

Isotypic analysis of grass pollen-specific antibodies in human plasma. 4. Biological activity of allergen-specific and autoanti-IgE antibody fractions on basophil histamine release

T. BATARD*†, A. WEYER†, A. LAROZE*, M.-R. ICKOVIC†, B. DAVID†, G. PELTRE† and B. BASUYAUX*

*Recherche et Développement, Laboratoire Français du Fractionnement et des Biotechnologies (LFB), Les Ulis and

†Unité d'Immuno-Allergie, Institut Pasteur, Paris, France

Summary

Background Blocking antibodies are defined as antibodies that compete with IgE for binding to allergens due to their specificity for those allergens. Thus, they may inhibit allergen-induced basophil and mast cell IgE-dependent mediator release both *in vivo* and *in vitro*.

Objective The present study was designed to evaluate the ability of antibodies isolated from human plasma samples on a *Dactylis glomerata* (Cocksfoot) pollen affinity-column to inhibit the *Dactylis* pollen-induced histamine release from human basophils (BHR) *in vitro*.

Methods Antibodies from Ig pools containing either high or low IgG4 anti-*Dactylis* pollen were purified on a *Dactylis* pollen affinity-column and then separated on an anti-human IgE column. Obtained Ig fractions were incubated for 30 min with *Dactylis* pollen allergens prior to incubation with basophils from *Dactylis* pollen-allergic donors. Cell supernatants were assessed for histamine content and the inhibition of BHR was calculated.

Results Unlike control non-isolated Igs, the antibodies isolated on the *Dactylis* pollen column were able to inhibit efficiently and in a dose-dependent manner *Dactylis* pollen-induced BHR. The inhibitory activity was increased in isolated antibody samples that had high IgG4 levels. Antibodies isolated on the *Dactylis* pollen column, however, consisted not only of true allergen-specific (potentially blocking) antibodies but also of autoanti-IgE binding to allergen-specific IgE and mistaken for allergen-specific antibodies, thus opening to question the involvement of the true allergen-specific antibodies in the BHR-inhibitory activity. Unlike the true allergen-specific antibodies, the autoanti-IgE were retained on and eluted from the anti-IgE column. Results showed that both the autoanti-IgE-depleted and the autoanti-IgE-containing fractions accounted for the inhibition observed with the related non-depleted sample that had been isolated on the *Dactylis* pollen column.

Conclusion For the first time, the true blocking activity of allergen-specific antibodies is demonstrated, that is, in the absence of the autoanti-IgE which can also inhibit BHR.

Keywords: basophil histamine release, grass pollen-specific antibodies, anti-IgE autoantibodies, blocking antibodies

Clinical and Experimental Allergy, Vol. 26, pp. 1308–1315. Submitted 8 September 1995; revised 15 February 1996; accepted 18 March 1996.

Introduction

The mechanisms by which specific allergen immunotherapy leads to the improvement of allergic symptoms are still

Correspondence: Dr B. Basuyaux, Recherche et Développement, Laboratoire Français du Fractionnement et des Biotechnologies, 3 avenue des Tropiques BP305, F-91958 Les Ulis Cedex, France.

unclear. Sixty years ago, Cooke *et al.* introduced the concept 'of a peculiar blocking or inhibiting type of antibody that prevented the action of allergen on the sensitizing antibody', that 'developed under treatment', and that 'appeared to be specific' [1] (our emphasis). Thus, due to their specificity for allergens, blocking antibodies can compete with IgE for allergen binding. IgE/IgG competition can result in decreased allergen-dependent cross-linking of basophil and mast cell-bound IgE and therefore lead to inhibition of mediator release from those cells.

Since then, many groups have searched for antibodies directed towards IgE-binding allergens, that could potentially have blocking activity on allergen-induced mast cell and basophil histamine release (BHR). According to a number of studies, potentially blocking antibodies are found mainly in the IgG4 subclass, although IgG1 might also be blocking (reviewed in [2]). Recently, we demonstrated that not only IgG4 and IgG1 but also IgA2 antibodies may have blocking activity due to their specificity for IgE-binding components of *Dactylis glomerata* pollen [3]. It has been shown that the inhibitory activity of human antibodies on antigen-induced BHR *in vitro* was due mainly to IgG4 [2,4] and to a lesser extent to IgG1 [2], both antibodies apparently being specific for the BHR-inducing antigens since they were retained by a gel to which those antigens were coupled. The exclusive role of allergen-specific antibodies in this inhibitory activity has become uncertain, however. Indeed, it has been demonstrated that autoantibodies to IgE (autoanti-IgE), which occur in normal and atopic human serum (reviewed in [5]), can be mistaken for allergen-specific antibodies since they bind to allergen-specific IgE [6]. Moreover, it has been shown recently that autoanti-IgE might also modulate allergen-induced BHR [7], therefore opening to question the whole blocking antibody concept. In this respect, and consistent with the findings of Jensen-Jarolim *et al.* [6], we have shown that Igs retained on a *Dactylis* pollen column consisted not only of antibodies strictly specific for *Dactylis* pollen antigens but also of autoanti-IgE [8]. Importantly, a very significant part of autoanti-IgE is of the IgG1 and IgG4 isotypes [5,8–12].

In order to appreciate the true nature of blocking activity, the present work was undertaken to determine the BHR-inhibiting activity of true allergen-specific and autoanti-IgE antibodies. First, antibodies isolated on a *Dactylis glomerata* (Cocksfoot) pollen affinity column [8] were studied using the *in vitro* human BHR model [13]. Antibodies were preincubated with *Dactylis* pollen allergens prior to incubation with the basophils to evaluate their ability to inhibit the subsequent allergen-induced BHR. Then, antibody fractions from which autoanti-IgE had been depleted were studied. Since, to our knowledge, the BHR-inhibitory activity of autoanti-IgE had not been investigated previously, we

also studied the autoanti-IgE-containing fractions in the same system.

Materials and methods

Pollen extract

The grass pollen extract used in this study was made using a previously described procedure [14]. Briefly, 50 mg of *Dactylis glomerata* pollen obtained from Institut Pasteur (Paris, France) were incubated with 450 μ L of deionized water for 1 h at room temperature, with constant agitation on a roller. The mixture was then centrifuged for 5 min at 9000 *g*. The protein concentration of the supernatant, hereafter called 'pollen extract', was 10 mg/mL according to a Lowry assay [15] using BSA as a standard. The *Dactylis* pollen extract has been characterized in a two-dimensional gel electrophoretic analysis [16].

Ig sources

Twenty-six plasma specimens previously selected on the basis of their having high levels of *Dactylis* pollen-specific IgG4, as detected by an ELISA, and referred to as 'IgG4⁺ plasma' [3] were pooled. They were mainly from desensitized grass pollen-allergic patients [3,17].

IgGAM [18] (BioTransfusion, Villepinte, France) is an Ig solution that had been prepared by ethanol fractionation of over 10 000 plasma samples according to Deutsch *et al.* [19] and Nitschmann and Kistler [20,21], as modified by Steinbuch *et al.* [22], and corresponds to the subfraction #3 of this fractionation. Most of the starting plasma samples were obtained from non-atopic subjects and therefore contained low levels of IgG4 anti-*Dactylis* pollen allergens [3,17]. IgGAM contained over 95% Ig [18]. Isotypic compositions of pooled IgG4⁺ plasma and IgGAM, as calculated from previously obtained data [8], are shown in Tables 1 and 2.

A control sample consisting of an intramuscular-injectable normal IgG preparation (Polygamma, Gamma TS, France) had been produced by the ethanol fractionation procedure described above, where it corresponds to the subfraction #2 [19–22]. It contained over 95% IgG. We used this pure Ig solution as a control, and not plasma samples, since the latter might contain either histamine releasing (HRF) or inhibiting (HIF) non-Ig factors [23].

Dactylis pollen and anti-IgE affinity-chromatography

Three hundred and fifty millilitres of pooled IgG4⁺ plasma and, in another experiment, 630 mL of IgGAM were passed over 25 mL of packed CNBr Sepharose 4B gel (Pharmacia, Sweden) coupled to 2.8 mg/mL of *Dactylis* pollen extract

Table 1. Distribution of Ig isotypes in antibody fractions isolated successively on a *Dactylis* pollen column and then on an anti-IgE column from a pool of 26 plasma samples with high levels of IgG4 anti-*Dactylis* pollen, mainly from desensitized grass pollen-allergic subjects (IgG4⁺ plasma)

	<i>Dactylis</i> pollen column		Anti-IgE column	
	Sample %	Eluate %	Flow-through %	Eluate %
IgG	74	62*	65†	67‡
IgA	13	8	6	8
IgM	13	30	29	24
IgE	0.01	0.6	0.001	1

*31% IgG1, 38% IgG2, 3% IgG3, 25% IgG4. †40% IgG1, 31% IgG2, 3% IgG3, 26% IgG4. ‡28% IgG1, 46% IgG2, 3% IgG3, 24% IgG4.

proteins, as previously described [24]. After extensive washing, elution was performed with 0.1 M citric acid/glycine buffer, pH 2.5, leading to the complete removal of the bound antibodies [24]. Eluted antibodies were immediately neutralized with solid Tris(hydroxymethyl)-aminomethane (Merck, Germany), concentrated six times and then dialysed against 0.1 M phosphate buffer pH 7.5 for 16 h at 4°C. They were >95% pure (data not shown). Eluted antibodies were then passed at 14 mL/h through 5.5 mL of CNBr Sepharose gel coupled to 2.5 mg/mL of goat polyclonal anti-human IgE Fc (Nordic Immunology, Norway) as previously described. The immunosorbent was washed and retained antibodies were eluted and neutralized as described above. In order to control the specificity of the anti-IgE column, a human Ig solution with no detectable IgE (Allergam, Gamma TS, France), obtained by the fractionation procedure described above from subfractions #1, #2, #3 [M. Steinbuch, pers. comm.], was passed over the column. None of the Igs bound to the immunosorbent (data not shown). Isotypic compositions of chromatographic fractions from the two columns, as calculated from previously obtained data [8], are given in Tables 1 and 2.

Autoanti-IgE ELISA

An ELISA modified from Nawata *et al.* [25] was used to assess the presence of autoanti-IgE. Briefly, 96-well microtitre plates were coated with purified myeloma IgE (PS) and blocked with 1% (m/v) BSA in PBS–0.1% (v/v) Tween 20. Antibody samples were added to the wells and, after washing, bound autoanti-IgE were detected using a horseradish peroxidase-anti-human Ig conjugate.

Table 2. Distribution of Ig isotypes in antibody fractions isolated successively on a *Dactylis* pollen column and then on an anti-human IgE column from an Ig pool with low levels of IgG4 anti-*Dactylis* pollen, mainly from nonatopic subjects (IgGAM)

	<i>Dactylis</i> pollen column		Anti-IgE column	
	Sample %	Eluate %	Flow-through %	Eluate %
IgG	65	46*	44†	51‡
IgA	15	12	15	3
IgM	20	42	41	46
IgE	0.000	0.02	0	0.05

*36% IgG1, 59% IgG2, 0.4% IgG3, 5% IgG4. †46% IgG1, 48% IgG2, 0.6% IgG3, 6% IgG4. ‡17% IgG1, 81% IgG2, 0.1% IgG3, 2% IgG4.

Cells and BHR experiments

Leucocytes of 13 *Dactylis* pollen-allergic patients were isolated out of the grass pollen season, in order to avoid high spontaneous BHR [26]. The allergen dilution eliciting maximal BHR was determined for every donor and used subsequently for these *in vitro* experiments, conducted as follows. Briefly, leucocytes were isolated according to May *et al.* [27]. Next, 0.1 mL of a dilution of allergen alone, allergen preincubated (30 min, room temperature) with the antibody sample to be evaluated, or antibody sample alone, was mixed with 0.9 mL of cell suspension. Duplicate samples were incubated for 30 min at 37°C, with constant agitation. The tubes were then centrifuged (400 g, 10 min, 4°C), except for the samples used for the determination of total histamine content. A volume of 0.9 mL of supernatant (or cell suspension) was mixed with 0.1 mL of 4 N perchloric acid and, after centrifugation, the histamine content was quantified by an automatized fluorometric method [28], based on the original technique of Shore *et al.* [29]. The percentage BHR was calculated with the formula $(H - H_{0\%}) / (H_{100\%} - H_{0\%}) \times 100$, where H = mean of fluorometric readings for the experimental supernatants, $H_{0\%}$ = spontaneous BHR, and $H_{100\%}$ = total histamine content. Inhibition of BHR obtained with a given concentration of antibody sample was calculated with the following formula $[(\%_{All} - \%_{(All+Ac)}) / \%_{All}] \times 100$, where $\%_{All}$ = percentage BHR due to the pollen extract only, and $\%_{(All+Ac)}$ = percentage BHR due to pollen extract preincubated with the antibody fraction.

Statistics

Regression line equations and Spearman's rank correlation

coefficients were assessed using StatView II software (Abacus Concept Inc., USA).

Results

Control Ig

The biological activity of the control Ig was evaluated with leucocytes of five different allergic donors (results not shown). The control Ig itself induced from 0 up to 91% BHR (mean = 39%) when incubated at 0.5 $\mu\text{g}/\text{mL}$ with these cells. When the control Ig was preincubated with *Dactylis* pollen extract, slight inhibition of allergen-induced BHR rarely occurred, was less than 12% (range = 0–12%; mean = 5%) with an Ig (μg):pollen protein (ng) ratio ranging from 0.01–10, and was not dose-dependent.

Antibodies isolated on the *Dactylis* pollen affinity column

The antibody fraction isolated on the *Dactylis* pollen column from pooled IgG4⁺ plasma was first incubated alone, at concentrations ranging from 0.5 to 5 $\mu\text{g}/\text{mL}$, with leucocytes from six different donors. In no case was BHR observed (results not shown). On the other hand, when this antibody fraction was preincubated with *Dactylis* pollen extract, a dose-dependent inhibition of allergen-induced BHR was observed, as shown in Fig. 1. As shown in Fig. 2, obtained from

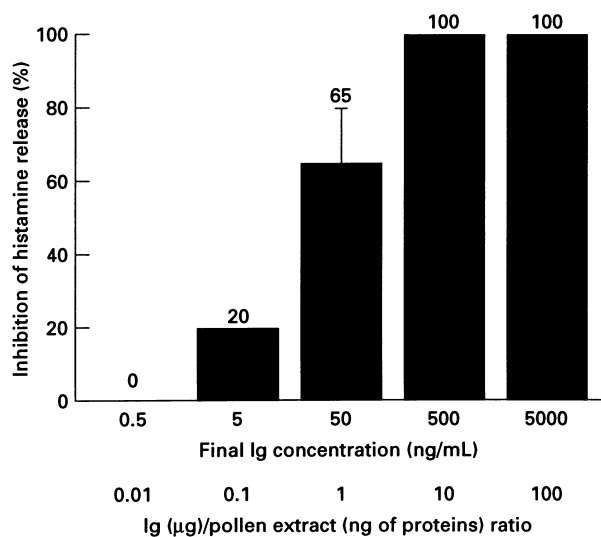


Fig. 1. Inhibition of *in vitro* BHR of a representative *Dactylis* pollen-allergic patient by antibodies isolated on a *Dactylis* pollen column from a pool of plasma samples with high levels of IgG4 anti-*Dactylis* pollen (IgG4⁺ plasma). Antibodies were preincubated at the indicated concentrations with a *Dactylis* pollen extract prior to incubation with the basophils.

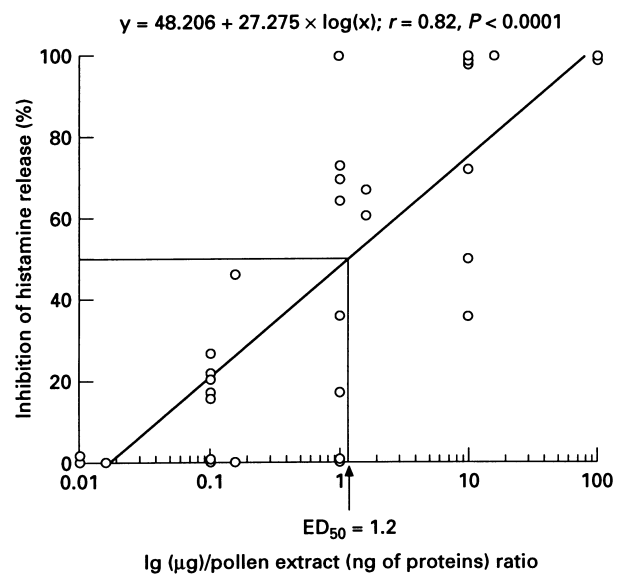


Fig. 2. Inhibition of *in vitro* BHR by antibodies isolated on a *Dactylis* pollen column from a pool of plasma samples with high levels of IgG4 anti-*Dactylis* pollen (IgG4⁺ plasma). Antibodies were preincubated at the indicated concentrations with a *Dactylis* pollen extract, prior to incubation with basophils of seven different *Dactylis* pollen-allergic patients (one experiment/patient). ED_{50} = average Ig (μg):pollen extract (ng of proteins) ratio eliciting 50% BHR-inhibition.

experiments performed with leucocytes from seven different patients, this inhibition could be total with an Ig (μg):pollen protein (ng) ratio as low as 1. For a given ratio, great variability in the inhibition rate, however, was observed from one leucocyte donor to another. On average, 50%-inhibition was observed at a ratio of 1.2 μg of Ig to 1 ng of pollen proteins.

The antibody sample isolated on the *Dactylis* pollen column from IgGAM was first incubated alone at concentrations ranging from 0.5 to 5 $\mu\text{g}/\text{mL}$ with leucocytes from four different donors. BHR was observed in only one case, the extent of which was 18%. When this antibody fraction was preincubated with *Dactylis* pollen extract, dose-dependent inhibition of allergen-induced BHR was observed, as shown in Fig. 3. However, as observed with antibodies isolated from the pooled IgG4⁺ plasma, great variability in the degree of inhibition was observed from one leucocyte donor to another (Fig. 4). On average, 50% inhibition was observed at a ratio of 35.8 μg of Ig to 1 ng of pollen proteins.

Antibody fractions of anti-IgE chromatography

Antibodies isolated on the *Dactylis* pollen column were compared with the antibodies that, among them, bound to and eluted from the anti-IgE column, and to the antibodies

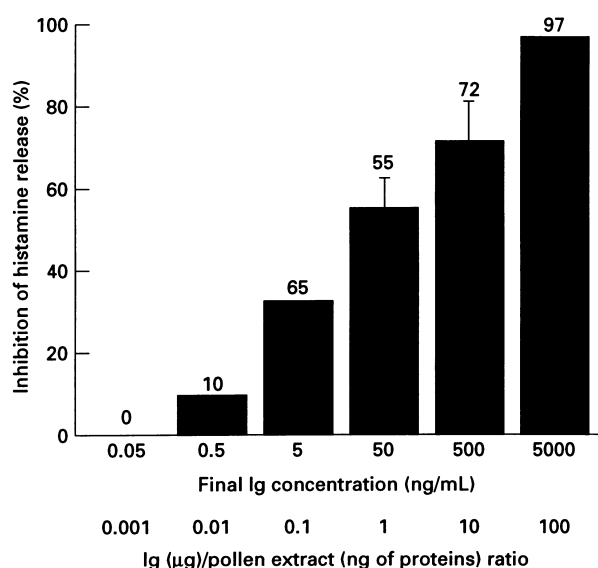


Fig. 3 Inhibition of *in vitro* BHR of a representative *Dactylis* pollen-allergic patient by antibodies isolated on a *Dactylis* pollen column from an Ig solution with low levels of IgG4 anti-*Dactylis* pollen (IgGAM; BioTransfusion, France). Antibodies were preincubated at the indicated concentrations with a *Dactylis* pollen extract prior to incubation with the basophils.

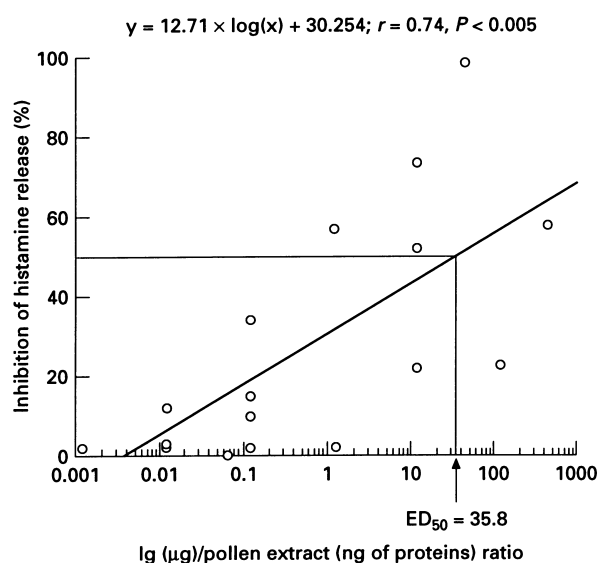


Fig. 4 Inhibition of *in vitro* BHR by antibodies isolated on a *Dactylis* pollen-column from an Ig solution with low levels of IgG4 anti-*Dactylis* pollen (IgGAM; BioTransfusion, France). Antibodies were preincubated at indicated concentrations with a *Dactylis* pollen extract prior to incubation with basophils of four different *Dactylis* pollen-allergic patients (one experiment/patient). ED₅₀ = average Ig (μg):pollen extract (ng of proteins) ratio eliciting 50% BHR-inhibition.

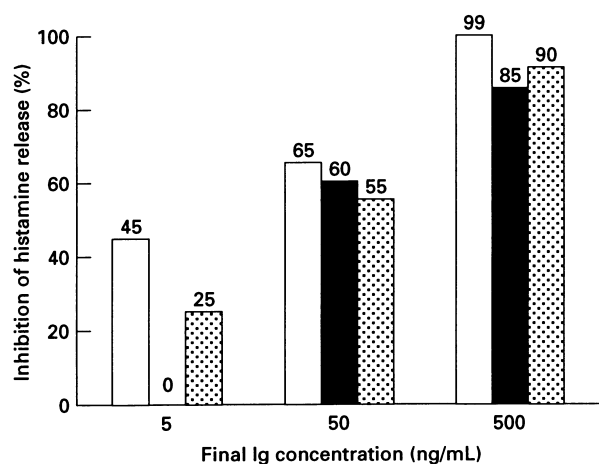


Fig. 5 Inhibition of *in vitro* BHR by: antibodies isolated on a *Dactylis* pollen-column from a pool of plasma samples with high levels of IgG4 anti-*Dactylis* pollen (IgG4⁺ plasma; □); antibodies isolated on the *Dactylis* pollen column that passed through and did not bind to an anti-human IgE column (flow-through; ■); antibodies isolated on the *Dactylis* pollen column that bound to and eluted from the anti-human IgE column (eluate; ▨). Antibodies were preincubated with a *Dactylis* pollen extract at the final concentration of 0.1 ng/mL prior to incubation with the basophils of a *Dactylis* pollen-allergic patient.

that passed through this column without binding (i.e. the flow-through). The latter contained no significant autoanti-IgE according to the autoanti-IgE ELISA (absorbance was <10 mDO, that is, on the order of the background level). Figure 5 is representative of the results obtained, in that all fractions tested inhibited BHR to a similar extent and in a dose-dependent manner.

Discussion

The control Ig, which was not passed through the *Dactylis* pollen column, itself induced up to 91% BHR. In contrast, antibodies isolated on the *Dactylis* pollen column did not induce much BHR (<20%) when incubated alone with the basophils at the same and even higher concentrations than the control Ig. Moreover, unlike the control Ig, they inhibited allergen-induced BHR effectively and in a dose-dependent manner. Thus, the *Dactylis* pollen affinity chromatography allowed basophil-inhibitory antibodies to be isolated in the eluate and appears to have eliminated basophil-stimulating antibodies in the flow-through. According to a recent report [30], at least part of the basophil-stimulating antibodies recovered in the flow-through of the *Dactylis* pollen affinity-column must be autoanti-idiotypic antibodies to grass pollen-specific IgE. Precisely, we have shown that the flow-through very probably contained autoanti-idiotypic antibodies, that

prevented some of the specific IgE from binding to the *Dactylis* pollen column (Batard et al., Allergy, in press).

When comparing the average Ig (μg):pollen protein (ng) ratios that elicited 50% inhibition, BHR-inhibiting activity was 30 times more efficient when the test antibodies were isolated from the Ig pool with high specific IgG4 (IgG4⁺ plasma pool) as compared with the Ig pool with low specific IgG4 (IgGAM). Interestingly, IgE was exactly 30 times more concentrated in the former than in the latter (see Tables 1 and 2). This might explain the 30-fold greater efficiency of the IgG4-enriched fraction in inhibiting allergen-induced BHR, provided that IgE from test antibodies that could compete with basophil membrane-bound IgE for binding to *Dactylis* pollen allergens themselves did not bind to the basophils. However, part of the inhibitory antibody samples was not retained on the anti-IgE-column, hence containing insignificant amount of IgE, but was still inhibitory and to the same extent as the inhibitory samples isolated on the *Dactylis* pollen column. This indicates that an isotype other than IgE was involved. IgG4 is the major candidate as it was the only isotype that displayed patterns of binding to *Dactylis* pollen antigens that paralleled those of specific IgE [3], a higher antibody concentration when isolated from IgG4⁺ plasma than when isolated from IgGAM, and a very similar proportion in the eluate of the *Dactylis* pollen-column and in the flow-through of the anti-IgE column (see Tables 1 and 2). This is further supported by previous works which indicate that IgG4, a priori specific for the IgE-binding antigens, is the main antibody involved in the inhibition of antigen-induced BHR [2,4].

It had been shown previously that antibodies, especially those of the IgG4 subclass, could act like and be mistaken for allergen-specific antibodies and that they are actually autoanti-IgE [5,6,8,10–12]. Others [6] and we (Batard et al., Allergy, in press) have shown that these autoanti-IgE appearing as *pseudo* allergen-specific antibodies were present in antibody samples that were retained on and eluted from allergen-immobilized columns. They might, at least in part, account for the inhibition of BHR observed with apparently specific antibodies [2,4]. The present study clearly shows that both the autoanti-IgE-depleted samples and the autoanti-IgE-containing samples accounted for the inhibitory activity of the related non-depleted samples. Therefore, the inhibition due to antibodies isolated on the *Dactylis* pollen column acted through at least two different mechanisms. One of the mechanisms can be considered as a truly blocking one, in that non-IgE antibodies strictly specific for *Dactylis* pollen allergens compete with IgE for binding to the allergens, thus decreasing allergen-induced BHR. As recently shown, it is not necessary that blocking antibodies bind to the same epitopes on the allergen molecules as IgE [31]. In this respect, it has been suggested that inhibition of BHR could be obtained by the co-aggregation

of Fc ϵ RI and Fc γ RII by an allergen molecule complexed to both a specific IgE and a specific IgG [32]. Such a specific IgG, albeit not directed towards the same epitope as the IgE, still meets the definition of 'blocking antibody' first given by Cooke *et al.* [1] (see Introduction).

It is the first time, to our knowledge, that a true blocking activity is clearly demonstrated, that is, in the absence of autoanti-IgE. In previous studies [2,4,33,34] autoanti-IgE which could also act as inhibiting antibodies [7] were not taken into account nor had they been removed from the true blocking antibodies, and they should not be considered as blocking antibodies since they are not specific for allergens. Therefore, they must act through different mechanism(s). They might prevent IgE from recognizing allergens either by binding to the paratope (anti-idiotypic antibodies) or by steric hindrance (anti-isotypic antibodies). The latter hypothesis is the more likely, as large Ig complexes the core of which is an IgE molecule can form [35] especially when IgE are immobilized [36], such as on the basophil membrane. Autoanti-IgE might also prevent basophils from releasing histamine by removing IgE from Fc ϵ receptors [37]. The mechanism(s) actually involved remain(s) to be elucidated. Nevertheless, IgG4 has been reported to be the main antibody involved in inhibition of allergen-dependent BHR, no matter if it was truly specific for the allergen or if it was an autoanti-IgE mistaken for allergen-specific antibody [2,4]. Therefore, BHR-inhibiting autoanti-IgE were very probably mainly of the IgG4 subclass. In this respect, it is noteworthy that among IgG autoanti-IgE, IgG4 was the subclass that complexed the most with IgE antibodies [11].

Attempts to obtain *in vitro* histamine release from basophils on challenge with affinity-purified or complexed autoanti-IgE were unsuccessful in almost all cases (reviewed in [38]). This indicates that autoanti-IgE is more likely to possess BHR-inhibiting activity [7]. Results we obtained support this hypothesis. Thus, although many roles for autoanti-IgE have been put forward, such as the possible regulation of IgE production at the B-cell level [37], they might act in a far more straightforward manner, i.e. directly on the effector cells of the allergic response. In this respect, it has been shown *in vitro* that anti-IgE can irreversibly desensitize rat mast cells [39] and human basophils [40] for all subsequent IgE-mediated histamine release. This suggests that those cells are both the main targets and the main actors in allergen immunotherapy. Indeed, basophils and mast cells are able to induce human IgE synthesis in B cells [41], and they may orientate helper T cells towards a 'TH₂-like' phenotype [42]. Therefore, 'deactivation' of those cells, as observed with autoanti-IgE or suboptimal doses of allergen [43–46], would first and foremost improve the allergic symptoms and then, at a later time, lead to decreased IgE production. The *in vivo*

relevance of the true blocking as well as autoanti-IgE has yet to be established.

Acknowledgements

The authors wish to thank Dr André Peyrouset and Philippe Adamowicz of the LFB, Les Ulis, France, for their constant interest and help. We also thank Françoise Marchand of Institut Pasteur, Paris, France, who assisted us in histamine release experiments. We are grateful to Professor Jean Bousquet and Professor David Levy for reviewing this manuscript.

References

- 1 Cooke RA, Barnard JH, Hebal S, Stull A. Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935; 62:733–51.
- 2 Hussain R, Poindexter RW, Ottesen EA. Control of allergic reactivity in human filariasis. Predominant localization of blocking antibody to the IgG4 subclass. *J Immunol* 1992; 148:2731–7.
- 3 Batard T, Basuyaux B, Lambin P et al. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma. 1. Specialization of certain classes and subclasses in the immune response. *Int Arch Allergy Immunol* 1993; 100:68–78.
- 4 Lambin P, Bouzoumou A, Murrieta M et al. Purification of human IgG4 subclass with allergen-specific blocking activity. *J Immunol Methods* 1993; 165:99–111.
- 5 Magnusson CGM, Johansson SGO. Clinical significance of anti-IgE autoantibodies and immune complexes containing IgE. *Clin Rev Allergy* 1989; 7:73–103.
- 6 Jensen-Jarolim E, Vogel M, de Weck AL, Stadler BM. Anti-IgE autoantibodies mistaken for specific IgG. *J Allergy Clin Immunol* 1992; 89:31–43.
- 7 Shakib F, Smith SJ. In vitro histamine-releasing activity of circulating IgG1 and IgG4 autoanti-IgE antibodies from asthma patients and the demonstration that anti-IgE modulates allergen-induced basophil activation. *Clin Exp Allergy* 1994; 24:270–5.
- 8 Batard T. Etude des anticorps humains anti-pollen de Graminée. PhD thesis, INA-PG, Paris, 1992:110–33.
- 9 Wilson PB, Fairfield JE, Beech N. Detection of IgG subclass-specific anti-IgE antibodies in normal and atopic individuals. *Int Arch Allergy Appl Immunol* 1987; 84:198–204.
- 10 Carini C, Fratazzi C. Detection of IgG subclasses with anti-IgE activity in patients with atopic diseases. *Int Arch Allergy Immunol* 1992; 98:227–32.
- 11 Shakib F, Mills CS, Powell-Richards A. The recognition of a recombinant human Fc ϵ fragment by the subclasses of IgG autoanti-IgE: disproportional subclass of complexed autoantibody. *Int Arch Allergy Immunol* 1992; 97:243–8.
- 12 Shakib F, Sihoe J, Smith SJ et al. Circulating levels of IgG1 and IgG4 anti-IgE antibodies and asthma severity. *Allergy* 1994; 49:192–5.
- 13 Weyer A, Dandeu JP, Marchand F, David B. In vitro histamine release from human basophils triggered by a purified allergen from *Dermatophagoides farinae*: bimodal aspect of the dose response curve. *Ann Immunol (Inst Pasteur)* 1982; 133D:87–94.
- 14 Peltre G, Lapeyre J, David B. Heterogeneity of grass pollen allergens (*Dactylis glomerata*) recognized by IgE antibodies in human patients sera by a new nitrocellulose immunoprint technique. *Immunol Lett* 1982; 5:127–31.
- 15 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193:265–75.
- 16 Brodard V, David B, Görg A, Peltre G. Two-dimensional gel electrophoretic analysis with immobilized pH gradients of *Dactylis glomerata* pollen allergens. *Int Arch Allergy Immunol* 1993; 102:72–80.
- 17 Lambin P, Debbia M, Bouzoumou A, Leynadier F. Estimation quantitative du taux des IgG4 anti-pollen de graminées par ELISA. *Rev Fr Allergol* 1991; 31:1.
- 18 Steinbuch M, Audran R, Pejaudier L, Blatrix C. Preparation of an IgM and IgA enriched fraction for clinical use. *Prep Biochem* 1973; 3:363–73.
- 19 Deutsch HF, Gosting LJ, Alberty RA, Williams W. Biophysical studies of blood plasma proteins. *J Biol Chem* 1946; 164:109–18.
- 20 Nitschmann H, Kistler P, Lergier W. Vereinfachtes Verfahren zur Gewinnung von humanem Albumin und g-Globin aus Blutplasma Mittels Alkoholfällung. *Helv Chim Acta* 1954; 107:866–73.
- 21 Kistler P, Nitschmann H. Large scale production of human plasma fractions. *Vox Sang* 1962; 7:414.
- 22 Steinbuch M, Audran R, Amouch P. The preparation of gamma-globulin for intravenous use in Paris. *Vox Sang* 1967; 13:103–6.
- 23 Grant JA, Alam R, Lett-Brown MA. Histamine-releasing factors and inhibitors: historical perspectives and possible implications in human illness. *J Allergy Clin Immunol* 1991; 88:683–93.
- 24 Batard T, Basuyaux B, Laroze A et al. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma. 2. Quantification of the IgE, IgM, IgA class and the IgG subclass antibodies. *Int Arch Allergy Immunol* 1993; 102:279–87.
- 25 Nawata Y, Koike T, Hosokawa H, Tomioka H, Yoshida S. Anti-IgE autoantibody in patients with atopic dermatitis. *J Immunol* 1985; 135:478–82.
- 26 Weyer A, Guinnepain MT, Sutra JP et al. Seasonal increase of spontaneous histamine release in washed leucocytes from rhinitis patients sensitive to grass pollen. *Clin Exp Immunol* 1990; 79:385–91.
- 27 May CD, Lyman M, Alberto R, Cheng J. Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J Allergy* 1970; 46:12–20.
- 28 Lebel B. A high-sampling-rate automated continuous-flow fluorometric technique for the analysis of nanogram levels of histamine in biological samples. *Anal Biochem* 1983; 133:16–29.
- 29 Shore PA, Burkhalter A, Cohn VH, Jr. Basophils histamine

- release. A method for the fluorometric assay of histamine in tissues. *J Pharmacol Exp Ther* 1959; 127:182–6.
- 30 Weyer A, Le Mao J, Etievant Met al. Human auto- anti-idiotypic antibodies to mite-specific IgE can degranulate human basophils in vitro. *Clin Exp Allergy* 1995; 25:935–41.
- 31 Van Ree R, Aalberse RC. Rabbit IgG directed to a synthetic C-terminal peptide of the major grass pollen allergen Lol pI inhibits human basophil histamine release induced by natural Lol p I. *Int Arch Allergy Immunol* 1995; 106:250–7.
- 32 Daëron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* 1995; 95:577–85.
- 33 Hofstetter M, Poindexter RW, Ruiz-Tiben E, Ottesen EA. Modulation of the host response in human schistosomiasis. III. Blocking antibodies specifically inhibit immediate hypersensitivity responses to parasite antigens. *Immunology* 1982; 46:777–85.
- 34 Lichtenstein LM, Norman PS, Winkenwerder WL. Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. *Am J Med* 1968; 44:514–24.
- 35 Shakib F. Aspects of the pathological significance of IgG4. *Exp Clin Immunogenet* 1987; 4:193–200.
- 36 Boluda L, Berrens L. Do IgE-IgG complexes occur in the circulation? *Clin Exp Immunol* 1995; 100:145–50.
- 37 Stadler BM. Anti-IgE autoantibodies: a possible specific feedback on the cytokine network in allergy? *Eur Cytokine Netw* 1992; 3:437–41.
- 38 Johansson SGO. Anti-IgE antibodies in human serum. *J Allergy Clin Immunol* 1986; 77:555–7.
- 39 Siraganian RP, Hazard KA. Mechanisms of mouse mast cell activation and inactivation for IgE-mediated histamine release. *J Immunol* 1979; 122:1719–25.
- 40 Mendoza G, Minagawa K. Subthreshold and suboptimal desensitization of human basophils. I. Kinetics of decay releasability. *Int Arch Allergy Immunol* 1982; 68:101–7.
- 41 Gauchat JF, Henchoz S, Mazzei G et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993; 365:340–3.
- 42 Frandji P, Oskéritzian C, Cacaraci F et al. Antigen-dependent stimulation by bone marrow-derived mast cells of MHC class II-restricted T cell hybridoma. *J Immunol* 1993; 151:6318–28.
- 43 Mendoza G, Minagawa K. Subthreshold and suboptimal desensitization of human basophils. II. Nonspecificity and irreversibility of desensitization. *Int Arch Allergy Appl Immunol* 1982; 69:282–4.
- 44 Siraganian RP, Hazard KA. Mechanisms of mouse mast cell activation and inactivation for IgE-mediated histamine release. *J Immunol* 1979; 122:1719–25.
- 45 Norn S, Stahl Skov P. Anaphylactic hyposensitization of rat mast cells in vitro by antigen. *Clin Exp Immunol* 1974; 18:431–7.
- 46 Shalit M, Levi-Schaffer F. Challenge of mast cells with increasing amounts of antigen induces desensitization. *Clin Exp Allergy* 1995; 25:896–902.