CHAPTER

DOWN-REGULATION OF MAST CELL RESPONSES THROUGH ITIM CONTAINING INHIBITORY RECEPTORS

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Abstract: The multiple cell types that comprise the immune system provide an efficient defense system against invading pathogens and microorganisms. In general, immune cells are activated for disparate functions, such as proliferation, production and release of mediators and chemotaxis, as a result of interactions between ligands and their matching immunoreceptors. This in turn leads to the recruitment and activation of a cascade of second messengers, via their regulators/adaptors, that determine the net effect of the initial response. However, activation of cells of the immune system must be tightly regulated by a finely tuned interplay between activation and inhibition to avoid excessive or inappropriate responsiveness and to maintain homeostasis. Loss of inhibitory signals may disrupt this balance, leading to various pathological processes such as allergic and auto-immune diseases. In this chapter, we will discuss down-regulating mechanisms of mast cells focusing on immunoreceptor tyrosine-based inhibition motifs (ITIM)-containing inhibitory receptors (IR).

INTRODUCTION

Mast cells are the main effector cells of allergic reactions as well as are important regulators of a number of pathophysiological processes. Mast cells can be activated by the binding of ligands to various receptors expressed on the cells. Nevertheless, the main mechanism of mast cell activation in allergy is through the binding of an allergen to the FcεRI-IgE complex. Positive or activation signals, which are transmitted through immunoreceptor tyrosine-based activation motifs (ITAM)-bearing receptors,

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are counterbalanced by negative or inhibition signals transmitted by immunoreceptor tyrosine-based inhibition motifs (ITIM). This is especially important to maintain the balance between activation and inhibition and to avoid hyperresponsiveness. In this chapter we will discuss in depth the down-regulation of IgE-mediated mast cell responses through ITIM-mediated inhibition signals.

CELL ACTIVATION

Cells of the immune system can be activated through a wide array of receptors, such as receptors for the Fc portion of different immunoglobulin subclasses (FcR), cytokines receptors, T-cell antigen receptor (TCR), the B-cell antigen receptor (BCR), Toll-like receptors (TLR), integrin receptors and G protein coupled receptors including those for specific chemokines, complement components, prostaglandin E_2 and adenosine. Ligands for the aforementioned receptors mediate their effects on cells through the activation of receptor-linked signal transduction pathways.

A pivotal mechanism of cell activation is carried out by Fc receptors. The extracellular domains of these receptors determine the specificity of the response and the types of cells involved. On the other hand, the intracellular domains of the Fc receptors are crucial for transducing signals into the cells and eventually for determining the duration and intensity of the initiated response.[1] The cytoplasmic portion of the Fc receptors contains immunoreceptor tyrosine-based activation motifs (ITAMs) through which the immune system transduces positive signals. Upon ligand recognition and receptor co-aggregation, tyrosine residues in the ITAMs are phosphorylated by Src family protein tyrosine kinase(s) (PTK(s)). The phosphorylated ITAMs serve as docking sites for the SH2 domains of Syk family PTKs, such as ZAP-70 or Syk. Syk family PTKs phosphorylate a series of substrates, leading to the formation of membrane-proximal scaffolds. In turn, important effector molecules, such as phospholipase C (PLC), are recruited leading to calcium signaling, as well as Ras activation, resulting in stimulation of the ERK pathway and cellular activation.[2]

In this chapter we will focus primarily on the IgE-mediated mast cell activation mechanism. The heterotetrameric high-affinity IgE receptor (FcεRI) on mast cells consists of three subunits: α, β and γ. The α subunit is unique to this receptor while the γ subunit is common to other receptors such as FcγRIIa. The β chain is important for stabilizing the receptor and for amplifying its signaling. Upon cross-linking of IgE bound to the FcεRI receptor with the multivalent antigen, FcεRI complexes aggregate and lead to a transphosphorylation of the ITAMs situated in the cytoplasmic regions of the β and γ chains. Tyrosine residues in the ITAMs are then phosphorylated by the src family kinase Lyn to initiate the signaling cascade[13] as above.

CELL INHIBITION/TERMINATION OF IMMUNE RESPONSES

As mentioned, immune responses are critical to fight pathogens and one of the main responses normally carried out is the classical inflammatory response. However, once the pathogen is removed, the inflammatory reaction must come to an end to avoid a chronic process. It is for this reason that multiple mechanisms exist to terminate or to down-regulate the activation and the recruitment of inflammatory cells and hence the inflammatory response. Activation of the immune system leads to the production and

Table 1. Human and murine inhibitory receptors expressed on mast cells

Subfamily	Human	Murine Counterpart	Ligand	Phosphatase Recruited	Inhibitory Component	Additional Cells Where Receptor Is Expressed
Ig-superfamily	FcγRIIB	FcγRIIB	Immune complexes lgG Abs	SHIP	1 ITIM	B-cells
	Siglec-8	-	6′sulfo-sLex	SHP-1	1 ITIM; 1 ITIM-like	Eosinophils
	CD300a	Lmir-1	Unknown	SHIP; SHPs	4 ITIMs	Eosinophils
	SIRP-α	-	CD47	SHP-1,2	2 ITIMs	Myeloid, hematopoetic neurons
	(LILRB4)*	Gp49B1	αvβ3	SHP-1,2	4 ITIMs	Neutrophils, Macrophages, NK
	ILT/CD85	PIR-B	MHC-1 molecules	SHP-1 (SHP-2)	4 ITIMs	B-cells, DC, Monocytes, Macrophages
	LAIR-1	LAIR-1	Collagens	SHPs	2 ITIMs	Most cells of the immune system
	PECAM-1	PECAM-1	CD38, αvβ3	SHP-2, SHP-1	2 ITIMs	Endothelial, Neutrophils, NK, Monocytes, Platelets
	CD200R	CD200R	CD200	SHIP	NPXY motif	Basophils
C-type lectin like	MAFA	-	E-cadherin	SHIP, SHP-2	1 ITIM	NK, Basophils, U937 (human) NK, CD8-T-cells (mouse)
	CD72	-	CD 100	SHP-1	2 ITIMs	B-cell, basophils

*Receptor was not found to be expressed on mature human mast cells at the protein level.[49]

secretion of arachidonic acid metabolites (i.e., prostaglandins, thromboxanes, leukotrienes) and of pro-inflammatory cytokines such as IL-8, IL-1 and TNF-α. Moreover, other specific mediators produced during inflammation include histamine from mast cells, elastase from neutrophils and perforin from NK cells. Therefore, one way to end the inflammatory response is to stop the production and/or secretion of such mediators. Indeed, a natural mechanism of self "extinguishing" the acute phase response occurs due to the relatively short half-lives of the lipid metabolites.[3] Similarly, production of anti-inflammatory cytokines (i.e., IL-10, TGF-β) and down-regulation and desensitization of immunoreceptors are also crucial for the termination of inflammation.

Apoptosis or programmed cell death can also end inflammation.[4,5] This can occur through two main signaling pathways: an intrinsic one mediated by the mitochondria; and an extrinsic one mediated by death receptors. Both pathways rely on a family of intracellular cysteine proteases called caspases.[6] The expression and function of two death receptors, FAS/CD95R and TRAIL-R, has been shown in both murine and human mast cells.[7] An additional mechanism to end an inflammatory process is through the resolution or catabasis process. This is an active process that leads to the reduction or removal of leukocytes and debris from inflamed sites. The resolution stage is initiated after an acute challenge by cellular pathways leading to the biosynthesis of anti-inflammatory, pro-resolution lipid mediators such as lipoxins, resolvins and protectins.[8]

In addition to the mechanisms mentioned above regulating immune responses, there has been a growing interest in a family of receptors that mediate inhibitory responses and are therefore named "inhibitory receptors". As opposed to the positive signals transmitted through ITAM-bearing receptors, negative regulation of the immune system is controlled by immunoreceptor tyrosine-based inhibition motifs (ITIM).

ACTIVATION AND DOWN-REGULATION OF MAST CELL RESPONSES: GENERAL

Mast cells are highly granulated, FcεRI-bearing tissue-dwelling cells that develop from myeloid progenitors expressing CD34, CD117 (Kit) and CD13, under the influence of stem cell factor (SCF). Mast cells have a "lead" role in allergic reactions, but are also involved in regulating fibrosis, in tissue responses to neoplastic diseases, in autoimmune diseases and in host-defense against bacterial and parasitic infections[9] (also reviewed in other chapters). By their activation and consequent release of mediators, mast cells cause the early symptoms of allergy and orchestrate and actively participate in the later chronic stages by releasing chemotactic factors for blood-borne inflammatory cells and by producing growth factors and activators for the same cells infiltrated in the tissues. Mast cells can be activated by various stimuli in addition to "the classical" binding of an allergen to the FcεRI-IgE complex. For example, SCFwhose receptor Kit is expressed on mast cells can induce cell differentiation, maturation, chemotaxis and survival; and also potentiate antigen-mediated mast cell activation.[10] Other examples of mast cell activation are through the binding of a ligand to other receptors such as: TLRs; and cytokine, chemokine, complement and neuropeptide receptors, all of which are also expressed on mast cells.[11,12] Moreover, basic or polybasic substances such as some endogenous mediators (i.e., substance P, bradykinin, neurotensin, eosinophil derived major basic protein, etc.) or drugs (i.e., codeine, morphine, desferroxamine), or synthetic peptides (compound 48/80) can similarly stimulate mast cells.[10]

INHIBITORY RECEPTORS: ITIM, ITAMi AND ITSM

Inhibitory receptors were initially identified and characterized on NK cells, where their ligands are primarily MHC-I molecules. The interaction of MHC-I with inhibitory receptors on NK cells leads to the abrogation of signaling pathways and therefore prevents the cell's cytotoxic activity against self.[13] Inhibitory receptors have now been shown to be expressed on multiple cell types in the immune system, including T-cells, B-cells, mast cells, eosinophils and neutrophils (Table 1).

The inhibitory receptors belong either to the Ig-receptor super-family or to the c-type (calcium dependent) lectin super-family. As mentioned above, inhibitory receptors contain one or more ITIM sequences. Classical ITIMs are defined as a consensus of a six amino acid sequence, I/V/LxYxxL/V (x denoting any amino acid), present in the intracytoplasmic domain of transmembrane molecules with inhibitory properties which, when phosphorylated, recruit SH2 domain-containing cytosolic phosphatases.[14,15] The Ig-like super-family inhibitory receptors are characterized by the presence of a single V-type Ig like domain in the extracellular portion. This family includes FcγRIIB, sialic acid binding Ig-like lectins (siglecs), signal regulatory protein α (SIRPα), Ig-like transcripts/leukocyte immunoglobulin receptors (ILTs/LIRs), killer cell Ig-like receptors (KIR), platelet endothelial cell adhesion molecule (PECAM-1), carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1), leukocyte-associated Ig-like receptors-1 (LAIR-1), CMRF-35H and paired Ig-like type 2 receptor α (PILR-A). Inhibitory receptors that belong to the C-type lectin superfamily derive their name from the homology in their extracellular domain to the C-type lectins. These are known to be calcium-dependent lectins which bind carbohydrates through a specific domain named carbohydrate-recognition domain (CRD) (Table 1).[16,17]

As shown in figure 1, typically, ligand engagement by inhibitory receptors containing one or several ITIMs, suppresses cell activation by promoting dephosphorylation reactions. Upon ligation/activation of ITIM-containing receptors, tyrosine residues within these motifs become phosphorylated after the activation of receptor tyrosine kinases or Src family member tyrosine kinases (SFKs). This leads to the recruitment of the protein phosphatases, Src homology 2 domain-containing tyrosine phosphatase (SHP)-1 and SHP-2, or the lipid phosphatase, Src homology 2 domain-containing inositol 5- phosphatase (SHIP) 1. SHP-1/2 dephosphorylate tyrosine-containing signaling molecules and therefore counterbalance the action of tyrosine kinases. On the other hand, SHIP1 dephosphorylates phosphatidylinositol 3,4,5 trisphosphate at the 5′ position, thereby terminating PI3K-mediated signaling pathways.[12] The dephosphorylation of these signaling proteins leads to the dampening of cellular activation. It is important to note that the different ITIM-bearing inhibitory receptors mediate their actions through different effector phosphatases. This partially determines the net effect of the inhibitory response. Moreover, although inhibitory receptors are usually characterized by an ITIM in their cytoplasmic tails, some of the newly discovered inhibitory receptors contain either an ITIM-like sequence or other tyrosine-containing motifs that are crucial for the inhibitory functions of the receptor.

Apart from ITIMs, certain immunoreceptors contain immunoreceptor tyrosine-based switch motifs (ITSMs). Like ITIMs, ITSMs can associate with SHP-1/2 or with SHIP. ITSMs can also bind adaptor molecules such as SH2-domain-containing protein 1A (SH2D1A) and EWS-activated transcript 2 (EAT-2). Moreover, ITSMs can bind to Src family kinases and the p85 regulatory subunit of PI3K.[18] Therefore, ITSMs can

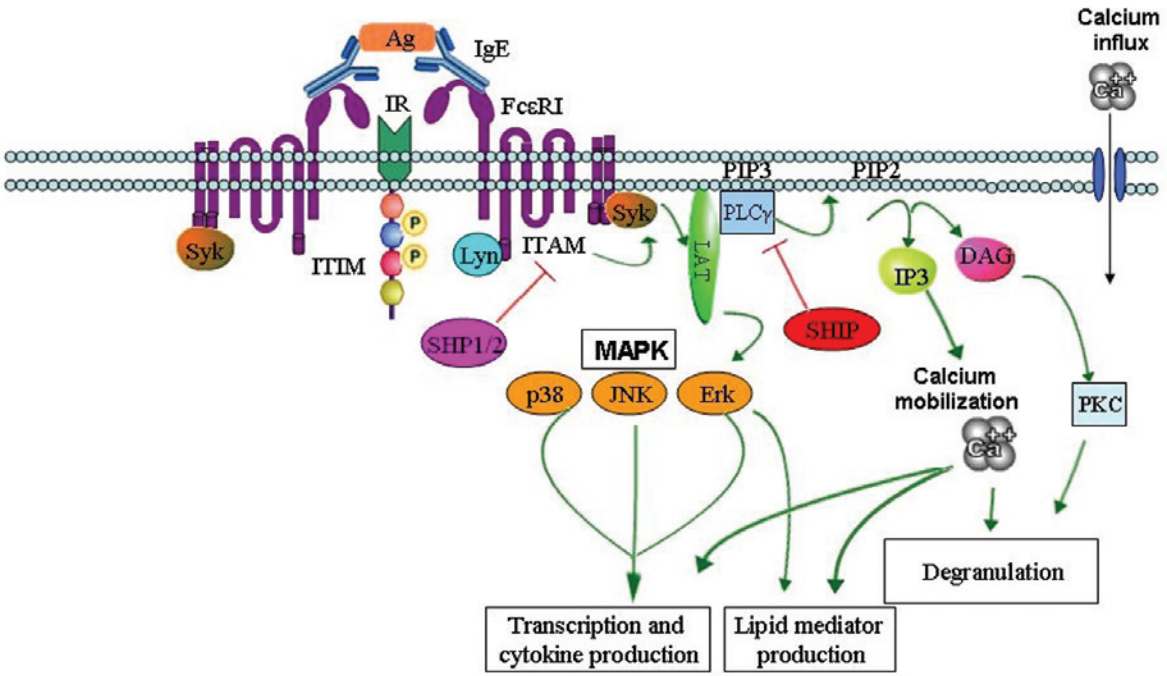

Figure 1. Schematic representation of ITIM-dependent inhibition of FcεRI-mediated activation. Mast cell FcεRI aggregation by receptor-bound IgE with an antigen leads to the phosphorylation of the receptors immunoreceptor tyrosine-based activation motifs (ITAMs). Tyrosine residues in the ITAMs are then phosphorylated by the kinase Lyn. Another tyrosine kinase (Syk) is also recruited to the complex and phosphorylates LAT. The latter activates PLCγ to degrade $PI(4,5)P_2$ to IP_3 and DAG. IP_3 induces intracellular Ca^{2+} mobilization followed by an extracellular influx. LAT also serves as a docking site for adaptor molecules leading to a downstream activation of MAPKs (JNK, p38, ERK). Subsequently, mast cell degranulation, cytokine and lipid mediator production occurs. Co-aggregation of an inhibitory receptor with FcεRI leads to the phosphorylation of the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the inhibitory receptor, leading to the recruitment of tyrosine phosphatases i.e., SHP-1/2 and SHIP. SHP-1/2 dephosphorylates the FcεRI β and γ chains as well as Syk. SHIP dephosphorylates PIP_3 to for PIP_2 thus abrogating downstream signaling and Ca^{2+} influx. Therefore, mast cell activation, i.e., cell degranulation as well as cytokine production, is abrogated. (The scheme was built using the "pathway builder" at www.proteinlounge.com).

transduce an activating or inhibitory signal depending on the immune receptor that they are associated with and the cell type in which they are expressed. The inhibitory receptor Lmir-3, which is expressed on murine mast cells and other myeloid cells, has been shown to impair mast cell cytokine production. Lmir-3 contains five tyrosine residues, two of which (Y241, Y289) are in the ITIM sequence, one in the ITSM and two (Y276, Y303) in the binding motifs for p85 or Grb2, respectively. The existence of the latter indicates a possible activating potential, in addition to its inhibitory one.

The 2B4 receptor, first described on NK cells, can also transduce activating and inhibitory signals. The inhibitory signals are mediated by the phosphorylation of its third ITSM and subsequent recruitment of SHP-1/2 and SHIP.[19] 2B4 has also been shown to be expressed (and functional) on murine mast cells.

In addition to the inhibitory ITIM mechanisms, it has been shown that under specific conditions, ITAMs can also transduce inhibitory signals. This led to the term inhibitory ITAMs or ITAMi. One of the known examples of such dual effects is mediated through immunoglobulin IgA and its receptor, FcαRI. Upon multimeric ligand binding and FcαRI aggregation, an inflammatory response is initiated. In contrast, following binding of a monomeric or low-valency ligand, with no sustained receptor aggregation, an inhibitory anti-inflammatory response is induced. Therefore, it seems that the type of interaction with the ligand determines the balance between inhibitory and activating functions of FcαRI. Moreover, ITAM-containing adaptors, FcRγ and DNAX-activating protein 12 (DAP12), have been shown to carry out dual functions. FcRγ has been shown to be involved in the inhibition of IgG-mediated phagocytosis in monocytes and IgE-mediated degranulation in mast cell transfectants.[20] Additionally, DAP12, associated with various immunoreceptors, mediated the inhibition of macrophages responses to pathogen-initiated signals through TLRs.[21]

Studies have demonstrated inhibition of activating responses of heterologous receptors such as FcγR or FcεRI, using anti-FcαRI Fab antibodies. These inhibitory effects are associated with the recruitment of SHP-1 as observed in ITIM-mediated inhibition, as well as a resultant decrease in FcεRI-induced ERK, LAT and Syk phosphorylation.[20]

In summary, although ITAM-activating responses are counter regulated by ITIM-regulated inhibition, ITAMi-mediated inhibition has emerged as a new aspect of negative regulation to cell activation. However, ITAMi inhibition appears to act as a more general mechanism in the long term maintenance of balance in the immune system. It is important to note that inhibitory receptors exist in human as well as murine cells, while some of the human receptors do not always have a murine counterpart.

MAST CELL-ASSOCIATED INHIBITORY RECEPTORS

The Ig-Like Superfamily

FcγRIIB

The FcγRIIB was the first inhibitory receptor in which the ITIM function was recognized, having been shown to inhibit B-cell receptor (BCR)-mediated activation in vitro.[22] It is a receptor for the immunoglobulin G constant (Fc) region and a type-I transmembrane protein consisting of 291 amino acids containing one ITIM which has been shown to be expressed in both murine bone marrow derived mast cells (BMMC)

and human cord blood derived mast cells (CBMC). FcγRIIB displays different inhibitory effects on MC proliferation and activation. In murine BMMC, co-aggregation of FcγRIIB with FcεRI results in the inhibition of IgE-mediated mast cell degranulation. The intact ITIM in the cytoplasmic tail of FcγRIIB is crucial to this effect.[23,24] Similarly, co-aggregation of FcγRIIB with FcεRI inhibits degranulation and cytokine secretion. This response is mediated by phosphorylation of tyrosine residues on FcγRIIB and subsequent recruitment of SHIP-1 but nor SHP-1/2.[14,24-26] FcγRIIB can also negatively regulate mast cell proliferation when co-aggregated with Kit[27] through the activity of SHIP-1.

Siglecs

Members of the siglec family have one variable (V-type) region located at the N-terminal domain which binds sialic acid residues and between 1-16 C2-type Ig-like domains in the extracellular portion.[22] The majority of the members of this family contain ITIM or ITIM-like domains. These receptors are expressed on various cell types of the innate and adaptive immune systems. However, the siglecs that are expressed on mast cells belong mainly to the CD33-related siglecs.[28] Human mast cells express siglec-2, siglec-3, siglec-5, siglec-6, siglec-8 and siglec-10.[29] Notably, a relatively high level of siglec-5, 6 and 8 proteins, all of which contain one ITIM motif, have been detected on mast cells during allergic inflammation.[30-32]

Among the members of this family that are expressed on human mast cells, siglec-8 is the one that has been most studied. This receptor contains one ITIM and one ITIM-like domain that upon antibody-induced coligation of the receptor, undergo activation, recruit SHP-1 and trigger downstream inhibitory events. Although it was initially identified on eosinophils, where self aggregation by a mAb led to apopotosis, ligated siglec-8 on mast cells was also described to significantly inhibit FcεRI-triggered histamine and prostaglandin D_2 release. Interestingly, it had no effect on the release of other synthesized mediators such as cytokines.[33,34] As with many of the inhibitory receptors expressed on human cells, murine counterparts also exist in the siglec family. However, the murine functional homolog of the human siglec-8, siglec-F, is not expressed on murine mast cells but is expressed on murine eosinophils. Although the natural ligand for siglec-8 has long been unknown, recent studies have proposed mucins as the potential ligand, based on their ability to engage with siglecs and to subsequently induce apopotosis on monocytes.[35,36] Moreover, in a more recent paper, it has been shown that eosinophils can undergo apoptosis when incubated not only with anti-siglec-8 Abs, but also with a soluble synthetic polymer displaying 6′-sulfo-sLe^x glycan.[37]

CD300a

CD300a (IRp60 or CRMF-35) is expressed on NK cells, MCs, T-cell subsets, granulocytes, monocytes and dendritic cells.[15,38-40] It is one of seven members in a family of stimulatory as well as inhibitory type I transmembrane glycoproteins that control and modulate leukocyte responses. CD300a contains four ITIMs in its cytoplasmic tail, three of them being "classical" (LHYANL, VEYSTV, LHYASV) and the fourth "non classical" (SDYSVI).[15] The function of CD300a was first described in NK cells where its co-aggregation resulted in down-regulation of the cells' cytolytic activity. Subsequently, CD300a's expression and function was further characterized in human mast cells. In CBMC, co-aggregation of CD300a with IgE-bound FcεRI, led to inhibition

of IgE-induced (but not of compound 48/80-induced) β-hexosaminidase, tryptase and IL-4 release. Concomitantly, an increase in CD300a phosphorylation, recruitment of SHP-1 and SHIP-1, decrease in Ca^{2+} influx and increase in syk dephosphorylation were detected. Moreover, CD300a cross-linking inhibited SCF-mediated CBMC survival.[38] Similarly, two bi-specific antibodies α-IgE/α-CD300a and α-Kit/α-CD300a, produced to crosslink the activating with the inhibitory receptors, abrogated Kit-mediated CBMC differentiation, survival and activation; and IgE dependent activation. In the context of mast cell-related disorders, when added to the malignant human mast cell line HMC-1, where Kit is constitutively activated, the CD300a bispecific antibody inhibited mediator release without affecting cells survival. In CBMCs, the inhibitory effect of CD300a on Kit was found to be mediated by a rapid phosphorylation of CD300a, recruitment of SHIP-1 but not of SHP-1 and by subsequent dephosphorylation of Syk and LAT.

CD300a and other members of this family have mouse orthologs, named CLM or CMRF-like molecules. The murine homologue of CD300a, LMIR-1 (or CLM-8), shares almost 80% homology with the human receptor and is expressed and functional on murine BMMC. In these cells, CD300a/Lmir-1 is capable of recruiting SHP-1, SHP-2 and SHIP.[41,42] CD300a has been shown to have a crucial role in the down-regulation or inhibition of allergic responses. This was demonstrated in murine models of allergic peritonitis, passive cutaneous anaphylaxis and acute asthma, where use of the murine bi-specific Ab α-CD300a(Lmir-1)/α-IgE proved to be effective in abrogating these allergic reactions.[43]

The ligand for CD300a remains unknown. However, evolutionary data reveals that CD300a is one of the human genes that shows strong positive selection[44,45] hinting that its potential ligand might have similarly gone through a strong positive selection as well.[46]

SIRP-α

SIRP-α belongs to the Ig-superfamily and is characterized by three Ig-like domains in the extracellular portion. The intracellular domain contains four tyrosine residues which form two ITIMs that recruit SHP-2 and SHP-1, thus negatively regulating signal transduction pathways.[47] SIRP-α has been shown to be expressed on human basophils and CBMC as well as on HMC-1 cells. A murine homologue of SIRP-α exists, but there is no evidence regarding its expression on mast cells. Functionally, SIRP-α inhibited mast cell degranulation when coligated with FcεRI and decreased the phosphorylation of FcεRI ITAMs. This was attributed to the recruitment of the tyrosine phosphatases SHP-1/2.[22] Moreover, it reduced intracellular Ca^{2+} mobilization, influx of extracellular Ca^{2+} and the activation of the MAP kinases Erk1 and Erk2. This resulted in inhibition of IgE-induced mast cell mediator release. The ligand SIRP-α has been identified as CD47, an integrin-associated transmembrane protein which is expressed on many cell populations. The interaction of the ligand with its receptor inhibits not only mast cell related responses but also FcγR dependent/independent phagocytosis by macrophages. Moreover, SIRP-α has been shown to inhibit production of IFN-γ by mature dendritic cells, suggesting that it inhibits the development of Th2 cytokines that drive allergic responses.

LILRs

LILRs belong to a family of receptors that are expressed on a wide range of cells such as B cells, dendritic cells, monocytes and NK cells. In humans, these receptors are

classified into three groups based on homology of Ig-like domains, gene architecture and organization: Ig-like transcripts (ILTs/CD85), killer-cell Ig-like receptors (KIRs) and leukocyte-associated Ig-like receptors (LAIRs (see below). It has been recently shown that LILRs are developmentally regulated in mast cells, suggesting a role in their maturation and differentiation. Human cord blood-derived progenitor mast cells (*hPrMCs*) express the inhibitory LILRB2, LILRB3 and LILRA2 on their surface. However, despite the presence of mRNA for multiple LILRs in mature CBMCs, LILR protein expression on the surface of these cells was not detected.[48] In contrast, flow cytometric analysis of mature human mast cells showed high intracellular expression of LILRB5 while none of the other inhibitory LILRs were detected.

Gp49B1 (mouse LILRB4), is a member of the Ig superfamily expressed constitutively on the surface of mast cells, neutrophils, macrophages[49] and natural killer (NK) cells. Gp49B1 is similar to FcγRIIB in that it contains two C2-type, Ig-like domains. However, gp49B1 has two cytoplasmic ITIMs.[50] The ITIM motifs in gp49B1 down-regulate NK cell and T-cell activation signals that lead to cytotoxic activity. In mouse BMMCs, gp49B1 binds SHP-1 and SHP-2 recruited from the cytoplasm upon tyrosine-phosphorylation. Gp49B1-mediated inhibition of FcεRI-driven activation is reduced in SHP-1-deficient BMMC.[51] Additionally, gp49B1 constitutively inhibits adaptive inflammation elicited by IgE-dependent mast cell activation in vivo.[52] It has also been shown that injection of LPS into gp49B1 null mice leads to hemorrhage, thrombosis and tissue neutrophilia. These results indicate that gp49B1 suppresses LPS-induced inflammation, thereby providing critical innate protection against a pathologic response to a bacterial component.[53] The ligand for gp49B1 has been identified as the integrin αvβ3, based on in vitro cell-cell and cell-protein binding studies. The interaction of αvβ3 with Gp49B1 on BMMC has been shown to inhibit antigen-induced IgE-mediated cell activation.[54]

Paired Ig-like Receptor B (PIR-B)

PIR-B is an inhibitory receptor, originally identified in mice that belong to the family of the immunoglobulin-like receptors. PIR-B is expressed by many types of hemopoietic cells, including B lymphocytes, dendritic cells, monocyte/macrophages, granulocytes, megakaryocytes/platelets and mast cells.[55] This inhibitory receptor, as well as its activated counterpart, is produced by murine mast cells. PIR-B was found to be preferentially expressed on the cell surface, where it is constitutively tyrosine phosphorylated and associated with iSHP-1. After coligation with FcεRI, PIR-B inhibited IgE -mediated mast cell activation and release of serotonin.[56] The human counterparts of PIR-A and PIR-B are considered to be the activating and inhibitory types of leukocyte Ig-like receptors/ CD85. Out of four ITIMs that the PIR-B receptor contains, two of them were found to be able to recruit SHP-1 and possibly SHP-2 when tyrosine phosphorylated and this leads to inhibition of cell activation.[57] The ligand for PIR-B appears to be various mouse major histocompatibility complex class I (H-2) molecules. Indeed, stimulation of PIR-B with H-2 tetramer on B cells, leads to intracellular phosphotyrosine signaling.[58]

LAIR-1

LAIR-1 (CD305) is a human type I transmembrane glycoprotein which contains a single extracellular C2-type Ig-like domain and two ITIMs in its cytoplasmic tail. Although most inhibitory receptors are usually restricted to specific cell types, LAIR-1

is expressed on almost all cells of the immune system. In the RBL-2H3 mast cell line, cross linking of LAIR-1 was able to inhibit FcεRI-mediated degranulation. Both ITIMs of this receptor are required for the full inhibition of degranulation in these cells, although the receptor is still partially active with one functional ITIM.[59] The murine homologue of LAIR-1 (mLAIR-1) shares 40% identity with the human receptor. Unlike many inhibitory receptors whose ligands have been identified, the ligand for LAIR-1 is not a cell-bound molecule. Collagens which are now known as the ligands for LAIR-1, define a new key role for extracellular matrix (ECM) proteins in immune regulation.[60] Human LAIR-1 recruits SHP-1 and SHP-2[61,62] but not SHIP.[63] However, mLAIR-1, recruits SHP-2 but not SHP-1. The inhibitory function of LAIR-1 is not completely phosphatase-dependent but can also be mediated through Csk, a negative effector that can inactivate Src family kinases, in cells where the phosphatases activity is abrogated or limited.[63]

CD200 Receptor (CD200R)

CD200R is a type-I membrane glycoprotein, containing two Ig-like domains and which is expressed on MC[64] and basophils.[65] Unlike the majority of immune inhibitory receptors bearing ITIMs, CD200R does not contain a classical ITIM motif but instead contains three tyrosine residues in its cytoplasmic tail which may be critical for function. Previous studies have shown the expression of CD200R on human CBMC and skin mast cells. CD200R has also been detected on murine bone marrow derived mast cells (BMMC) and murine skin mast cells.

In human mast cells, CD200R produced an inhibition in degranulation that was enhanced by cross linking the anti-hCD200R Abs. This inhibition did not require coligation to an activating receptor such as FcεRI, although this did further enhance inhibition. This differs from most inhibitory receptors whose inhibitory effect depends on co-aggregation/coligation to the balancing activating receptor. In murine mast cells, engagement of CD200R by its soluble ligand (CD200) did not lead to the inhibition of mast cell degranulation or cytokine production. However, overexpression of the receptor on these cells made them sensitive to anti-mCD200R Abs mediated inhibition. This demonstrates that CD200R activity depends on its density across the cells surface. Furthermore, the inhibitory effect of CD200R was also evident in vivo in a model of passive cutaneous anaphylaxis (PCA). Injection of anti-mCD200R Abs into mice (i.v) prior to mast cell activation significantly decreased PCA skin reactions in a dose dependent manner.

As mentioned, CD200R does not contain an ITIM sequence in its cytoplasmic domain. However, it has been shown that upon receptor engagement, CD200R is rapidly phosphorylated and recruits inhibitory adaptor proteins such as Dok1 and Dok2.[66] Two of the tyrosine residues of CD200R (Y286, Y297) have been found to be critical for the receptor- mediated inhibitory effect. One of these tyrosines is located in a conserved phosphotyrosine binding site ($NPXY^{297}$) for the signaling protein Shc. This motif mediates phosphorylation of Dok1 and Dok2 which subsequently bind RasGAP and SHIP, leading to a downstream inhibition of the RasMAPK pathways in the cell activation.

CD200 is strongly expressed on human and murine dermal fibroblasts, endothelial cells, dermal nerve bundles, hair follicles and subsets of glandular epithelial cells, but not on keratinocytes, Langerhans cells or the majority of mast cells and macrophages of the dermis in both murine and human skin sections.[67] Nevertheless, mast cells were

detected in close proximity to CD200-expressing cells in skin tissues of human skin and with a more significant expression in the murine skin.

(PECAM-1)

PECAM-1, also known as CD31, is a newly discovered member of the Ig superfamily containing six extracellular Ig domains and two ITIMs.[68,69] PECAM-1 was found to be expressed mostly at the lateral junctions of endothelial cells and at lower levels on neutrophils, monocytes, platelets, NK cells, T/B-cell subsets and on mast cells both of human and murine origin. This receptor has been shown to play a role in a number of biological processes, including leukocyte transmigration, cell migration, angiogenesis, cell adhesion, as well as modulation of intracellular signaling.[70]

In RBL-2H3 cells, following FcεRI clustering alone (without any need for receptor co-aggregation), PECAM-1 was shown to undergo rapid tyrosine phosphorylation on its ITIMs.[71] Based on plasmon resonance studies, it has been shown that PECAM's ITIMs bind SHP-2 with high affinity and SHP-1 with a lower affinity.[72] The ligands for PECAM-1 include the adhesion molecule $\alpha_v\beta_3$[73] (which is also the ligand for gp49B1 previously described) and CD38, a cell surface molecule involved in the regulation of lymphocyte adhesion to endothelial cells.[74]

The C-type lectin superfamily

Mast Cell Function-Associated Antigen (MAFA)

MAFA is an ITIM-containing (1 ITIM) membrane glycoprotein which was first described in RBL-2H3 cells in which most of its inhibitory effects have been studied. Mouse and human homologues of the rat MAFA were thereafter discovered. Mouse MAFA was renamed as killer cell lectin-like receptor subfamily G member 1 (KLRG1), which exhibited an 89% similarity at the amino acid level to rat MAFA. As opposed to the rat receptor, KLRG1 was found to be expressed only by lymphokine- activated NK cells and by virus-activated CD8 T-cells. It was not expressed on mice mast cells. Moreover, a human MAFA-like receptor has been identified as MAFA-L, sharing a 54% similarity to the rat receptor. The human receptor, differing from the rat MAFA, is expressed not only on mast cells and basophils, but also on various types of NK cells and the monocyte-like cell-line U937.

Human MAFA has been proposed to likely regulate responses to receptors other than the FcεRI.[75] In RBL-2H3 cells, MAFA has been shown to suppress the FcεRI secretory response and cytokine synthesis.[76] Aggregation of MAFA alone is sufficient to inhibit mast cell secretory responses prior to FcεRI activation. However, co-aggregation of MAFA with FcεRI has been recently shown to significantly increase the inhibition of mast cell degranulation.[77] Additionally, MAFA clustering has been found to negatively regulate the cell cycle of RBL-2H3 cells. This was evident by a significant increase in the numbers of cells arrested in the sub-G phase.[75] Using surface plasma resonance analysis it has been shown that the cytoplasmic tail of MAFA, containing an ITIM sequence, binds SHIP and SHP-2 but not SHP-1.[78] However, it has been shown that SHIP is the key phosphatase mediating the receptor's inhibitory effect. This involvement of SHIP is thought to mediate the recruitment of a multimolecular complex (Shc–SHIP–Dok–RasGAP) to the plasma membrane, where, RasGAP down-regulates the Ras-induced Raf-1/MEK/ERK signaling pathway by decreasing RasGTP levels. This in turn may eventually lead to a decrease in

gene transcription and in synthesis of cytokines regulated by Erk-1/2. On the other hand, according to various studies, MAFA does not interfere with the FcεRI-induced Fyn–Gab2–PI3K signaling pathway, which is essential for PKB and the Jnk pathway activation.

Regarding the ligand for MAFA, it was originally thought that the receptor binds MHC-I molecules. This hypothesis was suggested since there is a great homology between MAFA's extracellular domain and the CRD of other C-type lectins. However, there was no demonstrable interaction between murine MAFA and MHC-I or MHC-II molecules. It has also been implied that MAFA binds to saccharides following investigation of its binding capacity. Members of the classical cadherin family were shown to act as ligands for murine KLRG1[79-81] and the ligation of the receptor by E-cadherin led to an inhibition of CTLs induction as well as the lytic activity of an NK cell line in vitro. Recently, it has been demonstrated that the ligand for human MAFA is also the human E-cadherin.[82]

CD72

CD72, also termed Lyb-2, is an ITIM-containing, 45 kDa type II transmembrane protein[83] predominantly expressed on B-lineage cells in both mouse and human.[84] The precise function of CD72 remains unclear. However, studies have shown that anti-mouse-CD72 mAb induces an increase in the metabolism of phosphatidylinositol in purified small splenic B-cells.[85] Moreover, it induces an increase in MHC class II expression on B cells[86,87] and mobilization of small amounts of cytoplasmic free Ca^{2+} in those cells. The natural ligand for this receptor has been identified as CD100 or Semaphorin 4D (Sema4D).[88]

Recently, CD72 has been found to be expressed on human mast cell lines (LAD2, HMC1.1, HMC1.2) as well as on $CD34^{+}$ peripheral blood-derived mast cells. CD72 contains two ITIMs thus including this receptor in the inhibitory receptor family. Upon tyrosine phosphorylation, one of the receptor's ITIM binds SHP-1 and the other binds Grb2. On mast cells, coligation of CD72 with Kit by an anti-CD72 Ab (BU40) or the recombinant human CD100 (rCD100) and SCF respectively, led to CD72 phosphorylation and the subsequent recruitment of SHP-1. This resulted in dephosphorylation of SFKs and ERKs that are crucial in Kit-mediated human mast cell responses. CD72 ligation suppressed HMC1.2 growth and led to the reduction of Kit-dependent growth of human mast cells, SCF-induced chemotaxis, MCP-1(CCL2) production and SCF-enhancement of IgE-dependent degranulation. In contrast, CD72 ligation with FcεRI alone was unable to inhibit mast cell degranulation.[89]

Inhibitory Receptors as Therapeutic Tools?

Mast cell related diseases, such as allergic as well as non-allergic inflammatory diseases (asthma, rhinitis, conjunctivitis, etc.) affect a significant portion of the Western world's population with an increasing incidence each year. Some of these diseases might in certain cases lead to morbidity. Even with the proper use of current treatments such as glucocorticoids and several symptomatic drugs, symptoms and severe side effects may continue to occur and no ideal drug is yet available. Therefore, there remains a critical need for new approaches to treat mast cell-related diseases in a more useful and specific way. The expression of inhibitory receptors on mast cells makes them attractive targets for new drug designs for allergy and other mast cell-driven diseases. Indeed, some of the studies on inhibitory receptors aimed for therapeutic development are at an advanced stage.

With the discovery of the first inhibitory receptor, FcγRIIB, bi-specific antibodies recognizing both the receptor and FcεRI were generated. When tested in vitro, these antibodies showed a suppressive effect on basophil as well as on human mast cell activation.[90] Moreover, similarly to the bi-specific antibodies, the use of a fusion protein hFcγ-hFcε showed an inhibitory effect in both in vitro and in vivo studies using a murine passive cutaneous anaphylaxis model (PCA), as well as in skin test responses in Rhesus macaques allergic to dust mite.[91,92] For CD200R, in a murine model of PCA, treating the mice with specific antibodies targeting CD200R before challenge led to a significant dose-dependent decrease in cellular response.[67] Another member of the inhibitory receptors superfamily, CD300a has been an attractive target for the treatment of allergic diseases. Bi-specific antibodies targeting both mast cells and eosinophils have been generated and proven to be effective in abrogating allergic responses. Indeed, as described, in both in vitro and in vivo studies, the bi-specific antibodies α-IgE/α-CD300a and α-CCR3/α-CD300a abrogated allergic responses mediated by these cells.[39,42,43]

CONCLUSION

The field of inhibitory receptors continues to expand. This includes their application in the context of mast cells and allergic inflammatory diseases. Despite all the activity and research, many known inhibitory receptors are still not fully characterized on haematopoetic cells in general and on mast cells in particular. Therefore, there is a need for continued research to better characterize the existing inhibitory receptors, for example by studying their possible interactions with different activating receptors and discovering some of their unknown ligands. In addition, an effort should be made to identify other new inhibitory receptors on human mast cells.

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