

Roles of mast cells and basophils in innate and acquired immunity

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There have been several recent advances in knowledge about mast cells and basophils in immune responses, of which some are particularly important: a role has been found for heparin in the storage of certain proteases and other mediators in mast cell cytoplasmic granules; an important role for mast cells in the development of several chronic aspects of an asthma model in mice has been discovered; and a new approach has been developed, based on the generation of mast cells from embryonic stem cells *in vitro*, to investigate mast cell function *in vitro* or *in vivo*.

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Current Opinion in Immunology 2000, 12:624–631

0952-7915/00/\$ – see front matter

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Abbreviations

LPR late-phase reaction

LTC₄ leukotriene C₄

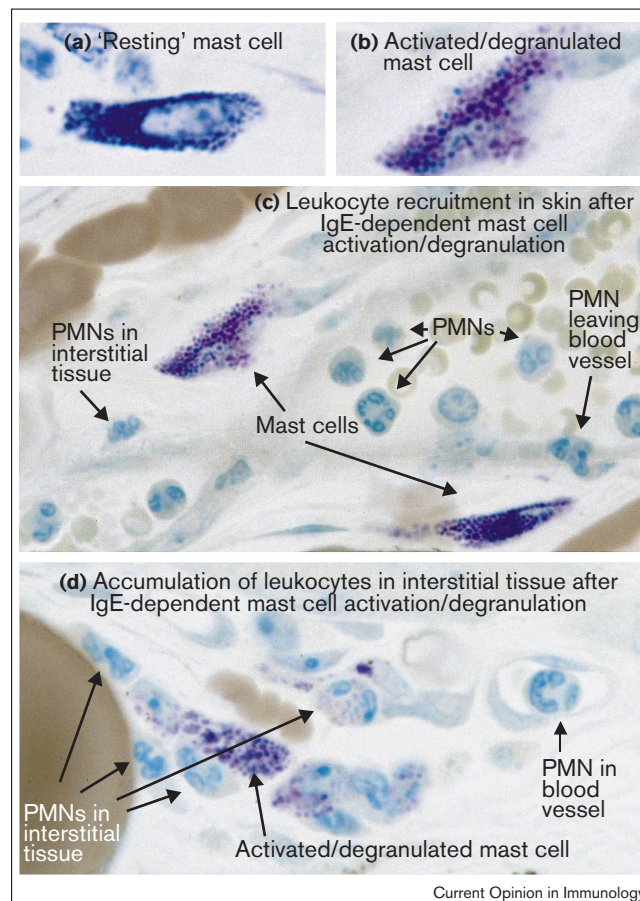
SCF stem cell factor

Introduction

Mast cells and basophils share a number of similarities but they also differ in many aspects of natural history and function. Both cell types express the $\alpha\beta\gamma_2$ form of the high-affinity receptor for IgE (Fc ϵ RI) on their surface and both mast cells and basophils can be activated to secrete diverse preformed mediators and lipid mediators (the latter are synthesized *de novo*), as well as cytokines, after crosslinking of Fc ϵ RI-bound IgE with bivalent or multivalent antigen. However, recent work shows that mast cells can also play a critical role in innate immunity to bacterial infection and that mast cells and basophils can be activated by viral proteins. This review will discuss recent advances in our understanding of the effector and the potential immunoregulatory functions of mast cells and basophils during innate or acquired immune responses.

Mast cells and basophils not only are important effector cells in acute IgE-associated allergic reactions but also may contribute significantly to the expression of aspects of acquired immune responses that develop over hours (e.g. late-phase reactions [LPRs]) or days to weeks (e.g. chronic allergic inflammation) [1–5] (Figure 1). Mast cells and basophils may also mediate immunoregulatory functions, both through their ability to produce certain cytokines and by other mechanisms [1–6]. Before we discuss the potential roles of mast cells and basophils in acquired or innate immunity, we will provide some background on the biology of these two cell types.

Figure 1



IgE-dependent activation of mast cells can induce leukocyte recruitment to sites of allergic responses. The figure shows the degranulation of mast cells and the subsequent recruitment of leukocytes (particularly polymorphonuclear leukocytes [PMNs]) from adjacent vessels. Mice were sensitized by intradermal injection of DNP-specific IgE and were challenged 24 hours later with DNP-HSA intravenously. (a) Resting and (b) activated mast cells can readily be distinguished. The figure illustrates that mast cells (c) not only contribute to leukocyte recruitment and migration out of blood vessels in the early phase of an allergic response but (d) are also importantly involved in orchestrating the accumulation of leukocytes in the interstitium of the dermis; these are also characteristic features of later stages of allergic inflammation in humans (i.e. during LPRs and chronic allergic inflammation). Photomicrographs (by JW) are of 1 μ m, Epon-embedded, Giemsa-stained sections of ear skin (a) before, (b,c) at 6 hours or (d) at 8 hours after induction of IgE-dependent passive cutaneous anaphylaxis. Original magnifications were $\sim 2000\times$ (a,b) and $\sim 1000\times$ (c,d).

Basic mast cell and basophil biology

Both mast cells and basophils are derived from CD34⁺ hematopoietic progenitor cells, such as those present in adult bone marrow; however, mast cells and basophils differ in natural history. Whereas basophils (like other granulocytes)

typically mature in the bone marrow and then circulate in the peripheral blood, from where they can then be recruited into the tissues, mature mast cells typically do not circulate in the blood but complete their differentiation in vascularized tissues [1–5]. Under physiological conditions, basophils have a short life-span of several days. IL-3 can promote the production and survival of human basophils *in vitro* and can induce basophilia *in vivo* [4,7,8]. Unlike basophils, mast cells can be very long-lived and mast cells that are apparently mature can proliferate under certain conditions [1,2]. Many aspects of mast cell development and survival are critically regulated by stem cell factor (SCF); this is the ligand for the c-kit receptor, a member of the receptor tyrosine kinase III family of growth factor receptors, which is expressed on the mast cell surface [1,9,10].

Upon appropriate stimulation (e.g. via the FcεRI), mast cells and basophils can secrete mediators that are either preformed and granule-associated (e.g. histamine, proteoglycans and neutral proteases) or are synthesized *de novo* (e.g. leukotriene C₄ [LTC₄], platelet activating factor [PAF] and [in mast cells only] prostaglandin D₂ [PGD₂]). Furthermore, mouse or human mast cells represent potential sources of many cytokines with effects in inflammation, immunity, hematopoiesis, tissue remodeling and diverse other biological processes (e.g. IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, TNF-α, bFGF, VEGF, TGF-β and several C-C chemokines, including MIP-1α and MCP-1); by contrast, the spectrum of basophil-derived cytokines appears to be more limited but includes IL-4 and IL-13 [11–13].

The cytoplasmic granules of both mast cells and basophils stain metachromatically with certain basic dyes, probably primarily reflecting their content of proteoglycans such as chondroitin sulfates (in basophils and mast cells) and heparin (which is thought to be produced exclusively by mast cells). The biological functions of basophil and mast cell proteoglycans are not fully understood; however, in mice, heparin is required for the normal packaging of certain neutral proteases in mast cell cytoplasmic granules [14•,15•]. Mast cells in different tissues or species, or at different stages of development, can vary in proteoglycan and/or protease content and in many other aspects of phenotype and function, a phenomenon called ‘mast cell heterogeneity’ [1–5]. Recent evidence suggests that basophils may be able to exhibit phenotypic heterogeneity as well [16].

Genetic approaches in studies of mast cell or basophil function

A particularly useful approach to study mast cell function *in vivo* is to employ genetically mast-cell-deficient WBB6F₁-*Kit^W/Kit^{Wv}* mice (*W/W^v* mice), which lack expression of a functional c-kit receptor due to spontaneous mutations in both copies of *c-kit* [9,17]. The mast cell activity in these *W/W^v* mice can be selectively reconstituted by the adoptive transfer of genetically compatible, *in-vitro*-derived mast cells; such mast cells can be derived

in at least two ways: firstly, from the bone marrow cells of either the congenic wild-type mice or various transgenic or knockout mice (bone-marrow-derived, cultured mast cells); or, secondly, directly from embryonic stem cells (embryonic-stem-cell-derived mast cells; Figure 2) [1,9,18•]. Such *in-vitro*-derived mast cells can be administered by intravenous, intraperitoneal or intradermal injection, or by direct injection into the anterior wall of the stomach, thus producing so-called ‘mast cell knockin’ mice [1,5,18•]. This approach permits one to assess directly how the expression of biological responses *in vivo* differs in the presence or absence of wild-type mast cells or mast cells with certain genetic alterations (including ‘embryonic lethal’ mutations) that affect mast cell phenotype or function [1,18•,19].

Unfortunately, there is no analogous ‘basophil knockin’ system available at this time. Even IL-3^{-/-} mice (which fail to develop basophilia during infections with certain intestinal nematodes) have normal baseline levels of bone marrow basophils and circulating basophils and therefore can not be regarded as truly ‘basophil-deficient’ [8].

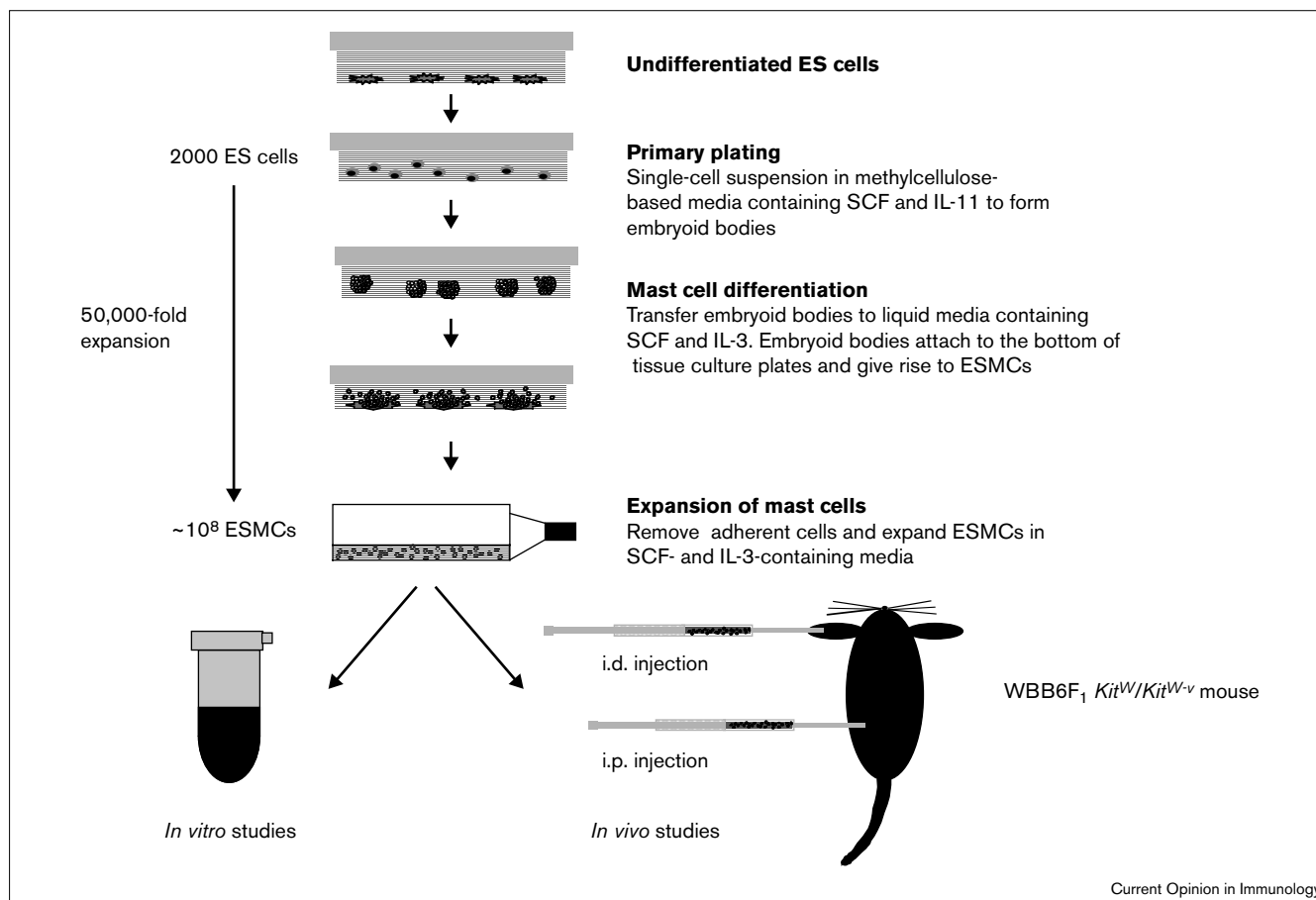
Mast cells and basophils in acquired immunity

The best-studied forms of acquired immunity involving mast cells and basophils are examples of IgE-associated immune responses. The aggregation of FcεRI that are occupied by IgE (e.g. following binding of IgE to multivalent antigens) is sufficient for initiating downstream signaling pathways that activate the cells to degranulate and also induce the *de novo* synthesis and secretion of lipid mediators and cytokines [20–22].

In vitro and *in vivo* studies in both mice and humans have revealed that levels of FcεRI surface expression on mast cells and basophils can be regulated by IgE [1,23–25] (Figure 3). IgE-dependent upregulation of FcεRI expression can permit mouse or human mast cells to secrete increased amounts of preformed and lipid mediators after administration of anti-IgE or antigen challenge, to exhibit IgE-dependent mediator release at lower concentrations of specific antigen and to secrete greatly enhanced amounts of cytokines and growth factors (including IL-4) [1,23,25]. Thus, basophils and mast cells in subjects with high levels of IgE (as typically characterizes patients with allergic disorders or parasite infections) can be significantly enhanced in their ability to express IgE-dependent effector and/or potential immunoregulatory functions.

In the mouse, mast cells also express FcγRIII and can undergo IgG₁- and FcγRIII-dependent activation in such settings as active anaphylaxis [26] and the Arthus reaction [27]. In certain mast cells, FcεRI-dependent mediator secretion can be downregulated by engagement of FcγRIIb [28] or gp49 [29] receptors. Beyond influencing immune responses via the secretion of cytokines, *in vitro* evidence has suggested that mast cells and/or basophils can function as antigen-presenting

Figure 2



In vitro differentiation of mast cells from embryonic stem (ES) cells. As recently reported in Tsai *et al.* [18**], $\sim 10^8$ ES cell-derived mast cells (ESMCs) can be derived from ~ 2000 ES cells in ~ 5 – 6 weeks, an expansion of $\sim 50,000$ -fold. ESMCs are an essentially pure population of mast cells that express functionally active Fc ϵ RI and c-kit receptors and that can survive, and express IgE-dependent function, after

adoptive transfer into the skin (after intradermal [i.d.] injection) or peritoneal cavity (after intraperitoneal [i.p.] injection) of genetically mast-cell-deficient WBB6F₁-Kit^W/Kit^{W-v} (W/W^v) mice. Thus, the effects of mutations of genes that are expressed in ESMCs, including many 'embryonic lethal' mutations, can be assessed either *in vitro* or, after adoptive transfer of the ESMCs to W/W^v mice, in adult animals *in vivo*.

cells [6] and/or represent sources of co-stimulatory activity (e.g. by expressing CD40 or its ligand) [30].

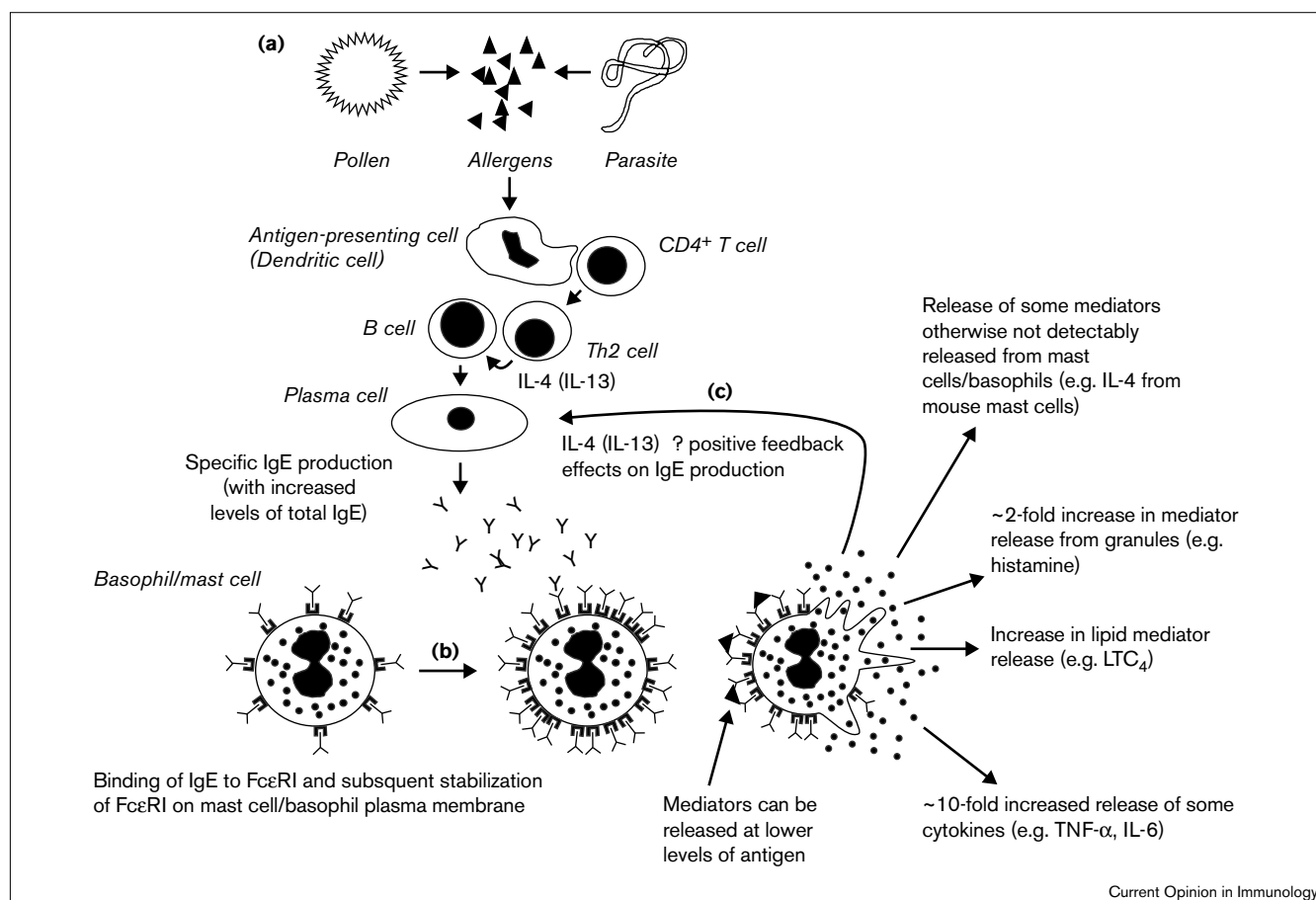
IgE-associated immune responses

Studies in 'mast cell knockin' mice show that mast cells are essential for virtually all of the augmented vascular permeability and tissue swelling that are associated with IgE-dependent passive cutaneous anaphylaxis reactions [1]. Several findings indicate that mast cells are critical for the immediate phase of IgE-associated 'Type I' reactions in humans as well [1–5,31]. However, in many allergic patients, the immediate reaction to cutaneous antigenic challenge is followed 4–8 hours later by persistent swelling and leukocyte infiltration (i.e. the LPR; see Figure 1) [1–5,32]. Many of the clinically significant consequences of IgE-dependent reactions, both in the respiratory tract and in the skin, are now thought to reflect the actions of the leukocytes recruited to these sites during LPRs rather than the direct effects of the mediators released by mast cells at early intervals after antigen challenge [1–5,32].

Several lines of evidence, derived from both clinical and animal studies, indicate that mast cell activation can contribute to the leukocyte infiltration associated with LPRs. Thus, studies in 'mast cell knockin' mice have demonstrated that mast cells are required for essentially all of the leukocyte infiltration that is associated with IgE-dependent reactions in the skin (see Figure 1) or stomach [33,34]. Both cytokines (e.g. TNF- α [33]) and neutral proteases [35], as well as biogenic amines and lipid mediators [1–5], may contribute to mast-cell-dependent leukocyte recruitment in such settings. The leukocytes recruited to sites of LPRs can include basophils, eosinophils, neutrophils, lymphocytes and monocytes/macrophages; all of these cells may influence the reactions by providing additional proinflammatory mediators and cytokines, with the relative importance of the various *potential* effector cells varying depending on the individual circumstances [1–5].

The release of cytokines and other mediators from mast cells and basophils has the potential to influence many

Figure 3



Enhanced IgE-dependent effector function and potential immunoregulatory function in mast cells or basophils after IgE-dependent upregulation of FcεRI surface expression. **(a)** Various factors can trigger immune responses whereby specific IgE is produced by plasma cells. **(b)** Binding of IgE upregulates its receptors and results in response at lower levels of antigen (e.g. allergens) and in increased release of mediators and cytokines. **(c)** There may also be a positive feedback via IL-4 and IL-13 that increases IgE production. Note that studies in IgE^{-/-}

mice indicate that low levels of FcεRI surface expression can be observed in mast cells/basophils in the complete absence of IgE and that low 'baseline' levels of blood IgE result in levels of FcεRI on peritoneal mast cells that are ~5-fold those observed in IgE^{-/-} mice [23]. The depicted consequences of IgE-dependent enhancement of FcεRI-dependent function are based primarily on *in vitro* studies with mouse [23] or human [25] mast cells or human basophils [24] and are supported by studies of human skin mast cells *in vivo* [24].

aspects of the pathophysiology at sites of allergic diseases, including some of the chronic changes (e.g. airway hyper-reactivity, connective tissue changes and enhanced mucus production) that are associated with these disorders [1–5,36]. Furthermore, given that mast cell degranulation results in the local release of proteoglycans that can bind a variety of growth factors [37], FcεRI-mediated mast cell activation may also regulate the function, concentration and spatial distribution of cytokines and growth factors within the tissue microenvironment. Studies in 'mast cell knockin' mice show that mast cells can contribute significantly to the development of at least three of the long-term features of asthma in mouse models of the disorder: allergen-induced bronchial hyper-reactivity to cholinergic stimulation [38*,39**]; eosinophil infiltration [39**]; and increased numbers of proliferating cells in the airway epithelium [39**]. Furthermore, IL-4 — which can be released from either

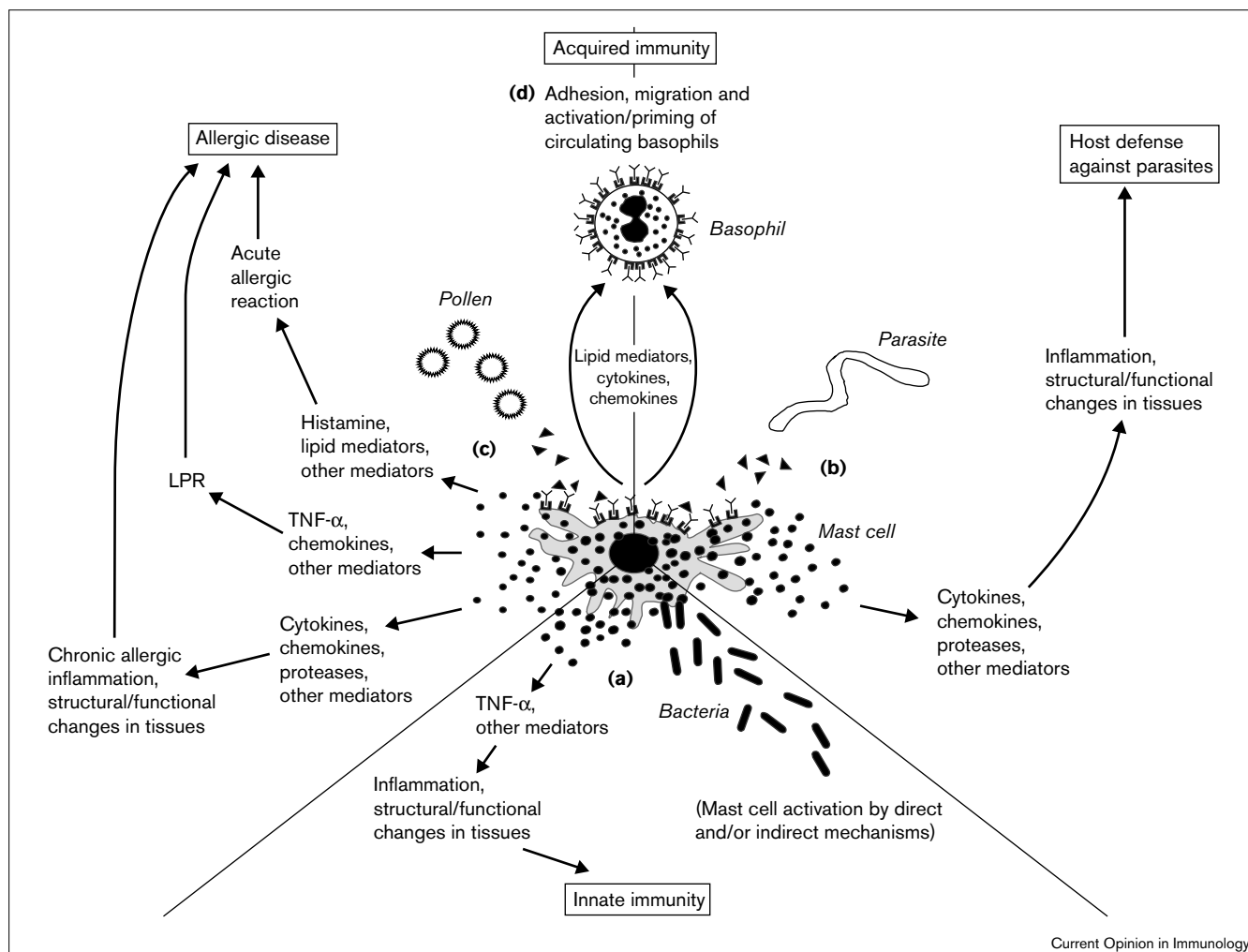
T cells or basophils, which are recruited to sites of allergic inflammation, as well as from at least some mast cells — may contribute to the local enhancement of mast cell effector function and proliferation [25,40*,41*].

Host defense against parasites

Parasite infections are often associated with increased levels of circulating basophils and eosinophils, markedly increased serum levels of IgE (and therefore enhanced expression of FcεRI on basophils and mast cells) and increased numbers of mast cells and/or basophils in the affected tissues [1–5].

Although it has been difficult to prove that individual components of IgE-associated immune responses to parasites are truly essential for the expression of host immunity, W/W^v mice exhibit a delayed clearance of certain parasites

Figure 4



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Roles of mast cells (and basophils) in acquired immune responses (including those involved in allergic disorders or in host defense against parasites) and in innate immunity. A mast cell is shown centrally here for simplicity but several similar functions can be carried out by basophils. Mast cells can exhibit protective functions (a) in the context of innate immunity (e.g. against bacteria) as well as (b) in acquired, IgE-associated responses (e.g. against helminths [or ticks]) by releasing TNF- α and other mediators that orchestrate local inflammatory responses with beneficial effects for the host. (c) However, when

IgE-dependent mast cell and/or basophil activation is induced in response to otherwise innocuous environmental antigens (e.g. allergens derived from pollen), this can contribute to the expression of allergic diseases (e.g. hay fever or asthma). (d) In IgE-associated responses, mast cell activation can induce the recruitment of leukocytes – including basophils – to the site of inflammation, providing additional Fc ϵ R1⁺ cells that then can function as additional effector and potential immunoregulatory cells. Not shown is mast cell and basophil activation by viral products, some of which bind to IgE (see text).

[1]. *W/W^v* mice that are also devoid of IL-3 (i.e. *W/W^v*, IL-3^{-/-} mice, which are profoundly mast cell deficient and fail to develop a basophilia during nematode infection) exhibit an especially striking impairment in their ability to expel a primary infection with the nematode *Strongyloides venezuelensis* (Sv) [8].

Basophils appear to be required for the full expression of immune resistance to infestation of the skin by larval ixodid ticks of the species *Amblyomma americanum* in guinea pigs [42] and may contribute to immune resistance to the feeding of larval ticks of the species *Dermacentor variabilis* in mice [43]; however, expression of IgE-dependent immune

resistance to cutaneous feeding by larval *Haemaphysalis longicornis* ticks in mice is dependent on mast cells [44]. These data suggest that mast cells and basophils may provide similar or overlapping effector functions in antiparasite immunity, with the relative contributions of one or the other cell type varying according to such factors as the species of the parasites, the specific host and the site of infection.

Mast cells and basophils in innate immunity

'Mast cell knockin' mice were used to show that mast cells can represent a central component of innate host defense against certain bacterial infections, that the recruitment of

circulating leukocytes with bactericidal properties is dependent on mast cells and that TNF- α is one important element of this response [10,45,46]. Whereas certain bacterial products — including lipopolysaccharide and at least one fimbrial adhesin [47^{*}] — can directly induce the release of some mast cell products, pathogens may also activate mast cells indirectly via activation of the complement system [10,48].

Maurer *et al.* [19] found that repetitive administration of SCF (which can increase mast cell numbers and also influence mast cell function) can improve the survival of normal mice subjected to cecal ligation and puncture (CLP), a model of acute bacterial peritonitis. SCF treatment not only increased numbers of peritoneal mast cells in C57BL/6 mice but also significantly enhanced the survival of these mice (and TNF- $\alpha^{-/-}$ mice) after CLP. Experiments in mast-cell-reconstituted *W/W^v* mice indicated that this effect of SCF treatment reflected, at least in part, the actions of SCF on mast cells.

Additional pathways for eliciting mast cell and/or basophil function during immune responses

Human basophils have been shown to respond to stimulation with immobilized secretory IgA (sIgA) by releasing both histamine and LTC₄; however, this only occurs if the cells had first been primed by pretreatment with IL-3, IL-5 or GM-CSF [49]. Since IgA is the most abundant immunoglobulin isotype in mucosal secretions, this finding suggests that sIgA may contribute to basophil activation during immune responses at mucosal sites [49].

Protein L of *Pneumococcus magnus* and Protein A of *Staphylococcus aureus* are bacterial superantigens that can interact with distinct regions of IgE and therefore induce mediator release from basophils and mast cells [50]. In addition, Patella *et al.* [51,52^{*}] recently demonstrated that mast cells and basophils can be activated by viral proteins. The HIV glycoprotein 120 (gp120), as well as Protein Fv (pFv) — which is released into the intestinal tract in patients with viral hepatitis — can interact with the V_H3 domain of IgE and thereby induce histamine, IL-4 and IL-13 release from human basophils and mast cells [51,52^{*}]. Accordingly, basophils and/or mast cells activated by gp120 may represent a source of IL-4 and IL-13 during the early stages of HIV-1 infections — which are characterized by a switch from a Th1-type towards a Th2-type pattern of cytokine production — as well as during later phases of HIV infection, when Th2 cells are reduced in number [52^{*}].

Many bacterial products have been shown to have direct effects on mast cell or basophil function, at least *in vitro* [10]. However, host responses to pathogens may also be associated with mast cell activation via other mechanisms; such mast cell activation in turn can have significant effects on the function of the affected organs. For example, mast cells can promote epithelial chloride secretion in a model of intestinal anaphylaxis [53], can promote colonic

mucin release in a mouse model of immobilization stress [54] and can contribute to both neutrophil recruitment and intestinal fluid secretion in a model of enteritis that is induced by *Clostridium difficile* toxin A [55].

Conclusions

It seems very likely that mast cells and basophils express complex — and partially overlapping — roles in acquired and innate immunity (Figure 4); these roles include both effector cell and, potentially, immunoregulatory activities.

Studies in mast-cell-reconstituted, genetically mast-cell-deficient mice — and correlative analyses in humans — indicate that mast cells can contribute to leukocyte infiltration, tissue remodeling and long-term functional changes in the context of IgE-associated acquired immunity and to innate immunity against certain bacteria. Basophils can be prominent in the leukocytic infiltrates elicited at sites of IgE-associated LPRs in the skin, nose and airways of humans, and may contribute to the expression of acquired immunity to certain nematodes or to the feeding of ectoparasites such as ticks.

Both basophils and certain mast cells can produce IL-4 and IL-13, which may influence IgE production, and mast cells can produce a large number of cytokines and growth factors that may contribute to the ability of these cells to influence many of the structural and functional changes that can be observed in tissues at sites of chronic allergic inflammation or other adaptive or pathological processes.

Finally, it is possible that some of the 'effector cells' of IgE-associated immunity, including the mast cell and basophil, can also express cytokine-dependent and other actions that help to limit the intensity or duration of such responses [1–5].

Acknowledgements

Some of the work reviewed herein was supported by United States Public Health Service grants CA 72074, AI 23990 and AI 41995 (project 1), by Deutsche Forschungsgemeinschaft grant WE 2300/1 and/or by AMGEN Inc. SJG consults for AMGEN Inc. under terms that are in accordance with Stanford University conflict-of-interest policies.

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