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Activated Oligoclonal CD4⁺ T Cells in the Lungs of Patients with Severe Emphysema

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Rationale: Within the lungs of patients with severe emphysema, inflammation continues despite smoking cessation and is characterized by the presence of T lymphocytes (T cells). Disease severity has been associated with increasing numbers of T cells within the small airways and alveolar walls of patients with chronic obstructive pulmonary disease (COPD). At present, the role of the T cell in this persistent inflammation remains unknown. One important question is whether these T cells are accumulating in the lung in response to an antigen or are being nonspecifically recruited. The aim of this study was to determine

if T cells recruited to the lungs of subjects with severe emphysema are composed of oligoclonal T-cell populations, suggesting their accumulation in response to conventional antigenic stimuli. **Methods:** Lung T-cell receptor (TCR) V β repertoire from seven patients with emphysema was evaluated at the time of tissue procurement (*ex vivo*) and after 2 weeks of culture with interleukin-2 (*in vitro*). Junctional region nucleotide sequencing of expanded TCR V β subsets was performed.

Results: No significantly expanded TCR V β subsets were identified in *ex vivo* samples. However, T cells grew from all emphysema (n = 7) but no control (n = 4) lung samples when exposed to interleukin-2. Within the cultured CD4⁺ T cells, a total of six major TCR V β subset expansions were identified in four of the seven patients with emphysema. Importantly, these T-cell expansions contained T-cell clones that had already been expanded *in vivo*.

Conclusion: From the lungs of subjects with severe emphysema, oligoclonal CD4⁺ T-cell populations were identified that preferentially proliferated in culture. Oligoclonal populations of T cells within the lungs of patients with severe emphysema suggest that response to an antigen plays an important role in the continuing inflammation characteristic of severe COPD.

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Mechanisms of Autoimmune Emphysema

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Although cigarette smoking is implicated in the pathogenesis of emphysema, the precise mechanisms of chronic progressive alveolar septal destruction are not well understood. Here we show in a novel animal model (1) that immune competent, but not athymic, nude rats injected intraperitoneally with xenogeneic endothelial cells produce antibodies against endothelial cells and develop emphysema. Anti-endothelial cell antibodies cause endothelial cell apoptosis *in vitro*. TUNEL assay and activated caspase-3 Western blot and immunostaining showed more cells undergoing cell death in immunized animal lungs than in control rat lungs. There is a significant up-regulation of matrix

metalloproteases (MMPs) MMP-9 and MMP-2 in the lungs of immunized animals when compared with control rat lungs. Anti-endothelial cell antibodies recognize several endothelial cell epitopes, including vascular endothelial growth factor receptor (VEGFR)-2 and angiotensin-converting enzyme (ACE). Mice injected with anti-endothelial cell serum also develop emphysema. Lung morphometry of mice injected with anti-endothelial cell antibody showed 20% enlargement of alveolar airspaces over a 5-wk period as compared with normal rat serum-injected controls. Immunization also causes accumulation of CD4⁺ T cells in the lung. Adoptive transfer of a pathogenic, spleen-derived CD4⁺ cell population into naive immune-competent animals also results in emphysema. Moreover, adoptive transfer of CD4⁺ spleen cells into secondary syngeneic rats resulted in emphysema, even though these secondary rats had not been immunized with human umbilical vein endothelial cells (HUVEC). In this study, we show for the first time that humoral and CD4⁺ cell-dependent mechanisms are sufficient to trigger the development of emphysema. Our data demonstrate that pathogenic CD4⁺ T lymphocytes are necessary and sufficient in breaking a regulatory tolerance and causing emphysema in naive immune-competent rats, suggesting the involvement of autoimmune mechanisms in alveolar septal cell destruction.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Altered T-Cell Phenotypes in Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States, and one of the few major diseases in which mortality continues to rise (1). There is a growing body of evidence that implies that adaptive immune responses could play a role in the pathogenesis of COPD (2–4). As with other immune-mediated diseases, activated effector lymphocytes can result in direct cytopathic effects, elaborate diverse proinflammatory mediators, and recruit and activate other “downstream” effector cells (5–8). Armed effector T cells have been specifically implicated in a variety of tissue injuries, including epithelial necrosis and fibroproliferation (8). Histologic and

morphometric analyses confirm an increase in cellular inflammation and further suggest a correlation between this response and advancing disease, as defined by GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria (9, 10). Subepithelial lymphoid aggregates (BAL) have an increased prevalence in advanced COPD (10). These reports are highly suggestive that an immune stimulus and corresponding response appear relevant to the pathogenesis of COPD. We characterized phenotypic alterations in T lymphocytes of subjects with COPD and demonstrated that they are indeed activated and elaborate proinflammatory cytokines capable of promoting tissue injuries.

Some of the results of these studies have been previously reported in the form of an abstract (11).

METHODS

Current and former smokers with no history of asthma and at least a 10-pack-year history of smoking were recruited through the Emphysema-COPD Research Registry at the University of Pittsburgh Medical Center. All subjects were rigorously characterized, including a complete history and physical, spirometry, diffusion capacity, plethysmography, sputum, and symptom scores, and chest computed tomography scan. Peripheral blood was obtained from each subject after informed consent. Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Histopaque 1.077; Sigma-Aldrich Biotechnology, St. Louis, MO), and stained for selected cell surface markers and intracellular cytokines (after stimulation with PMA and ionomycin in the presence of Golgistop) with monoclonal antibodies. Flow cytometry was used to determine expression of cell surface markers and cytokine elaboration. Expressions were quantified after gating on live lymphocyte populations (> 10,000 events). Blood samples obtained from age-matched nonsmokers (n = 20) were used as controls.

RESULTS

A total of 65 subjects have been recruited to date. The average age is 64 yr and 58% are male. Subjects had an average smoking history of 54 pack-years and 23% were currently smoking. Subjects were well distributed across GOLD stages, with 17 subjects with GOLD 0–1, 19 with GOLD 2, 14 with GOLD 3, and 15 with GOLD stage 4 disease.

CD4⁺ T cells from patients with COPD demonstrated significant down-regulation of CD28, a costimulatory molecule, as compared with control subjects. Similar findings have been reported in immune diseases characterized by chronic or repeated antigen exposure, including autoimmune disorders and lung transplant rejection (12, 13). A significantly greater number of CD4 lymphocytes from patients with COPD produced transforming growth factor (TGF)- β 1 as compared with those from control subjects (24 vs. 2%; $p < 0.0001$). When subjects were stratified by GOLD staging, TGF- β 1 elaboration positively correlated with disease severity. There was also an apparent trend for greater interleukin (IL)-2 production by CD4 T cells from patients with advanced COPD (GOLD stages 3 and 4), compared with those with less extensive disease ($p = 0.06$).

CD8⁺ T cells from subjects with COPD showed a higher degree of activation as assessed by expression of major histocompatibility class (MHC) II, and the magnitude of this abnormality was correlated with disease severity (as defined by GOLD criteria). A significantly greater number of COPD CD8⁺ T cells in patients with advanced GOLD stages also produced IFN- γ ($p < 0.05$). In addition, IL-8 and TGF- β 1 elaborations were also correlated with disease extent (Figure 1).

DISCUSSION

COPD is a complex disease with evidence of local tissue destruction as well as systemic effects. Although smoking is the primary