Alternatively Activated Macrophages and Airway Disease

Derek E. Byers, MD, PhD; and Michael J. Holtzman, MD, FCCP

Macrophages are the most abundant immune cell population in normal lung tissue and serve critical roles in innate and adaptive immune responses as well as the development of inflammatory airway disease. Studies in a mouse model of chronic obstructive lung disease and translational studies of humans with asthma and COPD have shown that a special subset of macrophages is required for disease progression. This subset is activated by an alternative pathway that depends on production of IL-4 and IL-13, in contrast to the classic pathway driven by interferon- γ . Recent and unexpected results indicate that alternatively activated macrophages (AAMs) can also become a major source of IL-13 production and, thereby, drive the increased mucus production and airway hyperreactivity that is characteristic of airway disease. Although the normal and abnormal functions of AAMs are still being defined, it is already apparent that markers of this immune cell subset can be useful to guide stratification and treatment of patients with chronic airway diseases. Here, we review basic and clinical research studies that highlight the importance of AAMs in the pathogenesis of asthma, COPD, and other chronic airway diseases.

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Abbreviations: AAM = alternatively activated macrophage; Chi3L1 = chitinase 3-like 1; Chi3L3/4 = chitinase 3-like 3 and chitinase 3-like 4; IFN- γ = interferon- γ ; iNKT = invariant natural killer T; MMP = matrix metalloproteinase; NKT = natural killer T; Th = T helper

Macrophages are critical for the normal function of the innate and adaptive immune responses during host defense and are implicated in the abnormal function of these same systems when they drive inflammatory disease.¹ Likely by design, lung macrophages reside at the interface of host and environment and stand ready to eradicate pathogens, noxious particles, and debris from necrotic and apoptotic cells. To accomplish their task, macrophages must be acti-

vated through ligand engagement of specific cell surface receptors. In particular, exposure to interferon- γ (IFN- γ) stimulates a classic pathway for activation that yields M1 macrophages with efficient capacities for phagocytosis and antigen presentation as well as production of T helper (Th) 1 cytokines, including IL-1 β , IL-6, IL-12, and tumor necrosis factor- α , that are all important for clearance of intracellular and bacterial pathogens. By contrast, a different type of activation occurs when macrophages encounter IL-4 and IL-13, two hallmark Th2 cytokines that are implicated in the development of chronic airway disease. This second pathway antagonizes the IFN- γ -induced events of the M1 pathway and instead drives the development of alternatively activated macrophages (AAMs) that are characterized by the expression of distinct surface receptors (eg, mannose receptor), biosynthetic enzymes (eg, arachidonate 12,15 lipoxygenases), and other proteins (eg, chitinases and chitinase-like proteins and matrix metalloproteinase [MMP]-12) that are not found in M1 macrophages.² In some definitions, AAMs include only so-called M2 macrophages that differentiate in response to IL-4

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Affiliations: From the Department of Pulmonary and Critical Care Medicine, Department of Medicine, and Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.

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Correspondence to: Michael J. Holtzman, MD, FCCP, Washington University School of Medicine, Campus Box 8052, 660 S Euclid Ave, St. Louis, MO 63110; e-mail: holtzmanm@wustl.edu

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and IL-13. In other broader definitions, the term AAM refers to all macrophage subtypes that respond to any stimulus other than IFN- γ , including engagement of Fc receptors or activation with glucocorticoids, IL-10, transforming growth factor- β , or macrophage colony-stimulating factor.³ M2 macrophages are often considered to assume antiinflammatory functions, but there is compelling evidence that these cells are also important in the eradication of extracellular parasites and the pathogenesis of inflammatory airway disease. In this review, we summarize the current understanding of the biology of AAMs in mouse models of airways disease and how these findings translate to COPD, asthma, and other chronic inflammatory lung diseases in humans.

AAMs IN MOUSE MODELS OF LUNG DISEASE

One of the first lines of evidence that AAMs contribute to chronic inflammatory disease of any kind comes from a mouse model of chronic airway disease that develops after an infection with a common type of respiratory virus.⁴ In this model, infection with mouse parainfluenza virus or Sendai virus leads to acute bronchiolitis followed by chronic airway disease that appears to mimic the sequence of events found in childhood asthma after infection with closely related human pathogens, such as respiratory syncytial virus. Thus, Sendai virus causes an acute illness with an immune response that includes activation of M1 macrophages and results in the eradication of infectious particles 2 weeks after infection.⁵ In genetically susceptible strains of mice, this acute illness is followed by a chronic disease that is detectable at 3 weeks after infection and is maximal at 7 weeks after infection. This chronic disease is characterized by immune cell infiltration of the airway and surrounding tissue, a marked increase in the number of mucous cells in the airways, and the development of airway hyperreactivity.⁴ This mouse model, therefore, appears to recapitulate a process that is relevant to the pathogenesis of airway disease in humans. The model does not require repeated administration of synthetic protein antigens, fungal spores, or allergen extracts to induce and maintain the airway disease, whereas the need for repeated administration is typical of allergen challenge models. The findings suggest instead that even a single immune event can switch on the immune response for a prolonged period of time to drive chronic inflammatory disease.

In a search of the underlying mechanism, it was discovered that the chronic postviral disease in this mouse model depended on persistent production of IL-13, and unexpectedly, that the IL-13 was produced by a subset of lung macrophages.^{4,6} Here again, the postviral model stands in contrast to allergen

challenge models. For example, in the commonly used ovalbumin challenge model, IL-13 production is attributed primarily to CD4⁺ Th2 cells and perhaps eosinophils and mast cells.⁷ In fact, IL-13-producing CD4⁺ Th2 cells are also found in the early phase of postviral disease at 3 weeks after infection, suggesting that this T-cell response may be more limited in time.⁸ However, these CD4⁺ Th2 cells are not essential for the development of chronic disease after viral infection, based on results in mice that are depleted of CD4⁺ T cells. Instead, CD11b⁺CD68⁺ macrophages are major producers of IL-13 at later time points, and macrophage depletion blocks the development of chronic disease.⁶ Relevant to subsequent studies of humans, the chronic postviral disease develops in concert with detection of AAM markers (as developed in the "AAMs in Human Lung Disease" section).

In a second unexpected development, it was found that AAMs were activated by another cellular component of the innate immune system. This finding was surprising because previous proposals predicted that the adaptive immune response was responsible for chronic inflammatory disease. However, in this model, it was found that invariant natural killer T (iNKT) cells, a component of the innate immune system, were required for the development of AAMs. In this case, natural killer T (NKT) cell-deficient mice failed to develop postviral lung disease.⁶ The mechanism for iNKT cell activation of macrophages depended on invariant T-cell receptor-CD1d and IL-13-IL-13R interactions. Thus, the development of AAMs in the context of chronic airway disease appears to be the result of paracrine effects of IL-13 from NKT cells as well as autocrine effects of IL-13 from AAMs themselves. This type of NKT-macrophage interaction may be special to the lung, since liver NKT cells do not as readily induce IL-13 production by lung macrophages.⁶ Together, the studies in mice identify a new immune axis comprising iNKT cells and AAMs responsible for the development of chronic airway disease (as diagrammed in Fig 1).⁹ The findings provide evidence that the immune alterations that lead to chronic postviral disease are likely to begin shortly after infection but do not result in maximal disease until 7 weeks later. The reason for delay in disease development as well as the basis for disease persistence need to be determined.

In addition to the role of AAMs in postviral airway disease, there is some evidence that this cell population is also activated in allergen challenge models of airway disease.¹⁰⁻¹³ For example, increased lung levels of AAMs were observed in an ovalbumin-induced model of lung disease and adoptive administration of AAMs at the time of allergen challenge worsened the severity of allergen-induced disease.⁷ However, there are no reports of the effect of macrophage depletion

FIGURE 1. Scheme for immune pathways leading to acute and chronic lung disease after viral infection or allergen challenge. A, For acute allergic disease, allergen exposure leads to production of allergen-specific IgE that is available to participate in allergen-driven FcεRI signaling, leading to activation of mast cells (and perhaps other cell types) with consequent recruitment of Th2 effector cells to the airway. Th2 cell production of IL-4 and/or IL-13 leads to AA Mac differentiation, whereas IL-5 generation leads to eosinophil (Eos) accumulation and further IL-13 production in the airway. The role of macrophages and any mechanism for positive feedback between Th2 cells, macrophages, eosinophils, and cDCs in the allergic response remains uncertain. B, For acute postviral disease, viruses cause AEC and pDC production of type 1 IFN. Subsequent IFNAR signaling leads to upregulation of FcεRI expression on resident lung cDCs. In turn, FcεRI activation by viral antigen and antiviral IgE leads to production of CCL28 and recruitment of CCR10-expressing IL-13-producing Th2 cells to the lung. IL-13 drives differentiation of AEC precursors toward mucous cells (mucous cell metaplasia) and airway smooth muscle cells to become more reactive to contractile agonists (AHR). C, For chronic postviral disease, viral remnants activate APCs and thereby facilitate CD1d-dependent antigen presentation and consequent activation of invariant CD4⁺ natural killer T (NKT) cells. NKT cells then interact directly with lung macrophages via IL-13 production and binding to the IL-13R as well as contact between invariant Vα14 TCR and glycolipid-loaded CD1d. This interaction leads to increased expression of IL-13R and production of IL-13 that drives a positive feedback loop to amplify IL-13 production and alternative activation of macrophages, including *Chi3l3/4*, *Mmp12*, *Alox12e*, *Arg1*, and *Relmα* gene expression in mice and *CHIT1*, *MMP12*, and *ALOX15* in humans. AA Mac = alternatively activated macrophage; AEC = airway epithelial cell; AHR = airway hyperreactivity; APC = antigen-presenting cell; cDC = conventional dendritic cell; IFN = interferon; IFNAR = interferon A receptor; IL-13R = IL-13 receptor; iNKT = invariant natural killer T; pDC = plasmacytoid dendritic cell; Th = T helper; TCR = T-cell receptor. (Modified with permission from Benoit and Holtzman.⁹)

or blockade in this model, so it is uncertain whether AAMs are the cause or the effect of the allergic response.¹⁴ In that regard, T-cell blockade with anti-CD4 antibodies markedly downregulates the response to ovalbumin and would presumably decrease

the level of AAMs in the lung.¹⁵ It is already recognized that IL-4-producing CD4⁺ T cells are required for the long-term maintenance of AAMs in mouse models of parasite infection.^{16,17} Yet other immune cells of the innate response, including eosinophils,

basophils, mast cells, NK cells, and NKT cells, also have the capacity to produce IL-4 or IL-13 and drive the development of AAMs.^{6,18-22} In fact, CD4⁺ T cells make up only 10% of the cells that produce IL-4 in the lungs of naive mice in the ovalbumin model, and most of the IL-4 is found in eosinophils and basophils.²³ In fact, T and B cells are not even required for the induction (as opposed to maintenance) of AAMs in the lung, based on observations during the pulmonary phase of nematode infections in *Rag1*-deficient and severe-combined immunodeficient mice.^{17,24} Given the importance of eosinophils, basophils, and mast cells in inflammatory lung disease, it will be critical to evaluate the role of these cell types in driving AAM development in allergen challenge and viral infection models of airway disease.

AAMs IN HUMAN LUNG DISEASE

Macrophages are implicated in the development of airway inflammation and mucus production as well as the alveolar destruction that are all characteristic of COPD. Studies of mouse models and humans with COPD support a role for M1 macrophages in alveolar destruction as a basis for pulmonary emphysema.²⁵ One study indicated that the pattern of gene expression in alveolar macrophages from smokers showed at least some overlap with genes found in mouse models of COPD, and this overlap included genes found in AAMs, such as *MMP12*.^{26,27} Another study showed that alveolar macrophages from smokers with mild to moderate COPD exhibit a further degree of M1 downregulation and M2 upregulation compared with alveolar macrophages from smokers without COPD.²⁸ Further support for the relationship between AAMs and COPD comes from studies of whole lung tissues obtained at the time of lung transplantation. In this case, increased numbers of AAMs that produce IL-13 are found in the lungs of patients with severe COPD compared with control subjects without COPD.^{6,29} These studies of lung tissue, along with the previous studies of BAL samples, constitute strong circumstantial evidence of a role for AAMs in the development of COPD. However, further functional studies are still needed that specifically monitor AAMs and the course of disease during immune strategies that block AAM activity. As developed in the “AAA Biomarkers in Airway Disease” section, it will be especially important to have validated methods to monitor AAM pathways in humans, since it appears that only a subset of patients may exhibit this mechanism as a component of their lung disease. Indeed, the failure of some previous studies to detect IL-13 production in COPD is likely attributable in part to heterogeneity among patients and to heterogeneity of lung disease within patients.³⁰ Thus,

in a given lung from a single patient even with GOLD (Global Initiative for Chronic Obstructive Lung Disease) stage IV disease, some parts of the lung may be fully preserved while others are entirely destroyed, and still others exhibit robust inflammation and repair while other sections do not. Thus, there is considerable sampling error in approaches that rely on relatively small endobronchial biopsies, brushings, or BAL fluids compared with a whole lung analysis.

Despite the drawbacks in sampling error, there are consistent reports of increased levels of IL-4 and IL-13 in airway samples from subjects with asthma, and a growing list of candidates (eg, IL-25, IL-33, and thymic stromal lymphopoietin) that might drive IL-13 production during airway disease.³¹ AAMs should thrive in this Th2 environment. Indeed, increased numbers of IL-13-expressing macrophages are found in the BAL samples from patients with severe asthma.⁶ Some studies suggest that AAM products, such as chitinase 3-like 1 (Chi3L1) and MMP-12, contribute to the pathogenesis of chronic airway disease.³²⁻³⁴ However, airway epithelial cells can also express Chi3L1, and any functional biology of MMP-12 in asthma remains uncertain, so the full characterization of AAMs in asthma still needs to be defined. Moreover, some have proposed a protective role for AAM products (including chitinases) under some circumstances.³⁵ Nonetheless, the initial findings in humans, in conjunction with the findings in mouse models, implicate an eventual role for AAMs in allergen- and virus-induced airway disease.

In addition to studies of COPD and asthma, the production of IL-13 and the generation of AAMs have also been linked to other airway diseases, such as bronchiolitis obliterans^{36,37} and cystic fibrosis,³⁸ and even pulmonary fibrosis.^{39,40} For example, serum and BAL levels as well as lung macrophage staining for Chi3L1 are increased in patients with idiopathic pulmonary fibrosis compared with healthy control subjects.⁴¹ In addition, increased expression of the mannose receptor is characteristic of AAMs and is found in airway macrophages from patients with cystic fibrosis.³⁸ These reports remain preliminary, but they point to a potential role for AAMs in the development of several chronic lung diseases in addition to COPD and asthma. A more complete profile of AAM phenotype and function will be needed to define whether they may assume an antiinflammatory role under some circumstances while driving inflammatory disease in others.

AAM BIOMARKERS IN AIRWAY DISEASE

To the extent that AAMs drive chronic airway disease, they also offer an opportunity to monitor and stratify patients and thereby personalize diagnosis and

treatment. Toward that end, an effort is being made to compile markers of AAM levels and/or activity that could be validated in experimental models and applied to patients with chronic obstructive lung disease. Two major challenges in this approach are the difficulties in predicting human orthologs of mouse genes and verifying that either mouse or human AAM markers are restricted to expression in macrophages. For example, arginase 1 is a highly informative marker of AAM levels in mice but does not appear to be part of the alternative pathway for activation of macrophages in humans.⁴² Similarly, chitinase 3-like 3 and chitinase 3-like 4 (Chi3l3/4) are prominently upregulated in AAMs in mice, but there is no human ortholog of Chi3l3/4 based on sequence homology. This circumstance forces a global search for other chitinase family members that might serve a similar marker function in human disease. This type of analysis revealed chitinase 1 as the most useful member of this family as a marker of AAM levels in humans with COPD^{29,43,44} and asthma as well.⁴⁵ Others identified chitinase A (also known as AMCase) and Chi3l1 as informative markers in the allergen challenge model in mice and then in patients with asthma.^{12,32,33,46-48} Expression levels of chitinase A and Chi3l1 are much lower than Chi3l3/4 in the allergen challenge or viral infection models in mice, and in initial studies, the levels of these proteins do not correlate quite as well as chitinase 1 (also known as CHIT1) with the severity of COPD in humans.^{29,43,44} For both asthma and COPD, it also appears that patients with the most severe disease are also the most likely to exhibit AAM signatures. However, a direct comparison with each of these potential biomarkers as well as others (eg, 15-lipoxygenase) is still in the process of being completed.⁴⁵ In addition, it will be useful to define the specificity of these and other biomarkers for AAMs, since the candidates are often expressed in other cell types (eg, airway epithelial cells and neutrophils) that may not be regulated in concert with the alternative pathway under all circumstances.

Another major issue in biomarker development is the capacity to discriminate between exposure to inflammatory stimuli and the onset of inflammatory disease. This issue is of particular significance in COPD wherein exposure to cigarette smoking must be discriminated from smoke-induced inflammatory disease. Here again, studies of chitinase and chitinase-like proteins are informative. Because these proteins are secreted out of the cell after production, it is possible to detect them in the airspaces of the lung and in the circulation. In fact, chitinase 1 may be found at increased levels in BAL samples from subjects who are current cigarette smokers but may or may not have significant lung disease.^{43,44} Circulating levels of chitinase 1 also correlate with the degree of

airway obstruction in subjects with COPD but have no relationship to current smoking status. Therefore, it appears that the increased lung levels of chitinase 1 found in smokers without lung disease are not necessarily detected in the circulation unless there is concomitant development of inflammatory lung disease. This observation is supported by other evidence that at least one determinant of increased circulating chitinase 1 level is the severity of COPD, which likely causes increased chitinase 1 production as well as greater movement of the protein into the circulation. Given the positive attributes of this approach, it would appear that future biomarker development should also target secreted proteins that will be detectable in the circulation in concert with disease severity.

FUTURE DIRECTIONS

In this brief review, we highlight evidence that AAMs contribute to the pathogenesis of chronic airway diseases. This disease mechanism is supported by evidence from experimental models of postviral airway disease and translational studies of asthma and COPD in humans. In both cases, ongoing work needs to further define the basis for AAM activation as a means to better understand pathogenesis. Even at this stage of understanding, investigators have begun to develop clinical biomarkers of AAM status. The application of these biomarkers indicates that AAM-driven pathologies are present in a subset of patients rather than all patients with common airway diseases, such as asthma and COPD. Future studies also need to address why the AAM pathway is active in some subjects and not others, and if that pattern is stable in individual patients over the course of their disease. It also appears likely that AAMs have the capacity to help in the control of inflammation and to promote repair of damaged tissues. It will, therefore, be useful to establish markers of AAM function that discriminate between inflammatory and reparative processes. The existing examples teach us that experimental models may direct general strategies, but specific markers may need to be specially tailored to the human condition. This combination of experimental and translational studies appears likely to continue to support a key role for AAMs in the pathogenesis of chronic obstructive lung diseases and to thereby provide a useful new target for diagnosis and treatment of chronic inflammatory airway disease.

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