Airway Inflammation

Moderator: James H. Shelhamer, MD; Discussants: Stewart J. Levine, MD; Tong Wu, MD; David B. Jacoby, MD; Michael A. Kaliner, MD; and Stephen I. Rennard, MD

Diseases characterized by airway inflammation, excessive airway secretion, and airway obstruction affect a substantial proportion of the population. These diseases include asthma, chronic bronchitis, bronchiectasis, and cystic fibrosis. Asthma and chronic bronchitis may affect 25 million persons in the United States. Much progress has been made in the last decade toward an understanding of the mechanisms underlying chronic airway inflammation; recent work has resulted in several new concepts of the initiation and maintenance of airway inflammation. Airway production of chemokines, cytokines, and growth factors in response to irritants, infectious agents, and inflammatory mediators may play an important role in the modulation of acute and chronic airway inflammation. Lipid mediators may be produced by resident airway cells and by inflammatory cells; production of these mediators may also be altered by inflammatory cytokines. Increased airway obstruction may be related to intercurrent viral respiratory infection and to the induction of airway inflammation and airway hyperreactivity that results from such infection. Furthermore, several models exist to explain the processes by which airway inflammation is perpetuated in diseases such as asthma and chronic bronchitis. These include neurogenic inflammation, the perpetuation of the acute inflammatory response, and cycles of airway epithelial cell–mediated and inflammatory cell–mediated recruitment and activation of inflammatory cells. An understanding of these mechanisms of airway inflammation may provide the clinician with new therapeutic approaches to the treatment of these common and chronic diseases.


Dr. James H. Shelhamer (National Institutes of Health [NIH], Bethesda, Maryland): Inflammation of the conducting airways is a feature of lung diseases characterized by airway obstruction and excessive airway secretions. These diseases include asthma, chronic bronchitis, cystic fibrosis, and bronchiectasis. In the United States, 25 million persons (13 million with chronic bronchitis and 12 million with asthma) may have these diseases (1). In patients with asthma, airway obstruction may be initiated by inflammatory events in the airway (2), and some investigators have suggested that there may be a correlation between indices of airway inflammation and airway hyperresponsiveness (3). In the patient with chronic bronchitis, there is a correlation between indices of airway inflammation and airway obstruction (4, 5). The mechanisms of this inflammatory response, the ways in which this response is propagated, and the effects of this response on airway function are subjects for investigation. To further explore the role of airway inflammation in chronic airway disease, we review current concepts of cytokine networks in the airways, modulation of lipid mediator metabolism in the airways, the potential role of viral infection in airway hyperresponsiveness, and mechanisms of chronic airway inflammation in asthma and chronic bronchitis.

Bronchial Epithelial Cell–Cytokine Interactions in Airway Inflammation

Dr. Stewart J. Levine (NIH): Airway epithelial cells can participate in local cytokine networks and regulate inflammatory airway events by synthesizing and secreting various cytokines that communicate in a paracrine manner with infiltrating inflammatory cells and structural airway cells. Furthermore, airway epithelial cells represent targets for numerous cytokines that regulate the expression of immune and inflammatory airway epithelial cell products. Interactions between airway epithelial cell cytokine products and inflammatory as well as structural airway cells enable the airway epithelium to orchestrate specific inflammatory and immune responses that are important both for normal host defense and the pathogenesis of inflammatory airway disorders such as asthma, acute and chronic bronchitis, bronchiectasis, and cystic fibrosis.

Cytokines produced by airway epithelial cells (Table 1) can either directly initiate or secondarily amplify acute and chronic inflammatory events by mediating the chemotaxis and recruitment of inflammatory cells to the airway. Interleukin-8, a member of the α-chemokine subfamily, is an important airway epithelial cell product that mediates neutrophil chemotaxis in airway disorders such as cystic fibrosis, chronic bronchitis, and bronchiectasis (6, 7). Furthermore, interleukin-8 has been reported to induce eosinophil and T-lymphocyte chemotaxis, to modulate basophil histamine release, and to inhibit interleukin-4–mediated B-lymphocyte IgE production (8). The α-chemokine subfamily members GRO-α and GRO-β, which mediate neutrophil chemotaxis and activation, and monocyte chemotactant protein-1, a member of the β-chemokine subfamily that mediates the chemotaxis and activation of monocytes and basophils and the chemotaxis of T-lym...
Table 1. Respiratory Epithelial Cell Cytokines*

<table>
<thead>
<tr>
<th>Chemokines</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Chemokine subfamily</td>
<td></td>
</tr>
<tr>
<td>Interleukin-8</td>
<td></td>
</tr>
<tr>
<td>GRO-α</td>
<td></td>
</tr>
<tr>
<td>GRO-γ</td>
<td></td>
</tr>
<tr>
<td>β-Chemokine subfamily</td>
<td></td>
</tr>
<tr>
<td>Monocyte chemoattractant protein-1</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte chemotactic factor</td>
<td></td>
</tr>
<tr>
<td>Colony-stimulating factors</td>
<td></td>
</tr>
<tr>
<td>Granulocyte macrophage colony-stimulating factor</td>
<td></td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor</td>
<td></td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor</td>
<td></td>
</tr>
<tr>
<td>Colony-stimulating factor-1</td>
<td></td>
</tr>
<tr>
<td>Pleiotropic cytokines</td>
<td></td>
</tr>
<tr>
<td>Interleukin-5</td>
<td></td>
</tr>
<tr>
<td>Interleukin-11</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td></td>
</tr>
<tr>
<td>Interleukin-1</td>
<td></td>
</tr>
</tbody>
</table>


phocyte subsets, have also been identified as airway epithelial cell products (9, 10). Lymphocyte chemoattractant factor is another important airway epithelial cytokine that may directly contribute to the pathogenesis of airway inflammation in asthma by inducing the chemotaxis of CD4+ cells such as T lymphocytes, eosinophils, and monocytes (11).

Once recruited to the local airway environment, airway epithelial cell colony-stimulating factors such as granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and colonystimulating factor-1 may promote the survival, activation, and differentiation of inflammatory cells such as eosinophils, neutrophils, and macrophages (12–15). In addition, airway epithelial cells can secrete multifunctional cytokines, such as interleukin-6, interleukin-10, tumor necrosis factor, and interleukin-1, that exert pleiotropic proinflammatory effects on various genes within several target cells, including inflammatory and structural airway cells (13, 16–18). For example, interleukin-6 can induce B-cell immunoglobulin production, mucosal IgA responses, T-lymphocyte proliferation, acute-phase protein synthesis, and cytotoxic T-cell, macrophage, and neuronal differentiation (19, 20). Airway epithelial cells also synthesize and secrete interleukin-11, another pleiotropic cytokine capable of regulating 1) the proliferation, differentiation, and activation of B lymphocytes, macrophages, and leukocytes; 2) neuronal differentiation; and 3) the induction of acute phase protein synthesis (16).

Cytokine secretion by airway epithelial cells can either primarily initiate local inflammatory responses in direct response to inflammatory stimuli or secondarily amplify inflammatory events previously initiated by activated macrophages, eosinophils, mast cells, or lymphocytes (Table 2). For example, airway epithelial cell production of cytokines can occur in direct response to viral and bacterial products; to sensitizing chemicals, such as toluene diisocyanate; and to noxious gases, such as nitrogen dioxide and ozone (15–18, 25, 26). Alternatively, airway epithelial cell secretion of cytokines may secondarily amplify inflammatory events in response to inflammatory cell products such as cytokines, transforming growth factor-β, histamine, and neutrophil elastase (12, 13, 15, 16, 27, 28). In addition, airway epithelial cell secretion of cytokines may represent a mechanism for the amplification of neurogenic inflammation, because vasoactive intestinal peptide

Table 2. Modulators of Airway Epithelial Cell Cytokine Production

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral</strong></td>
<td></td>
</tr>
<tr>
<td>Influenza type A</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>Interleukin-6, interleukin-8, interleukin-11, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td><strong>Bacterial products: Pseudomonas aeruginosa</strong></td>
<td></td>
</tr>
<tr>
<td>Rhamnolipids</td>
<td>Interleukin-6, interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>Mucoid exopolysaccharide</td>
<td>Interleukin-6, interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>1-kd product</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td><strong>Chemicals</strong></td>
<td></td>
</tr>
<tr>
<td>Toluene diisocyanate</td>
<td>Interleukin-1, interleukin-6</td>
</tr>
<tr>
<td><strong>Gases</strong></td>
<td></td>
</tr>
<tr>
<td>Ozone</td>
<td>Interleukin-6, interleukin-8</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>Interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td><strong>Drugs</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Interleukin-6, interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>Nedocromil</td>
<td>Interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>Cefodizime</td>
<td>Granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td><strong>Inflammatory cell products</strong></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>Interleukin-6, lymphocyte chemoattractant factor</td>
</tr>
<tr>
<td>Elastase (35)</td>
<td>Interleukin-6, interleukin-8, granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>Interleukin-6, interleukin-8, GRO-α, monocyte chemoattractant protein-1, granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>Interleukin-1</td>
<td>Interleukin-6, interleukin-11, granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Interleukin-6, interleukin-11</td>
</tr>
<tr>
<td><strong>Neuropeptides</strong></td>
<td></td>
</tr>
<tr>
<td>Vasoactive intestinal peptide</td>
<td>Interleukin-6</td>
</tr>
</tbody>
</table>

Table 3. Cytokine Regulation of Airway Epithelial Cell Functions*

<table>
<thead>
<tr>
<th>Cytokine Effector</th>
<th>Effect</th>
<th>Target Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon-γ, tumor necrosis factor-α, interleukin-1β, interleukin-4</td>
<td>↑</td>
<td>Intercellular adhesion molecule-1 expression</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, interleukin-1β</td>
<td>↑</td>
<td>Neutrophil adherence (21)</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>↑</td>
<td>HLA-DR expression</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>↑</td>
<td>Inducible NO synthase</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, interleukin-1, interleukin-2, interleukin-6</td>
<td>↑</td>
<td>Endothelin-1 secretion</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, interleukin-1β</td>
<td>↑</td>
<td>Interleukin-6 secretion</td>
</tr>
<tr>
<td>Interleukin-8 secretion</td>
<td>↑</td>
<td>Monocyte chemoattractant protein-1 expression</td>
</tr>
<tr>
<td>Interleukin-10, tumor necrosis factor-β</td>
<td>↑</td>
<td>Granulocyte macrophage colony-stimulating factor secretion</td>
</tr>
<tr>
<td>Interleukin-1</td>
<td>↑</td>
<td>Granulocyte colony-stimulating factor secretion</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>↑</td>
<td>Mucin secretion</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>↑</td>
<td>Cytokine phospholipase A&lt;sub&gt;2&lt;/sub&gt; expression</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>↑</td>
<td>15-lipoxygenase activity</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, interleukin-1β</td>
<td>↑</td>
<td>Chloride secretion (22)</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>↑</td>
<td>Complement (C3) expression (23)</td>
</tr>
<tr>
<td>Interferon-β</td>
<td>↑</td>
<td>Secretory leukocyte proteinase inhibitor secretion (24)</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>↑</td>
<td>Elastase-specific inhibitor secretion (24)</td>
</tr>
</tbody>
</table>


has been reported to induce the production of interleukin-6 by a transformed bronchial epithelial cell line (29).

Inhibition of respiratory epithelial cytokine secretion by corticosteroids is one mechanism by which airway inflammation may be attenuated. In vitro experiments have described the down-regulation of airway epithelial cell secretion of interleukin-6, interleukin-8, and granulocyte macrophage colony-stimulating factor by corticosteroids (12, 13, 15, 30). In addition, administering inhaled beclomethasone dipropionate for 8 weeks to patients with atopic asthma resulted in a substantial decrease in bronchial epithelial granulocyte macrophage colony-stimulating factor immunoreactivity, which correlated with decreased airflow obstruction and decreased bronchial hyperreactivity (31). Furthermore, the anti-inflammatory effects of nedocromil may be regulated in part by the inhibition of the secretion of interleukin-8 and granulocyte macrophage colony-stimulating factor bronchial epithelial cells (32).

The airway epithelium may also be able to modulate inflammatory responses by solubilizing cell-surface tumor necrosis factor receptors. Human airway epithelial cell lines release the p55 type 1 tumor necrosis factor receptor following the activation of protein kinase C by phorbol ester, which may then function as a tumor necrosis factor-binding protein and inhibit the biological activity of high concentrations of tumor necrosis factor (33). However, at low concentrations of tumor necrosis factor, soluble tumor necrosis factor-binding proteins may stabilize the trimeric structure of tumor necrosis factor and thereby serve as a biological reservoir of tumor necrosis factor (34). Therefore, solubilized tumor necrosis factor receptors from airway epithelial cells may prevent wide fluctuations in tumor necrosis factor concentrations within the airway microenvironment.

Airway epithelial cell–cytokine interactions may also contribute to the pathogenesis of acute and chronic inflammatory events by modulating airway epithelial cell inflammatory and immunoregulatory functions (Table 3). Interferon-γ and, to a lesser extent, tumor necrosis factor-α, interleukin-1β, and interleukin-4, have been reported to up-regulate bronchial epithelial cell intercellular adhesion molecule-1 expression, thereby promoting leukocyte recruitment and retention within the airway epithelium (36). Interferon-γ has also been reported to up-regulate airway epithelial cell MHC class II (HLA-DR) expression, raising the possibility that airway epithelial cells can present antigen to T lymphocytes, although less efficiently than monocytes (37). Cytokines can also induce the generation of airway epithelial cell products that regulate smooth-muscle tone, including endothelin-1, which is a bronchoconstrictor, and inducible nitric oxide synthase, which mediates bronchodilation through nitric oxide generation (38, 39). Lastly, tumor necrosis factor-α, interleukin-1β, and interleukin-6 have all been reported to induce human airway mucin hypersecretion (40–42).

Interactions between airway epithelial cell cytokine products and effector inflammatory cells may directly contribute to the pathogenesis of airway inflammation in disorders such as asthma and cystic fibrosis. Immunohistochemical studies done on bronchial mucosal biopsy specimens from patients with asthma have detected enhanced amounts of granulocyte macrophage colony-stimulating factor, monocyte chemoattractant protein-1, and tumor necrosis factor-α within bronchial epithelial cells (31, 43, 44). In addition, primary cultures of asthmatic bronchial epithelial cells secrete increased amounts of interleukin-6, interleukin-8, granulocyte macrophage colony-stimulating factor, and lymphocyte chemotactic factor (11, 45). These airway epithelial cell cytokines may then contribute to the recruitment, activation, differentiation, and survival of eosinophils, lymphocytes, and macrophages in asthmatic Airways. In cystic fibrosis, neutrophil elastase within epithelial lining fluid can induce interleukin-8 gene expression and the release of neutrophil chemotactic activity by a transformed human bronchial epithelial cell line; this represents a mechanism by which airway epithelial cells may amplify and perpetuate neu-
Arachidonic Acid Metabolites in the Airway*

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>Major Source</th>
<th>Major Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂</td>
<td>Epithelial cells</td>
<td>Smooth-muscle relaxation; ↓ mucus secretion</td>
</tr>
<tr>
<td>Prostaglandin E₂₉</td>
<td>Epithelial cells</td>
<td>Smooth-muscle contraction; sensory nerve activation</td>
</tr>
<tr>
<td>Prostaglandin D₂₉</td>
<td>Mast cells</td>
<td>Smooth-muscle contraction; airway hyperreactivity</td>
</tr>
<tr>
<td>Thromboxane A₂</td>
<td>Endothelium; platelets</td>
<td>Smooth-muscle contraction; airway hyperreactivity</td>
</tr>
<tr>
<td>Leukotriene B₄</td>
<td>Inflammatory cells</td>
<td>Chemostraction; lymphocyte activation</td>
</tr>
<tr>
<td>Leukotriene C₅₄, D₅₄, and E₄</td>
<td>Inflammatory cells</td>
<td>Smooth-muscle contraction; airway hyperreactivity</td>
</tr>
<tr>
<td>15-HETE</td>
<td>Epithelial cells; eosinphils</td>
<td>Stimulation of mucus secretion; ↑ others</td>
</tr>
<tr>
<td>8,15-DHETE</td>
<td>Epithelial cells; eosinphils</td>
<td>Chemostraction</td>
</tr>
</tbody>
</table>

* Data from references 51 and 52. DiHETE = dihydroxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid.

Modulation of Eicosanoid Production by the Airway Epithelium

Dr. Tong Wu (NIH): Metabolites of arachidonic acid may play a major role in modulating airway inflammation (51, 52). Arachidonic acid metabolites present in the airway, their major cellular sources, and their major effects in the airway are presented in Table 4. Airway cells are capable of producing arachidonic acid metabolites under baseline conditions and in response to various stimuli, such as calcium ionophore A23187, exogenous arachidonic acid, bradykinin, endothelin-1, or platelet-activating factor. The activity of cytosolic phospholipase A₂ is increased in the presence of Ca²⁺ or after phosphorylation at serine, threonine, or tyrosine residues. Platelet-derived growth factor, epidermal growth factor, adenosine triphosphate, thrombin, and phorbol 12-tetradecanoate 13-acetate cause the phosphorylation of the serine or threonine residues of cytosolic phospholipase A₂ and result in enhanced enzyme activity (56). Interleukin-1α, macrophage colony-stimulating factor, and tumor necrosis factor-α have recently been reported to induce the accumulation of cytosolic phospholipase A₂ in human fibroblasts, monocytes, and HeLa cells, respectively (57-59). Some cytokines have dual effects on cytosolic phospholipase A₂, such as activation of the enzyme by phosphorylation and induction of enzyme synthesis. For example, macrophage colony-stimulating factor and tumor necrosis factor-α (58, 59) regulate cytosolic phospholipase A₂ through the phosphorylation of the enzyme and through control enzyme synthesis in monocytes and epithelial cells (HeLa cells).

In a human bronchial epithelial cell line, the synthesis of cytosolic phospholipase A₂ protein can be induced by at least two cytokines, interferon-γ (60) and tumor necrosis factor-α (61). Treatment of the cells with interferon-γ increases the release of arachidonic acid from the cells and induces the synthesis of cytosolic phospholipase A₂ protein. Cells stimulated with interferon-γ show a mark-
edly increased release of arachidonic acid in response to
subsequent stimulation by calcium ionophore A23187.

Because cytosolic phospholipase A₂ plays a central role
in providing arachidonic acid and lysophospholipid for
subsequent metabolism to prostaglandins, leukotrienes,
HETES, and platelet-activating factor—all potent lipid
mediators of inflammation—the cytokine-induced synthe-
sis and activation of cytosolic phospholipase A₂ may play
an important role in modulating the airway inflammatory
response.

15-Lipoxygenase

The biological activities of 15-lipoxygenase attract spe-
cial interest because this enzyme represents a major path-
way for the metabolism of arachidonic acid in human lung
tissue. 15-Lipoxygenase catalyzes the insertion of mole-
cular oxygen into arachidonic acid at carbon 15, resulting
in 15-HETE production. 15-HETE is produced in large
amounts in human airway epithelial cells and eosinophils.
In human airways, freshly isolated epithelial cells exhibit
relatively high 15-lipoxygenase activity. Immunohisto-
chemical staining using antibodies against 15-lipoxygenase
indicates that 15-lipoxygenase is preferentially localized in
the airway epithelium (52). The enzymatic products of
15-lipoxygenase may act as specific mediators. It has been
shown that 15-HETE increases airway mucus secretion
(62). Other effects attributed to products of 15-lipoxy-
genase include neutrophil chemotaxis, modulation of the
host immune response, modification of other oxygenation
enzymes, induction of neuronal hypersensitivity, and mi-
tigen activity (51, 52). Because 15-lipoxygenase can oxida-
ze the fatty acids esterified in membrane phospholipids,
it is speculated that 15-lipoxygenase may modify mem-
brane composition and function. Expression of 15-lipoxy-
genase can be induced by inflammatory cytokines. Inter-
leukin-4 induces 15-lipoxygenase messenger RNA and
protein in cultured monocytes, which can be blocked by
cotreatment with interferon-γ, hydrocortisone, and endo-
toxin (63). In cultured human airway epithelial cells, in-
terleukin-4 has also been shown to increase 15-lipoxy-
genase activity (64).

Cyclooxygenase-2

The central enzyme in the prostaglandin synthetic path-
way is cyclooxygenase, or prostaglandin H synthase. Pre-
vious studies showed that the formation of cyclooxygenase
products can be regulated by growth factors and cyto-
kines. However, these studies did not usually exclude the
possibility that the agonist-stimulated formation of prosta-
glandins may actually result from the stimulation of phos-
pholipase activity. The recent identification of a sec-
ond isoform of cyclooxygenase, cyclooxygenase-2, has
prompted new questions about the regulation of prosta-
glandin synthesis. Now it is known that the cellular expres-
sion of cyclooxygenase is derived from two cyclooxy-
genase genes: cyclooxygenase-1, encoding a 2.8-kb transcript,
and cyclooxygenase-2, encoding a 4.1-kb messenger RNA.
These transcripts yield protein products, which share ap-
proximately 60% amino acid identity. Although the prod-
tects confer the same enzymatic function, data from in
vitro studies suggest that cyclooxygenase-1 is constitutively
expressed, whereas cyclooxygenase-2 is markedly induced
after stimulation with growth factors, cytokines, or tumor
promoters. In rheumatoid synovial tissues, interleukin-1β
or phorbol myristate acetate (PMA) induces the synthe-
sis of cyclooxygenase-2 but not cyclooxygenase-1. The
induced expression of cyclooxygenase-2 is suppressed by
cotreatment with dexamethasone (65).

Little is known about the expression and regulation of
cyclooxygenase isoforms in the lung. Recently, cyclooxy-
genase-2 was shown to be induced by endotoxin in rabbit
(66) and rat (67) alveolar macrophages. In mast cells, the
activation of protein kinase C with PMA enhances the
cyclooxygenase-2 messenger RNA level with increased
prostaglandin D₂ production (68). This effect of PMA is
blocked by dexamethasone. This study suggests that cy-
clooxygenase-2 may be important in airway inflammation
in which mast cells are activated.

Because arachidonic acid metabolites may play an im-
portant role in the pathogenesis of airway inflammation,
therapeutic approaches may be directed toward the pro-
duction of eicosanoids or toward their receptors. The
5-lipoxygenase enzyme that is present primarily in inflam-
atory cells is one of the targets. Inhibition of this en-
yme decreases the production of leukotriene B₄ and the
sulfidopeptide leukotrienes. The 5-lipoxygenase inhibitor
zileuton (AY64077) has been shown to have promise in
asthma. Inhibitors of 5-lipoxygenase-activating protein
and leukotriene D₄-receptor antagonists may have a
therapeutic role in some forms of asthma (69). The pro-
duction of eicosanoids may also be blocked by the inhi-
bition of phospholipase A₂ and cyclooxygenase. Glucocor-
ticoids inhibit the release of arachidonic acid from cell
membranes in part by inhibiting the gene expression of
both low-molecular-weight phospholipase A₂ (70) and cy-
tosolic phospholipase A₂ (57, 59), and cytokine-induced
production of both of these products may be blocked by
glucocorticoids. In addition, glucocorticoids may block
gene expression of cyclooxygenase-1 (70), cyclooxygen-
genase-2 (65), and 15-lipoxygenase (63).

In summary, recent studies have shown the interaction
between cytokines and eicosanoid-forming enzymes in res-
piratory cells. This complicated network of molecular
interactions may play an important role in modulating
airway inflammation. It is reasonable to speculate that
modulation of these interactions may provide new ap-
proaches to the treatment of inflammatory disorders.

Viral Respiratory Infection: A Mechanism for the
Induction of Airway Inflammation and Airway
Hyperreactivity

Dr. David B. Jacoby (Johns Hopkins School of Medi-
cine, Baltimore, Maryland): Viral infections of the airways
commonly cause exacerbations of chronic obstructive pul-
monary disease (71, 72) and asthma (73, 74). The associ-
ation of viral infections with exacerbations of asthma is
most striking in children, in whom at least 30% to 40% of
asthma exacerbations are caused by viral infections (75).
In fact, one recent study (76), in which children with
asthma were followed for 2 years, showed viral infections
in 75% of asthma exacerbations. Improved, highly sensi-
tive methods for detecting viral infections, coupled with
the collection of specimens at the earliest sign of exacer-
vation, probably account for the high rate of association found in this study.

Viral infections have many effects on the airways. Small increases in nonspecific airway responsiveness (to methacholine, for example) have been reported after viral infection, but changes of this magnitude would be unlikely, as an isolated mechanism, to result in clinical exacerbations of asthma. Other effects of viral infections include the potentiation of the airway response to tachykinins, an increase in vagally mediated reflex bronchoconstriction, and the recruitment and activation of inflammatory cells.

Loss of Neutral Endopeptidase Activity in the Airway

The tachykinins are a family of peptide neurotransmitters and include substance P, neurokinin A, neurokinin B, and other peptides with similar structures and functions. They are found in the afferent C-fiber nerve endings in the airways, where they have been shown to stimulate smooth muscle contraction (77), epithelial chloride secretion (78), ciliary beating (79), and mucous secretion (80). Tachykinins are also chemotactic for leukocytes (81-83) and increase airway vascular permeability (84). They are prime candidates to be mediators of neurogenic inflammation.

Viral infections have many effects on the airways. Small increases in nonspecific airway responsiveness (to methacholine, for example) have been reported after viral infection, but changes of this magnitude would be unlikely, as an isolated mechanism, to result in clinical exacerbations of asthma. Other effects of viral infections include the potentiation of the airway response to tachykinins, an increase in vagally mediated reflex bronchoconstriction, and the recruitment and activation of inflammatory cells.

Virus-induced airway hyperresponsiveness to tachykinins was first described by Saban and colleagues (85). They showed that airway tissues from guinea pigs infected with the parainfluenza virus showed enhanced contractile responses to both substance P and endogenous tachykinins released by capsaicin. We subsequently showed (86, 87) that this increased response to tachykinins results from the virus-induced loss of neutral endopeptidase, an enzyme that degrades tachykinins to inactive metabolites. This virus-induced loss of neutral endopeptidase also potentiates other responses to the tachykinins, particularly increased airway vascular permeability and neurogenic inflammation (84, 88).

Loss of neutral endopeptidase activity may also be significant in view of the production of bradykinin in virus-infected airways (89). Bradykinin is itself degraded by neutral endopeptidase and may therefore have greater effects where this enzyme is decreased. Furthermore, bradykinin may stimulate the release of tachykinins from airway C fibers (90).

The loss of neutral endopeptidase activity in virus-infected airways suggests several possible future therapeutic options in virus-induced asthma exacerbations. Recombinant neutral endopeptidase, delivered as an aerosol, attenuates airway responses to tachykinins in guinea pigs (91). Alternatively, the effects of tachykinins may be decreased by the use of neurokinin receptor antagonists.

Loss of Inhibitory Muscarinic Receptor Function

Several clinical studies (92) have suggested that increased, vagally mediated reflex bronchoconstriction contributes substantially to virus-induced hyperresponsiveness to histamine, exercise, and citric acid aerosols. It was initially thought that epithelial damage exposed irritant receptors, thereby potentiating the afferent limb of the vagal reflex arc. Although this may indeed contribute to enhanced vagal responses, Buckner and coworkers (93) showed that viral infections increased the bronchoconstriction response to electrical stimulation of the vagus nerve in guinea pigs without increasing the smooth muscle response to acetylcholine. Thus, viral infection potentiates the afferent limb of the reflex arc, increasing the release of acetylcholine from the airway vagal fibers.

In the airways, release of acetylcholine from the vagus nerves is under the local control of inhibitory muscarinic receptors on the postganglionic nerves (94). These are M2 muscarinic receptors, and the excitatory muscarinic receptors on airway smooth muscle are M3 muscarinic receptors. Thus, acetylcholine released from the vagus nerve stimulates both M2 muscarinic receptors on airway smooth muscle, causing contraction, and M3 muscarinic receptors on the nerves, decreasing the further release of acetylcholine.

Blocking the neuronal M2 receptors with selective antagonists eliminates this negative feedback and potentiates vagally induced bronchoconstriction as much as 10-fold. These neuronal inhibitory M2 muscarinic receptors have been shown in experimental animals and in humans (95) and may be hypofunctional in patients with asthma (96).

In the guinea pig, the function of these inhibitory receptors is markedly impaired after acute viral infection (97) and in other models of airway inflammation, including acute ozone exposure (98) and antigen challenge in sensitized animals (99). Several inflammatory cell products, including eosinophil major basic protein, can act as M3 receptor antagonists (100); thus, this may be a mechanism for the loss of M2 receptor function. However, it is clearly not the only mechanism for virus-induced loss of M2 receptor function, as was shown by the failure of leukocyte depletion to prevent such loss in guinea pigs with parainfluenza infection (101).

One possible leukocyte-dependent mechanism of virus-induced M2 receptor dysfunction is cleavage of sialic acid residues from M2 receptors by viral neuraminidase. Exposure of M2 receptors to the parainfluenza virus in vitro causes a 10-fold loss of agonist affinity. This effect can be mimicked by an equivalent concentration of purified neuraminidase, and the effect of the virus can be blocked by a neuraminidase inhibitor (102).

During recovery from viral infections, M2 receptor function returns to normal after 2 to 4 weeks (103). However, recent studies have shown that, although M2 receptor function in pathogen-free guinea pigs does not depend on the production of cyclooxygenase products, M2 receptor function can be completely blocked by indomethacin after recovery from viral infections (103). The mechanisms of this change in receptor coupling, and its possible significance in aspirin-sensitive asthma, are still unexplored.

Anticholinergic drugs may be expected to be effective in treating virus-induced parasympathetic hyperresponsiveness. Unfortunately, the currently available anticholinergics are all nonselective; that is, they bind to both the M3 receptor (decreasing the effect of acetylcholine on airway smooth muscle) and the M2 receptor (increasing the release of acetylcholine). The disadvantage of this nonsel ectivity can be shown with ipratropium, which can potentiate the response to vagal stimulation in guinea pigs by blocking the M2 receptor (104). A new anticholinergic
drug, tiotropium bromide, dissociates much more rapidly from the M₃ than from the M₂ receptor, making the drug effectively M₂ selective (105). This may be beneficial in the treatment of airway disease.

It may also be possible to reverse M₂ receptor dysfunction. The increased airway eosinophilia that may result from antigen inhalation after some viral infections (especially rhinovirus [see below]) may decrease M₂ receptor function because eosinophil major basic protein is an M₂ receptor antagonist (100). After antigen challenge in guinea pigs, M₂ receptor dysfunction (which is associated with eosinophilic inflammation of the airway nerves [106]) can be acutely reversed by polyanionic substances such as heparin and poly-I-glutamic acid (99), possibly because these substances are able to bind and neutralize eosinophil major basic protein.

Epithelial Cytokine Production

Both airway inflammation and the systemic activation and priming of leukocytes suggest that cytokines may be released in virus-infected airways. Because the primary target of most airway viruses is the epithelium, epithelial cells are logical candidates to be the source of those cytokines.

Airway epithelial cells are capable of producing various cytokines (Table 1). We showed (25) that influenza infection of primary cultures of human bronchial epithelial cells induced expression of the interleukin-8 gene and release of interleukin-8 and that rhinovirus infection of a transformed human bronchial epithelial cell line (BEAS-2B) caused the release of granulocyte macrophage colony-stimulating factor, interleukin-6, and interleukin-8 (107). Interleukin-8 is also generated by nasal epithelial cells and BEAS-2B cells in response to respiratory syncytial virus infection (108, 109).

The most obvious significance of epithelial cell cytokine release is that it may be a mechanism of airway inflammation. However, these cytokines may also be involved in the systemic activation of leukocytes that has been reported in the face of viral infections (110). Although the exact role of these processes in virus-induced exacerbations of asthma is unknown, it is worth noting that rhinovirus infection has been shown to predispose to delayed airway responses to antigen inhalation (110). These delayed responses, which occur in the airways of only a minority of uninfected persons, occur in most persons with ongoing rhinovirus infections. The physiologic similarities between asthma and the delayed airway response to antigen underscore the possible importance of this observation.

There may be many ways to attenuate either the synthesis or the effects of epithelial cytokines. In the case of viral infections, the possible role of epithelial oxidants in activating transcription factors (especially the transcription factor NF-κB) that induce cytokine gene expression may be a target for therapeutic intervention. Antioxidant treatment of epithelial cells attenuates the activation of NF-κB that would otherwise result from influenza infection (111). This may suggest a role for antioxidant therapy in the treatment of virus-induced airway inflammation.

Thus, viral infections, which are known to exacerbate asthma and chronic obstructive pulmonary disease, may exert various proinflammatory and bronchoconstrictive influences. Loss of neutral endopeptidase activity may potentiate both tachykinin-induced bronchoconstriction and neurogenic inflammation. Epithelial cytokines may promote airway inflammation and activate leukocytes, possibly contributing to enhanced late asthmatic responses to antigen. Loss of inhibitory muscarinic receptor function may increase reflex bronchoconstriction in response to any of these airway irritants.

Airway Inflammation and Airway Hyperreactivity in Asthma

Dr. Michael A. Kaliner (Institute for Asthma and Allergy, Washington, D.C.): During the past decade, since the late-phase allergic reaction was recognized as a sequela of mast cell activation (112), much attention has been directed toward the role that airway inflammation plays in asthma. Support for inflammation occurring in asthma has come primarily from models directed at cellular infiltration of the airway mucosa and the movement of inflammatory cells into the epithelial lining fluid, where they can be recovered by bronchoalveolar lavage. Substantial data have suggested that increased inflammation concomitantly increases airway reactivity and that aiming therapy at the inflammation reduces airway responsiveness. However, relatively little interest has been shown in airway edema and its role in asthma. I briefly discuss the data on each of the signs of inflammation, their pathogenesis, and their roles in asthma. In the end, vascular permeability and its contribution to both acute airflow obstruction and death from asthma is underscored.

A working definition of asthma might be the following: a disease of reversible airflow obstruction manifested as wheezing and caused by various combinations of airway mucosal edema and inflammation, increased secretions, and smooth muscle contraction. Patients with asthma show airway hyperreactivity, and the clinical course of asthma is variable (113). This definition stresses the four causes of airflow obstruction and emphasizes the influence of airway hyperresponsiveness.

The four cardinal signs of inflammation are edema (swelling), vasodilation (redness), cellular infiltration, and pain (increased airway responsiveness). I have reviewed each sign to determine whether sufficient evidence supports its role in asthma. Because the airways have no pain fibers, pain is defined as increased airway responsiveness, a definition that acknowledges that this phenomenon reflects increased sensory response.

Airway Edema

In the classic description of the pathology of fatal bronchial asthma, Dunnill (114) noted the presence of edema of the mucosa in 18 of 20 cases. The overlying mucosal denudation was attributed to the force of the mucosal edema, and the contribution of the plasma exudate to the excessive fluid in the bronchial lumen was discussed. The presence of large amounts of plasma proteins in the airway fluid has been confirmed many times (115) and was carefully documented in a recent study of bronchial lavage after allergen challenge (116). In the human nasal mucosa, a careful study of the dynamics of the formation
Table 5. Mediators Thought To Cause Microvascular Permeability in Asthma

<table>
<thead>
<tr>
<th>Mast cell mediators</th>
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<tbody>
<tr>
<td>Histamine</td>
</tr>
<tr>
<td>Bradykinin</td>
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<tr>
<td>Leukotrienes</td>
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<tr>
<td>Several prostaglandins</td>
</tr>
<tr>
<td>Chymase</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
</tr>
<tr>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Neuropeptides</td>
</tr>
<tr>
<td>Substance P</td>
</tr>
<tr>
<td>Neurokinin A</td>
</tr>
<tr>
<td>Calcitonin gene–related peptide</td>
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</table>

of respiratory epithelial lining fluid was recently published (117), documenting that movement of plasma-protein-rich fluid occurs within minutes of allergen exposure in allergic persons. The microvascular permeability leading to the plasma exudate causes both mucosal edema and a marked increase in nasal secretions. There is speculation about the precise causes of the plasma-protein exudation but, after allergen exposure, mast cell mediators are the most likely cause (Table 5). However, the release of mast cell mediators also causes secondary reflexes that might participate in microvascular permeability. The capacity of histamine antagonists to effectively reduce the acute response to allergen suggests that histamine release is an important factor in nasal allergic reactions.

In animal models, the process of microvascular permeability in the airways has been carefully studied and involves the following events: release of vasoactive substances, development of intercellular openings between postcapillary venule endothelial cells, escape of plasma-protein–rich fluid into the tissues surrounding the venule, movement of water into the tissues, and formation of mucosal edema. In the conducting airways, the responding postcapillary venules are part of an extensive plexus of sub-basement membrane vessels that probably act to warm and humidify inspired air. These vessels leak fluid into the area just beneath the basement membrane; this is precisely the area in which edema is found in asthmatic lungs. The edema may be cleared by two mechanisms: lymphatic clearance or epithelial secretion into the lumen. Lymphatic clearance undoubtedly contributes to the removal of the plasma exudation, but this process is slow and unproven. On the other hand, the process of paracellular transport of edema fluid between epithelial cells and into the bronchial or nasal lumen is well documented. This process occurs within seconds of the formation of edema and may participate in the development of increased luminal fluid, which is a major factor in the airflow obstruction of asthma. Thus, the idea that airway edema plays an important role in asthma is supported by morphologic evidence of its presence, proof that plasma proteins are increased in luminal fluid, and an understanding of the processes involved in the dynamics of both microvascular permeability and the movement of the edema fluid into the lumen.

It has been suggested that this process may play a protective role in asthma (118). The edema fluid that transverses the epithelium into the respiratory lining fluid would provide volume for increased mucociliary clearance; albumin to nonspecifically absorb proteins; IgG (and other plasma immunoglobulins) to interact with pathogens; inflammatory mediators, such as bradykinin and anaphylatoxins, to amplify the reaction; and enzyme inhibitors to limit the tissue destruction induced by pathogenic products. It seems more likely, however, that the process itself may be a useful primitive host defense mechanism. However, in asthma, the edema and increased luminal fluid are certain to be detrimental, contributing to airflow obstruction and the increased secretions thought to be responsible for death in many patients with severe asthma. The admixture of plasma proteins with mucus and dead and dying cells must contribute to the increased viscosity of secretions.

Thus, edema of the airways, such as that which occurs after allergic or inflammatory events, would result in narrowing of the airways, increased airway secretions, and thickening of the airway walls. Airway wall thickness might, in turn, increase airway hyperresponsiveness and further exacerbate the forces leading to airflow obstruction. The presence of increased intraluminal secretions would lead to increased resistance to airflow and trapping, requiring either mucociliary transport or the forces generated by coughing to be cleared. If the volume of secretions or the kinetics of their formation were sufficient, airways might become occluded, leading to ventilation-perfusion defects and hypoxemia. At the extreme, extensive acute vascular leakage could lead to severe hypoxia caused by retained secretions.

Vasodilation

Inflammatory responses always initiate concomitant hyperemia because of vasodilation; this can be seen in the skin or nasal mucosa after the application of inflammatory mediators. Several mediators that cause increased blood flow also cause increased vascular permeability (platelet-activating factor, bradykinin, leukotrienes, and histamine), and others primarily increase blood flow (prostaglandin E, vasoactive intestinal peptide, and calcitonin gene-related peptide). The addition of agents that increase blood flow to those causing vascular permeability potentiates the permeability (119). However, increased vasodilation alone does not lead to increased vascular permeability (120). Thus, increasing the blood flow through the mucosa does not by itself lead to plasma-protein exudation, but, in the presence of vasoactive amines, it potentiates the action of the amines.

Inflammation (Infiltration with Inflammatory Cells)

The association of asthma with airway inflammation has been acknowledged since the first recognition of eosinophils in the airways and sputum of patients with asthma. The classic descriptions of asthma pathology note the presence of eosinophils and neutrophils in both the lamina propria and the airway lumen of patients with asthma (121). However, understanding of the potential contribution of inflammatory events in asthma took a giant leap forward with the use of bronchoalveolar lavage and bronchial biopsy in ambulatory patients with mild asthma.

The presence of eosinophils in the bronchial wall, in
the absence of diseases associated with hypereosinophilia, is pathognomonic of asthma. Some reputable investigators now describe asthma as “chronic eosinophilic bronchitis” (122). Biopsy specimens from patients with mild asthma have confirmed the presence of eosinophils in the mucosa, often beneath the basement membrane and in the epithelium (123). Not only are eosinophils present, but their granule-derived proteins may be found in both the tissue and in bronchoalveolar lavage.

Other consistent findings are the presence of activated mucosal mast cells in the airways and an increased quantity of mast cells. The relation between mast cell degranulation and severe asthma was noted many years ago (121). The finding of increased numbers of mast cell mediators in bronchoalveolar lavage confirms the presence of ongoing mast cell activation even in apparently healthy persons with asthma (123).

Lymphocytes also appear in increased numbers in asthma, in both the epithelium and the lamina propria (124). It has been noted that these lymphocytes express the cell-surface marker for interleukin-2, which suggests that they have been activated (125). It has been suggested that these activated lymphocytes might produce cytokines (such as interleukin-3, interleukin-4, and interleukin-5) of the Th2 subtype, which might participate in airway hyperactivity and cellular infiltration in asthma (43).

Other changes that have been noted on bronchial biopsy specimens include the thickening of the basement membrane, caused by collagen and fibronectin deposition. Myofibroblasts along the basement membrane are increased in conjunction with this thickening and may be responsible for it (126). Mucous gland and goblet cell hyperplasia are also constant features.

Pain or Increased Irritability

The airways of patients with asthma express increased irritability in response to diverse, nonspecific stimuli. Because the airways have few pain fibers, this increased reactivity is taken to be the equivalent of the pain noted with cutaneous inflammation. The underlying causes of bronchial hyperresponsiveness have not been identified with precision, although many contributing factors have been suggested (127). Whereas the underlying hyperresponsiveness seen in all patients with asthma is unexplained, increases in hyperresponsiveness in relation to allergen challenge, natural allergy exposure, late-phase allergic reactions, viral infections, exposure to noxious fumes, inhalation of chemical sensitizers, and various other stimuli have been documented. One of the features of these diverse provocations, which all lead to airway reactivity, is the development of cellular infiltrates. The precise ways in which mast cell activation, eosinophil infiltration, lymphocyte activation, and other events actually cause increased reactivity are unclear. One recent study in a monkey model of asthma (128) suggested that mast cell activation and elaboration of tumor necrosis factor led to the generation of adhesion molecules, which then facilitated eosinophil infiltration and the generation of increased airway reactivity. Pretreatment with antibodies directed at tumor necrosis factor prevented the response. Compelling arguments, however, can also be made for the roles of neutral endopeptidase, increased or decreased amounts of neuropeptides, and the specific actions of mediators derived from mast cells, lymphocytes, or eosinophils.

Future targets for therapeutic intervention might include neuropeptide receptors as well as cytokines involved in the asthmatic inflammatory response. The use of recombinant human neutral endopeptidase was discussed earlier. Tachykinin receptor antagonists directed against the substance P receptor (NK1 receptor) or against the neurokinin A receptor (NK2 receptor) have been studied in guinea pig models of airway neurogenic inflammation (129, 130). In these models, NK1 blockade inhibits the development of airway edema associated with the release of endogenous tachykinins. Inhibition of cytokines such as interleukin-4 and interleukin-5, which amplify the allergic response, might also become a therapeutic option. Because of the central role played by interleukin-4 in the perpetuation of the Th2 lymphocyte phenotype and in the promotion of B-lymphocyte IgE production, this cytokine might be an important therapeutic target. However, selective inhibitors of its receptor are not yet available (131). Because interleukin-5 promotes eosinophil chemotraction and survival, it might also become a therapeutic target. In a guinea pig model of asthma, antibodies to interleukin-5 inhibit antigen-induced bronchoalveolar lavage eosinophilia and antigen-induced airway hyperreactivity to substance P and cholinergic stimulation (132, 133). Similarly, antibodies to interleukin-5 inhibit the development of virus-induced airway hyperresponsiveness to histamine in guinea pigs (134).

Conclusions

The cardinal features of inflammation are present and important in asthma. Cellular infiltration and activation participate in the airflow obstruction and increase airway reactivity. Edema is a major contributor to airflow obstruction and leads to increased airflow fluid, which is often the cause of death in patients with acute asthma. Vasodilation is part of asthma, and it has been suggested that it is an important part of exercise-induced asthma. Therapy aimed at reducing inflammation in asthma will be increasingly important, to reduce not only airflow obstruction but also airway reactivity. Currently, much attention is being directed toward reducing cellular infiltration in asthma, particularly eosinophilia. In the future, therapy aimed at reducing airway edema may also be available and may produce important new advantages.

Pathophysiology of Chronic Bronchitis

Dr. Stephen I. Rennard (University of Nebraska School of Medicine, Omaha, Nebraska): Chronic bronchitis is a clinical syndrome defined by the presence of cough on most days for 3 months in 2 consecutive years. It is aptly named, because airway inflammation is a chronic feature of this condition. The pathology of the process is nonspecific but consists of secretory cell hyperplasia, infiltration of the mucosa with variable numbers of acute and chronic inflammatory cells, focal submucosal fibrosis, and squamous metaplasia. In the peripheral airways, secretory cell metaplasia, mucus plugging, and peribronchial inflammation and fibrosis occur (135). Intraluminal inflamma-
tion is characterized by chronically increased numbers of neutrophils and macrophages. During exacerbations, markedly increased purulence of secretions caused predominantly by neutrophils is frequent.

The histologic features of chronic bronchitis, as noted above, are nonspecific and overlap those found in other disorders, including asthma, cystic fibrosis, and bronchiectasis (136). These overlaps are paralleled by many shared clinical features and are to be expected because these conditions share many pathophysiological pathways. Some distinctions, however, have been made. Asthma, for example, generally shows more eosinophil accumulation both in the airway wall and intraluminally, although eosinophils are also reported in chronic bronchitis. Peribronchial fibrosis, particularly that of the smaller airways, is more common in chronic bronchitis than in asthma, in which subepithelial fibrosis (a thickened lamina reticularis) is often present. Recent studies have begun to uncover the pathophysiologic mechanisms that lead to persistent inflammation of the airways and subsequently result in the architectural changes in airway structure that cause abnormal function. It is likely that such studies will better define both the features that distinguish chronic bronchitis and asthma and the features that will permit the subdivision of these syndromes.

As noted above, the accumulation of neutrophils and macrophages in the airway lumen is common in patients with chronic bronchitis. Although this finding is nonspecific, it may help to explain the pathophysiology of the disease process. The chemoattractant responsible for the neutrophil influx has been suggested to be C5a, leukotriene B4, or interleukin-8. Although all three of these may play some role, a recent study (7) has implicated interleukin-8 as a major chemoattractant in chronic bronchitis. Therefore, inhaled irritants or bacterial products might lead to the production of interleukin-8 by epithelial cells and alveolar macrophages. Interleukin-8 and other mediators attract neutrophils and other inflammatory cells to the airway (6). The effect of this inflammatory response is the subject of the following discussion.

The defining features of chronic bronchitis, cough, and sputum production might have been recognized by Celsus as “dolor” (that is, neural stimulation) of the airways, one of his four classic features of inflammation. Cough is thought to be mediated through numerous pathways, including reflex pathways initiated by both the activation of inflammatory pathways and sensory nerves within the airways themselves (135). Neuropeptides and kinins are thought to play important roles in signal transduction in these pathways (137). These peptides can be degraded by several enzymes, including angiotensin-converting enzyme (138) and neutral endopeptidase (139), which are present within the airways. These enzymatic activities may be lost through various mechanisms, including drugs and damage to the epithelial cells that normally express these enzymes. Loss of neutral endopeptidase has been associated with increased airway reactivity (87, 140). These pathways may involve the increased activity of neuropeptides such as tachykinin and substance P or, alternately, of inflammatory peptides such as bradykinin, the activity of which is increased when the enzymes responsible for its degradation are diminished (140–143).

Sputum is a complex secretion that is, by definition, pathologic. All persons normally secrete a complex mixture of secretory cell products onto the airway surface (143). These are cleared into the proximal airways without cough and expectoration. Numerous stimulatory and inhibitory pathways regulating the production of these secretions have been described. Importantly, several mediators released in inflammatory processes can play a prominent role in regulating the release of mucin glycoproteins and proteoglycans from secretory cells (Table 6). These mediators may result in secretory cell exocytosis, induction of mucin gene expression, or secretory cell hyperplasia. Those products most relevant to chronic bronchitis are the neutrophil products, elastase and cathepsin G, and the macrophage products, macrophage mucus secretagogue and tumor necrosis factor-α. Increased amounts of macrophage mucus secretagogue have been recovered from the bronchoalveolar lavage fluid of normal persons who smoke, and higher levels have been recovered from patients with chronic bronchitis (145).

<table>
<thead>
<tr>
<th>Table 6. Factors Affecting Mucus Secretion*</th>
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<tr>
<td><strong>Stimuli of secretory cell exocytosis</strong></td>
</tr>
<tr>
<td>Neurohormones and peptides</td>
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<tr>
<td>Cholinergic agonists</td>
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<tr>
<td>α-adrenergic agonists</td>
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<tr>
<td>β-adrenergic agonists</td>
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<tr>
<td>Nonadrenergic noncholinergic mediators</td>
</tr>
<tr>
<td>Neutrophils (elastase, cathepsin G)</td>
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<tr>
<td>Mast Cells (histamine, chymase)</td>
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<td>Eosinophils (eosinophil cationic protein)</td>
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<tr>
<td>Macrophages (macrophage mucus secretagogue, tumor necrosis factor-α)</td>
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<tr>
<td>Serum products (bradykinin, complement component 3a)</td>
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<tr>
<td>Exogenous products</td>
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<tr>
<td>Bacterial products (endotoxin, <em>Pseudomonas</em> organisms)</td>
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<td>alveolar protease, rhannolipids)</td>
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<tr>
<td>Irritant gases (reflex stimulation antidromic or spinal reflex pathways)</td>
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<tr>
<td>Factors that induce mucin gene expression</td>
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<tr>
<td>Tumor necrosis factor-α</td>
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<tr>
<td>Endotoxin</td>
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<tr>
<td>Factors that stimulate secretory cell hyperplasia</td>
</tr>
<tr>
<td>Neutrophil elastase, cathepsin G</td>
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<td>Endotoxin</td>
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* Reviewed in references 135 and 144. HETE = hydroxyeicosatetraenoic acid.
to syndromes resembling chronic bronchitis (147). Numerous genetic defects in cilia have been described; these can lead to the abnormal clearance of secretions and can predispose persons to bronchiectasis (148). Finally, recent studies have shown interactions between inflammatory cytokines and the ciliary apparatus (149). It seems likely, therefore, that cilia-mediated clearance can also be modulated by mediators released in an inflammatory milieu.

The abnormal environment of the airways in bronchitis appears to predispose to chronic bacterial colonization. Despite antibiotic therapy, many persons with chronic bronchitis remain colonized with bacteria. Either congenital or acquired immunoglobulin deficiencies, particularly IgA deficiencies, have been associated with chronic bacterial colonization of the airways (150, 151). The ability of bacteria to bind and adhere to the epithelial cells that line the airways may be important in maintaining the colonized state (152). In this regard, the airway epithelial surface can alter its properties. In particular, the injured airway epithelium and airway epithelial cells undergoing repair response after injury may express increased receptors that permit easier adhesion, at least for some strains of bacteria (152).

The chronically inflamed airway, even in the absence of toxin exposure, is susceptible to injury. Once injury occurs, repair responses are initiated and can lead to long-term alterations in airway architecture. Studies by Christensen and colleagues (153), for example, have shown that the injury of guinea pig airways from a single exposure to neutrophil elastase can result in goblet cell metaplasia, a lesion resembling chronic bronchitis. These changes appear to persist for the entire life span of the animal. Goblet cell metaplasia is a routine feature of airway alterations in both asthma and chronic bronchitis (154). Spurzem and colleagues (155) showed that the percentage of goblet cells recovered by bronchoalveolar lavage correlated strikingly with lung function in a group of persons with chronic bronchitis who were studied when their disease was in remission. This suggests that alterations in lung function may be caused by alterations in airway architecture, of which goblet cell metaplasia is part.

Altered airflow in chronic bronchitis is probably caused by airway narrowing due to peribronchiolar fibrosis (156). In this regard, mediators released by cells present within the lower respiratory tract, including epithelial cells (157) and macrophages, are capable of stimulating fibroblast recruitment and proliferation, collagen production, and subsequent tissue remodeling. As a result, the peribronchiolar fibrosis that leads to fixed airflow obstruction can be thought of as an ultimate result of repair responses initiated as a consequence of inflammation and injury.

Although all of the specific cells and mediators responsible for the development of chronic bronchitis remain to be delineated, these concepts suggest several clinically relevant strategies for the development of new therapies. First, the overlaps between chronic bronchitis and asthma suggest that bronchodilator therapy is rational in chronic bronchitis. Inasmuch as mechanisms leading to bronchospasm may differ, however, results from bronchodilator studies in asthma may not pertain to chronic bronchitis. More importantly, bronchospasm, although it is potentially important in some patients with chronic bronchitis, is certainly not the only valid therapeutic target. Strategies designed to reduce cough, to alter the volume and quality of secretions, to alter bacterial colonization, to reduce inflammation and tissue injury, and to augment effective tissue repair are all valid options. Current concepts of the pathophysiology of chronic bronchitis are beginning to define molecular targets for such approaches. Assessment of such therapeutic strategies may require novel paradigms for gauging clinical efficacy, and several consensus statements addressing this problem have been prepared (158). These new, mechanistically based therapies promise to advance the current level of
largely supportive care that is offered to patients with chronic bronchitis.

The Perpetuation of Airway Inflammation

Dr. Shelhamer: Because asthma and chronic bronchitis are chronic diseases, it is interesting to speculate on the mechanisms by which inflammation might be propagated in the airways. Various mechanisms by which the inflammatory response is perpetuated can be proposed; these include neurogenic inflammation, amplification of neutrophil recruitment and activation and via networks of inflammatory mediators, and macrophage-mediated or mast cell-mediated recruitment and activation of inflammatory cells. The models presented here are not meant to be complete or to define the relation between inflammation and disease but are intended to show how inflammatory cell activation may further support the recruitment and activation of inflammatory cells.

First, in a model of neurogenic inflammation, airway inflammation is initiated with epithelial cell injury caused by various stimuli (159) (Figure 1). This injury results in epithelial cell shedding and in reduced production of epithelial cell neutral endopeptidase. This reduction may result in prolonged effects of tachykinins, such as substance P and neurokinin A. Various products may then activate mucosal sensory nerve fibers, resulting in the antidromic release of nonadrenergic, noncholinergic neuropeptides, such as substance P, neurokinin A, and calcitonin gene–related peptide. These peptides may cause vascular permeability, mucosal edema, the release of plasma-derived inflammatory mediators into the submucosal space, and the recruitment and activation of inflammatory cells. As a result of plasma leakage, bradykinin may be released to the extravascular space where, in the absence of neutral endopeptidase, it can cause the release of arachidonic acid metabolites and further stimulate sensory nerve endings to release neuroptides. With reduced neutral endopeptidase levels, these neuroptides will have an enhanced effect on inflammatory cell recruitment and mucosal permeability.

A second model involves neutrophil recruitment and activation (Figure 2). In this model, the generation of interleukin-8 and granulocyte macrophage colony-stimulating factor by airway epithelial cells or activated macrophages results in neutrophil recruitment and activation. Activated neutrophils release neutrophil elastase, which stimulates additional production of interleukin-8 by the airway epithelium (46). The neutrophils also release leukotriene B4, which further activates neutrophils to produce interleukin-8 themselves (160). This cycle of elastase, leukotriene B4, and interleukin-8 production results in the amplified recruitment and activation of neutrophils in the inflamed airway.

A more complex model by which various cell types are recruited to the airway is initiated through macrophage activation and the release of tumor necrosis factor, interleukin-1, interleukin-8, and leukotriene B4 (Figure 3). This results in epithelial cell production of chemokines and growth factors, such as monocyte chemoattractant protein-1, interleukin-8, granulocyte macrophage colony-stimulating factor, and granulocyte colony-stimulating factor, as well as endothelial cell or epithelial cell production of cell adhesion molecules, such as intercellular adhesion molecule-1, E selectin, and vascular cell adhesion molecule-1 (21, 128). The chemokines and the cell adhesion molecule expression facilitate the recruitment of inflammatory cells (macrophages and neutrophils) to the mucosa and airway lumen. These newly recruited inflammatory cells may then produce various mediators, including cytokines, arachidonic acid metabolites, platelet-activating factor, oxygen radicals, and stored granule products. The cytokines and platelet-activating factor may induce epithelial cell arachidonic acid metabolism by either enzyme activation or the induction of gene expression for arachidonic acid–metabolizing enzymes. Newly produced arachi...
The action of arachidonic acid products, such as 15-HETE and 8-15-DiHETE (dihydroxyeicosatetraenoic acid), may be chemotactic for additional monocytes and neutrophils (161). These products may then serve to further recruit and activate migratory inflammatory cells to the airway.

Finally, a more specific model for allergic asthma might include a stimulus for mast cell activation resulting in the release of mast cell products, including histamine, leukotriene C4, mast cell proteases, and cytokines (Figure 4). Histamine release may then initiate the epithelial cell production of lymphocyte chemotactic factor. The lymphocyte chemotactic factor would serve to recruit additional CD4+ T cells. Th2-committed cells would then release additional cytokines and growth factors that support further Th2 differentiation (through interleukin-4), dendritic cell function (through interleukin-4), B-lymphocyte immunoglobulin production (through interleukin-4), and eosinophil survival (through interleukin-3, interleukin-5, and granulocyte macrophage colony-stimulating factor) (162). Mast cell–derived tumor necrosis factor may stimulate epithelial cell production of chemokines such as interleukin-8 and growth factors such as granulocyte macrophage colony-stimulating factor. In addition, mast cell–derived cytokines, such as interleukin-3, interleukin-5, and granulocyte macrophage colony-stimulating factor, might also support eosinophil survival. Eosinophil–derived products, including granule proteins and lipids (such as leukotriene C4 and platelet-activating factor) may stimulate inflammatory cell recruitment and activation. Furthermore, platelet-activating factor may activate airway epithelial cell arachidonic acid production.

These models are presented separately for the sake of simplicity; they are not assumed to occur in strict isolation from one another. Clearly, both in asthma and in chronic bronchitis, multiple processes (such as neurogenic inflammation, neutrophil recruitment and activation, and recruitment of other inflammatory and immune cells) may occur in concert. For example, epithelial cell damage associated with the development of neurogenic inflammation might be perpetuated by the release of proteases or oxygen radicals from neutrophils or by the release of major basic protein from eosinophils (159). It is hoped, however, that an understanding of these events will provide new opportunities for therapies aimed at interrupting these inflammatory cycles.

**Summary**

Airway inflammation may be initiated and in some cases perpetuated by inflammatory stimuli at the airway epithelial surface. These stimuli may activate resident structural or inflammatory cells to produce and release cytokines and lipid mediators. These stimuli may also induce sensory nerves to release neuropeptides and alter the production of the enzymes that degrade these neuropeptides. Once the inflammatory process is initiated, interactions between inflammatory cells, immune cells, and resident cells of the airway may serve to amplify and perpetuate the inflammatory process. The end result of this series of events may be airway obstruction and airway hyperreactivity in the patient with asthma or airway hypersecretion, and, in some cases, airway obstruction in the patient with chronic bronchitis. An increased understanding of the molecular mechanisms involved in these events will allow for the development of therapies targeted at specific events in this inflammatory cascade.

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Traditionally, the person best protected from death was the one whom society had condemned to die. Society felt threatened that the man on Death Row might use his tie to hang himself. Authority might be challenged if he took his life before the appointed hour. Today, the man best protected against setting the stage for his own dying is the sick person in critical condition. Society, acting through the medical system, decides when and after what indignities and mutilations he shall die. The medicalization of society has brought the epoch of the natural death to an end. Western man has lost the right to preside at his act of dying. Health, or the autonomous power to cope, has been expropriated down to the last breath. Technical death has won its victory over dying. Medical death has conquered and destroyed all other deaths.

Ivan Illich
*Medical Nemesis*

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