

Lung eosinophilic inflammation and airway hyperreactivity are enhanced by murine anaphylactic, but not nonanaphylactic, IgG1 antibodies

Maria Fernanda Macedo-Soares, PhD,^b Denise M. Itami, MSc,^b Carla Lima, PhD,^a Adenir Perini, PhD,^c Eliana L. Faquim-Mauro, PhD,^a Milton A. Martins, MD,^c and Mahasti S. Macedo, PhD^a São Paulo, Brazil

Background: Chronic airway inflammation is a fundamental feature of bronchial asthma, which is characterized by the accumulation and activation of inflammatory cells, such as mast cells and eosinophils, that are tightly regulated by T_H2 cytokines and chemokines. Recently, we demonstrated, in a murine model of asthma with immunosuppressed mice reconstituted with antigen-specific IgE or IgG1 antibodies, that IgE, but not IgG1, participates in potentiation of airway inflammation and induction of airway hyperreactivity (AHR). The IgG1 antibody, however, did not elicit passive cutaneous anaphylactic reactions, which was in contrast to IgE.

Objectives: Because 2 types of murine IgG1 have been demonstrated with regard to anaphylactic activity, the present experiments were undertaken to determine the role of anaphylactic and nonanaphylactic IgG1 antibodies in the development of antigen-induced eosinophilia and AHR in this model.

Methods: Dinitrophenyl-conjugated, heat-coagulated hen's egg white was implanted in immunosuppressed mice reconstituted with anaphylactic or nonanaphylactic IgG1. Intratracheal challenge with aggregated dinitrophenyl-ovalbumin was performed on day 14, and lung inflammatory and mechanical parameters were evaluated after 48 hours.

Results: Our results demonstrated that reconstitution of immunosuppressed mice with anaphylactic IgG1 antibodies in contrast to nonanaphylactic IgG1 antibodies potentiates their ability to have pulmonary eosinophilic inflammation and AHR. IL-5 and eotaxin levels in bronchoalveolar lavage fluid from anaphylactic IgG1-reconstituted mice were also higher than those in nonanaphylactic IgG1-reconstituted mice.

Conclusions: These results indicate that the anaphylactic property of murine IgG1 molecules is essential for their

capacity to enhance lung eosinophilic inflammation and to induce AHR. (J Allergy Clin Immunol 2004;114:97-104.)

Key words: Anaphylactic IgG1, nonanaphylactic IgG1, eosinophilic inflammation, airway hyperreactivity

Allergic asthma is characterized by airway hyperreactivity (AHR) to specific and nonspecific stimuli, chronic pulmonary eosinophilia, and increased serum IgE levels.¹⁻⁵ Cross-linking of allergen-specific IgE bound to high-affinity receptors on the surface of mast cells by antigen leads to activation and secretion of a wide variety of mediators, causing bronchoconstriction and enhancement of airway inflammation.^{6,7} Although it is clear that IgE is crucial for many allergic reactions, several rodent studies suggest the existence of alternative or additional pathways of hypersensitivity reactions.⁸⁻¹³ Thus allergen-specific IgG1, as well as IgE antibody, can be implicated in the allergic reaction in bronchial asthma because it binds to FcγRIII on the surface of murine mast cells and activates these cells.¹⁴⁻¹⁷ Recently, we investigated the role of antibodies on the development of airway eosinophilia and AHR induced by an implantation of heat-coagulated egg white coupled with the hapten dinitrophenyl (DNP-EWI). To do this, we used immunosuppressed mice treated with anti-μ and anti-δ mAbs that induce B-cell unresponsiveness¹⁸ and reconstituted with IgE or IgG1 antibodies. The results showed that the presence of IgE, but not IgG1, antibodies enhanced airway inflammation and AHR after intratracheal antigen challenge. However, passive cutaneous anaphylactic tests performed with the anti-DNP IgG1 used for reconstitution were negative.¹⁹ In 1999, Faquim-Mauro et al²⁰ demonstrated 2 types of murine IgG1 with regard to the anaphylactic activity that are differentially regulated by IL-4 and IL-12. The levels of anaphylactic-type and nonanaphylactic-type IgG1 antibodies produced in response to a particular antigen are modulated by adjuvants.²¹ Two IgG1 mAbs with the same antigenic specificity (anti-DNP) but different *in vivo* anaphylactic activities were also reported recently. The difference between these 2 types of IgG1 seems to be primarily caused by binding to the mast cell surface and closely related to a proper N-glycosylation of the molecules.²² The oligosaccharide chains of anaphylactic and nonanaphylactic IgG1 molecules are not similar, as demonstrated by the binding capacity to different lectins

From ^athe Department of Immunology, Institute of Biomedical Sciences, University of São Paulo; ^bthe Laboratory of Immunopathology, Butantan Institute, São Paulo; and ^cthe Department of Medicine, University of São Paulo.

Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). M.S.M. is a fellowship recipient of Conselho Nacional de Pesquisa (CNPq).

Received for publication October 30, 2003; revised March 17, 2004; accepted for publication March 18, 2004.

Reprint requests: Mahasti S. Macedo, PhD, Departamento de Imunologia, ICB/USP Av. Prof. Lineu Prestes 1730, 05508-900 São Paulo, SP, Brasil. E-mail: mamacedo@usp.br.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2004.03.033

Abbreviations used

AHR:	Airway hyperreactivity
BAL:	Bronchoalveolar lavage
Cr:	Dynamic compliance
DNP:	Dinitrophenyl
DNP-EWI:	Dinitrophenyl-coupled, egg white implant
EPO:	Eosinophil peroxidase
Grs:	Conductance of the respiratory system
OPD:	<i>o</i> -Phenylenediamine
OVA:	Ovalbumin

determined by means of chemoluminescence.²³ However, they do not seem to differ with regard to their protein sequence because it is known that there is only one C γ 1 gene, and using an RT-PCR strategy, we did not find alternatively spliced γ mRNAs that could encode different γ 1 heavy chains (data not published).

The purpose of the present study was to define the role of murine IgG1 antibodies in the development of AHR and pulmonary eosinophilia. To this end, mice immunized with DNP-EWI and submitted to a B-cell immunosuppressive protocol were reconstituted with anaphylactic or nonanaphylactic IgG1 antibody before challenge. Our results demonstrate that only anaphylactic IgG1-reconstituted mice recovered their former ability to have severe lung inflammation and AHR.

METHODS

Animals

Male BALB/c mice at 6 to 8 weeks of age were obtained from a colony at the Butantan Institute, São Paulo, Brazil, and maintained in our own animal facilities. All experimental protocols were approved by the Butantan Institute Ethical Committee for Animal Research.

Preparation of DNP-conjugated egg white implant and aggregated DNP-ovalbumin

Two hundred microliters of 1 mol/L 2,4 dinitrobenzenesulfonic acid sodium salt (Eastman Kodack Co, Rochester, NY) diluted in carbonate buffer, pH 9.2, were introduced into a chicken egg that was incubated overnight at room temperature with constant agitation. The hen's egg was then placed in a water bath at 100°C for 20 minutes. Solidified egg white coupled with DNP was washed several times in distilled water, dehydrated in ethanol for 24 hours, and cut into small blocks weighing 40 $\pm$ 5 mg. The egg white implants (DNP-EWI) were rehydrated by means of immersion in PBS at room temperature before use. Aggregated ovalbumin (OVA) was prepared according to the method of Titus and Chiller.²⁴ Type II OVA diluted in PBS, pH 7.2, was incubated for 1 hour at 80°C. After centrifugation at 3000g for 10 minutes, the pellet was resuspended with 1 mol/L 2,4 dinitrobenzenesulfonic acid sodium salt in carbonate buffer, pH 9.2, and incubated at room temperature overnight with constant agitation. The heat-aggregated DNP-OVA was then washed 3 times with sterile PBS and used for challenge.

Treatment of mice with anti-membrane Ig antibodies followed by reconstitution with anaphylactic or nonanaphylactic IgG1

Groups of mice were treated with 150 μ g of rat anti- μ (LO-MM-9) and anti- δ (LO-MD-6) mAbs administered intraperitoneally every

other day beginning 1 day before immunization and ending the day before challenge (immunosuppressed mice) to suppress the antibody production. The other group of mice received 300 μ g of IR-418 (nonsuppressed mice), which is a rat immunoglobulin of IgG2a-k isotype (same isotype as LO-MM-9 and LO-MD-6). On days 7 and 14, some immunosuppressed animals were reconstituted intravenously with 30 μ g of mouse anti-DNP mAb: anaphylactic IgG1, U7.6 (anaphylactic IgG1-reconstituted mice), or nonanaphylactic IgG1, B8401-H5 (nonanaphylactic IgG1-reconstituted mice). The rat mAb against mouse IgM and IgD and the rat mIg IR 418 were kindly provided by the Experimental Immunology Unit (UCL, Brussels, Belgium), and the mouse mAb B8401-H5 was a gift from Dr Van Snick (UCL). The mouse mAb U7.6 was a gift from Dr Robert Coffman (Dynavax Technologies, Berkeley, Calif).

Sensitization

Fragments of heat-coagulated hen's egg white coupled with DNP (DNP-EWI) were implanted in the subcutaneous tissue of the dorsal region of nonsuppressed, immunosuppressed, or reconstituted mice. After 14 days, the animals were challenged with 600 μ g of heat-aggregated DNP-OVA instilled into the trachea. Two days later, we analyzed lung tissue and bronchoalveolar lavage (BAL) fluid (AHR, differential cell count, and cytokine levels) and the antibody response. Nonsensitized animals that only received intratracheal challenge with DNP-OVA comprised the control group.

BAL and cell counts

Forty-eight hours after antigen challenge, mice were killed with an overdose of chloral hydrate, and the lungs were washed with 10 mL of saline with 10 mmol/L EDTA through the pulmonary artery. The tracheas were cannulated, and the airway lumina were washed with 6 $\times$ 0.5 mL of HBSS. The resulting BAL fluids were immediately centrifuged at 800 rpm and 4°C for 10 minutes. The supernatants were stored for cytokine and chemokine determination. The cell pellets were resuspended in 1 mL of HBSS for cell counts. Total cell counts were performed in a hemocytometer, and differential cell counts were performed in cytocentrifuge preparations stained with Wright-Giemsa. The lungs were then removed, weighed, and homogenized to give a 5% (wt/vol) suspension in HBSS and centrifuged (3000 rpm for 10 minutes). After lysis of red blood cells, the pellet was homogenized in HBSS plus hexadecyltrimethylammonium bromide for eosinophil peroxidase (EPO) assay.

Assay for EPO activity

EPO activity was measured in lung tissue according to the method of Strath et al.²⁵ The suspension of lung tissue was freeze-thawed 3 times with liquid nitrogen. After centrifugation at 4°C for 10 minutes, the lung extracts were serially diluted and placed in 96-well plates (75 μ L/well), followed by the addition of 150 μ L of substrate (1.5 mmol/L *o*-phenylenediamine [OPD] and 6.6 mmol/L H₂O₂ in 0.05 mol/L Tris-HCl, pH 8.0). After 30 minutes at room temperature, the reaction was stopped with the addition of 75 μ L of 0.2 mol/L citric acid, and absorbance of the samples was determined at 492 nm.

Quantification of IL-5 and eotaxin

IL-5 and eotaxin levels were measured in BAL fluid by using a specific 2-site sandwich ELISA with the following mAbs: for IL-5, TRFK5 and biotinylated TRFK4; for eotaxin, AF420NA and biotinylated BAF420. Binding of biotinylated mAbs was detected with streptavidin-biotinylated horseradish peroxidase complex (Amersham Int, Amersham, United Kingdom) and 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) or OPD (Sigma Chemical Co, St Louis, Mo) in 0.1 mol/L citrate buffer containing hydrogen peroxide. Samples were quantified by comparison with standard curves of recombinant mouse IL-5 or eotaxin. The results

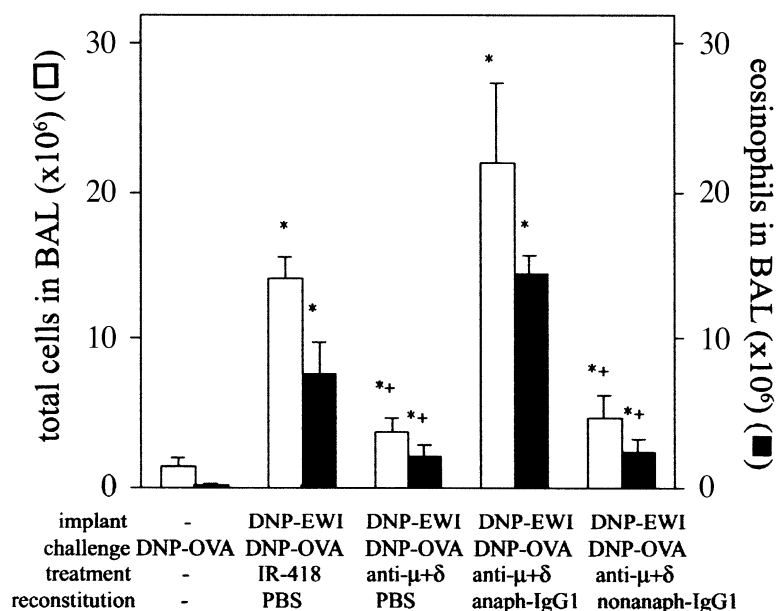


FIG 1. Effect of anaphylactic and nonanaphylactic IgG1 on airway eosinophilic inflammation. Mice were immunized and treated as depicted. Two days after challenge, BAL fluid was collected, and total cell and eosinophil numbers were estimated. Data represent means $\pm$ SEMs for 4 to 5 animals per group. $+P < .05$ compared with the IR-418-treated group; $*P < .05$ compared with untreated group.

were expressed as the arithmetic mean $\pm$ SEM for 5 samples per group. The detection limit of the assays was 15.62 pg/mL.

Measurement of serum antibodies

Determination of serum antibody levels was performed by means of ELISA with 96-well plates coated with OVA or DNP-BSA. Peroxidase-conjugated rat mAb against mouse κ chain (LO-MK-1) was used as a second antibody. The reactions were developed with OPD (Sigma Chemical Co) and H₂O₂ (Sicalab, São Paulo, Brazil). The plates were read at 492 nm on an automated ELISA reader (Titertek-Multiskan, Flow Labs). LO-MK-1 was developed in the Experimental Immunology Unit (UCL) and is commercially available (Serotec, Kidlington, United Kingdom).

Measurement of airway responsiveness

Conductance of the respiratory system (Grs) and dynamic compliance (Cr_s) were monitored in anesthetized, tracheostomized, and ventilated mice in a Harvard 386 apparatus. Different doses of methacholine (from 0.09 to 300 μ g in 30 μ L) were administered through a silastic catheter tied into the jugular vein, and data were stored at 30 seconds and 1, 2, 3, and 4 minutes after agonist injection. Plethysmograph pressure (from which changes in lung volume were derived) and tracheal pressure were transduced with differential pressure transducers. The outputs were amplified and transferred to a computer in which 6 to 10 consecutive breaths were stored and averaged. The Grs and Cr_s peak response to each agonist dose was expressed as a percentage of the baseline value obtained before the next dose of agonist. The estimated dose of methacholine required to reduce Grs to 50% and Cr_s to 40% of their control values was estimated from the log dose-peak response curve.

Statistical analysis

All values are expressed as means $\pm$ SEMs. Parametric data were evaluated by using 1-way ANOVA, followed by the Tukey test for

multiple comparisons. Nonparametric data were assessed by using Kruskal-Wallis 1-way ANOVA on ranks, followed by the Dunn test for multiple comparisons.²⁶ Differences were considered statistically significant at a *P* value of less than .05. The statistical software SigmaStat 2.0 for Windows (Jandel Scientific, Chicago, Ill) was used.

RESULTS

Only anaphylactic IgG1 had the ability to enhance eosinophil accumulation in BAL fluid and lung tissue

The role of IgG1 antibodies in the accumulation of inflammatory cells in response to intratracheal antigen challenge was analyzed in immunosuppressed mice reconstituted with either type of IgG1 and immunized with DNP-EWI (Fig 1). Nonsuppressed and immunosuppressed mice were immunized in a similar way as positive and negative controls for the humoral response. Airway challenge of nonsuppressed mice with DNP-OVA induced a significant increase in cell influx into BAL fluid compared with that seen in mice that were only challenged (control group), and 54% of the cells were eosinophils. In anaphylactic IgG1-reconstituted mice the total cell counts were also increased after DNP-OVA challenge compared with those seen in nonsuppressed mice or the control group, and eosinophils were the predominant cell type in BAL (~66%). Although immunosuppressed mice or nonanaphylactic IgG1-reconstituted mice had more eosinophils in BAL fluid compared with the control group, the number of eosinophils was less than that observed in nonsuppressed or anaphylactic IgG1-reconstituted mice. In the lung tissue the accumulation of

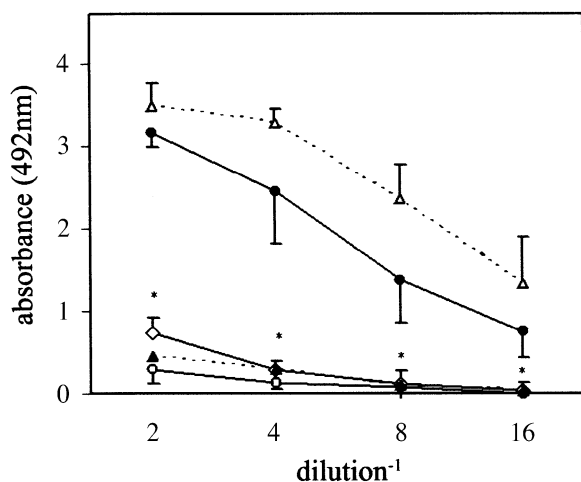


FIG 2. Comparison between EPO activity in the lung tissue from anaphylactic and nonanaphylactic IgG1-reconstituted mice. Lungs from mice described in Fig 1 were homogenized 48 hours after challenge. Data represent means $\pm$ SEMs for 4 to 5 animals per group. * $P < .05$ for the control, immunosuppressed, and nonanaphylactic IgG1-reconstituted groups compared with the nonsuppressed and anaphylactic IgG1-reconstituted groups. ○, Control group; ●, nonsuppressed group; ◇, immunosuppressed group; △, anaphylactic IgG1-reconstituted group; ▲, nonanaphylactic IgG1-reconstituted group.

eosinophils was evaluated on the basis of the levels of EPO (Fig 2). High EPO activity was only found in the homogenates of lung tissue from nonsuppressed and anaphylactic IgG1-reconstituted mice, indicating an intense lung eosinophilia in these groups but not in the others.

IL-5 and eotaxin in BAL fluid from IgG1-reconstituted mice

IL-5 and eotaxin, which are selective eosinophil chemoattractants,²⁷ were then measured in BAL fluid from IgG1-reconstituted mice. As shown in Fig 3, A, the levels of IL-5 were comparable with those of the nonsuppressed group only in the anaphylactic IgG1-reconstituted group. Immunosuppressed mice and nonanaphylactic IgG1-reconstituted mice displayed low levels of IL-5, but these levels were still significantly greater than those of the control group. Similar results were obtained with regard to the levels of eotaxin in BAL fluid from the same groups of mice (Fig 3, B).

Role of IgG1 antibodies in the development of airway hyperresponsiveness

The response of the groups of mice reported above to methacholine was determined *in vivo* 48 hours after antigen challenge to determine the contribution of IgG1 antibodies to the development of functional changes in the lung. The results in Fig 4 are expressed as the log of the methacholine dose that reduced Grs values to 50% and Crs values to 40% of baseline. Nonsuppressed mice showed AHR in comparison with the control group because lower doses of the agonist were required to reduce Grs (Fig 4, A)

and Crs (Fig 4, B) in the former group. Immunosuppressed or nonanaphylactic IgG1-reconstituted mice were unable to show AHR after antigen challenge, and their Grs and Crs values did not differ significantly from those of the control group. In contrast, the reconstitution with anaphylactic IgG1 antibodies restored AHR to the agonist.

Anaphylactic or nonanaphylactic IgG1 reconstitution restored the serum anti-DNP antibody levels in immunosuppressed mice

The discrepancies thus far observed between anaphylactic and nonanaphylactic reconstituted mice could be due to different levels of serum antibody and not due to their biologic activity. Therefore all groups of mice were bled 48 hours after antigen challenge, and their serum levels of OVA- and DNP-specific antibodies were determined by means of ELISA (Fig 5). As expected, the anti- μ and anti- δ mAb treatment induced an abrogation of the antibody response, and both OVA- and DNP-specific antibody levels in the immunosuppressed group were similar to those of the control group and quite less than those of the nonsuppressed group. The administration of anaphylactic or nonanaphylactic IgG1 equally restored the anti-DNP antibody levels in the immunosuppressed mice, whereas the OVA-specific levels remained low. In addition, the levels of DNP-specific anaphylactic or nonanaphylactic IgG1 in reconstituted mice were similar to those found in nonsuppressed mice that received DNP-EWI and produced DNP-specific IgM, IgG1, and IgE antibodies.¹⁹

DISCUSSION

In bronchial asthma cross-linking of allergen-specific IgE on the surface of mast cells activates these cells, leading to the release of inflammatory mediators that evoke bronchoconstriction, bronchial mucosal edema, and AHR.²⁸ In addition, increasing evidence indicates that IgG1 is capable of sensitizing murine mast cells independently of IgE, mediating not only immediate hypersensitivity but also AHR.^{11,29} The early phase of bronchoconstriction after allergen exposure in the mouse also requires allergen-specific IgG.¹³ In this study we demonstrated that only anaphylactic, but not nonanaphylactic, IgG1 had the ability to restore lung eosinophilic inflammation and AHR in mice submitted to a B-cell immunosuppressive protocol. This difference was neither due to their serum levels in reconstituted mice, as demonstrated here, nor to their antigen-binding capacities, as previously shown by the identical titration curves obtained in ELISA by using DNP-BSA-coated plates and biotinylated goat anti-mouse IgG1.²² The dose of antibody used for reconstitution was also 15-fold greater than the minimal dose of IgG1 used by Oshiba et al¹¹ to passively transfer AHR, and therefore the results obtained in nonanaphylactic IgG1-reconstituted mice cannot be ascribed to an insufficient amount of this antibody. The 2 types of IgG1 differ in their mast

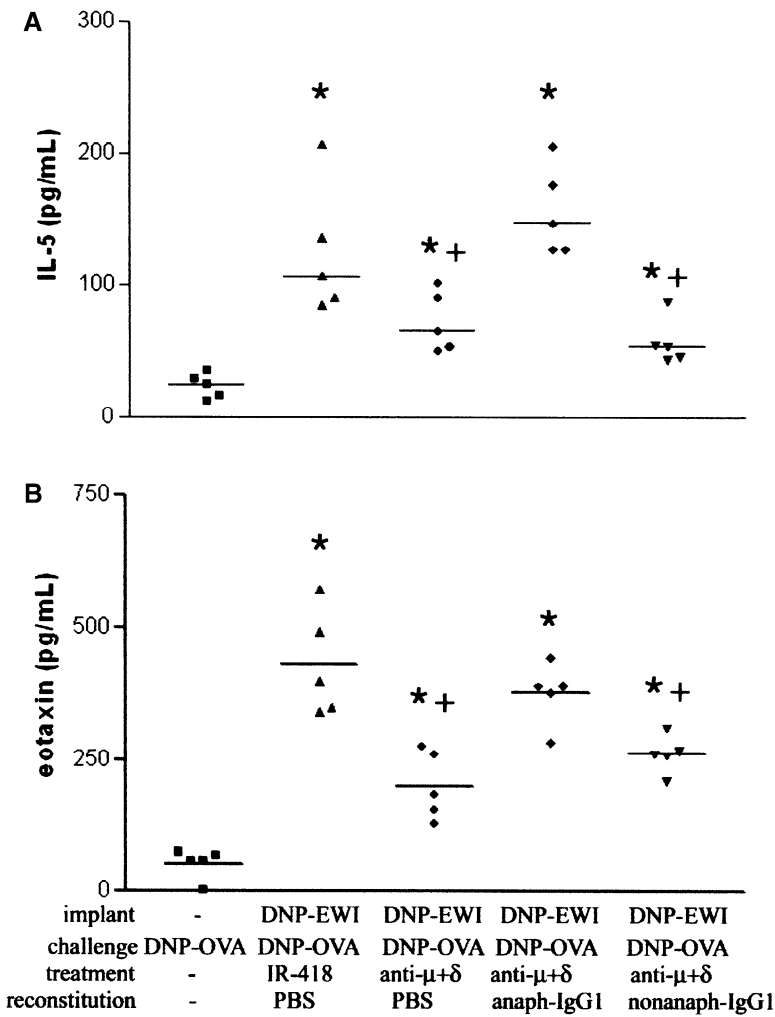


FIG 3. Comparison between IL-5 and eotaxin levels in BAL fluid from anaphylactic and nonanaphylactic IgG1-reconstituted mice. Two days after challenge, BAL fluid from all groups of mice were obtained for individual measurement of IL-5 (**A**) and eotaxin (**B**) by means of ELISA (horizontal bars, mean values). +*P* < .05 compared with the IR-418-treated group; **P* < .05 compared with the untreated group.

cell-binding and mast cell-activating properties and were identified in immunization protocols by using different antigens, as well as among IgG1-producing hybridoma cell lines.^{20,22} Reconstitution of immunosuppressed mice with anaphylactic IgG1 dramatically potentiated (more than 4-fold) airway eosinophilic inflammation, as did reconstitution with IgE,¹⁹ despite the significant number of eosinophils already present in BAL fluid from immunosuppressed or nonanaphylactic IgG1-reconstituted mice compared with nonimmunized mice. These latter results confirm that sensitization is sufficient for induction of late-phase inflammation, but the presence of antibodies, such as anaphylactic IgG1 and IgE, might determine the magnitude of this response. Likewise, Mayr et al,⁵ using FcεRIα chain-deficient mice in 2 models of murine asthma, demonstrated that IgE-dependent mast cell activation potentiates allergic airway inflammation and AHR. However, there is no doubt that late-phase reactions can be obtained in antibody-free models.^{4,30}

Eosinophil accumulation in tissues is orchestrated by IL-5 and eotaxin,^{31,32} and both IL-5 and eotaxin levels were found to be proportional to total cell and eosinophil numbers in BAL fluid from our experimental groups. Infiltration of T_H2 cells into the submucosal tissue of airways and release of T_H2-derived cytokines, mainly IL-4 and IL-5, control eosinophil recruitment and function in the asthma process.³³ In addition, beside their ability to secrete mediators that directly mediate inflammatory reactions, mast cells activated by cross-linking of their surface IgE or FcεRI can produce a variety of cytokines that partly overlap with the cytokine pattern of T_H2 cells.³⁴ IL-4 and TNF-α release by bone marrow-derived mast cells is also evoked by FcγRIII aggregation.³⁵ Because these cytokines increase the expression of vascular adhesion molecules, leading to a selective cell-type recruitment, activation of mast cells by cross-linking of anaphylactic IgG1 through antigen might be responsible for T_H2, and, consequently, eosinophil influx into the

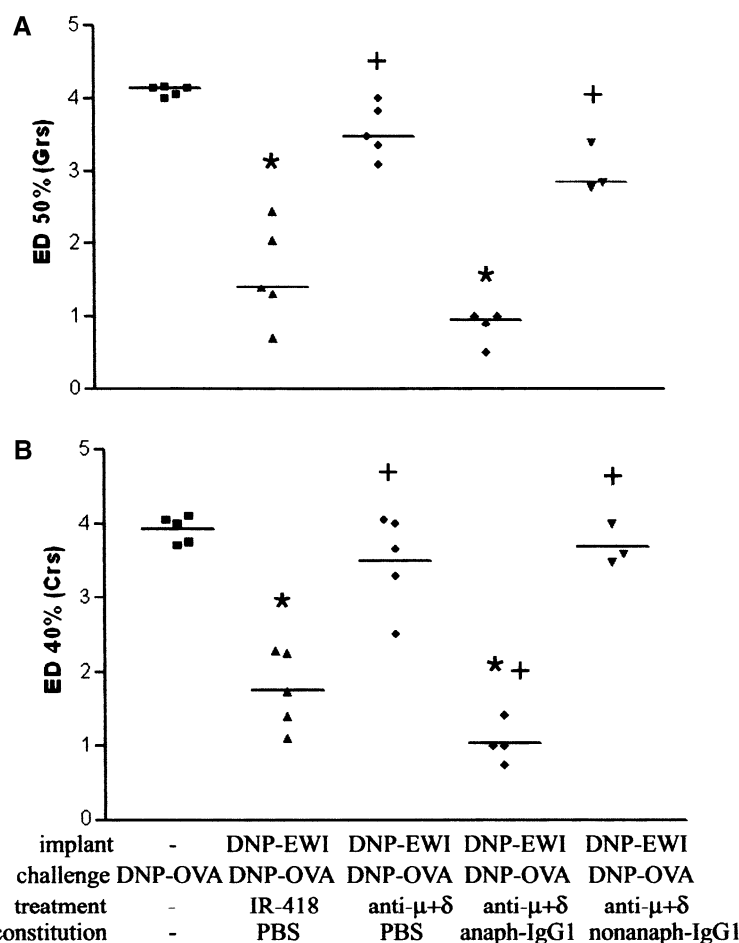


FIG 4. Effect of IgG1 reconstitution on conductance (**A**) and dynamic compliance (**B**) of the respiratory system. Two days after challenge, mice were intravenously injected with different doses of methacholine, and results were expressed as the dose (log) required to reduce Grs (ED50%) and Crs (ED40%; horizontal bars, mean values). + P < .05 compared with the IR-418-treated group; * P < .05 compared with the untreated group.

airways of immunosuppressed mice reconstituted with this type of antibody. Using IgE transgenic mice, Maezawa et al³⁶ showed that IgE-dependent mast cell activation recruits primarily CD4⁺ T cells and not eosinophils and further enhances antigen-specific T_H2-mediated eosinophil recruitment into the site of allergic inflammation. Therefore the presence of T_H2 cells among the cells infiltrating the lung tissue and airways from mice reconstituted with anaphylactic IgG1 and their further activation by antigen would favor a higher local concentration of IL-5 in this group compared with that seen in the nonanaphylactic IgG1-reconstituted group, potentiating the eosinophilic influx. As cellular sources of eotaxin production, strong eotaxin staining was found on sputum macrophages and epithelial cells from asthmatic patients, with weak staining seen on eosinophils.³⁷ In a mouse model of OVA-induced pulmonary eosinophilia, the principal cells producing eotaxin were also resident lung cells, mainly epithelial cells and alveolar macrophages, and CD4⁺ T cells did not regulate the expression of this chemokine.³⁸ On the basis of these findings and

taking into account the presence of high levels of eotaxin in our anaphylactic IgG1-reconstituted mice, mast cells seem to be good candidates capable of positively regulating eotaxin production by lung resident cells in our model.

Nevertheless, mast cells are not the only cells with Fc γ RIII in the lung. Strait et al¹² reported that systemic anaphylaxis in response to antigen challenge was obtained in mice injected with goat anti-mouse IgD through an alternative pathway involving IgG, Fc γ RIII, and macrophages. Although this mechanism has not been described to our knowledge in any model of murine asthma, we cannot exclude that alveolar macrophages might play a role in anaphylactic IgG1-reconstituted mice. Further experiments carried out in mast cell-deficient mice might elucidate this subject.

The correlative association between eosinophils and AHR has been documented in several mammalian species, including human subjects.³⁹⁻⁴¹ However, in mouse models the participation of eosinophils in the development of AHR is not clear. Anti-IL-5 antibody treatment

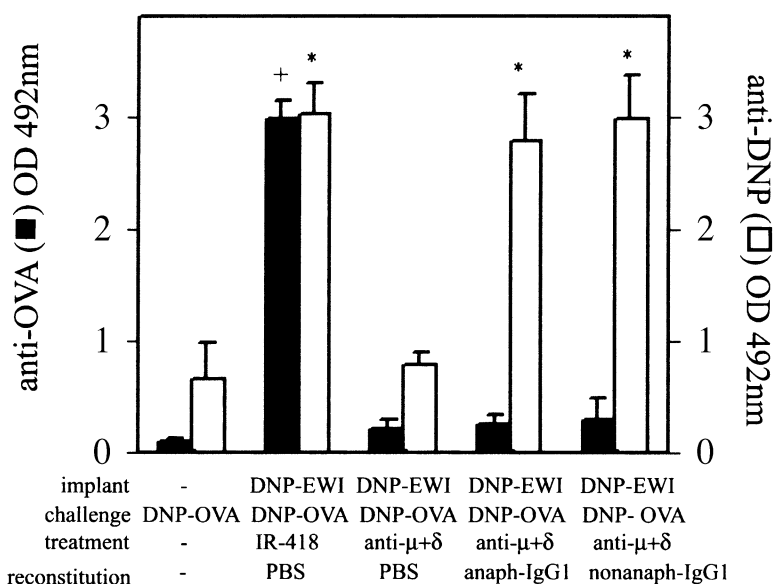


FIG 5. OVA- and DNP-specific antibody levels in immunosuppressed or IgG1-reconstituted mice. Two days after challenge, mice were bled, and antibody levels were determined by means of ELISA. Data represent the means $\pm$ SEMs for 4 to 5 animals per group. * $P < .05$ compared with the respective untreated group.

inhibits airway eosinophilia, but not AHR.⁴²⁻⁴⁴ In contrast, IL-5-deficient mice, in which eosinophilia does not develop, also do not have AHR, supporting a causal relationship between eosinophilic airway inflammation and AHR.⁴⁵ Hogan et al⁴⁶ proposed that other eosinophil-independent pathways might sometimes mask the contribution of these cells to the development of AHR. In our model the respiratory mechanical parameters obtained in anaphylactic IgG1-reconstituted mice showed increased lung responsiveness to methacholine similar to that obtained in nonsuppressed mice. It is interesting that although some eosinophilic inflammation was still present in immunosuppressed and nonanaphylactic IgG1-reconstituted mice, no AHR was observed in these groups. These findings seem to be related to the severity of local inflammation and indicate that the presence of anaphylactic antibody-sensitized mast cells is essential for the development of AHR in our model. As previously reported, the extent to which mast cells contribute to AHR in mice is highly dependent on the experimental model used to generate the airway response.^{44,47} Mayr et al⁵ showed recently in FcεRI-deficient mice that there might be a synergy between mast cells and eosinophils in the induction of AHR.

In conclusion, we demonstrated here for the first time that the anaphylactic property of murine IgG1 proved crucial to its capacity to potentiate lung eosinophilic inflammation and to induce AHR.

REFERENCES

- Wasserman SI. Basic mechanism in asthma. *Ann Allergy* 1988;60:477-82.
- Wasserman SI, Olivenstein R, Renzi P, Xu LJ, Martin JG. The relationship between late asthmatic responses and antigen-specific immunoglobulin. *J Allergy Clin Immunol* 1992;90:661-9.
- Zweiman B. The late-phase reaction: role of IgE, its receptor and cytokines. *Curr Opin Immunol* 1993;5:950-5.
- Hamelmann E, Takeda K, Oshiba A, Gelfand EW. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness—a murine model. *Allergy* 1999;54:297-305.
- Mayr SI, Zuberi RI, Zhang M, de Sousa-Hitzler J, Ngo K, Kuwabara Y, et al. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. *J Immunol* 2002;169:2061-8.
- Schleimer RP, Mac Glashan DW, Peters SP, Pinckard RN, Adkinson NF, Lichtenstein LM. Characterization of inflammatory mediator release from purified human lung mast cells. *Am Rev Respir Dis* 1986;133:614-7.
- Broide DH, Gleich GJ, Cuomo AJ, Coburn DA, Federman EC, Schwartz LB, et al. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J Allergy Clin Immunol* 1991;88:637-48.
- Watson JW, Conklyn MJ, Showell HJ. IgG1 mediated acute pulmonary hypersensitivity response in the guinea-pig. *Am Rev Respir Dis* 1990;142:1093-8.
- Oettgen HC, Martin TR, Wynshaw BA, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* 1994;370:367-70.
- Hazenbos WL, Gessner JE, Hofhuis FMA, Kuipers H, Meyer D, Heijnen IA, et al. Impaired IgG-dependent anaphylaxis and Arthus reaction in FcγRIII (CD16) deficient mice. *Immunity* 1996;5:181-8.
- Oshiba A, Hamelmann E, Takeda K, Bradley KL, Loader JE, Larsen GL, et al. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *J Clin Invest* 1996;97:1398-408.
- Strait RT, Morris SC, Yan M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109:658-68.
- Crosby JR, Cieslewicz G, Borchers M, Hines E, Carrigan P, Lee JJ, et al. Early phase bronchoconstriction in the mouse requires allergen-specific IgG. *J Immunol* 2002;168:4050-4.
- Mota I, Wong D, Sadun EH. Mouse homocytotropic antibodies: specific differentiation between 7S γ1 and mouse reagin-like antibodies. *Life Sci* 1968;7:1289-92.
- Daeron M, Provost DA, Voisin GA. Mast cell membrane antigens and Fc receptors in anaphylaxis. II. Functionally distinct receptors for IgG and IgE on mouse mast cells. *Cell Immunol* 1980;49:178-89.
- Miyajima I, Dombrowski D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. *J Clin Invest* 1997;99:901-14.

17. Wakayama H, Hasegawa Y, Kawabe T, Saito H, Kikutani H, Shimokata K. IgG-mediated anaphylaxis via Fc gamma receptor in CD40-deficient mice. *Clin Exp Immunol* 1998;114:154-60.
18. Gaur A, Yao X, Scott DW. B cell tolerance induction by cross-linking of membrane IgM but not IgD and synergy by cross-linking both isotypes. *J Immunol* 1993;150:1663-9.
19. Itami D, Latinne D, Bazin H, Garcia MLB, Perini A, Martins MA, et al. IgE is not required for but enhances airway inflammation and hyperresponsiveness. *Allergy* 2003;58:1117-24.
20. Faquim-Mauro EL, Coffman RL, Abrahamsohn IA, Macedo MS. Mouse IgG1 antibodies comprise two functionally distinct types that are differentially regulated by IL-4 and IL-12. *J Immunol* 1999;63:3572-6.
21. Faquim-Mauro EL, Macedo MS. Induction of IL-4-dependent, anaphylactic-type and IL-4-independent, non-anaphylactic-type IgG1 antibodies is modulated by adjuvants. *Int Immunol* 2000;12:1733-40.
22. Faquim-Mauro EL, Jacysyn JF, Macedo MS. Anaphylactic and non-anaphylactic murine IgG1 differ in their ability to bind to mast cells: relevance of proper glycosylation of the molecule. *Immunobiology* 2003;207:169-78.
23. Faquim-Mauro EL, Almeida IC, Macedo MS. Characterization of anaphylactic and non-anaphylactic IgG1 antibodies: differential binding to mast cell Fc gammaRIII and to lectins. *Rev Soc Bras Med Trop* 2001;34(suppl II):58.
24. Titus RG, Chiller JM. A simple and effective method to assess murine delayed-type hypersensitivity to proteins. *J Immunol Methods* 1981;45:65-78.
25. Strath M, Warren DJ, Sanderson CJ. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J Immunol Methods* 1985;83:209-15.
26. Zar JH. Biostatistical analysis. Englewood Cliffs (NJ): Prentice-Hall; 1984.
27. 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.
28. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434-57.
29. Mehlhop PD, Van De Rijn M, Goldberg AB, Brewer JP, Kurup VP, Martin TR, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci* 1997;94:1344-9.
30. Kaminuma O, Mori A, Ogawa K, Nakata A, Kikkawa H, Naito K, et al. Successful transfer of late phase eosinophil infiltration in the lung by infusion of helper T cell clones. *Am J Respir Cell Mol Biol* 1997;16:448-54.
31. Mould AW, Matthei KI, Young IG, Foster PS. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 1997;99:1064-71.
32. Wang J, Palmer K, Lotvall J, Milan S, Lei XF, Matthei KI, Gauldie J, et al. Circulating, but not local lung, IL-5 is required for the development of antigen-induced airways eosinophilia. *J Clin Invest* 1998;102:1132-41.
33. Kon OM, Kay AB. T cells and chronic asthma. *Int Arch Allergy Immunol* 1999;118:133-5.
34. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997;77:1033-97.
35. Yuasa T, Ono M, Watanabe T, Takai T. Lyn is essential for fc gamma receptor III-mediated systemic anaphylaxis but not for the Arthus reaction. *J Exp Med* 2001;193:563-72.
36. Maezawa Y, Nakajima H, Kumano K, Kubo S, Karasuyama H, Iwamoto I. Role of IgE in Th2 cell-mediated allergic airway inflammation. *Int Arch Allergy Immunol* 2003;131(suppl 1):2-6.
37. Park SW, Kim DJ, Chang HS, Park SJ, Lee YM, Park JS, et al. Association of interleukin-5 and eotaxin with acute exacerbation of asthma. *Int Arch Allergy Immunol* 2003;131:283-90.
38. Gonzalo J-A, Lloyd CM, Kremer L, Finger E, Martinez-A C, Siegelman MH, et al. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines and adhesion receptors. *J Clin Invest* 1996;98:2332-45.
39. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. *Annu Rev Med* 1993;44:85-101.
40. Lefort J, Nahori MA, Ruffie C, Vargaftig BB, Pretolani M. In vivo neutralization of eosinophil-derived major basic protein inhibits antigen-induced bronchial hyperreactivity in sensitized guinea pigs. *J Clin Invest* 1996;97:1117-21.
41. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470-3.
42. Nagai H, Yamaguchi S, Maeda Y, Tanaka H. Role of mast cells, eosinophils and IL-5 in development of airway hyperresponsiveness in sensitized mice. *Clin Exp Allergy* 1996;26:642-7.
43. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996;183:109-17.
44. Kobayashi T, Miura T, Haba T, Sato M, Serizawa I, Nagai H, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J Immunol* 2000;164:3855-61.
45. Foster PS, Hogan SP, Ramsay AJ, Matthei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
46. Hogan SP, Matthei KI, Young JM, Koshinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 1998;161:1501-9.
47. Williams CMM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000;192:455-62.