

Allergic to Autotaxin

A New Role for Lysophospholipase D and Lysophosphatidic Acid in Asthma?



In this issue of the *Journal*, Park and colleagues (pp. 928–940) make a convincing case that an enzyme known as autotaxin (ATX) is produced in the lung and plays an essential role in promoting allergic airway inflammation (1). What is an enzyme implicated in cancer growth and metastasis doing in the lung in asthma? To answer this question, a little background is in order. In 1992, Stracke and colleagues working at the National Cancer Institute identified a factor from the conditioned media of melanoma cells that potently induced autocrine cell motility and was unrelated to any known molecules at the time (2). The effects of this factor, which they termed ATX, could be inhibited by pretreating cells with pertussis toxin—which suggests that it acted through a G protein–coupled receptor. Cloning of the ATX complementary DNA revealed domains similar to the ectonucleotide phosphodiesterase and pyrophosphatase (ENPP) family, and ATX was designated ENPP2. However, an important breakthrough came from the discovery that the preferred substrate for ATX is not a nucleotide but actually a lipid moiety, namely lysophosphatidylcholine (3). ATX cleaves the choline group from lysophosphatidylcholine to generate the pluripotent extracellular mediator lysophosphatidic acid (LPA; see Figure 1), indicating that it was the sought-after extracellular lysophospholipase D. Subsequent research by several groups established a key role for ATX and LPA in promoting the growth and motility of different cancers (4). Twenty years after the original discovery of ATX, ATX antagonists are now under active development with the enormous potential to block tumor growth and metastasis.

In addition to its aberrant expression by cancer cells, ATX is expressed during embryogenesis, where it is essential for blood vessel formation and may regulate lung development (5). ATX-null mice are embryonic lethal, and consequently conditional deletion approaches will be required to dissect the role of this molecule *in vivo*. In adults, ATX is constitutively expressed in a tissue-specific manner, including in the lung, spleen, kidney, brain, and adipose tissue. Constitutive expression of ATX in these tissues generates plasma LPA, because heterozygous germ-line deletion or conditional knockout in adipose tissues reduces circulating LPA levels (5, 6). In addition to its enzymatic activity, ATX can bind to cells (e.g., lymphocytes and platelets) in an integrin-dependent manner, and the recent crystal structure of ATX provided insights into how ATX might function *in vivo* (7). Integrin binding requires the N-terminal SMB2 domain, which is adjacent to an unusual hydrophobic pocket and channel. These hydrophobic regions accommodate the binding of lysolipids with specific acyl side chains, and explain the preference of ATX for lipid substrates. Integrin binding is predicted to induce a conformational change in ATX, allowing it to deliver its bound lipids to LPA receptors in the immediate vicinity. This suggests that ATX can act at a distance from its site of production and that integrin-dependent tethering might target LPA signaling in a spatially restricted manner. Consequently one challenge in the field is to understand the molecular mechanisms underlying ATX tethering and/or activation *in vivo*.

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LPA binds to at least six cell-surface G protein–coupled receptors that vary in their tissue distribution and function (8). Experiments using gene-targeted mice and pathway inhibitors have uncovered novel roles for ATX and LPA in lung fibrosis acting at least in part via LPA1 expressed by fibroblasts (9, 10). Emerging evidence points to a role for LPA in asthma. For example, LPA enhances airway smooth muscle contractility (11), and induces bronchial epithelial cells to secrete thymic stromal lymphopoietin and chemokine (C-C) motif ligand 20 (12), which promote the recruitment and activation of leukocytes in asthma models. It seems likely that the ATX/LPA axis is more broadly involved in immune-mediated and inflammatory diseases. One interesting example stems from the discovery that ATX is constitutively produced by high endothelial venules in lymph nodes (13). High endothelial venules are unique cuboidal endothelial cells that express adhesion molecules and chemokines recognized by naive T cells, and facilitate steady-state recirculation of lymphocytes through the lymphatic system. Evidence is mounting that ATX-derived LPA regulates T-cell motility (14), and we speculate that locally generated LPA provides a “chemokinetic boost” to T cells traversing the lymph node vasculature, promoting transendothelial migration and trafficking to secondary lymphoid organs (Knowlden and Georas, unpublished data). Because entry into lymph nodes is a critical step in the initiation of immune responses, one implication of these studies is that ATX antagonists may have unanticipated immunosuppressive effects, which will need to be carefully evaluated as these compounds enter the clinical arena.

Park and colleagues conducted an elegant study that incorporates data from human subjects and a mouse model, and builds on our prior research indicating that LPA is enriched in the lung during allergen-induced reactions in humans (15). Similar to that prior report, Park and colleagues used a segmental bronchoprovocation allergen challenge model, and found that polyunsaturated LPA species including 22:5 and 22:6 LPA predominate in bronchoalveolar lavage (BAL) fluids during the last phase response. Importantly, Park and colleagues simultaneously measured LPA species in plasma, which were largely unchanged after allergen challenge. This strongly suggests that lung LPA species do not derive simply from plasma and that, instead, LPA production in the lung is compartmentalized from that in other tissues. Interestingly, using the absolute values of LPA reported by Park and colleagues and assuming a ~100-fold dilution during BAL (16), LPA levels in epithelial lining fluids may be higher than those in plasma. However, LPA is extensively protein bound, which affects its delivery to target tissues, and consequently the values obtained using high-pressure liquid chromatography and tandem mass spectrometry may not precisely capture bioactivity at different sites. Emerging data indicate that the composition of LPA acyl side chains can affect receptor affinity, which may translate into biological effects. For example, unsaturated LPA species preferentially induce chemotaxis of immature mouse dendritic cells (17). The mechanisms and consequences for compartmentalization in LPA generation in the

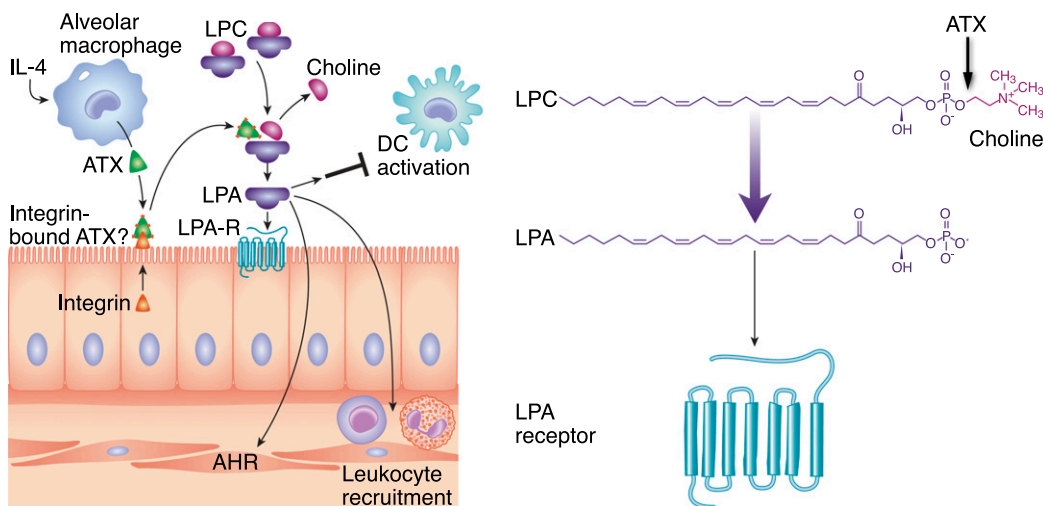


Figure 1. Cartoon diagram of the potential role of autotaxin (ATX) in asthma. ATX is secreted from alveolar macrophages exposed to the Th2 cytokine IL-4 and may be secreted into the airway. The activity of ATX will be increased when it is tethered in an integrin-dependent manner (red triangle), enhancing its lysophospholipase D activity, which results in the generation of lysophosphatidylcholine (LPC) from lysophosphatidic acid (LPA) by cleaving a choline moiety. Whether ATX binds integrins expressed on airway epithelial cells remains to be determined. LPA can interact with receptors

on different cell types and promote airway inflammation and hyperreactivity, but LPA also has potential inhibitory effects depending on cells and receptors engaged. The *right side* indicates the chemical structure of LPC and LPA and site of action of ATX. AHR = airway hyperreactivity; DC = dendritic cell.

systemic circulation versus the lung remain to be determined. Future research into the function of 22:5 and 22:6 LPA in the lung should prove insightful in this regard.

Park and colleagues also used a triple allergen challenge mouse model of asthma with extracts of house dust mites, ragweed, and *Aspergillus* species (DRA), which results in eosinophilic airway inflammation a few weeks after sensitization and challenge. Employing both gain-of-function (transgenic ATX driven by the α_1 -antitrypsin promoter) and loss-of-function (ATX $^{+/-}$ mice) approaches, the authors make a convincing case that ATX is required for Th2-dependent eosinophilic lung inflammation in the DRA model. Serum IgE levels were not increased in ATX-overexpressing mice, which argues against a role for ATX during allergen sensitization. However, it remains to be seen whether ATX deficiency or antagonism comprises allergen sensitization. Because the authors immunized mice by intraperitoneal injection of allergens plus alum, future research will be needed to determine whether ATX plays a role in the initiation of immune responses to inhaled allergens (e.g., by regulating dendritic cell activation or lymphocyte trafficking to lung-draining lymph nodes).

What lung cell types express ATX? Using a combination of immunohistochemistry and reverse transcription–polymerase chain reaction, Park and colleagues conclude that airway epithelial cells and alveolar macrophages are likely suspects. The observation that IL-4 induces low-level ATX secretion from bone marrow–derived macrophages provides a potential mechanism linking ATX with Th2-dependent responses, although the biological activity of macrophage-derived ATX remains to be determined. Future experiments in which the expression of ATX can be regulated in a cell type–specific manner will help define the precise source of ATX in models of allergen-induced airway inflammation and hyperreactivity. Using LPA2 gene–targeted mice, the authors conclude that some of the proinflammatory effects of ATX-derived LPA are dependent on this receptor (similar to studies by Zhao and colleagues [18]). However, we found that LPA2 transduces an inhibitory signal for dendritic cell activation and allergic lung inflammation using a different mouse model (19), and LPA2 has inhibitory effects in LPS-induced lung inflammation (Dr. Andrew Tager, personal communication). Thus more research will be needed to determine the specific cell types and receptors targeted by LPA in asthma models.

Finally, Park and colleagues used a new ATX inhibitor and showed that administration of this compound after sensitization but before challenge partially attenuated allergen-induced Th2

cytokine production in lung tissues and BAL fluids. This ATX antagonist (termed GWJ) appears to be relatively stable *in vivo* and demonstrated sustained inhibition of LPA levels for several hours after injection. Partial efficacy in the DRA model could be explained by the fact that GWJ does not fully inhibit ATX activity *in vivo*. This compound is an improvement over earlier ATX antagonists, which were much less stable, and suggests that ATX may be a viable therapeutic target in allergic airway inflammation and asthma.

Taken together with prior research, the study by Park and colleagues should firmly establish the ATX/LPA axis in the pathogenesis of allergen-driven airway inflammation and asthma. Not surprisingly, LPA production was variable in the human cohort, which suggests there may be subsets of individuals with asthma who are more LPA dependent. One interesting possibility is that the ATX/LPA axis may be more prominent in obese individuals with asthma, given the increasing evidence that ATX and LPA regulate adipocyte growth and differentiation (20). Several unanswered questions remain, including the precise mechanisms by which LPA enhances airway inflammation. In future studies, it will be important to determine whether LPA promotes leukocyte motility directly (e.g., acting on myeloid cells or T-cell subset LPA receptors), or indirectly (e.g., by inducing chemokine or cytokine production from epithelial cells). The potential for LPA to act directly on smooth cells and enhance airway hyperreactivity also deserves further study. We also need to reconcile the proinflammatory effects of LPA with the potential for this molecule to have a regulatory role in certain contexts. For example, if environmental exposures induce repeated cycles of LPA generation in the lung, then ATX/LPA-driven injury/repair responses may contribute to airway remodeling over time. Perhaps most excitingly, the work by Park and colleagues suggests that an entirely new class of compounds currently being designed for cancer therapeutics might also be useful in the asthma clinic.

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STEVE N. GEORAS, M.D.

Departments of Medicine, Environmental Medicine, and
Microbiology and Immunology
University of Rochester Medical Center
Rochester, New York

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The Future of Nature versus Nurture in Understanding Chronic Obstructive Pulmonary Disease



The burden of chronic obstructive pulmonary disease (COPD) is increasing and it is expected to become the third leading cause of death worldwide by 2030. Components of both nature and nurture are important in the development and progression of COPD, with the main risk factor in most of the developed world being tobacco smoke exposure (1). Yet about 25% of COPD occurs in people who have never smoked (2), with other environmental (occupational exposures and early-life infections) and genetic (personal and familial history of asthma) factors likely being important in this group.

Genetic factors are important contributors to disease risk and progression, and a large number of candidate gene studies have indicated that several genes are associated with COPD. However, these studies were not able to unequivocally point to causal genes or variants for COPD (3–5). The fact that COPD is a heterogeneous disorder comprising different phenotypes with components of airway obstruction, emphysema, bronchiectasis, and comorbidities, in addition to its occurrence in both smokers and never-smokers, complicates genetic research.

In recent years much effort has been put into unraveling the genetics of COPD (defined using lung function measurements). The

first hypothesis-free genome-wide association studies (GWAS) have revealed HHIP, CHRNA3/5, and FAM13A loci to be associated with COPD defined as airway obstruction ($FEV_1/FVC < 70\%$) (6–8) and BICD110 (9) loci to be associated with the emphysema phenotype that is not captured by lung function alone, but using radiographic measures. Recent studies confirmed these findings and indicated that these genes in fact play a role in both COPD phenotypes (10), suggesting potential underlying shared genetics.

These studies have in common that the explained variance in COPD due to the identified genes is very low, only up to a few percent. This “missing heritability” in COPD has been ascribed to rare variants and to gene-by-environment interactions. Gene-by-environment interactions underlying COPD can be either due to genetic susceptibility to cigarette smoking and other environmental exposures (e.g., passive smoke exposure and air pollution) or due to epigenetic modifications. Such epigenetic modifications do not change the DNA sequence, but refer to alterations in the accessibility of the DNA. By changing the accessibility of the DNA, these epigenetic modifications affect gene expression and can silence or activate genes.