

Lessons Learned from the ABCs of Granuloma Formation

Alveolar macrophages (AMs) are poised at the interface of the lung and the environment, where they encounter environmental antigens and selectively respond to potential threats to the host. AM-mediated immune responses are commonly triggered by potential pathogens (bacteria, fungi, viruses) or in response to foreign bodies. Ingestion of large, indigestible antigens by AMs can promote multicellular granuloma formation, wherein the antigen is sequestered within centrally located macrophages or multinucleated giant cells, representing a syncytium of numerous macrophages. In some individuals, the foreign body immune response can progress abnormally to promote inflammatory and fibrotic lung damage, with impaired lung function (1). The mechanisms contributing to progressive lung inflammation, damage, and fibrosis in response to foreign bodies are complex and poorly understood.

Using an established murine model of foreign body (i.e., multiwall carbon nanotube [MWCNT])-induced granulomatous lung disease, Dr. Thomassen's laboratory recently reported that peroxisome proliferator-activated receptor- γ (PPAR- γ) promotes the expression of specific ATP-binding cassette (ABC) lipid transporter genes (2). Enhanced expression of ABC transporters, particularly ATP-binding cassette subfamily G, member 1 (ABCG1), in response to PPAR- γ agonists was associated with reduced lipid accumulation in lung macrophages and suppression of macrophage activation and granuloma formation. However, the effects of PPAR- γ agonists are complex, and direct causality could not be established. On the basis of these compelling results, the authors further hypothesized that genetic knockout of the ATP-binding cassette subfamily A, member 1 (ABCA1), and/or ABCG1 lipid transporters would promote the uptake of antigens by AMs and would exacerbate MWCNT-induced lung granuloma formation in the murine model. The findings of that study, presented by McPeck and colleagues in this issue of the *Journal* (pp. 332–340), more conclusively implicate ABC transporters in the pathogenesis of foreign body-induced granulomatous lung disease (3). Instead of pharmacological manipulation of ABC transporters, as reported previously (2), this study used mice harboring selective and conditional gene knockouts of *Abca1* or *Abcg1* or both that were treated with inhaled MWCNT or surfactant (negative controls). As hypothesized, knockout of both *Abca1* and *Abcg1*, or selective knockdown of *Abcg1*, but not *Abca1*, resulted in the development of larger granulomas in the lung. AMs of *Abcg1* knockouts exhibited greater lipid accumulation and expressed markers promoting macrophage recruitment, including CCL2 and activation/enhancement of phagocytosis (osteopontin). *Ex vivo* analysis of the macrophages of *Abcg1* knockouts confirmed enhanced phagocytosis capacity. Regional mediastinal lymph node enlargement was observed in the *Abcg1*-knockout mice and was associated with greater tissue sequestration of MWCNT; however, the lymph node enlargement

was likely reactive to the lung inflammation because granuloma formation was not observed in the lymph nodes.

Although ABCG1 receptors were not shown to be essential for granuloma formation, these studies provide convincing evidence in support of the hypothesis that ABCG1 and related alterations of lipid homeostasis contribute significantly to granulomatous lung disease severity and progression. In addition to enhanced MWCNT uptake and related granuloma formation, BAL analysis of *Abcg1* (not *Abca1*)-knockout mice demonstrated higher gene expression levels of profibrotic mediators, platelet-derived growth factor- α , and transforming growth factor- β , but only transforming growth factor- β was found to be elevated in BAL by protein analysis. Gomori trichrome staining of the lung tissue of ABCG1-deficient mice treated with MWCNT confirmed the increased fibrosis in the lungs. Thus, ABCG1 receptors were implicated in the progression from granulomatous inflammation to fibrosis, such as is commonly observed in foreign body-induced granulomatous lung diseases.

Cre-lox gene manipulation, such as employed for these experiments, is a powerful tool to modify DNA such that genes are activated or suppressed in specific cell lineages. However, there are potential considerations that may confound the experimental results. For instance, transient germline activation of Cre-recombinase may occur, which is undetected by conventional genotyping methods, leading to off-target expression and unpredictable deletion efficiencies among littermates (4). Although appropriate knockout controls were included and no distinct phenotype was observed in untreated ABC transporter knockouts, more rigorous validation of the altered allele expression to screen for unexpected recombination or variable gene expression would have been reassuring.

On the basis of findings in the MWCNT granuloma model (3), the authors imply that ABCG1 could also play a role in the pathogenesis of other granulomatous disorders, such as sarcoidosis. This conclusion may be overreaching, given that the mechanisms by which foreign body granulomas form, such as induced by MWCNT, are mechanistically distinct from “immune granulomas,” such as sarcoidosis, Crohn's disease, or infectious granulomas. Foreign body granulomas are relatively deficient in APCs, as reflected by CD205 or human leukocyte antigen-DR (HLA-DR) expression, and lack associated T-cell proliferation (5). Biomaterials such as MWCNT are shown to directly activate macrophages via Toll-like receptors and/or scavenger receptors, eliciting an innate immune response (6). In contrast, immune-mediated granuloma formation depends on APC antigen recognition, processing, and presentation to T cells, leading to an adaptive immune response involving complex interactions among various immune cells, including but not limited to macrophages (5). Thus, the implications of myeloid cell ABC transporters in the pathogenesis of immune granulomatous disorders such as sarcoidosis remains to be determined.

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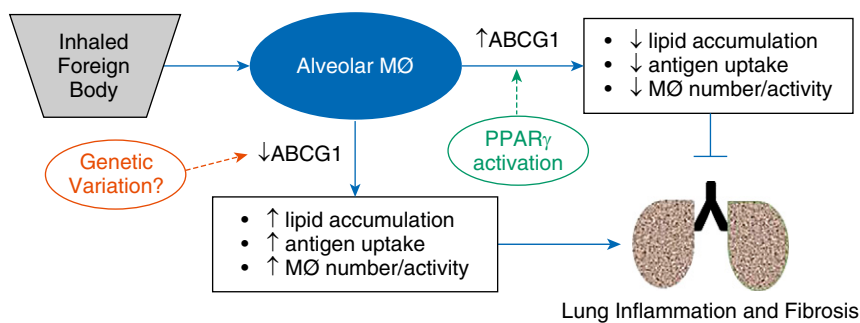


Figure 1. Schematic representation of mechanisms by which ATP-binding cassette subfamily G, member 1 (ABCG1), lipid transporters are proposed to regulate progression of foreign body granulomatous lung disease. Conditions favoring suppression of ABCG1 function in alveolar macrophages (MØ), including host factors such as genetic variability causing loss of ABCG1 function, are proposed to promote MØ recruitment and activation, resulting in more severe granulomatous lung inflammation and progression to lung fibrosis. Conversely, peroxisome proliferator-activated receptor- γ (PPAR γ) activation and related signaling pathways may be protective by promoting ABCG1 expression.

Despite these model limitations, the study by McPeek and colleagues (3) lends credence to a growing body of evidence linking ABC transporters to the pathogenesis of chronic inflammatory and fibrotic lung diseases. In keeping with the results of the MWCNT model, ABC transporters, particularly ABCG1, were shown to promote the clearance of lipids from macrophages in the setting of bleomycin-induced lung injury in mice, thereby attenuating progression to pulmonary fibrosis (7). Other murine studies show that defective macrophage ABCG1 function can lead to altered lipid homeostasis in the lung, promoting the formation of giant lipid- and cholesterol-filled AMs, abnormal pulmonary surfactant accumulation in alveoli, and the induction of lung inflammation. Moreover, a genetic variant in the *ABCG1* gene promoter was identified in humans presenting with idiopathic pulmonary alveolar proteinosis, a rare lung disease characterized by abnormal accumulation of surfactant and predisposing to the development of lung fibrosis (8). Together, these studies emphasize the importance of altered lipid homeostasis through ABCG1 as a novel mechanism driving chronic lung disease progression. However, additional investigations are needed to further elucidate the mechanisms linking altered AM lipid metabolism to inflammatory and fibrotic lung injury pathways.

On the basis of the intriguing results of the study by McPeek and colleagues (3) and other related investigations reviewed above, it is evident that factors influencing the function of the AM ABCG1 lipid transporter have important implications for the pathogenesis of environmental foreign body-mediated granulomatous lung disease (Figure 1). Further investigations are needed to determine if these mechanisms also apply to immune-mediated granulomatous disorders such as sarcoidosis or Crohn's disease and to consider the therapeutic implications. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Elliott D. Crouser, M.D.
 Division of Pulmonary, Critical Care and Sleep Medicine
 Ohio State University Wexner Medical Center
 Columbus, Ohio

ORCID ID: 0000-0003-3197-6746 (E.D.C.).

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