

Presence of IgG4 on the membrane of human basophils. Histamine release is induced by monoclonal antibodies directed against the Fab but not the Fc region of the IgG4 molecule

L. JIMENO, M. LOMBARDERO, J. CARREIRA
and J. MOSCOSO DEL PRADO

Alergia e Immunología Abelló SA, Madrid, Spain

Summary

We have studied the possible role of human IgG4 as an anaphylactic antibody. For that purpose, we have determined the induction of histamine release (HR) from human basophils by anti-IgE and anti-IgG4 monoclonal antibodies (MoAbs) recognizing different epitopes located at the Fc and Fab regions of the IgG4 molecule. The results show that anti-IgG4 (Fab) MoAb was able to induce HR in 93% of donors tested, with no differences between atopics and non-atopics. That HR is calcium dependent and is accompanied by the synthesis and release of leukotriene C₄. In contrast, no HR could be induced by anti-IgG4(Fc) MoAbs in any individual, even in the presence of D₂O or after a second challenge with a polyclonal goat anti-mouse IgG antibody. The results obtained suggest the presence of IgG4 on the basophil membrane and that the epitope recognized by the anti-IgG4 (Fc) MoAbs is probably hidden in cell-bound IgG4. This was demonstrated by immunofluorescence techniques: IgG4 bound to the basophil membrane could be detected with anti-IgG4(Fab) but not with anti-IgG4(Fc) MoAbs. In addition, we found that nine donors were unresponsive to an anti-IgE stimulus, while they released histamine efficiently after challenge with anti-IgG4(Fab), suggesting the existence of different receptors for both immunoglobulins.

Clinical and Experimental Allergy, Vol. 22, pp. 1007-1014. Submitted 29 July 1991; revised 24 April 1992; accepted 1 May 1992.

Introduction

Although the involvement of IgE in type I hypersensitivity is well established, it has been suggested that other immunoglobulins might also be implicated in that type of reaction. This suggestion stems from the observation that human IgG could act as short-term sensitizing antibodies in monkeys [1] and that anti-IgG antibodies could induce histamine release (HR) from human basophils [2].

Since those initial observations, different authors have tried to identify the nature of the IgG sensitizing antibodies. So far, most of the evidence points towards IgG4 as the subclass responsible for those non-IgE anaphylactic antibodies. The first indication for it was presented by Stanworth *et al.* who showed that a human IgG4 protein

inhibited IgE-mediated PCA reactions in baboons [3]. However, other authors [4] were unable to reproduce such results. Using a different approach, several authors found that IgG4 was capable of specifically sensitizing human leukocytes [5,6] and furthermore, it was also described that anti-IgG4 antibodies could induce HR from basophils [7,8]. Again other investigators failed to reproduce those results [9,10,11]. Thus, in view of these conflicting results, the possible role of IgG4 as anaphylactic antibodies remains purely hypothetical.

A common feature of the experiments mentioned above was the use of different polyclonal or monoclonal anti-IgG4 antibodies, sometimes with ill-defined specificities. However, the precise specificity of the antibodies could be of great importance. Thus, the ability of anti-IgE monoclonal antibodies to induce HR from basophils depends on the region of the IgE molecule they recognize

Correspondence: Dr L. Jimeno, Alergia e Immunología Abelló S.A., C/ Miguel Fleita 19, 28037 Madrid, Spain.

[12]. Similarly, a possible explanation for the contradictory results obtained when challenging basophils with anti-IgG4 antibodies could be the different specificities of the reagents used.

In an attempt to clarify the existing controversy on whether or not IgG4 antibodies participate in HR from basophils, we have taken advantage of two well defined anti-IgG4 monoclonal antibodies (MoAbs) which recognize two different epitopes located at the Fab and Fc regions of the molecule [13], hereafter designated anti-IgG4(Fab) and anti-IgG4(Fc), respectively. By challenging human leucocytes with these antibodies, we demonstrate that HR is readily induced by anti-IgG4(Fab) but not by anti-IgG4(Fc). Furthermore, we show by immunofluorescence that IgG4 is bound to basophils, thus demonstrating that IgG4 can mediate HR from human basophils. The fact that the ability of anti-IgG4 antibodies to induce HR depends on their specificity provides a plausible explanation for the contradictory results previously reported.

Materials and methods

Blood samples

Venous blood was drawn from a group of atopic and non-atopic volunteers by venipuncture, anticoagulated with 10 units/ml heparin, and used immediately in histamine release experiments as described below. Total IgG4 content was measured in all samples as described [13]. Samples from atopic donors had a total IgG4 content ranging from 0.065–1.9 mg/ml. Samples from non-atopics had a range of 0.040–2.2 mg ml⁻¹. Total IgE was measured using a commercial kit (HAMLET®, AIASA, Spain), resulting in a range of 14–1007 and 24–396 IU/ml for atopics and non-atopics, respectively.

Antibodies

Throughout this work we have employed two anti-IgG4 MoAbs obtained in our laboratory: G4.T2 and G4.T9 (both of IgG₁ isotype) which recognize epitopes located at the Fab and Fc regions of the IgG4 molecule, respectively, as shown by their pattern of reactivity against papain fragments of IgG4. They recognize both IgG4 isoallotypes (4a and 4b) and show no crossreactivity with other IgG subclasses, IgA, IgM, or IgE, nor they do recognize λ or κ chains. This was shown by ELISA and immunoblotting against five IgG4 paraproteins; four paraproteins of each IgG subclass; three IgE myeloma proteins and polyclonal IgA, IgM, as well as and Bence-Jones light chains. All these results were published elsewhere [13]. Other anti-IgG4(Fc) MoAbs tested in some experiments were G4.L; G4.T11; G4.T12 [13]; SK-

44 (ICN Immunologicals, U.S.A.) and RJ4, kindly provided by A. Jiménez (Hospital La Paz, Spain). All the antibodies were purified from ascitic fluid.

Other MoAbs employed were anti-human IgG1(Fc) (8C/6-39); IgG2(Fc) (GOM-1); IgG2(Fab) (HP6014); IgG3(Fab) (HP6050); IgM(Fc) (CH6) and IgA1 (M4F8), and were purified from ascitic fluid bought from The Binding Site Ltd (U.K.). As control we employed an anti-IgE MoAb, 3H5, which has been described in length elsewhere [12]. Goat anti-mouse IgG antibodies (GAM) were prepared by affinity chromatography on a mouse IgG-Sepharose column, from a serum pool from two goats previously immunized with mouse IgG.

Histamine release assay

Histamine was measured using a commercial radioimmunoassay (Immunotech, France), following the manufacturer's instructions. Briefly, blood anticoagulated with heparin was diluted (1:4) in the releasing buffer provided with the kit and 100 μ l challenged for 30 min at 37°C with 50 μ l of various concentrations of the anti-IgG4 or anti-IgE MoAbs, diluted in releasing buffer. In some experiments (see results) the releasing buffer contained either 40% D₂O or 10 mM EDTA. Histamine was measured in the diluted plasma after centrifugation of the samples. The kit has an intra-assay CV of less than 10%. The inter-assay CV is below 12%.

In a second series of experiments, cells were first challenged with the different MoAbs, and after centrifugation and withdrawal of the supernatants to measure their histamine content, the cells were resuspended in releasing buffer to the original volume and rechallenged with GAM (25 μ g/ml), measuring the histamine released as above.

Leukotriene C₄ assay

Mononuclear cells were purified from venous blood by centrifugation over Ficoll-Paque (Pharmacia, Sweden). After washing, the cells were resuspended in releasing buffer at a concentration of 10⁷ cells/ml. Based on toluidine blue staining, the preparations used contained an average of 2% basophils. One hundred microlitres of the cell suspension was incubated with 50 μ l of anti-IgG4(Fab) at 1 mg/ml. After 45 min at 37°C, the samples were centrifuged, and the supernatants were analysed for their content in LTC₄ using a commercial kit (Amersham, U.K.).

Fluoresceination of GAM

Ten milligrams of GAM in NaHCO₃ buffer (0.1 M, pH 8.5) was incubated with a ten-fold molar excess

of 5(6)-carboxyfluorescein-*N*-hydroxysuccinimide ester (FLUOS, Boehringer Mannheim, Germany) for 60 min at room temperature. The fluoresceinated antibody was separated from unbound FLUOS by gel filtration using a PD10 column (Pharmacia, Sweden) equilibrated with PBS. The conjugated GAM was aliquoted and stored frozen at -20°C in the presence of 1% BSA.

Immunofluorescent analysis of leucocytes

Venous blood from four different donors was collected in the presence of heparin (10 u/ml), diluted 1:1 in PBS, layered over Ficoll-Paque (Pharmacia, Sweden), and centrifuged at 800 *g* for 15 min. The interphase cells were collected and washed with PBS. After the final wash, the cells, resuspended in 250 μl of PBS, were stained and fixed simultaneously with a mixture of 500 μl toluidine blue (0.2% in PBS) and 500 μl paraformaldehyde (2% in PBS) [14]. After 10 min, the mixture was washed twice with PBS, and finally resuspended in 200 μl of PBS.

Aliquots of fixed and stained cells were incubated with anti-IgG4(Fab) or anti-IgG4(Fc) MoAbs at 100 $\mu\text{g}/\text{ml}$ in PBS for 60 min at room temperature. After washing, this was followed by an incubation with fluoresceinated GAM diluted 1:20 in PBS for an additional 60 min. After washing, cells were resuspended in a glycerol:carbonate buffer (9:1), pH 8.5 solution containing 2.5% 1,4-diazobicyclo(2,2,2)octane (Fluka, Switzerland) as anti-fading agent. Aliquots of that suspension were transferred to wells of immunofluorescence slides and examined with transmitted light to visualize basophils and epifluorescence using an Olympus BH2 fluorescence microscope (Olympus, Japan). A minimum of 20 basophils were examined from each donor.

Results

Blood was drawn from 27 volunteers (12 atopic and 15 non-atopic) and challenged in parallel with anti-IgG4(Fab), anti-IgG4(Fc) and anti-IgE MoAbs. Anti-IgG4 MoAbs were used at 1, 0.1 and 0.01 mg/ml; anti-IgE MoAb was used at 10 $\mu\text{g}/\text{ml}$, a concentration shown in preliminary experiments to induce HR optimally, in terms of the percentage of releasers. After 30 min challenge at 37°C , the released histamine was measured as described in Materials and Methods. The results are shown in Table 1. Spontaneous HR was measured in all blood samples, and ranged from 0.1 to 2.5% of the total histamine content. If over 5% HR (twice the maximum spontaneous release obtained) is considered significant, anti-IgG4(Fab) was able to induce HR in 59%, 93% and 85% of the samples at 0.01, 0.1 and 1 mg/ml, respectively, the maximum percentage of HR varying among releasers

Table 1. Histamine release induced by the different MoAbs. Histamine release is calculated as the percentage of the total histamine content in each sample. Spontaneous histamine release varied among individuals from 0.1 to 2.5%. The concentrations of the antibodies used are expressed in mg/ml

Donors	Anti-IgG4(Fab)			Anti-IgG4(Fc)			Anti-IgE
	1	0.1	0.01	1	0.1	0.01	0.01
<i>Non-atopics</i>							
JM	60	60	35	5	3	2	29
ML	50	38	19	1	1	1	21
FP	68	52	7	3	1	1	2
TM	48	48	6	2	1	1	6
PV	15	12	2	2	1	1	1
RG	40	18	3	3	1	1	2
LJ	53	33	5	2	1	1	2
IC	30	22	3	2	1	1	3
CC	3	3	2	2	1	1	2
LD	40	50	8	3	2	2	4
AC	45	40	8	5	3	3	6
RS	40	40	20	3	1	1	11
MV	5	10	3	1	1	1	2
MM	70	70	10	3	2	2	13
CV	60	63	25	4	2	1	15
<i>Atopics</i>							
CA	5	5	2	5	2	2	7
JR	10	10	6	3	1	1	5
PC	4	12	2	2	2	1	6
RA	70	65	40	3	3	2	26
CB	37	45	10	2	1	1	7
DB	40	41	7	1	2	1	11
JL	28	30	4	2	1	1	9
JM	29	28	5	3	2	2	11
CG	38	40	8	3	1	1	9
MG	80	60	20	3	2	2	15
AJ	10	25	7	2	1	1	3
CC	60	64	4	1	1	1	28

from 10 to 80%. The percentage of HR did not correlate with the total IgG4 or IgE content of the samples. Blood samples were taken from several donors in different days, and all of them remained constant in respect to their ability to release histamine or not, although in some cases it was observed a significant variation in the percentage of HR. The ability of anti-IgG4(Fab) to induce HR seemed independent of the clinical status of the donors, as similar percentage of releasers was found both in atopics and non-atopics (92% versus 93%, respectively). In contrast, no HR could be induced by anti-IgG4(Fc) in any individual, irrespective of the concentration tested. It is noteworthy that the anti-IgG4(Fc) MoAb has a high

Table 2. Histamine release from human basophils induced by secondary cross-linkage of anti-IgG4 and anti-IgE MoAbs with anti-murine IgG

First Ab	Challenge		%HR				
	mg/ml	Second Ab	samples				
			1	2	3	4	5
Anti-IgG4(Fab)*	1	None	12	5	45	50	50
	0.1	None	4	5	30	50	30
Anti-IgG4(Fab)†	1	GAM	19	5	50	50	50
	0.1	GAM	10	5	40	50	50
Anti-IgG4(Fc)*	1	None	2	5	2	4	3
	0.1	None	1	2	1	3	3
Anti-IgG4(Fc)†	1	GAM	2	4	2	4	3
	0.1	GAM	1	4	2	3	2
Anti-IgE*	0.01	None	n.d.	n.d.	n.d.	27	8
Anti-IgE†	0.01	GAM	n.d.	n.d.	n.d.	25	27

* Histamine release values after challenging with the MoAbs at the concentrations shown (in mg/ml).

† Additional histamine released (percentage of the total histamine present before challenge) by the same cells after a second challenge with a goat anti-mouse IgG antibody at 25 µg/ml.

n.d. means not determined.

Table 3. Effect of D₂O on the histamine release induced by anti-IgE and anti-IgG4 MoAbs. D₂O was included in the releasing buffer at a final concentration of 40%. Spontaneous histamine release in the presence of D₂O was increased in some of the samples (range 5–10%), and has been subtracted in the values shown

Samples		Anti-IgG4(Fab)			Anti-IgG4(Fc)			Anti-IgE
		100	10	1	100	10	1	10
1	–D ₂ O	28	6	1	2	1	1	8
	+D ₂ O	67	49	11	2	2	2	37
2	–D ₂ O	18	3	2	2	2	1	3
	+D ₂ O	32	18	4	3	2	2	24
3	–D ₂ O	9	5	1	2	1	1	1
	+D ₂ O	17	5	2	3	1	1	1
4	–D ₂ O	2	2	1	2	2	1	5
	+D ₂ O	2	1	1	2	2	2	7

* The concentrations of the antibodies used are expressed in µg/ml.

affinity constant for IgG4 ($4.93 \times 10^9/\text{M}$), which is an order of magnitude higher than that of the anti-IgG-4(Fab) [13]. Thus, the different results obtained with the two MoAbs cannot be explained in terms of their different affinity for IgG4, but rather, they seem related with their respective specificities, since five other anti-IgG4(Fc) MoAbs tested also failed to induce HR. Also, antibodies against IgG1(Fc), IgG2(Fab), IgG2(Fc), IgG3(Fab), IgM(Fc) and IgA1 were tested with negative results (data not shown).

On the other hand, the results obtained with anti-IgE MoAb seem independent of those obtained with anti-IgG4 antibodies. It has been described that not all subjects release histamine upon challenge with anti-IgE antibodies, and that a higher percentage of releasers is found among atopics [15,16]. Accordingly, we found only 63% of releasers among our study population, with an important, although non statistically significant, difference between atopics and non-atopics (83% and 47% of releasers, respectively). Again the ability to release histamine remained unchanged when the samples were retested on different occasions, but with significant variations in the percentage of HR in some of the samples.

Table 4. Sensitivity of the cells to different concentrations of anti-IgG4(Fab) and anti-IgE MoAbs. Blood from three different donors was challenged with different concentrations of anti-IgG4(Fab) and anti-IgE MoAbs. The numbers indicate the percentage of histamine released in each case

Samples	Anti-IgG4(Fab) ($\mu\text{g/ml}$)						Anti-IgE ($\mu\text{g/ml}$)					
	100	10	1	0.1	0.01	0.001	10	1	0.1	0.01	0.001	0.0001
1	52	18	2	1	1	1	20	20	14	14	6	12
2	20	2	1	1	1	1	18	22	10	2	2	7
3	11	3	1	2	1	2	3	1	1	2	2	2

Table 5. Release of preformed and non-preformed mediators after challenging with anti-IgG4(Fab) MoAb. Cells were challenged with anti-IgG4(Fab) at 1 mg/ml

Samples	Mediator release	
	Histamine	Leukotriene C ₄ †
1	60	105
2	40	56
3	5	28

* Histamine released expressed as percentage of the total histamine content of the samples.

† Values indicate picograms of LTC₄ released per 10⁶ cells. See Materials and Methods for details.

Nothing is known about the mechanisms through which anti-IgG4 antibodies induce HR. However, if a similar mechanism to that of anti-IgE antibodies is assumed (that is, crosslinking of receptor-bound molecules), then it could be possible that anti-IgG4(Fc) antibodies bound to IgG4 on the surface of basophils but were unable to crosslink them. In order to rule out this possibility, we measured HR after a first challenge with the different MoAbs and then, after a second challenge with a polyclonal goat anti-mouse IgG (GAM). If the MoAbs bound to membrane IgG4, then the second antibody should crosslink the receptor molecules and induce HR. The results are shown in Table 2. The addition of GAM resulted in a second pulse of HR in cells treated with anti-IgG4(Fab) or anti-IgE, but failed to produce significant HR in cells treated with anti-

IgG4(Fc). These results show that anti-IgG4(Fc) MoAbs are unable to bind surface-bound IgG4, and also, strongly indicate that the mechanism of HR induced by anti-IgG4 antibodies relies on the crosslinking of membrane-bound IgG4 molecules.

It has been described that basophils show an enhanced response to challenge with anti-IgE antibodies if deuterium oxide is present in the releasing buffer [17]. To investigate whether anti-IgG4(Fc) could induce HR in the presence of D₂O, we studied its effect in four donors previously shown to have different levels of response with anti-IgG4(Fab) and anti-IgE. The results are presented in Table 3. Cells challenged with either anti-IgG4(Fab) or anti-IgE showed an increased response with D₂O. In contrast, cells challenged with anti-IgG4(Fc) remained unresponsive even in the presence of D₂O.

Other authors who have described HR induced by anti-IgG antibodies found that they were between 10 to 1000 times less efficient in inducing HR than anti-IgE antibodies [2,6]. We have compared the efficiency of an anti-IgE MoAb ($K_a = 2.8 \times 10^9/\text{M}$) with that of the anti-IgG4(Fab) MoAb ($K_a = 2.1 \times 10^8/\text{M}$) in inducing HR. Cells from three individuals with different sensitivity to both anti-IgE and anti-IgG4 challenge were studied, and the results are shown in Table 4. In all cases, a much higher concentration of anti-IgG4 than anti-IgE MoAb was needed to induce significant HR, even correcting for the different affinities of both MoAbs. Although the magnitude of that difference varied among individuals, it seemed to be between one or two orders of magnitude.

It is known that HR induced by anti-IgE is a calcium dependent process. Similarly, we found that the presence of EDTA (10 mM) when challenging the cells with anti-IgG4(Fab) completely abolished the induction of HR (data not shown). The increase in intracellular calcium also leads to the induction of synthesis and release of other non-preformed mediators such as leukotriene C₄.

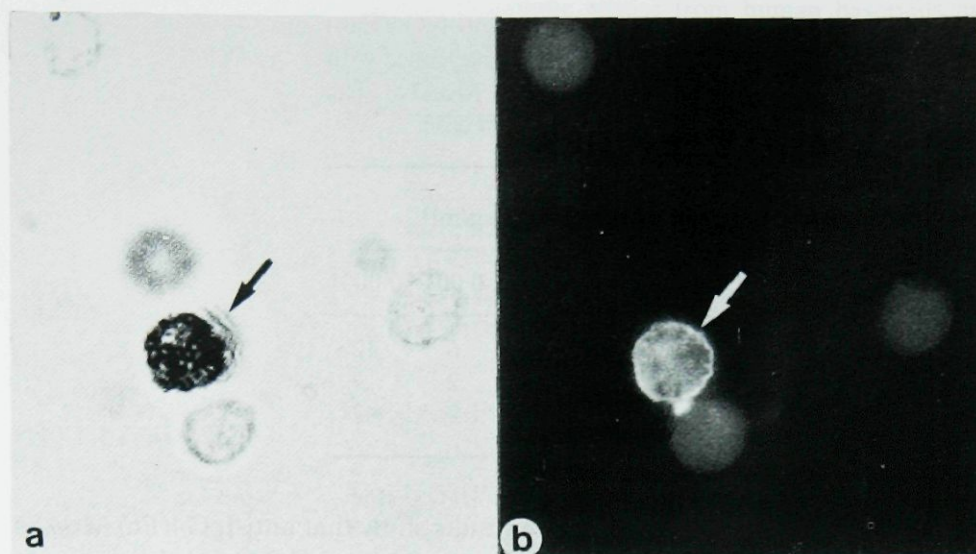


Fig. 1. Fixed and toluidine blue stained cells were incubated with anti-IgG4(Fab) and fluoresceinated GAM as described in Material and Methods. (a) Microphotograph of cells stained with toluidine blue. The arrow shows a metachromatically stained basophil. (b) The same field photographed under the appropriate u.v. light. The arrow shows the specific fluorescence of the basophil.

That mediator was also induced after challenging the cells with anti-IgG4(Fab), as shown in Table 5.

The results presented so far demonstrate that an anti-IgG4(Fab) antibody can induce mediator release from basophils whereas anti-IgG4(Fc) antibodies (at least those with specificities like the ones we have employed) are unable to do so, suggesting that the epitope(s) recognized by the latter is(are) hidden on basophil membrane-bound IgG4. To obtain additional evidence of the presence of IgG4 on the membrane of basophils, we performed indirect immunofluorescence studies of cells treated with either of both MoAbs and a second goat anti-mouse antibody labelled with fluorescein. Whereas anti-IgG4(Fab)-treated basophils readily showed fluorescence, no specific fluorescence could be observed in basophils treated with anti-IgG4(Fc) MoAb, irrespective of the concentrations tested (Fig. 1). This result clearly demonstrates the presence of IgG4 molecules bound to the membrane of basophils and also gives additional evidence that the epitope recognized by anti-IgG4(Fc) MoAb remains inaccessible in those membrane-bound IgG4 molecules.

Discussion

The view that IgG4 antibodies might be implicated in the allergic response is mainly based on two different pieces of evidence: the observation that IgG4 could inhibit IgE-mediated PCA reactions in baboons, and on reports that IgG4 could mediate HR from basophils [3,5–8]. However, both observations have been questioned by several authors who failed to reproduce them [4,9–11], leading to a controversy that has not been resolved yet.

Taking advantage of two well-characterized MoAbs that recognize different epitopes on the human IgG4 molecule, located in the Fab and the Fc regions of that

immunoglobulin, respectively, we have addressed the question of whether IgG4 can mediate HR from basophils. It is known that in the case of antibodies against IgE, their ability to trigger HR is dependent on the region of the IgE molecule they recognize [12]. A similar explanation could account for the discrepant results obtained when studying the ability of anti-IgG4 antibodies to induce HR, since different authors employed different antibodies, often with not well-defined specificities. Another element that has contributed to the controversy is that most of the experiments reporting anti-IgG4 induced HR were conducted under non-physiological conditions, such as in the presence of D₂O and usually the results were not compared with controls in its absence. Thus, we have performed our study in the absence of such HR enhancers.

Our study population consisted of 27 donors (12 atopic and 15 non-atopic), and the results have been unequivocal; cells from up to 93% of the population released histamine when challenged with an antibody directed against the Fab region. Furthermore, we show that, as expected, HR depends on the calcium influx into the cell, since it is inhibited by EDTA, and is accompanied by the release of other non-preformed mediators such as LTC₄. In contrast, antibodies against the Fc region were unable to induce significant HR in any of the samples tested, even in the presence of a potent enhancer of HR such as D₂O. A possible explanation for that behaviour could be that anti-IgG4(Fc) antibodies bind basophil-bound IgG4, but are unable to crosslink it. This explanation is highly improbable since even after a second challenge with polyclonal anti-mouse IgG, no HR could be detected if the first antibody was directed against the IgG4 Fc. Moreover, with the use of immunofluorescence techniques we have demonstrated the existence of IgG4 bound to the basophil membrane, but that IgG4 can be

detected with antibodies against the Fab, but not Fc. It should be pointed out, however, that all the anti-IgG4(Fc) MoAbs tested by us seem to recognize the same or very closely related epitopes, as judged from cross-inhibition experiments, and from the pattern of recognition of protease-fragmented IgG4 [13]. It is possible, in principle, that other antibodies with different specificities within the Fc region could induce HR.

Previous studies showed that a fraction of the population is unresponsive to anti-IgE challenge [15,16]. We have confirmed that result: 35% of the donors are non-releasers to anti-IgE stimulus, with a marked, although non-statistically significant difference between atopics and non-atopics (17% and 53% non-releasers, respectively). In contrast, only 7% of the donors fell under the category of non-releasers when challenged with anti-IgG4(Fab), with no differences found between atopics and non-atopics. Recent data suggest that non-releasers (with anti-IgE) have a defect in some early step linking the IgE receptor and the machinery involved in signal transduction [16]. If that proves to be true, then the fact that nine out of 10 non-releasers with anti-IgE release histamine upon challenge with anti-IgG4 suggests the existence of independent receptors for both immunoglobulins in the basophil membrane, or, more unlikely, that there is a unique receptor with different transduction pathways for each ligand. The identity of that putative IgG4 receptor is not clear, however. It has been described that basophils express only one of the known IgG receptors, Fc γ RII [18,19], which only weakly binds IgG4. Alternatively, the existence of a different receptor specific for IgG4, not yet identified, cannot be ruled out and has been suggested by others [6].

Several authors have described the presence of anti-IgE autoantibodies in some sera [20,21]. Thus, it could be argued that anti-IgG4-mediated HR is caused by the presence of IgG4 autoantibodies bound to membrane-linked IgE. This explanation is, however, highly unlikely. First, such autoantibodies have been described in only a fraction of patients suffering atopic diseases [20,21], while we find that over 90% of both normal and atopic individuals release histamine after challenge with anti-IgG4(Fab) MoAb. Second, that explanation would not account for the failure of anti-IgG4(Fc) and the other anti-Igs MoAbs to induce HR.

IgG4-dependent HR seems to be more efficient than its IgE counterpart at least in terms of the percentage of releasers and, apparently, in the magnitude of HR (although others have reported higher percentages of HR using different anti-IgE antibodies [16]). However, on a molar basis, anti-IgG4 antibodies are much less efficient in inducing HR than anti-IgE, even after correcting for their different affinities. The reason for that difference is

not known, but we can think, among others, of three most likely explanations. First IgG4, when linked to its receptor, can undergo conformational modifications that may impair the affinity of antibodies against free IgG4. Second, the number of receptors of IgG4 might be lower than that of IgE. Although we have not performed a systematic and quantitative study, our immunofluorescence experiments do not support that view; the intensity of fluorescence of basophils labelled with either anti-IgE or anti-IgG4 does not seem to correlate with their susceptibility to HR in response to either stimulus. In our view a more plausible explanation would be that the IgG4 receptor has a lower affinity than the IgE receptor for their respective ligands. It can be speculated that the much higher concentration of IgG4 than IgE present in serum could compensate *in vivo* for that difference in affinity.

To summarize, we show that IgG4 can mediate the release of mediators from basophils. The conflicting results previously reported could be explained by our observation that this phenomenon can be detected only with the use of antibodies against particular regions of the IgG4 molecule, such as the Fab. We do not know about the physiological role of that basophil-bound IgG4. It is, however tempting to speculate about a possible anaphylactic role for that immunoglobulin. Although other data such as the co-regulation of IgE and IgG4 by interleukin-4 and γ -interferon may support that hypothesis [22,23], clearly, that point is far from being established.

Acknowledgments

We wish to thank Dr C. Audera for critically reading the manuscript, Mr E. Soler for his technical assistance to carry out this work and Mrs I. Courinha for her expert secretarial assistance.

References

- 1 Parish WE. Short-term anaphylactic IgG antibodies in human sera. *Lancet* 1970; 2:590-2.
- 2 Ishizaka T, De Bernardo R, Tomioka H, Lichtenstein LM, Ishizaka K. Identification of basophil granulocytes as a site of allergic histamine release. *J Immunol* 1972; 108:1000-8.
- 3 Stanworth DR, Smith AK. Inhibition of reagin-mediated PCA reactions in baboons by the human IgG4 subclass. *Clin Allergy* 1973; 3:37-41.
- 4 Malley A, Beacher L, Porter G, Gerding R. Competitive inhibition of reagin-mediated histamine released by a human IgG4 myeloma protein. *Int Arch Allergy Appl Immunol* 1974; 47:194-201.
- 5 Vijay HM, Perelmutter L. Inhibition of reagin-mediated PCA reactions in monkeys and histamine release from human leukocytes by human IgG4 subclass. *Int Arch Allergy Appl Immunol* 1977; 53:78-87.

- 6 Nakagawa T, de Weck AL. Membrane receptors for the IgG4 subclass on human basophils and mast cells. *Clin Rev Allergy* 1983; 1:197-206.
- 7 Nakagawa T, Stadler BM, Heiner DC, Skvaril F, de Weck AL. Flow-cytometric analysis of human basophil degranulation. II Degranulation induced by anti-IgE, anti-IgG4 and the calcium ionophore A23187. *Clin Allergy* 1981; 11:21-30.
- 8 Fagan DL, Slaughter CA, Capra DC, Sullivan TJ. Monoclonal antibodies to immunoglobulin G₄ induce histamine release from human basophils *in vitro*. *J Allergy Clin Immunol* 1982; 70:399-404.
- 9 van Toorenbergen AW, Aalberse RC. IgG4 and passive sensitization of basophil leukocytes. *Int Arch Allergy Appl Immunol* 1981; 65:432-40.
- 10 van Toorenbergen AW, Aalberse RC. IgG4 and release of histamine from human peripheral blood leukocytes. *Int Arch Allergy Appl Immunol* 1982; 67:117-22.
- 11 Devey ME, Panzani R. The IgG subclass of antibodies to castor bean allergen in patients with allergic asthma; detection of a high incidence of antibodies of the IgG4 subclass. *Clin Allergy* 1975; 5:353-61.
- 12 Moscoso del Prado J, Jimeno L, Obispo T, Carreira J. Monoclonal antibodies against human IgE. Identification of an epitope sharing properties with the high affinity receptor binding site. *Mol Immunol* 1991; 28:839-44.
- 13 Jimeno L, Carreira J, Lombardero M. Obtention of monoclonal antibodies against human IgG4 using two different immunization strategies. Development of a biotin-based ELISA for IgG4 quantitation. *Int Arch Allergy Appl Immunol* 1990; 92:175-82.
- 14 Stallman JC, Aalberse RC. Estimation of basophil-bound IgE by quantitative immunofluorescence microscopy. *Int Arch Allergy Appl Immunol* 1977; 54:9-18.
- 15 Conroy MC, Adkinson NFJ, Sobotka AK, Lichtenstein LM. Releasability of histamine from human basophils. *Fed Proc* 1978; 37:1216.
- 16 Nguyen KL, Gillis S, MacGlashan DW. A comparative study of releasing and non-releasing human basophils: non-releasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol* 1990; 85:1020-9.
- 17 Gillespie E, Lichtenstein LM. Histamine release from human leukocytes: studies with deuterium oxide, colchicine and cytochalasin B. *J Clin Invest* 1972; 51:2941-7.
- 18 Anselmo LM, Perussia B, Thomas LL. Human basophils selectively express the Fc γ RII (CDW32) subtype of IgG receptor. *J Allergy Clin Immunol* 1989; 84:907-14.
- 19 Valent P, Majdic O, Maurer D, Botger M, Muhm M, Bettelheim P. Further characterization of surface membrane structures expressed in human basophils and mast cells. *Int Arch Allergy Appl Immunol* 1990; 91:198-203.
- 20 Inganäs M, Johansson SGO, Bennich H. Anti-IgE antibodies in human sera: occurrence and specificity. *Int Arch Allergy Appl Immunol* 1981; 65:51-61.
- 21 Carini C, Fratazzi C, Barbato M. IgG autoantibody to IgE in atopic patients. *Ann Allergy* 1988; 60:48-52.
- 22 Lundgren M, Persson U, Larsson P, Magnusson C, Edvard Smith CI, Hammarström L, Severinson E. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. *Eur J Immunol* 1989; 19:1311-13.
- 23 Spiegelberg HL. The role of interleukin-4 in IgE and IgG subclass formation. *Springer Semin Immunopathol* 1990; 12:365-83.

Copyright of Clinical & Experimental Allergy is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.