

The mast cell: an antenna of the microenvironment that directs the immune response

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Abstract: Mast cells (MCs) have long been considered as critical effector cells during immunoglobulin (Ig)E-mediated allergic disease and immune response to parasites. Recent studies, however, suggest that this understanding of MC function is incomplete and does not consider the complex roles that MCs play in adaptive and innate immunity. The added function gives an innovative vision of regulation of immune responses and the development of autoimmune diseases. It had been assumed that the aggregation of Fc ϵ receptor I with IgE and specific antigen is the main stimulus able to induce the MC activation, degranulation, release, and generation of mediators of the allergic reaction. However, MCs exhibit an array of molecules involved in cell–cell and cell–extracellular matrix adhesion, mediating delivery of costimulatory signals that empower those cells with an ability to react to multiple nonspecific and specific stimuli. Their tissue distribution and their capability to release many cytokines after stimulation indicate MCs as potential regulatory linkers between innate and acquired immunity. In this review, we will summarize some findings on the roles of MCs in innate and acquired immunity, on the molecular mechanism and signaling pathways, and on selective signals that induce discrete MC response and its ability to polarize adaptive-immune response. *J. Leukoc. Biol.* 75: 579–585; 2004.

Key Words: signal transduction · polarization · cytokines · Fc ϵ receptor

ROLE OF MAST CELLS (MCs) IN INNATE AND ACQUIRED IMMUNITY

MCs have long been considered for their initiation and maintenance of anaphylaxis and as effector functions in immunoglobulin (Ig)E-associated allergic disorders and immune responses to parasites. However, recently, these cells and their multiple preformed and de novo-synthesized mediators have been considered to play a wide role in many aspects of natural and acquired immunity [1, 2].

MCs are multifunctional, tissue-dwelling cells. In the bone marrow, under the influence of growth factors, they begin their differentiation from CD34⁺ hematopoietic progenitors [3] to

later migrate in the bloodstream as committed progenitors. Finally, MCs reach the peripheral tissue [4], where they mature under the influence of stem cell factor (SCF) in human [5] and SCF and interleukin (IL)-3 in rodents [6, 7]. However, several molecules are known to promote or augment MC proliferation, such as IL-4 [8], IL-6 [9], IL-9 [10], and IL-10 [11], or to prevent the apoptosis as nerve growth factor [12, 13]. Thus, the local cytokine milieu, in addition to the interaction with neighboring cells or matrix components in the peripheral tissue, leads to the final differentiation into MCs. Although MCs share many common characteristics, MC subpopulations can be differentiated on the basis of tissue localization, size, staining properties, histamine content, as well as proteoglycan and neutral protease composition. These differences in histochemical, biochemical, and functional characteristics result in complex phenotypes describing an extremely heterogeneous population [14]. Furthermore, these subtypes show differences in function, responsiveness to various stimuli that implicate MCs in innate immunity, host-defense mechanism against parasites and bacteria, and the genesis of disease states.

Similar functional abilities are shown also by basophils, which evolve from a CD34⁺/IL-3 receptor α^+ /IL-5⁺ eosinophil/basophil progenitor. Unlike MCs, basophils differentiate completely in the bone marrow, subsequently enter the bloodstream, and reach inflammatory tissues only upon selective recruitment. MC and basophils are well-suited to initiate and propagate immunological and inflammatory responses [15]. However, although MCs are known to release several mediators, such as proteases, arachidonic acid derivatives, cytokines, and chemokines, basophils appear to produce a more restricted subset of such factors, including preferentially IL-4, IL-13, and leukotriene C₄ (LTC₄) [16]. Therefore, by virtue of their tissue distribution and of the broader secretion of mediators upon activation, MCs may represent an important modulatory element of potential interest to the outcome of adaptive-immune response.

The best-studied forms of acquired immunity involving MCs are examples of IgE-associated immune responses including IgE-mediated allergic reactions and host-defense responses against parasites. Moreover, through the ability to produce

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cytokines that mediate immunoregulatory functions, MCs contribute to the expression of acquired-immune responses that develop over hours or days to weeks. Recent studies consider MCs as front line cells of the innate immunity against certain bacterial infections, as when activated, these cells then synthesize and release key immunoregulatory cytokines, one consequence of which is to mobilize a rapid and vigorous inflammatory response. Tumor necrosis factor α (TNF- α) release and recruitment of circulating leukocytes are critical elements of response against pathogens, and characteristically, MCs are the only cell type that can store TNF- α in a preformed state and release it upon activation [17]. These biological characteristics and the strategic location at the host-environment interface, as a sentinel posted in serosal cavities and immediately beneath epithelial surfaces as well as near blood vessels, nerves, and glands, may provide an early defense against invading pathogens. In addition, they express an array of adhesion and immune receptors that may assist in the recognition of invading pathogens and in the sampling of different stimuli coming from the microenvironment, suggesting that these cells could be considered a relevant link between innate and acquired immunity [18].

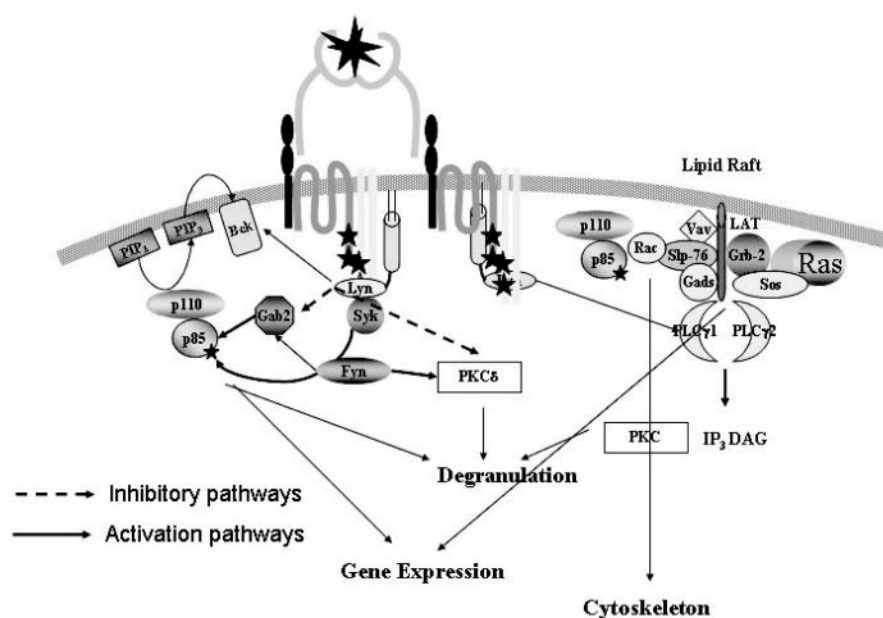
MOLECULAR MECHANISM AND SIGNALING PATHWAYS THAT REGULATE IgE-DEPENDENT MC ACTIVATION

MCs exhibit an array of immune-response receptors and other surface molecules that confer the advanced capability to react to multiple, nonspecific and specific stimuli. Classically, the multivalent binding of antigen (Ag) to receptor-bound IgE and subsequent aggregation of the Fc ϵ receptor I (Fc ϵ RI) provide triggers for MC activation. Ag aggregation of IgE-occupied Fc ϵ RI results in phosphorylation of immunoreceptor tyrosine-based activation motifs by Lyn kinase (Lyn), receptor associ-

ation and activation of Syk kinase (Syk), and inclusion of the Fc ϵ RI in lipid rafts. [19]. Downstream of Lyn and Fyn, multiple adaptor proteins or proteins containing adaptor-like regions have been identified in MCs [20]. These proteins function in the organization of the molecular components required for signaling and can directly influence signal generation and in some cases, MC responses (**Fig. 1**). Among the various adaptors expressed in MCs, Gab2, LAT, and SLP-76 are of particular interest, as their absence has severe consequences on MC responses. When modified by Fc ϵ RI-induced phosphorylation, LAT forms a molecular complex that includes the adapters SLP-76, Grb2, phospholipase C γ 1 (PLC γ 1), and the guanine nucleotide exchange factor, Vav1 [21]. In vivo, LAT-deficient mice are refractive to an anaphylactic challenge, and in vitro LAT deficiency in MCs causes the loss of calcium signals and degranulation because of inhibition of PLC γ activation [22]. However, not all signals or MC responses are lost in the absence of LAT [22]. Thus, whereas LAT is important in IgE-dependent, MC activation, other pathways still exist.

PI-3K and PKC activation increase intracellular calcium levels, allowing this pathway to regulate later events of the signaling cascade. The intracellular calcium flux is required for induction of the mitogen-activated protein kinase (MAPK), c-jun NH $_2$ -terminal kinase (JNK), and p38 cascades and for the calcium-dependent transcription factors nuclear factor of activated T cells and nuclear factor- κ B, which are important components of transcriptionally active complexes in the regulation of MC gene expression [23]. Recently, an alternative pathway of MC activation has been described, which uses a Fyn kinase-dependent pathway that does not require Lyn kinase or the adaptor LAT for its initiation but is necessary for MC degranulation [24]. Fyn seems to play a key role in MC degranulation, similar to that reported for Gab2, LAT, SLP-76, and Syk. In fact, Lyn deficiency enhanced Fyn-dependent signals and degranulation but inhibited the calcium response. Fyn deficiency impaired degranulation, whereas Lyn-mediated

Fig. 1. Fc ϵ RI signaling. Following the Ag aggregation of IgE-occupied Fc ϵ RI, some of the first events to occur are the phosphorylation of receptor by Lyn, receptor association and activation of Syk, and inclusion of Fc ϵ RI in lipid rafts. Moreover, the aggregation of Fc ϵ RI also induces the rapid phosphorylation of Grb2-associated, binder-like protein 2 (Gab2), an event that requires Fyn kinase (p59Fyn). Lyn kinase negatively regulates the Gab2 pathway and MC degranulation but positively regulates the activation of Syk and phosphorylation of linker for activation of T cells (LAT), a complex that is required for normal MC calcium responses. Fyn kinase positively regulates Gab2 and its association with phosphatidylinositol 3-kinase (PI-3K) and is essential for initiation of the degranulation response. It is suggested that LAT signals contribute to the activation of Tec family kinase activation (Btk/Itk). Signals generated by the two distinct adaptor complexes (LAT and Gab2) may be integrated via adhesion and degranulation-promoting adapter protein, a protein that binds Fyn and Src homology 2-containing leukocyte-specific protein of 76 kDa (SLP-76) and regulates cytokine production, MC degranulation, and integrin clustering. PIP, PI phosphate; PKC, protein kinase C; IP $_3$, inositol triphosphate; DAG, diacylglycerol.



signaling and calcium were normal. Thus, Lyn is required for immediate events leading to calcium flux, and Fyn is responsible for complementary signals that are essential for MC degranulation and cytokine production. A distinct key point of cross-talk between Fyn- and Lyn-mediated pathways may exist, and different molecules could affect a Fc ϵ RI-generated response at various steps of the signal cascade.

The existence of multiple signal-transduction pathways, which may appear to constitute redundancies, instead represents means for modulating gene expression by triggering different receptors or combinations of receptors and at varied degrees of receptor occupancy.

Differences in the extent of Fc ϵ RI occupancy with Ag-specific IgE and thus, in receptor, aggregate size upon binding the Ag could cause selective, MC responses. Aggregation of Fc ϵ RI-bound IgE by a low-affinity Ag is ineffective at stimulating the distal steps of the signaling cascade, and many of the early events such as receptor phosphorylation still occur [25]. In contrast, high-affinity Ags are competent in transducing signals that result in cell degranulation. In murine bone marrow MCs, varying the concentration of Ag induced different profiles of cytokine mRNA. The accumulation of macrophage-inflammatory protein-1 α (MIP-1 α), MIP-1 β , monocyte chemoattractant protein-1 (MCP-1), IL-2, IL-4, and IL-6 mRNAs reaches maximal levels at significantly lower concentrations of Ag than are required for maximal induction of IL-10, leukemia inhibitory factor, MIP-2, IL-3, and interferon- γ (IFN- γ). Low receptor occupancy is relatively effective in stimulating the phosphorylation of Akt, suggesting that the Fyn/Gab2 pathway is preferentially engaged at low Ag concentration. The different profile of cytokine production seen at low receptor occupancy is also accompanied by p38 MAPK phosphorylation, whereas intermediate or high receptor occupancy is required for JNK1 and extracellular-regulated kinase (ERK)2 activation [26]. It is thus possible that MCs possess different secretory phenotypes depending on the intensity of the stimulus and modify the profile of released molecules, adapting to the ongoing immune response and potentially contributing to its regulation. In fact, lymphokines that promote allergic inflammation, such as MCP-1, are potently induced at low Ag concentrations or at low receptor occupancy with IgE, whereas some that down-regulate this response, such as IL-10, require high receptor occupancy. MC activation via high-affinity IgE receptors can be down-regulated by inhibitory pathways with physiological as well as clinical relevance. Examples include the cyclic adenosine monophosphate production induced by β_2 -receptor stimulation that inhibits the release of histamine and leukotrienes [27] and the triggering of low-affinity receptors for IgG (Fc γ RII), which when cross-linked to Fc ϵ RI, down-regulate mediator and cytokine release following Fc ϵ RI aggregation [28]. However, not all agonists evoke a distinct positive or negative signaling. Cannabinoids and endocannabinoids can act on two different receptors, CB1 and CB2, whose expression has been assessed recently on MC lines. CB1 and CB2 mediate distinct responses potentiating or antagonizing IgE-dependent MC activation, respectively. The observation that MCs are able to secrete endocannabinoids suggests a potential autocrine, regulatory loop during cell activation and inflammatory reaction [29].

SELECTIVE IgE-INDEPENDENT SIGNALS INDUCE DISCRETE MC RESPONSES

Tissue-resident MCs are exposed in the inflammatory sites to allergens leading to the aggregation of IgE bound to the Fc ϵ RI as well as the products of a variety of infectious agents by opsonin-dependent mechanisms or via direct binding to parasites or bacteria [30–32]. In addition, autoantibodies and complement bind to MCs, which prompt them to dump their cytokines and other inflammatory chemicals, thus calling inflammatory cells into the scene [30, 33]. These data suggest that MCs might be triggered by stimuli other than those induced by the Fc ϵ RI aggregation. Moreover, Fc ϵ RI-deficient mice display a normal T helper cell type 2 (Th2) response, demonstrating that IL-4 production induced by triggering Fc ϵ RI in non-B non-T spleen cells is not essential for initiating a Th2 response [34].

One alternative to IgE-dependent activation may involve the high-affinity IgG receptor, which was demonstrated to be expressed on human MC surface after IFN- γ treatment [35]. Aggregation of Fc γ RI results in MC degranulation with histamine release and generation of arachidonic acid metabolites. Moreover, IFN- γ -treated MCs synthesize and secrete chemokines and cytokines after Fc γ RI aggregation [36]. The signaling pathways activated by triggering the IgG receptor generally resemble those observed following Fc ϵ RI aggregation that induces phosphorylation of Src kinases and p72 and subsequent activation of multiple substrates. The one exception is that although PI-3K is phosphorylated after Fc ϵ RI and Fc γ RI engagement, only Fc γ RI signaling requires this molecule for degranulation [37]. The induction of MC activation through Fc γ RI in addition to Fc ϵ RI in an IFN- γ -rich environment appears to present an additional mechanism by which MCs may be recruited to produce diversity of inflammatory mediators. Thus, IFN- γ , by up-regulating Fc γ RI on human MCs, allows these cells to be recruited by an IgG-dependent mechanism in a Th1-like environment, and in a Th2 environment, Fc ϵ RI–IgE-mediated activation would predominate. These data indicate that MC responses may occur in a Th1 and Th2 microenvironment, although responsiveness is modulated through receptor expression and specific mediator production.

Recently, highly selective production of different classes of MC mediators in response to distinct Toll-like receptor (TLR) activators of potential importance to the host response to bacterial or fungal pathogens has been demonstrated [38, 39]. TLR are a family of pattern-recognition receptors that play an important role in host defense, as they participate in microbe detection by interacting with specific, nonself, structural elements shared by different pathogens. Different TLR are involved in the specific recognition of different pathogens: Gram-positive and -negative bacteria are recognized by TLR2 and TLR4, respectively, and flagellin is recognized by TLR5, dsRNA is recognized by TLR3, and unmethylated CpG DNA is recognized by TLR9. Surface expression of TLR2 and TLR4 was recently evidenced on human cord blood-derived MCs (CBMCs) [40]. The differential stimulation of MCs via TLR2 and TLR4 induces different effects in terms of histamine release and cytokine production. Natural ligands of TLR2 and TLR4 such as peptidoglycan (PGN) and lipopolysaccharide

(LPS), respectively, are able to induce the production of IL-5, IL-10, and IL-13, and TLR2 stimulation by PGN but not TLR4 stimulation with LPS induces histamine release by CBMCs in a dose-dependent manner. LPS up-regulates the production of some Th2 cytokines, such as IL-5, IL-10, IL-13, but not IL-4, in addition to IL-6 and TNF- α from MCs and potentiates the secretion of these cytokines when combined with Ag binding to Fc ϵ RI. LPS-induced expression of IL-5, IL-10, and IL-13 is mediated by TLR4 and is distinctly regulated by MAPK proteins [41]. The differential expression of inflammatory molecules by selective TLR signaling may account for a different outcome of immune response depending on the type of the offending pathogen. Thus, distinct MC responses may be elicited in MCs as well as in macrophage [42].

CD48, a mannose containing glycosylphosphatidylinositol-anchored molecule expressed on MCs, can bind and uptake not only the fimbrial adhesion molecule FimH of *Escherichia coli* [43] but also *Mycobacterium tuberculosis* and its Ags *M. tuberculosis*-specific, secreted Ag-10, MPT-63, and early secreted Ag target-6 [44]. MC activation via CD48 involves lipid rafts and results in degranulation, bacterial internalization, and cytokine production. The overall effect of *Mycobacterium* on MC is the release of prestored mediators, such as histamine and hexosaminidase, and secretion of TNF- α and IL-6 [44].

Recently, oxidative stress signaling was indicated as a possible pathway of MC activation. Mercuric chloride (HgCl₂) treatment was used to induce an autoimmune disease with a predominant Th2 phenotype in Brown Norway rats [45]. In this rat model, HgCl₂ and hydrogen peroxide (H₂O₂) caused MC degranulation and enhanced IL-4 mRNA production, therefore

suggesting that both stimuli might activate similar signaling pathways. Moreover, studies aimed at understanding the effects of environmental heavy metals on the immune system demonstrated that silver nitrate promotes degranulation and LTC₄ production in rat basophilic leukemia cell line (RBL)-2H3 cells but does not affect IL-4 and TNF- α production. Silver induces MC activation through an alternative pathway that induces tyrosine phosphorylation of ERK1 and ERK2, bypassing the early signaling events [46].

We have demonstrated that oxidative stimuli could trigger cytokine expression and secretion and could strengthen Fc ϵ RI stimulation in the RBL-2H3 cell line. Our findings show that nontoxic concentrations of H₂O₂ activate MCs to produce IL-4 and IL-6 and weakly affect IL-5 mRNA expression but are unable to induce the production of IL-10 [47]. Furthermore, this phenomenon is not species-restricted, as the human basophilic KU812 cell line and murine bone marrow MCs treated with H₂O₂ show the same behavior as the RBL-2H3 cell line. We demonstrated that different stimuli, such as IgE + Ag and H₂O₂ treatment, are able to induce selective patterns of cytokine expression (**Fig. 2A and B**). Oxidative stimulation may also have physiological relevance, as BMMC treated with different H₂O₂ concentration are able to release discrete levels of IL-4 in the supernatants, as shown in Figure 2B, indicating that the increase of cytokine expression is not limited to mRNA. Moreover, the concomitant IgE cross-linking and H₂O₂ treatment augment the Ag-induced cytokine production, indicating the additive effect of both stimuli on IL-4 secretion. H₂O₂-induced cytokine production uses the Fyn pathway preferentially and requires p38 phosphorylation [47], suggesting once

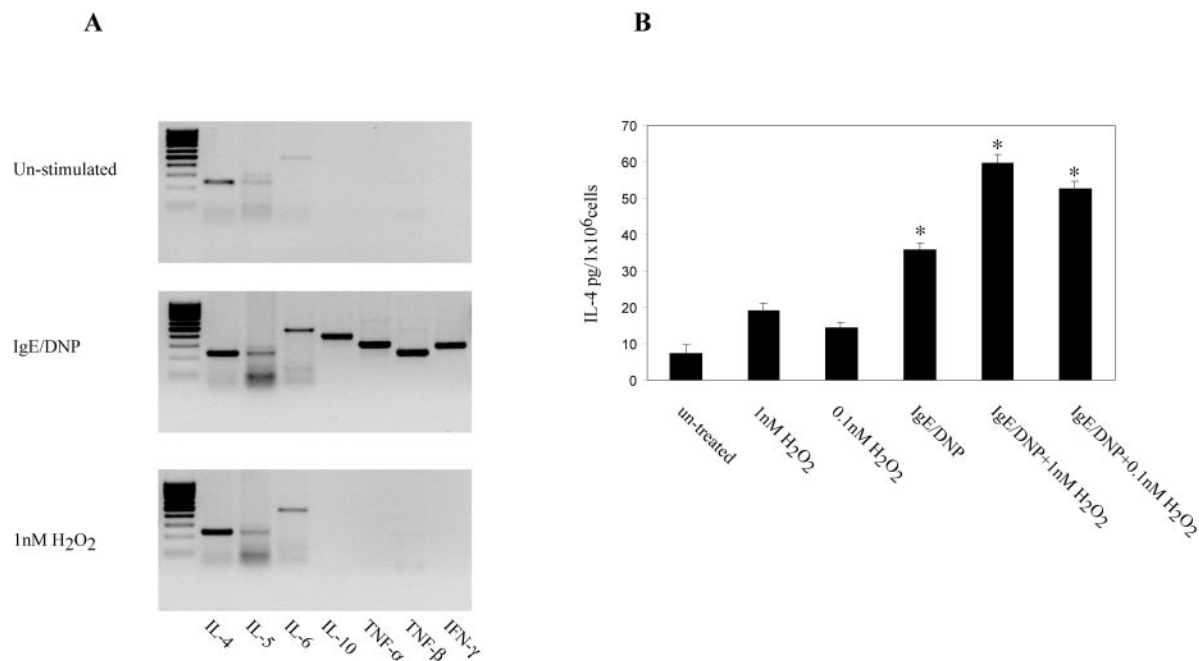


Fig. 2. Regulation of cytokines in H₂O₂ or IgE + Ag-stimulated bone marrow mononuclear cells (BMMC). Total RNA from untreated BMMC together with IgE/dinitrophenyl (DNP) or 1 nM H₂O₂-treated cells was analyzed by reverse transcriptase-polymerase chain reaction for the indicated cytokine mRNA expression. cDNA was first normalized using primer for glyceraldehyde 3-phosphate dehydrogenase (A). BMMC were stimulated with the same indicated H₂O₂ concentrations, with IgE/DNP alone and with H₂O₂ + IgE/DNP. After 24 h, IL-4 released in the culture supernatants was detected by specific IL-4 enzyme-linked immunosorbent assay. Results are expressed as pg of cytokine measured from 1×10^6 cells. Results are the means SD from three independent experiments. *, Statistically significant difference ($P < 0.005$) versus IgE/DNP-treated cells (B).

TABLE 1. Different Stimuli Induce Discrete Cytokine Production by MCs

Stimulus	Pathway	Effects			Ref.
		Histamine release	Degranulation	Cytokine production	
High antigen dose	Lyn/Syk all MAPKs	+	+	IL-13, IL-10, IFN- γ , TNF- α	[24]
Low antigen dose	Fyn p38	–	–	IL-2, IL-4, IL-6, MIP, MCP	[24]
H ₂ O ₂	Fyn p38	–	+	IL-4, IL-5, IL-6	[40, 42]
LPS	Via TLR4 all MAPKs	–	–	IL-5, IL-10, IL-13, IL-6, TNF- α	[36]
Peptidoglican	Via TLR2	+	–	IL-5, IL-10, IL-13	[35]
AgNO ₃	ERK1, ERK2	+	+	–	[41]
Micobacterium tuberculosis	Via CD48 protein Gi α	+	+	IL-6, TNF- α	[38, 39]

again, the ability of MCs to respond in different ways to different signals. These data support the hypothesis that the oxidative environment plays an important role in the modulation of MC function also in vivo.

ROLE OF MCs IN THE POLARIZATION OF ADAPTIVE-IMMUNE RESPONSE

MCs are thought to be one of the first inflammatory cells to make contact with the invading pathogens, and the extremely diverse MC responses to different stimuli suggest that specific signals can activate different pathways, leading to different MC responses (**Table 1**). The complex interplay of signals influenced by the molecular properties and the concentration of Ag(s) modulate MC function, which is primarily directed to secrete a broad spectrum of preformed and newly synthesized mediators. These inflammatory mediators are key molecules in mobilizing and recruiting various other cells to the site of inflammation and ultimately in directing T cell response. Differences in the cocktail of molecules released by MCs have a profound influence on T cell differentiation and hence, on the characteristics of adaptive-immune responses. Since the initial observation of Paul and co-workers [48], Fc ϵ RI⁺ (al posto di MC) cells have been considered the principle source of IL-4 during immune responses that prime Th2 differentiation. However, to date, no other physiological stimulus has been described, apart from IgE cross-linking, which is able to induce IL-4 production by MCs and basophils. IgE-dependent IL-4 production appears to be important in amplifying and maintaining an already established Th2 response [48, 49], and agents other than IgE + Ag must induce IL-4-driven Th2 differentiation by non-T non-B cells during primary immune response. In agreement with this concept, it has been shown that Fc ϵ RI knockout mice infected with *Schistosoma mansoni* develop a normal Th2 response despite lacking Fc ϵ RI-dependent IL-4 production by non-T non-B cells [34]. Moreover, it was demonstrated that in an established human in vivo model of allergic rhinitis, cells belonging to the MC/basophil lineage residing in the nasal mucosa represent the initial source of production of IL-4, which drives the subsequent immune response toward Th2 polarity [50]. From this point of view, oxidative stimulus may represent an important activator signal for IL-4 production by MCs, as those are exposed to an oxidative environment in the course of allergic and inflammatory

reactions. IL-4 is an essential cytokine in directing CD4 cell phenotype, but other signals delivered from Ag-presenting cells can control the development of Th subsets [51]. Prostaglandin E₂ (PGE₂), a product of MC activation, is a relevant, polarizing factor for type 2 dendritic cells (DC2), the subset able to up-regulate Th2 cytokines in CD4 cells, and it has been reported that histamine also induces DC2 differentiation by stimulating H1 and H2 receptors [52]. Therefore, an oxidative environment has the potential to induce MC release of IL-4, PGE₂, and histamine, promoting DC2 polarization and priming T cells toward Th2 differentiation. The concept that oxidative stress is an important, physiological alternative pathway is strengthened by the different pattern of cytokine expression that RBL-2H3 cells display when stimulated by H₂O₂ or by triggering Fc ϵ RI [47]. The lack of IL-10 expression, in fact, seems to be a distinctive feature of H₂O₂-stimulated cells, as TLR2 and TLR4 pathways elicit IL-10 production by CBMCs. IL-10 has important, suppressive activities, and its absence in the microenvironment is relevant for T cell proliferation and subsequent cytokine production [53]. It is tempting to speculate that oxidative stress-mediated MC activation may be important in influencing primary immune response when DC cells acquire information about Ag(s) from environmental signals. The lack of IL-10 secretion by MC during primary immune response may have important effects in breaking tolerance to autologous and exogenous Ags.

By sensing the microenvironment, reacting to specific and nonspecific stimuli, and secreting a broad spectrum of preformed and newly synthesized factors, MCs may represent the most adaptable cell type that functions in orchestrating the first response to foreign Ags and in directing adaptive-immune responses.

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