Commentary

Sorting Out the Cytokines of Asthma

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Asthma is a complex disorder characterized by intermittent, reversible airway obstruction, and by airway hyperresponsiveness and inflammation. Although its cause(s) remain unknown, we now recognize that asthma is a syndrome whose common pathologic expression is inflammation of the airways. The airways of patients with even mild asthma are inflamed, and some data suggest that the severity of the asthma parallels the degree of inflammation (1–3).

The airway inflammation of asthma is unique in that the airway wall is infiltrated by T lymphocytes bearing the T helper type 2 phenotype (4) and by eosinophils and mast cells (2, 5, 6). Each of these cells is thought to contribute to the physiologic changes that characterize asthma (Fig. 1). The TH_2 lymphocytes produce a limited panel of cytokines including IL-3, IL-4, IL-5, and GM-CSF. Although the primary signals resulting in the infiltration of the asthmatic airways by this lymphocyte subset have not yet been identified, a number of models have been proposed based on the concept that the inflammtory process is triggered by the presentation of a restricted panel of antigens in the presence of appropriate cytokines. The net effect of antigen presentation in this microenvironment is to promote the synthesis of IgE through the actions of IL-4 on Ig isotype switching (7) and to enhance the differentiation, migration, and pathobiologic capacity of eosinophils through the actions of GM-CSF, IL-3, and IL-5 (8, 9). The IgE produced in asthmatic airways binds to $Fc_\epsilon RI$ on mast cells, priming them for activation by antigen. Mast cells arise from bone marrow precursors, enter the circulation as non–metachromatically staining, agranular, $CD34^+$, $c\text{-}kit^+$, and $Fc_\epsilon RI^+$ mononuclear leukocytes (10), and localize to mucosal and submucosal sites such as the bronchi. Once localized, these lineage-committed immature mast cells undergo tissue-specific differentiation along with maturation and expansion. Their development and movement involves growth factors and cytokines derived from both structural and hematopoietic cells in their microenvironment. The mast cells that further migrate into the luminal space, and which can be recovered by bronchoalveolar lavage, are primed for an augmented activation response (11). Mast cells also elaborate IL-4 (12, 13), which favors conversion of T cells to the TH_2 phenotype, and IL-5 (14), which contributes to eosinophilopoiesis and to the priming of eosinophils for augmented responses. Once recruited from the circulation, mature eosinophils, in the presence of "asthmatic" cytokines, convert to an autoaggressive phenotype (15, 16), termed "hy-

podense" because of a change in eosinophil density gradient sedimentation characteristics. Hypodense eosinophils are primed for ligand-initiated generation of increased amounts of superoxide and cysteinyl leukotrienes and for cytotoxic effects on bystander target cells (15, 16). Furthermore, eosinophils may participate in a positive feedback loop by generating eosinophil-active cytokines, including IL-5, IL-3, and GM-CSF (17, 18).

Although these anatomic characteristics of the asthmatic airway wall are well established, we can only speculate on the mechanisms that link airway inflammation and the altered physiology of asthma; our fullest understanding of these mechanisms is that of the allergen-driven asthmatic reaction. As described above, exposure to allergen favors the synthesis of IgE; the binding of IgE results in immunologically specific sensitization of mast cells, monocytes/macrophages, eosinophils, and basophils. It is important to note that expression of the high affinity receptor for IgE, $Fc_\epsilon RI$, by eosinophils and monocytes appears to be limited to particular pathobiologic states, of which atopy is prominent (19–21). When activated, these cells elaborate mediators of inflammation, including histamine, leukotrienes, lipoxins, platelet-activating factor, and various proteases into the local microenvironment (22–29). They also secrete cytokines, which amplify the response and perpetuate the asthmatic phenotype by aiding in the recruitment of more inflammatory bone marrow–derived cells and by having additional proinflammatory effects on smooth muscle and other resident cells (30–36). Together, these mediators and cytokines transduce the physiologic changes that we recognize as asthma, namely, airway obstruction and hyperresponsiveness. The latter is an important phenotypic characteristic of asthma and is recognized clinically as an obstructive physiologic response to a bronchoconstrictor stimulus that would be innocuous in an individual without asthma. Airway hyperresponsiveness is thought to result from some combination of submucosal edema, airway epithelial infiltration with eosinophils and lymphocytes, damage to bronchial epithelial cells with loss of regulatory mechanisms for mediator inactivation, and direct effects of mast cell–derived mediators, especially secretory granule neutral proteases and the cysteinyl leukotrienes.

Although the chain of events leading to asthma is clearly complex, IL-4 and IL-5 have emerged as important participating cytokines. Their relative importance in linking the cellular events noted above with airway hyperresponsive-
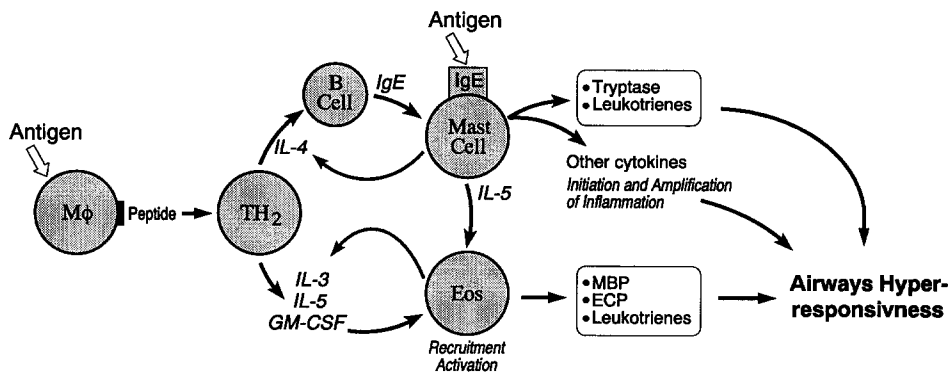
Figure 1. Putative mechanisms of mast cell– and eosinophil-dependent airway hyperresponsiveness. In the sensitization phase, antigen is processed by professional APC such as the macrophage (MΦ). Presentation of certain peptides by APC, in the context of the appropriate cytokines and MHC, drives the clonal expansion of TH_2 cells that elaborate IL-4, IL-3, IL-5, and GM-CSF. IL-4 directs a switch in the Ig synthesis by B cells to IgE, which is released and bound to mast cells, and under certain conditions to eosinophils and monocytes, through the high affinity IgE receptor. In the activation phase, after challenge with the relevant antigen, mast cells release preformed constituents of their secretory granules, newly synthesized bioactive lipids, and induced cytokines. Of these mediators, tryptase and the cysteinyl leukotrienes may elicit airway hyperresponsiveness. Mast cell–derived TNF-α may contribute to the inflammatory response through the upregulation of endothelial cell adhesion molecules to localize eosinophils and T cells. IL-3, IL-5, and GM-CSF, which potentially activate, prime, and recruit eosinophils (Eos) to the site of allergic inflammation, are predominantly T cell–derived, but the mast cell can contribute IL-5 and the eosinophil the entire triad. Eosinophil-derived granule contents, such as major basic protein (MBP) and eosinophil cationic protein (ECP), which damage the epithelium, and leukotrienes may elicit airway hyperresponsiveness. Both strain-related genetic factors and the sensitization procedures will determine the microenvironmental factors that regulate the prominence and pathobiologic function of the critical cell types in allergic inflammation.

ness, however, has not been established. Indeed, there is currently no way to definitively test their role in human asthma since such tests require the ability to prevent either the formation or the action of these moieties at critical states in the development of atopy. Because of the importance of understanding the role of these cytokines in asthma, investigators have turned to animal models of asthma. Murine models are currently the most useful because mice develop airway hyperresponsiveness after systemic allergen sensitization followed by repeated aerosol challenge and because specific reagents are available for manipulation of the murine immune system, including immunologically relevant transgenic or "knockout" mice.

Two papers in this issue (37, 38) report studies in which the roles of IL-4 and IL-5 in the pathologic and physiologic changes of asthma were explored in murine models. In both studies, airway responsiveness was used as an outcome indicator. It is generally accepted that credible animal models of asthma must express airway hyperresponsiveness (39) and that experimental manipulations that modify airway responsiveness can provide useful information on the importance of specific mechanisms in modifying airway responsiveness. Investigators in each study altered airway responsiveness by manipulating the cytokines in the microenvironment of the airways. What is intriguing is that Corry et al. implicate IL-4 as the key cytokine and provide data indicating that hyperresponsiveness can occur independently of IL-5 and eosinophils, whereas Foster et al. provide equally convincing evidence that IL-5 is the critical cytokine. Resolution of this apparent paradox provides clues about the mechanisms that link both cytokines to airway responsiveness.

Foster and colleagues probe the role of IL-5 in airway responsiveness. They first demonstrate that they can induce airway hyperresponsiveness in C57BL/6 mice by intraperitoneal injection of antigen (50 μg OVA in Alhydrogel) followed by repeated exposure to aerosols generated from

antigen solutions (10 μg/ml OVA). They show that IL-5 knockout mice fail to develop airway hyperresponsiveness when sensitized with the same regimen and that when these mice are "reconstituted" for IL-5 with the use of recombinant vaccinia viruses expressing a cDNA for this cytokine, the eosinophils return. The investigators do not state whether the mice reacquired the capacity to develop airway hyperresponsiveness after antigen sensitization. The investigators did not report experiments in which IL-4 was manipulated, but they demonstrated that the IL-5 knockout mice produced normal amounts of IgE in response to antigen sensitization. Because the modifications in airway responsiveness were paralleled by changes in airway eosinophilia, the investigators conclude "that IL-5 and eosinophils are central mediators in the pathogenesis of allergic lung disease."

Corry and coworkers took a similar approach to creating experimental inflammation; BALB/c mice were sensitized by the subcutaneous injection of antigen (25 μg OVA with alum weekly for 4 wk) followed by repeated airway exposure to aerosols. This regimen induced airway hyperresponsiveness. Mice that were treated either with an mAb that depleted CD4$^+$ T cells or with an antibody against IL-4 during the sensitization phase of the protocol, but not during subsequent inhalation challenge, failed to develop airway responsiveness. In contrast, mice treated with a neutralizing anti–IL-5 mAb had marked inhibition of airway eosinophilia but developed airway hyperresponsiveness when appropriately sensitized. They conclude "the data . . . do not support a role for either IL-5 or eosinophils in mediating the acute airway hyperactivity."

How can these two seemingly conflicting studies be resolved? The answer, we believe, lies in understanding that the same physiologic phenotype may be achieved through at least two distinct cellular mechanisms. One such mechanism could be through the IgE-stimulated activation of mast cells. For example, airway hyperresponsiveness can be induced in mice by anti-IgE by a mechanism that is mast cell

dependent (40). This indicates that IgE–mediated activation of mast cells initiates a sequence of events that can produce airway hyperresponsiveness. We believe that this sequence of events occurred in the model used by Corry et al. They used BALB/c mice, which are "high IgE" responders, as their primary experimental animal. Their sensitization protocols increased the levels of IL-4 and IgE in these mice, and treatment with an anti–IL-4 mAb prevented the increase in IL-4 and airway hyperresponsiveness. Importantly, they also showed that this sequence of events leading to airway hyperresponsiveness is independent of eosinophils and IL-5.

If only one cellular mechanism could lead to airway hyperresponsiveness, there would be little need for further discussion. Foster et al., however, clearly demonstrate that airway hyperresponsiveness can be induced in the mouse by an eosinophil- and IL-5–dependent mechanism, thereby elucidating an alternative cellular pathway leading to the same physiological response. We believe that the choice of the C57BL/6 mouse as the experimental species by Foster et al. was a fortunate one, as this strain has a number of genetic defects that potentially prevented induction of airway hyperresponsiveness by the pathway documented by Corry et al. and thus allowed recognition of the eosinophil-dependent pathway.

What is special about the C57BL/6 mouse? It has been established, through linkage analysis in a cross with the naturally hyperresponsive A/J mouse, that a locus on mouse chromosome 17, *Bhr3*, which maps close to the gene for mouse mast cell protease 7, can confer constitutive airway hyperresponsiveness on the naturally hyporesponsive C57BL/6 mouse (41). Analysis of the gene encoding murine mast cell protease 7, which is a tryptase-like molecule (42, 43), has demonstrated a point mutation that prevents the C57BL/6 mouse from producing this mast cell protease (44, 45). Exogenously admininstered tryptase can induce airway hyperresponsiveness (46), in part through its enzymatic capacity to cleave and inactivate vasoactive intestinal peptide, an endogenous bronchodilator (47). The C57BL/6 strain is also deficient in the low molecular weight secretory phospholipase A_2 (48); this enzyme has been implicated in facilitating exocytosis in mast cells by generating lysophospholipids for fusion of the perigranular and plasma membranes (49) and in providing some of the arachidonic acid used for eicosanoid biosynthesis (50, 51). We therefore speculate that because of these and perhaps other genetic deficiencies, the C57BL/6 mouse is resistant to the induc-

tion of mast cell–dependent airway hyperresponsiveness. Perhaps because the mechanisms discovered by Corry et al. were genetically attenuated in the host species used by Foster et al., the latter group came upon a series of sensitization maneuvers that recruited eosinophils into the airways and activated them in such a way as to induce hyperresponsiveness. Such mechanisms include the production of leukotrienes or the release of major basic protein (52). Had the C57BL/6 mouse not been deficient in critical constituents, it is likely the capacity of eosinophils to mediate such an effect in an allergen model would have gone undiscovered.

The attempt to reconcile the differences in the two models of airway hyperreactivity based on the role of the mast cell is not entirely satisfactory. One still has to explain the absence of hyperresponsiveness in the BALB/c strain in the presence of marked tissue eosinophilia after treatment with antibodies to IL-4 during the sensitization protocol. Perhaps the eosinophils are not primed because of a deficiency of the requisite cytokine(s) at the critical tissue sites that results from differences in the strain, the sensitization protocols, or both. The sensitization regimen used by Foster et al. (37) induced airway hyperresponsiveness in the C57BL/6 strain, whereas that used by Corry et al. (38) did not. The differences in the relative contributions of IL-4 and IL-5 to the two models may reside in some combination of events, both inborn and acquired through sensitization, which regulate the cytokine profile and hence the critical cellular elements of the acquired airway hyperreactivity. Despite the frustration of our failure to completely reconcile the two studies, together they demonstrate clearly that there are multiple cellular pathways to the same physiologic phenotype.

If we extend this line of thinking to human asthma, we must conclude that multiple pathways can lead to the same clinical phenotype, namely airway hyperresponsiveness. Airway hyperresponsiveness can be induced in humans by allergen exposure, and this phenotype also can be expressed in the absence of eosinophilia by infection with certain viruses (53) or exposure to ozone (54). Thus, the reports by Foster et al. and Corry et al., through a fortunate choice of experimental strain and sensitization protocols, provide relatively "pure" examples of eosinophil-dependent and eosinophil-independent pathways to the same clinical phenotype. Since individuals with asthma differ in their development of airway hyperresponsiveness, we now have a firm biologic basis for such differences.

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