

Review article

Mast-cell–leukocyte cytokine cascades in allergic inflammation

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Despite decades of intensive study, the generation of a wide variety of imaginative hypotheses, and the revelation of many interesting and potentially important findings, the role of the mast cell in allergic inflammation is still not fully understood. This statement is especially true in regard to those clinically significant examples of allergic inflammation that occur in man, such as allergic asthma and atopic dermatitis. That the mast cell *may* have critically important roles in such disorders is, of course, not in question. The issue is not what mast cells *might* do in these responses but what they *actually* do, *how* they do it, and, beyond that, the *relative importance* of the mast cell's role(s) in these reactions compared to those of other cell types that may express functions that are similar to, or overlap, those of the mast cell.

The purpose of this brief review is not to consider all of the many potential roles of the mast cell and its diverse mediators in various components of allergic inflammatory responses. Instead, using asthma as the main example, the discussion will focus primarily on the possibility that mast-cell cytokine production represents an important (and therapeutically accessible) link between mast-cell activation and many of the clinically significant features and consequences of allergic inflammation.

Inflammation, mast cells, and cytokines in the pathogenesis of allergic asthma

Clinically, asthma is characterized by episodic reversible airway narrowing, with associated bronchial hyperresponsiveness to many stimuli (26, 44). Allergic asthma often begins during childhood in subjects with a family history of atopic disorders, and is characterized by allergen-dependent, frequently seasonal, symptoms and by both positive skin tests to allergens and elevated serum levels of total and allergen-specific IgE (25, 31). Because IgE-dependent activation of mast cells via the high-affinity IgE receptor ($Fc_\epsilon RI$) can induce these cells to release a panel of preformed or newly synthesized bronchoactive mediators (e.g., histamine, tryptase, prostaglandin (PG) D_2 , leukotriene (LT) C_4), mast cells have long been implicated in the early phase of bronchoconstriction that follows allergen challenge in appropriately sensitized individuals (21, 55).

However, it has become increasingly clear that the clinical and pathologic features of allergic asthma cannot be understood solely in terms of the bronchoactive mediators that are released acutely by mast cells activated via the $Fc_\epsilon RI$. Thus, several lines of evidence, including morphologic and immunohistochemical (IHC) analyses of endobronchial biopsy and bronchoalveolar lavage fluid (BALF) specimens,

support the now widely held view that allergic asthma can be considered a disease of inflammation of the airways (6, 21, 26, 44, 69, 83, 92). Indeed, it has been proposed that corticosteroids, which have little or no ability to inhibit IgE-dependent release of mast-cell histamine or leukotrienes, confer clinical benefit in asthma largely because of their ability to suppress inflammation in the respiratory tract (7, 93, 99). Yet, despite these important changes in our thinking about the nature and treatment of allergic asthma, a fully coherent model of the pathogenesis of allergic inflammation in this disorder, and, in particular, a complete understanding of the mechanisms that initiate and perpetuate the mucosal inflammation in this disease, have proven to be quite elusive.

This is not because of a lack of candidate effector cells and mediators. Indeed, if anything, there is an overabundance of potential key players. In addition to the mast cell, eosinophils, basophils, T cells, monocytes/macrophages, neutrophils, and platelets have been put forward as critical participants in the pathogenesis of allergic asthma (reviewed in refs. 6, 17, 26, 38, 92, 107). Clearly, since all of these cells are present in the affected tissues, it is possible that each may contribute to this disorder. Similarly, a wide variety of mediators can be detected in BALF obtained from patients with active disease or after experimental provocation with allergen (6, 26). In addition to "classical" mast-cell-associated mediators such as histamine, tryptase, PGD₂ and LTC₄, and proteins derived from eosinophil cytoplasmic granules, considerable attention is now focused on cytokines (26, 36, 44, 94).

Recent work by many groups has identified a potentially bewildering number of cytokines, and some of the biologic effects of these molecules have been characterized. These studies indicate that the many individual cytokine proteins:

- 1) generally are synthesized and secreted only upon appropriate cellular stimulation
- 2) often can have several distinct effects in a variety of biologic processes
- 3) frequently can be produced by many different cell types
- 4) may be produced by cells upon which they act, mediating autocrine effects.

Moreover, most cytokines have actions that partially overlap, synergize with, or antagonize those of other cytokines (84). While space does not permit a detailed discussion of all of the cytokine-dependent processes that have been implicated in allergic inflammation, these include the upregulation of the IgE response (e.g., interleukin (IL)-4, IL-5, IL-6, IL-13), the enhancement or induction of basophil recruitment (e.g., tumor necrosis factor- α (TNF- α), IL-4) or mediator production (e.g., IL-3, IL-4, macrophage inflammatory protein (MIP)-1 α),

the promotion of eosinophil development/survival (e.g., IL-5, IL-3, granulocyte/macrophage-colony stimulating factor (GM-CSF) and recruitment (e.g., IL-3, IL-5, GM-CSF, lymphocyte chemotactic factor (LCF), RANTES, MIP-1 α), and the recruitment of monocytes and T cells (e.g., MIP-1 α , RANTES) (15, 26, 28, 36, 60, 88, 92, 94, 96, 97, 108, 111, 114, 117, 118).

Much of the ability of certain cytokines (including IL-1 and TNF- α) to promote allergic inflammation is thought to reflect the ability of these agents to enhance the recruitment of leukocytes by inducing the increased expression of adhesion molecules, such as P- and E-selectin, VCAM-1, and ICAM-1, on vascular endothelial cells (11, 15, 106, 117). However, cytokines may critically influence many other stages in the development of allergic inflammation, as well as regulate some of the local consequences of these responses. For example, several cytokines (including TNF- α and transforming growth factor (TGF)- β) can regulate various aspects of tissue remodeling, including the production of extracellular matrix proteins, through effects on fibroblasts and other cell types (66). And some cytokines, including TNF- α , can also induce upregulation of epithelial cell expression of type II, or inducible, nitric oxide synthase (iNOS) and thus contribute to the development of NO-dependent damage to the airway epithelium (5, 51, 90, 91).

Glucocorticoids can inhibit cytokine production in many cell types, as can cyclosporin A (CsA), and these effects have been proposed as one of the important mechanisms of action of these agents in patients with asthma (3, 92, 93, 99).

Mast cells as a source of cytokines

It is now apparent that mast cells *might* influence many important aspects of the pathogenesis of allergic inflammation in asthma and other allergic disorders via the elaboration of multifunctional cytokines. Indeed, we have proposed that the production of cytokines represents one of the critical links between the IgE-dependent mast-cell activation that occurs immediately after allergen challenge in atopic subjects, the inflammation that develops during the subsequent late-phase reactions to such provocation, and the persistent inflammation and associated tissue changes that are characteristic of chronic allergic disorders (36, 41, 45).

Mice

On the basis of the work of several groups, the first cytokine bioactivity to be clearly associated with normal mast cells was TNF- α (reviewed in refs. 45, 128). Moreover, certain mouse mast-cell populations

constitutively contain TNF- α bioactivity, and at least some of this TNF- α is, upon cell fractionation analysis, associated with the cytoplasmic granules (46, 47, 128). These findings illustrate an important point: the biology of cytokine production by mast cells may differ significantly from that in other cell types. Indeed, mast cells probably represent the *initial* source of TNF- α in IgE-dependent reactions, since other cellular elements of allergic inflammation, such as macrophages, T cells, and B cells, apparently contain little or no preformed TNF- α bioactivity, whereas mature, "resting" (nonactivated) mouse mast cells contain preformed stores of TNF- α available for immediate release upon appropriate stimulation of the cells (reviewed by Galli (36)).

However, mast cells represent a potential source of many cytokines, in addition to TNF- α , that might influence allergic inflammation, and the synthesis and release of these products can be induced via IgE-dependent mechanisms. Thus, Plaut et al. (87) demonstrated that IgE-dependent stimulation of long-term IL-3-dependent mouse mast-cell lines resulted in increased levels of mRNA for IL-3, IL-4, IL-5, and IL-6 and the release of bioactivity for IL-3, IL-4, and IL-6; Wodnar-Filipowicz et al. (124) reported that primary cultures of IL-3-dependent mouse mast cells stimulated with IgE and antigen exhibited increased levels of IL-3 and GM-CSF mRNA and released IL-3 and GM-CSF bioactivity; and Burd et al. (24) showed that long-term IL-3-dependent or -independent mouse mast-cell lines activated by IgE and antigen exhibited increased levels of mRNA for IL-1 α , IL-3, IL-5, IL-6, GM-CSF, interferon-gamma (IFN- γ), and four members of the C-C chemokine family (MIP-1 α , MIP-1 β , JE [MCP-1], and TCA3). We also showed that IgE-dependent activation of mast-cell lines or primary cultures of BMCMC resulted in release of bioactivity for IL-1, IL-4, and IL-6 (24). Certain mouse mast-cell populations can also generate the C-C chemokine, MARC (67), as well as IL-9 and IL-13 (57).

Humans

In part because of the difficulty of obtaining highly purified preparations of human mast cells, studies of *human* mast-cell cytokine production have been rather limited. Much of this work has focused on TNF- α . Klein et al. (65) showed that activation of mast cells in fragments of human skin *in vitro* resulted in increased expression of E-selectin on adjacent vascular endothelial cells. E-selectin is one of the adhesion molecules whose expression can be upregulated by TNF- α (11), and Klein et al. (65) reported that antibodies to TNF- α inhibited the augmented E-selectin expression observed after mast-cell activation. While the cellular source of TNF- α

in this study was not established, the same group later reported that TNF- α was detectable by IHC in the cytoplasmic granules of mast cells in normal neonatal foreskin, adult skin, and oral mucosa, and in mast cells purified from normal skin, but not in any other contaminating cell type; that exposure to morphine sulfate *in vitro* induced dermal mast cells to release immunoreactive TNF- α ; and that TNF- α mRNA was detectable by *in situ* hybridization (ISH) in many mast cells in lesions of cutaneous delayed hypersensitivity (115).

Subsequently, Benyon et al. (9) demonstrated that stimulation of purified human skin mast cells with anti-IgE induced these cells to release TNF- α bioactivity, and Gordon et al. (49) showed that, upon challenge with anti-IgE, highly purified human lung mast cells released TNF- α bioactivity and exhibited increased steady-state levels of TNF- α mRNA. Ohkawara et al. (80) showed that challenge of human lung fragments with anti-IgE antibodies *in vitro* induced both lung mast cells and lung macrophages to express higher levels of TNF- α mRNA by ISH. While Ohkawara et al. (80) did not determine whether the stimulation of increased levels of TNF- α mRNA in the macrophages was a direct or indirect effect of treatment with anti-IgE, these findings suggest that resident macrophages also represent a source of TNF- α during IgE-dependent inflammatory responses in human airways.

Mast-cell heterogeneity

In contrast to mouse or rat peritoneal mast cells, certain populations of normal human mast cells may constitutively contain relatively low levels of TNF- α (9, 80). Moreover, human skin mast cells can exhibit *decreased* TNF- α immunoreactivity after activation (115). These observations illustrate another very important point: the phenotypic characteristics of individual mast-cell populations can vary considerably, both between species and among various anatomic sites in a single species, and also in "resting" compared with recently activated (degranulated) mast cells (14, 32, 35, 64). This "mast-cell heterogeneity" can encompass significant differences in the cells' mediator content, responses to proliferation/survival factors, and patterns of sensitivity to agents that induce or pharmacologically modulate degranulation and mediator release. Consequently, to understand the relevance of mast-cell cytokine production/secretion to a particular biologic response (e.g., allergic inflammation in the respiratory tract), one ideally should study those mast cells that actually participate in that response *in vivo*.

Notably, both the cytokine content of mouse or human mast cells and their ability to release cytokines and other mediators in response to appropri-

ate stimulation can themselves be regulated by cytokines present in the cells' microenvironment (28, 40, 104). Several lines of evidence indicate that the c-kit ligand, stem cell factor (SCF) (see below), may represent one of the most important of the factors that regulate the phenotype and mediator content of mast cells, and that also can influence the ability of these cells to release cytokines and other mediators, in response to both IgE-dependent and other forms of stimulation (reviewed in 40, 42, 76).

Mast-cell-leukocyte cytokine cascades in allergic inflammation

Despite exhibiting heterogeneity in many other characteristics, all mature mast-cell populations so far studied in mice, rats, or man express the $Fc_\epsilon RI$, and can be activated to release cytokines and other me-

diators by stimulation with IgE and specific antigen (8, 28, 55, 62). Accordingly, we have formulated the hypothesis that a "mast-cell-leukocyte cytokine cascade" critically contributes to the initiation and perpetuation of IgE-dependent allergic inflammation in the airways and other sites (36, 41, 45). Specifically, we propose that the activation of mast cells through the $Fc_\epsilon RI$ initiates the response, in part through the release of $TNF-\alpha$ and other cytokines that can influence the recruitment and function of additional effector cells (Fig. 1). These recruited cells then influence the further progression of the inflammatory response by providing additional sources of certain cytokines (e.g., $TNF-\alpha$) that can also be produced by mast cells stimulated by ongoing exposure to allergen, as well as new sources of cytokines and other mediators that may not be produced by mast cells. Finally, mast-cell activation may directly or indi-

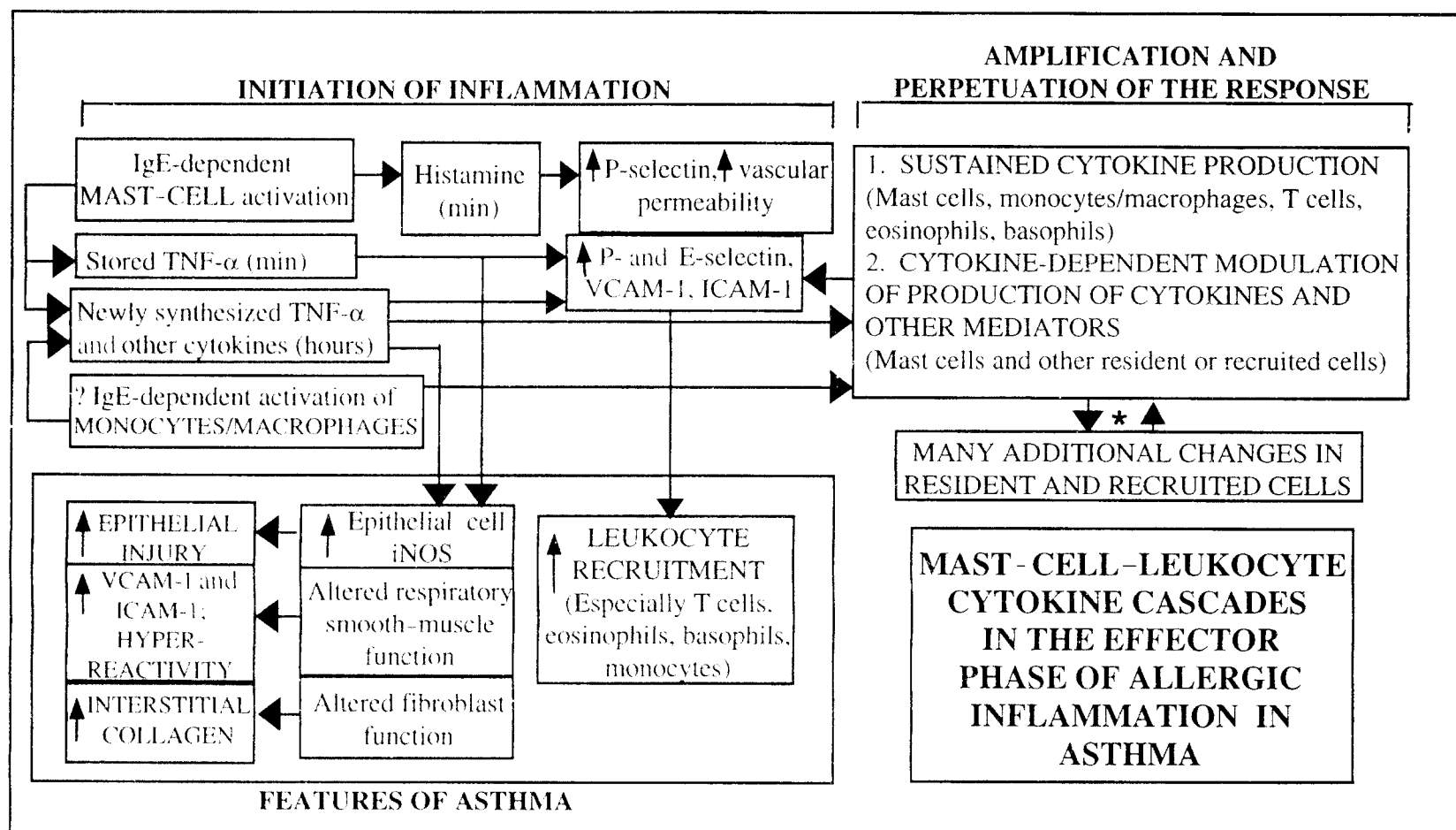


Fig. 1. A greatly simplified diagram of the pathogenesis of allergic inflammation in asthma and some of its consequences. The diagram is limited to the effector phase of allergic inflammation, and focuses primarily on the roles of IgE, mast cells, and cytokines, especially $TNF-\alpha$. In sensitized subjects, allergic inflammation is initiated by the responses of mast cells, monocytes/macrophages, and perhaps other resident cells (e.g., T cells) to a specific allergen, and can then be amplified and sustained, especially in response to persistent or repeated allergen exposure, by the actions of both resident and recruited cells. $TNF-\alpha$ has effects on both resident cells (e.g., vascular endothelial cells, epithelial cells, smooth-muscle cells, and fibroblasts) and recruited leukocytes which can contribute to several of the clinical and pathologic features of asthma. Mast cells and resident monocytes/macrophages may represent the most important initial sources of $TNF-\alpha$ after allergen exposure, but many other effector cells, including T cells, eosinophils, and neutrophils, may provide additional sources of $TNF-\alpha$ during later phases of the inflammatory response. * The cytokine production associated with allergic inflammation in asthma can result in many changes in the phenotype, function, and, in some cases, numbers of resident cells, and some of these changes in turn can influence cytokine production by mast cells and other effector cells at these sites. Moreover, many mediators other than cytokines, such as histamine (derived from mast cells or basophils), mast-cell tryptase, and lipid mediators (from many cell types) can have actions that overlap and/or modulate certain cytokine-dependent effects. Note that mast cells (and mast-cell-derived cytokines) have also been implicated in the *afferent limb* of allergic responses and even in the *resolution* of allergic inflammation (see text).

rectly promote the release of cytokines from certain resident cells in the respiratory tract, such as alveolar macrophages and bronchial epithelial cells. Bronchial epithelial cells do not bind IgE, but can express TNF- α after anti-IgE challenge of lung *in vitro* (80), findings that suggest that TNF- α expression by these cells probably reflects an indirect consequence of anti-IgE challenge. In contrast, recent findings with blood monocytes (75) raise the possibility that alveolar macrophages can be activated *directly* via Fc ϵ RI-dependent mechanisms.

The mast-cell-leukocyte cytokine cascade hypothesis thus proposes that mast-cell-derived cytokines have a critical role in the initiation of the effector limb of IgE-dependent allergic inflammation, and importantly contribute to the perpetuation of the response, but that the manifestations of allergic inflammation, once the response is established, reflect the contributions of the mast cell and many other resident or recruited cell types.

TNF- α in mast-cell-leukocyte cytokine cascades

As indicated in Fig. 1, we have been particularly interested in TNF- α as one of the potentially critical participants in mast-cell-leukocyte cytokine cascades during allergic inflammation. This cytokine can be produced both by mast cells and by other cells present in allergic inflammation, including T cells, eosinophils, monocytes/macrophages, and neutrophils (reviewed in refs. 10, 29, 33, 36). TNF- α can increase bronchial hyperresponsiveness in animal models (63). TNF- α also has many "proinflammatory" effects, including the ability to:

- 1) induce other cell types to produce cytokines that promote inflammation (e.g., IL-8, refs. 10, 113)
- 2) upregulate four adhesion molecules involved in leukocyte recruitment during immunologic and nonimmunologic forms of inflammation: P-selectin, E-selectin, VCAM-1, and ICAM-1 (12, 15, 106)
- 3) upregulate cytotoxic effector function in eosinophils and other leukocytes (113)
- 4) increase microvascular permeability (2)
- 5) upregulate epithelial cell iNOS expression, especially in combination with other cytokines such as IL-1 β or IFN- γ (5, 51, 90, 91)
- 6) alter respiratory tract smooth-muscle function, e.g., by increasing expression of ICAM-1 and VCAM-1 and increasing adherence of activated T cells (70, 82)
- 7) promote tissue remodeling via effects on fibroblasts and other cell types (66).

Moreover, TNF- α can synergize with other cytokines, e.g., IL-1, in mediating many of the specific steps in the pathogenesis of allergic inflammation,

including the upregulation of adhesion molecules (10, 71, 113).

However, cell types other than the mast cell (including some that can produce TNF- α) can also interact with IgE, either via the Fc ϵ RI, e.g., basophils (8, 62), epidermal Langerhans' cells (13, 116), and at least some eosinophils (1) and monocytes (75), or through other interactions (e.g., those mediated through the low-affinity IgE receptor, CD23) (reviewed by Sutton & Gould (108)). Accordingly, to investigate the *specific* contributions of mast cells and mast-cell-derived TNF- α in allergic inflammation, we developed a mouse model in which allergic inflammation can be studied *in vivo* in the presence or virtual absence of the mast cell.

Mast-cell-deficient W/W^c (Kit^W/Kit^{W-c}) or Sl/Sl^d (Mgf^{Sl}/Mgf^{Sl-d}) mice and stem cell factor (SCF)

It has long been known that WBB6F $_1$ - W/W^c mice and WCB6F $_1$ - Sl/Sl^d mice have similar phenotypes: they virtually lack tissue mast cells (<1.0% the number present in +/+ mice in the skin; none in the respiratory tract), are anemic, lack melanocytes in the skin, and are sterile, but contain basophils and also exhibit normal circulating levels of other granulocytes, T cells, B cells, and platelets (reviewed in refs. 37, 39, 42, 64). However, adoptive i.v. transfer of bone-marrow cells of congenic +/+ origin can correct the anemia, and result in the development of tissue mast-cell populations of +/+ origin, in W/W^c , but not Sl/Sl^d , mice (64).

Because of exciting recent progress, the similarities and differences in the phenotypes of W/W^c and Sl/Sl^d mice can now be understood in molecular terms. The mouse W locus is allelic with *c-kit*, which encodes a member of the receptor tyrosine kinase III family (herein designated the SCF receptor, or SCFR) that is expressed on the surface of hematopoietic progenitor cells, mast cells, melanocytes, germ cells, and certain other cells (reviewed by Galli et al. (42)), whereas the mouse Sl locus encodes the ligand for that receptor, a growth factor herein designated SCF (129), but also called mast-cell growth factor (MGF, ref. 27), kit ligand (KL, ref. 56), or steel factor (SL, ref. 123). Thus, mice with mutations at the W locus (e.g., W locus (e.g., W/W^c mice, which should now be designated Kit^W/Kit^{W-c} mice (122)) express defects in the SCFR $^+$ cell lineages themselves, whereas mice with mutations at the Sl locus (e.g., Sl/Sl^d mice, which should now be designated Mgf^{Sl}/Mgf^{Sl-d} mice (122)) express abnormalities in the microenvironments necessary for the normal survival and/or development of these lineages.

“Mast-cell knock-in mice”

Although the *c-kit* mutations in *Kit^{fl}/Kit^{fl}* mice result in a virtual absence of tissue mast cells, this defect can be *selectively* repaired. In collaboration with Yukihiro Kitamura, we showed that when *Kit^{fl}/Kit^{fl}* mice were injected i.d., i.p., or i.v. with phenotypically immature mast cells (BMCMC, bone-marrow-derived cultured mast cells) which had been generated *in vitro* from the bone-marrow cells of either the congenic *+/+* mice or “beige” mice (whose mast cells can be identified by their giant cytoplasmic granules), such *+/+* or “beige” BMCMC → *Kit^{fl}/Kit^{fl}* “mast-cell knock-in mice” developed tissue mast-cell populations of donor origin whose anatomic distribution and histochemical characteristics were similar to those of the corresponding mast-cell populations in the tissues of normal mice (78).

Subsequently, we used *Kit^{fl}/Kit^{fl}* and *+/+* BMCMC → *Kit^{fl}/Kit^{fl}* mice to characterize and quantify the specific contributions of mast cells to the expression of IgE-dependent reactions and other biologic responses *in vivo*. We found that mast cells are essential for virtually all of the increases in vascular permeability, tissue swelling, and leukocyte recruitment at sites of IgE-dependent cutaneous reactions (120, 121), are essential for the cardiopulmonary changes and death associated with IgE-dependent *passive* systemic anaphylaxis response (109), and are required for the increased bronchial responsiveness to methacholine which is observed 20 min after the i.v. administration of anti-IgE (74). Moreover, Kung et al. (68) used *+/+* BMCMC → *Kit^{fl}/Kit^{fl}* mice to show that mast cells can significantly contribute to aerosol allergen-induced eosinophil recruitment into BALF and lung tissue of sensitized mice.

Mast-cell-derived TNF- α importantly contributes to IgE-dependent cutaneous late-phase reactions in mice

Our studies in “mast-cell knock-in mice” showed that mast cells were required for essentially all of the leukocyte infiltration observed in the skin after challenge with IgE and specific antigen (121). We then demonstrated that ~50% of such IgE- and mast-cell-dependent leukocyte infiltration was inhibitable with an antibody to recombinant mouse TNF- α (rmTNF- α) (121). Moreover, mast cells accounted for virtually all of the upregulated expression of TNF- α mRNA observed within 60–90 min of antigen challenge at cutaneous sites that had been injected with IgE (47). More recently, we established that IgE-dependent mouse mast-cell activation both promotes the TNF- α -dependent recruitment of granulocytes which themselves exhibit TNF- α

mRNA according to *in situ* hybridization (Wershil & Galli, unpublished data), and also induces, by a mechanism that is in part TNF- α -dependent, increased type I collagen production by dermal fibroblasts (48). Finally, we showed that either dexamethasone or CsA can substantially suppress:

- 1) the IgE-dependent secretion of TNF- α by mouse mast cells *in vitro*
- 2) the leukocyte recruitment induced by the injection of recombinant mouse TNF- α into mouse skin *in vivo*
- 3) the mast-cell-, IgE-, and TNF- α -dependent leukocyte infiltration observed at cutaneous reaction sites in mice *in vivo* (119).

Taken together, these findings offer direct support for the existence of a mast-cell–leukocyte cytokine cascade during IgE-dependent allergic cutaneous inflammation in mice, and demonstrate the importance of TNF- α in the late-phase component of this response. Moreover, antibodies to TNF- α and TGF- β can virtually eliminate the ability of supernatants from IgE- and antigen-stimulated mouse mast cells to upregulate steady-state levels of type I collagen mRNA in dermal fibroblasts *in vitro* (48). This finding suggests one mechanism by which mast-cell-derived TNF- α and TGF- β may contribute to the increased deposition of extracellular matrix proteins that is observed in certain chronic allergic disorders, such as that which occurs beneath the respiratory epithelial basement membrane in allergic asthma (20). Our findings also raise the possibility that at least some of the therapeutic benefit of drugs such as corticosteroids and CsA, both in asthma and in other inflammatory disorders, may reflect the ability of these agents to interfere with multiple steps in the pathogenesis of mast-cell–leukocyte cytokine cascades, including the mast-cell-dependent production of TNF- α and other cytokines (119).

TNF- α and allergic asthma

Studies in “mast-cell knock-in mice” can provide compelling evidence of the role of mast cells and mast-cell-derived cytokines in murine biologic responses, but what is the relevance of these observations to human allergic diseases? Several groups have provided evidence that TNF- α may indeed contribute to the initiation and perpetuation of the inflammation associated with allergic asthma, and that the mast cell may represent an important source of this cytokine. Ying et al. (127) found that TNF- α mRNA-expressing cells were markedly increased in the BALF of stable atopic asthmatic subjects when compared with controls, but the specific cell type that expressed the TNF- α transcripts was not identified. Broide et al. (22) showed that levels of BALF TNF- α bioactivity, but not IL-4, were markedly in-

creased in subjects with active as opposed to inactive atopic asthma. Bradding et al. (19) used IHC in glycol methacrylate-embedded sections to assess the distribution and identity of cells that expressed IL-4, IL-5, IL-6, and TNF- α in the airways of normal individuals and subjects with mildly symptomatic atopic asthma. In biopsies of both the normal and asthmatic airways, many cells stained for each of the four cytokines, and most of these cells were identified as mast cells according to their tryptase content. However, there were no significant differences between the total numbers of IL-5- or IL-6-positive cells (mast cells plus other cell types) in the normal or asthmatic airways, and a significant increase in the number of IL-4-positive cells in the airways of asthmatic individuals was detected with only one of the two anti-IL-4 antibodies tested.

In contrast, there was a highly significant, seven-fold mean increase in the number of cells staining for TNF- α in the biopsies of asthmatic as opposed to normal subjects. And approximately twice as many mast cells were positive for TNF- α by IHC in the biopsies of subjects with asthma than in the biopsies of normal subjects. Moreover, most of the TNF- α ⁺ cells in the biopsies were mast cells, both in the airways of normal subjects (range = 50–100%) and in the airways of subjects with asthma (range = 75–100%). On the basis of histologic analysis, the authors felt that most or all of the remaining TNF- α ⁺ cells were monocytes/macrophages. The latter result was consistent with the findings of Ohkawara et al. (80), as well as with earlier reports indicating that human peripheral blood monocytes can release TNF- α in an IgE-dependent manner (16), and identifying alveolar macrophages as one source of TNF- α during late-phase reactions in asthma (50).

The IHC findings of Bradding et al. (19) indicate that TNF- α expression is upregulated in the airway mucosa of subjects with allergic asthma and that this expression of TNF- α occurs predominantly in mast cells and monocytes/macrophages. Remarkably, no TNF- α immunoreactivity was localized in either T cells or eosinophils in these specimens. From these results, the authors suggested that the upregulation of TNF- α expression in mast cells may be an important characteristic of allergic asthma (19). On the other hand, it is not yet clear whether the increased expression of TNF- α protein by mast cells and other cells in the respiratory tract of patients with symptomatic allergic asthma reflects a *consequence* of the ongoing disease in these individuals or instead represents a “baseline” characteristic of the mast cells of patients with atopic asthma.

Moreover, it is not yet apparent to what extent the mast cells of asthmatic individuals, as opposed to other potential sources of TNF- α in these subjects, such as eosinophils, T cells, or monocytes, express

mRNA for TNF- α , as would be consistent with the ongoing synthesis and release of newly formed cytokine by these cells. This is an important point, since TNF- α stored in mast-cell granules (probably accounting for the TNF- α detected in these cells by IHC) does not mediate signals to surrounding target cells until it is released by the mast cell, and since the ability of mast cells and other cell types to synthesize and release TNF- α may be reflected by upregulated expression of TNF- α mRNA under circumstances where the levels of immunoreactive TNF- α protein detected in these cells by IHC does not increase or may even decrease (e.g., due to release of stored TNF- α). For example, Walsh et al. (115) found that human skin mast-cell immunoreactivity for TNF- α *decreased* in association with mast-cell activation, and Finotto et al. (33) found that eosinophils infiltrating nasal polyps, as well as eosinophils secreting TNF- α *in vitro*, exhibited little or no TNF- α by IHC even though most of these cells were strongly positive for TNF- α mRNA by ISH. Moreover, Ohkawara et al. (80) found that *three* resident cell populations in human lung fragments expressed TNF- α by IHC 4 h after anti-IgE challenge *in vitro*: mast cells, tissue and alveolar macrophages, and bronchial epithelial cells, whereas *no cells* exhibited TNF- α immunoreactivity in specimens incubated with medium alone. This work suggested that “normal” human lung mast cells might exhibit low levels of stored TNF- α , but that expression of the cytokine can be upregulated in mast cells challenged via the Fc ϵ RI.

Do mast cells contribute to the afferent limb of allergic responses?

While this review has focused primarily on the effector limb of the allergic response, the possibility that mast cells might contribute to the regulation of IgE production was suggested by observations that mouse mast cells could release IL-4 upon Fc ϵ RI-dependent stimulation (24, 87). More recent data, indicating that human mast cells can express both IL-4 (18, 19, 126) and the CD40 ligand (43), provide additional evidence that mast cells may have a role in the afferent limb of the allergic response.

On the other hand, the *relative importance* of the mast cell in the development of IgE responses, as opposed to other cell types that can produce IL-4 and/or express the CD40 ligand (including the basophil) (refs. 4, 23, 43, 72, 101), remains to be determined. For example, initial studies have shown that genetically mast-cell-deficient mice apparently have no defect in their ability to generate an IgE response after certain forms of immunization (53, 109) and can even express slightly higher circulating levels of IgE under “baseline” conditions than do the

congenic +/+ mice (109). The extent to which the ability of mice that virtually lack mature mast cells to orchestrate an IgE response reflects the contribution of basophils, as opposed to other factors, is not yet clear. Nevertheless, it is tempting to speculate, much as others have (85, 95), that the mast cell and/or the basophil, upon direct, "nonimmunologic" stimulation by an allergen (perhaps in association with a "priming" factor such as a cytokine or neuropeptide), can generate sufficient IL-4 or IL-13 to initiate a Th2-type response and thus can regulate both the onset and the "organ specificity" of allergic responses. Of course, once even small amounts of allergen-specific IgE have been generated at a site of allergen exposure, it is possible that mast cells (and/or basophils) may promote the further development of the IgE response via cytokine- and CD40 ligand-dependent effects on local B cells (28, 43, 98).

Conclusions

Several lines of evidence derived from animal models, including "mast-cell knock-in mice", indicate that mast cells importantly contribute to the pathogenesis of both acute and more chronic components of allergic inflammation, and that certain features of these responses, such as the associated leukocyte recruitment, are significantly influenced by TNF- α . The results of studies in human subjects suggest that mast-cell-derived TNF- α contributes to the development of allergic inflammation in asthma as well. However, it is very unlikely that TNF- α represents the only mast-cell-derived cytokine that significantly contributes to allergic inflammation. Nor should the potential contributions of the many other types of mast-cell-derived mediators be overlooked.

Indeed, while this brief review has emphasized the role of mast cells and TNF- α in allergic inflammation, the redundancy in the effector mechanisms that potentially participate in allergic inflammation is considerable: many cells can express Fc ϵ RI and/or CD23, many cells can elaborate TNF- α or other cytokines, many cytokines have similar or overlapping function, and many mediators in addition to cytokines (notably including certain additional mast-cell-derived mediators) have actions that may be very important in the pathogenesis of these responses. Moreover, it is clear that some "allergic responses" can occur in the virtual absence of mast cells, at least in mice. For example, even though mast cells appear to be essential for IgE-dependent *passive* systemic anaphylaxis responses (109), mast cells are *not* required for the changes in pulmonary function (73, 109), or death (52, 53, 59, 73, 109), associated with certain forms of *active* anaphylaxis in mice. Whether the persistence of fatal active anaphylactic responses in genetically mast-cell-deficient mice reflects the

contribution of basophils, as opposed to other cell types, has not yet been determined, but it has been shown that a form of fatal active anaphylaxis can also be elicited in IgE-null mice (79). Taken together, these findings indicate that the effector mechanisms responsible for immunologic responses that are thought to involve IgE and mast cells can be highly redundant. They also illustrate that the extent of such redundancy may only become apparent in the context of *in vivo* "deletion experiments", such as those based on the use of animals which genetically lack individual elements of these immunologic responses.

The redundancy engineered into immunologic and inflammatory responses extends even to the level of individual effector cells, each of which represents a source of multiple mediators that may have overlapping, synergistic, or even antagonistic effects in allergic inflammation. To cite one example, mast cells represent a potentially unique source of tryptase (58), which has a number of actions that can contribute to the pathogenesis of allergic inflammation and airways hyperreactivity (77, 100, 102, 105). However, the relative importance of tryptase, as opposed to TNF- α or other mediators, as a component of allergic inflammation in human asthma (or other disorders) is not yet clear. In the clinical setting, the best current approximation of a "deletion experiment" to address this issue may be the use of highly specific inhibitors of the individual candidate effector molecules. Specific inhibitors of human mast-cell tryptase have recently been reported (110), and these agents may help to assess the importance of tryptase in various clinically significant examples of allergic inflammation. Similarly, highly specific neutralizing antibodies to human TNF- α (30, 34) may help in efforts to probe the relative importance of TNF- α in the pathogenesis of allergic asthma.

However, only time and appropriate studies will determine whether, and under what circumstances, the administration of single or multiple agents with actions that may be highly specific for individual candidate mediators (e.g., tryptase inhibitors, anti-cytokine antibodies) will be useful as treatments for asthma and other allergic disorders. It will be particularly important to compare the efficacy and safety of such approaches to those based on the use of agents that have broader actions (e.g., corticosteroids and other immunosuppressive drugs), but which thereby can interdict many of the diverse effector mechanisms that may contribute to the pathogenesis of these diseases. Similarly, it will be of interest to determine the extent to which the clinical efficacy of broadly "immunosuppressive" agents reflects their actions on specific effector cells. For example, *in vitro* data and animal studies indicate that both corticosteroids and CsA can suppress mast-cell cytokine

production (24, 54, 61, 119). While these findings raise the possibility that effects on mast-cell cytokine production contribute to the clinical benefit of these agents, at least in some disorders, they do not clarify the relative importance of the actions of these agents on mast cells, as opposed to other cell types.

No discussion of allergic inflammation is complete without a brief consideration of the possibility that some of the cells and mediators that participate in these processes may help to *downregulate* the responses. Even cytokines that mediate certain "pro-inflammatory" effects may have other actions that are "anti-inflammatory" in nature. For example, studies of TGF- β 1-null mice indicate that the anti-inflammatory effects of TGF- β may be quite important *in vivo* (103), and both mast cells (48, 86, 112) and eosinophils (81, 125), which are recruited in large numbers to sites of allergic inflammation, represent potential sources of this cytokine. It is thus possible that certain "effectors" of allergic inflammation, including the mast cell and the eosinophil, can express some actions which help to limit the duration or intensity of the reactions. If major cellular "effectors" of allergic responses can indeed also express functions that are "anti-inflammatory" or "reparative" in nature, then the identification of factors which can instruct these cells to abandon their destructive tendencies in favor of more protective actions would be of considerable interest.

In summary, despite much recent progress, the "riddle of the mast cell", that is, its actual functions in health and disease (89), remains to be completely solved. On the other hand, many of the experimental tools and conceptual frameworks necessary to solve this problem are already in hand, and we can look forward to further progress with some confidence and considerable excitement.

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