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Relationship between facilitated allergen presentation and the presence of allergen-specific IgE in serum of atopic patients

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SUMMARY

Allergen presentation to allergen-specific T cells can be facilitated when IgE–allergen complexes are endocytosed by antigen-presenting cells (APC) after binding to the low-affinity FcεR type II (CD23). Here we present a study on the relative capabilities of sera of atopic patients to mediate facilitated antigen presentation (FAP). To this aim FAP was studied in an *in vitro* model in which CD23-expressing Epstein–Barr virus (EBV)-B cells act as APC to T lymphocyte clones (TLC) that are specific for *Der p* 2, a major allergen of housedust mite *Dermatophagoïdes pteronyssinus* (Dp). *Der p* 2 is immune-complexed by preincubation in sera from atopic patients, containing allergen-specific IgE. If EBV-B cells are preincubated with these complexes before using the cells as APC, the allergen-specific TLC proliferate at 100–1000-fold lower allergen concentration than required for T cell activation after presentation of uncomplexed allergen. The relative capability of various sera to mediate FAP was correlated with total serum IgE, and especially with *Der p* 2-specific serum IgE. In the model used, a high FAP capacity could be demonstrated only in sera with a total serum IgE concentration above approximately 2 μg/ml or with *Der p* 2-specific IgE above approximately 100 ng/ml. Maximal FAP, i.e. the ability to induce maximal proliferation of the TLC, was obtained in the presence of more than ± 600 ng *Der p* 2-specific IgE/ml. At 100–600 ng/ml *Der p* 2-specific IgE the level of FAP was correlated with the concentration of allergen-specific IgE, whereas at lower concentrations FAP was low or absent. All tested sera from eczema patients, all having serum anti-*Der p* 2-IgE concentrations > 600 ng/ml, showed a high FAP capacity, whereas all tested sera from atopic patients without eczema, which had serum anti-*Der p* 2-IgE levels < 600 ng/ml, showed no or a low FAP capacity. The association of high FAP capacity with eczema may reflect a functional role of FAP in the pathogenesis of atopic dermatitis.

Keywords atopy IgE facilitated allergen presentation

INTRODUCTION

Antigen presentation to T cells is highly facilitated by receptor-mediated endocytosis of antigen by antigen-presenting cells (APC). Facilitated antigen presentation (FAP) has been described *in vitro* for antigen-presenting B cells binding antigen via the B cell antigen receptor [1] and for non-B cell APC binding IgG-complexed antigen via FcγR [2]. In these cases FAP enables T cell activation at antigen concentrations that are 100–1000-fold lower than with fluid phase endocytosis of the antigen. Recently, antigen-specific IgE-mediated FAP was described, implying a more efficient trapping of IgE-complexed antigen by FcεRII (CD23)-expressing APC [3,4]. Elevated levels of allergen-specific serum IgE and increased numbers of CD23-expressing cells in atopic patients [5] suggest that such a FAP mechanism for the presentation of allergen to allergen-specific $CD4^+$ T cells in atopic disease may occur. We previously reported this for the presentation of *Der p* 2, a major allergen of housedust mite *Dermatophagoïdes pteronyssinus* (Dp) in an *in vitro* model [6]. Serum from *Der p* 2-allergic patients was preincubated with low doses of *Der p* 2 to allow the formation of IgE–allergen complexes. The complexed serum was subsequently incubated with $CD23^+$ APC to allow binding of immune complex. FAP, as a result of receptor-mediated endocytosis, could be demonstrated to occur by the capacity of these APC to induce proliferation in *Der p* 2-specific T lymphocyte clones (TLC), after preincubation with extremely low allergen concentrations. The occurrence of high levels of allergen-specific IgE in interstitial fluid and serum in atopic patients implies that allergen-specific T cells may be activated already at very low doses of inhaled or ingested allergen.

Data from literature indicate that atopic patients without dermatoses, e.g. allergic rhinitis or allergic bronchial

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asthma patients, have serum IgE levels below approximately 1 μg/ml, whereas atopic patients with a manifestation of eczema have elevated serum IgE concentrations higher than 1 μg/ml [7]. Furthermore, Dp is an important allergen in atopic dermatitis patients [8]. In this study we investigated the relationship between FAP and the presence of allergen-specific IgE in sera derived from atopic patients with variously elevated levels of serum IgE and with special reference to the capacity of facilitated presentation of *Der p* 2 using sera from atopic patients with and without dermatitis.

PATIENTS AND METHODS

T cell clones and APC

TLC PBA-1 expresses $CD4^+$ and recognizes peptide 20–33 of *Der p* 2. TLC JBA2 recognizes peptide 116–129 of *Der p* 2. As APC, autologous or MHC class II-matched Epstein–Barr virus (EBV)-B cells were used. The expression of CD23 by EBV-B cells varied between 60% and 80%.

Culture medium and cell culturing

TLC were cultured in Iscove's modified Dulbecco's medium (IMDM) (GIBCO, Paisley, UK), supplemented with rHuIL-2 (10 U/ml; Cetus, Emeryville, CA) and 10% C-inactivated normal human serum (CLB, Amsterdam, The Netherlands), containing no detectable levels of IgE. EBV-transformed B cell lines were maintained in IMDM supplemented with 10% bovine calf serum (HyClone, Logan, UT). All cultures were performed at 37°C in a humidified atmosphere containing 5% CO_2.

Sera and RAST

Sera were obtained from healthy volunteers and from atopic patients suffering from atopic dermatitis or allergic asthma. Sera were acquired from the Central Laboratory Blood Transfusion Service (CLB, Dr G. Hakkaart) and the Department of Dermatology AMC Amsterdam (Dr M. de Rie). Dp-specific radio-allergosorbent tests (RASTs) and levels of total and *Der p* 2-specific IgE were measured by Dr S. Stapel (CLB). Levels of *Der p* 2-specific IgE were expressed as the percentage of the total serum IgE binding to solid-phase-coupled *Der p* 2.

Allergen

Der p 2 was used unpurified at 10% w/v protein extract of lyophilized Dp, containing 0·32 mg/ml *Der p* 2. The extract was a kind gift of HAL (Haarlem, The Netherlands).

T cell proliferation assays

In the standard T cell proliferation assay, 4×10^4 TLC cells were cultured in 200 μl culture medium with 1×10^4 irradiated (30 Gy) autologous EBV-B in the presence of 4 μg to 0·4 ng/ml *Der p* 2. FAP was studied with EBV-B cells that were preincubated with immunoglobulin-complexed allergen. Cells were cultured for 40 h in 96-well flat-bottomed culture plates (Costar, Cambridge, MA), the last 16 h in the presence of 0·3 μCi/well of ^{3}H-TdR (Radiochemical Centre, Amersham, UK). Incorporation of ^{3}H-TdR was determined by liquid scintillation spectroscopy and expressed as mean ct/min of triplicate cultures. Standard deviations were in all cases less than 15%.

Demonstration of FAP

To demonstrate FAP, sera of atopic patients and non-atopic control individuals were first incubated for 1 h at 37°C with allergen in serial dilutions. Subsequently, EBV-B cells were added at a concentration of 3×10^5 cells/ml to allergen-complexed serum and incubated for 1 h at 4°C or 37°C. After incubation, the cells were washed three times in Hanks' balanced salt solution (HBSS) with 2% bovine calf serum (HyClone), irradiated (30 Gy), and washed again. To determine the relative FAP capacity, sera in serial dilutions were preincubated with a low *Der p* 2 concentration (10 ng/ml), sufficient only for T cell proliferation under FAP conditions. After incubating EBV-B cells with the allergen-preincubated serum, cells were extensively washed and added to TLC. The FAP capacity of serum sample was arbitrarily defined as the serum concentration which, after this procedure, resulted in a proliferation of the T cells as high as 25% of the maximum proliferation (QMP) as achieved in the standard T cell proliferation assay in the presence of 4 μg/ml *Der p* 2, the optimal concentration without preincubation.

RESULTS

Relation between FAP effect and RAST values for Dp

The ability of sera from *Der p* 2-allergic patients to facilitate allergen presentation was studied in an *in vitro* model, using *Der p* 2-specific TLC PBA-1 and autologous CD23 expressing EBV-B cells as APC. Varying concentrations of *Der p* 2 were incubated with 1 : 2 diluted sera, derived from several allergic patients with different Dp-specific RAST values, to allow complex formation with *Der p* 2-specific IgE. As a control, sera from non-allergic donors were used. After preincubation with 20 different sera, a high FAP capacity could be demonstrated with all sera from patients with a Dp-RAST of 3 or more, although the FAP level could vary considerably. FAP was low or absent in sera with lower RAST classification and sera from control donors (Fig. 1).

An arbitrary parameter was introduced to quantify the FAP capacity. To this aim, serially diluted serum was preincubated with a fixed amount of *Der p* 2 dilution, requiring FAP to activate the TLC. The FAP capacity was expressed as the concentration of serum able to induce QMP as obtained with an optimal allergen concentration present in the wells (Fig. 2).

Correlation between Der p *2-specific IgE and FAP*

In a test with a large panel of sera, sera with a RAST of 4 and 5 showed a wide range of FAP capacities. Sera with lower RAST classifications, showing low or no FAP, scored negative in this assay (data not shown). We subsequently tried to explain the variance of the FAP capacity of sera from patients by the occurrence of different concentrations of allergen-specific IgE in these sera.

A weak correlation was found between the serum concentration resulting in QMP and the total serum IgE levels (Fig. 3a). The curvefit coefficient R-squared was 0·6 (R-squared = 1 indicates a perfect fit). The experimental model suggests a threshold of IgE concentration at approximately 2 μg/ml required for maximal FAP, whereas the FAP capacity below this level was variable. A better linear correlation and more clear thresholds, however, were found

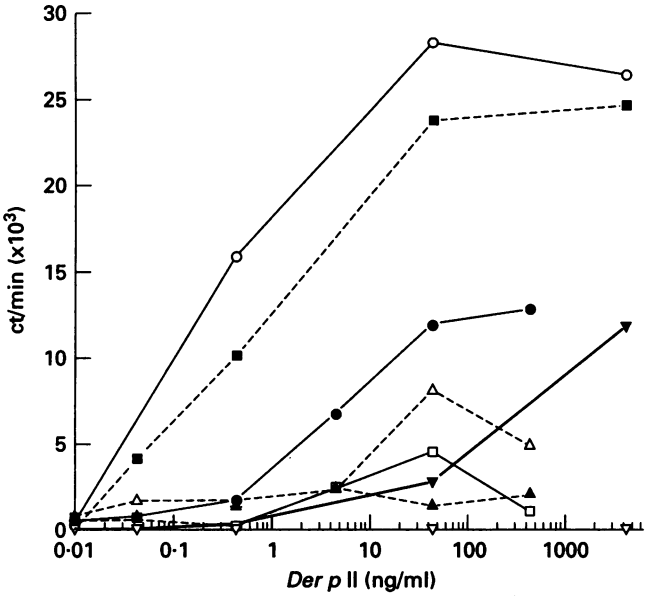

Fig. 1. Representative experiment showing facilitated antigen presentation (FAP) to *Der p* 2-specific T lymphocyte clone (TLC) PBA1 after preincubating *Der p* 2 with atopic sera and Epstein–Barr virus (EBV)-B cells. Serum was incubated with *Der p* 2 in serial dilution for 1 h at 37°C. After 1 h, EBV-B cells were added and incubated for 1 h at 4°C. After washing, EBV-B cells were added to TLC. Atopic sera, with Dp-RAST 5 (■), 4 (○, □, ●), 3 (△), 2 (▲), and normal control serum (▽). ▼, Proliferation without preincubation.

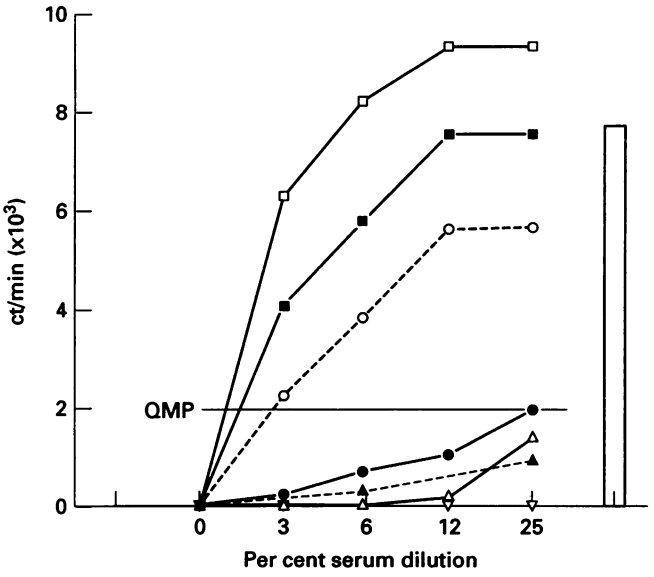

Fig. 2. Quantification of the facilitated antigen presentation (FAP) capacity of sera using the percentage serum giving rise to a quarter of the maximal proliferation (QMP, horizontal line) as obtained after incubation without FAP conditions. FAP was established after preincubating Epstein–Barr virus (EBV)-B cells in allergen-incubated sera in a serial dilution. Atopic sera with Dp RAST 5 (○), 4 (●, □, ■), 3 (△), 2 (▲) and normal control serum (▽).

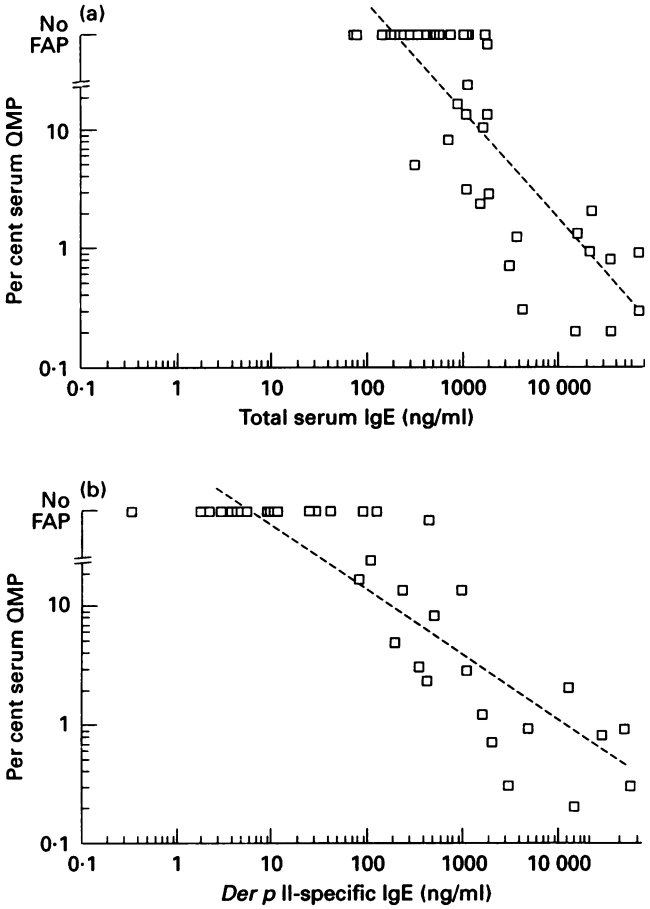

Fig. 3. Percentage of serum needed during preincubation to obtain a quarter of the maximal proliferation (QMP) in relation with the total serum IgE concentration (a) and with the *Der p* 2-specific IgE concentration (b).

when the FAP capacity of the sera was related to the contents of *Der p* 2-specific IgE (Fig. 3b). The curvefit coefficient was 0·8. Above the *Der p* 2-specific IgE concentration of ±600 ng/ml (250 U) the FAP effect is maximal (QMP < 2%) irrespective of the concentration of *Der p* 2-specific IgE. Below the concentration of 600 ng/ml allergen-specific IgE there is a small concentration range of 100–600 ng/ml with highly variable QMP values, all higher than 2. Allergen-specific IgE concentrations below this range elicit no or, at high serum concentrations, a very low FAP effect that is registered as negative in this assay. Similar observations were made using the T cell clone JBA-2 (data not shown) that recognizes a different peptide of *Der p* 2, indicating that the FAP capacity is not associated with T cell epitope specificity.

FAP capacity of sera from patients with and without atopic dermatitis

Atopic dermatitis (AD) is associated with high serum IgE levels and high RAST values, indicating high levels of allergen-specific IgE [9]. To investigate whether in the experimental model AD sera have a higher FAP capacity than non-AD sera we separated the QMP values of sera from patients with eczema from those of sera from atopic asthma patients without dermatitis. In Fig. 4 it is shown that all tested sera from the

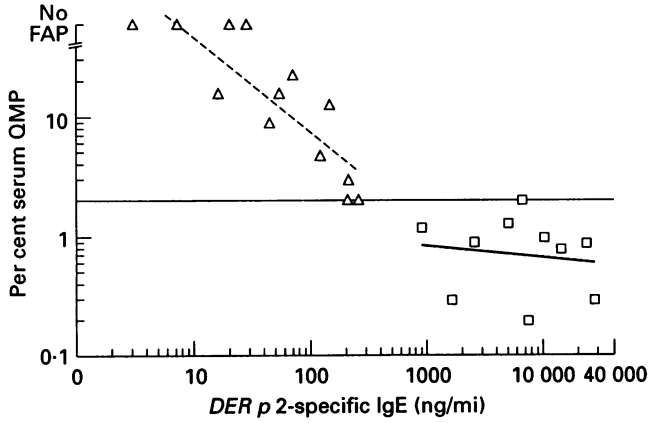

Fig. 4. Percentage of serum needed during preincubation to obtain a quarter of the maximal proliferation (QMP) in relation to the existence of dermatitis. Atopic patients without (△) or with dermatitis (□). The horizontal line indicates a QMP level of 2% serum.

AD patients showed a high, maximal FAP capacity with QMP values of less than 2. The sera from the non-AD patients all showed lower FAP capacities, in accordance with the fact that these atopic asthma patients without eczema all had *Der p* 2-specific IgE serum concentrations below 600 ng/ml.

DISCUSSION

In the present study facilitated presentation of allergen by sera from patients with various RAST values was investigated in an *in vitro* model. As most allergic atopic patients are hyper-reactive to housedust mite allergens [10,11], we focused on FAP by IgE against *Der p* 2, a major allergen of Dp. Strong FAP of *Der p* 2 was only found with sera from patients with Dp-specific RAST values of 4 and 5. The FAP capacity of the sera was compared using an arbitrary parameter, i.e. the serum dilution which, after preincubation with allergen, resulted in the same proliferation of allergen-specific TLC as 25% of the maximal proliferation obtained without FAP conditions, but with an optimal allergen concentration present in the well during culture. This 25% proliferation level was chosen, because below the 25% level variances in reactivity of TLC during culture and small non-specific differences in the sera could obscure FAP effects, whereas above this level the FAP capacity turned out to be consistently high. The variance of FAP capacity within one RAST classification appeared to be explained by the variance of allergen-specific IgE present in the sera. The experimental model revealed a clear correlation between serum concentration of 100–600 ng/ml *Der p* 2-specific IgE and the FAP capacity. A limited study suggests a relation between maximal FAP and the occurrence of AD. Sera from atopic patients with active dermatitis all contained *Der p* 2-specific IgE levels above 600 ng/ml and thus all showed maximal FAP in this assay, whereas sera from atopic patients without dermatitis contained *Der p* 2-specific serum IgE levels lower than 600 ng/ml and had lower or no IgE-mediated FAP capacities. Indeed, the occurrence of AD is associated with high (>1 μg/ml) IgE serum levels [7,12,13]. However, high IgE levels are not associated with dermatitis regularly, e.g. in Schistosomiasis. Patients with atopic respiratory diseases, without dermatitis, have much lower IgE serum levels. Most atopic patients from this study also showed allergic relations to other proteins such as grasspollen, pet animals, etc., and it should be taken into account that in patients without dermatitis maximal FAP might occur with other allergens. There is no consensus on the correlation between the severity of dermatitis and the total serum IgE level [7,14], but there is some evidence for a relation between the occurrence of eczema and high *Der p* 2-specific IgE concentration [15]. Results of our study indicate that for this particular allergen, *Der p* 2, there might be a relation between FAP of *Der p* 2 and atopic dermatitis. In our panel of patients, the percentage of *Der p* 2-specific IgE is about three times higher in AD than in atopic asthmatic patients, and even though in some atopic asthmatic patients FAP for the *Der p* 2-specific response exists, it is possible that activation of allergen-specific T cells, resulting in inflammation, is unsufficient. In patients with allergic rhinitis, who have low elevated serum IgE, no relation between severity of disease and titres of serum allergen-specific IgE was found [16].

The present study was focused on the relationship between FAP of *Der p* 2 and atopic allergy. The association between maximal FAP and AD may be causal. We [17] and others [18] have demonstrated that in active eczematous skin areas allergen-specific T cells are accumulated. Atopic dermatitis probably develops upon the allergen-specific activation of these T cells, and it may be hypothesized that FAP plays a role herein. Thus, due to FAP, the activation of one or more allergen-specific dermal T cells may be expected at extremely low allergen protein concentrations, provided CD23-expressing APC and a concentration of more than 600 ng/ml of allergen-specific IgE are present.

Besides CD23-mediated FAP, other forms of FAP may be expected to occur, such as FAP mediated by high affinity FcεR as expressed by dendritic cells (Langerhans cells) [19,20] and monocytes/macrophages and FAP mediated by FcγR types. The relative contributions of each of these types of FAP are unclear at the moment, and the role of FcεRI and IgG-mediated FAP in atopy is under investigation now.

The data on IgE-mediated FAP fit well in the hypothesis that IgE is potentiating allergen-specific responses by its special role in antigen capture, as postulated by Mudde *et al.* [21] and experimentally confirmed by us recently [6]. The present study suggests a relation between strong FAP and the occurrence of AD, and a possible role of FAP in the onset and maintenance of inflammation in skin was discussed. Definitive conclusions concerning the correlation between CD23-mediated FAP and the development of eczematous skin require more extended panels of sera and further study of the relative roles of other types of FAP.

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