

Antigen-specific Immunoglobulin-A Prevents Increased Airway Responsiveness and Lung Eosinophilia after Airway Challenge in Sensitized Mice

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Aeroallergens such as *Amb a 1* from short ragweed are important in the development of allergic airway disease. We tested the ability of a human monoclonal immunoglobulin-A (IgA) antibody specific for *Amb a 1* (A-IgA) to modulate airway responsiveness and lung eosinophilia after airway challenge with nebulized *Amb a 1* or ragweed extract in mice sensitized to *Amb a 1* or ragweed extract, respectively. A-IgA or nonspecific IgA (C-IgA) were given intranasally up to 8 h before each challenge. Allergen challenge resulted in increases in airway responsiveness, in numbers of lung eosinophils, and in *Amb a 1*-specific IgE levels. These were prevented by pretreatment with A-IgA but not with C-IgA. Decreases in IFN- γ and increases in IL-4 and IL-5 production following challenge with *Amb a 1* were also reduced by A-IgA treatment. In contrast, increases in total IgE and total IgG and in numbers of lung neutrophils after challenge were not significantly affected by A-IgA, which additionally induced increased levels of *Amb a 1*-specific IgG2a antibodies. In mice sensitized and challenged with ovalbumin (OVA), A-IgA did not affect airway responsiveness, lung eosinophilia, cytokine production, or immunoglobulin levels. These data indicate that allergen-specific IgA can prevent airway hyperresponsiveness and reduce eosinophil influx into the lungs following allergen challenge via the airways in sensitized mice, and these effects are allergen-specific. Neutralization of allergen may contribute to the effects of IgA, but the induction of allergen-specific IgG2a in A-IgA-treated mice suggests an immunomodulatory action for A-IgA. Schwarze J, Cieslewicz G, Joetham A, Sun LK, Sun WN, Chang TW, Hamelmann E, Gelfand EW. Antigen-specific immunoglobulin-A prevents increased airway responsiveness and lung eosinophilia after airway challenge in sensitized mice.

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On mucosal surfaces, secretory immunoglobulin-A (IgA) is the most abundant immunoglobulin (1), and it provides the initial specific immunologic barrier to pathogens such as viruses or to other antigens (2). Secretory IgA can specifically bind antigens and pathogens on the mucosal surface and, as a result, prevent their uptake across the mucosal membrane (3). Additionally, secretory IgA has been shown to form complexes with antigens in the lamina propria of the mucous membrane which are subsequently secreted onto the mucosal surface (4) as well as to interact intracellularly with viral antigens, preventing their replication (5). Resistance to respiratory viruses correlates with the presence of IgA antibodies specific for these pathogens in the respiratory secretions (6, 7), and IgA antibodies directed against Sendai virus were shown to neutralize this virus *in vitro* and to protect against Sendai virus infection after passive administration of the antibody to

the respiratory tract in a murine model (8). These data indicate that IgA antibodies themselves can effectively neutralize viruses on mucosal surfaces, thus preventing infection, and that this defense mechanism is of importance in naturally occurring host defense. Similarly, monoclonal IgA antibodies directed against cholera toxin have been shown to prevent both toxin binding to intestinal epithelial cells and the toxin-induced chloride secretory response (9). Considering these findings, it seems logical that IgA antibodies specific for antigens other than viruses may be used to prevent their uptake across mucous membranes, preventing access to lymphoid tissue.

Aeroallergens such as pollens, animal dander, and house dust mite all play an important role in the development and the triggering of allergic rhinitis and allergic asthma. Hypersensitivity to these allergens develops after inhalation, contact with respiratory secretions, and presumably uptake by the respiratory mucous membranes. Repeated exposure to allergen leads to inflammatory changes in the nasal and bronchial mucosa and the surrounding tissues, resulting in allergic rhinitis and asthma. Consequently, a mainstay of treatment of these diseases is the avoidance of the relevant allergens, often a difficult task. Taking this into account, the concept of "molecular avoidance" by immune exclusion using allergen-specific antibodies has recently been proposed, and a human recombinant dimeric IgA monoclonal antibody directed against *Amb a 1* (A-IgA) has been generated (10). *Amb a 1* is the major aller-

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gen in the pollen of short ragweed, a prevalent and important airborne allergen in North America (11).

In the present study, we determined whether IgA antibodies specific for this aeroallergen were capable of modifying allergic airway sensitization and the immunologic and respiratory consequences. To investigate these issues, we administered A-IgA in the respiratory tract in a murine model of airway sensitization which enabled us to assess airway responsiveness to provocation with methacholine, pulmonary inflammation, local cytokine production, and serum immunoglobulin levels.

METHODS

Animals

Female BALB/c mice, 8 to 12 wk of age, free of specific pathogens, were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were maintained on *Amb a I*-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Experimental Protocols

Mice were sensitized by intraperitoneal injection with 10 μ g of *Amb a I* obtained from Greer Laboratories (Lenoir, NC) emulsified in 2.25 mg of aluminum hydroxide from Pierce (Rockford, IL) on Days 0 and 10. They were challenged with nebulized *Amb a I* solution (500 μ g/ml, 7 ml in phosphate-buffered saline [PBS]) or with PBS as a control, using an AeroSonic ultrasonic nebulizer obtained from DeVilbiss (Somerset, PA) for 20 min daily on Days 24, 25, and 26. In separate sets of experiments, mice were immunized with ragweed extract containing 10 μ g *Amb a I* or with ovalbumin (both emulsified in alum hydroxide) and challenged with these antigens respectively in the same manner as described previously (12). In the treatment groups, a human recombinant IgA1 (α 1, κ) antibody specific for *Amb a I* from Tanox Biosystems (Houston, TX) (10) or a nonspecific human secretory IgA antibody as a control from Sigma (St. Louis, MO) were used. The purified A-IgA preparation was a mixture of mostly dimeric IgA (approximately 80% as shown by sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS–PAGE]) and monomeric IgA. Monomers were linked by murine J chains expressed by the parental myeloma Sp2/0 cell line, without secretory component. Both antibodies, in a dose of 75 μ g in 50 μ l, were administered intranasally under light anesthesia 3 or 8 h before each challenge with *Amb a I*. On Day 28, airway responsiveness was assessed and animals were killed the following day for collection of blood and the removal of peribronchial lymph nodes (PBLN) and lungs.

Determination of Airway Responsiveness

Airway responsiveness was assessed by a method we have described recently (12), using a single-chamber whole-body plethysmograph obtained from Buxco (Troy, NY). In this system, an unrestrained, spontaneously breathing mouse is placed into the main chamber of the plethysmograph and pressure differences between this chamber and a reference chamber are recorded. The resulting box pressure signal is caused by volume and resultant pressure changes during the respiratory cycle of the animal. A low-pass filter in the wall of the main chamber allows thermal compensation. From these box pressure signals the phases of the respiratory cycle, tidal volumes, and the enhanced pause (Penh) can be calculated. Penh is a dimensionless value which represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated animals (12). Penh was used as the measure of airway responsiveness in this study. In the plethysmograph, mice were exposed for 3 min to nebulized PBS and subsequently to increasing concentrations of nebulized methacholine (Sigma) in PBS using the AeroSonic ultrasonic nebulizer. After each nebulization, recordings were taken for 3 min. The Penh values measured during each 3-min sequence were averaged and are expressed for each methacholine concentration as the percentage of baseline Penh values following PBS exposure.

Lung Cell Isolation

Lung cells were isolated by collagenase digestion as previously described (13, 14) and counted with a hemocytometer. Cytospin slides were stained with Leukostat from Fisher Diagnostics (Pittsburgh, PA), and differential cell counts were performed in a blinded fashion, counting at least 300 cells under light microscopy.

Cell Preparation

Peribronchial lymph nodes were harvested and mononuclear cells were purified by passing the tissue through a stainless steel mesh, followed by density-gradient centrifugation (Organon Teknika, Durham, NC). Cells were washed three times with PBS and resuspended in RPMI 1640 medium from GIBCO (Gaithersburg, MD).

In Vitro Cytokine Production

Mononuclear cells were cultured for 48 h in 96-well round-bottom plates at a concentration of 4×10^5 cells/well in the presence of the combination of phorbol-12,13-dibutyrate (10 ng/ml) (Sigma) and ionomycin (0.5 μ M) from Calbiochem (La Jolla, CA) (P/I). Culture supernates were harvested and frozen at -20° C. The concentrations of interferon gamma (IFN- γ), interleukin-4 (IL-4), and IL-5 in the supernates were assessed by ELISA as described (15). Briefly, Immulon 2 plates from Dynatech Lab (Chantilly, VA) were coated with anti-IFN- γ -(R4-6A2), anti-IL-4- (11B11), both from Pharmingen (San Diego, CA), or anti-IL-5-antibodies (TRFK-5) kindly provided by Dr. R. Coffman (Palo Alto, CA), and blocked with PBS/10% fetal calf serum (FCS) overnight. Samples were added, biotinylated anti-IFN- γ - (XMG 1.2), anti-IL-4- (BVD6-24G2), or anti-IL-5-antibodies (TRFK-4) (all from Pharmingen) were used as detecting antibodies and the reactions were amplified with avidin–horseradish–peroxidase from Sigma. Cytokine levels were calculated by comparison with known cytokine standards from Pharmingen. The limits of detection in the assays were 4 pg/ml for each cytokine.

Measurement of Anti-*Amb a I* or Antiovalbumin (Anti-OVA) Antibody Levels

Total immunoglobulin (Ig) G and IgE levels and anti-*Amb a I*-specific or anti-OVA-specific IgE, IgG₁, and IgG_{2a} antibody levels in the serum were measured by ELISA in a modified fashion of the assay previously described for allergen-specific antibodies (14). Briefly, Immulon 2 plates were coated with 5 μ g/ml of *Amb a I* or 5 μ g/ml of OVA. After addition of serum samples, a biotinylated anti-IgE antibody (02122D) obtained from Pharmingen was used as detecting antibody and the reaction was amplified with avidin–horseradish–peroxidase from Sigma. To detect IgG₁ and IgG_{2a}, alkaline phosphatase labeled antibodies (02003 E and 02013 E) from Pharmingen were used. The anti-*Amb a I* or anti-OVA antibody titers of samples were related to an internal pooled standard which was arbitrarily assigned to be 100 ELISA units (EU). Total IgG and IgE levels were calculated by comparison with known mouse IgG or IgE standards from Pharmingen. The limits of detection were 100 pg/ml for IgE and 1 ng/ml for IgG.

Statistical Analysis

Single pairs of groups were compared by Student's *t* test; comparison of more than two groups was performed by the Tukey-Kramer honest significant difference (HSD) test, and Pearson correlations were performed. Probability values of < 0.05 were considered statistically significant. Values for all measurements are expressed as mean $\pm$ SD except for values of airway responsiveness (Penh) which are expressed as mean $\pm$ SEM.

RESULTS

Pretreatment with A-IgA Antibody Prior to Allergen Challenge Prevents Airway Hyperresponsiveness

As shown in response to sensitization and challenge with other allergens, sensitization and challenge with *Amb a I* resulted in altered airway responsiveness. Airway responsiveness to methacholine was significantly increased in mice following sensitization and airway challenge; a 10.5 ± 2.9 -fold

increase in Penh values over the response to PBS was detected following inhalation of 50 mg/ml of methacholine (Figure 1A). Mice sensitized without challenge or challenged without sensitization showed little increase in Penh. Pretreatment with A-IgA 3 h before each challenge prevented the increase in airway responsiveness caused by exposure to *Amb a 1* in sensitized mice. In contrast, pretreatment with C-IgA did not significantly alter the response to methacholine (Figure 1A). A-IgA was still effective in preventing airway hyperresponsiveness if the interval between pretreatment and challenge was increased to 8 h (Figure 1B). If whole ragweed extract was used instead of *Amb a 1*, A-IgA also prevented the increase in airway responsiveness after sensitization and challenge (Figure 1C). In mice sensitized and challenged with OVA, an unrelated allergen, A-IgA treatment did not have any effect on the development of airway hyperresponsiveness (Figure 1D).

A-IgA Treatment before Airway Challenge Reduces Eosinophil Influx into the Lung

Allergen challenge via the airways with *Amb a 1* in *Amb a 1*-sensitized mice resulted in a 3-fold increase in the number of lung eosinophils compared with mice sensitized alone and receiving PBS challenge (Figure 2). Pretreatment with A-IgA prior to each airway challenge significantly reduced the number of lung eosinophils. In mice pretreated with C-IgA, no such effect was observed (Figure 2). The reduction in numbers of eosinophils correlated with the reduction in airway responsiveness following A-IgA pretreatment ($r = 0.64$, $p < 0.001$). The numbers of neutrophils in the lungs also increased significantly after allergen challenge, but these numbers were not affected by pretreatment with A-IgA. The numbers of total lung cells, mononuclear cells, and macrophages in the lungs did not differ significantly between the groups. A-IgA pretreatment

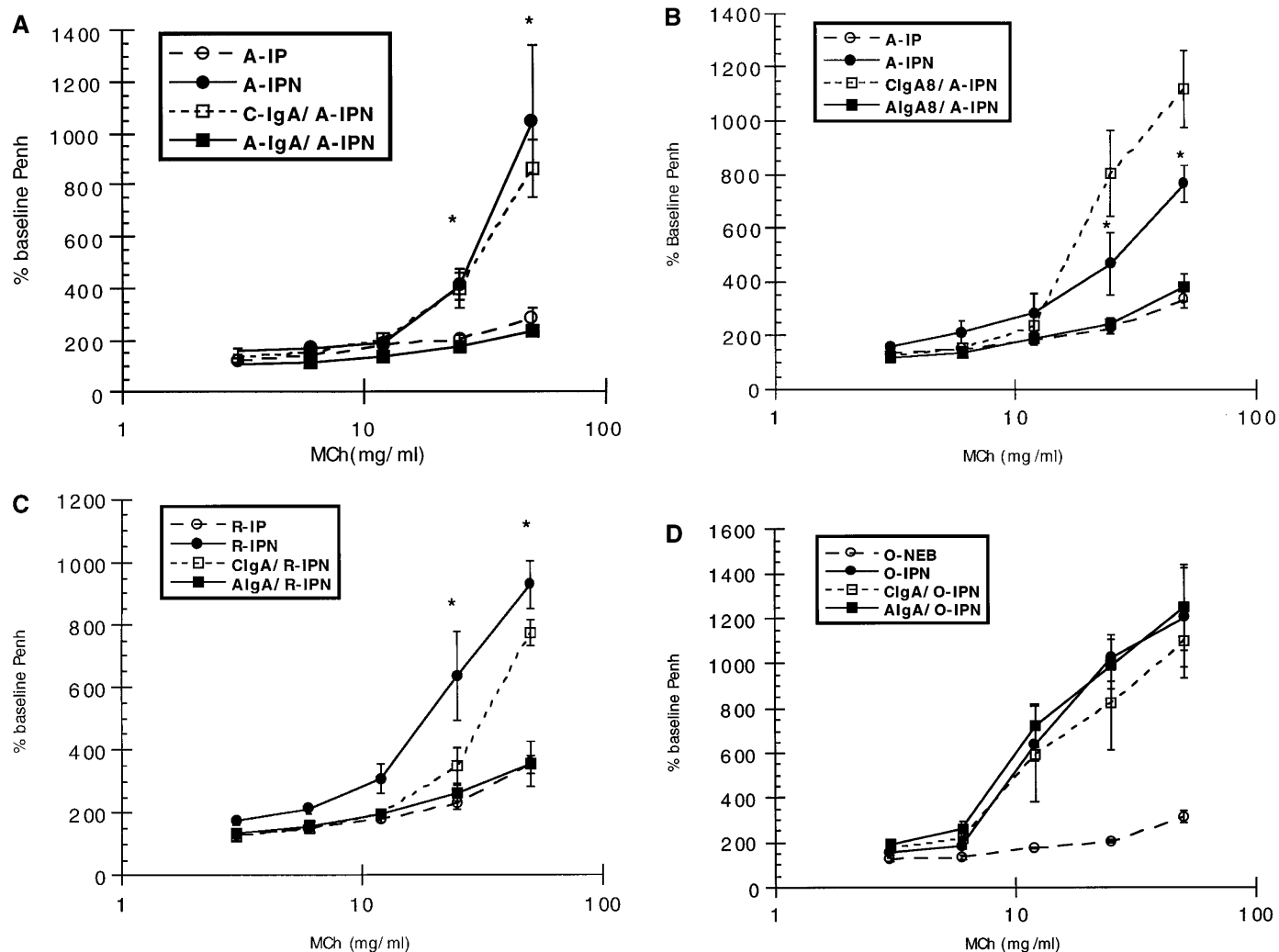


Figure 1. A-IgA prevents increases in airway responsiveness after airway challenge with *Amb a 1* or ragweed extract in mice sensitized to *Amb a 1* or ragweed extract. Mice were sensitized to *Amb a 1* (panel A: A-IP, $n = 12$) or whole ragweed extract (panel C: R-IP, $n = 8$) by two intraperitoneal injections and subsequently challenged with nebulized *Amb a 1* (A-IPN, $n = 12$) or ragweed extract (R-IPN, $n = 8$) via the airways. Three hours before each challenge, A-IgA (A-IgA/A-IPN, panel A: $n = 12$; A-IgA/R-IPN, panel C: $n = 8$) or C-IgA (C-IgA/A-IPN, panel A: $n = 12$; C-IgA/R-IPN, panel C: $n = 8$) were administered intranasally under light anesthesia. In some mice sensitized to *Amb a 1* (panel B: A-IgA8/A-IPN and C-IgA8/A-IPN, both $n = 8$), the interval between IgA treatment and challenge was increased to 8 h. Additionally, mice challenged via the airways alone (O-Neb, $n = 8$) or sensitized and challenged (O-IPN, $n = 8$) with OVA were treated with A-IgA (A-IgA/O-IPN, $n = 8$) or C-IgA (C-IgA/O-IPN, $n = 8$) in the same manner (panel D). Forty-eight hours after the last challenge, airway responsiveness to increasing concentrations of methacholine (3–50 mg/ml) was assessed by barometric whole-body plethysmography, and Penh values were calculated. Means $\pm$ SEM of Penh values from three independent experiments are expressed as the percentage of baseline Penh values observed after PBS exposure. *Significant differences, $p < 0.05$, between IPN and A-IgA/A-IPN.

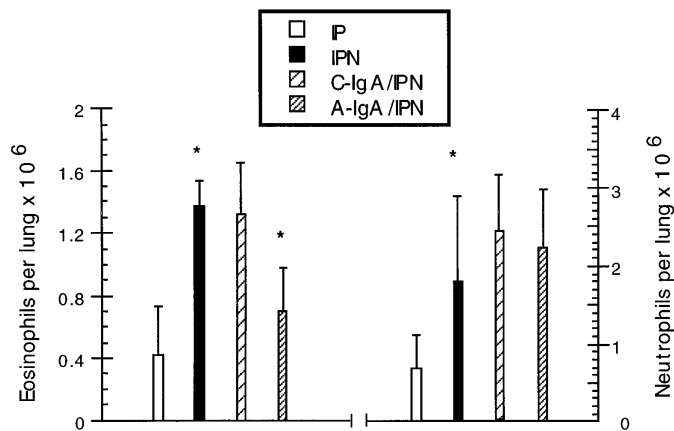


Figure 2. A-IgA reduces the increase in lung eosinophils after airway challenge with *Amb a I* in *Amb a I*-sensitized mice. Lung cells were isolated from the same mice described in Figure 1, and were either sensitized intraperitoneally to *Amb a I* alone (IP, *n* = 9), sensitized and challenged via the airways (IPN, *n* = 9), or sensitized and treated intranasally with A-IgA (A-IgA/IPN, *n* = 12) or C-IgA (C-IgA/IPN, *n* = 12) before the challenges. Numbers of eosinophils and neutrophils were determined. Illustrated are means $\pm$ SD of the numbers of these cells from three independent experiments. *Significant differences, *p* < 0.05.

8 h before challenge and A-IgA treatment in mice sensitized and challenged with ragweed extract also resulted in significant decreases in lung eosinophil numbers. The increase in eosinophil influx into the lung following sensitization and challenge with OVA was not affected by A-IgA pretreatment (Table 1).

A-IgA Prevents Increases in *Amb a I*-specific IgE and Results in Increased *Amb a I*-specific IgG2a Levels

Intraperitoneal sensitization with *Amb a I* and alum triggered the production of IgE antibodies specific for *Amb a I* (Tables 2 and 3). Subsequent challenge with *Amb a I* via the airways resulted in further increases in the serum levels of IgE antibodies specific for the allergen and the levels of total IgE and total IgG. Treatment with A-IgA but not with C-IgA before the airway challenge prevented these secondary increases in allergen-specific IgE; the increases in total IgE and total IgG concentrations were reduced, but the differences did not reach significance. Serum levels of IgG₁ specific for *Amb a I* were elevated following sensitization, but did not increase further with allergen challenge and were not significantly altered by

TABLE 1

A-IgA REDUCES EOSINOPHIL INFLUX IN THE LUNGS IN AN ALLERGEN-SPECIFIC MANNER AND IS EFFECTIVE AS LONG AS 8 h BEFORE CHALLENGE*

Allergen	Eosinophils ($\times 10^6$) per Lung			
	IP	IPN	A-IgA/IPN	C-IgA/IPN
<i>Amb a I</i>				
(A-IgA/8 h)	0.45 $\pm$ 0.11	1.75 $\pm$ 0.42 [†]	0.42 $\pm$ 0.25 [‡]	2.01 $\pm$ 0.87
Ragweed extract	0.41 $\pm$ 0.32	1.95 $\pm$ 0.58 [†]	0.61 $\pm$ 0.32 [‡]	3.01 $\pm$ 1.73
OVA	0.73 $\pm$ 0.35	4.04 $\pm$ 2.20 [†]	3.57 $\pm$ 1.32	3.67 $\pm$ 1.61

* Lung cells were isolated from mice either sensitized intraperitoneally alone (IP, *n* = 8 per group), or sensitized and challenged via the airways (IPN, *n* = 8 per group), or sensitized and treated intranasally with A-IgA (A-IgA/IPN, *n* = 8 per group) or C-IgA (C-IgA/IPN, *n* = 8 per group) before the challenges. Either *Amb a I*, ragweed extract, or OVA were used for sensitization and challenge. In *Amb a I*-sensitized mice, A-IgA or C-IgA was administered 8 h prior to challenge. Numbers of eosinophils were determined. Expressed are means $\pm$ SD of the numbers of these cells from two independent experiments.

[†] Significant differences between IP and IPN, *p* < 0.05.

[‡] Significant differences between A-IgA/IPN and IPN, *p* < 0.05.

IgA treatment (data not shown). Concentrations of *Amb a I*-specific IgG2a were not detectable after sensitization and challenge. However, they were significantly increased after treatment with A-IgA, but not with C-IgA. The concentrations of antigen-specific IgG2a were negatively correlated with levels of *Amb a I*-specific IgE (*r* = -0.59, *p* < 0.01). In mice sensitized and challenged with OVA, treatment with A-IgA did not have a significant effect on allergen-specific or total immunoglobulin levels.

Pretreatment with A-IgA Prevents Increases in Th2-type Cytokine Production

Cytokine production was assessed in cultures of PBLN cells stimulated with either the combination of P/I or with *Amb a I* for 48 h. After airway challenge with *Amb a I* in sensitized mice, a shift to the production of T-helper cell type 2 (Th2) cytokines was observed (Table 4); the concentrations of IFN- γ in the supernates of P/I-stimulated cultures were significantly reduced and the levels of IL-4 and IL-5 in supernates of allergen-stimulated cultures were significantly increased when compared with cultures of PBLN cells obtained from mice that were sensitized alone. These changes in cytokine production were largely prevented by pretreatment with A-IgA but not with C-IgA. IFN- γ concentrations were restored to the same range as in mice that had been sensitized alone without airway challenge, whereas the levels of IL-4 and IL-5 in the culture supernates were significantly decreased by A-IgA treatment

TABLE 2

A-IgA PREVENTS THE INCREASE IN *Amb a I*-SPECIFIC IgE AND RESULTS IN AN INCREASE OF *Amb a I*-SPECIFIC IgG2a*

Group	Total IgE (ng/ml)	Total IgG (ng/ml)	<i>Amb a I</i> -specific IgE (EU/ml)	<i>Amb a I</i> -specific IgG2a (EU/ml)
NS	13.9 $\pm$ 2.7	167.2 $\pm$ 15.9	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
A-IP	35.9 $\pm$ 8.5 [†]	223.1 $\pm$ 30.9 [†]	44.1 $\pm$ 16.0 [†]	0.0 $\pm$ 0.0
A-IPN	44.5 $\pm$ 5.8 [†]	277.3 $\pm$ 38.4 [†]	73.7 $\pm$ 28.3 [†]	0.0 $\pm$ 0.0
A-IgA/A-IPN	38.5 $\pm$ 6.6	255.6 $\pm$ 30.4	46.6 $\pm$ 10.9	50.8 $\pm$ 40.5 [‡]
C-IgA/A-IPN	44.9 $\pm$ 4.1	279.7 $\pm$ 40.7	69.1 $\pm$ 18.4	4.4 $\pm$ 7.5 [§]

* Serum was collected 72 h after the last challenge from nonsensitized mice (NS, *n* = 8) or mice either sensitized intraperitoneally to *Amb a I* alone (A-IP, *n* = 9), or sensitized and challenged via the airways (A-IPN, *n* = 9), or sensitized and treated intranasally with A-IgA (A-IgA/A-IPN, *n* = 12) or with C-IgA (C-IgA/A-IPN, *n* = 12) before the challenges. Concentrations of total IgE, total IgG, and *Amb a I*-specific IgE and IgG2a were assessed by ELISA. Expressed are the means $\pm$ SD of immunoglobulin concentrations from three independent experiments.

[†] Significant differences between NS and A-IP and A-IPN.

[‡] Significant differences between A-IgA/A-IPN and A-IPN.

[§] Significant differences between C-IgA/A-IPN and A-IgA/A-IPN, *p* < 0.05.

TABLE 3
A-IgA DOES NOT CHANGE LEVELS OF OVA-SPECIFIC IMMUNOGLOBULINS
IN MICE SENSITIZED TO OVA, AN UNRELATED ALLERGEN*

Group	Total IgE (ng/ml)	Total IgG (ng/ml)	OVA-specific IgE (EU/ml)	OVA-specific IgG2a (EU/ml)
O-Neb	5.8 ± 1.2	183.4 ± 23.6	0.0 ± 0.0	0.0 ± 0.0
O-IPN	55.7 ± 21.6 [†]	291.3 ± 50.4 [†]	27.6 ± 10.7 [†]	13.8 ± 11.4 [†]
A-IgA/O-IPN	39.4 ± 14.7	275.1 ± 42.1	23.6 ± 17.9	10.3 ± 5.2
C-IgA/O-IPN	52.1 ± 7.4	269.7 ± 37.8	20.3 ± 11.4	19.3 ± 7.9

* Serum was collected 72 h after the last challenge from mice either challenged with OVA via the airways alone (O-Neb, n = 8), or sensitized intraperitoneally to OVA and challenged (O-IPN, n = 8), or sensitized and treated intranasally with A-IgA (A-IgA/O-IPN, n = 8) or with C-IgA (C-IgA/O-IPN, n = 8) before the challenges. Concentrations of total IgE, total IgG, and *Amb a I*-specific IgE and IgG2a were assessed by ELISA. Expressed are the means ± SD of immunoglobulin concentrations from two independent experiments.

[†] Significant differences between O-Neb and O-IPN, p < 0.05.

compared with treatment with C-IgA or compared with sensitization and challenge without IgA pretreatment. After sensitization and challenge with OVA, A-IgA treatment failed to alter the production of cytokines.

DISCUSSION

In the present study we monitored airway responsiveness following airway challenge with *Amb a I* in mice systemically sensitized to this allergen. This approach was used to assess the influence of A-IgA on the consequences of airway challenge with *Amb a I* or whole ragweed extract by aerosol inhalation. Mice systemically sensitized to *Amb a I* or ragweed extract were treated intranasally with A-IgA or with C-IgA 3 or 8 h prior to each of three airway challenges with *Amb a I*. Additionally, to assess the specificity of this treatment, mice sensitized to ovalbumin, an unrelated allergen, were treated in the same way with A-IgA before challenge with the respective allergen. Airway responsiveness to aerosolized methacholine was assessed using barometric whole-body plethysmography in unrestrained animals. Further, pulmonary inflammatory changes, cytokine production in the local draining lymph nodes of the lung, the PBLN, and serum levels of allergen-specific immunoglobulins were monitored and related to airway responsiveness.

Airway challenge with *Amb a I* in mice systemically sensitized to *Amb a I* resulted in increased airway responsiveness to methacholine and in the influx of eosinophils into the lung. These changes were associated with increased concentrations of IL-4 and IL-5 and decreased levels of IFN- γ production in cultures of PBLN cells. Further, concentrations of *Amb a I*-specific IgE antibodies in the serum exceeding those found in animals sensitized alone were also detected. These responses to airway challenge of sensitized animals parallel those reported in other models of systemic sensitization and subsequent airway challenge using a different allergen such as ovalbumin (12, 16). In addition, challenge of sensitized mice resulted in increases in levels of total IgE and total IgG and in the accumulation of neutrophils in the lungs.

Intranasal administration of A-IgA either 3 or 8 h before each of the airway challenges completely prevented the development of airway hyperresponsiveness to methacholine in this model and significantly reduced the number of eosinophils detected in the lungs. The same results were observed after A-IgA treatment in mice sensitized and challenged with ragweed extract. In contrast, A-IgA treatment of mice sensitized and challenged with OVA had no effect on airway responsiveness and eosinophil influx into the lungs. Additionally, the increases in IL-4 and IL-5 and the decreases in IFN- γ production observed in cultured PBLN cells following sensitization

and challenge with *Amb a I* were prevented by A-IgA treatment. IFN- γ levels were restored to the same concentrations as in nonchallenged mice and IL-4 and IL-5 levels were elevated to a lesser degree. In mice sensitized and challenged with OVA, A-IgA treatment did not result in changes in cytokine production. After A-IgA pretreatment and airway challenge, no secondary increases in *Amb a I*-specific IgE serum levels as a result of challenge were observed. In contrast to A-IgA, pretreatment with C-IgA did not result in any significant changes in the parameters monitored. Surprisingly, the changes observed after sensitization and challenge in total IgE and total IgG levels and in neutrophil numbers in the lungs were not prevented by A-IgA treatment. In addition, in mice pretreated with the specific IgA antibody, serum levels of allergen-specific IgG2a were significantly increased. This was not observed after treatment with A-IgA in mice sensitized to OVA, an unrelated antigen.

These findings clearly show that pretreatment with allergen-specific IgA antibodies can modify the respiratory and immunologic responses to allergen challenge in sensitized animals in an allergen-specific manner. It is perhaps not surprising that the IgA antibodies to the recombinant antigen *Amb a I* were also effective when whole ragweed extract was used as the sensitizing and challenge antigen. It is well recognized that the recombinant protein is the major allergic constituent of whole ragweed (11). Indeed, the influx of eosinophils and the

TABLE 4
A-IgA PREVENTS THE SHIFT TO PREDOMINANT PRODUCTION
OF Th2 CYTOKINES IN PBLN CELLS*

Group	IFN- γ	IL-4	IL-5
A-IP	8,241.0 ± 553.6	0.0 ± 0.0	136.8 ± 144.3
A-IPN	4,643.3 ± 366.0 [†]	64.0 ± 8.3 [†]	726.8 ± 122.5 [†]
A-IgA/A-IPN	8,203.4 ± 794.5 [‡]	23.1 ± 39.9 [‡]	435.1 ± 180.5 [‡]
C-IgA/A-IPN	6,859.1 ± 2,681.6	85.0 ± 22.3 [§]	788.2 ± 116.0 [§]
O-IPN	5,767.2 ± 574.5	58.2 ± 10.7	1,269.2 ± 236.4
A-IgA/O-IPN	5,522.2 ± 692.3	55.0 ± 13.4	1,483.9 ± 352.2
C-IgA/O-IPN	4,309.7 ± 521.0	61.8 ± 24.6	1,137.9 ± 170.3

* PBLN were harvested 72 h after the last challenge from mice either sensitized intraperitoneally to *Amb a I* alone (A-IP, n = 9), or sensitized and challenged via the airways (A-IPN, n = 9), or sensitized and treated intranasally with A-IgA (A-IgA/A-IPN, n = 12) or with C-IgA (C-IgA/A-IPN, n = 12) before the challenges. Additionally, PBLN were harvested from mice sensitized and challenged with OVA (O-IPN, n = 8) or sensitized and treated intranasally with A-IgA (A-IgA/O-IPN, n = 8) or with C-IgA (C-IgA/O-IPN, n = 8) before challenge. Mononuclear cells were isolated and cultured in the presence of *Amb a I* or P/I for 48 h. Concentrations of IFN- γ , IL-4, and IL-5 were assessed in culture supernatants by ELISA. Expressed are the means ± SD of cytokine concentrations (pg/ml) from three independent experiments (two for ovalbumin sensitization).

[†] Significant differences between A-IP and A-IPN.

[‡] Significant differences between A-IgA/A-IPN and A-IPN.

[§] Significant differences between C-IgA/A-IPN and A-IgA/A-IPN, p < 0.05.

development of airway hyperresponsiveness were inhibited comparably when sensitization and challenge were carried out with either *Amb a 1* or whole ragweed extract.

A simple explanation for the action of A-IgA in these studies is the binding to the allergen on the mucosal surface or in the mucosa, thereby preventing access of the inhaled allergen to the mucosal immune system as has been reported in other studies that have investigated the mechanism of action of secretory IgA (3, 9, 17, 18). However, several of our findings are not explained by this "passive" mode of action of A-IgA (i.e., simple neutralization of the effects of the allergen). In contrast, the findings following A-IgA treatment are also consistent with an "active" element of immune regulation. The effects of A-IgA treatment were evident even if allergen challenge was carried out 8 h later, when persistence of antibodies would be unlikely. Further, if the effects of A-IgA treatment were simply due to the binding of allergen and the prevention of access to the immune system, it would be expected that all of the consequences of the airway challenge in sensitized animals would be prevented or at least markedly reduced by pretreatment with allergen-specific IgA. The increases in total IgE and total IgG concentrations and in the numbers of lung neutrophils following challenge, however, were not significantly affected by A-IgA. At a minimum, these observations indicate that complete allergen neutralization was not achieved and that at least some of the effects of allergen challenge were not prevented. Additionally, the increase in allergen-specific IgG2a concentrations following A-IgA treatment cannot be explained by allergen neutralization, because *Amb a 1*-specific IgG2a was not detected following sensitization alone but was only observed after challenge of sensitized animals pretreated with A-IgA.

Production of IgG2a antibodies in mice is generally observed during immune responses associated with induction of Th1-type cytokines, particularly IFN- γ (19). The detection of allergen-specific IgG2a antibodies in A-IgA-treated mice supports the notion that administration of A-IgA together with allergen "actively" modified the immune response to allergen challenge, possibly by inducing an antigen-specific Th1 cytokine response. Such a response could also explain the diminished influx of eosinophils into the lung and the normalization of airway responsiveness (20, 21). Interestingly, oral vaccination against influenza antigens in mice has been reported to induce a strong antigen-specific IgA response combined with an increased frequency of IFN- γ -producing cells in tissues exposed to the antigen (22). The cellular mechanisms by which allergen-specific IgA antibodies might have modified the immune response in our model remain to be defined. Nonspecific (23) and antigen-specific (24) activation of murine T cells can result in the increased expression of Fc α receptors on their surface. Upregulation of these receptors can also be achieved with IgA antibodies (25, 26) and has proven to be especially strong if murine cells are exposed to human monoclonal IgA antibodies or to antigen-IgA immune complexes (27). Both enhancing (28) and suppressing (29) regulatory effects of T-cell Fc α receptor upregulation have been reported. Further, large numbers of CD8⁺ T cells expressing Fc α receptor were found in mice with IgA-secreting plasmacytomas (30), and CD8⁺ T cells expressing Fc α receptors can suppress proliferation and IgA production of plasmacytoma cells (31). Considering that cytokines produced by Th2 cells have been implicated in the upregulation of IgA production (32–34), it seems possible that Th1 cytokines are produced by CD8⁺ T cells expressing Fc α receptors which are able to downregulate IgA production. Whether binding of excessive amounts of IgA complexed to antigen can induce cytokine responses that have the potential

to counteract allergic sensitization, as appears to have been the case in this study, has not been previously reported.

In summary, we present a murine model of airway sensitization to allergen in which pretreatment with allergen-specific IgA prior to inhalation of an airborne allergen can prevent the development of airway hyperresponsiveness, pulmonary eosinophilic inflammation, increased production of allergen-specific IgE, and the increase in local Th2 cytokine production, while at the same time inducing the production of allergen-specific IgG2a antibodies. These findings demonstrate that allergen-specific IgA can modify the respiratory and immunological consequences of airway challenge in sensitized mice. This approach using allergen-specific IgA could prove useful in modifying human allergic airway diseases in which airborne allergens play an important role in triggering exacerbations.

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