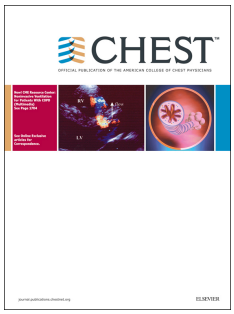
Accepted Manuscript



The A's have it: Developing Apolipoprotein A-I Mimetic Peptides into a Novel Treatment for Asthma

Xianglan Yao, M.D., Ph.D., Elizabeth M. Gordon, Ph.D., Amisha V. Barochia, M.B.B.S., Alan T. Remaley, M.D., Ph.D., Stewart J. Levine, M.D.

PII: S0012-3692(16)50294-3

DOI: 10.1016/j.chest.2016.05.035

Reference: CHEST 521

To appear in: *CHEST*

Received Date: 25 April 2016

Revised Date: 28 April 2016

Accepted Date: 2 May 2016

Please cite this article as: Yao X, Gordon EM, Barochia AV, Remaley AT, Levine SJ, The A's have it: Developing Apolipoprotein A-I Mimetic Peptides into a Novel Treatment for Asthma, *CHEST* (2016), doi: 10.1016/j.chest.2016.05.035.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Word Count: Abstract 225; Text 2421

Submitted for: Ahead of the Curve

The A's have it: Developing Apolipoprotein A-I Mimetic Peptides into a Novel Treatment for Asthma

Xianglan Yao, M.D., Ph.D.[1]
Elizabeth M. Gordon, Ph.D.[1]
Amisha V. Barochia, M.B.B.S.[1]
Alan T. Remaley, M.D., Ph.D.[2]
Stewart J. Levine, M.D.[1]

From the Laboratory of Asthma and Lung Inflammation[1] and the Lipoprotein Metabolism Section[2], Cardiovascular and Pulmonary Branch, Division of Intramural Research, NHLBI, NIH, Bethesda, Maryland, 20892-1590.

Corresponding Author: Stewart J. Levine, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 10, Room 6D03, MSC 1590, Bethesda, Maryland 20892-1590; email: levines@nhlbi.nih.gov

Conflict of Interest Statement: Alan T. Remaley is the holder of patents issued regarding the 5A apoA-I mimetic peptide. None of the other authors have conflicts of interest to disclose.

Funding: This work was funded by the Division of Intramural Research, NHLBI, NIH, Bethesda, Maryland (Project Number: 1ZIAHL006054-06).

Abstract

New treatments are needed for asthmatics who are refractory to standard therapies, such as individuals with a phenotype of "type 2 low" inflammation. This important clinical problem could potentially be addressed by the development of apolipoprotein A-I (apoA-I) mimetic peptides. Apolipoprotein A-I interacts with its cellular receptor, the ATP-binding cassette subfamily A, member 1 (ABCA1), to facilitate cholesterol efflux out of cells to form nascent HDL particles. The ability of the apoA-I/ABCA1 pathway to efflux cholesterol from cells that mediate adaptive immunity, such as antigen-presenting cells, can attenuate their function. Data from experimental murine models have demonstrated that the apoA-I/ABCA1 pathway can reduce neutrophilic airway inflammation primarily by suppressing the production of granulocyte-colony stimulating factor. Furthermore, administration of apoA-I mimetic peptides to experimental murine models of allergic asthma has decreased both neutrophilic and eosinophilic airway inflammation, as well as airway hyperresponsiveness and mucous cell metaplasia. Higher serum levels of apoA-I have also been associated with less severe airflow obstruction in asthmatics. Collectively, these results suggest that the apoA-I/ABCA1 pathway may have a protective effect in asthma and support the concept of advancing inhaled apoA-I mimetic peptides to clinical trials that can assess their safety and effectiveness. Thus, we propose that the development of inhaled apoA-I mimetic peptides as a new treatment could represent a clinical advance for severe asthmatics who are unresponsive to other therapies.

ACCEPTED MANUSCRIPT

Asthma is a common disease that affects over 7% of the population in the United States[1]. Since the 1970s, administration of inhaled corticosteroids to suppress airway inflammation has been the mainstay of asthma treatment[2]. However, inhaled corticosteroids will only improve lung function in asthmatics with a phenotype of "type 2 high" inflammation, as indicated by the increased expression of IL-13-inducible genes in airway epithelial cells[3]. These "type 2 high" asthmatics have eosinophilic airway inflammation, as well as increases in airway hyperresponsiveness (AHR), mucin gene expression, subepithelial fibrosis, and total IgE levels. In contrast, almost half of asthmatics have a phenotype of "type 2 low" inflammation and do not show improvements in airflow obstruction in response to treatment with corticosteroids. Therefore, new therapeutic approaches are needed for asthmatics who do not respond to standard therapies, especially those with severe disease. This clinical problem could be addressed by the development of apolipoprotein A-I (apoA-I)-based therapies.

Apolipoprotein A-I is the major structural protein of high-density lipoproteins (HDL), which has an important protective role in atherosclerotic vascular disease by mediating reverse cholesterol transport out of cells[4, 5]. In particular, apoA-I, which is synthesized in the liver and small intestine and secreted into the blood, interacts with the ATP-binding cassette subfamily A, member 1 (ABCA1) on cells, such as macrophages, to facilitate the transfer of excess cellular cholesterol and phospholipids to nascent, discoidal HDL particles. Nascent HDL then matures into spheroidal particles by a process involving the esterification and transfer of cholesterol to the hydrophobic HDL particle core by lecithin-cholesterol acyltransferase. These spheroidal HDL particles then efflux additional cholesterol and phospholipids from peripheral cells via the ATP-binding cassette subfamily G, member 1 (ABCG1) and the scavenger receptor class B member 1 (SRB1). In addition to its role in reverse cholesterol transport, HDL particles have increasingly been recognized to modulate innate and adaptive immune responses, as well as to have anti-inflammatory, anti-oxidant, anti-thrombotic and anti-fibrotic properties[6-8].

This Ahead of the Curve article will review the evidence supporting the concept of developing apoA-I mimetic peptides for the treatment of asthma.

Insights from murine studies regarding the role of apoA-I in asthma.

Experimental results from murine models of experimental asthma have identified an unexpected role for the apoA-I/ABCA1 pathway in the pathogenesis of asthma. Apolipoprotein A-I and ABCA1 are both expressed by alveolar epithelial cells and alveolar macrophages in the lung, whereas pulmonary vascular endothelial cells and airway smooth muscle cells also express ABCA1[9-14]. Mice with a genetic deletion of apoA-I have a phenotype of increased lung inflammation and oxidative stress, as well as enhanced AHR and collagen deposition[15]. In addition, levels of HDL, as well as the anti-oxidant protein, paraoxonase 1, are reduced in *Apoa1*-deficient mice.

Ovalbumin (OVA)-challenged *Apoa1*-deficient mice have a phenotype consistent with neutrophilic-predominant, "type 2 low" asthma with an increase in airway neutrophils that involved multiple pathways, including type 1 cytokines (IFN-γ, TNF-α), a type 17 cytokine (IL-17A), a CXC chemokine (CXCL5), a vascular adhesion molecule (vascular cell adhesion molecule-1) and granulocyte-colony stimulating factor (G-CSF)[9]. In contrast, type 2 cytokines (IL-4, IL-5 and IL-13) were not increased in this model. The enhanced neutrophilic airway inflammation in OVA-challenged *Apoa1*-deficient mice was primarily mediated by G-CSF, as administration of a neutralizing anti-GSF antibody significantly reduced OVA-induced bronchoalveolar lavage fluid (BALF) neutrophil numbers. This shows that apoA-I can attenuate neutrophilic airway inflammation in type 2-low experimental murine asthma via a G-CSF-dependent mechanism.

Apolipoprotein A-I can also attenuate lipopolysaccharide (LPS)-mediated neutrophilic airway inflammation. This is relevant for asthma, as inhalational LPS exposure has been associated with an increased risk for asthma and wheezing in the

United States, whereas LPS inhalation by asthmatics increases sputum neutrophils and CXCL8 levels[16-18]. Furthermore, LPS signaling via Toll-like receptor 4 mediates neutrophilic, as well as eosinophilic, airway inflammatory responses to inhaled antigens in murine models of experimental asthma[19-21]. Several mechanisms have been identified by which apoA-I can suppress LPS-mediated neutrophilic airway inflammation. LPS-challenged *Apoa1*-deficient mice have increased numbers of BALF neutrophils as compared to wild type mice[22]. Suppression of CXCR2-mediated neutrophil chemotaxis in response to CCL2 was identified as the mechanism by which apoA-I attenuated LPS-induced neutrophil recruitment to the lung[22]. Additionally, apoA-I can directly bind and neutralize LPS, and it can also associate with the LPS-binding protein as another mechanism to neutralize LPS activity[23-25]. Furthermore, apoA-I can directly suppress neutrophil activation, with associated reductions in cellular adhesion, oxidative burst, and degranulation[26, 27].

Murine models of experimental asthma have also provided insights into the role of ATP-binding cassette transporters in the pathogenesis of asthma. OVA-challenged *Tie2-hABCA1* transgenic mice, which conditionally over-express the human ABCA1 transporter under the Tie2 promoter in vascular endothelial cells and macrophages, have attenuated neutrophilic airway inflammation, airway epithelial wall thickness, and serum levels of OVA-specific IgE[11]. The attenuated neutrophilic airway inflammation in OVA-challenged *Tie2-hABCA1* mice was associated with significant reductions in G-CSF protein expression by pulmonary vascular endothelial cells and alveolar macrophages, as well as reduced G-CSF protein levels in BALF. This suggests that ABCA1 expression by vascular endothelial cells and macrophages may reduce allergen-induced neutrophilic airway inflammation by suppressing G-CSF production.

Experiments performed by several laboratories have independently shown that administration of apoA-I or apoA-I mimetic peptides can attenuate the manifestations of allergen-induced experimental asthma. First, intranasal administration of the 5A apoA-I mimetic peptide to OVA-challenged *Apoa-I*

knockout mice suppressed increases in neutrophilic airway inflammation[9]. Similarly, administration of the apoA-I mimetic peptide, L-4F, to wild type mice that received inhaled LPS reduced the number of BALF neutrophils[22]. Second, systemic administration of the 5A apoA-I mimetic peptide coincident with intranasal administration of HDM to wild type A/J mice significantly reduced the induction of airway inflammation with decreased numbers of BALF eosinophils, lymphocytes, and neutrophils. This reduction in airway inflammation was associated with decreases in type 2 cytokines (IL-4, IL-5 and IL-13), a type 17 cytokine (IL-17A), C-C chemokines (CCL7, CCL11, CCL17 and CCL24), alternative macrophage activation, AHR and mucous cell metaplasia[28]. Third, intranasal administration of the D4F apoA-I mimetic peptide in a murine model of OVA-induced experimental asthma reduced airway inflammation, AHR, TGF-β and lung collagen deposition, as well as total IgE and pro-inflammatory HDL in plasma[29]. Lastly, intranasal administration of full-length human apoA-I to HDM-challenged mice reduced airway inflammation with decreases in BALF eosinophils, neutrophils, lymphocytes and macrophages, as well as AHR and lung levels of the airway epithelial cell-derived cytokines, IL-25, IL-33 and TSLP, that promote allergic inflammation[30]. Administration of apoA-I also increased the expression of airway epithelial cell tight junction proteins, as well as levels of the anti-inflammatory and pro-resolving lipid mediator, lipoxin A4[30]. Collectively, these experiments support the concept of developing apoA-I-based treatment approaches for asthma (Figure 1).

What are the potential cellular mechanisms by which the apoA-I/ABCA1 pathway may modulate asthma pathogenesis?

An important mechanism by which the apoA-I/ABCA1 pathway may modulate the pathogenesis of asthma involves cholesterol efflux out of antigen-presenting cells that mediate adaptive immunity. Reduction of cellular cholesterol levels diminishes receptor localization to lipid raft domains and thereby attenuates signaling. For example, *Abca1*/*Abcg1* double knockout mice have enhanced signaling via the IL-

3/GM-CSF receptor with resultant increases in blood neutrophils and monocytes, as well as bone marrow hematopoietic stem cells, common myeloid progenitor cells and granulocyte-monocyte progenitor cells[31, 32]. The enhanced responsiveness to IL-3 and GM-CSF was caused by increased clustering of the common β subunit of the IL-3/GM-CSF receptor within lipid rafts that formed in response to excess cellular cholesterol. Treatment of murine antigen-presenting cells (e.g., dendritic cells, macrophages and B cells) with apoA-I or HDL increases cholesterol efflux and decreases the abundance of membrane lipid raft domains, which thereby reduces MHCII density and attenuates T cell activation[33]. Similarly, treatment of dendritic cells with recombinant HDL comprised of apoA-I and lipids in a murine model of antigen-induced arthritis reduced cell surface expression of co-stimulatory molecules and attenuated the ability of dendritic cells to activate IFN-γ-producing Th1 cells, as well as IL-17-producing Th17 cells, via an ABCA1- and SRB1-dependent pathway[34].

Additional mechanisms have been identified by which apoA-I may modulate immune cell function. Apolipoprotein A-I can inhibit the differentiation of human peripheral blood mononuclear cells into dendritic cells by increasing the production of IL-10 and prostaglandin E2[35]. In hypercholesterolemic *Ldlr/Apoa1* double knockout mice, administration of lipid-free apoA-I increased the number of regulatory T cells (Tregs) and decreased the number of effector/effector memory T cells in lymph nodes[36]. However, in a murine model of normocholesterolemic systemic lupus erythematosus, apoA-I over-expressing transgenic mice had a reduction in Th1 cells that was independent of cholesterol or changes in Tregs or dendritic cells[37]. Instead, apoA-I increased the production of oxidized metabolites of linoleic acid (e.g., HODEs) and arachidonic acid (e.g., HETEs) that activate peroxisome proliferator-activated receptor-γ. These studies suggest that the mechanism by which apoA-I regulates T cell function may be context-dependent and modified in the setting of hypercholesterolemia and obesity. In addition, apoA-I blocks the contact-mediated activation of monocytes by stimulated T cells, with a

resultant decrease in TNF-α and IL-1β[38]. The role of the apoA-I/ABCA1 pathway in modifying innate and adaptive immune responses in asthmatic patients, however, has yet to be defined.

The apoA-I/ABCA1 pathway may also modulate the function of airway structural cells in asthma. For example, ABCA1 mediates the efflux of cholesterol and phospholipids from human airway smooth muscle cells in response to activation of liver X receptors, LXR-α and LXR-β, which are nuclear receptors that induce the expression of both ABCA1 and ABCG1[14]. This suggests that modulation of cholesterol homeostasis in airway smooth muscle cells by ABCA1 may directly influence the pathological manifestations of asthma. Taken collectively, the ability of apoA-I to interact with the ABCA1 transporter and efflux cholesterol out of both immune and structural cells represents a mechanism by which asthma severity may be modified.

Insights from human studies regarding the role of apoA-I in asthma.

Recent studies have begun to investigate the role of apoA-I in human subjects with asthma. For example, apoA-I levels in BALF were found to be decreased in mild-to-moderate asthmatics who were not receiving asthma medications[30]. Similarly, protein levels of apoA-I were reduced in the lungs of wild type mice that had been sensitized and challenged with OVA[9]. The reduction of apoA-I in BALF from mice and humans suggests that disease severity might be augmented in asthmatics due to lower levels of apoA-I in the lung. In addition, apoA-I has been shown to attenuate IL-25, IL-33 and TSLP production by normal human bronchial epithelial cells in response to cockroach extract or the house dust mite allergen, Der p1[30].

Clinical studies have also demonstrated an association between higher serum apoA-I levels and less severe airflow obstruction in asthmatics. An analysis of 14,135 subjects without respiratory disease who participated in the Third National Health

and Nutrition Examination Survey found that serum levels of both apoA-I and HDL were positively correlated with FEV_1[39]. Recently, we reported a cross-sectional analysis of 159 atopic asthmatic subjects, which similarly showed a positive correlation between FEV_1 and serum levels of apoA-I, as well as HDL-C (HDL-cholesterol)[40]. Furthermore, serum samples were analyzed by nuclear magnetic resonance (NMR) spectroscopy to identify the subset of HDL particles that mediated the association with FEV_1. This technique measures the NMR proton signal from the terminal methyl group on lipids to determine the amount of each type of lipoprotein particle and also categorizes lipoprotein particles based upon size[41]. This analysis identified that the subset of large HDL particles, but not the small- or medium-sized HDL particles, were specifically associated with higher FEV_1 in asthmatic subjects. Interestingly, the concentration of large HDL particles has also been associated with a reduction in risk of cardiovascular disease in healthy women[42].

Can apoA-I be developed into a novel therapeutic approach for asthma?

We propose that the available data from animal and human studies support the concept of developing apoA-I-based therapies for asthma. One approach for the delivery of apoA-I to the lung for the treatment of asthma is to use apoA-I mimetic peptides, which have successfully attenuated disease activity in murine asthma models[28, 29]. ApoA-I mimetic peptides have an amphipathic α helical structure that resembles the secondary structure of the native apoA-I protein, which is a tandem array of 10 class A amphipathic α helices that mediate interactions with lipids[43, 44]. ApoA-I mimetic peptides can facilitate reverse cholesterol transfer out of cells, as well as promote anti-inflammatory, anti-oxidant and other anti-atherogenic effects[45]. Advantages of apoA-I mimetic peptides over full-length apoA-I relate to the relative ease and lower cost of synthesis[4]. Murine asthma studies have used the D4F and 5A apoA-I mimetic peptides, while additional apoA-I mimetic peptides are actively being developed[45, 46]. The D4F apoA-I mimetic peptide is an 18 amino acid peptide comprised of D-amino acids[47]. The 5A apoA-I mimetic peptide is comprised

of two 18 amino acid peptides linked by a proline, one of which is a high lipid affinity helix, while the other is a low lipid affinity helix[48]. This design allows the 5A peptide to specifically mediate phospholipid efflux from cells via ABCA1 with low-associated cellular toxicity. The D4F apoA-I mimetic peptide has already been safely administered by oral administration in clinical trials to human subjects with cardiovascular disease, which provides evidence to support the concept that apoA-I mimetic peptide therapy for asthma is potentially feasible[49, 50]. Furthermore, the 5A peptide is currently being developed for human clinical trials of atherosclerotic vascular disease by intravenous administration. Since medications can be directly delivered to the respiratory system by inhalation, it would be reasonable for clinical trials of apoA-I mimetic peptides in asthma to utilize this approach, where they could potentially directly interact with ABCA1 transporters expressed by lung cells.

Based upon the emerging body of evidence from pre-clinical murine models regarding the protective role of apoA-I in asthma, as well as human translational studies that have shown an association between serum apoA-I levels and FEV_1 in asthmatics, we propose that a reasonable next step is to advance apoA-I mimetic peptides from the lab to clinical trials where their safety and efficacy can be evaluated. Furthermore, clinical trials of inhaled apoA-I mimetic peptides can establish whether this represents an effective therapeutic approach for asthma. In particular, we propose that the development of apoA-I mimetic peptides could represent an important clinical advance as a new treatment for severe asthmatics who are refractory to standard treatment with corticosteroids. This could be particularly relevant for asthmatics with a "type 2 low" phenotype of neutrophilic-predominant airway inflammation, as therapies do not currently exist for these individuals. ApoA-I mimetic peptides could additionally represent a treatment option for "type 2 high" asthmatics with eosinophilic-predominant airway inflammation.

ACCEPTED MANUSCRIPT

Figure Legends

Figure 1. Administration of apolipoprotein A-I mimetic peptides or human apolipoprotein A-I to murine models of experimental asthma attenuates both neutrophilic and eosinophilic airway inflammation, as well as airway hyperresponsiveness and airway remodeling.

ACCEPTED MANUSCRIPT

References

1. National Current Asthma Prevalence (2013). http://www.cdc.gov/asthma/most_recent_data.htm.

2. Gauthier M, Ray A, Wenzel SE. Evolving Concepts of Asthma. *Am J Respir Crit Care Med* 2015; **192**(6)**:** 660-668.

3. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009; **180**(5)**:** 388-395.

4. Kingwell BA, Chapman MJ, Kontush A, Miller NE. HDL-targeted therapies: progress, failures and future. *Nat Rev Drug Discov* 2014; **13**(6)**:** 445-464.

5. Rosenson RS, Brewer HB, Jr., Davidson WS, Fayad ZA, Fuster V, Goldstein J *et al.* Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation* 2012; **125**(15)**:** 1905-1919.

6. Catapano AL, Pirillo A, Bonacina F, Norata GD. HDL in innate and adaptive immunity. *Cardiovasc Res* 2014; **103**(3)**:** 372-383.

7. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. *Nature Reviews Cardiology* 2011; **8**(4)**:** 222-232.

8. Osei-Hwedieh DO, Amar M, Sviridov D, Remaley AT. Apolipoprotein mimetic peptides: Mechanisms of action as anti-atherogenic agents. *Pharmacol Ther* 2011; **130**(1)**:** 83-91.

9. Dai C, Yao X, Keeran KJ, Zywicke GJ, Qu X, Yu ZX *et al.* Apolipoprotein A-I Attenuates Ovalbumin-Induced Neutrophilic Airway Inflammation via a Granulocyte Colony-Stimulating Factor-Dependent Mechanism. *Am J Respir Cell Mol Biol* 2012; **47**(2)**:** 186-195.

10. Kim TH, Lee YH, Kim KH, Lee SH, Cha JY, Shin EK *et al.* Role of lung apolipoprotein A-I in idiopathic pulmonary fibrosis: antiinflammatory and antifibrotic effect on experimental lung injury and fibrosis. *Am J Respir Crit Care Med* 2010; **182**(5)**:** 633-642.

11. Dai C, Yao X, Vaisman B, Brenner T, Meyer KS, Gao M *et al.* The ABCA1 Transporter Attenuates Ovalbumin-induced Neutrophilic Airway Inflammation. *Am J Respir Cell Mol Biol* 2014.

12. Bates SR, Tao JQ, Yu KJ, Borok Z, Crandall ED, Collins HL *et al.* Expression and biological activity of ABCA1 in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 2008; **38**(3)**:** 283-292.

13. Bortnick AE, Favari E, Tao JQ, Francone OL, Reilly M, Zhang Y *et al.* Identification and characterization of rodent ABCA1 in isolated type II pneumocytes. *Am J Physiol Lung Cell Mol Physiol* 2003; **285**(4)**:** L869-878.

14. Delvecchio CJ, Bilan P, Nair P, Capone JP. LXR-induced reverse cholesterol transport in human airway smooth muscle is mediated exclusively by ABCA1. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**(5)**:** L949-957.

15. Wang W, Xu H, Shi Y, Nandedkar S, Zhang H, Gao H *et al.* Genetic deletion of apolipoprotein A-I increases airway hyperresponsiveness, inflammation, and collagen deposition in the lung. *J Lipid Res* 2010; **51**(9)**:** 2560-2570.

16. Nightingale JA, Rogers DF, Hart LA, Kharitonov SA, Chung KF, Barnes PJ. Effect of inhaled endotoxin on induced sputum in normal, atopic, and atopic asthmatic subjects. *Thorax* 1998; **53**(7)**:** 563-571.

17. Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes SJ, Jr., Zeldin DC. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. *Am J Respir Crit Care Med* 2005; **172**(11)**:** 1371-1377.

18. Thorne PS, Mendy A, Metwali N, Salo P, Co C, Jaramillo R *et al.* Endotoxin Exposure: Predictors and Prevalence of Associated Asthma Outcomes in the United States. *Am J Respir Crit Care Med* 2015; **192**(11)**:** 1287-1297.

19. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; **196**(12)**:** 1645-1651.

20. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; **15**(4)**:** 410-416.

21. McAlees JW, Whitehead GS, Harley IT, Cappelletti M, Rewerts CL, Holdcroft AM *et al.* Distinct Tlr4-expressing cell compartments control neutrophilic and eosinophilic airway inflammation. *Mucosal Immunol* 2015; **8**(4)**:** 863-873.

22. Madenspacher JH, Azzam KM, Gong W, Gowdy KM, Vitek MP, Laskowitz DT *et al.* Apolipoproteins and apolipoprotein mimetic peptides modulate phagocyte trafficking through chemotactic activity. *J Biol Chem* 2012; **287**(52)**:** 43730-43740.

23. Emancipator K, Csako G, Elin RJ. In vitro inactivation of bacterial endotoxin by human lipoproteins and apolipoproteins. *Infect Immun* 1992; **60**(2)**:** 596-601.

24. Henning MF, Herlax V, Bakas L. Contribution of the C-terminal end of apolipoprotein AI to neutralization of lipopolysaccharide endotoxic effect. *Innate immunity* 2011; **17**(3)**:** 327-337.

25. Wurfel MM, Kunitake ST, Lichenstein H, Kane JP, Wright SD. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J Exp Med* 1994; **180**(3)**:** 1025-1035.

26. Liao XL, Lou B, Ma J, Wu MP. Neutrophils activation can be diminished by apolipoprotein A-I. *Life Sci* 2005; **77**(3)**:** 325-335.

27. Blackburn WD, Jr., Dohlman JG, Venkatachalapathi YV, Pillion DJ, Koopman WJ, Segrest JP *et al.* Apolipoprotein A-I decreases neutrophil degranulation and superoxide production. *J Lipid Res* 1991; **32**(12)**:** 1911-1918.

28. Yao X, Dai C, Fredriksson K, Dagur PK, McCoy JP, Qu X *et al.* 5A, an apolipoprotein A-I mimetic peptide, attenuates the induction of house dust mite-induced asthma. *J Immunol* 2011; **186**(1)**:** 576-583.

29. Nandedkar SD, Weihrauch D, Xu H, Shi Y, Feroah T, Hutchins W *et al.* D-4F, an apoA-1 mimetic, decreases airway hyperresponsiveness, inflammation, and oxidative stress in a murine model of asthma. *J Lipid Res* 2011; **52**(3)**:** 499-508.

30. Park SW, Lee EH, Lee EJ, Kim HJ, Bae DJ, Han S *et al.* Apolipoprotein A1 potentiates lipoxin A4 synthesis and recovery of allergen-induced disrupted tight junctions in the airway epithelium. *Clinical and Experimental Allergy* 2013; **43**(8)**:** 914-927.

31. Hansson GK, Bjorkholm M. Tackling two diseases with HDL. *Science* 2010; **328**(5986)**:** 1641-1642.

32. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S *et al.* ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science* 2010; **328**(5986)**:** 1689-1693.

33. Wang SH, Yuan SG, Peng DQ, Zhao SP. HDL and ApoA-I inhibit antigen presentation-mediated T cell activation by disrupting lipid rafts in antigen presenting cells. *Atherosclerosis* 2012; **225**(1)**:** 105-114.

34. Tiniakou I, Drakos E, Sinatkas V, Van Eck M, Zannis VI, Boumpas D *et al.* High-density lipoprotein attenuates Th1 and th17 autoimmune responses by modulating dendritic cell maturation and function. *J Immunol* 2015; **194**(10)**:** 4676-4687.

35. Kim KD, Lim HY, Lee HG, Yoon DY, Choe YK, Choi I *et al.* Apolipoprotein A-I induces IL-10 and PGE2 production in human monocytes and inhibits dendritic cell differentiation and maturation. *Biochem Biophys Res Commun* 2005; **338**(2)**:** 1126-1136.

36. Wilhelm AJ, Zabalawi M, Owen JS, Shah D, Grayson JM, Major AS *et al.* Apolipoprotein A-I modulates regulatory T cells in autoimmune LDLr-/-, ApoA-I-/- mice. *J Biol Chem* 2010; **285**(46)**:** 36158-36169.

37. Black LL, Srivastava R, Schoeb TR, Moore RD, Barnes S, Kabarowski JH. Cholesterol-Independent Suppression of Lymphocyte Activation, Autoimmunity, and Glomerulonephritis by Apolipoprotein A-I in Normocholesterolemic Lupus-Prone Mice. *J Immunol* 2015; **195**(10)**:** 4685-4698.

38. Hyka N, Dayer JM, Modoux C, Kohno T, Edwards CK, 3rd, Roux-Lombard P *et al.* Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 2001; **97**(8)**:** 2381-2389.

39. Cirillo DJ, Agrawal Y, Cassano PA. Lipids and pulmonary function in the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 2002; **155**(9)**:** 842-848.

40. Barochia AV, Kaler M, Cuento RA, Gordon EM, Weir NA, Sampson M *et al.* Serum apolipoprotein a-I and large high-density lipoprotein particles are positively correlated with FEV1 in atopic asthma. *Am J Respir Crit Care Med* 2015; **191**(9)**:** 990-1000.

41. Rosenson RS, Brewer HB, Jr., Chapman MJ, Fazio S, Hussain MM, Kontush A *et al.* HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin Chem* 2011; **57**(3)**:** 392-410.

42. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009; **119**(7)**:** 931-939.

43. Getz GS, Wool GD, Reardon CA. Apoprotein A-I mimetic peptides and their potential anti-atherogenic mechanisms of action. *Curr Opin Lipidol* 2009; **20**(3)**:** 171-175.

44. Segrest JP, Jones MK, De Loof H, Brouillette CG, Venkatachalapathi YV, Anantharamaiah GM. The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. *J Lipid Res* 1992; **33**(2)**:** 141-166.

45. Leman LJ, Maryanoff BE, Ghadiri MR. Molecules that mimic apolipoprotein A-I: potential agents for treating atherosclerosis. *J Med Chem* 2014; **57**(6)**:** 2169-2196.

46. Reddy ST, Navab M, Anantharamaiah GM, Fogelman AM. Apolipoprotein A-I mimetics. *Curr Opin Lipidol* 2014; **25**(4)**:** 304-308.

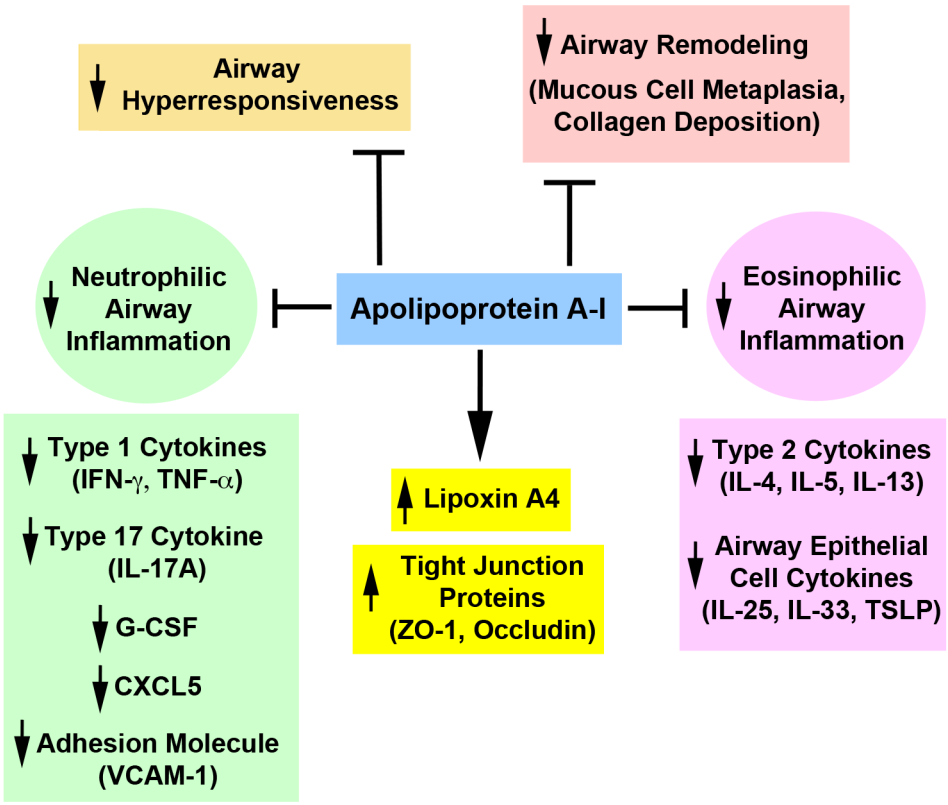
47. Navab M, Anantharamaiah GM, Hama S, Garber DW, Chaddha M, Hough G *et al.* Oral administration of an Apo A-I mimetic Peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. *Circulation* 2002; **105**(3)**:** 290-292.

48. Sethi AA, Stonik JA, Thomas F, Demosky SJ, Amar M, Neufeld E *et al.* Asymmetry in the lipid affinity of bihelical amphipathic peptides. A structural determinant for the specificity of ABCA1-dependent cholesterol efflux by peptides. *J Biol Chem* 2008; **283**(47)**:** 32273-32282.

49. Bloedon LT, Dunbar R, Duffy D, Pinell-Salles P, Norris R, DeGroot BJ *et al.* Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients. *J Lipid Res* 2008; **49**(6)**:** 1344-1352.

50. Dunbar RL, Bloedon LT, Duffy D, Norris RB, Movva R, Navab M *et al.* Daily oral administration of the apolipoprotein A-I mimetic peptide D-4F in patients with heart disease or equivalent risk improves high-density lipoprotein anti-inflammatory function (Abstract). *J Am Coll Cardiol* 2007; **49:** 366A.

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abbreviation List

ABCA1	ATP-binding cassette subfamily A, member 1
ABCG1	ATP-binding cassette subfamily G, member 1
AHR	airway hyperresponsiveness
apoA-I	apolipoprotein A-I
BALF	bronchoalveolar lavage fluid
G-CSF	granulocyte-colony stimulating factor
HDL	high-density lipoproteins
LPS	lipopolysaccharide
NMR	nuclear magnetic resonance
OVA	ovalbumin
SRB1	scavenger receptor class B member 1
Tregs	regulatory T cells

ACCEPTED MANUSCRIPT