Review Article

Human mast cell activation through Fc receptors and Toll-like receptors

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ABSTRACT

Mast cells express high-affinity IgE receptors (FcεRI) on their surface and can be activated to secrete a variety of biologically active mediators by cross-linking of receptor-bound IgE. Recent studies in animal models indicate that mouse mast cells may play a protective role in host defense against bacteria through the production of tumor necrosis factor-α, mainly as a result of Toll-like receptor (TLR) 4- or CD48-mediated activation. Moreover, several recent observations in animal models have indicated that mast cells may also play a pivotal role in coordinating the early phases of autoimmune diseases, particularly those involving auto-antibodies. We recently identified functional TLR4 and FcγRI on human mast cells, in which their expression had been upregulated by interferon-γ. We compared each of the receptor-mediated gene expression profiles with the FcεRI-mediated gene expression profile using high-density oligonucleotide probe arrays and discovered that human mast cells may modulate the immune system in a receptor-specific manner.

Key words: adaptive immunity, FcεRI, FcγRI, GeneChip, innate immunity, mast cell, Toll-like receptor 4.

INTRODUCTION

Mast cells are major effector cells in allergic inflammation and are located at the host–environment interface, where they encounter not only antigens, but also invading pathogens. Recent studies in animal models indicate that mouse mast cells may also play a protective role in host defense against bacteria through the production of tumor necrosis factor (TNF)-α, mainly as a result of Toll-like receptor (TLR)4- or CD48-mediated activation. Tissue mast cells contain TNF-α in their granules and release it by triggered exocytosis. The rapid release of TNF-α is of note because of the pleiotropic pro-inflammatory effects of this cytokine and because its release is more rapid than by other cell systems, which require induction of synthesis after cell activation. The rapid release of TNF-α by mast cells may be important not only for innate immunity, but also for the initiation of adaptive immunity in infection and autoimmune diseases. The present review describes the function of mast cells in infection and autoimmune diseases in animal models, as well as discussing the roles of FcγRI and TLR4 expressed on human mast cells.

MAST CELLS IN ALLERGIC INFLAMMATION

FcεRI is expressed with α-, β-, and γ-chains on mast cells. It binds monomeric IgE with a $K_a$ of $10^{-10}$ mol/L and is believed to essentially be responsible for allergen-dependent allergic responses. Immediate hypersensitivity is an immune reaction that is initiated by antigen binding to IgE preattached to mast cells that leads to the secretion of inflammatory mediators. Following aggregation of FcεRI, mast cells release biogenic
amines, such as histamine and proteases, including tryptase and chymase. Activated mast cells also synthesize prostaglandin D$_2$ and leukotriene C$_4$, as well as other mediators known to cause bronchoconstriction, mucus secretion and edema formation. The role of mast cells in potentiating the late allergic response has been attributed, in part, to mast cell-dependent secretion of pro-inflammatory cytokines and chemokines and the inflammatory infiltrates of the late phase reaction are rich in eosinophils, basophils and Th2 cells (Fig. 1a).

**Mast Cells in Innate Immunity**

Mast cells are inflammatory cells typically found in relatively large numbers in the mucosa of the respiratory, gastrointestinal and urinary tracts, as well as near blood and lymphatic vessels. Because these sites are also common portals of infection, mast cells are likely to be one of the first inflammatory cells to come into contact with invading pathogens and, after activation, they release a myriad of pro-inflammatory cytokines, proteases and inflammatory mediators.

In addition to these inflammatory mediators, mast cells have been reported to contain antimicrobial peptides. Peptide antibiotics have been isolated from all types of organisms, from plants to mammals, and a novel family of peptide antibiotics called ‘piscidins’ has recently been isolated from fish. Interestingly, piscidins have potent, broad-spectrum in vitro activity against many pathogens, including multidrug-resistant bacteria. Interestingly, piscidins reside in mast cells, which are also known as eosinophilic granule cells and are morphologically and functionally similar to their mammalian counterparts. However, whether these fish mast cells are derived from the same lineage as mammalian mast cells is unclear.

Cultured murine mast cells contain cathelicidins and their expression is inducible by lipopolysaccharide (LPS) or lipoteichoic acid. Human skin mast cells have also been reported to express cathelicidins (LL-37) as a result of their detection by immunohistochemical analysis. Thus, mast cells may participate directly in killing microbes.

A number of studies have confirmed that mast cells play a critical role in host immune defense against Gram-negative bacteria through the release of TNF-α. As shown in two different models of acute bacterial infection, mast cell-deficient W/W$^{v}$ mice are less efficient in clearing the pathogenic bacteria in cecal ligation and puncture (CLP)-induced peritonitis or from the lungs of mice challenged intranasally with *Klebsiella pneumoniae*. CD48 has been demonstrated to be the mast cell membrane receptor for *Escherichia coli* expressing the fimbrial adhesion molecule FimH. The interaction between CD48 and bacterial FimH results in mast cell degranulation and concomitant bacterial uptake via lipid rafts.

Murine mast cells have been reported to express TLR2 and TLR4. Peptidoglycan (PGN) from *Staphylococcus aureus* stimulates murine bone marrow-derived mast cells (mBMMC) to produce TNF-α, interleukin (IL)-4, IL-5, IL-6 and IL-13 in a TLR2-dependent manner, but not IL-1β, and TLR2-dependent mast cell stimulation results in mast cell degranulation and Ca$^{2+}$ mobilization. The mBMMC and the murine mast cell line MC/9 express TLR4 mRNA and, when activated with *E. coli*-derived LPS, murine mast cells produce TNF-α, IL-1β, IL-6 and IL-13, but not IL-4 or IL-5. A study using a mast cell-dependent model of acute sepsis revealed higher mortality by TLR4-mutated mBMMC-reconstituted W/W$^{v}$ mice and that TLR4 deficiency in bone marrow mast cells in mice results in significantly higher mortality because of defective neutrophil recruitment and production of pro-inflammatory cytokines in the peritoneal cavity. These findings in murine models suggest that mast cells play important roles in the expression of innate immunity through TLR4.

In humans, cord blood-derived mast cells express mRNA for TLR1, TLR2 and TLR6, but not TLR4. Bacterial peptidoglycan and yeast zymosan, the putative TLR2/TLR6 activators, are potent inducers of granulocyte–macrophage colony stimulating factor (GM-CSF) and IL-1β and also induce substantial short-term cysteinyl leukotriene generation. In contrast, a synthetic triacylated lipopeptide, the putative TLR2/TLR1 activator, induces short-term degranulation but does not induce cysteinyl leukotriene production. Varadaradjalou et al. have reported that both LPS from *E. coli* and PGN induce significant release by cord blood-derived mast cells of not only TNF-α, but also histamine, IL-5, IL-10 and IL-13. Stimulation with PGN induces histamine release.

We investigated the expression of TLR4 on human adult peripheral blood-derived cultured mast cells and discovered functional expression of TLR4 that is upregulated following IFN-γ exposure. To explore systematically how human mast cells modulate the immune system in response to pathogen and antigen, we compared the LPS-induced gene expression profiles in mast cells with the FceRI-mediated profiles using high-density
oligonucleotide probe arrays (GeneChip; Affimetrix, Santa Clara, CA, USA). The results showed both a shared core response and LPS- or antigen-specific programs of gene expression in the mast cells. Mast cells also exhibited an antiviral-response gene program in response to IFN-γ and LPS sustained this expression. (c) FcγRI-mediated human mast cell activation. Human mast cells express the high-affinity IgG receptor FcγRI on their surface and the expression of FcγRI is upregulated by IFN-γ. Aggregation of FcγRI on human mast cells results in histamine release, the generation of prostaglandin D₂ and leukotriene C₄ and the production of a variety of cytokines. Pro-inflammatory cytokines, such as TNF-α and IL-1β, in particular, were found to be upregulated in mast cells following the aggregation of FcγRI compared with aggregation of FcεRI.⁵²

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**Fig. 1** (a) FcεRI-mediated mast cell activation. Mast cells express high-affinity IgE receptors (FcεRI) on their surface and can be activated to secrete a variety of biologically active mediators, such as histamine, lipid mediators and cytokines, by cross-linking of receptor-bound IgE. These mediators cause bronchoconstriction, mucus secretion and edema formation,¹¹ as well as activation of inflammatory cells, such as eosinophils and Th2 cells. (b) Toll-like receptor (TLR) 4-mediated mast cell activation. The TLR4 on human mast cells is upregulated by interferon (IFN)-γ. Lipopolysaccharide (LPS) activates mast cells through TLR4 and induces the expression of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, and the cytokines, in turn, activate macrophages and recruit neutrophils. Mast cells also express an antiviral-response gene program in response to IFN-γ and LPS sustained this expression. (c) FcγRI-mediated human mast cell activation. Human mast cells express the high-affinity IgG receptor FcγRI on their surface and the expression of FcγRI is upregulated by IFN-γ. Aggregation of FcγRI on human mast cells results in histamine release, the generation of prostaglandin D₂ and leukotriene C₄ and the production of a variety of cytokines. Pro-inflammatory cytokines, such as TNF-α and IL-1β, in particular, were found to be upregulated in mast cells following the aggregation of FcγRI compared with aggregation of FcεRI.⁵²
antigen-specific immune responses and that human mast cells may have an important role in innate immunity as well as adaptive immunity.

**Mast Cells in Autoimmune Diseases**

Numerous studies have reported a correlation between the number and/or distribution of mast cells and the development of autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis. As an example, evidence for mast cell activation in the course of multiple sclerosis came from an immunohistochemical demonstration of increasing degranulation in the brain in an animal model and increased amounts of tryptase in the cerebrospinal fluid. Gene expression profiling of multiple sclerosis brain lesions detected a high contribution of transcripts, such as proteases and other inflammatory mediators, derived from mast cells. It is also well known that mast cells accumulate in the synovial tissues and fluids of humans with rheumatoid arthritis. Experimental allergic encephalomyelitis (EAE) is an experimental model of multiple sclerosis, a chronic inflammatory disorder of the central nervous system that is characterized by a breach of the blood–brain barrier, mononuclear cell infiltration of white matter and eventual demyelination. Secor et al. showed that mice lacking mast cells (W/Wv mice) develop EAE later and less severely than control wild-type mice in response to injection of different myelin components, such as myelin oligodendrocyte glycoprotein. Complementation of W/Wv mice with immature mast cells derived in vitro restored typical EAE susceptibility. Mast cell function appears to be dependent on the expression of FcγR, in particular FcγRII, by mast cells. This is true in animal models of rheumatoid arthritis and bullous pemphigoid. Immune complexes may aggregate FcγR to release TNF-α, which recruits neutrophils.

**Expression of the Low-Affinity IgG Receptors FcγRII and FcγRIII on Murine Mast Cells**

There have been extensive studies on the expression of FcγRII and FcγRIII on murine mast cells. Interleukin-3-dependent mBMMC express the low-affinity IgG receptors FcγRIIb1 and FcγRIIb2, and mouse serosal mast cells also express FcγRII. Mature serosal mast cells degranulate when exposed to IgG complexes, whereas mBMMC appear to internalize aggregated IgG without release of substantial amounts of histamine. In later studies, it was reported that FcγRIIB inhibits FcγRI-mediated degranulation of mBMMC and rat basophilic leukemia cells because of the ability of FcγRIIB to recruit Src homology 2 domain-bearing inositol 5-phosphatase. Mouse mast cells alter the surface expression of certain IgG receptors. The mBMMC upregulate the surface expression of FcγRIII when cultured with 3T3 fibroblasts, which may be functionally important because cocultured mBMMC degranulate and generate various lipid mediators when their FcγRII receptors are cross-linked. Mast cells have also been shown to play a role in immune complex-induced injury related to the expression of IgG receptors in a mouse model. As mentioned above, several recent observations indicate that mast cells may also have a key role in coordinating the early phase of autoimmune diseases, particularly those involving auto-antibodies. However, the roles of FcγR in human mast cells in autoimmune diseases remain to be determined.

**Human FcγRI Family Gene Products and Structure**

Three FcγRI genes (i.e., FcγRIα, FcγRIβ and FcγRIc) have been identified as encoding at least six transcripts: FcγRIα1, FcγRIα2, FcγRIβ1, FcγRIβ2, FcγRIβ3 and FcγRIc. The FcγRIα gene product FcγRIα1 uniquely contains a third extracellular domain (EC3), as well as transmembrane and cytoplasmic domains. The presence of these domains, together with the presence of EC2, allows this gene product to bind monomeric IgG with high affinity and to initiate IgG-mediated cellular responses. Two transcripts (b1 and c) have stop codons in EC3 and are believed to only code for soluble secreted proteins. Three transcripts are alternatively spliced isoforms, including one (a2) from gene A and two (b2 and b3) from gene B. FcγRIβ2 lacks EC3 and is not expressed on the cell surface. FcγRIβ3 lacks the signal sequences (S) 2, EC1 and EC3. FcγRIα2 lacks the S2 and EC1. The protein product and function of FcγRIα2 and FcγRIβ3 are unknown. Thus, FcγRIα1 is the only full-length gene product and binds IgG with high affinity (Kd 10^-8 to 10^-9 mol/L). It is expressed at the cell surface as an αγ2 complex. The full-length FcγRI presents three extracellular Ig-like domains on the extracellular portion of its α chain. FcγR chains are required for surface expression of FcγRI on monocytes, macrophages, neutrophils, dendritic cells and eosinophils and for...
signaling mediated by FcγRI. FcγRI expression has been shown to be influenced by immune cytokines. For example, monocytes, macrophages and neutrophils constitutively express FcγRI, but surface expression of FcγRI on neutrophils is strongly increased by IFN-γ.

**Expression of a Functional High-Affinity IgG Receptor (FcγRI) on Human Mast Cells**

Because of the increasing body of evidence in animal models that mast cells may be recruited into allergic reactions by non-IgE-dependent mechanisms and the fact that FcγRIII has been shown to induce murine mast cell degranulation, we hypothesized that human mast cells may also express Fcγ receptors and that the expression of these receptors may be affected by specific factors produced in the microenvironment. Human mast cells derived from CD34+ progenitors in peripheral blood were obtained by culture of progenitor cells with recombinant human (rh) IL-3, rhIL-6 and stem cell factor (SCF) and then for 6–8 weeks with rhIL-6 and SCF. These culture conditions yielded a cell population that was 97% human mast cells, as assessed by Toluidine blue staining, and the mast cell cultures were then enriched to greater than 99.9% mast cells by removing monocytes by adherence. We first determined using reverse transcription–polymerase chain reaction (RT-PCR) that resting human mast cells contain mRNA for FcγRIα1, FcγRIb2, FcγRIIA, FcγRIIB1, FcγRIIB2 and FcγRIII, and then investigated whether pro-inflammatory cytokines can influence the expression of Fcγ receptors on human mast cells. Mast cells were incubated with IL-4, IL-5, IL-10, GM-CSF, nerve growth factor or IFN-γ, but only IFN-γ upregulated the expression of FcγRIα1 and FcγRIIB2. As described above, FcγRIIB2 appears not to be present on the surface membrane, not to bind to either monomeric or complexed IgG and not to be recognized by antibodies to the high-affinity IgG receptor. Thus, according to the results of RT-PCR, human mast cells express the high-affinity IgG receptor, the gene product of FcγRIA, and FcγRI mRNA expression was maximal between 4 and 8 h, when the increase was approximately 10-fold of the baseline level. This was confirmed by flow cytometry, which showed that IFN-γ exposure increased FcγRI expression on human mast cells from approximately 2 to 44%. The intensity of surface expression of FcγRI was maximal at 24 h and appeared to plateau from 24 h through to 48 h. The expression of FcεRI, FcγRII and FcγRIII was unaffected. Although murine mast cells express a functional FcγRIII, FcγRIII protein expression was minimal and unaltered by permeabilization. The presence of FcγRI on the mast cell surface was further confirmed by immunoprecipitation. The human mast cell FcγRI had a molecular mass of approximately 72 kDa, the same as FcγRI expressed by human monocytes. Scatchard plots of FcγRI using 125I-labeled human IgG1 were consistent with these data and the average number of binding sites for monomeric IgG1 (Kd = 4–5 × 10^-8 mol/L) increased from approximately 2400 to 12 000–17 300 per cell. Thus, human mast cells express the high-affinity IgG receptor FcγRI and a low-affinity IgG receptor (FcγRII) on their surface and the expression of FcγRI is upregulated by IFN-γ. Aggregation of FcγRI on human mast cells resulted in histamine release, the generation of prostaglandin D2 and leukotriene C4, and the production of a variety of cytokines. Pro-inflammatory cytokines, such as TNF-α and IL-1β, were particularly upregulated in mast cells following the aggregation of FcγRI compared with the aggregation of FcεRI (Fig. 1c). Because both the FcεRI α chain and the FcγRI α chain require Fcγ (common γ chains) for signal transduction, the FcεRI- and FcγRI-induced gene expression profile appeared to be similar. However, we did find some differences (Y Okayama et al., unpubl. data, 2004), and we are now confirming the data at the protein level.

**Conclusions**

We recently identified a subset of genes that is specifically induced by stimulation through TLR4 and not stimulated by aggregation of FcεRI. Interferon-γ induced a variety of receptors, such as FcγRI, in mast cells and antiviral-response genes were also induced. In addition, we identified a subset of genes that is specifically induced in FcγRI-mediated mast cells. Thus, human mast cells exhibit tailored pathogen- and antigen-specific immune responses, suggesting that human mast cells may play important roles in innate and adaptive immunity.

**Acknowledgments**

This work was supported, in part, by grants from the RIKEN Yokohama Institute, the Grants-in-Aid for
Scientific Research (C) program of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (project no. 14570402 to YO), the Hokuriku Seiyaku Co. Ltd–Japanese Allergy Foundation to YO), the AstraZeneca Asthma Research Award 2002 (to YO), and the Organization for Pharmaceutical Safety and Research of the Ministry of Health, Labour and Welfare (Millennium Genome Project; MPJ-5).

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