

The Concept of Airway Inflammation

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The generation of an inflammatory response is a critical mechanism through which mammalian species respond to and protect themselves from infectious as well as noninfectious insults (1). In terms of the lung, both nonspecific and antigen-specific (immune) mechanisms may lead to an inflammatory response. While this response is usually protective and beneficial, inflammation also has the potential to injure tissue, including the airways, within the lung. This brief communication focuses in general terms on basic aspects of inflammation within airways. The issue of what we need to know if we are to better understand and more effectively treat children with respiratory disorders in which inflammation plays a role is addressed. Emphasis is placed on providing information and examples that are most relevant to childhood asthma. Although data from animal models cannot be reliably extrapolated to humans, animal studies that provide insight into the inflammatory process are included in this review.

WHAT DO WE KNOW?

Inflammation is broadly defined as a nonspecific protective reaction of vascularized tissues to injury. The classic clinical features of this phenomenon are related to an increase in blood flow in vessels (calor and rubor), an increase in vascular permeability (tumor), an infiltration of cells into tissue (tumor), and a release of materials at the site of inflammation leading to pain (dolor). In general, this process is self-limiting and leads to a return of the tissue and organ to a normal state both structurally and functionally.

The histological picture seen in an inflammatory reaction within the lung is somewhat stereotypical and is independent of the initial insult. The sequence of increased permeability, granulocyte accumulation, and, later, mononuclear phagocyte infiltration that occurs before resolution of the process, is evident with stimuli as diverse as bacterial pathogens, immune complexes, and highly purified phlogistic agents such as fragments of the fifth component of complement (1). In addition, a similar histological evolution was seen in large as well as smaller airways when an allergen-driven process was examined (2). In terms of the timing of this latter process, alterations in permeability in larger airways occur within minutes of allergen exposure, while the granulocytic response develops over several hours. There is an early influx of neutrophils and eosinophils, with the eosinophil influx predominating as time after challenge increases. The mononuclear component is evident within 24 to 48 h, and persists for days even in the absence of further allergen exposure.

The hemodynamic changes associated with inflammation are often the first to manifest and are of central importance if the process is to fully develop. Vasodilation, increased blood flow, and enhanced permeability are fundamental elements of

inflammation, and these changes maximize the opportunity to recruit inflammatory cells and bring plasma proteins to the site of injury. This has potential importance in terms of both mounting an appropriate response and limiting the process when it is no longer needed. In this respect, plasma proteins that exit vessels to become part of the inflammatory reaction may help resolve the process by bringing proteinase inhibitors from the circulation to the site of inflammation (*see below*).

The specific histology of an inflammatory response is a result of the actions of several mediators. Inflammatory mediators may be broadly defined as chemical messengers that act on blood vessels and/or cells to produce or contribute to an inflammatory response. Many agents fulfill this definition. Among those mediators most important early in an inflammatory response, histamine and various eicosanoids (products of arachidonic acid metabolism) are involved in the regulation of local blood flow and may also alter vessel permeability. However, although prostaglandins exert part of their effect by modulating blood flow, it is now apparent that vasodilator prostaglandins (PGE_2 and PGI_2) have additional proinflammatory actions in some models and may potentiate the effect of other mediators including chemotactic factors (3). In addition, vasodilatory prostaglandins such as PGE_2 may also have effects that are antiinflammatory; they may inhibit leukocyte and mast cell secretion, thus limiting an inflammatory response. Indeed, in a study involving allergen challenge of patients with atopic asthma, inhalation of PGE_2 immediately before allergen exposure attenuated the accumulation of eosinophils in sputum and blunted allergen-induced changes in airway function (4). Thus, an individual mediator may have both pro- and antiinflammatory effects, with the net result dependent on the type of insult and timing of mediator release, as well as on other factors that are less clearly defined.

It should be noted that some products of the cyclooxygenase pathway of arachidonic acid metabolism such as thromboxane A_2 and PGD_2 have bronchoconstricting properties while vasodilator prostaglandins such as PGE_2 and PGI_2 have a "bronchoprotective" function. Thus, eicosanoids generated during an inflammatory process may have contrasting actions on airway patency. In a study involving patients with atopic asthma, patients with nonatopic asthma, nonasthmatic patients with atopy, and normal individuals, the ratio of combined bronchoconstrictors (PGD_2 and thromboxane) to combined bronchoprotectors (PGE_2 and the PGI_2 metabolite 6-keto- $\text{PGF}_{1\alpha}$) generated after allergen challenge best differentiated these groups. The patients with atopic asthma had the greatest increase in this ratio (5). Therefore, the concentration and mix of mediators generated at a site within the lung may help define the overall effect on the tissue.

One of the most noticeable histological features of an inflammatory response is the accumulation of cells within the pulmonary tissue. In terms of the acute changes, polymorphonuclear leukocytes (neutrophils, eosinophils) are normally present in small numbers within the conducting airways and alveolar spaces, and are thought to be virtually absent from the interstitial spaces of lung parenchyma. However, a large number of neutrophils are margined in the pulmonary vas-

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cular bed. This pool of cells, as well as the circulating cells, can migrate into the lung. For both types of granulocytes, the number of mediators/chemokines that may attract them into the lung and the cellular sources of these chemoattractants is large. The eosinophil influx and activation, central to the pathogenesis of asthma, is achieved by overlapping and complementary processes. Murine models of allergen-induced airway dysfunction have been used to assess the relative contribution of factors such as interleukin 5 (IL-5) (6), eotaxin, and other chemokines (7) to the pathological and physiological processes. Although a detailed analysis has not yet been conducted in humans, clinical studies in adult patients with asthma have shown that inhalation of IL-5 leads to increases in airway eosinophilia and airway responsiveness (8).

The molecular mechanisms by which leukocytes migrate out of blood vessels have been explored in great detail. Recruitment of leukocytes to an inflammatory reaction is regulated by the sequential engagement of leukocyte-endothelial cell adhesion molecules. The β_2 -integrins lymphocyte function-associated antigen 1 (LFA-1) and membrane attack complex 1 (MAC-1) are thought to be particularly important in leukocyte adhesion and transmigration across the endothelium. LFA-1 is expressed by all leukocytes and binds to the immunoglobulin superfamily molecules intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 on the endothelium. In a rat model of allergic lung inflammation, eosinophil influx into the lung was significantly diminished by use of monoclonal antibodies against various integrins (9). In addition, use of ICAM-1-deficient mice has been associated with a decrease in the number of eosinophils within the lung after allergen challenge (10, 11). Activation of pulmonary endothelial adhesion molecules is accompanied by shedding of the soluble form into the circulation, providing a potential marker of this inflammatory process. The sensitivity and specificity of these markers for the asthmatic process in young children have not been explored.

The mechanisms mobilized through the inflammatory response to defend the lung also have the potential to injure this organ. Two general mechanisms of defense employed by granulocytes and other inflammatory cells are production of oxygen radicals and use of proteins and proteases found within intracellular granules. With respect to the production of oxygen radicals, the enzyme NADPH oxidase, located on the plasma membrane of granulocytes, is able to generate a family of reactive oxidizing chemicals including superoxide anion, hydrogen peroxide, and the hydroxyl radical. The bulk of superoxide generated by the cell dismutates to hydrogen peroxide. Work suggests that reactive oxygen species such as superoxide may damage neural elements within airways, altering the responsiveness of airways to cholinergic stimuli (12). Another mechanism by which products of the respiratory burst may participate in lung inflammation is through production of mediators that magnify the inflammatory response (*see above*). In this respect, there is a link between the occurrence of aggressive oxygen species and stimulation of eicosanoid biosynthesis (13). Recently it has been noted that the oxidative stress seen in asthma can be monitored noninvasively by assessing exhaled hydrogen peroxide and nitric oxide. Both are increased in patients with asthma compared with normal individuals (14). These investigators noted that in unstable steroid-treated patients with asthma, hydrogen peroxide levels remained elevated while exhaled nitric oxide did not, suggesting that the former may be more reflective of disease activity in the face of antiinflammatory therapy. The overall utility of measuring hydrogen peroxide in exhaled air in young children with recurrent wheezing remains to be defined.

As noted above, neutrophils and eosinophils also contain

granules that are the source of microbicidal substances and digestive enzymes. Some of the most important granular products of the eosinophil, which may serve as markers of inflammation, include eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil protein X (EPX), and major basic protein (MBP). Although highly charged proteins may have desirable properties in terms of host defense, they are also able to injure and/or disrupt the normal functions of airway epithelial cells (15). Their utility as potential markers of disease activity in asthma has been reviewed (16).

Stimuli that produce inflammation may do so through immunologic and/or nonimmunologic mechanisms. For example, inhalation of endotoxin or pollutant gases such as ozone may lead to inflammation through direct effects of the agents on sentinel cells within the lung (airway epithelial cells, pulmonary macrophages, mast cells) without participation of antigen-specific cellular or humoral mechanisms. Gram-negative bacteria such as *Pseudomonas aeruginosa*, to which the host has been previously exposed, may produce inflammation through a combination of processes. For example, endotoxin within the cell wall may serve as a stimulus for migration of polymorphonuclear leukocytes into the site of infection. When bacteria-specific antibodies are also present, an antigen-antibody complex may also initiate an inflammatory reaction through activation of complement. Both processes are critical in lung defense and may be effective deterrents only when combined.

Most material with antigenic potential is effectively limited from producing an immunologic response by pulmonary defense mechanisms including cough and mucociliary clearance. Thus, for an immune response to occur, material must breach defense barriers and reach responsive lymphoid tissue. When this occurs, a complex series of events transpires which subsequently provides specificity to host defenses within the lung. This specificity is conferred by an elaborate system composed of receptors on T and B lymphocytes and through antibodies (1). In the context of antigen specificity, a basic function of the immune system is to differentiate "self" from "nonself" at a molecular level, thus providing additional layers of defense against foreign entities.

Concerns about the increasing incidence of asthma has focused attention on maturation of immune responses early in life (17). The expression of a helper T cell type 2 (Th2)-skewed immunity against soluble protein antigens present in the environment is thought to be a primary cause of allergic inflammation in atopic individuals. Conversely, nonallergic normal individuals have a Th1-skewed immunity against the same antigens. Thus, much attention is now being given to how this Th1 versus Th2 balance is achieved early in life. Prescott and coworkers (18) reported that Th2-skewed responses to common environmental antigens (allergens), comprising IL-4, IL-5, IL-6, IL-9, and IL-13, are present in virtually all newborn infants and are dominated by high-level production of IL-10. These observations suggest that the key etiological factor in atopic disease may not be the initial acquisition of allergen-specific Th2-skewed immunity, but instead may be the efficiency of the mechanisms that normally redirect these fetal immune responses toward a Th1 cytokine phenotype. Additional work by Prescott and coworkers (19) demonstrated that there was rapid suppression of Th2 responses during the first year of life in nonatopic children, while there was consolidation of Th2 responses in atopic children associated with defective neonatal interferon γ (IFN- γ) production. Earlier studies of peripheral blood mononuclear cells from infants (20) and neonates (21) suggest that genetic predisposition to atopic sensitization is associated with delayed postnatal maturation

sufficient to produce IFN- γ ; however, whether this is causally related to an inefficiency in immune deviation in Th2 responses in at-risk infants or is a marker for a more fundamental underlying defect in Th1 function remains to be determined.

It is also feasible that variations in the postnatal maturation of airway epithelial dendritic cells (DCs), the principal antigen-presenting cells that regulate immune responses to inhaled antigens, may contribute to the differing patterns of Th cell memory against inhalant allergens that develop in atopic and nonatopic children. Direct information on the ontogeny of these cells in humans is lacking. However, in the rat it has been shown that this DC network develops numerically and functionally slowly between birth and weaning (22). It has also been argued (23) that the observed Th2 polarity of the resting mucosal immune system may be an inherent property of the resident DC population. Employing rat respiratory tract DCs, Stumbles and coworkers found that mobilization of Th1 immunity might rely on the presence of appropriate microenvironmental costimuli (e.g., tumor necrosis factor α and CD40 ligand). A possible role in the maturation of antigen-presenting cells has also been proposed for CD14, a glycoprotein that functions as a high-affinity receptor for endotoxin and other structurally related components of microbial pathogens (17, 24). This may explain some of the observed epidemiological links between microbial burden in early life and susceptibility to the development of atopy (25).

Understanding the ontogeny of inflammatory responses within the lung is important when focusing on young children. However, it must be remembered that many of these studies have been performed in animal models and their relevance to humans remains to be established. However, a few additional observations are cited to stress that the inflammatory response has age-dependent features. In terms of inflammatory cells, some studies suggest that cellular functions (intracellular killing of organisms, oxidative metabolism, migration of human neutrophils) are immature in the neonate (26). In addition, newborn and perinatal animals have been found to be hyporesponsive to several vasoactive mediators and mediators that produce directed migration (chemotaxis) of inflammatory cells (27). In terms of antigen-specific pulmonary immunity, there is currently no information available regarding age-related changes in levels of immunoglobulins within the human respiratory tract. However, studies in experimental animals, which indicate that major developmental changes occur during the preweaning period in the airway intraepithelial DC populations (22), suggest that age-dependent changes in local antibody production within the human airway mucosa may occur.

Although a great deal of information is available on both antigen-specific and nonspecific mechanisms that initiate and perpetuate inflammation, much less is known about resolution of this response. However, this is critical if injury to the lung is to be avoided. For an inflammatory response to resolve, the influx of cells must cease and injurious oxygen radicals and proteases must be inactivated. Fluids and proteins must also be removed or reabsorbed. In addition, inflammatory cells and debris must be removed and damaged cells (such as epithelial cells) replaced. As part of the reparative process, new basement membrane material may have to be laid down and epithelial cells induced to replicate. The host has several mechanisms in place to contain an inflammatory reaction and to repair damaged tissue. Systems known to exist for these purposes include inactivators of chemotactic factors and circulating inhibitors of neutrophil proteinases. Macrophages also play an important role in the resolution of acute inflammation. These cells accumulate at sites of inflammation and act as scavengers to remove debris. Macrophages may also help maintain

normal lung architecture through their ability to release parenchymal cell growth factors (platelet-derived growth factor, fibronectin, etc.). Yet, despite these and other mechanisms of repair, some disease processes lead to chronic and irreversible tissue injury.

There are many factors that determine whether a pulmonary inflammatory response resolves after protecting the lung, or persists and damages the host. One critical determinant is the nature of the insult. The physical characteristics of the agent help determine how the inflammatory response is initiated (direct stimulation to lung parenchymal, immune, or inflammatory cells, antigen presentation to immunocompetent cells, direct activation of complement, etc.). Other important factors include the concentration as well as the length and frequency of exposure to the foreign agent, and of potential critical importance is the age of the host at the time of the initial exposure. The provoking agent may also have an effect on processes that control resolution of a normal inflammatory reaction. For example, inflammation may become chronic because of persistence of the etiologic agent within the host (tuberculosis, etc.). The genetically programmed response of the host to environmental factors is also of importance. In terms of asthma, factors responsible for remodeling of chronically inflamed airways are currently the focus of important work into asthma pathogenesis (28–30). A growing body of opinion favors a critical role for mechanisms that control the inflammation-induced “repair” response in this remodeling process and in the subsequent expression of airway hyperresponsiveness (AHR) (31). Indeed, one report suggested that mucosal dwelling cells of the innate immune system potentially control the development of AHR by regulating the repair response of airway epithelial cells to immunoinflammatory damage (32).

To summarize, an inflammatory reaction is of critical importance in defense of the lung and can be initiated by both antigen-specific and nonspecific mechanisms. In addition, a fundamental feature of inflammation is the redundant nature of the process. Interactions of the kinin, clotting, fibrinolytic, and complement pathways are in part responsible for this redundancy as generation of inflammatory mediators can occur through any of these systems and the response can then be amplified by recruitment of mediators from any of the other systems. This redundancy is also manifest by the fact that many different cells within the lung can produce mediators with similar actions. This built-in redundancy amplifies the response in a normal individual and preserves the response if one system is deficient. However, it also complicates therapeutic approaches to diseases in which the inflammatory response is exuberant, as a specific inhibitor or antagonist may be less effective than a more nonspecific antiinflammatory agent. For asthma, one likely consequence of the redundant nature of inflammation is the superiority of the response to corticosteroids compared with more selective agents (antihistamines, drugs active against leukotrienes, etc.). While inflammation generally resolves without sequelae because of an extensive array of checks and balances, problems arise when some of the checks and balances are lacking (deficiency of α_1 -proteinase inhibitor). In addition, if the programmed response of an individual goes awry (collagen vascular disorders) or is prolonged or inappropriate in magnitude (asthma, cystic fibrosis), lung dysfunction and irreversible injury may be produced.

WHAT DO WE NEED TO KNOW?

1. How early is inflammation present within the airways of subjects with asthma? Does this occur only after the onset of symptoms?

2. If analysis of cells in bronchoalveolar lavage suggests that there are two different "forms" of childhood asthma (33), how many additional subclasses might be identified with a more complete analysis of lavage fluid or tissue from airways?

3. What are the critical targets of the inflammatory reaction that alter airway function? For example, is the damage to neural elements within the airway more detrimental than damage to the airway epithelium, smooth muscle, and/or blood vessels?

4. Are there markers of airway remodeling that can be assessed via the blood or urine?

5. Activation of pulmonary endothelial adhesion molecules is accompanied by shedding of the soluble form into the circulation, providing a potential marker of this pulmonary inflammatory process. What are the sensitivity and specificity of these markers for the asthmatic process in young children?

6. What is the overall utility of measuring hydrogen peroxide in exhaled air in young children with recurrent wheezing? Does this offer technical and/or scientific advantages compared with assessment of exhaled nitric oxide?

7. The relationship between cellular indices of airway inflammation and airway function (obstruction, responsiveness) is quite variable in studies performed in older patients with asthma (34). Will there be a closer relationship between these variables when studying the disease closer to its onset?

8. What role should animal models play in the study of childhood lung diseases in which inflammation contributes to the pathologic process?

HOW CAN WE ACHIEVE THIS?

1. Studies of an invasive nature that involve bronchoscopy with bronchoalveolar lavage plus additional attempts to obtain tissue from an airway (brushings) must be approached in a careful manner in children with lung disease. The challenge of enrolling "normal control subjects" in such studies remains formidable.

2. Given the difficulty of enrolling adequate numbers of young subjects for meaningful studies at a single site, multicenter projects should be encouraged. The recent establishment of the Childhood Asthma Research and Education (CARE) Network under the auspices of the National Heart, Lung, and Blood Institute is an example that may allow investigation of critical issues in pediatric asthma in a more efficient manner.

3. Many of the points listed in the preceding section require studies that cannot be performed in infants and small children. One practical consequence of this fact is that we will continue to rely on work in mammalian models and *in vitro* systems to address certain issues. While this approach and the information obtained in this manner can be questioned in terms of relevance to the human condition, many insights have been gained in various disease processes by using a similar tactic. Unique genetically engineered species (e.g., transgenics, knockouts) are now providing new methods to investigate biological processes.

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