Bronchiolitis in Chronic Obstructive Pulmonary Disease

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This presentation concerns the nature of the lesions in the smaller conducting airways less than 2 mm in internal diameter, which become the major site of airway obstruction in chronic obstructive pulmonary disease (COPD) (1–3). The 2-mm airways are located between the 4th and the 14th generation of tracheobronchial tree branching, depending on the length of the pathway followed (4). Unpublished data from our laboratory on more than 200 cases suggest that 80% of the conducting airways beyond this point are nonrespiratory bronchioles and the remaining 20% are smaller bronchi identified by the cartilage in their walls. Both the total number of airways and their collective cross-sectional area increase rapidly in this region of the lung (4), and although there has been controversy about their contribution to the resistance to airflow in the normal lung (2), there is complete agreement that they become the major site of obstruction in COPD (1–3). The tobacco smoking habit is associated with a chronic inflammatory immune response in the lungs of every smoker and this response increases substantially in those who develop COPD (5, 6). It is also associated with the appearance of lymphoid follicles that document the presence of an adaptive immune response that increases sharply in severe (GOLD [Global Initiative for Chronic Obstructive Lung Disease] stage 3) and very severe (GOLD stage 4) COPD (5). A multivariate analysis of all of the data collected on these airways, however, showed that airflow limitation was most closely associated with the severity of the lumen occlusion by inflammatory exudates and the thickening of the walls of the small airways by a repair and remodeling process (5). The purpose of this presentation is to briefly review the nature of these inflammatory immune repair and remodeling processes as a basis for discussing the nature of the pathology at the site of airway obstruction in persons with COPD.

THE INNATE AND ADAPTIVE INFLAMMATORY IMMUNE RESPONSE

The lower airways are protected from the inhalation of the toxic particles and gases by the innate and adaptive inflammatory immune systems (5, 7, 8). The innate system consists of mucociliary transport and cough that clear the airway surface (9) and the physical barrier provided by the tight junctions that join the epithelial cells together. After challenge with toxic gases and particles, mucociliary clearance slows (9), the production of mucus is increased (10), and the epithelial barrier is disrupted (11,

12). Epithelial cells and resident monocytes/macrophages respond to this challenge by generating a wide variety of cytokines and chemokines that control the movement of migrating innate inflammatory immune cells into the injured tissue (13). These migrating cells all originate in the bone marrow and include polymorphonuclear leukocytes (PMNs); monocytes/macrophages; eosinophils; CD-4, CD-8, and B-cell lymphocytes; as well as smaller numbers of natural killer and dendritic cells. These cells migrate out of the blood into the tissue where they infiltrate the airways and parenchyma in the peripheral lung. CD-4, CD-8, and B cells aggregate to form lymphoid follicles in the bronchial-associated lymphoid tissue (BALT).

BALT differs from regional lymph nodes in that the lymphoid aggregates have no capsule and are not supplied with afferent lymphatics (13, 14). Antigen can be transported across the epithelial surface of the BALT by specialized M cells but these cells do not present antigens to T and B lymphocytes. The antigen transported by the M cells is probably taken up by Langerhans dendritic cells and presented to T and B lymphocytes. Furthermore, because these dendritic cells are scattered throughout the epithelium, they probably pick up antigen deposited away from the BALT structures and find their way to the BALT or enter the local lymphatic vessels that transport them to regional lymph nodes. Cross-sectional study of groups of patients with different levels of COPD severity shows an overall increase in natural killer cells and Langerhans dendritic cells as well as CD-4, CD-8, and B cells in association with the level of COPD severity (5). It is the formation of the lymphoid follicles, however, that defines appearance of the adaptive immune response. Furthermore, the sharp increase in their numbers in cases of advanced COPD suggests an increase in antigen load in the later stages of the disease (5). Figure 1 shows a BALT collection in a small airway that provides the venue for the accumulation of large numbers of T and B cells. This accumulation increases the probability that CD-4 T cells will meet and interact with B cells that have recognized the same antigen. This interaction between CD-4 T and B cells provides the facultative help that initiates B-cell proliferation in the germinal center and results in the production of high-affinity antibodies by processes of isotype switching and affinity maturation (8). We suspect that most of this antibody production is directed at the microbes that colonize and infect the lower airways in the later stages of COPD (5). The recent hypothesis, however, that COPD is caused by an autoimmune response which might result from incremental development of overlapping activity against self-determinants (i.e., autoantigens) is currently under active investigation (15).

SMALL AIRWAY PATHOLOGY

The symptoms of chronic cough and sputum production are strongly associated with the tobacco smoking habit and, in the mid-1960s, the British Medical Research Council suggested that a diagnosis of chronic bronchitis should be made when these symptoms are present on most days of the month for at least 3 months in 2 consecutive years without any other underlying

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explanation (16). Reid was the first to associate the mucus production in chronic bronchitis with hypersecretion of the mucous glands and used the size of the mucous glands as a yardstick for the postmortem diagnosis of chronic bronchitis (17). Although she did not implicate the inflammatory process as the cause of either the gland enlargement or the excess mucus production, a reevaluation of this problem in surgically resected lung tissue showed that the symptoms of chronic bronchitis are associated with inflammatory response in the mucus-secreting apparatus of the bronchi between 2 and 4 mm in diameter (18, 19).

The hypothesis that there was a natural progression from chronic bronchitis to chronic airflow limitation was tested in an important longitudinal study of its natural history by Fletcher and associates (20). Interestingly, they rejected this hypothesis when they discovered that a considerable proportion of the men that developed airflow obstruction over the 8 years of their study could not, by the Medical Research Council definition, be considered to have obstructive bronchitis. On the basis of this important finding, they went on to conclude that "it seems likely that emphysema and intrinsic disease of the small airways were involved to different degrees in these obstructed men." Unfortunately, the term "chronic bronchitis" continues to be used interchangeably with airway obstruction by those who are either unaware or unwilling to accept the results of the Fletcher study.

A seminal study by Macklem and Mead (21) introduced the concept of small airway disease, and subsequent studies showed that smaller bronchi and bronchioles less than 2 mm in diameter were the major site of airway obstruction in COPD (1–3). These studies also established that the small airway obstruction was due to intrinsic disease within the airways themselves and could not be accounted for by decreased parenchymal support of these airways (1). If decreased support were the problem, inflating the lungs should have allowed the remaining support to reduce the

contribution of the small airways to total resistance as the lungs were inflated and it did not. These data suggested that disease in the lumen and walls of the small airways was the cause of the obstruction, and recent quantitative study of these airways has provided insight into the nature of these lesions (5). Although the airways of the smokers who maintain normal lung function (GOLD stage 0) were infiltrated by inflammatory immune cells, this infiltration was more extensive (judged by the percentage of airways involved) and severe (judged by the accumulated volume of each cell type) as the severity of COPD progressed from GOLD-0 to GOLD-4 disease. Furthermore, the formation of follicles with germinal centers by the infiltrating lymphocytes indicated the presence of an adaptive immune response.

OCCCLUSION OF THE LUMEN BY MUCOUS EXUDATES

The data in Figure 2 show the relationship between the different levels of FEV₁ used to define the GOLD categories of COPD and the severity of the occlusion of the inflammatory exudates containing mucus. Furthermore, data presented elsewhere in this issue by Sciurba and colleagues (see pages 533–534) showed that the quartile of cases with the most severe occlusion also had a reduced 72-month survival after surgery. Because these premature deaths were not associated with symptoms of chronic bronchitis, we assume that the mucus-containing exudates were formed in the small airways rather than aspirated from the larger, more central airways affected by bronchitis. Several studies have shown that the number of goblet cells increases in the small airways in COPD and an early investigation into this metaplastic process showed human PMN elastase stimulated the release of mucus from secretory cells (22). These observations were subsequently confirmed and extended in experiments showing that PMN elastase triggers the cleavage of membrane-tethered

Figure 1. (A) A collection of bronchial-associated lymphoid tissue with a lymphoid follicle containing a germinal center (GC) surrounded by a rim of darker staining lymphocytes that extend to the epithelium of both the small airway and alveolar surface. (B) Another follicle where the germinal center stains strongly for B cells. (C) A serial section of the same airway stained for CD-4 cells that scatter around the edge of the follicle and in the airway wall. (D) An airway that has been extensively remodeled by connective tissue deposition in the subepithelial and adventitial compartments of the airway wall. (The arrow points to the smooth muscle that separates the subepithelial from the adventitial compartments; Movats stain.)

Figure 2. The relationship between the median value for lumen occlusion by inflammatory exudates containing mucus and the decline in FEV₁ in 159 cases where lung function was measured just before lung resection for small peripheral tumor or lung volume resection for advanced tumor. ●, Gold 4; ■, Gold 3; △, Gold 2; □, Gold 1; ◇, Gold 0.

tissue growth factor- α (TGF- α), allowing it to bind to and consequently activate the epidermal growth factor receptor (EGFR) (23). This activation of EGFR then results in stimulation of downstream signaling pathways that culminate in the expression of MUC5AC. These and other experiments established that EGFR and its ligands provide a regulatory axis for mucin production where membrane-bound ligands of EGFR, such as TGF- α (24), and a reactive oxygen species-dependent pathway that bypass surface EGFR to activate its intracellular domain directly control the secretion of the mucus (25). Other studies have also shown that exposure of isolated airway epithelial cells to cigarette smoke results in increased EGFR mRNA expression and induces EGFR-specific tyrosine phosphorylation followed by MUC5AC mRNA expression and protein production (26, 27). Furthermore, these effects were inhibited by selective EGFR tyrosine kinase inhibitors and antioxidants *in vitro*. Other experiments in intact animals have shown that cigarette smoke inhalation increases MUC5AC mRNA expression and goblet cell production and that these effects could be prevented by kinase inhibitor pretreatment (28). Collectively, these data support the

theory that cigarette smoke-induced inflammation causes excess mucus production in the small airways by stimulating an inflammatory response that brings in cells capable of releasing enzymes that cleave ligands for EGFR from the cell surface and that these ligands stimulate EGFR in an autocrine fashion to initiate mucus production. They also suggest that reactive oxygen species can bypass EGFR and stimulate mucus production by interacting with the intracellular domain of this receptor, and that up-regulation of EGFR can stimulate the entire cascade.

REMODELING OF THE AIRWAY WALL

Fibrocytes/fibroblasts have an octopus-like shape with long projections that extend from the endothelial to the epithelial basement membranes that form the boundaries of the interstitial compartment. They also send short projections through preformed holes in these basement membranes that come into close contact with both endothelial and epithelial cells. Elegant three-dimensional reconstructions of the interstitial spaces of the lung indicate that migrating inflammatory cells enter the interstitial

Figure 3. The relationship between volume of the airway wall and surface area of the basement membrane (estimated as total wall area divided by basement membrane length) and FEV₁ from the same cases shown in Figure 1. These data show that the airway wall tends to thicken as FEV₁ declines. ●, Gold 4; ■, Gold 3; △, Gold 2; □, Gold 1; ◇, Gold 0.

compartment through these preformed holes and use the surface of the fibroblast to guide their movement within the interstitial compartment (29). They then leave the interstitial compartment by migrating through similar preformed holes in the epithelial basement membrane before passing between epithelial cells into the airspace. Presumably, growth factors generated during the inflammatory immune response stimulate the fibroblasts to develop into myofibroblasts, which have mobility required to infiltrate the provisional matrix at the damaged site, as well as the ability to contract and close the wound, before secreting the proteoglycans and collagen fibrils that eventually form a permanent matrix during the remodeling phase of the repair process.

There is an association between airway wall thickening and the level of airflow limitation in COPD (Figure 3) that has been attributed to the repair and remodeling process that is briefly described above (5). A striking feature of the lesions that develop in COPD is the deposition of connective tissue in the adventitia of the airway wall in a manner that appears to restrict the opening of the airway lumen. This finding confirmed earlier work showing that peribronchial and peribronchiolar fibrosis is an important feature of small airway pathology in advanced emphysema (30). We are currently examining the hypothesis that interleukin (IL)-13 and IL-1 β stimulate growth and differentiation of the lung fibroblasts by a coordinated up-regulation of platelet-derived growth factor (PDGF)-AA and PDGF-R α (31, 32). According to this hypothesis, IL-13 stimulation up-regulates PDGF-AA through the STAT-6 pathway and IL-1 β stimulation up-regulates PDGF-R α through the p-38 pathway, and the interaction between PDGF-AA and PDGF-R α stimulates myofibroblast proliferation through a mitogen-activated protein kinase (MAPK)-dependent extracellular signal-regulated kinase (ERK) pathway. According to this hypothesis the role of transforming growth factor- β is to inhibit the expression of TGF-R α to shut down fibroblast proliferation and initiate collagen synthesis. Our preliminary studies indicate that many of the relevant cytokines and growth factors believed to be involved in this process are expressed either at the mRNA or protein level. Much more work is required to determine the precise mechanism by which connective tissue is laid down in the small airway walls.

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Cigarette Smoke Causes Small Airway Remodeling by Direct Growth Factor Induction and Release

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Small airway remodeling (SAR) is one of the anatomic causes of airflow obstruction in patients with chronic obstructive pulmonary disease. The pathogenesis of SAR is poorly understood, but there are two general theories: (1) SAR is caused by smoke-induced inflammatory cells that damage the airway and evoke a repair process and (2) SAR is caused by direct induction of growth factors by cigarette smoke. To investigate this process, we briefly exposed rat tracheal explants, a model system free of exogenous inflammatory cells, to whole cigarette smoke, and then maintained the explants in organ culture for varying periods. With explants cultured in air, smoke induced up-regulation of both transforming growth factor (TGF)- β_1 and procollagen gene expression at 24 h after initial exposure. Increased procollagen gene expression was prevented in a dose-response fashion by the TGF- β antagonist fetuin, indicating that TGF- β was driving fibrosis. Collected supernatant from explants exposed to smoke and cultured in fluid medium showed increased release of TGF- β_1 , and this was abolished by the oxidant scavenger tetramethylthiourea. Gene expression of connective tissue growth factor (CTGF) was sharply increased 2 h after smoke exposure. Using pure recombinant proteins, smoke-conditioned medium oxidized TGF- β latency-associated peptide and also caused release of active TGF- β_1 from latent TGF- β_1 . We conclude that cigarette smoke directly induces release of preformed TGF- β_1 by oxidizing the latency-associated peptide in rat tracheal explants, and that release must be extremely rapid, since gene expression of CTGF, the downstream fibrogenic driver of TGF- β effects on collagen synthesis, is elevated very shortly after smoke exposure. Although tracheal explants are only an approximate model of small airways, these findings suggest that smoke-induced SAR may reflect direct induction of growth factor release and signaling and does not require smoke-evoked inflammatory cells.

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Regulation of Airway Mucin Secretion by MARCKS Protein Involves the Chaperones Heat Shock Protein 70 and Cysteine String Protein

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We have reported previously that MARCKS protein is a key regulatory molecule controlling mucin secretion by airway epithelial cells *in vitro* and *in vivo*, and presented evidence supporting a mechanism whereby MARCKS regulates secretion by translocating from plasma membrane to cytoplasm, where it binds to membranes of intracellular mucin granules (1, 2). The actual mechanism whereby MARCKS is targeted to and attaches onto membranes of mucin granules has not been elucidated. We hypothesized that two specific chaperone proteins, cysteine string protein (CSP) and heat shock protein 70 (Hsp/Hsc70), play important roles in this process, and do so by complexing with MARCKS on the mucin granule membrane. Both Hsp/Hsc70 and CSP were found to be present in normal human bronchial epithelial (NHBE) cells. CSP was localized to membranes of intracellular mucin granules, as determined by ultrastructural immunohistochemistry and Western blotting of isolated mucin granule membranes. Coimmunoprecipitation and Western blotting showed that CSP within these cells complexed with both MARCKS and Hsp/Hsc70. A peptide against the C-terminus of CSP added to NHBE cells attenuated mucin secretion in a concentration-dependent manner, whereas a control missense peptide had no effect. This C-terminal peptide also blocked binding of MARCKS to mucin granule membranes. Transfection of HBE1 cells, a virally transformed human airway epithelial cell line, with siRNA constructs against MARCKS, CSP, or Hsp70 attenuated mucin secretion, indicating that these proteins are involved in the process. The results suggest that CSP, and specifically its C-terminus, associates with membranes of mucin granules in airway secretory cells and, apparently in conjunction with Hsp/Hsc70, plays a role in MARCKS attachment to granule membranes during the secretory process.

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