Does the Mast Cell Still Have a Key Role in Asthma?*

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In recent years, the emerging concept of bronchial inflammation as a prominent histopathologic characteristic of asthma has profoundly modified the view of the role of the mast cell, which was traditionally thought to be linked to the release of soluble chemical mediators substantially involved in the genesis of acute, immediate bronchospasm. The finding that the production of proinflammatory cytokines by mast cells in asthmatic airways is comparable, in some circumstances, to that of T-cell origin, has led to the hypothesis that mast cells, along with T lymphocytes and eosinophils, may also contribute to the genesis of chronic, persistent asthma. This hypothesis is further supported by the finding that mast cells are able to functionally interact with B cells (promoting IgE synthesis) and T lymphocytes (acting as antigen presenting cells), thus taking part in the immune network. Moreover, mast cells produce an exclusive family of proteases (tryptases and chymases) that exert many biological actions relevant to airways inflammation and remodeling. Future studies will better explain the role of mast cells in asthma and, more specifically, the links with bone marrow—where mast cell progenitors originate—and the airways, where mast cells develop, differentiate, and assume the functions of mature cells. This article reviews recent data available on these topics.

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Abbreviations: BALF=BAL fluid; IL=interleukin; MC=mast cell; mRNA=messenger RNA; SCF=stem cell factor; TH2=T-helper 2; TNF-α=tumor necrosis factor-α; VCAM-1=vascular cell adhesion molecule-1

In the recent report of a workshop on global strategy for the management and prevention of asthma, supported by The National Heart, Lung, and Blood Institute and World Health Organization, an operational description of the disease is provided that underscores the importance of bronchial inflammation: “asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils and T lymphocytes, etc.”1 Considering asthma as an inflammatory disorder carries profound implications for the management of the disease. Consequently, in recent years, much effort has been dedicated to the study of the cellular mechanisms of bronchial inflammation.

The aims of this article are to focus on the role of one of the various cellular types involved in the pathogenesis of asthma, ie, the mast cell (MC), to stress the changing perception of its role in light of the results of recent studies and to discuss the MC contribution to different clinical stages of the disease, and more specifically, to persistent, chronic asthma.

MC MEDIATORS AND INTERMITTENT ASTHMA

The MC has long been considered to be involved in the pathogenesis of acute bronchoconstriction through IgE-mediated release of preformed and newly generated mediators. Many of these, such as histamine, sulfido-peptide leukotrienes, and prostaglandin D2, exert direct spasmodic activity on the smooth muscle of the airways.2 This type of immediate reaction plays a prominent role in the genesis of intermittent asthma, in which patients are asymptomatic and have normal lung function between exacerbations (step 1 in the classification of asthma severity).1

The classification and the biological activity of the numerous soluble chemical mediators originating from MCs and released after immunologic or non-immunologic stimuli have been extensively reviewed.2-4

The relevance of MC-derived mediators to the pathogenesis of asthma was confirmed by the finding that histamine (a MC/basophil-specific amine) can
be recovered after specific bronchoprovocation with allergens. Moreover, elevated levels of MC mediators in BAL fluid (BALF) of allergic asthmatics, both spontaneously and experimentally induced, are correlated with nonspecific bronchial hyperresponsiveness.

**MC and Persistent Asthma**

Throughout the last decade, many studies have been made on the morphologic features of asthmatic airways, with the application of fiberoptic bronchoscopy to obtain lavage fluids and tissue directly from the airways (Fig 1). Consequently, much information has been added to our knowledge of the structural changes induced by the disease. Particularly, the cellular components of bronchial inflammation have been characterized according to the above-reported definition of asthma, which in turn has led to the concept that asthma is a chronic inflammatory disorder of the airways.

Although inflammation is considered to be the pathologic hallmark of asthma, whatever the severity, it is conceivable that in clinically persistent asthma (of mild, moderate, or severe degree), the flogistic reaction of the airways is more remarkable than in intermittent asthma. As mentioned above, the MC, the eosinophil, and the T lymphocyte are the three cellular types implicated in the genesis of bronchial inflammation in asthma. Nevertheless, T cells have been placed by some authors as pivotal in orchestrating the inflammatory response through the release of multifunctional cytokines. Indeed, there is considerable evidence that T cells are activated in asthma and, in animal models, the inhibition of T-cell costimulation abrogates allergen-induced airway hyperresponsiveness. Also, eosinophils have been found to be the most abundant inflammatory cell type in asthma and their number correlates with the ability to mount a late-phase bronchial response and to express bronchial hyperresponsiveness. As regards MCs, many authors reported that they are significantly more numerous in asthmatics than in nonasthmatics, even in patients with newly diagnosed asthma. Moreover, progressive MC degranulation occurs from lamina propria toward the airway lumen. The observation that histamine and tryptase (a human MC-specific protease) are present in BALF of patients with chronic asthma suggests that MC mediators, along with mediators originated from eosinophils, such as major basic protein, may also play a role in the pathogenesis of persistent asthma.

**MC-Derived Cytokines**

Parallel to *in vivo* studies in BALF and bronchial biopsy specimens, *in vitro* studies have continued to expand our understanding of the biology and functions of MCs. In 1989, several independent investigators reported that murine MCs were able to elaborate an array of cytokines, most of these with a pronounced proinflammatory activity. Interestingly, the range of cytokines originated by MCs was similar to that produced by T-helper 2 (Th12) cells—the subset of T cells considered to play a central role in atopic asthma.

The observation that murine MCs, *in vitro*, produced a panel of multifunctional cytokines supported the hypothesis that MCs might be implicated not only in the genesis of acute bronchoconstriction, but also in bronchial inflammation. Consequently, the capacity of human MCs to elaborate cytokines and the relative importance of MCs compared to other cell types in producing cytokines was further assessed. Human lung MCs were shown to produce tumor necrosis factor-α (TNF-α), interleukin 4 (IL-4), and IL-5 after IgE-mediated stimulation *in vitro*. Interestingly, Bradding et al. using immunohistochemical analysis of endobronchial biopsy specimens, showed that MCs were the major source
of IL-4, IL-5, IL-6, and TNF-α, both in normal individuals and in subjects with mild atopic asthma. Moreover, MC-associated TNF-α was significantly increased in asthmatics when compared to normal subjects, while no TNF-α immunoreactivity was present in either T cells or eosinophils. On the contrary, Ying et al. investigated the phenotype of cells expressing messenger RNA (mRNA) for IL-4 and IL-5 in BALP and bronchial biopsy specimens of atopic asthma and normal subjects, using immunocytochemistry followed by in situ hybridization. They found that >70% of IL-4 and IL-5 mRNA-positive cells in asthmatics were activated T cells, while MC and eosinophil accounted for the remaining positive cells. Thus, at present, although there is wide evidence that human MCs produce mRNA and express protein for IL-4, IL-5, IL-6, and TNF-α, debate continues on what the relative contributions of MCs and TH2 cells are to the production of cytokines in the airways of asthmatic patients.

Progress of Inflammation in Asthma

Apart from considering which cellular source of cytokines is most important—probably T cells, MCs, and eosinophils contribute with different quantities, different times, and different kinetics of production—the role of cytokines is pivotal in establishing the peculiar type of inflammation that is present in asthma. Specifically, TNF-α and IL-4 can potentially upregulate the expression of vascular cell adhesion molecule-1 (VCAM-1)—an adhesion molecule member of the immunoglobulin superfamily—in the endothelial layer of the bronchial vasculature. Eosinophils, basophils, and mononuclear cells display the very late activation antigen 4 (VLA-4) integrin on their cellular surfaces, which interacts with VCAM-1. Thus (through the interaction VLA-4/VCAM-1) TNF-α and IL-4 facilitate the recruitment of circulating leukocytes. TNF-α can also induce the expression of E-selectin, which is implicated in the recruitment of neutrophils and, along with IL-1β, can modulate the airway smooth muscle contractility. IL-5 acts as a potent chemotactic factor and activator for eosinophils and promotes their development and survival. IL-4 and IL-13 upregulate the synthesis of IgE, by promoting the isotype switching of B cells from IgM to IgE production. IL-4 also plays a dominant role in the differentiation of precursors (TH0 cells) into cytokine-secreting TH2 cells.

The capacity of MCs to release preformed cytokines (TNF-α) on IgE-mediated stimulus or to rapidly synthetize others (IL-4, IL-5) could be the initial event leading to bronchial inflammation. In fact, the induction and activation of TH2 clones, through a further production of cytokines, facilitates the activation and recruitment of the eosinophils, which act as the terminal effectors of the inflammatory reaction. In turn, the cytokines produced by leukocytes (TH2 cells in particular) profoundly affect the development, activation, and priming of mucosal MCs, thus promoting a positive proinflammatory loop.

The recent findings that human MCs produce IL-5 and that murine pulmonary-derived MCs express both C-X-C (ENA-78) and C-C (RANTES), monocyte chemoattractant protein-1, macrophage inflammatory protein-1α) chemokines, suggest that besides the cytokines classically involved in leukocyte recruitment (IL-4, IL-5, TNF-α), MCs also elaborate additional, potent chemoattractants in the airways, acting on polymorphonuclear leukocytes (IL-8) and eosinophil (RANTES). Moreover, since chemokines—acting as histamine-releasing factors—elicit MC degranulation, they might further sustain an autocrine activating loop.

MC Interaction With Other Immune Cells

MCs were recently shown to produce B-cell growth and differentiation factors and to provide—like basophils—the cell contact signal to B cells (CD 40 ligand) that is required, along with IL-4, for IgE synthesis in vitro. This finding suggests that MCs may directly regulate the production of IgE independently of T cells. It has been speculated that MCs and/or basophils, upon direct, nonimmunologic stimulation—MCs can be activated by a variety of agonists such as nucleotides, neuropeptides, opiates, adhesion molecules—or upon IgE cross-linking, can generate a sufficient amount of IL-4 to initiate a local TH2 response. Alternatively, MCs, interacting with B cells, might directly induce a local production of IgE. This last phenomenon could take place outside lymph node germinal centers, for example in the airway mucosa, where a local production of IgE could dramatically affect the allergic response. Moreover, MCs can also act as an antigen presenting cell to T lymphocytes, suggesting an even larger role for MCs in the immune network.

MC Proteases and Airways Remodeling

Besides the production of soluble chemical mediators and cytokines and the interaction with other cells of the immune system, MCs may exert other biological actions relevant to the pathogenesis of asthma, attributable to their peculiar functional versatility. In recent years, a growing interest has developed in MC-derived proteases: chymases—found in human connective tissue MCs—and tryptases—present in all human MCs and activated by coexpressed heparin. These enzymes are able to de-
grade a variety of extracellular peptides and proteins, including vasoactive intestinal peptide, a bronchodilating neuropeptide.\textsuperscript{56} In addition, trypstases inactivating procoagulant proteins, prevent the deposition of fibrin, and activate urokinase, thereby accelerating fibrin lysis. In this way, trypstases may facilitate the influx of leukocytes into inflamed tissues.\textsuperscript{57} Interestingly, by activating matrix metalloproteinases,\textsuperscript{57} stimulating the growth of fibroblasts\textsuperscript{58} and airways smooth muscle cells,\textsuperscript{59} trypstases may play a critical role in the processes of airways remodeling in asthma. Particularly, the mitogenic activity on fibroblasts and smooth muscle cells could influence the subbasement membrane deposition of types 3 and 5 collagen, as well as the thickening of the airway wall, which are both peculiar morphologic characteristics of the disease.\textsuperscript{10}

**The Role of MC in the Genesis of Atopy and Asthma**

Genetic studies have indicated a possible association between atopy and specific variants of the gene encoding for the β-subunit of the high-affinity IgE receptor.\textsuperscript{60,61} This receptor is typically expressed on MCs and basophils and is also present in a limited number of other cells, such as the monocyte/macrophage, the eosinophil, and the Langerhans’ cells. The very recent finding that genetic variants of MC chymase are associated with an atopic disorder (eczema)\textsuperscript{62} may confirm that the genetic basis of atopy is strictly related to MC function.

Additionally, Humbert et al\textsuperscript{63} have recently reported that cells expressing high-affinity IgE receptors (identified as MCs and macrophages) are significantly increased in bronchial biopsy specimens of both atopic and nonatopic asthmatics when compared to normal subjects. This finding suggests that the IgE-mediated activation of MCs also could be implicated in the genesis of “intrinsic” asthma and may be the trigger that sets in motion the chain of events that leads to bronchial inflammation.

**Future Perspectives**

There is some evidence that bronchial asthma, although specifically involving the airways, could be—owing to some of its features—a “systemic” disease. More precisely, it has been shown that asthma can be transmitted with bone marrow transplantation\textsuperscript{64} and that a bidirectional communication exists between the airways and the bone marrow in asthmatics.\textsuperscript{65} Consequently, it is likely that cells originating from the bone marrow—in mature form or, more probably, as immature progenitors—reach the airways through the bloodstream and there, cooperating with local factors, predispose them to develop the asthmatic inflammation upon interaction with external environmental factors. The preponderance of experimental data available would suggest the view that the putative candidate for this role is a cell of the MC or lymphocyte lineage. In fact, the third cellular type specifically implicated in asthma, the eosinophil, although producing an array of cytokines and enzymes that are determinant in damaging the bronchial epithelium and in inducing bronchial hyperreactivity,\textsuperscript{12-42} is believed to be a “second line” actor, recruited by cytokines and chemokines originated from MCs or lymphocytes. Therefore, in order to explain the role of the MC—compared to that of the T cell—in orchestrating the airway inflammation, it would be of interest to investigate the links between circulating MC progenitors and the airways. In particular, a better characterization of human MC precursors is needed, similar to what was done in mice.\textsuperscript{66} Furthermore, it would be of interest to locate MC precursors in the airways.

The recently developed techniques for primary human MC cultures\textsuperscript{67-69} should help us understand the functional changes—for example, the expression of adhesion molecules—occurring during human MC differentiation. Additionally, the study of local factors in the airway, particularly the production of stem cell factor (SCF) (a growth factor of fibroblast origin that supports both the development and the activation of human MCs\textsuperscript{71}) as well as the study of the characteristics of the extracellular matrix (which interacts with the adhesion molecules expressed by MCs),\textsuperscript{47} may explain whether specific conditions exist in asthmatics or in individuals with genetic predisposition to bronchial asthma, which facilitate the homing and/or the differentiation of circulating MC progenitors in the airways.

Finally, animal models—such as MC-deficient mice—provide a promising avenue of investigation. These “knock out” mice have a mutation in the W locus (c-kit) that encodes for the c-kit receptor, a member of the receptor tyrosine kinase family that is expressed on the surface of hematopoietic progenitor cells, MCs, and other selected cell types (melanocytes, germ cells) and that interacts with SCF (c-kit ligand).\textsuperscript{71} Thus, mice with mutation in both copies of the W locus (W/W\textsuperscript{c}) show a virtual absence of tissue MCs, since they are unable to respond to SCF. MC-deficient mice have proved to be of great value for studying, in vivo, the contribution of MCs to IgE-dependent cutaneous late-phase reactions\textsuperscript{72} and to host defense from bacterial infections.\textsuperscript{73} Recent data show that W/W\textsuperscript{c} mice have an impaired capacity to recruit eosinophils into BALF and lung tissues or to develop bronchial hyperreactivity after direct
The exciting advances in the knowledge of the biology of the MC obtained in the last few years have radically changed our view of the role played by this cell in asthma. More specifically, it is now clear that beyond the well-known action of MC mediators in the genesis of immediate bronchoconstriction—which is a peculiar feature of clinical intermittent asthma—MC-derived cytokines, chemokines, and proteases, interacting with other immune cells (T lymphocytes and eosinophils), substantially contribute to establishing the chronic inflammation and the remodeling of the airways that are typically present in the various stages of persistent asthma. Additionally, the new techniques of cellular and molecular biology and genetic studies are broadening our understanding of the complex relationships between the various cell types involved in the immune network and will enable us in the future to more completely comprehend the pathogenesis of asthma.

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