Amb a 1–linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma

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Background: Recently, it has been demonstrated that immunostimulatory DNA sequences (ISS) containing CpG motifs prevent the development of allergic airway responses in murine models of disease. However, few studies have addressed the issue of whether these agents will reverse established T_H2-driven allergic airway responses.

Objective: The aim of this study was to determine whether intradermal delivery of an immunogenic protein of ragweed pollen linked to an immunostimulatory DNA sequence could reverse an established allergic response in the mouse lung.

Methods: Mice sensitized and challenged with ragweed pollen extract were treated intradermally twice at 1-week intervals with an ISS chemically linked to Amb a 1 (Amb a 1-ISS). One week after the Amb a 1-ISS treatment, mice were rechallenged intratracheally with ragweed extract, and airway responses were assessed.

Results: Amb a 1-ISS treatment of ragweed-sensitized and ragweed-challenged mice significantly reversed allergen-induced airway hyperresponsiveness and suppressed the total number of eosinophils in bronchoalveolar lavage fluid. The inhibitory effect of Amb a 1-ISS was associated with a marked increase in IFN-γ levels by Amb a 1–stimulated splenocytes and a shift in the antibody profile from a T_H2-directed IgG1 response to a T_H1-directed IgG2a response. Interestingly, the inhibitory effect of Amb a 1-ISS on allergen-driven airway hyperresponsiveness was independent of suppression of T_H2 cytokine production.

Conclusion: These results demonstrate that intradermal delivery of allergen-specific DNA conjugates can reverse established allergic responses in the murine lung, supporting their potential use in the treatment of human asthma. (J Allergy Clin Immunol 2002;109:455-62.)

Key words: *Allergy, T cells, immunotherapy, cytokines, CpG*

The morbidity and mortality of asthma has increased worldwide, despite increased use of currently available medications, underscoring the need for the development of novel therapies.[1,2] Asthma is a chronic inflammatory disease of the lung characterized by airway hyperresponsiveness (AHR) to a variety of stimuli, eosinophilic inflammation of the airways, mucus hypersecretion, and elevated serum IgE levels.[3,4] Numerous experimental and clinical studies support the concept that $CD4^+$ T cells producing a T_H2 cytokine profile (IL-4, IL-13, IL-5, and IL-9) are responsible for the pathophysiologic manifestations of this disease.[5,6] Previous studies have shown that blockade of T_H2 cytokines[7,8] or induction of T_H1 cytokines[9-11] can ameliorate the asthmatic response in mice. Thus it has been hypothesized that agents that selectively elicit T_H1 immune responses to known allergens may be useful in inhibiting the pathologic consequences of T_H2-mediated T-cell responses in asthmatic individuals.

DNA-containing unmethylated CpG motifs common in bacterial but not in mammalian DNA have been shown to induce strong T_H1-polarized immune responses both in vitro[12-15] and in vivo.[16-18] The induction of T_H1 responses is thought to be a result of the ability of immunostimulatory sequences containing CpG (CpG-oligodeoxynucleotides [ODN]) to induce activation and secretion of IL-12 and IL-18 by macrophages and dendritic cells.[19-24] These cytokines then synergize to induce IFN-γ production by natural killer and T cells.[25-28] In addition, CpG-ODNs cause immature dendritic cells to mature to professional antigen-presenting cells able to activate antigen-reactive naive T cells. CpG-ODNs are also capable of directly driving B lymphocytes to proliferate and to trigger immunoglobulin production.[29-32] Together, these features of CpG-ODNs suggest that they may have promise as novel therapies for the treatment of asthma. Indeed, several studies have shown that delivery of immunostimulatory DNA sequences (ISS), either

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Abbreviations used

AHR: Airway hyperresponsiveness
Amb a 1-ISS: Immunostimulatory oligodeoxynucleotide chemically conjugated to Amb a 1
Amb a 1–non-ISS: Mutated immunostimulatory oligodeoxynucleotide chemically conjugated to Amb a 1
BAL: Bronchoalveolar lavage
ISS: Immunostimulatory DNA sequence
ODN: Oligodeoxynucleotide
PMA: Phorbol myristate acetate
RWP: Ragweed pollen

intraperitoneally or intratracheally, to mice at the time of sensitization or before local antigen challenge prevents the development of allergic airway responses.[16-18] However, few studies have examined the effect of delivery of these agents in the context of an established T_H2-mediated immune response.

Recent studies suggest that the immunogenicity and specificity of CpG can be improved when immunostimulatory sequences are conjugated to a specific antigenic peptide.[33,34] Specifically, Tighe et al[33] have shown that the ISS conjugated to the major short ragweed allergen Amb a 1 was far more potent at inducing T_H1 immune responses (IFN-γ and IgG2a antibody titers) than when the ISS was delivered as a mixture with the antigen. Moreover, the ability of the conjugate to induce a strong T_H1 response was retained, even when administered after an initial immunization with Amb a 1 and alum, which induces a potent T_H2 response. Furthermore, the antigen conjugated with ISS was far less reactive with human IgE antibodies from patients with ragweed allergy than with antigen alone. Together, these results suggest that delivery of ISS conjugated to relevant antigens, such as Amb a 1, may be an optimal means of modulating asthmatic responses in vivo.

In the present study we examined the ability of an ISS chemically conjugated to Amb a 1 (Amb a 1-ISS) to reverse established manifestations of asthma in a ragweed-induced model of allergic asthma. We demonstrate that Amb a 1-ISS delivery significantly reversed established allergic airway responses concomitant with induction of T_H1 immune responses. Interestingly, the beneficial effects of ISS delivery were independent of suppression of T_H2-mediated immune responses. These data suggest that conjugation of CpG-ODNs to an allergenic peptide of a common allergen may provide a specific and effective means of combating the symptoms of allergic asthma.

METHODS

Preparation of conjugates

Defatted ragweed pollen (RWP; *Ambrosia artemissifolia*) was purchased from Greer Laboratories (Lenoir, NC). The ISS 5′-TGACTGTGAACGTTCGAGATGA-3′ phosphorothioate ODN (used to prepare Amb a 1-ISS) and the mutated 5′-TGACTGTGAACCTAGAGATGA-3′ phosphorothioate ODN (used to prepare a control conjugate, Amb a 1–non-ISS) were purchased from Avecia BioTechnologies (Milford, Mass). Amb a 1 isolation and conjugation was done as previously described.[33] Endotoxin levels were less than 40 EU/mg, resulting in less than 0.2 EU in the 50 μL injected per mouse.

Animals

Four-week-old BALB/cj female mice were purchased from Jackson Laboratories (Bar Harbor, Me) and were housed under laminar flow hoods in an environmentally controlled specific pathogen-free animal facility for the duration of the experiment. The studies reported conformed to the principles for laboratory animal research, as outlined by the Animal Welfare Act and the Department of Health, Education, and Welfare (National Institutes of Health) for the experimental use of animals.

Experimental protocol

Mice were sensitized by means of an intraperitoneal injection of 150 μg of endotoxin-free RWP extract plus alum (1:3 ratio) on days 0 and 3. On day 10, mice were anesthetized with a mixture of ketamine and xylazine (45 and 8 mg/kg body weight, respectively) and challenged intratracheally with a solution containing 200 μg of RWP or an equivalent volume (40 μL) of PBS as a control. On days 14 and 21, mice were injected intradermally at the base of the tail with 50 μL of PBS containing either 10 μg of Amb a 1 alone, Amb a 1-ISS conjugate, or Amb a 1–non-ISS. On day 28, mice were challenged again intratracheally with either RWP or PBS. Characterization of the allergic phenotype was performed 72 hours after the second antigen challenge.

Airway responsiveness measurements

Airway reactivity to the intravenous administration of acetylcholine was measured, as previously described.[35] Briefly, 3 days after the second antigen challenge, mice were anesthesized with sodium pentobarbital (90 mg/kg), intubated, and ventilated at a rate of 120 breaths/min with a constant tidal volume (0.2 mL). After a stable baseline airway pressure was recorded, mice were paralyzed with decamethonium (25 mg/kg), and acetylcholine was injected intravenously (50 μg/kg).

Assessment of airway inflammation

After airway responsiveness measurements, lungs were lavaged thoroughly with 1 mL of cold HBSS solution without calcium or magnesium. The lavage fluid was centrifuged (1800 rpm × 10 minutes), and cell pellets were resuspended in 10% FBS/PBS and counted with a hemocytometer. Slide preparations were stained with Diff-Quick (Baxter, McGraw Park, Ill) and bronchoalveolar lavage (BAL) cell differential percentages were determined on the basis of light microscopic evaluation of 500 cells per slide.

Organ histology

Lungs were excised and fixed in 10% formalin, washed in methanol, dehydrated, embedded in paraffin, and cut into 10-μm sections. Sections were mounted on slides and stained with hematoxylin and eosin and periodic acid–Schiff. Three cross-sections were cut from each lung. Kidneys, livers, and sections of the tail at the site of injection were also fixed and stained by using the same protocol.

Antibody analysis

Terminal bleedings were collected from all animals 72 hours after the second intratracheal antigen challenge. Mouse serum samples were analyzed by means of ELISA, with plates coated with 50

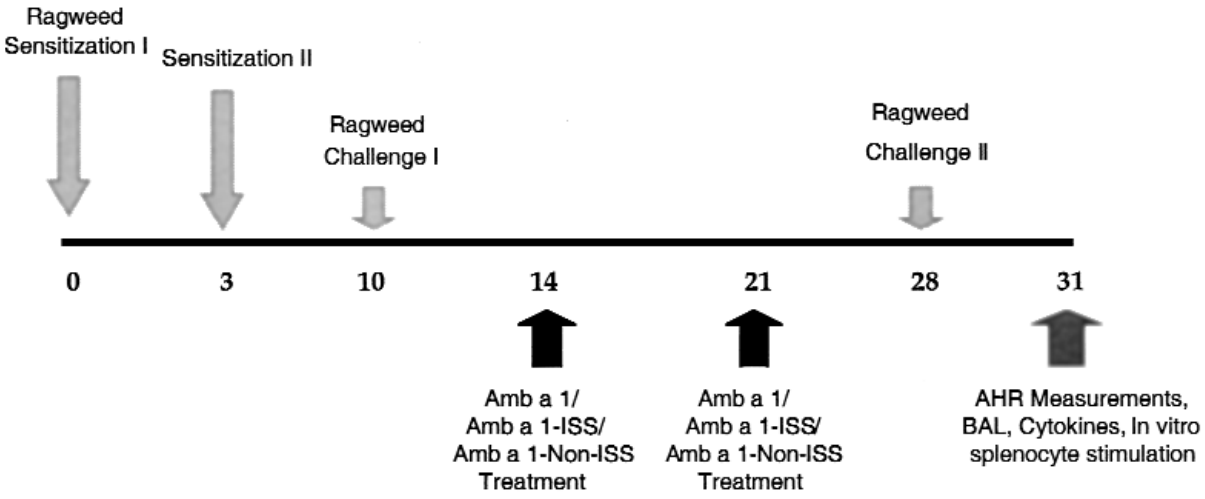

FIG 1. Experimental protocol. BALB/cj mice were sensitized by means of an intraperitoneal injection of RWP in alum on days 0 and 3. On day 10, mice were challenged intratracheally with RWP or PBS as a control. On days 14 and 21, mice were injected intradermally with 10 μg of either Amb a 1 alone, Amb a 1-ISS conjugate, or Amb a 1–non-ISS control conjugate. On day 28, mice were rechallenged intratracheally with either RWP or PBS. Characterization of the allergic phenotype was performed 72 hours after the second antigen challenge.

μL per well of Amb a 1 antigen at 1 μg/mL (pH 9.0). Goat anti-mouse IgG1, IgG2a, or IgE biotin-conjugated antibody was used as the secondary antibody (PharMingen, San Diego, Calif). Streptavidin-horseradish peroxidase conjugate was used for detection, and the assay was developed with TMB (KPL, Gaithersburg, Md). The absorbance values were determined at 450 nm, with background subtraction at 650 nm. The titer was defined as the reciprocal of the serum dilution that gave an ELISA absorbance of 0.5 OD by using 4-parameter analysis. All samples were tested in duplicate wells on separate plates.

Splenocyte cytokine analysis

After airway measurements, spleens were harvested and dissociated, and cell suspensions were made. For plating, cells were diluted to 5×10^6 cells/mL and added to triplicate wells in a total volume of 200 μL and stimulated with either 25 μg of Amb a 1, phorbol myristate acetate (PMA), or medium as a negative control. Cell cultures were incubated at 37°C with 5% to 7% CO_2 and water-saturated conditions. Supernatants were harvested 96 hours after plating and frozen at –80°C. Splenocyte supernatants were tested for IL-5 and IFN-γ by means of capture ELISA on anticytokine mAb-coated plates. Biotinylated anticytokine antibodies were used as secondary antibodies. Streptavidin-horseradish peroxidase conjugate was used for detection, and the assay was developed with TMB. Concentration was calculated from a standard curve assayed on each plate. The absorbance values were determined at 450 nm, with background subtraction at 650 nm. All samples were tested in duplicate wells on separate plates.

Data analysis

Data are presented as means ± SEM. Significant treatment effects were determined by using 1-way ANOVA. If differences among groups were significant ($P < .05$), the Fisher least-significant-difference test was used to distinguish between pairs of groups. Statistical analysis for antibody and splenocyte cytokine responses were done with log-transformed data by using ANOVA with 1-way ANOVA. A P value of less than .05 was considered significant.

RESULTS

Effects of Amb a 1-ISS on ragweed-induced airway hyperreactivity

To assess the ability of Amb a 1-ISS treatment to reverse established allergen-driven airway responsiveness, we treated ragweed-sensitized mice with PBS, Amb a 1 alone, Amb a 1-ISS, or AMB a 1–non-ISS after a primary allergen challenge, according to the protocol shown in Fig 1. A control group was sensitized, challenged, and treated with PBS. Mice were rechallenged with antigen, and characterization of the allergic phenotype was performed 72 hours after the second ragweed challenge. Ragweed sensitization and challenge induced significant increases in airway reactivity to acetylcholine compared with responses in PBS-sensitized and PBS-challenged control animals ($P < .0001$; Fig 2, *A*). Administration of Amb a 1-ISS intradermally after the first antigen challenge significantly reduced the increase in reactivity to acetylcholine after the second ragweed challenge of sensitized mice ($P < .0005$). Interestingly, Amb a 1 treatment also reduced ragweed-induced AHR, although to a lesser extent ($P < .03$). In additional studies we tested the effect of a nonimmunostimulatory oligonucleotide conjugate and found that Amb a 1–non-ISS had no significant effect on airway reactivity in either PBS- or ragweed-sensitized and challenged mice (Fig 2, *B*).

Effect of Amb a 1-ISS on airway inflammation

Ragweed sensitization and challenge significantly increased the total number of cells recovered in the lavage fluids when compared with those in PBS-sensitized and PBS-challenged control groups ($P < .005$, Table I). Amb a

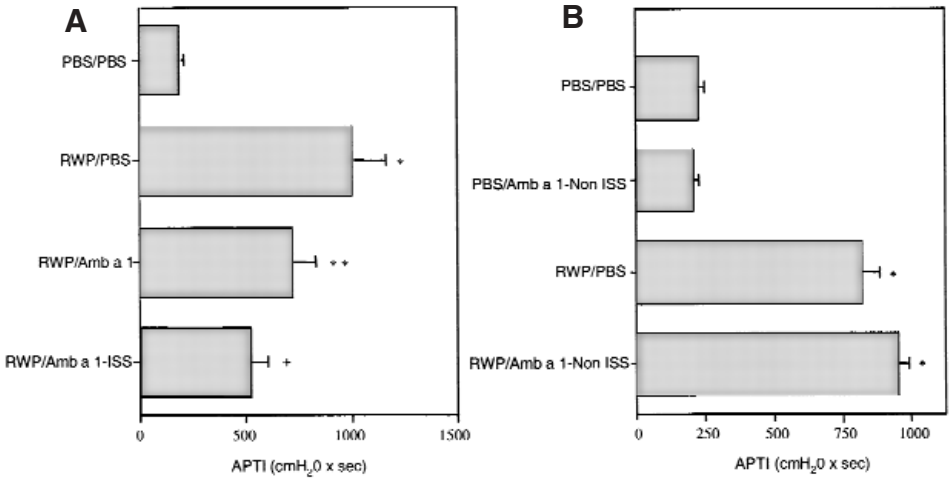

FIG 2. A, Amb a 1-ISS treatment inhibits ragweed-induced AHR. AHR was defined by the time-integrated change in peak airway pressure (airway pressure-time index *[APTI]*; in centimeters of water times seconds). Values shown are means ± SE of 8 to 10 animals per group. **P* < .0001 compared with PBS control; ***P* = .03 compared with the RWP-sensitized and RWP-challenged group; and +*P* = .0005 compared with the RWP-sensitized and RWP-challenged group. **B**, Administration of Amb a 1–non-ISS did not suppress ragweed-induced AHR. Protocol is as described in Fig 1. Values shown are means ± SE of 8 to 10 animals per group. **P* < .0001 compared with PBS control.

TABLE I. Effect of Amb a 1-ISS on total cells and cell differentials from BAL fluid in ragweed-sensitized and ragweed-challenged mice

Groups	Total cells	Epithelial cells	Macrophages	Eosinophils	Lymphocytes	Neutrophils
PBS/PBS	24,375 ± 3,801	19,162 ± 3,445	4,900 ± 2,075	0	67 ± 43	25 ± 16
RWP/PBS	85,500 ± 9,529*	23,738 ± 4,164	25,798 ± 5,584*	29,141 ± 5,375*	609 ± 287*	1,814 ± 738
RWP/Amb a 1	104,750 ± 15,190	33,822 ± 5,660	29,690 ± 5,234	41,477 ± 11,789*†	495 ± 266	5,829 ± 1,700‡
RWP/Amb a 1-ISS	63,250 ± 14,162	23,008 ± 3,943	18,359 ± 7,003	13,638 ± 4,730‡	355 ± 149	2,393 ± 926§

Values are means (in cells per milliliter) ± SE of 8 to 10 animals per group. See Fig 1 for experimental design.
**P* < .005 compared with the PBS/PBS group.
†*P* = .02 compared with the RWP/PBS group.
‡*P* < .05 compared with the RWP/PBS group.
§*P* = .006 compared with the RWP/Amb a 1 group.

1 treatment of ragweed-sensitized mice elevated total cell counts in the lavage fluid, whereas Amb a 1-ISS treatment suppressed the total number of cells recovered in ragweed-sensitized mice. Treatment with Amb a 1–non-ISS did not affect the number of total cells recovered in BAL fluid in antigen-sensitized mice (RWP/PBS, 196,000 ± 18,451; RWP/Amb a 1–non-ISS, 164,444 ± 21,254).

Because pulmonary eosinophilia is thought to be a hallmark of the allergic airway response, we assessed the ability of Amb a 1-ISS to inhibit eosinophilic inflammation in the lung. We showed that ragweed sensitization and challenge induced significant elevations in the numbers of eosinophils in the lavage fluids ($P < .005$, Table I). Amb a 1-ISS treatment significantly reduced the degree of airway eosinophilia in the allergen-exposed groups ($P < .05$). In contrast, Amb a 1 treatment of ragweed-challenged animals enhanced the eosinophil response induced by ragweed sensitization and challenge ($P = .02$). Amb a 1–non-ISS did not affect the number of eosinophils recovered in BAL fluid (RWP/PBS, 25,412 ± 6,506; RWP/Amb a 1–non-ISS, 31,078 ± 7,201).

Histologic examinations of lung tissues and other organs

Lung sections were stained with hematoxylin and eosin and periodic acid–Schiff to detect inflammatory cells and mucus-containing cells and determine whether Amb a 1-ISS had a significant effect on the influx of inflammatory cells into the lung tissue after ragweed challenge. No signs of inflammation and few mucus-containing cells were observed in the lung sections from PBS-sensitized and PBS-challenged control mice (Fig 3, *A*). In contrast, mice that were ragweed sensitized and challenged had significant cellular infiltrates in the airway wall extending to the nearby vessels (Fig 3, *B*). These changes were observed in both small and large airways. In addition, ragweed challenge significantly increased the number of mucus-containing cells in the airway epithelium. The degree of inflammation and mucus-containing cells was not different between the RWP/PBS, RWP/Amb a 1 and RWP/Amb a 1-ISS groups.

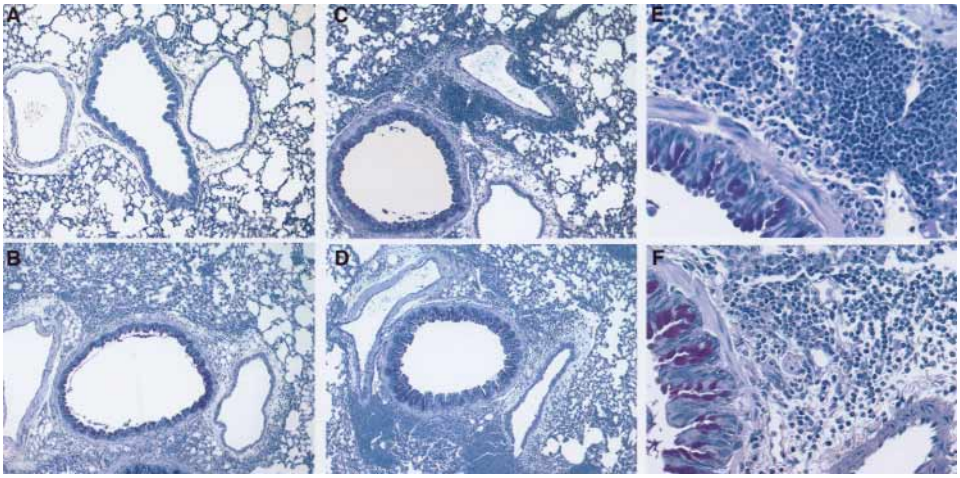

FIG 3. Effects of ragweed sensitization and challenge on cellular infiltration and mucus-containing cells in the airway wall. Ten-micrometer lung sections were fixed in formalin and stained with hematoxylin, eosin, and periodic acid–Schiff. Representative sections were obtained from the following treatment groups: **A**, PBS/PBS control group with few mucus-containing cells and no interstitial inflammatory cells; **B**, RWP/PBS group with ragweed-induced marked increases in interstitial inflammatory cells and intense periodic acid–Schiff staining in airway epithelium; **C**, RWP/Amb a 1; **D**, RWP/Amb a 1-ISS with inflammatory infiltrates and mucus-containing cells; **E**, high-power view (40×) of section from RWP/PBS; and **F**, high-power view of section from RWP/Amb a 1-ISS with no significant suppression of ragweed-induced inflammatory cells or mucus-containing cells.

To evaluate the possible induction of a systemic inflammatory response to the conjugate, we performed a histologic examination of different organs (tail at the site of injection, liver, and kidney). We did not find any macroscopic or microscopic evidence of inflammation in any of the organs collected from Amb a 1-ISS–treated mice (data not shown).

Serum antibody responses

We examined the effects of Amb a 1-ISS treatment on serum levels of Amb a 1–specific IgG1 and IgG2a antibodies in ragweed-sensitized and ragweed-challenged mice. This method has been used previously to assess the relative influence of T_H1 versus T_H2 cytokines in vivo because the production of the mouse IgG1 subclass is augmented by IL-4 and inhibited by IFN-γ, whereas that of IgG2 is augmented by IFN-γ and inhibited by IL-4.[36] In ragweed-sensitized and ragweed-challenged mice, elevated levels of both IgG1 and IgG2a were observed compared with those in the PBS control groups; however, IgG1 titers were predominant (Table II). Treatment with Amb a 1 alone enhanced IgG1 levels with no effect on IgG2a titers when compared with those of the sensitized mice receiving only PBS. In contrast, Amb a 1-ISS significantly increased both anti-Amb a 1 IgG1 titers and anti-Amb a 1 IgG2a titers compared with levels seen in ragweed-sensitized and PBS-treated mice. However, Amb a 1-ISS induced a 425-fold increase in IgG2a levels while inducing only a 9-fold increase in IgG1 titers. In contrast, treatment with a nonactive conjugate induced only a 1.8- and 1.7-fold increase in anti-Amb a 1 IgG1 levels and IgG2a, respectively. These data suggest that Amb a 1-ISS treatment shifts the antibody profile from one directed by T_H2 cytokines toward that of a T_H1-dominated response. All IgE titers, both total and Amb a 1–specific titers, were low and not significantly different between groups.

TABLE II. Effect of Amb a 1-ISS on Anti-Amb a 1 serum antibody titers in ragweed-sensitized and ragweed-challenged mice

Groups	Anti-Amb a 1 IgG1	Anti-Amb a 1 IgG2a
PBS/PBS	60 ± 19	45 ± 8
RWP/PBS	82,269 ± 20,209	492 ± 125
RWP/Amb a 1	107,612 ± 21,953	562 ± 232
RWP/Amb a 1-ISS	784,852 ± 66,320*	209,140 ± 15,310*

The titer was defined as the reciprocal of the serum dilution that gave an ELISA absorbance of 0.5 OD by using 4-parameter analysis. Values are means ± SE of 8 to 10 animals per group. See Fig 1 for experimental design.
*$P < .0001$ versus RWP/PBS group, RWP/Amb a 1 group.

Cytokine production by splenocytes

IFN-γ and IL-5 levels were assessed in splenocytes cultured with Amb a 1 or the T-cell mitogen PMA to determine the effect of Amb a 1-ISS conjugate treatment on T-cell cytokine production. Splenocytes from control PBS/PBS animals produced low to undetectable IFN-γ levels after in vitro exposure to Amb a 1 (Fig 4, *A*). Ragweed-sensitized and ragweed-challenged animals that were treated with PBS or Amb a 1 showed similar IFN-γ responses on exposure to Amb a 1. Ragweed-sensitized and ragweed-challenged mice that were treated in vivo with Amb a 1-ISS produced significantly higher levels of IFN-γ in response to Amb a 1 stimulation compared with

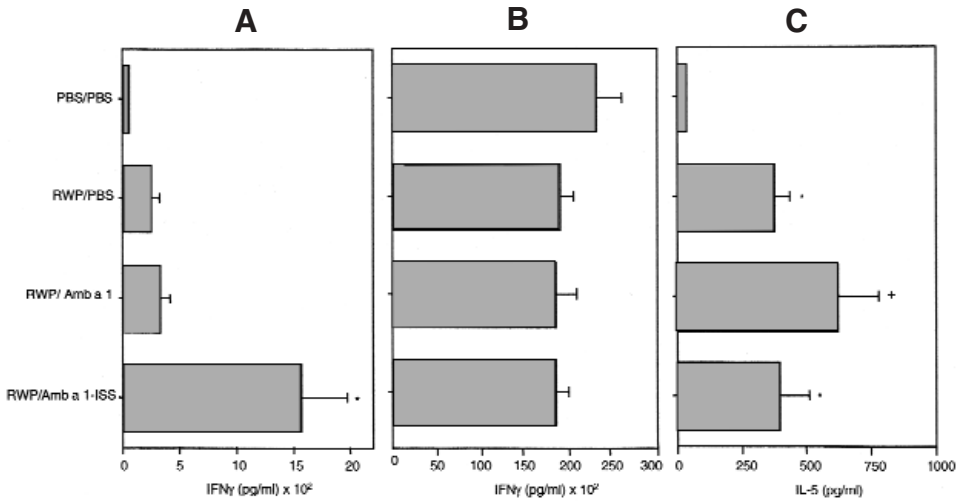

FIG 4. A, Amb a 1-ISS treatment increases IFN-γ–productive capacity of murine splenocytes stimulated in vitro with Amb a 1. Spleens were harvested 72 hours after antigen challenge, and splenocytes from individual mice were cultured for 96 hours in the presence of 25 μg of Amb a 1. Values shown are means ± SE of 8 to 10 animals per group. **P* < .005 compared with the RWP/PBS and RWP/Amb a 1 groups. **B**, Splenocytes stimulated in vitro with PMA show similar IFN-γ responses. Splenocytes from individual mice were cultured for 96 hours in the presence of 10 ng/mL PMA. **C**, In vivo Amb a 1-ISS treatment of ragweed-sensitized mice does not suppress the IL-5–productive capacity of their splenocytes when stimulated with Amb a 1 in vitro. IL-5 levels in supernatants were measured by means of ELISA. **P* < .04 versus PBS/PBS and +*P* = .001 versus PBS/PBS.

levels in the RWP/PBS and RWP/Amb a 1 groups ($P < .005$). Mice treated with Amb a 1–non-ISS showed a significant increase in IFN-γ levels (RWP/PBS, 70 ± 15.6 pg/mL; RWP/Amb a 1–non-ISS, 185 ± 35.8 pg/mL) but to a lesser extent compared with that in Amb a 1-ISS group. Splenocytes were cultured in the presence of PMA to evaluate the specificity of the induction of IFN-γ by in vivo Amb a 1-ISS treatment (Fig 4, *B*). The levels of IFN-γ produced in response to PMA were similar in all groups. Taken together, these data suggest that in vivo Amb a 1-ISS treatment enhances the IFN-γ–productive capacity of splenocytes in an antigen-specific manner.

To assess the effect of in vivo Amb a 1-ISS treatment on T_H2 cytokine production, we measured IL-5 levels in splenocytes stimulated in vitro with Amb a 1 (Fig 4, *C*). Splenocytes from PBS/PBS control animals produced undetectable levels of IL-5 on exposure to either Amb a 1 or ragweed extract. Ragweed sensitization and challenge induced significant increases in the ability of splenocytes to make IL-5 compared with those from PBS/PBS control animals ($P < .04$). Interestingly, treatment with Amb a 1 tended to increase IL-5 levels above those seen in ragweed-sensitized and ragweed-challenged mice treated with PBS, although this increase did not reach statistical significance. Surprisingly, in vivo Amb a 1-ISS treatment did not affect the IL-5–productive capacity of splenocytes from ragweed-sensitized and ragweed-challenged mice. Similarly, Amb a 1–non-ISS had no effect on IL-5 production (RWP/PBS, 213 ± 19 pg/mL; RWP/Amb a 1–non-ISS, 298 ± 88 pg/mL). Although the amount of IL-5 secreted was not significantly suppressed by Amb a 1-ISS treatment in vivo, the conjugate greatly enhanced IFN-γ production, such that the IFN-γ/IL-5 ratio was likely to be sufficient to decrease the effects of the IL-5 produced.

DISCUSSION

In this study we demonstrated that in the context of a T_H2-dominant response to RWP, intradermal delivery of DNA linked to the dominant peptide of ragweed (Amb a 1) markedly suppressed an ongoing eosinophilic inflammatory response and antigen-induced AHR. The Amb a 1-ISS–induced suppression in AHR was associated with an increase in antigen-stimulated splenocyte IFN-γ production and an increase in the levels of the T_H1-directed IgG2a antibody subclass. Interestingly, the beneficial effects of ISS occur in the absence of suppression of T_H2-mediated immune responses because Amb a 1–specific IL-5 production and T_H2-directed IgG1 antibody formation were not suppressed by Amb a 1-ISS treatment. These data suggest that linkage of ISS-ODN to an allergenic peptide of a common allergen may provide a specific and effective means of combating the symptoms of allergic asthma.

The majority of previous investigations into the effect of CpG on allergic responses have examined the effects of delivery of the ISS either at the time of antigen sensitization[16] or before lung challenge with antigen.[17,18] Uniformly, these studies have shown that CpG-ODN treatment before the initiation of allergic responses prevents the development of allergen-induced AHR concomitant with inhibition of the inflammatory response. Because sensitization has occurred in most asthmatic subjects

before clinical presentation with the disease, we examined the ability of CpG treatment to reverse allergic responses once they have been established in the lung. We have previously noted that in our model a single ragweed challenge induces a T_H2-mediated inflammatory response and AHR (data not shown). We find that even after establishment of T_H2-mediated inflammation in the murine lung, Amb a 1-ISS markedly suppresses subsequent allergen-induced AHR and eosinophilic inflammation. Our findings are consistent with the findings of Serebrisky et al,[37] who showed that administration of CpG-ODN shortly after the initial antigen challenge (24 hours) significantly reduced AHR and BAL fluid eosinophil numbers. Taken together with the studies that demonstrate that CpG-ODN treatment has prolonged protective effects against allergic responses,[38] these studies support the therapeutic potential of CpG-ODNs in the treatment of allergic asthma.

The mechanism of Amb a 1-ISS's inhibitory effect on allergen-induced AHR and eosinophilic inflammation is currently unknown. However, the effects of CpG-ODNs in a variety of immune models are thought to be mediated by their ability to induce T_H1 immune responses.[16-18,34,37,38] Consistent with previous reports, we found that Amb a 1-ISS induced a marked T_H1 response, as evidenced by the upregulation of the IFN-γ–productive capacity of splenocytes and upregulation of the T_H1-directed IgG2a antibody subclass. Amb a 1-ISS's inhibitory effect on allergen-induced airway hyperreactivity in our study is not likely to be a result of suppression of T_H2 differentiation by means of T_H1 cytokines because Amb a 1-ISS treatment did not suppress IL-5 production or the production of the T_H2-directed IgG1 antibody. Although other groups have clearly shown that T_H2 cytokines are suppressed when CpG is given before or at the time of sensitization,[16] few studies have shown reversal of established T_H2 immune responses by CpG-ODN. For example, Peng et al[39] found that coadministration of CpG-ODN with antigen to preimmunized mice failed to downregulate ongoing IgE responses, despite inducing significant elevations in IgG2a antibody levels. Likewise, CpG-ODN treatment was unable to redirect a neonatally established T_H2 response to tetanus toxoid antigen.[40] Finally, Jahn-Schmid et al[41] reported that although ISS-ODN treatment of mice induced a dramatic increase in birch pollen antigen (bet v 1)–specific IgG2a antibody titers, it did not suppress IgG1 levels.

Another potential mechanism by which CpG-ODN may confer protection from allergic airway responses is through induction of IgG antibodies. It has been reported that ISS induces significant polyclonal activation of B lymphocytes, including proliferation and immunoglobulin production in both mice and human subjects.[29-32] Our finding that Amb a 1-ISS markedly elevated the secretion of IgG2a is in line with these observations. Antigen-specific CpG-ODNs have been shown to enhance the expression of a number of cytokine (IL-2 and IFN-γ) receptors on B cells, which may enhance the in vivo B-cell isotype switching to IgG2a on exposure to the corresponding cytokines. Bohle et al[15] have shown that CpG-ODN exposure of PBMCs from allergic patients induced increases in both total and allergen-specific levels of IgM and IgG, while reducing both total and allergen-specific IgE levels. Increases in IgG levels have been observed in patients undergoing standard immunotherapy. It has been proposed that IgG may act as a blocking antibody, and several groups have attributed the clinical efficacy of standard immunotherapy to this shift in antibody production. Whether this contributes directly to the beneficial effects of Amb a 1-ISS or whether the shift is simply a marker of T_H1 cytokine production awaits further investigation.

Our previous studies with the Amb a 1-ISS conjugate suggest that the conjugate was substantially more potent at inducing IFN-γ and T_H1-directed antibody production in mice than when ISS was delivered in a mixture with the antigen.[33] Shirota et al[34] found a similar relationship with an ovalbumin-ISS conjugate. This group has hypothesized that targeting of antigen and ISS to the same antigen-presenting cell may enhance immune responses to the antigen. A recent report by Shirota et al[42] supports this hypothesis because they demonstrated that CpG tagging of antigen enhances the efficiency of antigen uptake by dendritic cells. Taken together, these studies suggest that conjugation of the antigen with ISS may optimize the T_H1 response to the antigen of interest.

In summary, we report that an allergen-specific DNA conjugate containing ISS significantly reverses an ongoing allergic response to a clinically relevant allergen. These studies suggest that delivery of the ISS linked to common aeroallergens may provide a safe and effective therapy for asthma and other allergic disorders.

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