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5A, an Apolipoprotein A-I Mimetic Peptide, Attenuates the Induction of House Dust Mite-Induced Asthma

Xianglan Yao,* Cuilian Dai,* Karin Fredriksson,* Pradeep K. Dagur,† J. Philip McCoy,† Xuan Qu,‡ Zu-Xi Yu,‡ Karen J. Keeran,§ Gayle J. Zywicke,§ Marcelo J. A. Amar,* Alan T. Remaley,* and Stewart J. Levine*

New treatment approaches are needed for patients with asthma. Apolipoprotein A-I (apoA-I), the major structural protein of high-density lipoproteins, mediates reverse cholesterol transport and has atheroprotective and anti-inflammatory effects. In this study, we hypothesized that an apoA-I mimetic peptide might be effective at inhibiting asthmatic airway inflammation. A 5A peptide, which is a synthetic, bimetal apoA-I mimetic, was administered to wild-type A/J mice via osmotic mini-pump prior to the induction of house dust mite (HDM)-induced asthma. HDM-challenged mice that received the 5A apoA-I mimetic peptide had significant reductions in the number of bronchoalveolar lavage fluid eosinophils, lymphocytes, and neutrophils, as well as in histopathological evidence of airway inflammation. The reduction in airway inflammation was mediated by a reduction in the expression of Th2- and Th17-type cytokines, as well as in chemokines that promote T cell and eosinophil chemotaxis, including CCL7, CCL17, CCL11, and CCL24. Furthermore, the 5A apoA-I mimetic peptide inhibited the alternative activation of pulmonary macrophages in the lungs of HDM-challenged mice. It also abrogated the development of airway hyperresponsiveness and reduced several key features of airway remodeling, including goblet cell hyperplasia and the expression of collagen genes (Col1a1 and Col3a1). Our results demonstrate that the 5A apoA-I mimetic peptide attenuates the development of airway inflammation and airway hyperresponsiveness in an experimental murine model of HDM-induced asthma. These data support the conclusion that strategies using apoA-I mimetic peptides, such as 5A, might be developed further as a possible new treatment approach for asthma. The Journal of Immunology, 2011, 186: 576–583.
Because airway inflammation is a key pathogenic manifestation of asthma, we hypothesized that apoA-I mimetic peptides might be effective as a therapeutic approach for asthma. In this article, we show that administration of a 5A peptide, which is a synthetic, bihelical apoA-I mimetic, attenuates the key manifestations of house dust mite (HDM)-induced asthma, such as airway inflammation and airway hyperresponsiveness (AHR) (3, 5).

Materials and Methods

HDM-induced asthma

Six- to eight-week-old female A/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Asthma was induced by nasal inhalation of HDM (Dermatophagoides pteronyssinus) extract (Greer, Lenoir, NC), 25 μg protein in 10 μl saline, for 5 d each week for 4 wk (22). The HDM extract contained 0.05 U/μl endotoxin. Osmotic mini-pumps (Model 2004; ALZET, Cupertino, CA), which administered the 5A apoA-I mimetic peptide (1 mg/kg/d) or a control peptide (1 mg/kg/d), were implanted 3 d prior to the initial nasal HDM challenge to give the animals sufficient time to recover from surgery prior to the induction of asthma. The control peptide, which is known to be functionally inactive, corresponded to the scrambled peptide (1 mg/kg/d) or a control peptide (1 mg/kg/d), were implanted 3 d prior to the initial nasal HDM challenge to give the animals sufficient time to recover from surgery prior to the induction of asthma. The control peptide, which is known to be functionally inactive, corresponded to the scrambled peptide.

Analysis of lung histology revealed intra- and interanimal heterogeneity with regard to the presence of goblet cell hyperplasia within individual airways. To quantify goblet cell hyperplasia throughout the entire lung of each animal, all of the airways present (large [conducting], medium airways. To quantify goblet cell hyperplasia throughout the entire lung (Roche Diagnostics, Indianapolis, IN) were used for differential cell counts. Lungs were inflated to a pressure of 25 cm H2O prior to fixation in 10% formalin for 24 h and were dehydrated through gradient alcohol and embedded in paraffin prior to the cutting of sagittal sections at a thickness of 5 μm.

Bronschoalveolar lavage and lung histopathologic examination

Bronchoalveolar lavage was performed using three instillations of 0.5 ml PBS. RBCs were lysed with ACK buffer for 2 min at 4°C, and cells were resuspended in 0.3 ml RPMI 1640 containing 10% FBS. Total cells were counted using a hemocytometer, and Diff-Quik–stained cytospin slides (Signet–Medical, Dedham, MA) were used to determine the differential cell counts. Lungs were inflated to a pressure of 25 cm H2O prior to fixation in 10% formalin for 24 h and were dehydrated through gradient alcohol and embedded in paraffin prior to the cutting of sagittal sections at a thickness of 5 μm. Sections were stained with H&E or periodic acid–Schiff (PAS).

Analysis of lung histology revealed intra- and interanimal heterogeneity with regard to the presence of goblet cell hyperplasia within individual airways. To quantify goblet cell hyperplasia throughout the entire lung of each animal, all of the airways present (large [conducting], medium [central], and small [distal]) within representative lung sections were analyzed, and the number of airways containing PAS+ cells was recorded. Goblet cell hyperplasia is presented as the percentage of airways containing PAS+ cells. The number of airways inspected in each animal is also given. The quantification of goblet cell hyperplasia was performed by one of the investigators who was blinded to the identity of the animals.

Quantitative RT-PCR

Lungs were minced into 1-mm pieces, placed in RNAlater (Ambion, Austin, TX), and stored at −80°C until total RNA was isolated using the mirVana kit (Ambion). RNA was treated with 10 U DNase I per 20 μg RNA, reverse transcribed using the High-capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA), and amplified using TaqMan Universal PCR Master Mix, FAM dye-labeled TaqMan MGB probes, and a 7500 Real Time PCR System running Sequence Detector version 2.1 software. Gene expression was quantified relative to the expression of 18S rRNA using the comparative ΔΔCT method.

Flow cytometry

Lung cells were isolated, and flow-cytometric analysis of alternatively activated macrophages (AAMs) was performed using modifications of methods described by Lewkowich et al. (24) and Lee et al. (25). Briefly, lungs were minced with ice-cold PBS, minced into small pieces, and incubated at 37°C for 30 min in RPMI 1640 containing 26 U Liberase TH (Roche Diagnostics, Indianapolis, IN) and 0.5 mg/ml DNase I (Sigma-Aldrich, St. Louis, MO). Lungs were disrupted by passage through a 100-μm cell strainer, followed by lysis of RBCs using ACK lysis buffer (BioSource International, Camarillo, CA). Lung cells were washed with RPMI 1640 containing 10% FBS, pelleted by centrifugation, and resuspended at a concentration of 1 × 10^6 cells/ml. Staining reactions were performed at 4°C using anti-CD11b-PerCP (eBioscience, San Diego, CA), F4/80-PE (eBioscience), and CD206-Alexa Fluor 647 (BioLegend, San Diego, CA). Flow cytometry was performed using an LSR-II SORP flow cytometer (BD Biosciences, San Jose, CA). After excluding cellular debris using a forward light scatter/side scatter plot, a macrophage gate was determined based upon light scatter properties. Alveolar macrophages with high autofluorescence were identified, and the number of CD11b+/F4/80+/CD206+ cells was counted.

Airway hyperreactivity

Airway resistance was measured in anesthetized mice using an Elan RC Fine Pointe system (Buxco Research Systems, Wilmington, NC), which contains a fully automated built-in ventilator and an in-line aerosol controller. Following anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg), a midline incision was made in the skin extending from the point of the jaw to just above the thoracic inlet. The skin was reflected laterally to show the underlying sternohyoideus and sternothyroideus muscles. Using blunt dissection and continuing on the midline, these two muscle groups were separated laterally to expose the underlying trachea. Following tracheal cannulation with a 19-gauge beveled metal catheter, mice were mechanically ventilated with a constant inspiratory flow, and a second dose of ketamine (100 mg/kg) or increasing doses of methacholine, which included 0.1875, 0.375, 0.75, and 1.5 mg/ml, were given prior to nebulization of PBS. Airway resistance was recorded at 10-s intervals for 5 min, and average values are presented as cm H2O/ml/s.

Measurement of serum IgE

Total serum IgE was measured with an OptEIA (BD Pharmingen, San Diego, CA).

Statistics

Results are presented as mean ± SEM. A one-way ANOVA with a Bonferroni multiple-comparison test or a two-way ANOVA with a Bonferroni posttest test (GraphPad Prism, version 5.0a; GraphPad Software, La Jolla, CA) was used; a p value < 0.05 was considered significant.

Results

The 5A apoA-I mimetic peptide inhibits airway inflammation in a murine model of HDM-induced asthma

Intranasal administration of HDM for 5 d per week for 4 wk induced airway inflammation characterized by an increase in the total number of inflammatory cells present in bronchoalveolar lavage fluid (BALF), as well as in the number of eosinophils, lymphocytes, and neutrophils (Fig. 1). Systemic administration of the 5A apoA-I mimetic peptide by osmotic mini-pump attenuated the total number of BALF inflammatory cells in HDM-challenged mice compared with those that received the control peptide (Fig. 1A). Furthermore, the numbers of BALF eosinophils, lymphocytes, and neutrophils were significantly reduced in HDM-challenged mice that received the 5A peptide but not in those that received the control peptide (Fig. 2A).

The 5A apoA-I mimetic peptide inhibits AHR in HDM-induced asthma

Administration of the 5A peptide to HDM-challenged mice also completely inhibited the induction of AHR. As shown in Fig. 2B, levels of airway resistance in HDM-challenged mice that received the 5A peptide were similar to those in saline-challenged mice. In contrast, levels of airway resistance in HDM-challenged mice that received the control peptide were elevated to levels similar to those in HDM-challenged mice. This demonstrates that the 5A apoA-I mimetic peptide inhibits the induction of AHR in HDM-induced asthma.

The 5A apoA-I mimetic peptide attenuates manifestations of airway remodeling in HDM-induced asthma

Having shown that the 5A apoA-I mimetic peptide inhibited airway inflammation and AHR, we assessed its effect on airway-
remodeling responses, such as mucin gene expression and goblet cell hyperplasia. As shown in Fig. 3, HDM-challenged mice that received the 5A peptide had reductions in mRNA encoding the MUC5AC mucin gene and Clca3, a calcium-activated chloride channel that is associated with goblet cell hyperplasia, as compared with HDM-challenged mice (26). Similarly, goblet cell hyperplasia was reduced in HDM-challenged mice that received the 5A peptides compared with those that did not (Figs. 2, 3). The effect of the 5A peptide on the expression of collagen genes that contribute to subepithelial collagen deposition was also assessed (27). As shown in Fig. 3, the 5A peptide significantly reduced the expression of the genes encoding type I (Col1a1) and type III (Col3a1) collagens. These data demonstrated that the 5A apoA-I mimetic peptide reduced several key manifestations of airway remodeling in HDM-induced asthma, such as goblet cell hyperplasia, as well as the expression of genes encoding airway mucins and collagens.

Administration of the 5A apoA-I mimetic peptide inhibits the expression of Th2 and Th17 cytokines in HDM-induced asthma

We next investigated the mechanisms by which the 5A apoA-I mimetic peptide attenuates HDM-induced airway inflammation. Th2 CD4+ T cells are increased in asthmatic airways and produce canonical Th2 cytokines, such as IL-4 and IL-13 (28). IL-4 promotes the differentiation and proliferation of Th2 cells and IgE production, whereas IL-13 is an effector cytokine that mediates mucin production and AHR in asthma (29). IL-17A, a product of Th17 cells, is required during the induction of allergic asthma; it

FIGURE 2. Effect of the 5A apoA-I mimetic peptide on lung histology and AHR in a murine model of HDM-induced asthma. A, Histologic sections of lung were stained with H&E or PAS stains, and images were obtained at ×200 or ×1000. Scale bars, 100 μm (×200 images) and 25 μm (×1000 images). A representative image is shown. B, Airway resistance was measured following nebulization of increasing doses of methacholine (n = 8–10 mice). A representative result from three independent experiments is shown. *p < 0.05 versus saline; **p < 0.001 HDM + 5A versus HDM.
can also mediate neutrophil-mediated inflammation (28, 30). As shown in Fig. 4, mRNA levels of IL-4, IL-5, IL-13, and IL-17A were reduced in lung homogenates from HDM-challenged mice that had been treated with the 5A apoA-I mimetic peptide. Administration of the 5A apoA-I mimetic peptide also inhibited HDM-induced increases in IL-10 mRNA expression. This result is consistent with the conclusion that the 5A apoA-I mimetic peptide inhibits the induction of inflammatory responses mediated by Th2 and Th17 cytokines in HDM-mediated asthma.

The 5A apoA-I mimetic peptide inhibits lung CC chemokine expression in HDM-induced asthma

Because the recruitment of inflammatory cells to the lungs of HDM-challenged mice was significantly reduced by the 5A apoA-I mimetic peptide, we investigated whether this effect might be mediated by a reduction in chemokine expression. CCL11 (eotaxin-1) and CCL24 (eotaxin-2) are important chemotactic factors for eosinophils and basophils, whereas CCL7 (MCP-3) has chemotactic
activity toward eosinophils, basophils, and monocytes via binding to CCR3 (28, 31). CCL11 (eotaxin-1), CCL24 (eotaxin-2), and CCL7 (MCP-3) also mediate T cell recruitment to the lung in the setting of asthma (32). CCL17 thymus and activation-regulated chemokine, which is produced by dendritic cells and airway epithelial cells, also mediates the chemotaxis of Th2 T cells to the lung during allergic inflammation via CCR4 (28, 33). As shown in Fig. 5, mice that had been treated with the 5A apoA-I mimetic peptide had significantly reduced expression of mRNA encoding CCL7, CCL11, CCL17, and CCL24. This shows that the suppression of key CC chemokines is a mechanism by which the 5A apoA-I mimetic peptide attenuates HDM-mediated increases in T cells and eosinophils.

Administration of the 5A apoA-I mimetic peptide does not inhibit serum IgE

Because treatment with the 5A peptide was initiated prior to sensitization with HDM, we measured total serum IgE levels to assess whether the ability of 5A to attenuate airway inflammation in HDM-induced asthma was a consequence of inhibition of the allergic-sensitization process. As shown in Fig. 6, administration of the 5A peptide did not reduce total serum IgE levels. This suggests that the mechanism by which the 5A peptide attenuates airway inflammation in asthma does not involve impaired allergic sensitization to HDM Ags.

The 5A apoA-I mimetic peptide attenuates alternative macrophage activation in HDM-challenged mice

In contrast to the classical pathway of macrophage activation by IFN-γ, macrophages can undergo alternative activation by the Th2 cytokines IL-4 and IL-13 in the setting of allergic and parasitic inflammation (34, 35). Consistent with this, AAMs have been implicated in the pathogenesis of allergic asthma and mediate the recruitment of eosinophils to sites of parasitic infection (25, 34). AAMs express the macrophage mannose receptor (Mrc1, CD206), arginase 1, and resistin-like α (Fizz1/RELM-α) (36). The macrophage mannose receptor is a C-type lectin that binds pathogenic viruses, bacteria, and fungi (37). Chi3L3 (Ym1, eosinophil chemotactic factor L) and the closely related Chi3L4 (Ym2) are enzymatically inactive chitinases that function as eosinophil chemotactic factors in asthma (35, 38, 39). Arginase 1 converts arginine to ornithine and, thereby, reduces available substrate for inducible NO synthase (40). Fizz1 (resistin-like α) is upregulated in parasitic pulmonary inflammation and functions as an endogenous negative regulator of Th2 inflammatory responses (41). In this study, we showed that treatment with the 5A apoA-I mimetic peptide attenuated the HDM-mediated increases in the total number of CD11b+/F4-80+/CD206+ alveolar macrophages (Fig. 7). Similarly, the expression of mRNA encoding arginase 1, Chi3L3, Chi3L4 (data not shown), and Fizz1 was reduced in the lungs of HDM-challenged mice that were treated with the 5A apoA-I mimetic peptide. These data are consistent with the conclusion that the 5A apoA-I mimetic peptide inhibits the alternative activation of macrophages in HDM-challenged mice.

Discussion

More than 22 million individuals in the United States have asthma (42). Of these, ~5–10% have severe disease that is difficult to control, despite treatment with high doses of inhaled corticosteroids plus long-acting β2-agonists or oral corticosteroids (43, 44). Limited alternative treatment options exist for these individuals who are refractory to standard therapies. Additional controller medications that can be used as add-on therapy are limited to anti-IgE mAbs and leukotriene modifiers, such as leukotriene receptor antagonists and 5-lipoxygenase inhibitors (42). Therefore, new treatment options are needed for asthmatics, especially for those...
with severe disease who experience significant morbidity and have high health care-related costs.

ApoA-I, a major constituent of HDLs, can prevent and reverse atherosclerosis by mediating cholesterol efflux from lipid-laden macrophages (1, 3). ApoA-I also attenuates inflammation in atherosclerosis by removing proinflammatory oxidized phospholipids from low-density lipoproteins and arterial cell walls (1, 45). It was also shown to have anti-inflammatory effects on a variety of cell types that play important roles in the pathogenesis of asthma, such as dendritic cells, T cells, neutrophils, and macrophages. For example, apoA-I prevents dendritic cell maturation, reduces T lymphocyte and neutrophil activation, suppresses macrophage cytokine production, and blocks T cell–monocyte interactions (46–51). Taken together, these findings suggest that the anti-inflammatory effects of apoA-I might be used in a therapeutic fashion to attenuate airway inflammation in asthma.

The expense and difficulty in preparing sufficient quantities of pure, pharmaceutical-quality apoA-I protein have limited its development as a therapeutic agent (3). To address this problem, several apoA-I mimetic peptides that retain the beneficial effects of apoA-I and HDL on cholesterol efflux and atherosclerosis have been developed (1, 3). Consistent with this, the administration of apoA-I mimetic peptides was shown to have anti-inflammatory effects in models of atherosclerosis and cardiac ischemia–reperfusion injury, as well as to attenuate endothelial dysfunction (52–56). Furthermore, apoA-I mimetic peptides demonstrated anti-inflammatory properties in murine models of viral infection and collagen-induced arthritis (57, 58).

Because airway inflammation plays a major role in the pathogenesis of asthma, we assessed whether the administration of an apoA-I mimetic peptide could suppress inflammatory and immune responses in a HDM-challenge model of asthma. We used the 5A apoA-I mimetic peptide, which is a bivalent amphipathic peptide that mediates cholesterol efflux and reduces atherosclerosis via the ABCA1 transporter (3, 5, 59). Each helix is composed of 18 aa linked by a proline (5). In contrast to other apoA-I mimetic peptides that are cytotoxic based upon their ability to insert into cell membranes and disrupt the lipid bilayer, the 5A peptide does not induce hemolysis of RBCs (5). In this article, we demonstrated that the 5A apoA-I mimetic peptide dramatically inhibits the induction of many of the key pathologic features of HDM-induced asthma, including airway inflammation and AHR. It also reduced the severity of several key manifestations of airway remodeling, such as goblet cell hyperplasia and expression of the MUC5AC mucin gene expression and genes encoding types I and III collagens. The ability of the 5A apoA-I mimetic peptide to inhibit airway inflammation was mediated by multiple mechanisms that included the attenuated expression of Th2- and Th17-type cytokines, as well as the reduced expression of chemokines that promote the chemotaxis of T cells, dendritic cells, and eosinophils. Furthermore, the 5A apoA-I mimetic peptide inhibited the recruitment of AAMs to the lungs of HDM-challenged mice. In contrast, it did not inhibit HDM-induced increases in serum IgE levels, which is consistent with the conclusion that the mechanism by which 5A attenuates asthma is not a consequence of impaired allergic sensitization.

The mechanism by which the 5A peptide mediates its inhibitory effects on the induction of asthma may be mediated by its interaction with ABCA1. Consistent with this, the 5A peptide was shown to mediate enhanced lipid efflux from HeLa cells, as well as inhibit TNF-mediated NF-κB activation in vascular endothelial cells, in an ABCA1-dependent fashion (5, 60). ABCA1 is expressed by several cell types in the lung, including airway smooth

**FIGURE 6.** The 5A apoA-I mimetic peptide does not inhibit HDM-induced increases in serum IgE. Quantification of serum IgE levels (n = 8). A representative result from three independent experiments is shown.

**FIGURE 7.** The 5A apoA-I mimetic peptide inhibits alternative macrophage activation in a murine model of HDM-induced asthma. A. Quantification of the total number of CD11b<sup>+</sup>F4-80<sup>+</sup>CD206<sup>−</sup> macrophages in whole-lung digests (n = 10). Pooled data from two independent experiments are shown. *p < 0.05, HDM versus saline; **p < 0.05, HDM versus HDM + 5A. B. Quantification of lung mRNA levels for arginase 1 (Arg1) (B), Chi3L3 (C), and Fizz1 (D) (n = 6 mice). A representative result from three independent experiments is shown. *p < 0.001, HDM versus saline; **p < 0.001, HDM versus HDM + 5A.
5A APOA-I MIMETIC PEPTIDE ATTENUATES EXPERIMENTAL ASTHMA

References


