

Gas6'ing the Innate Immune Response during Experimental Asthma

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Abstract

Growth arrest–specific gene 6 (Gas6) binds Tyro3, Axl, and Mertk (TAM) receptors and exerts prominent effects in many diseases, but little is known about its role in asthma. Herein, we examined the role of Gas6 and TAM receptors differentially in an experimental asthma model driven by *Aspergillus fumigatus*. *A. fumigatus*–sensitized mice were challenged with live *A. fumigatus* conidia, and airway hyperresponsiveness and airway remodeling were determined 28 days later. When administered to mice from Days 14 to 28

after conidia challenge, anti-Axl monoclonal antibody, but not anti-Mertk monoclonal antibody, treatment significantly inhibited airway hyperresponsiveness and airway remodeling compared with the appropriate control IgG group. These results demonstrate that Gas6 has modulatory functions in fungal asthma via Axl receptor activation in immune and nonimmune cells.

Keywords: allergic inflammation; *Aspergillus*; tyrosine kinase receptors

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A major question perplexing clinicians and academics alike is the seemingly unabated rise in the incidence and prevalence of asthma worldwide. Many hypotheses are currently under rigorous testing, including one that is currently under investigation in our laboratory: the planet is becoming a more hospitable place for pathogens that provoke mucosal allergic responses that drive asthma. This hypothesis has gained credence with the publication of high-profile papers reporting on the data showing that emerging infectious diseases caused by fungi are increasingly recognized as presenting a worldwide threat to food security and human health (1). Although not all asthmatic responses are allergen driven, allergens are among the most potent stimuli in the asthmatic airway. Our research group focuses on the fungus *Aspergillus fumigatus*. Aside from patients with allergic bronchopulmonary aspergillosis who exhibit fungal colonization and

asthmatic disease, sensitization to *Aspergillus* affects up to 10% of all patients with asthma and is correlated with incidence and severity. Very recently, David Denning and colleagues reported that antifungal therapy reverses asthmatic features in patients with severe asthma with fungal sensitization (2). Finally, it is well documented that increased asthma hospitalizations have been linked to high airborne conidia or spore counts (3).

The immune response evoked by *Aspergillus* can be very effectively modeled in mice. The nature of the immune response varies with time in the model beginning as a proinflammatory, Th1-type response dominated by neutrophils that is followed by a Th2-type response dominated by eosinophils and T cells. Key remodeling events in the lungs of mice with fungal asthma include mucus cell metaplasia and peribronchial inflammation. This immunopathology is the direct result of the retention of fungal material in the lung.

The retention of fungus also drives the persistent changes in airway physiology, including enhanced responsiveness to bronchoconstricting signals (4). We have had the opportunity to explore a number of nodes of the immune response that dictate the multiple parameters highlighted, but much of our attention has been directed to the role of Toll-like receptors (TLR) in fungal asthma (5). We have highlighted in a number of published reports that the loss of TLR function leads to exacerbated lung disease, leading us to speculate that there must be some active inhibitory pathway present in asthma that impairs the function of this critical innate immune process.

This speculation led us to growth arrest–specific 6 (Gas6) and Tyro3, Axl, and Mertk (TAM) receptors. The role of Gas6 and TAM has been explored in several settings, and the interactions contribute to the clearance of apoptotic cells, the proliferation of stromal cells, and, most recently, the inhibition of

TLR function. Another tantalizing feature of Gas6 is that it is present in subjects with severe asthma with exacerbated disease. TAM receptors belong to a distinct receptor protein-tyrosine kinase subfamily and are expressed in various cells and tissues. First described as critical receptors for the clearance of apoptotic cells (6–8) and as proproliferative mediators on smooth muscle and fibroblasts (9, 10), it is now known that TAM receptors have potent immunosuppressive functions related to their induction of immunoregulatory factors, including suppressor of cytokine signaling 1 (SOCS1), SOCS3, and Twist, that inhibit both TLR- and cytokine-driven immune responses (11, 12). Vitamin K-dependent protein Gas6 and protein S are the two ligands that bind and activate TAM receptor (13). More is known about Gas6, which is a soluble factor implicated that binds to Axl and Tyro3 with equal affinity but binds Mertk with an affinity that is 3- to 10-fold lower than the other TAM receptors (14, 15).

In the present study, we addressed the expression and role of Gas6/Axl and Gas6/Mertk signaling in a well-described model of chronic fungal asthma. Our data show that Gas6 via Axl activation modulates airway inflammation and promotes airway remodeling in this model.

Results

We first addressed whether Gas6 was altered in this fungal asthma model. Both ELISA and immunohistochemical analysis showed that the expression of Gas6 protein changed with time, and the highest levels of this protein were present during the chronic phase of the model. Although it was apparent that epithelial cells stained for Gas6 at Day 28 after conidia challenge, we also noted that myeloid cells, such as macrophages and possibly dendritic cells (DCs), strongly expressed Gas6 in the fungal asthma model. Equally important, these myeloid cells present in the lung at Day 28 after the conidia challenge also expressed Axl and Mertk. The expression of Gas6 increased with time in alternatively activated or M2 macrophages from this asthma model. The association of Gas6 with M2 macrophages was confirmed *in vitro* as demonstrated by the fact that M2 and not M1 macrophages expressed Gas6, Axl, and Mertk.

Gas6 also impacted DC function during fungal asthma, driving Th2 responses and suppressing Th1 responses

in vitro. Specifically, Gas6 impaired the generation of IFN- γ -generating T cells, but promoted both IL-4- and IL-13-generating T cells. There was no effect of this molecule on IL-10 generation in these coculture systems. Gas6 also had marked effects on the generation of chemokines by DCs generated from asthmatic mice. In these studies, bone marrow was isolated from control or Gas6-treated asthmatic mice at Day 28 after conidia challenge. Fully differentiated DCs from both groups were then activated with various TLR ligands for 24 hours. As shown here, the response to TLR ligands was differentially affected by the Gas6 treatment. Accordingly, Gas6 inhibited the generation of Th1-type chemokines, such as CXCL9 and CXCL10, whereas the TLR-mediated generation of the Th2-type chemokine CCL22 was enhanced in this *in vitro* system. Together, these data showed that Gas6 had a modulatory effect on TLR activation but only on the Th1 side of the equation.

Clearly, further work is required to explore the mechanism behind this divergent effect, but we have had the opportunity to move forward with a monoclonal antibody from Genentech that effectively neutralizes the bioactivity of Axl. With this antibody we were able to show that when targeting Axl in a therapeutic manner, there was a statistically significant reduction in methacholine-induced airway hyperresponsiveness. Histologically, the lungs from the anti-Axl-treated mice were markedly improved and showed markedly less goblet cell metaplasia and

peribronchial fibrosis. Our observation that targeting Axl attenuated chronic fungal disease was motivation to further explore whether this TAM receptor impacted other pathways leading to asthmatic lung disease. Specifically, we focused on the impact of respiratory syncytial virus as a major culprit leading to the dramatic overproduction of mucus in the airways of patients with asthma. This response again appears to be due in part to a failure in TLR signaling, particularly those TLRs that recognize viral RNA and DNA components. There was another reason to be interested in this pathway, because Morizono and colleagues (16) showed that Gas6 bridges the entry of viruses into target cells, and this group demonstrated that a wide variety of viruses, including lentiviral vectors and vaccinia virus to name a few, use Gas6/Axl interactions for cell entry. Our studies indicated that viral infection increased Axl expression in both the lung and the lymph node, and targeting Axl during the asthma phase before viral infection dramatically reduced airway inflammation. Finally, the anti-Axl monoclonal antibody-mediated approach also completely ablated the mucus cell metaplasia as revealed by histopathology and quantitative polymerase chain reaction.

Discussion

Gas6 has been previously shown to be elevated in clinical asthma, particularly during exacerbation of this disease, but

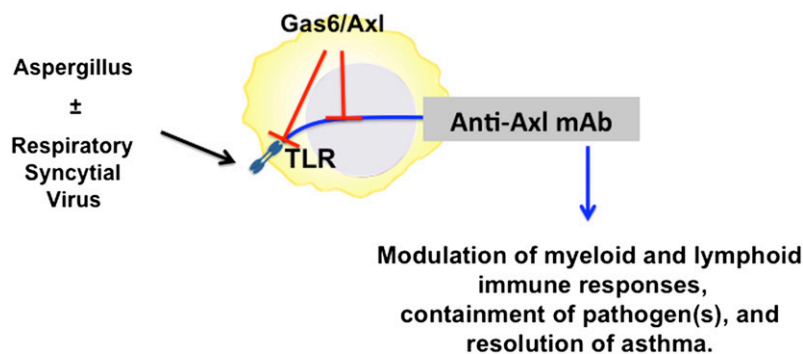


Figure 1. Growth arrest-specific gene 6 (Gas6)–Axl interactions mediate airway inflammatory, reactivity, and remodeling during *Aspergillus*-induced allergic airway disease and respiratory syncytial virus (RSV)-induced exacerbation of this disease in mice. The activation of myeloid cells, including macrophages and dendritic cells, is modulated by Gas6–Axl, and these cells in turn promote Th2-type responses in the allergic lung. Blockade of Axl using a monoclonal antibody reversed all features of primary and RSV-exacerbated *Aspergillus*-induced allergic airway disease. mAb = monoclonal antibody; TLR = Toll-like receptor.

its role was previously unknown (17). Elucidating the role of Gas6 in a model of chronic allergic asthma was the goal of the present study, and the data generated in this regard indicate that this TAM receptor ligand is involved in various aspects of the maintenance and regulation of this airway disease. Transcript and protein levels of Gas6, Axl, and MerTK were significantly elevated in whole lung

samples but only at the most distal time point (i.e., Day 28 after conidia challenge) analyzed in this model. Blocking Axl via a monoclonal antibody approach *in vivo* significantly attenuated all features of asthmatic airway disease. Together, these data demonstrate that Gas6, via its effects through Axl, contributes to the lung pathology associated with experimental asthma. In summary, Gas6 is a proallergic

factor that has exacerbating effects on airway hyperresponsiveness, airway inflammation, and airway and blood vessel remodeling during chronic fungal asthma via Axl (Figure 1). In light of these findings, targeting Axl might provide clear therapeutic effects in asthma. ■

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

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