldehyde to prevent Ag processing (15). Fixation of EBV-B after preincubation for 1 h at 4°C with precomplexed *Der p* II eliminated Ag presentation to JBA2 completely (Fig. 7). Fixation of EBV-B cells, preincubated with IgE-*Der p* II at 37°C, allowing allergen processing before fixation, had only a marginal effect. These findings demonstrate that the absorbed IgE-*Der p* II complexes had to undergo lysosomal degradation for successful presenta-

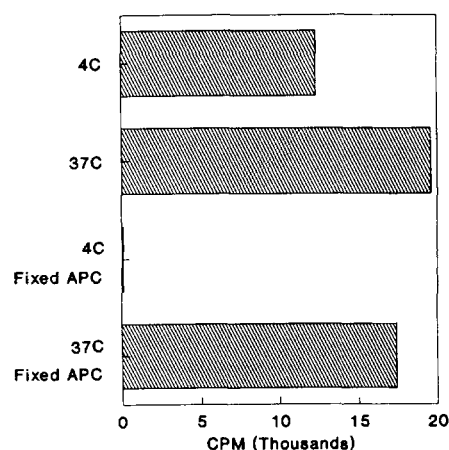


FIGURE 7. Effect of EBV-B fixation after preincubating the cells in allergen-incubated atopic serum. Atopic serum was preincubated with 400 ng *Der p* II/ml. EBV-B cells were incubated at 4 or 37°C to compare circumstances with and without internalization.

tion. After processing, *Der p* II was indeed presented to and recognized by the *Der p* II-specific T cells in association with HLA-DR, because anti-HLA-DR mAb B8.11.2 completely blocked T cell proliferation when added during the proliferation assay (Table IV).

Discussion

The present study indicates that atopic patients express factors that enable functional presentation of allergen, especially house dust mite proteins, at doses as low as several ng/ml. Although full evidence is lacking, the experiments strongly suggest that FAP is based on receptor-mediated endocytosis, i.e., facilitated binding of preformed IgE-allergen complexes mediated by CD23 expressed by APC and subsequent endocytosis of this complex. Internalization is followed by processing and functional re-exposure of peptide fragments of allergen in association with class II MHC molecules. Receptor-mediated phagocytosis results in 100- to 1000-fold more efficient endocytosis of protein, when compared to fluid phase endocytosis (16), as also demonstrated for FAP in this study.

It may be expected that other types of Fc ϵ R, such as the high affinity Fc ϵ R that can bind uncomplexed IgE, are also able to mediate FAP. For instance, epidermal Langerhans cells and dermal dendritic cells in lesional skin of atopic dermatitis patients (17–19) and dendritic cells in the epithelium of alveoli in asthma patients may express cell surface IgE. Indeed, expression of the high affinity Fc ϵ R-I has recently been reported for Langerhans cells in the epidermis (20, 21). In favor of FAP mediated by this high affinity Fc ϵ R is the observation of Mudde et al. (22) that only IgE⁺ Langerhans cells, obtained from lesional skin, could induce a specific proliferative response to exogenous house dust

Table IV
Effect of anti-HLA-DR on FAP to *Der p II*-specific JBA2 cells

Blocking Antiserum	Proliferative Response of JBA2 (cpm $\times 10^{-3}$)
No antibody	47.6
Anti-HLA-DR ^a (dilution)	
1000 $\times$	36.5
200 $\times$	0.4

^a Monoclonal anti-HLA-DR (B 8.11.2) was added to the culture at the onset of the proliferation assay.

mite Ag in autologous peripheral blood T cells. This observation is suggestive for the occurrence of FAP as a result of Ag binding to uncomplexed, house dust mite-specific IgE bound to Langerhans cells.

The present experiments do not argue for a role for IgG in facilitated presentation of allergen in atopic disease, inasmuch as preincubating atopic serum with polyclonal anti-IgG could not inhibit FAP, whereas blocking experiments with anti-CD23 mAb and anti-IgE antiserum indicate a role for IgE and its low affinity FcR. A substantial role of IgG-mediated FAP, comparable to the IgE-potentiated FAP, is not likely, inasmuch as the allergen-specific IgG percentage of total IgG is much lower than allergen-specific IgE percentage of total IgE (23). Allergen-specific IgG1 antibodies may be present in the serum of most atopics and nonatopics, but only IgG4 levels are significantly increased in atopics, an antibody type for which it is not clear whether it is a sensitizing or a blocking antibody (24). However, the increase in IgG4 levels is only moderate, and the total IgG concentration remains within normal limits (25). Moreover, IL-4, although it stimulates the expression of FcεRII, suppresses the expression of the low affinity FcγRII (26).

Recently, it was demonstrated that also the low affinity FcγRII (CD32) can play a role in binding complexed IgE (27). However, blocking experiments (data not shown) using CD32 mAb only resulted in marginal blocking (up to 20%) of FAP compared to the effect of CD23 mAb (up to 80%), suggesting that CD32 is less important in the FAP of allergens.

The FAP presently described seems to be Ag-selective, that is, predominantly house dust mite protein is subject to FAP. FAP proved to be strictly dependent on IgE with the relevant specificity, inasmuch as it could only be demonstrated with the sera from atopic patients with a positive RAST for specific IgE. In case of *Der p II*, our experiments showed that there is no clear correlation with the total IgE serum level, but with the RAST value. In case of cat proteins, many RAST-positive sera did not mediate FAP. This may be a reflection of levels of cat allergen-specific IgE too low to allow FAP in the in vitro model. So far, we were unable to quantify the level of allergen-specific IgE in the sera used more accurately than in the semi-quantitative RAST. The relation between FAP, the quantities of allergen-

specific IgE in the sera used and category of atopic patient are presently studied in more detail.

In atopic subjects, Dp-specific TLC from atopic donors are Th2 cells as defined by their cytokine secretion profiles (1, 2). Activation of these T cells leads to secretion of large quantities of IL-4, inducing allergen-specific IgE synthesis (1) and expression of the CD23b isoform by APC, including normal B lymphocytes (28, 29), monocytes (4), and Langerhans cells (5, 30), and they are associated with late phase responses to allergen. Evidence is accumulating that atopic lesional skin areas contain elevated numbers of allergen-specific, IL-4-producing CD4⁺ T cells (3, 31). The actual activation of these Th2 cells is suggested by the presence of high CD23-expressing cells in lesional skin of atopic dermatitis patients (7, 8) and the increased frequency of high CD23-expressing cells in the PBMC fractions of atopic patients (6, 7).

The presence of elevated numbers of cells with high CD23 expression in atopy gives support to the concept of in vivo occurrence of FAP, as presently described in vitro. An increased efficiency of allergen presentation in vivo may have important significance for the pathology of atopic allergy. As a result of FAP, as presently found for house dust mite Dp protein in particular, inhalation of only minute doses of Dp protein may keep allergic reactions going on in atopic disease by activation of Dp-specific T cells.

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References

1. Wierenga, E. A., M. Snoek, C. de Groot, I. Chrétien, J. D. Bos, H. M. Jansen, and M. L. Kapsenberg. 1990. Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J. Immunol.* 144:4651.
2. Parronchi, P., D. Macchia, M. Piccinini, P. Biswas, C. Simonelli, E. Maggi, M. Ricci, A. Ansari, and S. Romagnani. 1991. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc. Natl. Acad. Sci. USA* 88:4538.
3. Van der Heijden, F. L., E. A. Wierenga, J. D. Bos, and M. L. Kapsenberg. 1991. High frequency of IL-4-producing CD4⁺ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J. Invest. Dermatol.* 97:389.
4. Vercelli, D., H. H. Jabara, B. Lee, N. Woodland, R. S. Geha, and D. Y. M. Leung. 1988. Human recombinant interleukin 4 induces FcεR2/CD23 on normal human monocytes. *J. Exp. Med.* 167:1406.
5. Bieber, T., A. Rieger, C. Neuchrist, J. C. Prinz, E. P. Rieber, G. Boltz-Nitulescu, and G. Stingl. 1989. Induction of FcεR2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and γ interferon. *J. Exp. Med.* 170:309.

6. Bujanowski-Weber, J., B. Brings, I. Knöller, T. Pfeil, and W. König. 1989. Expression of low-affinity receptor for IgE (FcεRII, CD23) and IgE-BF (soluble CD23) release by lymphoblastoid B-cell line RPMI-8866 and human peripheral lymphocytes of normal and atopic donors. *Immunology* 66: 505.
7. Takigawa, M., T. Tamamori, D. Horiguchi, T. Sakamoto, M. Yamada, A. Yoshioka, K. Toda, S. Imamura, and J. Yodo. 1991. Fcε receptor II/CD23-positive lymphocytes in atopic dermatitis. I. The proportion of FcεRII⁺ lymphocytes correlates with the extent of skin lesion. *Clin. Exp. Immunol.* 84:275.
8. Sugiura, H., M. Uehara, and T. Maeda. 1990. IgE-positive epidermal Langerhans cells in allergic contact dermatitis lesions provoked in patients with atopic dermatitis. *Arch. Dermatol. Res.* 282:295.
9. Kehry, M. R., and L. C. Yamashita. 1989. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. *Proc. Natl. Acad. Sci USA* 86: 7556.
10. Pirron, U., T. Schlunck, J. C. Prinz, and E. P. Rieber. 1990. IgE-dependent antigen focusing by human B lymphocytes is mediated by the low-affinity receptor for IgE. *Eur. J. Immunol.* 20:1547.
11. van 't Hof, W., P. C. Driedijk, M. van den Berg, A. G. Beck-sicking, G. Jung, and R. C. Aalberse. 1991. Epitope mapping of the *Dermatophagoides pteronyssinus* house dust mite major allergen Der p II using overlapping synthetic peptides. *Mol. Immunol.* 28:1225.
12. Chrétien, I., A. van Kimmenade, M. K. Pearce, J. Banchereau, and J. S. Abrams. 1989. Development of polyclonal and monoclonal antibodies for immunoassay and neutralization of human interleukin-4. *J. Immunol. Methods* 117:67.
13. Van der Meide, P. H., M. Dubbeld, and H. Schellekens. 1985. Monoclonal antibodies to human immune interferon and their use in a sensitive solid-phase ELISA. *J. Immunol. Methods* 79:293.
14. Richards, M. L., J. F. Marcelletti, and D. H. Katz. 1988. IgE-antigen complexes enhance FcεR and Ia expression by murine B lymphocytes. *J. Exp. Med.* 168:571.
15. Shimonkevitz, R., J. Kappler, P. Marrack, and H. Grey. 1983. Antigen recognition by H-2-restricted T cells. *J. Exp. Med.* 158:303.
16. Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature* 314:537.
17. Bruynzeel-Koomen, C., D. F. van Wichen, J. Toonstra, L. Berrens, and P. L. Bruyn zeel. 1986. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch. Dermatol. Res.* 278:199.
18. Barker, J. N., V. A. Alegre, and D. M. MacDonald. 1988. Surface-bound immunoglobulin E on antigen-presenting cells in cutaneous tissue of atopic dermatitis. *J. Invest. Dermatol.* 90:117.
19. Bieber, T., B. Dannenberg, J. C. Prinz, E. P. Rieber, W. Stolz, O. Braun-Falco, and J. Ring. 1989. Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. *J. Invest. Dermatol.* 92:215.
20. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Födinger, J.-P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J. Exp. Med.* 175:1353.
21. Bieber, T., H. de la Salle, A. Wollenberg, J. Hakimi, R. Chiz-zonite, J. Ring, D. Hanau, and C. de la Salle. 1992. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). *J. Exp. Med.* 175:1285.
22. Mudde, G. C., F. C. van Reijssen, G. J. Boland, G. C. de Gast, P. L. Bruynzeel, and C. A. Bruijnzeel-Koomen. 1990. Allergen-presentation by epidermal Langerhans cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 69:335.
23. Merrett, J., R. S. C. Barnetson, M. L. Burr, and T. G. Merrett. 1984. Total and specific IgG4 antibody levels in atopic eczema. *Clin. Exp. Immunol.* 56:645.
24. Aalberse, R. C., P. H. Dieges, V. Knul-Bretlova, P. Vooren, M. Aalberse, and J. van Leeuwen. 1983. IgG4 as blocking antibody. *Clin. Rev. Allergy* 1:289.
25. Shakib, F., P. McLaughlan, D. R. Stanworth, E. Smith, and E. Fairburn. 1977. Elevated serum IgE and IgG4 in patients with atopic dermatitis. *Br. J. Dermatol.* 97:59.
26. Lutzker, S., P. Rothman, R. Pollock, R. Coffman, and F. W. Alt. 1988. Mitogen and IL-4-regulated expression of germ-line Ig γ2b transcripts: evidence for directed heavy chain class switching. *Cell* 53:177.
27. Takizawa, F., M. Adamczewski, and J.-P. Kinet. 1992. Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as FcγRII and FcγRIII. *J. Exp. Med.* 176:469.
28. Hivroz, C., A. Vallé, J. C. Brouet, J. Banchereau, and C. Grillot-Courvalin. Regulation by interleukin 2 of CD23 expression of leukemic and normal B cells: comparison with interleukin 4. *Eur. J. Immunol.* 19:1025.
29. Delespesse, G., and M. Sarfati. 1991. An update on human CD23 (FcεRII). FcεRII and IgE-BFs (soluble CD23) play an essential role in the regulation of human IgE synthesis. *Clin. Exp. Allergy* 21:153.
30. Torresani, C., G. C. Manara, C. Ferrari, and G. De Panfilis. 1991. Immunoelectron microscopic characterization of a subpopulation of freshly isolated epidermal Langerhans cells that reacts with anti-CD23 monoclonal antibody. *Br. J. Dermatol.* 124:533.
31. Ramb-Lindhauer, C., A. Feldmann, M. Rotte, and C. Neumann. 1991. Characterization of grass pollen reactive T-cell lines derived from lesional atopic skin. *Arch. Dermatol. Res.* 283:71.