

Research Article

The effectiveness of different rat IgG subclasses as IgE-blocking antibodies in the rat basophil leukaemia cell model

JR PHILIPS,¹ W BROUWER,¹ M EDWARDS,¹ S MAHLER,² J RUHNO³ and AM COLLINS¹

¹School of Microbiology and Immunology and ²Department of Biotechnology, University of New South Wales and ³Royal North Shore Hospital, Sydney, New South Wales, Australia

Summary The degranulation of mast cells in an allergic response is initiated by the aggregation of high-affinity IgE receptors (FcεRI) by IgE and antigen. Recently it has been shown that such degranulation can be inhibited by cross-linking FcεRI and low-affinity IgG receptors (FcγRII) which are also expressed by mast cells. The ability of various monoclonal antibodies to block the degranulation of rat basophil leukaemia (RBL) cells sensitized with IgE antidinitrophenyl (DNP) antibodies has been investigated. Sensitized cells were challenged with immune complexes formed using varying concentrations of antigen, and of both high- and low-valency antigen. It is reported here that rat IgG1 antibodies, which are associated in the rat with a Th1-type response, act as highly effective blocking antibodies over a wide concentration range. Rat IgG2a antibodies, which are associated with a Th2-type response, were able only to inhibit degranulation when immune complexes were formed with very low concentrations of high-valency antigen (DNP₃₂-HSA). Under these conditions, some inhibitory activity was seen with high-affinity murine IgA anti-DNP but not with low-affinity rat IgG2b anti-DNP antibody-containing immune complexes. In addition to this inhibitory activity, IgG2a antibodies were shown to be capable of inducing degranulation of cells via unoccupied FcεRI. These results demonstrate that blocking activity may arise via both inhibitory receptors and by masking of antigen.

Key words: allergy, blocking antibody, epitope, IgE, mast cell, valency.

Introduction

The mast cell is recognized as being the cell at the heart of the allergic response, and the cross-linking of cell-surface IgE is the principal pathway to mast cell activation. Studies of antibody-mediated mast cell function have therefore focused mainly upon such cell-bound IgE. However, the function of mast cells *in vivo* is complicated by their residence in an immunoglobulin-rich environment.

Allergen-specific antibodies other than IgE antibodies are often described as blocking antibodies, for they have been thought to be capable of interfering with IgE-mediated functions. The mechanism of this inhibition has usually been ascribed to direct competition for antigen binding between cell-bound IgE antibodies, and IgG antibodies in solution.¹

More recently an alternative FcγR-mediated mechanism of inhibition of IgE activity has been described. Using mast cell-like rat basophil leukaemia (RBL) cells transfected with a number of different murine FcγR isoforms, Dæron *et al.* have shown that the cross-linking of the wild-type FcεRI and transfected murine FcγRIIb1 prevents IgE-mediated RBL cell degranulation.² These experiments are likely to be relevant to normal mast cell function in many species, because in addition to the high-affinity FcεRI, mast cells have been reported

to express a variety of Fcγ receptors, including FcγRIIb1. Mouse mast cells, for example, express three low-affinity receptors for complexed IgG: FcγRIIb1, FcγRIIb2 and FcγRIII.³ Similarly, RBL cells have been shown to express the rat FcγRIIb1 isoform,⁴ the FcγRIIb2 isoform,⁵ and a number of FcγRIII isoforms.⁶

The existence of FcγR-mediated inhibition does not preclude the possibility that inhibitory antibodies may also function by simple competition for antigen. In the present report we describe a further development of the RBL model for the *in vitro* study of mast cell function⁷ in the presence of both IgE and non-IgE antibodies. We report that the degranulation of RBL cells is strongly inhibited by antibodies of the IgG1 subclass. Inhibition was effective over a wide range of antigen/antibody concentrations. Other antibody isotypes were only able to inhibit degranulation when sensitized cells were challenged with immune complexes formed in overwhelming antibody excess, and with high-valency antigen. These results suggest that in addition to FcγR-mediated inhibition, in certain circumstances FcγR-independent mechanisms may be responsible for blocking antibody activity.

Materials and Methods

Preparation of antigens

A total of 100 mg ovalbumin (OVA; Sigma Chemical Co., St Louis, MO, USA) was dissolved in 1 mL PBS, and 10 μL 25% glutaraldehyde (Ajax, Auburn, NSW, Australia) was added. The solution was

Correspondence: Dr AM Collins, School of Microbiology and Immunology, University of New South Wales, Sydney, NSW 2052, Australia.

Received 1 September 1998; accepted 29 October 1998.

mixed for 2 h on a rotary mixer and then dialysed extensively against PBS.

Dinitrophenylated human serum albumin (HSA) was prepared by the addition of 2 μL dinitrofluorobenzene (DNFB; Sigma) to 1 mL of a 100 mg/mL solution of HSA (Sigma) in 1 mol/L NaHCO_3 . The solution was mixed on a rotary mixer for 45 min at room temperature, and then dialysed against PBS. The substitution ratio was determined spectrophotometrically as previously described.⁸ Highly substituted DNP-BSA was similarly prepared.

Production of antibodies

H-1 ϵ anti-DNP and OVA6B anti-OVA IgE monoclonal antibodies were raised as ascites, and antibody concentrations were quantified by RBL degranulation assay as previously described.⁹ MOPC-315 murine IgA was raised as ascites. Antibodies were purified by ion exclusion chromatography. Purity was confirmed by SDS-PAGE and antibody concentration was quantified by ELISA assay against an IgA standard.⁸ Polyclonal anti-DNP and anti-HSA antisera were raised by immunization of outbred Wistar rats with 100 μg DNP-BSA or 100 μg HSA in complete Freund's adjuvant. Animals were boosted after 14 days with antigen in incomplete Freund's adjuvant, and serum was collected at day 28. The DNP-1 (IgG1), DNP-16 (IgG2a) and DNP-11 (IgG2b) rat monoclonal anti-DNP antibodies were purchased from Biogenesis Ltd (Poole, UK).

Rat basophil leukaemia assays

Cells were cultured in Dulbecco Modified Eagle's Medium (DMEM, Sigma), containing 10% foetal bovine serum (Trace, Castle Hill, NSW, Australia), 1% L-glutamine (Trace), and 0.5% penicillin/streptomycin (Trace) in 75 cm^2 cell culture flasks (Greiner GmbH, Frickenhausen, Germany). The cells were incubated in a humidified 37°C 5% CO_2 incubator, harvested using Trypsin-Versene solution (Trace) and spun for 10 min at 200 g in a Beckman model TJ-6 centrifuge (Beckman, Palo Alto, CA, USA). Nunclon (Nunc, Roskilde, Denmark) sterile flat-bottomed 96-well cell culture plates were seeded with 5×10^4 cells/well in supplemented DMEM, also containing 2 μL ^3H -serotonin (hydroxytryptamine creatine sulphate, 1.1×10^{12} Bq/mmol, Dupont NEN, Boston, MA, USA) per mL of medium. The plates were incubated for 24 h in a humidified 37°C 5% CO_2 incubator. The medium was then discarded, and cells were sensitized with 100 μL /well anti-DNP or anti-OVA IgE (1 $\mu\text{g}/\text{mL}$) diluted in DMEM and incubated for 3 h. The medium was removed and 100 μL /well challenge solution was added. Challenge solutions included antigen alone (DNP-HSA), as well as immune complexes formed by the pre-incubation of antigen and various antibody preparations. Plates were then incubated for 30 min. Twenty- μL samples were removed from each well and placed in the corresponding well of a Wallac 96-well sample plate (Wallac Oy, Turku, Finland). The remaining medium in the Nunc cell culture plate was discarded and 100 μL /well 1% Triton X-100 (Sigma) was added to lyse cells and to disperse unreleased serotonin. A 20- μL sample of lysate was then removed from each well and placed in the corresponding well of a second Wallac sample plate. A total of 200 μL OptiPhase 'HiSafe' scintillant (Wallac) was then added to each well of the two plates. The plates were sealed and shaken gently for 15 min before reading with a 1450 Microbeta Plus liquid scintillation counter (Wallac Oy). The percentage of serotonin released upon antigen challenge was then calculated. Four-fold replicates were set up for each antibody-antigen combination, from which the means and standard errors were calculated. All plates included wells challenged with A23187 ionophore and anti-IgE antibodies as positive controls. Negative controls included unsensitized cells and sensitized cells challenged with medium alone.

ELISA assays

An ELISA was used to determine the saturating concentration of antibody for use in determinations of antibody affinity. Flat-bottomed 96-well PolySorp plates (Nunc) were coated overnight at 4°C with 100 μL /well 10 $\mu\text{g}/\text{mL}$ DNP₃₂-HSA (Sigma) diluted in carbonate coating buffer (pH 9.6). The plates were washed with 0.1% Tween 20/PBS and blocked for 1 h at 37°C with coating buffer containing 1% skim milk powder (SMP). After washing the plates, doubling dilutions of antibody were added to the plates and incubated for 1 h at 37°C. The plates were then washed and incubated for a further 45 min at 37°C with peroxidase-labelled goat anti-mouse or goat anti-rat IgG (Kierkegaard and Perry Laboratories, Gaithersburg, MD, USA), at a dilution of 1:1000 in diluting buffer (1% SMP/PBS/Tween 20). The plates were thoroughly washed with washing buffer and then distilled water, and finally incubated for 10 min at room temperature with 5'-5' tetramethylbenzidine (TMB; Sigma) substrate. The reaction was stopped using 2 mol/L H_2SO_4 and the optical density at 450 nm ($\text{OD}_{450\text{nm}}$) was measured using a BioRad 3550 Microplate reader (Bio-Rad, Hercules, CA, USA). Triplicate wells were set up for all antibody concentrations, and mean OD values for each sample were determined.

Thiocyanate elution assay

The affinity of antibodies for their specific antigen was determined by a modification of the aforementioned assay, in which plate-bound antibodies were incubated for 15 min at room temperature in the presence of chaotropic thiocyanate ions at differing molarities.⁸ Ammonium thiocyanate in 0.1 mol/L phosphate buffer, pH 6.0, was used at molarities ranging from 0 to 6.5 mol/L. The plates were then washed and the assay was then completed as described here. The affinity of the antibodies (the I_{50}) was calculated as the molar concentration of thiocyanate ions producing a 50% reduction in absorbance when compared with thiocyanate-free wells. A high antibody affinity is reflected by the high concentration of thiocyanate ions required to disrupt antigen-antibody binding.

Statistics

Results were analysed using the unpaired *t*-test, and statistical significance was accepted at the 5% level.

Results

Determination of antibody affinity

The affinities of the monoclonal antibodies (H-1 ϵ IgE, MOPC-315 IgA, DNP-1 IgG1, DNP-16 IgG2a, DNP-11 IgG2b) for the hapten DNP was determined by thiocyanate elution assay, and the thiocyanate I_{50} concentrations are presented in Table 1.

The murine H-1 ϵ IgE antibodies were shown to be of relatively low affinity, with an I_{50} of between 2 and 2.5 mol/L. MOPC-315, a mouse IgA anti-DNP antibody, had the highest affinity with an I_{50} of between 6 and 6.5 mol/L. Two rat IgG monoclonals, DNP-1 and DNP-16, were of moderate affinity while DNP-11 was of low affinity.

Inhibition with polyclonal antibodies

Initial experiments investigated the ability of polyclonal antibody preparations to inhibit degranulation of IgE-sensitized

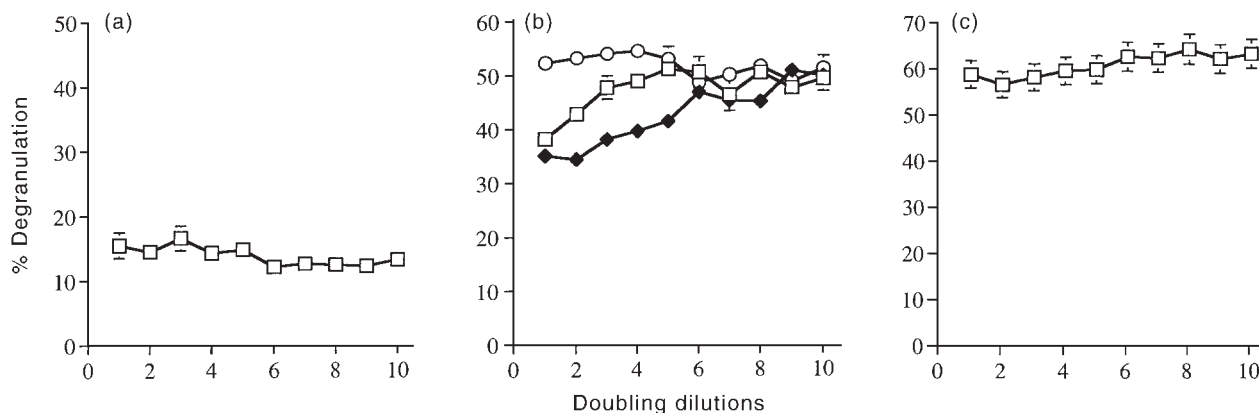


Figure 1 Inhibition of degranulation of anti-DNP IgE-sensitized cells by polyclonal antibody preparations. (a) Effect of the addition of normal rat serum (NRS) to unsensitized cells; (b) the effect of the addition of (○) NRS, (□) anti-DNP and (◆) anti-HSA polyclonal antibodies to sensitized cells. The addition of immune complexes, formed by the incubation of doubling dilutions of polyclonal anti-DNP anti-serum and 100 ng/mL DNP₈-HSA, in the presence of challenge-aggregated OVA (1 µg/mL) to IgE anti-OVA sensitized rat basophil leukaemia (RBL) cells is seen in (c). All error bars are the standard error of the mean. Error bars not visible in (a,b) are too small to be seen.

Table 1 Antibody affinities as determined by ammonium thiocyanate elution

Antibody	Species	Isotype	Thiocyanate I ₅₀
H-1ε	Mouse	IgE	2 < I ₅₀ < 2.5 mol/L
MOPC-315	Mouse	IgA	6 < I ₅₀ < 6.5 mol/L
DNP-1	Rat	IgG1	4 < I ₅₀ < 4.5 mol/L
DNP-16	Rat	IgG2a	2.5 < I ₅₀ < 3 mol/L
DNP-11	Rat	IgG2b	I ₅₀ < 1 mol/L

A high antibody affinity is reflected by a high thiocyanate I₅₀.

cells. The results are presented as Fig. 1. Neither antisera nor normal rat serum was able to induce degranulation of unsensitized cells (Fig. 1a). Antibodies directed against both hapten and carrier determinants complexed with DNP-HSA were tested on cells sensitized with anti-DNP IgE. Both antisera led to a significant inhibition of RBL cell degranulation (Fig. 1b). No such inhibition was seen when cells were challenged with antigen and normal rat serum.

To determine whether or not the inhibitory effect observed was FcγR mediated, we next investigated whether IgG-containing immune complexes were able to interfere with IgE directed against a second antigen. The RBL cells were sensitized with IgE anti-OVA antibodies, and challenged with aggregated OVA (1 µg/mL) in the presence of immune complexes formed by the incubation of DNP₈-HSA (100 ng/mL) with varying concentrations of rat anti-DNP antibodies. Doubling dilutions of polyclonal anti-DNP anti-serum were used from an initial dilution of 1:10. A representative assay is shown as Fig. 1(c). No inhibition was observed. A similar lack of inhibition was also seen when anti-DNP sensitized RBL cells were challenged with specific antigen in the presence of aggregated IgG or OVA/anti-OVA immune complexes (data not shown).

Inhibition with monoclonal antibodies

The ability of IgG anti-DNP antibodies of differing subclasses and of high-affinity murine IgA MOPC-315 anti-DNP anti-

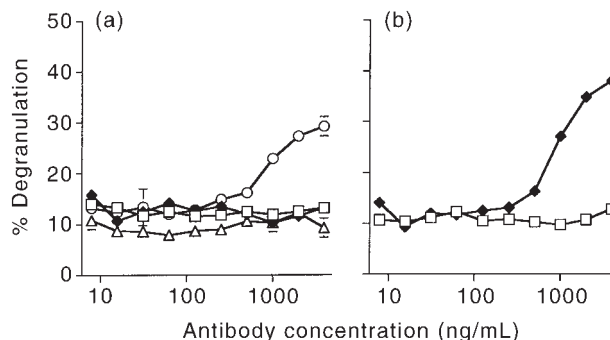


Figure 2 Addition of immune complexes to rat basophil leukaemia (RBL) cell cultures. In (a) the immune complexes were formed by the incubation of varying concentrations of rat (□) IgG1, (○) IgG2a, (△) IgG2b and (◆) mouse IgA anti-DNP antibodies and constant concentrations of DNP₈-HSA. Complexes were then added to unsensitized RBL cell cultures. (b) shows the addition of IgG2a-containing immune complexes of DNP₈-HSA to RBL cells (◆) unsensitized and (□) sensitized with irrelevant (anti-ovalbumin) IgE. All error bars are the standard error of the mean. Error bars not visible in (a,b) are too small to be seen.

bodies to induce cell degranulation was then investigated by challenging RBL cells with immune complexes. These immune complexes were formed by the pre-incubation of varying concentrations of rat IgG1, IgG2a or IgG2b, or MOPC 315 anti-DNP antibodies and constant concentrations of DNP₈-HSA. The immune complexes so formed were then added to cultures of RBL cells. The results showed that IgG2a-containing immune complexes were able to directly induce RBL cell degranulation (Fig. 2a). This degranulation appeared to be FcεRI-mediated, because no such degranulation occurred when RBL IgE receptors were first saturated with anti-OVA IgE (Fig. 2b).

The ability of the various antibodies to block IgE-mediated degranulation was then investigated by challenging H-1ε IgE anti-DNP sensitized RBL cells with immune complexes. A striking inhibition was seen in the case of DNP-1

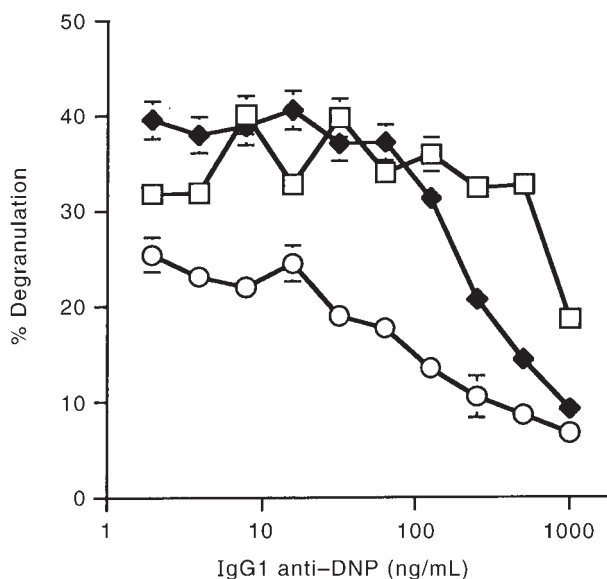


Figure 3 Challenge of H-1 ϵ IgE anti-DNP sensitized rat basophil leukaemia (RBL) cells with immune complexes formed with varying concentrations of DNP-1 IgG1 anti-DNP antibodies and (\square) 1000 ng/mL DNP₈-HSA, (\blacklozenge) 250 ng/mL DNP₈-HSA and (\circ) 100 ng/mL DNP₈-HSA. All error bars are the standard error of the mean. Error bars not visible are too small to be seen.

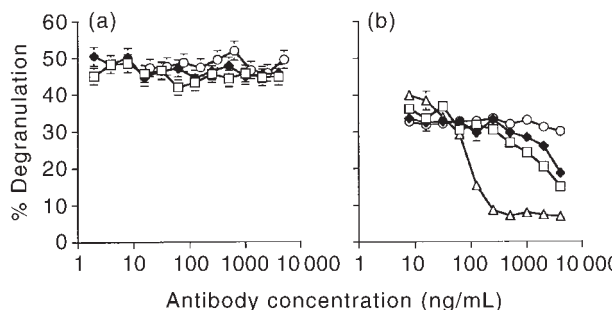


Figure 4 Challenge of H-1 ϵ IgE anti-DNP sensitized rat basophil leukaemia (RBL) cells with immune complexes containing (a) 250 ng/mL DNP₈-HSA and varying concentrations of anti-DNP rat (\square) IgG2a, (\blacklozenge) IgG2b and (\circ) mouse IgA antibodies; and (b) 1 ng/mL DNP₃₂-HSA and varying concentrations of anti-DNP (\square) mouse IgA, (\blacklozenge) rat IgG2a, (\circ) IgG2b and (\triangle) IgG1 antibodies. All error bars are the standard error of the mean. Error bars not visible in (a,b) are too small to be seen.

IgG1 anti-DNP antibodies (Fig. 3). Inhibition was particularly effective using immune complexes formed in the presence of lower concentrations (100 ng/mL) of DNP₈-HSA. Significant inhibition was seen when cells were challenged with antigen in the presence of just 32 ng/mL DNP-1, while 1000 ng/mL DNP-1 reduced degranulation to background levels. Immune complexes formed in the presence of the highest concentration of DNP₈-HSA (1000 ng/mL) were only inhibitory at the highest antibody concentrations tested. In the presence of 1000 ng/mL DNP-1, the percentage degranulation was reduced to 18.6%. Similar trends were seen when sensitized cells were challenged with immune complexes containing lower valency DNP₄-HSA (data not shown).

Neither rat IgG2a, rat IgG2b nor murine IgA antibodies were able to inhibit IgE-mediated degranulation over the same range of DNP₈-HSA concentrations. Figure 4(a) shows the results of experiments where sensitized cells were challenged with rat IgG-containing immune complexes or mouse IgA-containing immune complexes formed in the presence of 250 ng/mL DNP₈-HSA. No inhibition was seen. Similar results were obtained with antigen concentrations of 100 ng/mL and 1000 ng/mL (data not shown). However, significant inhibition was seen when sensitized cells were challenged with immune complexes formed from very low concentrations of high-valency DNP₃₂-HSA (1 ng/mL) and high concentrations of antibodies (Fig. 4b). Under these conditions, the high-affinity MOPC-315 murine IgA antibodies showed good levels of blocking activity, while the low-affinity IgG2b antibodies failed to significantly inhibit degranulation (Fig. 4b). IgG1 antibodies remained the most effective blocking antibodies.

Discussion

Desensitization therapy, in which allergic individuals are repeatedly challenged with very low doses of allergen, has been an effective therapy for allergies for many decades. The therapy was originally conceived at a time when the most basic aspects of the allergic reaction were unknown. Allergies were hypothesized to result from the action of environmental toxins. The success of vaccines targeting bacterial toxins led to the hope that appropriate exposure to allergens could similarly lead to the production of protective antibodies against allergy-producing toxins.¹⁰ Despite this misunderstanding, the therapy has proven successful, particularly in the treatment of insect sting anaphylaxis, allergic rhinitis and asthma.¹¹

Following the identification of IgE antibodies and of their role in the allergic reaction, desensitization therapy was shown to be accompanied by elevated allergen-specific IgG antibodies (reviewed in¹²). Such IgG antibodies have long been believed to function by virtue of their direct competition with cell-bound IgE for allergen binding.¹ *In vitro* experiments of Daeron *et al.* have recently challenged this view by demonstrating the ability of Fc γ R to inhibit IgE-mediated cell functions.² The low-affinity Fc γ RIIB isoform has an intracytoplasmic domain containing an immunoreceptor tyrosine-based inhibition motif (ITIM).¹³ Coaggregation of this receptor with Fc ϵ RI promotes the phosphorylation of Fc γ RIIB by Fc ϵ RI-associated lyn and the subsequent binding of the inhibitory inositol polyphosphate-5-phosphatase SHIP to the ITIM.¹⁴

The existence of such a regulatory mechanism may help explain the mechanism of allergen desensitization therapy, but it does not rule out the possibility that blocking antibodies could also function in a purely competitive fashion. The studies reported here were undertaken to address this issue.

The studies of Daeron *et al.* were conducted using RBL cell lines transfected with a number of murine Fc γ R constructs.² In our simpler model, we have demonstrated that IgG antibodies are capable of inhibiting IgE-mediated degranulation of untransfected RBL cells. The fact that anti-serum containing IgG anti-HSA antibodies was able to

influence anti-DNP IgE-mediated function, and that inhibition was seen only when IgG and IgE antibodies were both directed against a single antigen, suggest that the inhibition could result from the cross-linking of Fc γ R and Fc ϵ R.

We next examined blocking activity in the RBL model using a wide range of antigen-antibody concentrations and using monoclonal antibodies of different isotypes and affinities. By using carrier proteins substituted to varying degrees with hapten moieties, we were also able to model the consequences of antigen valency upon their activity.

IgG1 antibodies were shown to have the strongest blocking activity. Inhibition was seen with both low- and high-valency antigens, and was particularly evident at lower concentrations of challenge antigen, where more IgG is available to bind to each antigen molecule. It may be that this activity is a result of the higher affinity of rat IgG1 for Fc γ RIIb1, though the affinities of the rat IgG subclasses for this receptor have not been reported in the literature. Certainly among the human IgG subclasses, IgG1 antibodies bind with the highest affinity to Fc γ RIIb1.¹⁵

The inhibitory activity of other competing isotypes was only evident when immune complexes were formed using extremely low concentrations of very-high-valency antigen. The inhibition effected by murine IgA and rat IgG2a antibodies was seen only when using DNP₃₂-HSA (1 ng/mL) as the challenge antigen. In these conditions, where immune complexes were formed in conditions of substantial antibody excess, sufficient masking of antigenic determinants may have limited interactions between the immune complexes and cell-bound IgE. Such masking could equally occur with lower valency antigens, but low-valency antigens are unable to induce degranulation at low antigen concentrations.¹⁶ The failure of DNP-11 antibodies to similarly inhibit degranulation may be a reflection of the low affinity of these antibodies, and the possibility that higher affinity antibodies of the rat IgG2b isotype could function as blocking antibodies cannot be excluded.

The activity of rat IgG2a antibodies is clearly complex. These antibodies are able to bind to Fc ϵ RI with low affinity,¹⁷ and we have shown here that they can induce the degranulation of RBL cells when Fc ϵ RI are unoccupied by IgE. Mast cells bearing free Fc ϵ RI have been reported, for example, in the peritoneal cavity of rats,¹⁸ despite the very high affinity of Fc ϵ RI for IgE. Paradoxically, when Fc ϵ RI are saturated with specific IgE, IgG2a antibodies in some circumstances are able to inhibit IgE-mediated degranulation.

In the rat, IgG1 is a Th1 cytokine-driven antibody subclass, while IgG2a, IgG2b and IgE arise during a Th2-type response.¹⁹ It would therefore appear that a switch from a Th2 to a Th1 response not only leads to a down-regulation of IgE production, but may also lead to the appearance of antibodies that can inhibit IgE-mediated function. In certain circumstances, however, it appears that other antibody isotypes including Th2 cytokine-regulated isotypes are able to influence mast cell function by epitope masking.

In the human, IgG1 antibodies bind with the highest affinity of the IgG subclasses to Fc γ RIIb1,¹⁵ and clinical studies have shown that some blocking activity is the result of the action of IgG1 antibodies. For example, rising allergen-specific IgG1 concentrations have been associated with clinical improvements in patients undergoing immunotherapy

for allergic disease.²⁰ Such changes in antibody production may result from a switch from a Th2 to a Th1 response. Although the relationship between IgG subclass production and Th1/Th2 activity has not been fully characterized, during infections such as tuberculosis and tuberculoid leprosy, which are associated with a strong Th1-type response, high IgG1 antibody concentrations have been reported.²¹ On the other hand, infections which are associated with Th2 activity suggest that Th2 activity promotes IgG2 and IgG4 production. IgG2 antibodies are conspicuous in lepromatous leprosy²¹ and human filariasis has been associated with IgG2 and IgG4 antibodies.²² Yet IgG4 antibodies, which bind to human Fc γ R with a lower affinity than IgG1 antibodies, have also been reported to display IgE-blocking activity.¹

The results presented here suggest that in the RBL model, antibodies can mediate blocking activity through both an Fc γ R-dependent mechanism and by Fc γ R-independent epitope masking. It is possible that in the human, both blocking mechanisms may also operate. A number of studies have shown greater effectiveness of allergen desensitization for inhalant allergens in exposures of low allergen concentration, and that allergen desensitization is less effective in environments of higher allergen exposure.¹¹ The present study suggests that in the latter circumstance, only Fc γ R-dependent blocking antibodies may substantially inhibit IgE antibody activity.

Acknowledgement

JP and WB contributed equally to this study, which was supported by a grant from the NH & MRC.

References

- 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.
- Daeron 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.
- Daeron M, Malbec O, Latour S, Bonnerot C, Segal DM, Fridman WH. Distinct intracytoplasmic sequences are required for endocytosis and phagocytosis via murine Fc gamma RII in mast cells. *Int. Immunol.* 1993; **5**: 1393–401.
- Bocek PJ, Draberova L, Draber P, Pecht I. Characterization of Fc gamma receptors on rat mucosal mast cells using a mutant Fc epsilon RI-deficient rat basophilic leukemia line. *Eur. J. Immunol.* 1995; **25**: 2948–55.
- Bocek P, Pecht I. Cloning and sequence of the cDNA coding for rat type II Fc gamma receptor of mast cells. *FEBS Lett.* 1993; **331**: 86–90.
- Farber DL, Giorda R, Nettleton MY, Trucco M, Kochan JP, Sears DW. Rat class III Fc gamma receptor isoforms differ in IgG subclass-binding specificity and fail to associate productively with rat CD3 zeta. *J. Immunol.* 1993; **150**: 4364–75.
- Seldin DC, Adelman S, Austen KF *et al.* Homology of the rat basophilic leukemia cell and the rat mucosal mast cell. *Proc. Natl Acad. Sci. USA* 1985; **82**: 3871–5.
- Collins AM, Basil M, Nguyen K, Thelian D. Rat basophil leukaemia (rbl) cells sensitized with low affinity IgE respond to high valency antigen. *Clin. Exp. Allergy* 1996; **26**: 964–70.

- 9 Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J. Exp. Med.* 1995; **182**: 1169–74.
- 10 Noon L, Cantab BC. Prophylactic inoculation against hay fever. *Lancet* 1910; **i**: 1572–3.
- 11 Malling HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998; **53**: 461–72.
- 12 Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy [Review]. *Allergy* 1985; **40**: 469–86.
- 13 Fong DC, Malbec O, Arock M, Cambier JC, Fridman WH, Daeron M. Selective in vivo recruitment of the phosphatidylinositol phosphatase ship by phosphorylated fc-gamma-riib during negative regulation of ige-dependent mouse mast cell activation. *Immunol. Lett.* 1996; **54**: 83–91.
- 14 Malbec O, Fong DC, Turner M *et al.* Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. *J. Immunol.* 1998; **160**: 1647–58.
- 15 Warmerdam PA, van den Herik-Oudijk IE, Parren PW *et al.* Interaction of a human Fc gamma RIIb1 (CD32) isoform with murine and human IgG subclasses. *Int. Immunol.* 1993; **5**: 239–47.
- 16 Collins AM, Thelian D, Basil M. Antigen valency as a determinant of the responsiveness of IgE-sensitised rat basophil leukemia cells. *Int. Arch. Allergy Immunol.* 1995; **107**: 547–56.
- 17 Benhamou M, Berenstein EH, Jouvin MH, Siraganian RP. The receptor with high affinity for IgE on rat mast cells is a functional receptor for rat IgG2a. *Mol. Immunol.* 1994; **31**: 1089–97.
- 18 Chen XJ, Enerback L. IgE receptors, IgE content and secretory response of mast cells in athymic rats. *Immunology* 1994; **83**: 595–600.
- 19 Cuturi MC, Josien R, Cantarovich D *et al.* Decreased anti-donor major histocompatibility complex class I and increased class II alloantibody response in allograft tolerance in adult rats. *Eur. J. Immunol.* 1994; **24**: 1627–31.
- 20 Moss RB, Hsu YP, Kwasnicki JM, Sullivan MM, Reid MJ. Isotypic and antigenic restriction of the blocking antibody response to ryegrass pollen: Correlation of rye group I antigen-specific IgG1 with clinical response. *J. Allergy Clin. Immunol.* 1987; **79**: 387–98.
- 21 Sousa AO, Henry S, Maroja FM *et al.* IgG subclass distribution of antibody responses to protein and polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. *Clin. Exp. Immunol.* 1998; **111**: 48–55.
- 22 Yazdanbakhsh M, Paxton WA, Brandenburg A *et al.* Differential antibody isotype reactivity to specific antigens in human lymphatic filariasis: gp15/400 preferentially induces immunoglobulin E (IgE), IgG4, and IgG2. *Infect. Immun.* 1995; **63**: 3772–9.