MINIREVIEW

T-Lymphocyte Dysregulation in Asthma

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Asthma, derived from the Greek word αστθμα, meaning gasping or panting, is a condition characterized by intermittent obstruction of the airways resulting in dyspnea, an uncomfortable increase in the work of breathing. This disease has been recognized since antiquity. The first description of asthma, including its seasonality and association with respiratory infection, was reported in the ancient Chinese text on medicine, the *Nei Ching*, ascribed to the Emperor Huang-Ti (2698–2598 B.C.) (1). Treatment options for asthma, including frankincense, juniper berries, beer, and cumin, were summarized in the Egyptian medical tract, the Ebers Papyrus (1550 B.C.) (2). The Greeks also were familiar with asthma. Hippocrates (460–375 B.C.) described the symptoms of panting and wheezing, and Galen (130–200 A.D.) specified that this description was valid only in the absence of a fever (2). These classical descriptions of the signs and symptoms of asthma have led to the modern approach to this disease, which is now being explored at a cellular and molecular level.

It is important to improve our understanding of asthma; its cost is high in both human suffering and economic losses. It has been estimated that between 9 and 12 million people have asthma in the United States alone (3), resulting in 454,000 admissions in 1987 (4) and 5,000 deaths (5). The patients in whom mortality and hospital admissions are increasing most rapidly are less than 17 years old (6). Economic costs are more difficult to evaluate, but a recent study estimated that the direct and indirect costs due to asthma were $6.2 billion in 1990, with the largest direct cost ($1.6 billion) due to inpatient hospitalizations, and the bulk of the indirect costs secondary to decreased productivity (7). Despite vigilant searches for new therapies, the death toll from asthma has increased, worldwide, over the past two decades (8, 9). Explanations for this disturbing trend have implicated causes as disparate as the increasingly polluted environment, poor socioeconomic status and overtreatment with sympathomimetic agents. Whatever the cause(s), these observations have triggered an urgent stimulus for research into the pathophysiology of asthma. It is hoped that further characterization of the asthmatic response will result in specific therapies to alter the natural history of this disease.

Asthma was long thought to be a disorder whose pathognomonic feature was bronchospasm: only in recent years has it been recognized as an inflammatory disorder. Studies focusing upon the inflammation of asthma at a cellular and molecular level have led to the development of a number of theories of pathogenesis of asthma. Investigators early noted eosinophils and the by-products of their fragmentation, Charcot-Leyden crystals, in the airways of patients with asthma, and concluded that eosinophil activation was the *sine qua non* of this disease. Others turned their attention to the influence of histamine and other vasoactive mediators, and concluded, alternately, that mast cell degranulation was the most significant event in the pathogenesis of asthma. Attention also has been paid to abnormalities in regulation of platelets, macrophages, neutrophils, and epithelial, neural, and airway smooth muscle cells. As is usually the case with such

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a wealth of theories, one may conclude that the pathogenesis of asthma is complex and incompletely understood.

The T lymphocyte appears to play a central role in the pathogenesis of asthma. Although it is not the most numerous inflammatory cell in either the alveolae or the airways of normal subjects, the T cell is present in both pulmonary compartments in relatively large numbers, and these populations expand rapidly in response to inflammatory signals. The T lymphocyte is a key member of the immune response in both normal and asthmatic individuals, and can communicate with and activate other inflammatory cells by release of cytokines; T cells obtained from asthmatic patients are activated and spontaneously release cytokines which stimulate eosinophils and mast cells (10–13). T-lymphocyte populations are increased in asthmatic lungs, especially during exacerbations of asthma. They play an especially prominent role in the large population of asthmatics with atopy, in whom the T cell's regulation of B-lymphocyte IgE production controls many of the manifestations of allergic disease. This paper will explore how dysregulation of T lymphocytes may impact the pathogenesis of asthma.

Clinical Aspects

Although a detailed discussion of the diagnosis and management of asthma is beyond the scope of this paper, it is important to review some manifestations of this disease in order to appreciate its pathophysiology. The most recent definitions of asthma have recognized important components of the disease, including reversible airway obstruction, airway hyperresponsiveness to a variety of specific and nonspecific stimuli, and airway inflammation (14, 15). The disease may present acutely, with mild to life-threatening respiratory failure, but more often it has an insidious onset and is recognized by the presence of prolonged wheezing and cough, which may follow an upper respiratory infection.

The diagnosis of asthma is frequently easy for the clinician, as patients often have diminished airflow on pulmonary function testing, which is, at least partially, reversible with the use of inhaled bronchodilators. Occasionally, atypical presentations consist of persistent cough, exercise-, cold-, or workplace-induced symptoms, or unexplained dyspnea. These cases may require more sophisticated studies, including bronchoprovocation with cholinergic agents or (rarely) allergens, exercise testing, workplace monitoring, or a therapeutic trial with anti-inflammatory agents.

An important aspect of evaluating the asthmatic patient is determination whether an element of atopy exists. Beeson and Michael wrote, in 1954, "It is well known, even to laymen, that some persons develop symptoms from contact with substances which are quite harmless to most individuals. These are commonly referred to as allergic manifestations (16)." Atopic asthma, also known as extrinsic or allergic asthma, is most common among younger patients. According to some studies, up to 90% of asthmatic patients under the age of 16 are atopic, and this percentage decreases to less than half of patients over the age of 30 (17). The diagnosis of an atopic condition, thus, may be suggested by early presentation of asthma or, alternatively, by a family history of allergic disorders, seasonal exacerbations of asthma, coexisting allergic disorders such as rhinitis or dermatitis, or blood or sputum eosinophilia (17). These patients often have allergen-specific IgE and positive skin tests; however, positive skin tests do not prove that asthma is caused by allergies. In some instances, atopic asthma will respond to immunotherapy (desensitization), but it is most important to recommend environmental control and allergen avoidance.

Initial treatment of moderate to severe cases of asthma consists of inhaled anti-inflammatory agents, particularly corticosteroids, with the additional use of sympathomimetic inhaled bronchodilators, as required for the management of symptoms. Patients with mild asthma may be treated by intermittent use of inhaled bronchodilators. If diminished airflows persist, most clinicians initiate systemic corticosteroids as a means of controlling the inflammatory response. In infrequent and unfortunate cases, patients may require long-term or even life-long corticosteroids and, thus, are at risk to suffer the many adverse consequences of this type of treatment. This overview of therapy illustrates that asthma is an inflammatory disease of the airways and that state-of-the-art asthma treatment is anti-inflammatory. Better understanding of the pathogenesis of this inflammation will aid the development of more specific means of therapy.

Diagnostic and Investigative Procedures in Asthma

Early data on the inflammation of asthma were compiled from autopsy reports of fatal asthma. These cases were clearly not representative of most asthmatics with mild asthma or asthma not in exacerbation. Recently, the development of fiberoptic bronchoscopy has allowed safe study of the airways of milder asthmatics. Although original reports warned of the dangers of bronchospasm and ventilatory failure caused by bronchoscopy of asthmatics (18, 19), subsequent studies revealed that, with cautious technique, these dangers did not prohibit diagnostic or research examinations. This work has confirmed that epithelia from acutely inflamed asthmatic airways are abnormal, with edema, separation, and shedding (20). A number of differences between asthmatic and normal epithelia can be found. Notable inflammatory cells present

within the epithelia of asthmatics are mast cells and eosinophils. Tissue mast cells and eosinophils have been noted in increased numbers relative to normal controls in mild as well as severe asthmatics (21, 22). In addition, T cells were prominent in the biopsies obtained from asthmatics in a number of studies (23, 24).

Knowledge of the immune responses in asthma has been greatly enhanced by the use of bronchoalveolar lavage (BAL) (25). This technique may be used to harvest alveolar inflammatory cells by instilling saline through a bronchoscope which has been wedged into a distal airway, and collecting the returned fluid under gentle suction. BAL may be performed after inhaled or bronchoscopically delivered antigenic challenge to determine the response to allergens (26–28). A modification of BAL, bronchial washing, uses balloon-tipped catheters to specifically lavage the proximal airways (29, 30).

Studies of atopic asthmatics not in exacerbation have compared total and differential cell counts obtained by bronchial washing, BAL, and peripheral blood with cells from normal control subjects. Kirby *et al.* found no difference in the total cell counts between the atopic asthmatic and control groups, but did note a significant increase in the numbers of eosinophils in both the washings and BAL fluids, but not the peripheral blood, from asthmatics; the pulmonary eosinophil counts from the asthmatic subjects correlated with their methacholine sensitivity (31). The eosinophils obtained from asthmatic subjects are degranulated (28). Metzger *et al.* noted no differences in total or differential BAL cell counts between atopic asthmatic and normal control subjects at baseline, but found their responses to local antigen challenge to differ markedly (26, 32). When evaluated serially, atopic asthmatic subjects exhibited an increase in neutrophils, eosinophils and helper T lymphocytes following antigen challenge (26, 32). By 96 hr after challenge, the neutrophils returned to baseline, the eosinophils remained moderately elevated, and the T helper lymphocytes were still significantly elevated. This observation suggests that T-lymphocyte responses to allergen challenge persist for longer periods of time than do the responses of other types of cells.

Bronchial biopsies have also been used to evaluate the nature of the asthmatic response to disease exacerbation (21, 23, 24). Although it is not universally accepted, some authors have reported more marked T-lymphocyte inflammatory changes in the bronchial biopsies than are seen in BAL specimens (24). Because of these differences, there has been interest in less invasive methods of obtaining biopsy for further study. One possible solution, for research purposes, is the use of nasal mucosal biopsies. A similar inflammatory reaction is present in the nasal mucosa and airways of asthmatic patients (23); this finding may allow for further study of the pathogenesis of asthma without resorting to transbronchoscopic biopsy.

Pathology

In the early reports of cases of fatal asthma, pulmonary hyperinflation, focal saccular bronchiectasis, epithelial desquamation, and widespread mucous plugging were prominent (33–37). The airway epithelium and basement membranes were thickened and edematous, smooth muscle hyperplasia was evident, and intraluminal and infiltrating eosinophils were present. The inflammatory infiltrate present in the airway walls was notable for increased numbers of lymphocytes with some focal aggregations. These findings were similar whether or not the patients suffered atopic asthma.

Mast cells have received attention in the study of the pathogenesis of asthma, especially allergic asthma, since this cell type is one of the most active histamine-releasing cells in the airway. Mast cells may be found at all levels of the bronchial tree (38) and are usually located above the basement membrane, sometimes within the airway lumen. Most frequently, mast cells are located in close proximity to capillaries and larger blood vessels; mast cells typically have a short (usually less than 5-hr) circulating life span before they move into tissue. Numbers of mast cells seen in biopsies and obtained through bronchoalveolar lavage are increased in asthma, particularly during exacerbation (39). It has been suggested that the early inflammatory response following inhaled allergen is due to mast cell activation and mediator release. Within minutes of antigen challenge, serum and BAL histamine levels are increased, as are the BAL levels of mast cell tryptase, a specific marker of mast cell degranulation (40). Mast cells are frequently degranulated when recovered from asthmatic but not from control subjects' airways, and this is associated with increased bronchoalveolar lavage fluid histamine levels (41). High-affinity IgE receptors on the mast cells are probably responsible for their degranulation following allergen exposure. Interestingly, disodium cromoglycate, a mast cell stabilizer which prevents IgE-dependent degranulation, is effective in preventing nonallergic as well as atopic asthma attacks. This suggests a role for mast cells in the pathogenesis of atopic and nonatopic asthma.

Basophils, another important cellular source of histamine, may also be involved in asthmatic inflammation. They have been identified in increased numbers in the sputum of asthmatics (42), and these basophils may release histamine more readily than those from nonasthmatics (43). Like mast cells, basophils possess high-affinity IgE receptors. Despite these findings, the role of basophils in asthma is still poorly understood.

Eosinophils are also prominent in the airways inflammation of asthma; they may form up to half of the cellular infiltrate in acute asthma (44). Eosinophils may extensively infiltrate the airway mucosa, alveoli, and occasionally even the pleura. The eosinophil responds vigorously to chemotactic and activation factors, such as the cytokines IL-4 and IL-5, and leukotriene B4. It contains granules of preformed mediators including major basic protein, eosinophilic cationic protein, neurotoxin, and peroxidase and other enzymes including lysolecithinase, which when crystallized forms Charcot-Leyden crystals (45). It also has the ability, when activated, to release cyclooxygenase (e.g., PGE_2 and PGI_2) and 5-lipoxygenase (e.g., LTC_4) products and superoxide. In pathologic studies, including autopsies of patients dying in status asthmaticus, prominent infiltration of eosinophils is reported (46). In addition, studies of atopic asthmatics following instilled or inhaled allergen have confirmed that bronchoalveolar eosinophilia is a prominent response in this group of patients (27, 32, 47).

The presence of neutrophils in the setting of acute inflammation is well established. Their accumulation is both a response to and a cause of cellular damage. Neutrophils may cause tissue injury through the elaboration of a number of proteases, oxygen radical release, and prostaglandin metabolites (48). Like other inflammatory cells, neutrophils can be found in increased numbers and with an increased degree of activation in the airways of asthmatic patients than in control subjects (22, 49, 50). Studies have demonstrated that the presence of neutrophils is mandatory for development of bronchial hyperreactivity and the late phase response in several animal models (51–53). That these findings may apply to humans is suggested by the recent study of Sur *et al.* (54), which proposed that asthma deaths with sudden onset of symptoms, as opposed to those with a longer duration, may represent a different inflammatory mechanism, one with a more prominent neutrophil response. Alternatively, these cases may represent an earlier time point in the same continuum, with neutrophils accumulating occurring earlier than eosinophils.

Lymphocyte-Mediated Responses in Asthma

Lymphocytes have long been known to be involved in the allergic response of asthma because of their role in degranulation of mast cells and basophils. This is mediated by antigen-specific IgE. The regulation of IgE production involves complex interactions between antigen-presenting cells, and B and T lymphocytes. It is thought that antigen-presenting cells present allergens to antigen-specific CD4+ T helper lymphocytes in the setting of Class II major histocompatibility complex interactions (55). These activated T cells subsequently interact with B cells and, in the context of the presented antigen and cytokine secretion, may activate the B cell to secrete antibody, including IgE.

Although not as well understood as T helper cells, T suppressor cells are also clearly important in the regulation of the immune response. T suppressor cells may influence the B cell response to allergen, as some investigators have suggested that low T-suppressor activity may lead to an exaggerated IgE response to allergen exposure (56). Thus, specific IgE production and release by B lymphocytes requires interactions with T cells, most clearly T helper lymphocytes.

A number of authors have evaluated changes in ratios of T helper (CD4+) and T suppressor (CD8+) T lymphocytes and other markers of T-cell function in asthma and atopic disease. These studies may conveniently be divided into those which examine baseline differences between asthmatic subjects not in exacerbation and nonasthmatic individuals, studies of the atopic/asthmatic response to allergen challenge, and studies of patients with asthma in exacerbation.

At baseline, there appears to be an overall increased ratio of CD4+/CD8+ T lymphocytes in the peripheral blood of patients with atopic dermatitis. In some cases, this is due to increased numbers of CD4+ T cells (57), whereas other studies have noted decreased numbers of CD8+ T cells (58, 59). Although this has not consistently been found in atopic asthma (60), it is interesting that several studies have reported an increased peripheral blood CD4+/CD8+ ratio in nonatopic asthmatics (61–63). Most studies of bronchoalveolar lavage T cells have failed to detect consistent alterations in the ratio of T helper and T suppressor cells in asthmatic subjects. Other baseline differences between asthmatic and nonasthmatic subjects do exist, however. Walker *et al.* compared the expression of lymphocyte activation markers on peripheral blood cells from atopic and nonatopic asthmatics and normal controls (63). They found that both allergic and nonallergic asthmatics had significantly increased numbers of IL-24 positive cells compared with normal controls, and the allergic asthmatics also had increased numbers of B cells bearing the low-affinity IgE receptor, CD23, which is evidence of B-cell activation. In a follow-up study which additionally used BAL to examine these groups, nonallergic asthmatics also had increased expression of IL-2R, HLA-DR, and VLA-1 on both T helper and suppressor cells obtained from BAL (64). This group also found that the degree of lymphocyte activation in BAL specimens correlated with the severity of the underlying asthma (65). Activation of T lymphocytes is not confined to the peripheral blood or within the airways; activated T lymphocytes have also been identified in bronchial biopsies of

asymptomatic asthmatic patients (66), underscoring the fact that both systemic and local inflammation are important in asthma.

To understand the pathogenesis of inflammation in atopic asthmatics, several investigators have employed allergen challenges. Some of these studies examined changes in peripheral blood T lymphocytes after allergen inhalation. Gerblich *et al.* found that allergen bronchoprovocation in atopic asthmatics caused a decline in numbers of peripheral blood CD4+ T cells immediately after challenge, which persisted for up to 72 hr (67). Circulating T cells expressing activation markers were increased 48 hr after the challenge, and no significant difference was seen in numbers of circulating CD8+ T cells. None of these changes were caused by bronchial challenge with methacholine. The same group of investigators also examined peripheral blood T helper and suppressor population changes following bronchoprovocation in a group of symptom-free atopic asthmatic subjects (67, 68). Compared with normal control subjects, CD4+ cell numbers decreased after allergen challenge and remained depressed for a prolonged period; this response did not occur after bronchoprovocation with inhaled methacholine.

T lymphocyte changes in the lung have also been examined, using bronchoalveolar lavage, after allergen inhalation. Metzger *et al.* reported that allergic asthmatics with a previously documented late phase response responded to allergen inhalation by a rapid increase in the number and percentage of helper T lymphocytes in the lung (32). The BAL helper T-cell count remained elevated for up to 96 hr following the challenge. Although the T suppressor cell count also increased, albeit modestly, at 48 hr after allergen inhalation, these cells diminished rapidly in number. Gonzalez *et al.* found that, following allergen challenge, a group of atopic asthmatics known to have a single early response to inhaled allergen, developed significantly increased numbers of peripheral blood CD4+ cells along with a significantly lower percentage of CD4+ and a higher percentage of CD8+ BAL cells when compared with dual responders or normal control subjects (69). In addition, following allergen challenge, there were fewer CD4+ cells and a lower CD4/CD8 ratio in the BAL cells obtained from single responders compared with those from dual responders. It has been suggested that these findings may be explained by a protective effect derived from the migration of CD8+ cells following allergen exposure; this population may prevent the development of a late phase inflammatory response in this group of patients (70). Gerblich *et al.* compared the changes in T-cell populations of peripheral blood and BAL specimens obtained from atopic asthmatics, and again detected a decline in peripheral blood T helper lymphocytes which correlated with an increase in BAL T helper lymphocytes, after allergen challenge (68). Gratziou *et al.* also compared the response of BAL T-lymphocyte populations to local allergen challenge between allergic asthmatics and nonatopic control subjects (71). Again, at baseline, no difference between the asthmatic and control populations was seen in the percentages of T helper and suppressor lymphocyte populations. Following challenge, however, a rapid (less than 10 min) decrease in CD4+ cells with a decline in the CD4/CD8 ratio was detected in the BAL from asthmatic but not control subjects.

Because allergen challenge creates an artificial situation, examination of asthmas in exacerbation, and studying their responses to therapy may provide the most relevant information about the mechanisms of inflammation in these patients. In a study of moderately symptomatic atopic asthmatics who were treated with a single dose of corticosteroids, Gerblich *et al.* reported a significant decrease in peripheral T helper and increase in T suppressor lymphocytes, resulting in a decrease in the T helper/T suppressor ratio compared with normal controls; like the earlier studies, the pretreatment T-lymphocyte subset populations were similar (72). Other markers of T-cell activation in peripheral blood and BAL specimens from symptomatic atopic asthmatics also appear to be increased (61, 62, 65, 73). Corrigan and Kay compared patients with acute severe asthma with a variety of control groups and found that expression of the IL-2 receptor, HLA-DR, and very late antigen 1 (VLA-1), all of which are known to signify activation of T lymphocytes, were significantly increased on CD4+ cells peripheral blood lymphocytes from the asthmatics in exacerbation (61, 62).

These studies emphasize the importance of the T lymphocyte to the inflammation of asthma. A wide variety of differences between asthmatic and nonasthmatic T-lymphocyte populations have been reported, at baseline, following allergen challenge, and particularly develops asthma exacerbations. These changes appear to be strongly affected by steroid therapy. The variability of findings from all of these studies, however, underscores the point that changes in peripheral blood cell populations do not necessarily reflect processes which are occurring in the lung.

Th1 and Th2 T Helper Lymphocytes in Asthma

Mossmann *et al.* first described Th1 and Th2 CD4+ T cells in mice (74). Th1 cells produce interleukin 2 (IL-2) and γ-interferon (IFN-γ) but no IL-4 or IL-5, and Th2 cells produce IL-4, IL-5, IL-6, and IL-10, but no IL-2 or IFN-γ. Both cell types produce IL-3 and granulocyte-macrophage colony-stimulating fac-

tor (GM-CSF). Some investigators have also described a population of T helper lymphocytes with an unrestricted ability to release cytokines (75–77). These cells produce both IL-2 and IL-4, and their functional significance is not yet clearly understood (77). It seems likely that these CD4+ T-cell subsets differentiate from precursor cells. Expression of the Th1 or Th2 phenotypes may depend, in part, on exposure to antigen; for example, exposure to helminthic antigens promotes expansion of Th2 clones, and exposure to mycobacterial antigen leads to expansion of Th1 clones. It is possible that the cells designated as Th0 represent, in fact, the pool of uncommitted Th1 or Th2 precursors, and that the presence of Th1 or Th2 cytokines or even prostaglandin products may control this maturation (78–80).

The categorization of T helper cells into these groups appears to have functional relevance, as Th1 cells regulate delayed-type hypersensitivity reactions, and Th2 cells are prominent in allergic reactions. Th1 and Th2 cells interact in a counterregulatory fashion; for example, IL-10 downregulates cytokine production by Th1 cells (81) and IFN-γ inhibits the proliferation of Th2 cells (82). In addition, each cell type has the ability for autoregulation; IL-4 promotes the development

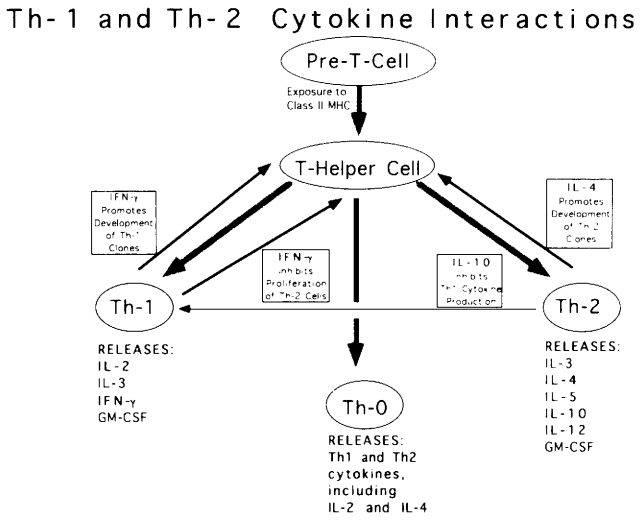

Figure 1. Th1 and Th2 T helper lymphocytes release cytokines which effect both Th1 and Th2 clones. Th1 lymphocytes are characterized by the production of IL-2 and IFN-γ, and are associated with the development of delayed hypersensitivity reactions. Th2 lymphocytes typically release IL-4, IL-5, IL-10 and IL-12, and are considered an integral part of the atopic response; they promote B-cell IgE production and chemotaxis and proliferation of eosinophils. Both types of T helper cells release IL-3 and GM-CSF. Th0 cells seems to have an unrestricted cytokine profile, and release cytokines associated with both Th1 and Th2 cells. Cytokines released by both Th1 and Th2 cells inhibit the development or proliferation of the opposing response. The Th1 cytokine, IFN-γ, not only promotes the development of Th1 clones from uncommitted precursor cells but also inhibits the proliferation of Th2 cells. The Th2 cytokine, IL-4, promotes the development of Th2 clones, while IL-10 inhibits the production of cytokines by Th1 cells.

of Th2 clones from uncommitted precursor cells (83, 84) and disruption of the IL-4 gene prevents the development of cells with any of the cytokine release characteristics of Th2 cells (85). IFN-γ influences uncommitted cells to developing a Th1 cytokine profile (84). Although some investigators have contended that Th1 and Th2 cells, well accepted in murine immunology, have never been proven to exist in humans (86), evidence against these arguments is mounting. A number of independent studies have convincingly demonstrated Th1 and Th2 clones *in vitro* as well as *in vivo* (84, 87–92); these clones have been isolated from patients with disease as diverse as systemic eosinophilia and tuberculous leprosy.

The cytokines released by Th1 and Th2 cells are known to have functions which may influence the asthmatic response. The most important Th1 cytokines are IL-2 and IFN-γ. IL-2, although not classically considered central to the inflammation seen in asthma, is released by T cells after they interact with antigen complexed onto an antigen-presenting cell (93). Generally considered an arm of the delayed type hypersensitivity reaction, this Th1 cytokine may explain the "late response" of inflammation seen in asthma if the presented antigen were a classic allergen. In addition, IL-2 can promote B-cell growth and differentiation (94), and thus may be an arm of the antibody response in atopic asthma. IL-2 may also increase the airway response to inhaled allergens; administered to rats sensitized to ovalbumin, IL-2 enhanced the early and late responses to ovalbumin without changing airway responsiveness to methacholine or IgE levels (95). IFN-γ is important as an activator of macrophage function; macrophage activation is a frequent finding in studies of airways of asthmatic patients. IFN-γ is also significant in its ability to down-regulate the Th2 response by decreasing proliferation of and cytokine production by Th2 cells. Its function in decreasing IL-4-induced IgE production may be particularly relevant in maintaining homeostasis in asthma (96).

The Th2 cytokines which have been most implicated in the inflammation of asthma have been IL-4 and IL-5. IL-4 was originally characterized as a B-cell differentiation and growth factor (97, 98). In high concentrations, it is known to switch B-cell immunoglobulin production to IgE (99) and thus to amplify allergic responses. It also may act as a growth factor for mast cells (100), an important cell type for the production of asthma mediators. Its soluble receptor, acting as an antagonist, can decrease the allergic response and IgE production in experimental models (101, 102). Like IL-4, IL-5 stimulates B-cell growth and immunoglobulin secretion (103, 104). IL-5 also has well-characterized roles in the proliferation and differentiation of eosino-

phils (105), as well as their activation (106, 107). Finally, IL-5 can also promote granule release from basophils (108), an important source of histamine.

In asthma, a large body of evidence has accumulated implicating Th2 cells in the pathogenesis of the atopic inflammatory response. Preliminary studies examined the relevance of Th2 cells to atopic disease and found increased expression of mRNA for the Th2 cytokines IL-4 and IL-5 in skin biopsies of patients with atopic dermatitis (109), as well as in the nasal mucosa of patients with atopic rhinitis after nasal allergen challenge (110). This work supported the theory that Th2 lymphocytes are important in atopic disease.

Based on these studies and other earlier work investigating changes in the composition of peripheral blood T lymphocytes of atopic patients, Parronchi *et al.* examined cytokine release from T-cell clones obtained from atopic donors in order to evaluate their response to antigen challenge (90). They found that although a majority of the T-cell clones produced IL-4, IL-5, and IFN-γ in response to bacterial antigens, all allergen-specific clones released IL-4 and IL-5, but no or minimal IFN-γ. This suggested that Th2 cells interact specifically with allergen and thus release cytokines which promote important components of the allergic response, such as IgE production and eosinophilia. Walker *et al.* examined the correlation between T-cell activation and eosinophilia in asthma. They found that T-cell activation, quantitated by numbers of T lymphocytes expressing the IL-2 receptor, correlated with peripheral blood eosinophilia in both allergic and nonallergic asthmatics, and that purified T lymphocytes spontaneously released factors which prolonged the *in vitro* life of eosinophils. These factors were also present in the sera of asthmatic patients; this activity was significantly eliminated by the use of neutralizing antibodies against IL-5 and GM-CSF. T lymphocytes from normal control subjects only displayed similar characteristics after stimulation with an anti-CD3 antibody. This provided further evidence that T-cell products, especially those associated with the Th2 response, can influence expression of clinical manifestations of asthma. Corrigan *et al.* recently investigated the influence of steroid therapy on the activation of, and cytokine release by, peripheral blood T lymphocytes from asthmatic patients (111). T lymphocytes were isolated from asthmatic patients prior to, and after 7 days of, treatment with glucocorticoids for exacerbation of asthma. Initially, the asthmatic patients had significantly elevated expression of the T lymphocyte activation markers CD25, CD45RO, and HLA-DR, and increased serum levels of IL-5, compared with the matched controls. These differences were abolished after a week of steroid treatment, leading to the conclusion that steroid treatment of asthma exacerbations results in diminished activation of T lymphocytes, and decreased release of IL-5. This data is consistent with the theory that asthma exacerbations and activation of Th2 cells are related.

T lymphocytes harvested by bronchoalveolar lavage have also been shown to release cytokines in a Th2-like pattern. Robinson *et al.* obtained cells by BAL T cells from patients with mild atopic asthma and normal controls, and examined their expression of IL-2, IL-3, IL-4, IL-5, IFN-γ, and GM-CSF mRNA. They found that the asthmatic subjects had increased numbers of BAL cells which expressed IL-2, IL-3, IL-4, IL-5, and GM-CSF, in comparison with control subjects. These cytokines were localized to T cells by simultaneous examination of T-cell markers. Although the Th1 cytokine, IL-2 was increased in this study, the majority of cytokine expression was in the Th2 pattern (112). This same group of investigators later examined the effect of allergen inhalation on bronchoalveolar lavage T-lymphocyte cytokine expression, and found that allergen challenge led to activation of T helper lymphocytes and increased expression of mRNA for the Th2 cytokines IL-4, IL-5, and GM-CSF, but not the Th1 cytokines IL-2 or IFN-γ (113). In a separate study, Robinson *et al.* also examined the relationship between BAL cell cytokine expression, asthma symptoms, and methacholine responsiveness (114). They found differences in expression of IL-3, IL-4, IL-5, and GM-CSF but not IL-2 or IFN-γ by BAL cells, with increased expression of these cytokines by cells obtained from symptomatic relative to asymptomatic asthmatics. Expression of IL-4, IL-5, and GM-CSF mRNA correlated significantly with patients' symptoms and their sensitivity to methacholine challenge. Thus, expression of the Th2 cytokine phenotype by T lymphocytes harvested by BAL is associated with atopic asthma; allergen challenge increases this expression, and this pattern of cytokine production is associated with increased numbers of symptoms and increased bronchial hyperreactivity.

The evidence suggesting Th2 activation in atopic asthma is further supported by several studies which evaluate the expression of cytokine mRNA in bronchial biopsies. Since it has been proposed that Th2 lymphocytes are recruited from the peripheral blood into the airways of asthmatics in exacerbation, increased expression Th2 cytokines should be detectable in tissue lymphocytes. Hamid *et al.* detected IL-5 mRNA in the biopsies from 6/10 asthmatics but from none of nine healthy, nonatopic control subjects (115, 116). Those asthmatics expressing IL-5 had increased asthma symptoms and worse pulmonary function, compared with those asthmatics who did not express IL-5. Bentley *et al.* later evaluated cytokine mRNA expression in bronchial biopsies from mild atopic asth-

matics after allergen or diluent inhalation (117). They found that the allergen challenge caused a significant increase in IL-5 and GM-CSF mRNA. These studies support the data from BAL that Th2 cytokines may be upregulated during asthma exacerbations.

An association between atopic asthma, characterized by formation of allergen-specific IgE, and the Th2 response is easily accepted, since IL-4 promotes IgE production by B cells and additionally stimulates mast cell growth. It is less clear that nonatopic, or "intrinsic" asthma would have a similar pathogenesis. Several groups have accordingly examined the cytokine profile of BAL lymphocytes obtained from nonatopic asthmatics. Walker *et al.* compared the release of cytokines by BAL T-lymphocytes obtained from nonatopic and atopic asthmatics, and found increased release of IL-5 by both groups, and also increased IL-2 release from the nonatopic group (64). Marini *et al.* similarly found increased expression of IL-5 mRNA from nonatopic asthmatic subjects; this study did not examine IL-2 expression (118). The IL-5 correlated with increased BAL (118) or peripheral blood (64) eosinophilia in each of these studies. The fact that IL-2 as well as IL-5 is up-regulated in BAL lymphocytes from nonatopic asthmatics suggests either a Th0, or unrestricted cytokine profile, component of inflammation, or participation of Th1 cells in the nonatopic asthmatic response.

Several investigators have also shown increased levels of the Th1 cytokine IL-2 in the airways, suggesting a Th1 or, perhaps, a Th0 response. Garssen *et al.* have proposed a model of Th1 induced asthma, which suggests that certain forms of non-IgE-mediated asthmatic inflammation may resemble a delayed type hypersensitivity reaction; in their model Th1 cell-dependent immune responses were required for the induction of airway hyperresponsiveness in mice sensitized to picryl chloride (119). A Th1 response may be more common in asthma than this model would suggest. Corrigan and Kay reported that serum levels of IFN-γ are elevated in patients with exacerbations of asthma (61, 62), and others have noted increased release of IFN-γ by BAL cells from asthmatics (119, 120). IL-2 may be elevated in BAL fluid of symptomatic asthmatics (121), and the soluble form of the IL-2 receptor, sIL-2R, is significantly elevated in acute asthma (122). In animal models, parenteral administration of IL-2 has been associated with bronchovascular inflammation and increased airway methacholine responsiveness (123); subsequent studies revealed that parenteral IL-2 administration increased the allergic response to inhaled allergen (95).

Summary

In this paper, we have reviewed the evidence suggesting that T-cell dysregulation is important in the pathogenesis of asthma. The history, clinical presentation, and an overview of the appropriate management of asthma have been briefly reviewed. T cells obtained from the airways of asthmatics display signs of activation; these changes mirror the intramural inflammation found at biopsy. There is debate about the importance of T helper/suppressor ratios in this population of cells, but alterations in these ratios have been noted with experimental allergen exposure, as well as during acute asthma attacks; some of these changes revert toward normal with steroid therapy. The division of T helper lymphocytes into Th1 and Th2 cells, first described in studies of murine immunology, appear to be relevant in humans, particularly in allergic disease. Although IL-4 and IL-5, prototypical Th2 cytokines, have been most clearly implicated in asthma, there is some evidence supporting a role for Th1 cells/cytokines as well.

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