

IgE-independent activation of human mast cells indicates their role in the late phase reaction of allergic inflammation

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Received 2 January 2002; accepted 4 June 2003

Key words: mast cells, eosinophils, tryptase, chymase, cytokines

Abstract

Mast cells are tissue dwelling cells that have a clear-cut pathologic role in allergy. Besides that, they participate in several chronic inflammatory conditions, helminthic parasitosis, and in some solid tumor reactions, but also in physiological situations, such as wound healing and innate immunity. Mast cells produce and release various mediators after activation induced by either IgE-dependent or IgE-independent mechanisms. Although much information has been gathered on the immunological (IgE-dependent) mast cell activation both *in vivo* and *in vitro*, not much is known about the non-immunological (IgE-independent) activation particularly in human mast cells. Mast cell IgE-independent activation is mediated through Gi3alpha which has been identified in rat mast cells as the pertussis toxin (Ptx)-sensitive heterotrimeric G protein that interacts with cationic secretagogues inducing PLC-independent mast cell exocytosis. Mast cell IgE-independent activation in allergy most likely occurs when mast cells encounter eosinophils, the main inflammatory cells that persist throughout the late and chronic phases of the allergic reaction. This review summarizes the influence of eosinophils on mast cell activation demonstrating that IgE-independent activation has a significant role in pathophysiological processes.

Introduction

Mast cells are tissue dwelling cells containing prominent cytoplasmic granules. These proteoglycan and protease-rich granules have led to the recognition of mast cells by Paul Ehrlich in the late 1800s (Ehrlich 1877). Mast cells have a clear role in allergy, but also in other physiological situations such as wound healing, fibrosis and native immunity (Church et al. 1997). Mast cells are derived from hematopoietic stem cells (Kitamura, et al. 1978), although, unlike other leukocytes, they do not mature in the bone marrow and circulate as committed progenitors. These progenitors complete their maturation with phenotypic diversity after entering different tissues (Gurish and Austen 2001).

In rodents, two main mast cell subtypes have been described according to their tissue localization. The

first subtype, the T-cell dependent mucosal mast cell, is mainly found in the mucosa of the gastrointestinal tract and in the lamina propria of the respiratory tract. The second subtype, the T-cell independent connective tissue mast cell, is mainly located in the submucosa of the gastrointestinal tract, in the skin and in the peritoneum (Coffman 1989).

In humans, mast cells have been further classified according to their neutral protease content. One phenotype contains only tryptase (MC_T) and the other one contains both tryptase and chymase (MC_{TC}) (Church et al. 1997). MC_T appear to be immune system-related, whereas MC_{TC} appear to have functions in tissue remodeling and angiogenesis (Church et al. 1997). Both phenotypes express FcεRI and participate in IgE-dependent allergic or parasitic reactions. Human mast cells display heterogeneity in respect to cytokine content too. IL-4 is distributed between both phenotypes,

Table 1. Human mast cells: Characteristics of MC_{TC} and MC_T phenotypes

MC _T	MC _{TC}
Immune system associated	Non immune system associated
Tryptase	Tryptase, Chymase, Cathepsin G
IL-4, IL-5, IL-6	IL-4, low IL-5, low IL-6
Increased in allergic and parasitic diseases	Unchanged in allergic and parasitic diseases
Increased near sites of Th-cell activation	Increased in tissue remodeling and angiogenesis

whereas IL-5 and IL-6 are restricted almost exclusively to the MC_T subset (Church et al. 1997) (Table 1).

Mast cells release a large number of pro-inflammatory, immunoregulatory and tissue regulatory mediators after activation. Some are granule associated and preformed, such as histamine, proteoglycans (heparin and chondroitin sulfate) and neutral proteases. Newly generated lipid mediators comprise PGD₂, LTC₄, PAF, and to a lesser extent LTB₄. Rodent and human mast cells also synthesize and release a wide variety of cytokines and chemokines, including IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, RANTES, IFN- γ , TGF- β , TNF- α and GM-CSF (Church et al. 1997; Gordon et al. 1990).

IgE-dependent and IgE-independent activation

The study of human mast cells has been long proved to be difficult. Culturing human mast cells from tissues yields insufficient numbers of cells in order to perform growth, differentiation and functional studies. A possible source for culturing human mast cells is human cord blood (Saito et al. 1996). Progenitor cells bearing CD34+ and *c-kit* (CD117) are cultured in the presence of *c-kit* ligand (SCF) (Rottem et al. 1994), IL-6 and PGE₂. Following a period of 8–10 weeks the human cord blood-derived mast cells are mature and mostly express the MC_{TC} phenotype.

Cord blood-derived mast cells can be activated by IgE-dependent, IgG-dependent, and by non immunologic pathways. The IgE-dependent activation is initiated upon interaction of an allergen with its specific IgE antibody attached to the cell membrane via its high-affinity receptor Fc ϵ RI. Cross-linkage of IgE specific Fab fragments with the multivalent antigen initiates mast cell degranulation, mediator generation and cytokine release (Metcalf et al. 1997). The release of

histamine and other preformed lipid mediators from mast cells by immunological activation is a rapid process that is completed in less than 30 min. There is an absolute requirement for the presence of extracellular calcium, and optimal release takes place at 37°C (Foreman and Mongar 1972).

Human cord blood-derived mast cells are sensitive also to IgE-independent activation. This type of stimulation is caused by polybasic compounds (Lagunoff et al. 1983) that share similar structural features essential for their activity. These include positive charges associated with a hydrophobic structure such as the carboxy terminus of substance P or the aromatic ring of compound 48/80 (Aridor et al. 1990). The polybasic compounds appear to release histamine by a common mechanism (Sagi-Eisenberg et al. 1985). They activate directly a pertussis toxin-sensitive G protein that is coupled to PLC activation (Gsp) and another pertussis toxin-sensitive G protein that is coupled to exocytosis (Gi3 α), which is PLC-independent (Aridor et al. 1993).

The activation of mast cells by compound 48/80 or substance P, as observed in mouse bone marrow-derived mast cells (Ogasawara et al. 1997) and in the mouse cell line CFTL (Ansel et al. 1993), elicits not only exocytosis, but also eicosanoid generation and cytokine production, both of which are prominent responses following IgE-dependent activation of mast cells. These observations are related to recent results in which it has been demonstrated that the stimulation of G-proteins by the basic secretagogues, results in the propagation of two distinct signalling pathways involving Gi3 and PTK that induce histamine release and arachidonic acid metabolites, respectively (Shefler et al. 1998). Since PTK are also involved in MAPK activation that finally leads to cytokine gene expression, it is probable that G-protein stimulation induces the synthesis and secretion of cytokines in mast cells following a non-immunologic activation.

We have recently found that eosinophil-derived major basic protein (MBP) causes histamine release from IgE-activated rat peritoneal mast cells (Piliponsky et al. 2001) and from resting human lung mast cells after co-culture with fibroblasts (Piliponsky et al. 2001a).

These recent findings indicate that an important cross-talk exists between mast cells and eosinophils. Indeed, human cord blood-derived mast cells are activated by MBP and compound 48/80 to release a variety of mediators such as histamine, PGD₂, and TNF- α (Piliponsky et al. 2001b). Moreover, cord blood-derived mast cells are also activated by MBP

and compound 48/80 to release GM-CSF and IL-8. Similarly, it has been shown that human heart mast cells are activated by ECP and MBP to release histamine and LTC₄ (Patella et al. 1996).

Mast cells can also influence eosinophils for example mast cell mediators such as histamine and PGD₂ activate human eosinophils *in vitro* (Raible et al. 1992). In addition, mast cell derived TNF- α enhance eosinophil survival partially by inducing the autocrine production of GM-CSF (Levi-Schaffer et al. 1998) which in turn elicits eosinophil survival and ECP release (Okayama et al. 1998). Tryptase is another preformed mediator present in all mast cells. Tryptase can induce ECP release from eosinophils (Numerof et al. 1996) and act as one of their chemoattractants (Jung et al. 1995). Recently, we have shown that tryptase activates the MAPK/AP-1 pathway in human peripheral blood eosinophils thus, inducing IL-8 and IL-6 release (Temkin et al. 2002).

When eosinophils encounter mast cells in the tissue during the late phase of the allergic response they find a vast majority of mast cells that have already been activated by IgE-dependent mechanisms and that therefore underwent a partial desensitization to the antigen (Jung et al. 1995). However, at this time mast cells are still capable of residual histamine release together with other preformed mediators upon a non-IgE dependent activation (Sullivan 1982). Therefore, we suggest that the interactions between mast cells and eosinophils are bilateral. Mast cells initiate the early-phase response, causing an infiltration of inflammatory cells, notably the eosinophils. The cross-talk between these two cells causes a positive loop of mediator release which aggravates and prolongs the allergic response.

Conclusions

All of the findings reported here showing that eosinophil-derived products can activate human mast cells, suggest that *in vivo* this interaction can occur during the late phase response of allergic reactions. Therefore, we hypothesize that mast cells are both initiators of the early phase response, as well as still effector cells of the late phase by delivering mediators to the inflamed tissue.

We believe that the interactions between mast cells and eosinophils can be a major cause of the perpetuation of allergic inflammatory reactions and therefore a target for immunopharmacological modulations of these reactions.

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