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$\gamma\delta$ T cells and Th17 cytokines in Hypersensitivity Pneumonitis and Lung Fibrosis

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Abstract

Hypersensitivity pneumonitis (HP) is an inflammatory lung disease caused by repeated inhalation of aerosolized antigens. With chronic exposure to an inhaled antigen, patients are at risk of developing irreversible pulmonary fibrosis and increased morbidity and mortality. Although $\alpha\beta$ T cells have been shown to be important in the pathogenesis of HP, $\gamma\delta$ T cells also accumulate in the bronchoalveolar lavage of patients with HP. $\gamma\delta$ T cells represent a distinct lymphocyte subset, whose primary function is not well understood. In contrast to $\alpha\beta$ T cells, $\gamma\delta$ T cells recognize unprocessed antigens such as those upregulated on injured or stressed epithelial cells. In a murine model of HP induced by exposure to the ubiquitous microorganism, *Bacillus subtilis*, $\gamma\delta$ T cells expressing the canonical V γ 6/V δ 1 T cell receptor were dramatically expanded in the lung. The predominant cytokines expressed by this $\gamma\delta$ T cell subset were Th17 cytokines that were critical for bacterial clearance and the resolution of lung inflammation. Th17-expressing $\gamma\delta$ T cells are also expanded in other murine models of lung infection and inflammation, suggesting that these cells play a sentinel role in mucosal immunity. Thus, an increased understanding of $\gamma\delta$ T cells that express Th17 cytokines in HP and other inflammatory lung diseases may lead to the development of novel therapeutic and clinical strategies that prevent the development of fibrotic lung disease.

INTRODUCTION

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is an environmental lung disease that results from repeated inhalation of aerosolized antigens (1). The etiologic agents are composed of a wide variety of organic particles (e.g., mammalian and avian proteins, fungi, and thermophilic bacteria) and certain small molecular weight volatile and nonvolatile chemical compounds. A classic example of HP is Farmer's lung, which is caused by the thermophilic actinomycete, *Saccharopolyspora rectivirgula*. This disorder occurs in genetically susceptible individuals who are repeatedly exposed to moldy hay. For example, when workers directly handle hay, spores are released from the packed vegetation in clouds, and in poorly ventilated areas, workers can inhale and retain approximately 750,000 spores (0.5 to 1.3 μ m in size) per minute (2). HP occurs in several clinical forms (e.g., acute,

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subacute, and chronic), depending on the nature of the antigen, the quantity and duration of exposure, and host/environment interactions (1). The acute form of disease is characterized by cough and dyspnea and is typically nonprogressive in nature with spontaneous improvement after cessation of antigen exposure. The subacute and chronic forms of disease result from continued low-level exposure to inhaled antigens. In patients with chronic disease, pulmonary fibrosis occurs in up to 41% of cases, resulting in irreversible pulmonary dysfunction and right heart failure (3–6). In fact, lung fibrosis has been shown to be an independent predictor of mortality in these patients, with a 5-year mortality of 27% and a median survival of 12.8 years (7). Histopathologically, this disorder is characterized by a diffuse mononuclear cell infiltration of the lung with poorly-formed granulomas located along the bronchovascular bundle (8). The bronchoalveolar lavage of HP patients is characterized by a T cell alveolitis (9).

IMMUNOPATHOGENESIS OF HP

The immunopathogenesis of hypersensitivity pneumonitis remains poorly understood. Early studies suggested an immune complex (type III) hypersensitivity resulting in lung inflammation (10). However, recent evidence supports a prominent role of a T cell-mediated, delayed-type hypersensitivity immune response in the pathogenesis of HP. Supportive evidence in humans includes the presence of a T cell alveolitis and granulomatous inflammation as well as the production of Th1-type cytokines by T cells stimulated with mitogens (11–13). In order to further delineate the immunopathogenesis of HP, several murine models of disease have been developed. The best known of these models is the Farmer's lung model in which C57BL/6 mice are repeatedly exposed to *S. rectivirgula*. This exposure results in the development of mononuclear infiltrates and collagen deposition in a peribronchovascular distribution that approximates the human disease (Figure 1) (14–18). The mononuclear infiltrates are predominantly composed of T and B cells and macrophages. T cells have been shown to be important in the immunopathogenesis of this disease as athymic nude mice that lack T cells develop significantly less severe HP (17). In addition, the adoptive transfer of memory CD4⁺ T cells, but not CD8⁺ T cells, into T cell-deficient mice recapitulates both lung inflammation and fibrosis after intratracheal instillation of antigen (16,17,19). Thus, CD4⁺ T cells are the critical $\alpha\beta$ -expressing T cell subset in this murine model of HP. Although type 1 and 2 cytokines have been implicated in the inflammatory lung infiltrate that develops in mice treated with *S. rectivirgula* (20–23), only low levels of type 1 (IFN- γ) and type 2 (IL-5) cytokines were detected in the lung after repeated exposure to *S. rectivirgula* (16,24). Conversely, large concentrations of Th17 cytokines (e.g., IL-17A and IL-22) were found in the lung, and these cytokines were differentially expressed by CD4⁺ T cells (16). In the absence of IL-17 or IL-17 receptor signaling, mice exposed to *S. rectivirgula* had less lung inflammation and fibrosis, indicating that Th17 cytokines are critical for the development of mononuclear infiltrates and lung fibrosis in this model (16,24). These data suggest that although Th1 and Th2 cytokines have historically been implicated in the pathogenesis of HP and lung fibrosis, Th17-polarized CD4⁺ T cells mediate the development of lung inflammation and fibrosis in this murine model of HP, consistent with murine models of autoimmune disease such as experimental autoimmune encephalitis (25).

Exposure of C57BL/6 mice to the ubiquitous soil organism, *Bacillus subtilis*, also results in the development of mononuclear cell infiltrates and lung fibrosis in a bronchovascular distribution similar to the human disease. The mononuclear cells are composed of CD4⁺ and CD8⁺ T cells along with B cells, macrophages and neutrophils (26). However, in contrast to the *S. rectivirgula*-induced model of Farmer's lung, mice exposed to *B. subtilis* develop a large expansion of $\gamma\delta$ T cells that express a unique T cell receptor, V γ 6/V δ 1. In the absence of $\gamma\delta$ T cells, mice develop increased numbers of CD4⁺ and CD8⁺ T cells with accelerated pulmonary fibrosis, suggesting that $\alpha\beta$ T cells may promote while $\gamma\delta$ T lymphocytes protect against fibrotic lung disease caused by chronic exposure to *B. subtilis* (26). To delineate the regulatory role of

V γ 6/V δ 1⁺ $\gamma\delta$ T cells in *B. subtilis*-induced lung fibrosis, we exposed transgenic V γ 6/V δ 1 mice to this microorganism and found decreased collagen content in the lung compared to wild-type C57BL/6 mice (27). Cytokine analysis of lung homogenates from wild-type mice demonstrated increased IL-17A concentrations with repeated exposure to *B. subtilis*. In the absence of IL-17 receptor signaling, IL-17ra^{-/-} mice had delayed clearance of *B. subtilis* with increased lung inflammation and fibrosis. Importantly, IL-17A was predominantly expressed by V γ 6/V δ 1⁺ T cells, suggesting an important role for IL-17A-expressing $\gamma\delta$ T cells in bacterial clearance, thus preventing excessive inflammation and eventual lung fibrosis in this murine model of *B. subtilis*-induced HP. Although $\gamma\delta$ T cells have been described in patients with hypersensitivity pneumonitis and other granulomatous diseases such as sarcoidosis (28–31), their role in the pathogenesis of the human disease remains unknown.

The divergent findings between these two models likely relate to the lack of an expanded $\gamma\delta$ T cell population in the lung in response to *S. rectivirgula* exposure. However, in the absence of IL-17 receptor signaling, IL-17ra^{-/-} mice exposed to *S. rectivirgula* developed a heterogeneous expansion of $\gamma\delta$ T cells that correlated with decreased numbers of macrophages and CD4⁺ T cells and lung fibrosis (P.L.S and A.P.F, unpublished observation). These data further suggest that the presence of $\gamma\delta$ T cells may attenuate lung fibrosis by limiting expansion of inflammatory cells in the lung in response to microbial invasion which is independent of IL-17A.

$\gamma\delta$ T CELLS IN LUNG DISEASE

$\gamma\delta$ T cells represent a separate lymphocyte subset, whose primary function is not well understood (32,33). In contrast to $\alpha\beta$ T cells which recognize processed peptide antigens in the context of major histocompatibility complex (MHC) molecules, $\gamma\delta$ T cells recognize highly conserved non-protein antigens (34,35) as well as stress-induced MHC class I-like molecules (36). Although $\gamma\delta$ T cells constitute a minor component of the overall T cell population in peripheral lymphoid organs (<5% of total T cells), these cells are enriched in many epithelial-containing organs such as skin, lung, intestine and genitourinary tract (37). Within the normal lung of C57BL/6 mice, $\gamma\delta$ T cells represent 5–10% of all T lymphocytes, with the majority of these cells expressing either TCR V γ 4 or V γ 1 (38). V γ 6-expressing $\gamma\delta$ T cells are quite rare in lung (38). Importantly, these $\gamma\delta$ T cell subsets express different functional capabilities. For example, V γ 1⁺ $\gamma\delta$ T cells enhance airway hyperresponsiveness while V γ 4⁺ $\gamma\delta$ T cells suppress this response in a murine model of asthma (39).

The epithelium of the lung like other organ systems such as the gastrointestinal tract is constantly exposed to pathogenic microorganisms and toxins from the environment. The fact that $\gamma\delta$ T cells are found in the subepithelium of alveolar and non-alveolar regions of the lung (38) suggests an important role of these cells in the immune response directed against inhaled particles such as microbial pathogens and toxic substances (37). Therefore, $\gamma\delta$ T cells may prevent epithelial injury and damage by attenuating an excessive inflammatory response to an invading pathogen or toxin (37). Numerous studies support this hypothesis. For example, mice intratracheally infected with *Cryptococcus neoformans* develop a transient increase in $\gamma\delta$ T cells in the lung that peaks 3–6 days after infection. In the absence of $\gamma\delta$ T cells, *C. neoformans* is cleared more rapidly from the lung (40). Similar to pulmonary infection with *C. neoformans*, mice infected with *Pneumocystis carinii* and *Bordetella pertussis* have a transient increase in $\gamma\delta$ T cells in the lung, again with enhanced clearance of these microbial pathogens in the absence of $\gamma\delta$ T lymphocytes (41,42). These data suggest that $\gamma\delta$ T cells in these models of lung infection downregulate the immune response generated against the inhaled pathogen which prevents tissue damage at the expense of delayed clearance of the pathogen from the lung. Therefore, $\gamma\delta$ T cells may help regulate the delicate balance between excessive inflammation resulting in tissue damage and microbial persistence. Conversely, in

the absence of $\gamma\delta$ T cells, mice infected with *Nocardia asteroides* have delayed clearance of this microorganism and increased mortality (43,44) indicating that at least in this model of infectious lung disease, $\gamma\delta$ T cells are necessary for microbial destruction that prevents death of the animal. These studies suggest that $\gamma\delta$ T cells are important in pulmonary infections due to a variety of inhaled microorganisms, but the immunoregulatory role of this T cell subset is dependent on the specific microbial pathogen. In HP, investigators recently found a decrease in the percentage of $\gamma\delta$ T cells in the lung by bronchoalveolar lavage in both subacute and chronic disease compared to controls (31). Interestingly, there was a further decline in $\gamma\delta$ T cells in the lung of patients with chronic HP suggesting that loss of $\gamma\delta$ T cells may increase the risk of developing pulmonary fibrosis in humans.

$\gamma\delta$ T cells are also important in protecting the lung epithelium against noninfectious insults such as ozone. After ozone exposure, $\gamma\delta$ T cell-deficient mice fail to recruit inflammatory cells, resulting in a decreased clearance of necrotic epithelial cells in the terminal airways (44). Thus, in response to infectious and noninfectious insults, $\gamma\delta$ T cells are capable of monitoring the integrity of the lung epithelium and responding to the expression of stress-induced self-molecules.

TH17 CELLS IN LUNG DISEASE

Th17 cells are a new lineage of T cells that express novel cytokines such as IL-17A, IL-17F and IL-22 (Figure 2). As described above, these cytokines have recently been described in a murine model of HP (16,24) as well as in humans (45). IL-17A and IL-17F are critical for host defense against bacterial invasion by recruiting neutrophils to areas of tissue inflammation for pathogen clearance (46). In addition, IL-17A and IL-22 regulate the production of antimicrobial proteins in mucosal epithelium (47). Therefore, T cells that express Th17 cytokines are critical for mucosal host defense against bacteria (48). Numerous cell types including CD4⁺, CD8⁺ and $\gamma\delta$ T cells have been shown to express IL-17 (46). $\gamma\delta$ T cells that express IL-17 have been shown to be important in lung diseases such as pulmonary tuberculosis (49). Interestingly, $\gamma\delta$ T lymphocytes are the major cell type producing IL-17 in the murine model of pulmonary tuberculosis and may promote inflammation by recruiting cells to the site of infection although in the absence of $\gamma\delta$ T cells, TCR $\delta^{-/-}$ mice infected with *M. tuberculosis* did not demonstrate differences in bacterial load compared to wild-type mice (50,51). Therefore, IL-17A is most likely important for clearance of most microorganisms from the lung, consistent with recent reports showing the persistence of *K. pneumonia* (52) and *B. subtilis* (27) in the lung of mice deficient in IL-17. In addition, the receptor for IL-17 has been shown to be up-regulated in lung of patients with HP further suggesting that the Th17 pathway is also relevant in human disease (45). Interestingly, TGF- β and IL-6 are necessary for the differentiation of naive CD4⁺ T lymphocytes into Th17 cells in mice via the transcription factor ROR γ t (53–57). Whether TGF- β is also required for expression of IL-17A by $\gamma\delta$ T cells, however, is unknown. TGF- β has been shown to be a critical cytokine for the development of pulmonary fibrosis (58,59) and is elevated in the lung in response to both *S. rectivirgula* (16) and *B. subtilis* (27). Therefore, TGF- β may promote differentiation of Th17 cells with the adverse consequence of promoting collagen deposition in the lung in response to chronic exposure to microbial pathogens.

CONCLUSION

HP is an inflammatory lung disease caused by repeated inhalation of aerosolized antigens. With chronic exposure to an inhaled antigen, patients are at risk of developing irreversible pulmonary fibrosis and increased morbidity and mortality. Although $\alpha\beta$ T cells have been shown to be important in the pathogenesis of HP, $\gamma\delta$ T cells are also found and may be protect against lung damage and fibrosis due to chronic exposure to an inhaled pathogen. In addition to type 1

cytokines, Th17 cytokines such as IL-17A and IL-22 expressed by T cells may be important in the pathogenesis of these lung diseases and protect against chronic exposure to microbial pathogens through enhanced elimination that prevents chronic immune activation and thus, lung fibrosis. Considerable work is still needed to further our understanding of the role of these unique T cells and Th17 cytokines in both HP and lung fibrosis.

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Figure 1.

C57BL/6 mice repeatedly exposed to *S. rectivirgula* develop mononuclear infiltrates and pulmonary fibrosis. **A.** H&E staining of lungs from C57BL/6 mice repeatedly treated with inhaled *S. rectivirgula*. Arrowheads denote mononuclear infiltrates in the peribronchovascular space. (100x) **B.** Masson Trichrome staining of lungs from C57BL/6 mice repeatedly treated with *S. rectivirgula*. Arrow denotes collagen deposition in a peribronchovascular distribution. (100x)

Figure 2. Model of Th1, Th17, and Th2 lineage development

Naive T cells are activated by antigen-presenting cells, and the cytokine environment in which the T cell resides determines the terminal lineage commitment. IL-12 potentiates IFN- γ expression, through a STAT-1 and T-bet dependent mechanism, IL-4 increases IL-4 production in a STAT-4 and GATA-3 dependent fashion. Retinoid-related orphan receptor γ t (ROR γ t) is the critical transcription factor involved in Th17 differentiation. IFN- γ inhibits Th2 and Th17 development while IL-4 inhibits Th1 and Th17 development. TGF- β inhibits Th1 and Th2 differentiation, but is necessary for initiation of Th17 differentiation.