

Original article

Immunoglobulin E is not required for but enhances airway inflammation and hyperresponsiveness

The aim of this study was to investigate the role of immunoglobulin E (IgE) in the late phase reaction (LPR) of murine experimental asthma. Our model consisted of an implant of DNP-conjugated, heat-coagulated hen's egg white (DNP-EWI), followed 14 days later by an intratracheal challenge with aggregated DNP-ovalbumin. Airway inflammation was analyzed 48 h after challenge and compared with a similarly immunized group of mice with highly suppressed humoral response due to anti- μ and anti- δ antibody treatment. Total number of cells in the bronchoalveolar lavage (BAL) (with predominance of eosinophils) and EPO activity in the lung homogenate were increased in the DNP-EWI-immunized group compared with immunosuppressed or nonimmunized mice. However, the cellular infiltration and EPO activity observed in the immunosuppressed group were still significantly above those obtained in the nonimmunized group, indicating that inhibition of antibody production did not completely prevent the inflammatory manifestations in BAL and lung. Airway hyperresponsiveness to methacholine was obtained in DNP-EWI-immunized mice, but the respiratory mechanical parameters returned to normal levels in the immunosuppressed group. When these mice were reconstituted with monoclonal anti-DNP antibodies, only IgE, but not IgG1, restored lung inflammation and decreased the conductance of the respiratory system, therefore, increasing hyperresponsiveness. These results indicate that antibodies are not essential for induction of LPR in the lung. However, IgE enhances pulmonary inflammation and hyperresponsiveness.

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Clinical manifestations of asthma are the result of three basic physiopathologic events within the airways: reversible obstruction, augmented bronchial responsiveness to a variety of physical and chemical stimuli (airway hyper-reactivity), and inflammation. It is now widely accepted that chronic inflammation of the bronchial mucosal lining plays a fundamental role in the genesis of these clinical manifestations. The most striking feature of the histopathology of asthma is the intense infiltration of the bronchial mucosa with many inflammatory cells like eosinophils, neutrophils, macrophages, and lymphocytes. These cells and their products account for the chronic inflammation and tissue damage observed in the airways. The pathology of asthma is thought to be mediated by CD4⁺T lymphocytes producing the type-2 cytokines interleukin (IL)-4 and IL-5 that are elevated in bronchial biopsies (1), bronchoalveolar lavage (BAL) (2), and blood

(3) of allergic patients. These cytokines promote the accumulation and activation of eosinophils (4, 5) as well as IgE synthesis by B cells (6).

In contrast to the recognized importance of Th2 cells, cytokines, and eosinophils, the role of IgE in chronic or persistent airway inflammation and hyperresponsiveness is not well defined. It is well documented that bronchial hyperreactivity and airway inflammation in asthmatic patients are associated with high levels of total and antigen-specific IgE (7, 8). A number of investigations based on murine models have pointed toward a central role of IgE in asthma pathogenesis. For example, the passive transfer of IgE⁺ B cells or anti-ovalbumin (OVA) IgE antibody resulted in OVA-inducible bronchial hyper-reactivity upon challenge with nebulized OVA (9, 10). Other studies have shown that eosinophilic inflammation and bronchial hyperreactivity occur even in the absence of IgE (11, 12).

The purpose of this study was to correlate the levels of serum antibodies with pulmonary cell infiltration and airway responsiveness. Using a new murine model of asthma (13) we investigated the effect of different antibody isotypes on the induction of pulmonary

Abbreviations: BAL, bronchoalveolar lavage; Crs, compliance of respiratory system; DNP-EWI, 2,4 dinitrophenol-coupled, egg white implant; Grs, conductance of respiratory system; LPR, Late-phase reaction; mIg, membrane immunoglobulin; OVA, Ovalbumin; PBS, Phosphate buffered saline.

hypersensitivity in immunosuppressed mice. These mice were obtained by anti- μ plus anti- δ antibody treatment prior to and during immunization. B cell tolerance by crosslinking of sIg has been extensively studied since the early 1980s. In adult mice, a synergic effect of simultaneous crosslinking with anti- μ and anti- δ antibodies on the induction of B cell unresponsiveness was observed (14).

Material and methods

Animals

Male BALB/c mice (6–8 weeks of age) were obtained from a colony at the Instituto Butantan, São Paulo, Brazil, and maintained in our own animal facilities.

Preparation of DNP conjugated EWI and aggregated DNP-OVA

Two hundred microliters of 1 M 2,4 dinitrobenzenesulfonic acid sodium salt (Eastman Kodack Co, Rochester, NY) diluted in carbonate buffer pH 9.2 was introduced into a chicken egg that was incubated overnight at room temperature with constant agitation. The egg was then placed in a water bath at 100°C for 20 min. Solidified egg white coupled with DNP was washed several times in distilled water, dehydrated in ethanol for 24 h and cut into small blocks with 40 ± 5 mg. The egg white implants (DNP-EWI) were re-hydrated by immersion in phosphate buffered saline (PBS) at room temperature before use.

Aggregated OVA was prepared according to Titus and Chiller (15). Type II OVA diluted in PBS pH 7.2 was incubated for 1 h at 80°C. After centrifugation at 3000 *g* for 10 min, the pellet was resuspended with 1 M 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 three times with sterile PBS and used for challenge.

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 immunosuppressed or normal 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 the serum antibody response and the cellular infiltration in lung and BAL. The control group consisted of animals not implanted with DNP-EWI.

Treatment of mice with anti-membrane Ig monoclonal antibodies

In order to suppress the antibody production, groups of BALB/c mice were treated with 150 μ g of anti- μ (LO-MM-9) and anti- δ (LO-MD-6) monoclonal antibodies, i.p., each other day, beginning 1 day before immunization. A control group received 300 μ g of IR-418, which is a rat immunoglobulin of IgG2a-*k* isotype (same isotype as LO-MM-9 and LO-MD-6). On day 7 and 14, some animals were injected intravenously with 30 μ g of mouse monoclonal IgG1 (MADNP-1) or IgE (LB4) anti-DNP antibody.

The rat MoAb against mouse IgM or IgD and the rat MoIg IR-418 were provided by the Experimental Immunology Unit (UCL, Brussels) and mouse MoAbs against DNP were a gift from Dr Van Snick (UCL, Brussels).

Bronchoalveolar lavage

The animals were killed on the second day after challenge by an overdose of chloral hydrate i.p. The trachea was cannulated and BAL obtained by washing the airway lumina with 6×0.5 ml of sterile PBS at 4°C.

Total and differential cell counts

The BAL fluid was centrifuged at 170 *g* for 10 min and the cell pellet was resuspended in 0.5 ml of sterile PBS. The total number of viable cells was determined in a Neubauer counting chamber using trypan blue solution. Differential cell counts were performed in cytocentrifuge preparations of BAL stained with HEMA 3 (Biochemical Sciences Inc., Swedesboro, NJ).

Measurement of serum antibodies

Determination of serum antibody levels was performed by enzyme-linked immunosorbent assay (ELISA) using 96-well plates coated with OVA or DNP-BSA. Peroxidase conjugated rat monoclonal antibodies against mouse kappa chain (LO-MK-1), γ 1 chain (LO-MG1-3), γ 2a chain (LO-MG2a-1), γ 2b chain (LO-MG2b-2), γ 3 chain (LO-MG3-13), or μ chain (LO-MM-8) were used as secondary antibody. The reactions were developed with orthophenylenediamine (OPD; Sigma Chemical Co., St Louis, MA) and H_2O_2 (Sicalab, SP, Brazil). The plates were read at 492 nm on an automated ELISA reader (Titertek-Multiskan, Flow Labs, Helsinki, Finland). All rat monoclonal antibodies against mouse Ig used were developed in the Experimental Immunology Unit (UCL, Brussels) and are commercially available (Serotec, Kidlington, UK or Zymed Laboratory, San Francisco, CA).

Assay of eosinophil peroxidase activity

The EPO activity in lung homogenates was measured according to the method described by Strath et al. (16). Briefly, after perfusion with 10 ml of PBS containing 10 mM ethylenediaminetetraacetic acid, lungs were homogenized with 5% of Hank's balanced salt solution containing 10 mM of HEPES (Sigma). The cellular suspension was then centrifuged at 1050 *g* for 10 min and the pellet was resuspended with 5% of Hank's Balanced Salt Solution (HBSS) containing 0.5% of hexadecyldimethylammonium bromide (Sigma). The cellular membrane was disrupted by heat shock (37 and -70°C , three times).

To measure the enzymatic activity, 150 μ l of each sample was added to a 96-well microplate with 75 μ l of substrate containing 1.5 mM OPD and 6.6 mM H_2O_2 . The plates were left at room temperature for 30 min before stopping the reaction by the addition of 75 μ l of 4 M sulfuric acid. The absorbance was read at 492 nm using a spectrophotometer Titertek Multiskan (Flow Labs.).

Pulmonary function measurements

Conductance of the respiratory system (Grs) and dynamic compliance (Crs) were monitored in anesthetized, tracheotomized, ventilated mice in a Harvard 683 apparatus (Harvard Apparatus, South Natick, MA). Different doses of methacholine (from 0.01 to 10 mg/kg) were administered via a silastic catheter tied into a jugular vein and data were stored at 30 s, 1, 2, 3 and 4 min after agonist injection. Whole body plethysmograph pressure (from which changes in lung volume were derived) and tracheal pressure were transduced by differential pressure transducers. The outputs were amplified and

transferred to a computer in which six to 10 consecutive breaths were stored to average one data point. The Grs and Crs peak response to each agonist dose was expressed as percentage of the baseline value obtained before the next dose of agonist. The dose of methacholine required to reduce Grs and Crs to 60% of their control values was estimated from the log dose–peak response curve and chosen as our data point.

Statistical analysis

All values are expressed as mean $\pm$ SEM. Parametric data were evaluated using one way analysis of variance, followed by the Tukey test for multiple comparisons. Nonparametric data were assessed using Kruskal–Wallis one-way analysis of variance on ranks, followed by Dunn's test for multiple comparisons. A P value ≤ 0.05 was accepted as statistically significant. The statistical software SigmaStat 2.0 for Windows (Jandel Scientific, CA) was used.

Results

Isotype profile of antibody response induced by DNP-EWI implant

In order to determine the isotypes produced in our model, mice implanted with DNP-EWI and challenged with DNP-OVA or PBS on day 14 were bled 2 days later and the sera were analyzed by ELISA (Fig. 1).

The results showed a preferential production of IgM, IgG1 and IgE antibodies against OVA and DNP. We also observed that 48 h after challenge there was no difference between groups of mice that received DNP-OVA or PBS,

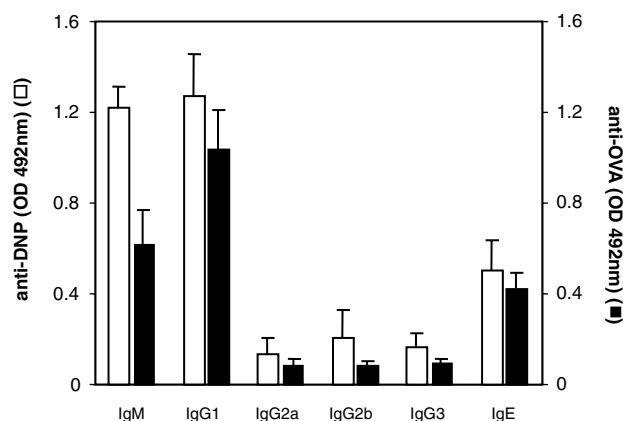


Figure 1. Profile of antibody production in murine experimental asthma. Groups of mice were implanted with solidified, DNP-conjugated hen egg-white (DNP-EWI) and 14 days later received an intratracheal instillation of 0.6 mg of aggregated DNP-OVA (□) or PBS (■). The animals were bled 48 h after challenge and serum levels of different isotypes of anti-OVA (A) or anti-DNP (B) antibodies were tested by enzyme-linked immunosorbent assay. Data represent mean and SEM (five to six mice per group) of 1 : 20 [immunoglobulin M (IgM), IgG2a IgG2b and IgG3], 1 : 80 (IgE), or 1 : 640 (IgG1) serum dilutions.

indicating that challenge had not affected the primary antibody response yet (data not shown).

Effect of the presence of antibodies on pulmonary inflammation

Groups of BALB/c mice implanted with DNP-EWI were treated with anti-membrane immunoglobulin (mIg) or IR-418 MoAb. Two groups treated with the former MoAb were reconstituted with IgE or IgG1 anti-DNP antibodies. Forty-eight hours after challenge with DNP-OVA, the animals were bled and BAL was obtained. The total number of cells on BAL as well as the serum antibody levels are presented in Fig. 2.

Animals implanted with DNP-EWI and challenged with DNP-OVA that received only control Ig (IR-418) had a marked anti-DNP antibody production as well as pulmonary inflammation measured by the high number of cells in BAL compared with the control group.

Treatment with anti-mIg results in a great dampening of the antibody serum level and a marked reduction of cellular infiltration in the BAL. It is interesting to notice that although there was no difference in antibody production between suppressed and control group, the number of cells in BAL was significantly different.

When the suppressed animals were reconstituted with different antibody isotypes, IgE, but not IgG1 antibodies, promoted a great cellular migration to the lung. In the presence of IgE, the number of cells in BAL was

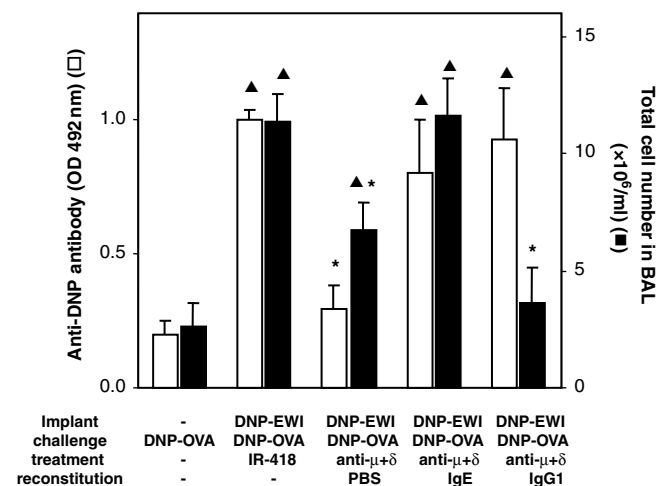


Figure 2. Effect of antibodies on late phase reaction of murine asthma. Anti-DNP antibody level (□) and number of cells in the bronchoalveolar lavage (■) of groups of BALB/c mice implanted with DNP-coupled, egg white implant (s.c.) and 14 days later challenged with aggregated DNP-ovalbumin (i.t.). Some animals were also treated (i.v.) with anti-μ + anti-δ MoAb or control Ig (IR-418). The mice treated with anti-mIg MoAb was divided into three groups injected with phosphate buffered saline or anti-DNP antibody [immunoglobulin E (IgE) or IgG1]. Data represent mean $\pm$ SEM of four to five animals per group. * $P < 0.05$ compared with IR-418-treated group. $\blacktriangle P < 0.05$ compared with control group.

Table 1. Cellular infiltration in the bronchoalveolar lavage (BAL)

Implant	Challenge	Treatment	MoAb	Cell number ($\times 10^6$)			
				Total	Mononuclear	Eosinophil	Neutrophil
—	DNP-OVA	—	—	3.25 ± 1.20	2.75 ± 1.01	0.20 ± 0.07	0.30 ± 0.11
DNP-EWI	DNP-OVA	IR-418	—	$14.1 \pm 1.46^\dagger$	4.89 ± 1.37	$7.63 \pm 2.13^\dagger$	$1.58 \pm 0.44^\dagger$
DNP-EWI	DNP-OVA	anti- $\mu + \delta$	—	$8.30 \pm 1.55^{*\dagger}$	3.54 ± 0.66	$3.15 \pm 0.59^{*\dagger}$	$1.61 \pm 0.30^\dagger$
DNP-EWI	DNP-OVA	anti- $\mu + \delta$	IgE	$14.4 \pm 2.00^\dagger$	4.93 ± 0.69	$6.40 \pm 0.90^\dagger$	$3.07 \pm 0.43^{*\dagger}$
DNP-EWI	DNP-OVA	anti- $\mu + \delta$	IgG1	$4.70 \pm 1.50^*$	1.30 ± 0.41	$2.45 \pm 0.78^{*\dagger}$	$0.95 \pm 0.30^\dagger$

Total and differential cell numbers in the BAL of BALB/c mice sensitized subcutaneously with EWI-DNP, i.t. challenged with aggregated DNP-OVA and treated with anti-IgM and anti-IgD MoAbs or with control Ig alone. The immunosuppressed mice (treated with anti- $\mu + \delta$) received i.v. injection with anti-DNP immunoglobulin E (IgE), IgG1 mAbs or phosphate buffered saline. The control group consisted of only challenged animals. The BAL was obtained 48 h after challenge and cells were counted. Data represent mean $\pm$ SD for four to five animals per group.

* $P < 0.05$ compared with IR-418-treated group.

$^\dagger P < 0.05$ compared with control mice.

DNP-EWI, DNP-coupled, egg white implant; DNP-OVA, DNP-ovalbumin.

quite the same as that observed in nonsuppressed animals (treated with IR-418) and significantly higher than the number of cells observed in the group treated with anti-mIg MoAb.

The increase of cellular infiltration in BAL of IgE-reconstituted mice was due basically to the migration of eosinophils and neutrophils (Table 1). Administration of IgG1 anti-DNP antibodies resulted in a cellular migration in BAL ($4.70 \pm 1.50 \times 10^6$) comparable with that observed in the control group ($3.25 \pm 1.20 \times 10^6$), that was significantly lower than the immunosuppressed group ($8.30 \pm 1.55 \times 10^6$). However, analysis of the cell type in BAL showed the prevalence of mononuclear cells in control animals, whereas animals injected with IgG1 anti-DNP MoAb had a typical inflammatory infiltrate with prevalence of polymorphonuclear cells. The difference between the IgG1-reconstituted group, and immunosuppressive but nonreconstituted group was due to the inhibition of mononuclear cell migration only (Table 1).

Effect of the presence of antibodies on eosinophil migration to the lung

In the lung tissue, the accumulation of eosinophils was evaluated by the levels of EPO. The EPO activity in lung homogenates of the different groups was compared with the total number of eosinophils in BAL (Fig. 3). The results showed that treatment with anti-mIg resulted in dampening of EPO activity in the lung when compared with non-suppressed animals, similar to the decrease of eosinophil numbers in BAL. The EPO activity in the lung tissue of immunosuppressed animals was significantly higher when compared with the control group. A similar result was obtained with regard to the eosinophil numbers in BAL.

In addition, injection of IgE anti-DNP antibodies promoted a significant enhancement of EPO activity in the lung tissue similar to the nonsuppressed group. The IgG1 antibodies did not change the EPO activity.

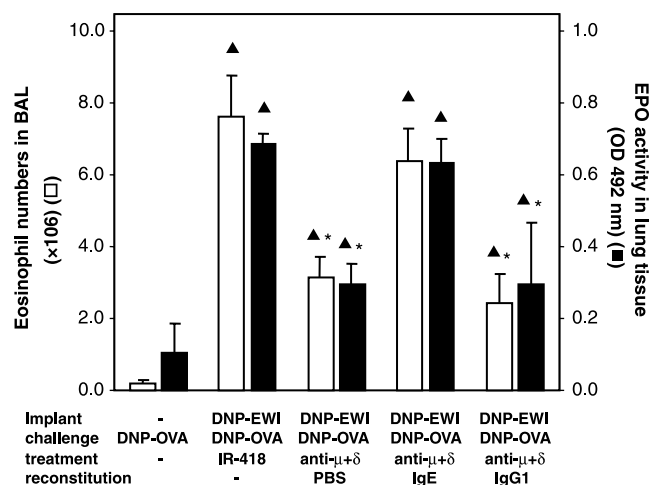


Figure 3. Effect of antibodies on the EPO activity in the lung tissue. Groups of BALB/c were implanted with DNP-EWI, challenged (i.t.) with aggregated DNP-OVA and treated with anti- μ MoAb (LO-MM9) plus anti- δ MoAb (LO-MD6) or with control Ig (IR-418). Immunosuppressed animals (treated with anti-mIg) were injected with IgE or IgG1 anti-DNP MoAb or phosphate buffered saline, 7 and 14 days after immunization. In the control group mice were only challenged. The EPO activity was determined in the homogenate of the lung tissue 48 h after challenge. The data represent mean $\pm$ SEM of five animals per group. * $P < 0.05$ compared with IR-418-treated group. $^\Delta P < 0.05$ compared with control group.

Role of IgE antibodies in airway responsiveness

Airway hyperreactivity was evaluated in a dose-response curve to methacholine. Values of compliance (Cr_s), which represents distal bronchial response and conductance (Gr_s), which represents airway mechanical data, were obtained by volume, airflow, and pressure data. The results in Fig. 4 are expressed as log of the dose of methacholine that reduced Cr_s and Gr_s to 60% of the baseline values. It can be observed that lower

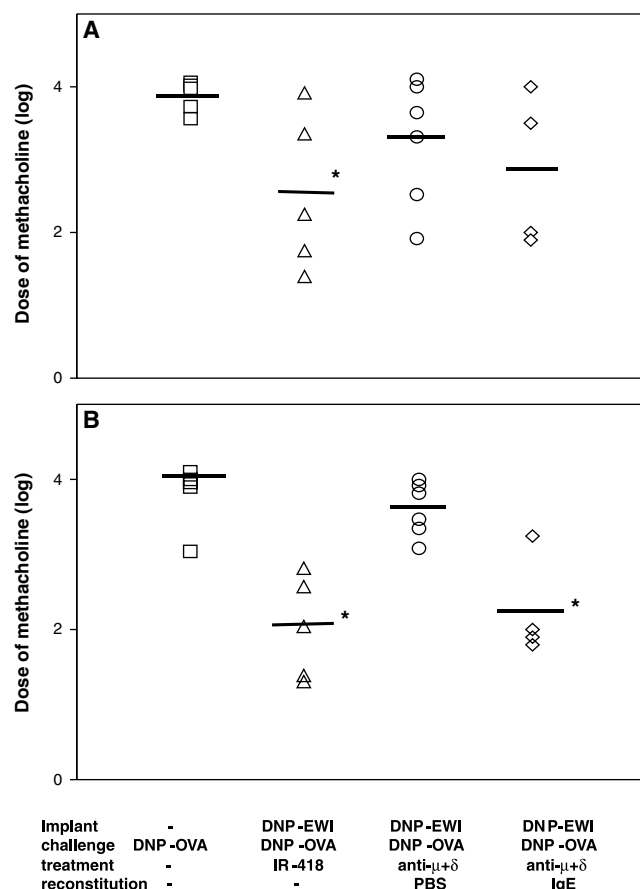


Figure 4. Effect of immunoglobulin E on dynamic compliance (A) and conductance (B) of the respiratory system. BALB/c mice sensitized with DNP-EWI were challenged 14 days later with aggregated DNP-OVA and treated with anti- μ and anti- δ MoAb or IR-418 for suppression of the humoral immune response ($n = 4-6$). All the animals were anesthetized and ventilated 48 h after challenge and different doses of methacholine (0.01–10.0 mg/Kg) were injected into the jugular vein. The results represent the concentration of intravenous methacholine required to reduce Crs and Grs to 60% of baseline values. * $P < 0.05$ compared with control and immunosuppressed groups.

doses of the agonist were required to reduce Crs (Fig. 4A) and Grs (Fig. 4B) in the DNP-EWI-immunized, nonsuppressed mice compared with the control mice, indicating the induction of lung hyperresponsiveness in the former group. In contrast, the immunosuppressed mice did not show significantly different Crs60% and Grs60% values compared with the same control group. When the immunosuppressed animals were reconstituted with IgE anti-DNP antibodies, they again needed lower doses of methacholine to promote a reduction of Grs to 60% of baseline values, pointing out the enhancing activity of IgE in airway hyperresponsiveness. The Crs60% value in this group showed only a lower tendency.

Discussion

In order to analyze the role of antibodies in the late phase of immediate hypersensitivity reactions, we used a murine protocol of asthma consisting of implants of a fragment of heat-coagulated egg white coupled with the hapten DNP. In this kind of protocol, the antigen persists for a longer period in the animals, which resembles the continuous exposure to allergens present in the environment. In addition, it induces persistent lung lesions due to the presence of a large number of eosinophils in the parenchyma around the small bronchi and also in the alveoli (13). In our experimental groups, the analysis of pulmonary inflammation based on cellular infiltration in BAL showed that 48 h after challenge sensitized animals had a total number of cells four to fivefold greater than the control group (not implanted), consisting predominantly of eosinophils (50%). High levels of EPO activity in the lung tissue confirmed the eosinophilic infiltration in the parenchyma at the same time.

The profile of antibodies produced in this model (predominately IgG1, IgE, and IgM) as well as the eosinophilic inflammation in the lung indicates that type 2 CD4+ T cells are implicated in the observed immune response. The pattern of cytokines produced by Th2 cells promotes proliferation of mast cells, stimulates IgE and IgG1 production and facilitates eosinophil migration, activation, and survival (17). The blockage of Th2 development by IL-12 administration *in vivo* inhibits both the allergen-induced eosinophilia and bronchial hyperactivity (18).

The effect of different antibody isotypes on airway inflammation and hyperresponsiveness was further tested by introducing a B-cell immunosuppressive protocol that consisted of several injections of anti- μ plus anti- δ antibodies (14, 19). Thus, in the immunosuppressed animals, airway inflammation was significantly reduced in comparison with nonsuppressed animals, but remained above the control group (regarding total number of cells in BAL and EPO activity in the lung tissue), indicating that antibody is not absolutely required for induction of inflammation. However, the presence of IgE, but not IgG1, antibodies enhanced airway inflammation dramatically. Furthermore, the kind of inflammation obtained in IgE-reconstituted mice was selective, because eosinophils were predominant among the migrated cells. The number of neutrophils increased slightly in this group too. It is known that IgE-mediated mast cell triggering plays a role in several aspects of the allergic response, including neutrophil and eosinophil infiltration (20) and increased collagen gene expression (21), which are in part dependent on tumor necrosis factor- α (TNF- α) production by the mast cell. Therefore, an exacerbation of this mechanism could account for the enhancement of neutrophils in the IgE-reconstituted group compared with the IR-418-treated group of mice.

The IgE antibodies bound to high affinity IgE receptors activate mast cells after cross-linking by specific antigens,

resulting in degranulation, and synthesis and release of proinflammatory mediators and cytokines (22, 23). However, mast cell degranulation can occur even in the absence of IgE (24). Moreover, no difference was found in the eosinophilic infiltration in BAL between mast cell knockout and wild-type mice (25). Therefore, other mechanisms such as cytokine or eotaxin release by macrophages, epithelial cells, neutrophils, or eosinophils (26) could be involved in the residual inflammation that persists in our model in the absence of IgE. There is no doubt, however, that the presence of IgE antibodies enhances the eosinophilic infiltration in the lung of immunosuppressed mice to the levels observed in non-suppressed mice that received the implant.

It should be emphasized at this point that although results show that late phase reaction (LPR) is induced in the presence or absence of IgE, in line with others (9–11), the main difference is regarding the magnitude of this response which, in this model, can be compared between the immunosuppressed mice that were or were not reconstituted with IgE, under the same immunization protocol. In addition, IgE can play an important role in airway inflammation and hyperreactivity even after systemic sensitization, in contrast to conclusions drawn from other murine asthma models (12).

The severity of bronchial hyperreactivity, which is one of the major characteristics of asthma, parallels the intensity of pulmonary eosinophil infiltration (27). In the present model, mechanical lung parameters pointed out an increased responsiveness in immunized, nonsuppressed mice and in the immunosuppressed, IgE-reconstituted mice. The lung hyperresponsiveness in these groups was demonstrated by the need of lower doses of methacholine to induce the same decrease in conductance of the respiratory system (Grs60%). The same was observed with regard to dynamic lung compliance (Crs60%) in the nonsuppressed group, although a lower tendency was displayed by the IgE-reconstituted group. This result may be due to three factors: first, asthma and all pulmonary allergic diseases are characterized by symptoms predominantly in the airways, such as bronchospasm and wheeze; secondly, methacholine agonist acts predominantly in the airways and less frequently in the peripheral pulmonary tissue; and thirdly, in this experimental model, mice were immunosuppressed in adult life and antibody serum levels showed a significantly dampened pattern, but were not totally abolished, allowing our data to be underestimated. Consistent with these explanations is the observation that inflammatory infiltration by eosinophils is found not only in the airways but also in the septa (data not shown), suggesting that inflammation occurred in the central and peripheral areas as well in the lung.

Mechanical parameters are less sensitive than histologic analysis for the diagnosis of inflammation, but functional data such as lung mechanics *in vivo* represent what is implicated in patients' worsening quality of life. Therefore, the link between functional data and histologic and serum

results is fundamental in the elucidation of physiopathology of hyperreactivity and for therapeutic implications.

The correlation between Th2 cells, IgE, eosinophils and lung hyperresponsiveness has been extensively investigated (28–30). However, the participation of IgG antibodies (IgG1 in the mouse) in allergic reactions cannot be ruled out. For example, Oettgen et al. (31), working with SJL/mice, an IgE-deficient strain of mice, observed active anaphylaxis. The development of eosinophilic inflammation and airway hyperresponsiveness can occur in IgE-deficient mice, but this may result from allergen-specific IgG1 production (32). Ujike et al. (33) demonstrated that mice deficient in the inhibitory receptor for IgG (Fc γ RIIB) display enhanced IgE-mediated anaphylactic responses, whereas those deficient in Fc γ RIII have a corresponding attenuated response. Therefore, the presence of both immunoglobulins would be responsible for the modulation of anaphylactic responses.

In our system, the reconstitution of immunosuppressed mice with IgG1 antibodies did not restore lung eosinophilic inflammation or hyperreactivity, as did IgE antibodies. However, negative cutaneous tests performed with the anti-DNP IgG1 (B8401-H5) showed that this antibody is not anaphylactic (data not shown). Faquim-Mauro et al. (34) demonstrated two types of murine IgG1 as regards anaphylactic activity, a finding that could explain our results. In this sense, experiments of reconstitution of immunosuppressed mice with anaphylactic or nonanaphylactic IgG1 are now underway.

In immunosuppressed mice reconstituted with the nonanaphylactic IgG1 antibody, the total number of infiltrating cells was diminished compared with the immunosuppressed, nonreconstituted mice. This result was due to the inhibition of mononuclear cell, rather than polymorphonuclear, migration. Immunoglobulin G antibodies can interact with three cellular IgG receptors – two of which lead to cellular activation and one to inhibition. The balance of Fc γ R stimulatory and inhibitory inputs likely determines the threshold of cell activation (35). Using Fc γ RII-deficient mice, the role of this receptor in the inhibition of inflammatory responses mediated by mast cells and macrophages was demonstrated (36, 37). In mice, IgG1 binds with higher affinity to Fc γ RII than IgG2a and b (38). Therefore, in our experiments, the triggering of alveolar macrophages through this receptor could explain the dampening of mononuclear cell migration in the IgG1-reconstituted mice. As regards the absence of this effect on polymorphonuclear cell migration, mast cells may be activated through the stimulatory Fc γ RI and synthesize more chemokines and cytokines like regulated upon activation normal T expressed and secreted (RANTES), TNF- α , IL-5 and IL-8 (39), thus creating proper conditions for migration of eosinophils and neutrophils.

In conclusion, our results indicate that antibodies are not essential for the induction of LPR in the lung, but IgE enhances the magnitude of pulmonary inflammation and hyperresponsiveness.

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