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New developments in mast cell biology

Janet Kalesnikoff[1] and **Stephen J. Galli**[1,2]

[1] Department of Pathology, Stanford University, School of Medicine, Stanford, California 94305-5324, USA

[2] Department of Microbiology & Immunology, Stanford University, School of Medicine, Stanford, California 94305-5324, USA

Abstract

Mast cells can function as effector and immunoregulatory cells in IgE-associated allergic disorders, as well as in certain innate and adaptive immune responses. This review will focus on exciting new developments in the field of mast cell biology published within the last year. It will highlight advances in the understanding of FcεRI-mediated signaling and mast cell activation events, as well as in the use of genetic models to study mast cell function *in vivo*. Finally, we will discuss newly identified roles of mast cells or individual mast cell products, such as proteases and IL-10, in host defense, cardiovascular disease and tumor biology, and in settings in which mast cells have anti-inflammatory or immunosuppressive functions.

Mast cells are derived from hematopoietic progenitor cells but do not ordinarily circulate in mature form; instead, differentiation and maturation of mast cells occurs locally, following migration of their precursors to the vascularized tissues or serosal cavities in which mast cells will ultimately reside[1–6]. Mast cells are key effector cells in IgE-associated immune responses, including allergic disorders and certain protective immune responses to parasites[2, 6–8]. IgE-dependent mast cell activation leads to the secretion of three classes of mediators; degranulation results in secretion of preformed mediators that are stored in the cells' cytoplasmic granules (e.g., vasoactive amines and neutral proteases), proinflammatory lipid mediators are synthesized *de novo*, and growth factors, cytokines, and chemokines are synthesized and secreted. However, mast cells can be activated to express important effector and immunomodulatory functions by many mechanisms that are independent of IgE, and the kinetics, amounts and/or spectrum of mediators released can be stimulus-dependent[6, 7, 9].

FcεRI signalling events

Mast cells can respond to many different stimuli, and thereby participate in a wide variety of physiological and pathological processes, as a result of their activation by any of an array of receptors. However, the best-studied mechanism by which mast cells perform immunologically specific function is through antigen- and IgE-dependent aggregation of the high affinity IgE receptor, FcεRI[7, 10–15] (Fig. 1).

FcεRI is expressed on mast cells as a heterotetrameric receptor comprised of an IgE-binding α subunit, the membrane tetraspanning β subunit, and two identical disulphide linked γ subunits (which are important for initiating signalling events downstream of this receptor because they each contain one immunoreceptor tyrosine-based activation motif [ITAM])[7, 11, 12, 14]. The stability of FcεRI on the mast cell surface, which is a major determinant of

Address correspondence to: Stephen J. Galli, Department of Pathology, L-235, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5324, Tel: 650-723-7975, Fax: 650-725-6902, sgalli@stanford.edu.

FcεRI expression levels, can be influenced both by external factors (e.g., IgE binding[2, 7])and intracellular molecules (e.g., Rabaptin-5[16]). Antigen- and IgE-induced crosslinking of cell surface FcεRI induces activation of Lyn, which phosphorylates FcεRI ITAMs and activates Syk following ITAM binding. Lyn and Syk phosphorylate several adaptor molecules and enzymes to regulate mast cell activation. In addition to Lyn, FcεRI aggregation activates a second Src family kinase, Fyn, which phosphorylates the adaptor Gab2 to activate the PI3K pathway.

In addition to its aforementioned signal initiating activity, Lyn also negatively regulates FcεRI-induced signalling events (including Fyn activation)[12]. Indeed, Lyn knockout mast cells and mice are hyperresponsive to IgE plus antigen stimulation[12, 17, 18]. Hong et al.[19] reported that a third Src family kinase, Hck, plays a positive regulatory role in FcεRI-induced mast cell degranulation and cytokine release by both Lyn-dependent and Lyn-independent mechanisms (both of which are dependent, at least in part, on phosphorylation of the FcεRI β chain). The Lyn-dependent mechanism involves Hck-mediated suppression of Lyn's negative regulatory kinase activity (*i.e*., Lyn activity and the phosphorylation of various Lyn targets [e.g., SHIP] were increased, while the phosphorylation of various positive regulatory molecules [e.g., Syk] was reduced, in $hck^{-/-}$ bone marrow-derived cultured mast cells [BMCMCs])[19]. The authors proposed a hierarchical relationship among the Src family kinases downstream of FcεRI: Hck negatively regulates Lyn, which negatively regulates Fyn[19]. Additional studies are required to understand more fully the interplay among Src family kinases downstream of FcεRI (*e.g*., why was Fyn activity normal in $hck^{-/-}$ BMCMCs despite increased Lyn activity?).

PLCγ hydrolyzes PI-4,5-P_2 (phosphatidyl inositol 4,5-bisphosphate) to form soluble IP_3 (inositol 1,4,5-trisphosphate) and membrane bound DAG (diacylglycerol) [7, 11–14]; IP_3 binding to its receptor in the endoplasmic reticulum (ER) rapidly induces the first stage of calcium (Ca^{2+}) mobilization, the transient release of Ca^{2+} from ER stores, which in turn induces prolonged influx of Ca^{2+} through store-operated calcium release-activated calcium (CRAC) channels in the plasma membrane (Fig. 1). The recent identification of STIM1, a sensor of ER Ca^{2+} concentrations that couples depletion of ER Ca^{2+} stores with activation of CRAC channels[20, 21], and CRACM1 (aka Orai1), the pore-forming subunit of the CRAC channel[22–25], has increased our understanding of CRAC currents at the molecular level. Using STIM1-deficient mice, Baba et al.[26] showed that STIM1 is required for FcεRI-induced Ca^{2+} influx, degranulation, transcription factor (i.e., NFκB and NFAT) activation, and IgE-dependent anaphylaxis *in vivo* and Vig et al.[27] used CRACM1-deficient mice to show that CRACM1 is required for FcεRI-induced degranulation, lipid mediator synthesis, cytokine release and IgE-dependent allergic responses *in vivo*. These studies demonstrate conclusively that the second stage of FcεRI-induced Ca^{2+} mobilization, the influx of Ca^{2+} mediated by STIM1 and CRACM1, is essential for mast cell activation *in vitro* and *in vivo*[26,27].

Recent work indicates that canonical transient receptor potential channels (TRPC), which are Ca^{2+}-permeable nonselective cation channels, may associate with STIM1 and CRACM1 to enhance Ca^{2+} entry[28, 29]. Using inhibitory RNA in a rat mast cell (RBL-2H3) line, Ma et al.[30] showed that TRPC5, in addition to STIM1 and CRACM1, is required for optimal FcεRI-induced Ca^{2+} influx and degranulation. They proposed that strontium-permeable TRPC5 associates with STIM1 and CRACM1 in a stoichiometric manner to enhance FcεRI-induced Ca^{2+} influx and degranulation in mast cells[30].

The rate of Ca^{2+} influx through store-operated channels is also dependent on the membrane potential, which is regulated by calcium-activated nonselective (CAN) cation channels[31], such as TRPM4 (transient receptor potential cation channel, subfamily M, member 4). Using

TRPM4-deficient mice, Vennekens et al.[32] showed that TRPM4 activates a CAN current which depolarizes membrane potential and limits the driving force for Ca^{2+} entry through CRAC channels in BMCMCs; FcεRI-induced degranulation, leukotriene release and TNF (but not IL-6) production was increased in TRPM4-deficient BMCMCs and TRPM4-deficient mice exhibited more severe acute (but not late-phase) inflammation during IgE-mediated passive cutaneous anaphylaxis responses. Accordingly, the authors proposed that TRPM4 acts as a "molecular brake" on Ca^{2+} influx after FcεRI-induced mast cell activation *in vitro* and *in vivo*[32].

Downstream of early FcεRI-induced signaling events (such as Ca^{2+} influx), the final stages of mast cell degranulation require membrane fusion events. The exocytosis of mast cell granules, or secretory lysosomes, is mediated by membrane fusion proteins called SNAREs (soluble N-ethyl-maleimide-sensitive factor [NSF] attachment protein receptors)[33–37]; these are divided into t-SNAREs, localized on the target membrane (e.g., syntaxins and soluble NSF attachment proteins [SNAPs]) and v-SNAREs, localized on the vesicle membrane (e.g., vesicle-associated membrane proteins [VAMPs]). Murine rodent and human mast cells express VAMP-2, -3, -7 and -8, and both VAMP-7 and -8 colocalize with secretory granules in RBL-2H3 cells[35, 38]. Two groups recently showed that FcεRI-induced exocytosis is reduced in VAMP-8-deficient mast cells[39, 40]. Puri & Roche[39] reported that this defect was limited to a distinct subset of secretory granules (i.e., those containing serotonin and cathepsin D). They did not observe any defects in the regulated exocytosis of granules containing histamine[39]. By contrast, Tiwari et al.[40] reported an approximately 50% reduction in FcεRI-induced β-hexosaminidase and histamine release *in vitro* in the absence of VAMP-8. Moreover, they observed reduced blood histamine levels in VAMP-8-deficient mice during passive systemic anaphylaxis[40]. Although these groups used different VAMP-8-deficient mice, the reason for the discrepancies in their findings remains to be determined. Finally, Sander et al.[41] reported that inhibition of syntaxin 4, SNAP-23, VAMP-7 or VAMP-8, but not VAMP-2 or VAMP-3, inhibited FcεRI-induced histamine release in primary human mast cells.

The t-SNAREs syntaxin 4 and SNAP23 regulate FcεRI-induced exocytosis from mast cells[33, 35] and the phosphorylation of SNAP23 (on Ser120 and Ser95) has been shown to modulate exocytic events[42]. Suzuki & Verma[43] recently reported that IKKβ (inhibitory κB kinase β; also termed IKK2), one of two catalytically active subunits of the IKK complex, phosphorylates SNAP23 on Ser120 and Ser95. Although the IKK complex is best known for its role in activating the transcription factor NFκB, this IKKβ/IKK2-mediated phosphorylation of SNAP23 was shown to upregulate FcεRI-induced degranulation *in vitro* in an NFκB-independent manner[43]. Moreover, they showed that IKKβ/IKK2 in mast cells plays a critical role in enhancing IgE-mediated acute local or systemic reactions, as well as an example of a cutaneous late phase reaction (in an NFκB-dependent manner), *in vivo*. These exciting results suggest that IKKβ/IKK2 may have additional substrates that allow this kinase to regulate NFκB-independent mast cell activation events, such as SNARE complex formation.

In addition to SNAREs, Rab GTPases regulate exocytic events[34, 36, 37]; for example, Rab27a and its effector, Munc13–4, enhance FcεRI-induced mast cell degranulation[44]. Higashio et al.[45] reported that Doc2α, which was thought to be a brain specific isoform of the Doc2 family that regulates Ca^{2+}-dependent synaptic vesicle exocytosis, is expressed in mast cells. They showed that Doc2α colocalized with Munc13–4 on secretory granules in RBL-2H3s and that FcεRI-induced Ca^{2+}-dependent secretory lysosome exocytosis was reduced in BMCMCs from Doc2α-deficient mice[45].

Negative regulation of FcεRI-dependent mast cell activation

Several negative intracellular regulators can diminish FcεRI-induced signaling events (e.g., the lipid phosphatase SHIP; Fig. 2). Some signaling molecules initiate both activating and inhibitory signals (e.g., Lyn phosphorylates FcεRI ITAMs as well as inhibitory receptor immunoreceptor tyrosine based inhibitory motifs [ITIMs], the latter leads to recruitment of inhibitory signaling molecules, such as SHIP). Other signaling molecules can negatively regulate FcεRI-induced mast cell activation events by altering the rate of FcεRI internalization (e.g., one function of RabGEF1 is to enhance FcεRI internalization)[46, 47]. Bansal et al.[48] recently identified regulator of G protein signaling (RGS) 13 as a novel negative regulator of FcεRI-induced degranulation (but not cytokine [TNF, IL-6 or IL-13] production) *in vitro* and IgE-dependent passive cutaneous or systemic anaphylaxis *in vivo*. RGS proteins typically inhibit G protein coupled receptor (GPCR) signaling events through GTPase-accelerating protein (GAP) activity on Gα subunits. Although GPCR signaling can amplify FcεRI-mediated responses through activation of PI3Kγ[49], RGS13-mediated inhibition of FcεRI-induced activation occurs independently of RGS13's GAP activity[48]. Instead, Bansal et al.[48] proposed that RGS13, which is upregulated following antigen stimulation, binds to the p85α subunit of PI3K and disrupts its association with an FcεRI-activated signaling complex containing Gab2 and Grb2[48] (Fig. 2).

In addition to negative intracellular regulators, signalling events initiated by FcεRI and other ITAM-containing immunoreceptors are negatively regulated by their coaggregation with ITIM-containing receptors. Mast cells express several inhibitory receptors, including FcγRIIB, gp49B1, MAFA and PIR-B[7, 14]. FcγRIIB, the first identified ITIM-containing receptor, is an attractive therapeutic target for mast cell activation events because it recruits the lipid phosphatase SHIP following coaggregation with FcεRI *in vivo*[50] (Fig. 2).

Melendez et al.[51] reported that ES-62, a glycoprotein secreted by filarial nematodes, might also be investigated as a novel therapeutic for allergy. They showed that ES-62, which inhibits the activation of many immune cells (*e.g.*, B and T cells, dendritic cells and macrophages), also inhibited FcεRI-induced degranulation, arachidonic acid metabolism, and the production of TNF, IL-3 and IL-6 (but not IL-13 or IL-5) in human BMCMCs. ES-62 mediated these effects by forming a complex with Toll-like receptor 4, which caused sequestration and proteasome-independent degradation of PKCα, resulting in decreased phospholipase D-coupled, sphingosine kinase-mediated Ca^{2+} influx and NFκB activation[51] (Fig. 2). Moreover, they showed that ES-62 significantly reduced the magnitude of both a model of cutaneous immediate-type hypersensitivity to oxazolone and a model of OVA-induced airway hyperreactivity to Methacholine, and airway allergic inflammation, in mice sensitized to OVA admixed with aluminum hydroxide as an adjuvant. The authors suggested that ES-62-mediated suppression of mast cell activation may contribute to the reduced incidence of allergic disorders in people harboring worms and, since ES-62 appears to be well tolerated by millions of infected people, that ES-62 derivatives might represent a new type of therapeutic agent for diseases such as asthma.

New developments in mast cell models

Human mast cell populations and mouse mast cells derived from bone marrow (BMCMCs), other hematopoietic tissues (e.g., fetal liver; FLCMCs) or embryonic stem cells (ESCMCs) are powerful tools for investigating the mechanisms by which mast cells might influence various immunological or other biological responses *in vitro*. Although there are many similarities between mast cell populations in humans and in mice, there are also some differences in their anatomical distribution, phenotype and function that may influence the particular roles of mast cells in various biological responses in the two species[2, 52].

However, the ability to manipulate human mast cell numbers, phenotype or function *in vivo* is quite limited. Accordingly, many investigators have attempted to analyze mast cell function using more tractable experimental species, especially the mouse.

The *in vivo* relevance and biological importance of *in vitro* observations about mast cell function, as well as the contributions of mast cells towards the expression of particular biological responses *in vivo*, can be assessed using c-*kit* mutant mice (e.g., $WBB6F_1$-*Kit*$^{W/W\text{-}v}$ or C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice) that virtually lack mast cell populations[53–58]. The mast cell deficiency of these mice can be selectively repaired by the adoptive transfer of genetically compatible, *in vitro* derived mast cells from congenic wild-type mice or various transgenic or mutant mice[55, 57, 58] or from mouse embryonic stem cells[56], or mast cells that have been transduced with short hairpin (sh)RNA to reduce expression of proteins of interest[59]. These mast cell knockin mice are now widely used to assess the contributions of mast cells or specific mast cell products in diverse biological responses *in vivo*.

C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice are gaining popularity for such studies because these mice have fewer, or less severe, phenotypic abnormalities than are observed in $WBB6F_1$-*Kit*$^{W/W\text{-}v}$ mice. For example, unlike $WBB6F_1$-*Kit*$^{W/W\text{-}v}$ mice, C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice are neither anemic nor sterile, and they appear to have normal numbers of bone marrow and blood neutrophils[57, 60]. It is important to consider the genetic background of the mice and the effects of the different c-*kit* mutations on other cell lineages when studying various disease models. For example, while Lee et al.[61] showed that $WBB6F_1$-*Kit*$^{W/W\text{-}v}$ mice were resistant to the development of joint inflammation in one model of autoantibody-induced arthritis, Zhuo et al.[60] recently reported that mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$, but not mast cell-deficient $WBB6F_1$-*Kit*$^{W/W\text{-}v}$, mice developed autoantibody-mediated, neutrophil-dependent immune complex arthritis. They attributed this difference to the relative neutrophil deficiency observed in $WBB6F_1$-*Kit*$^{W/W\text{-}v}$ mice[60].

Two groups recently reported the generation of mast cell-specific Cre mice. Scholten et al.[62] generated transgenic mice expressing Cre recombinase under the control of the *mast cell protease (Mcpt) 5* promoter; using ROSA26-EYFP mice, they showed efficient Cre-mediated recombination in mast cells from the peritoneal cavity and skin while only minimal reporter gene expression was detected outside the mast cell compartment. Musch et al.[63] expressed Cre recombinase under the control of the baboon α-chymase promoter; using ROSA26R mice, they showed efficient Cre-mediated recombination in lung and colon tissue mast cells, but not in mast cells isolated from the peritoneal cavity or *in vitro* generated BMCMCs. It will be important to exercise care both in characterizing the phenotypic features of such mice (as expressed in mast cells and possibly in other cell types) and in interpreting the results of experiments using such animals. However, we think that validated mast cell-specific Cre mice, and inducible mast cell-specific Cre mice, may well become powerful genetic models for investigating the contributions of mast cells or mast cell-specific products to health and disease.

Mast cell proteases

Helping out in host defense

Mast cells are strategically located very near sites where the body comes in contact with the external environment; a prime location for the initiation and modulation of innate immune responses. Indeed, Malaviya et al.[64] and Echtenacher et al.[65] showed that mast cells can contribute importantly to innate bacterial clearance, at least in part by enhancing the recruitment of neutrophils to the site of infection. Since then, many *in vitro* or *in vivo* studies have provided additional evidence that mast cells can enhance host defense through direct

effects on pathogens, by initiating and modulating the inflammation associated with innate immune responses, and perhaps by initiating adaptive immune responses to pathogens.

Another protective function of mast cells during innate responses to bacterial infection is to limit the toxicity of certain products generated by the host, which can have adverse effects at high concentrations. For example, mast cells can limit the toxicity of the peptide endothelin (ET)-1, whose levels are markedly elevated during acute bacterial peritonitis and sepsis, by releasing proteases stored in their granules that can degrade this peptide[66, 67]. ET-1 exhibits high homology with sarafotoxins (the most toxic components of Israeli mole viper [*Atractaspis engaddensis*] venom), and Metz et al.[59] showed that mast cells can substantially enhance resistance to the pathology and mortality induced in mice by the venoms of *A. engaddensis* and two other poisonous snakes, and that of the honeybee. Metz et al.[59] used shRNA and pharmacological methods to show that this mast cell-mediated reduction in endogenous (ET-1) and exogenous (sarafotoxin) toxic peptides was dependent on carboxypeptidase A3 (CPA3) activity; however, mast cells that lack CPA3 concomitantly lack mast cell protease (MCP)-5[59, 67]. In an elegant study, Schneider et al.[67] generated a mutant mouse (*Mc-cpa*^{Y356L,E378A}) bearing two amino acid mutations that rendered CPA3 catalytically inactive without affecting expression of other proteases. Using this mutant, they confirmed that mast cell-mediated innate defense against ET-1 and sarafotoxin is dependent on CPA3 activity and defined the molecular mechanism by which CPA3 inactivates these toxins[67].

In addition to ET-1, Piliponsky et al.[68] recently reported that levels of neurotensin (NT; a peptide known to induce hypotension) are increased in a mouse model of sepsis. They showed that, in mice, NT can contribute to sepsis-related mortality, that mast cells can reduce NT levels *in vivo*, that mast cells can degrade NT via the protease neurolysin, and that mast cells can reduce NT-induced hypotension, as well as sepsis-related mortality[68]. Moreover, in a pilot study of human patients with sepsis (or with cardiogenic shock), they found that plasma concentrations of NT were elevated to levels similar to those observed in mice with acute bacterial peritonitis[68]. Because sepsis is the most common cause of death in intensive care units in the United States, there is considerable interest in identifying additional biomarkers and therapeutic targets in this disorder. The findings of Piliponsky et al.[68] raise the possibility that NT might contribute to the pathology in patients with sepsis; therefore, inhibiting the pathological actions of NT may confer benefit in this setting.

Although IL-15 is known to play a critical role in innate immunity, Orinska et al.[69] found that IL-15-deficient mice are actually less susceptible to sepsis-related mortality. Moreover, WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice engrafted with IL-15-deficient mast cells survived better than those engrafted with wild-type mast cells. Orinska et al.[69] showed that mast cells express intracellular IL-15 (both constitutively and following stimulation with lipopolysaccharide), which appears to function as a negative transcriptional regulator of a mast cell chymase, MCP-2. The authors proposed that this IL-15-mediated repression of MCP-2 activity limits the bactericidal activity of mast cells and the recruitment of neutrophils needed to clear the bacterial infection.

Further highlighting the importance of mast cell proteases in innate host defense, Thakurdas et al.[70] reported that the mast cell tryptase, MCP-6 (also known as tryptase β2 [Tpsb2]), can play a critical protective role in bacterial infections (i.e., MCP-6/Tpsb2-deficient mice have reduced ability to clear *Klebsiella pneumoniae* injected into their peritoneal cavity, probably because of diminished recruitment of neutrophils. The authors suggested that MCP-6/Tpsb2 is the primary preformed granule mediator of mast cells that can protect mice during acute bacterial infections[70]; however, the mechanism by which MCP-6/Tpsb2 induces neutrophil recruitment to the site of infection remains to be determined.

Mast cell proteases also can contribute to resistance to parasite infections. For example, Knight et al.[71] reported delayed expulsion of the adult helminth and increased deposition of muscle larvae in MCP-1-deficient mice. Shin et al.[72] recently showed that MCP-6 is important for clearance of chronic *Trichinella spiralis* infection; i.e., the recruitment of eosinophils to *T. spiralis* larvae and the elimination of larvae in chronically infected skeletal muscle were decreased in MCP-6/Tpsb2-deficient mice. Because eosinophil infiltration around *T. spiralis* larvae was also decreased in IgE-deficient mice, the authors suggested that mast cells and, more specifically, MCP-6/Tpsb2 link adaptive and innate immunity in the chronic phase of *T. spiralis* infection[72].

Helping out in allergic reactions

Mast cells are typically thought of as troublesome cells due to their prominent role in IgE-dependent allergic hypersensitivity reactions, such as allergic rhinitis (hay fever), atopic dermatitis (eczema), allergic (or "atopic") asthma, and some food allergies. However, Rauter et al.[73] showed that β-tryptase, a protease released by mast cells, can cleave IgE. They detected IgE cleavage products in tissue fluids collected from sites of allergic inflammation and showed that protamine (an inhibitor of heparin-dependent proteases) enhanced IgE-mediated allergic skin inflammation induced by skin prick testing in human subjects[73]. Although protamine treatment may have had other effects that influenced the magnitude of the biological responses analyzed, the findings suggest that mast cell protease-dependent degradation of IgE may help to limit this type of allergic inflammation. However, the extent to which this mechanism can contribute to the reduction of local (or systemic) IgE levels during other examples of allergic inflammation remains to be determined.

Immunomodulatory roles of mast cells

Slowing things down

Mast cells can exert positive or negative immunomodulatory functions on immune cells (i.e., influence the recruitment, survival, development, phenotype or function of immune cells) and thereby enhance or suppress the initiation, magnitude and/or duration of immune responses[6]. Because they might at first seem counter intuitive, given the mast cell's well-deserved reputation as a promoter of inflammation, we will first discuss some negative immunomodulatory functions of this cell. Hart et al.[74] showed that the ability of ultraviolet B (UVB) irradiation of the skin to induce systemic immunosuppression of contact hypersensitivity (CHS) responses was mast cell dependent. Byrne et al.[75] recently showed that UV-induced mast cell migration from the skin to the draining lymph nodes, which is mediated by CXCR4 on mast cells, is a key step in the induction of UV-induced immunosuppression. Mast cells have also been shown to mediate immunosuppressive functions following *Anopheles* mosquito bites[76], and in peripheral tolerance to skin allografts (which requires the participation of $CD4^+CD25^+FoxP3^+$ T_{Reg} cells)[77]; however, the mechanism(s) by which mast cells mediate immunosuppressive functions in each of these studies remains to be elucidated.

Grimbaldeston et al.[78] showed that mast cells can mediate negative immunomodulatory functions *in vivo* by producing IL-10. Mast cells and mast cell-derived IL-10 limited the magnitude, and promoted the resolution, of CHS responses induced in response to the hapten 2,4-dinitro-1-fluorobenzene (DNFB) or urushiol (Fig. 3), which is the hapten-containing sap of poison ivy (*Toxicodendron radicans*) or poison oak (*T. diversilobum*)[78]. Mast cells and mast cell-derived IL-10 also suppressed innate cutaneous responses to chronic low-dose UVB-irradiation[78]. Although mast cells limited multiple aspects of these responses, including inflammation, epidermal hyperplasia, and skin ulceration, the pathways that link mast cell-derived IL-10 (or other mast cell mediators that are relevant in this

setting) to the observed tissue changes remain to be defined (i.e., mast cells and mast cell-derived IL-10 may influence these responses through a complex combination of direct and indirect effector and immunomoregulatory functions).

Revving things up

Mast cells are involved in the development of various T cell-associated immune responses in mice, including models of multiple sclerosis (i.e., experimental autoimmune encephalomyelitis)[79] and bullous pemphigoid[80], and mast cells (and mast cell-derived TNF) were shown to contribute to disease pathology in a model of T_H17 cell-dependent, neutrophil-associated lung inflammation in ovalbumin (OVA)-challenged, OVA-specific T cell receptor transgenic mice[81]. Moreover, mast cells contribute to the development of delayed type CHS (*aka* allergic contact dermatitis) under some, but not all, experimental conditions[5, 82, 83]. Using different immunizing doses of the hapten oxazolone, Norman et al.[84] recently showed that mast cells can act to reduce or enhance the inflammatory response in CHS reactions depending on the concentration of hapten used for immunization.

Several mast cell-derived products can influence T cell development, recruitment, phenotype, proliferation and activation *in vitro* and *in vivo*[5, 6, 79, 81, 85, 86] and mast cells can promote T cell activation indirectly through the stimulation of antigen-presenting cells (APCs) *in vivo* (i.e., mast cells induce the migration of dendritic cells[86] and Langerhans cells[85, 87] to draining lymph nodes where antigen presentation occurs). Kambayashi et al.[88] reported that antigen incorporated into mast cells via FcεRI can activate antigen-specific T cell responses *in vitro*; this mechanism is independent of mast cell MHC class II expression, but requires that such mast cells undergo apoptosis and then ingestion by APCs.

Because mast cells can help to initiate adaptive immune responses by inducing or enhancing the migration of APCs to draining lymph nodes, and via lymphocyte activation, McLachlan et al.[89] hypothesized that the administration of small-molecule mast cell activators (e.g., compound 48/80) with vaccine antigens might enhance the development of a protective antigen-specific immune response. Indeed, they found that subcutaneous or nasal administration of these activators enhanced dendritic cell and lymphocyte trafficking to draining lymph nodes and increased antigen-specific serum IgG responses[89]. Moreover, nasal administration of compound 48/80 with B5R poxvirus protein, but not B5R poxvirus protein alone, protected the immunized mice against infection with vaccinia virus *in vivo*. The authors showed that mast cells and mast cell-derived TNF were required for the enhancement of immune responses in $WBB6F_1$-$Kit^{W/W\text{-}v}$ mice that had been engrafted with mast cells in the footpad and then vaccinated at the same site, however, their efforts to engraft mast cells in the nasal cavity of mast cell-deficient mice were unsuccessful. As noted above, $WBB6F_1$-$Kit^{W/W\text{-}v}$ mice have other defects besides a profound deficiency of mast cells, including neutrophil defects, and mast cell activators can have effects on cell types other than mast cells. However, from a clinical perspective, if this novel vaccination approach can be shown to be effective and, as importantly, safe (since mast cell activation in the context of vaccination could result in clinical toxicities), this approach may be of considerable value even if the method works because of effects on cells in addition to (or other than) mast cells.

Mast cells in models of disease

Cardiovascular disorders

Several lines of evidence have implicated mast cells in the development of a variety of chronic inflammatory disorders, including cardiovascular diseases. Because mast cells are found in the heart and, in humans, around coronary arteries and within atherosclerotic lesions, several groups have proposed that mast cells may contribute to the pathogenesis of

atherogenesis[90–92]. Indeed, Bot et al.[90] showed that targeted activation of perivascular mast cells promoted atherogenesis and plaque destabilization in apolipoprotein E-deficient mice. By crossing atherosclerosis-prone low-density lipoprotein receptor-deficient mice with C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, Sun et al.[92] provided *in vivo* evidence that mast cells can contribute to atherosclerosis; i.e., they observed smaller lesions with fewer inflammatory cell (macrophage and T cell) infiltrates in the absence of mast cells. They provided evidence that mast cells promote atherosclerosis in this setting by releasing proinflammatory cytokines (IL-6 and IFNγ), which augment the expression of matrix-degrading proteases. This group also reported that mast cells contribute to the pathogenesis of elastase-induced abdominal aortic aneurysms (AAA) in mice (i.e., C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice failed to develop AAA)[93]. They showed that AAA formation in this model required mast cell-derived IL-6 and IFNγ, but not TNF, and that mast cells increased matrix-degrading protease expression, smooth muscle cell apoptosis, and microvessel growth[93]. Similarly, Tsuruda et al.[94] showed that AAA formation following periaortic application of calcium chloride (accompanied by increased numbers of mast cells and T cells, activation of matrix metalloproteinase 9, and angiogenesis in the aortic tissue) was impaired in mast cell-deficient *Ws/Ws* rats.

Cancer

The importance of a possible functional link between chronic inflammation and cancer has long been recognized; for example, treatment with non-steroidal anti-inflammatory drugs, which can inhibit chronic inflammation, reduces the risk of several cancers[95]. The majority of tumors contain inflammatory cells, including mast cells, which have potential effects that might either benefit the tumor or contribute to tumor resistance or rejection. Using WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice, Coussens et al.[96] provided evidence that mast cells can facilitate angiogenesis during early stages of skin carcinogenesis. Soucek et al.[97] recently used pharmacological (cromolyn) and genetic (C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice) approaches *in vivo* to provide evidence that mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic β-cell tumors. Although cromolyn is widely characterized as a ‘mast cell-stabilizer’ (i.e., an agent that blocks the release of mast cell mediators following appropriate activation of the cell) that can suppress mouse mast cell function *in vivo*, its molecular targets are neither fully defined nor restricted to mast cells[6]. Moreover, because Kit signaling has been shown to be important for angiogenesis[98] and cell lineages other than mast cells are affected by the c-*kit* mutation in C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, it will be of interest to assess whether engraftment of the C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice with mast cells can restore wild-type responsiveness in this model of tumor progression. However, taken together, these results and others indicate that certain tumors may hijack certain functions of mast cells which facilitate angiogenesis and contribute to tumor survival.

Mutations of the tumor suppressor, adenomatous polyposis coli (APC) gene, are necessary and sufficient for the initiation of hereditary and many spontaneous human colorectal cancers[99, 100]. However, angiogenesis and tissue remodeling are also required for tumor expansion. Gouaris et al.[99] recently reported that mast cells accumulate in adenomatous polyps (in a lympocyte-independent manner) and are required for polyp formation, the initiating step of colon cancer. Polyp-prone APC mutant (APC$^{\Delta 468}$) mice reconstituted with bone marrow from wild-type, C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$, or *Cd34*$^{-/-}$*Cd43*$^{-/-}$ mice showed a tight correlation between the number of mast cells and mast cell progenitors with the frequency and size of polyps (wild-type > C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ > *Cd34*$^{-/-}$*Cd43*$^{-/-}$) and blood vessel density (wild-type > *Cd34*$^{-/-}$*Cd43*$^{-/-}$)[99]. TNF was required for adenomatous polyp growth, and the authors proposed that mast cell-derived TNF acts in an autocrine fashion to amplify the local mast cell pool at the site of tumor formation[99]. They concluded that mast cells contribute importantly to the development of colon cancer. These results are

consistent with those in a study reporting the reduced susceptibility of WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice to chemically-induced intestinal tumors[101]. Engraftment of the WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice with wild-type bone marrow cells increased carcinogen-induced tumorigenesis to nearly wild-type levels, consistent with a role of mast cells in this process. However, engraftment of wild-type BMCMCs failed to result in mucosal mast cells in WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ recipients, and also failed to "normalize" the animals' response to the carcinogen[101].

Although other evidence also suggests that mast cells can promote tumorigenesis and tumor progression, there are some tumor models in which mast cells appear to have roles that favor the host. For example, Sinnamon et al.[100] reported a protective role for mast cells in colorectal tumorigenesis. They crossed C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ with Min (multiple intestinal neoplasia; a model for early intestinal tumorigenesis) mice and reported that the frequency and size of adenomas was increased in such mice, whereas tumor cell apoptosis and eosinophil infiltration were decreased[100]. Sinnamon et al.[100] suggested that the net contributions of mast cells in various tumor models may favor the host or the tumor, depending on the specific tumor models, genetic variables (both germline and tumor-specific) and microenvironmental factors (e.g., in the case of gastrointestinal tumors, intestinal flora). The story is likely to be as (if not more) complicated in humans, given that humans, as well as their colonic neoplasms, are so diverse.

Conclusions

The new evidence indicating that mast cells can contribute to the pathology of cardiovascular diseases and certain cancers (at least in rodents) continues to tarnish the reputation of this enigmatic cell. However, this "bad guy" image is increasingly being challenged, and to some extent overshadowed, by the identification of an impressive number of protective roles that mast cells can play in both innate and adaptive immune responses, and even in host responses to some tumors (Fig. 4). But many questions in mast cell biology remain to be resolved. For example, certain features of mouse mast cell phenotype and/or function can vary substantially among different strains of mice[102, 103]. It will be of interest to define how, and to what extent, various genetic factors can influence aspects mast cell biology in humans. However, this is likely to be a challenging topic to investigate. Another current goal is to understand how the mast cell lineage can perform so many distinct functions, e.g., depending on the setting either promoting or limiting innate or adaptive immune responses[6, 104, 105]. Can mast cells, like T cells, generate developmentally, phenotypically and functionally distinct "subsets", or do individual mast cells have sufficient plasticity to exhibit distinct features based on their responsiveness to particular local and/or systemic environmental signals? Do both mechanisms occur? Finally, it will be important to assess how our understanding of mast cell biology can be exploited clinically. Defining to what extent one can safely enhance the positive functions of mast cells, or inhibit their harmful activities, will continue to represent important goals, both to achieve a fuller understanding of this fascinating cell and to exploit such knowledge to reduce disease and promote health.

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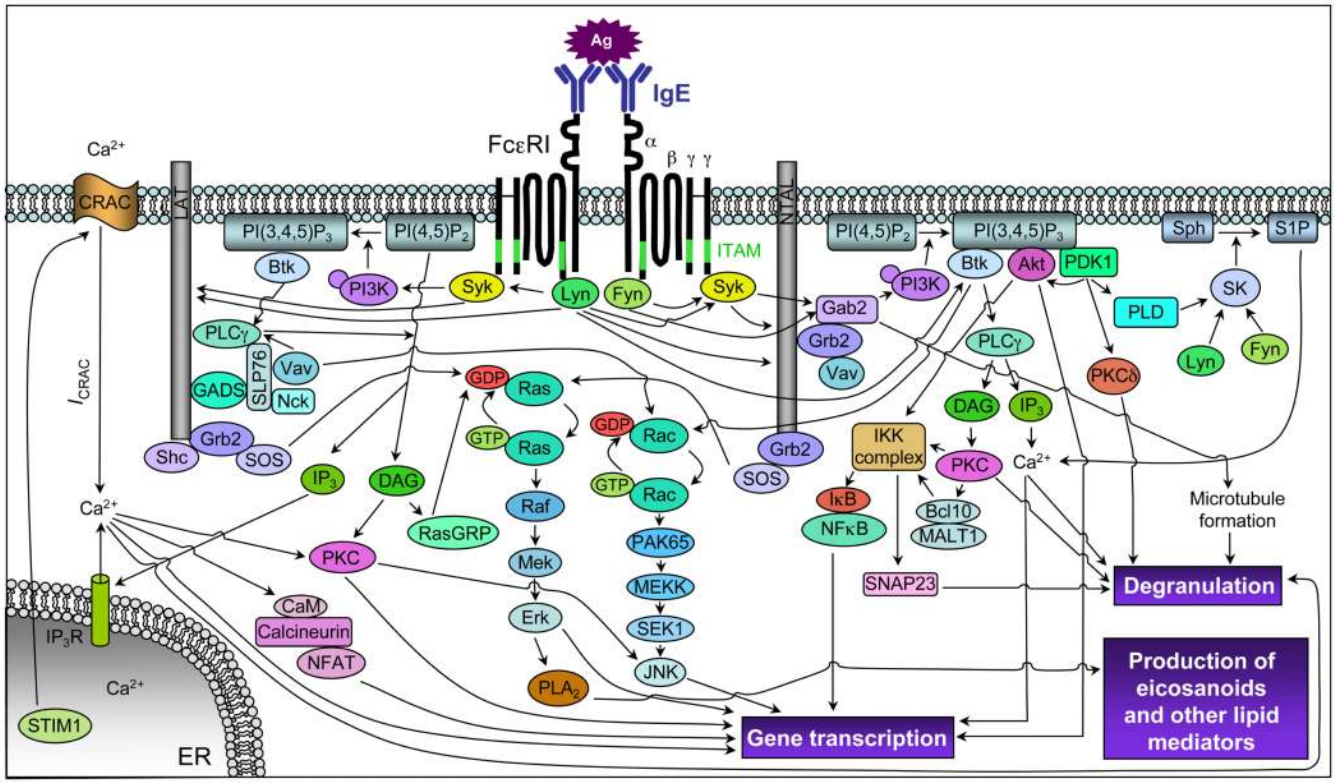

Figure 1.

Simplified scheme of early FcεRI-mediated signaling events. Ag-induced crosslinking of FcεRI induces activation of Lyn, which phosphorylates FcεRI ITAMs (green) and activates Syk following ITAM binding, and Fyn, which phosphorylates the adaptor Gab2 to activate the PI3K pathway. Lyn and Syk phosphorylate many adaptor molecules, e.g., LAT and NTAL, and enzymes to regulate activation of the Ras, PLCγ, PI3K and other pathways. Grb2 and SOS activate the Ras/Erk pathway, which regulates transcription factor activation and arachidonic acid metabolism (through PLA_2 activation). PLCγ can either be activated through the coordinated function of LAT/Gads/SLP-76/Vav and Btk or independently of LAT through a PI3K/Btk-dependent pathway. PLCγ activation regulates classical PKC activation (through DAG generation) and calcium responses (through the generation of IP_3). IP_3 binding to the IP_3R triggers Ca^{2+} release from the ER; STIM1 couples ER Ca^{2+} store depletion with the activation of CRAC channels, leading to the influx of extracellular Ca^{2+} and I_{CRAC}. The PI3K product, $PI(3,4,5)P_3$, is an important lipid mediator that regulates the activity of various enzymes, e.g., Btk, Akt, PDK1, PLD and SK, and the formation of other lipid mediators, e.g., DAG and S1P. S1P can act intracellulary, to regulate Ca^{2+} influx and degranulation (independently of PLC and IP_3), and extracellularly (following secretion from the cell) by binding to surface $S1P_1$ or $S1P_2$ receptors and thereby inducing cytoskeletal rearrangement or enhancing degranulation, respectively. The IKK complex consists of two catalytic subunits, IKKα/IKK1 and IKKβ/IKK2, and a regulatory subunit, NEMO/IKKγ; this complex phosphorylates IκB to activate the transcription factor NFκB. IKKβ/IKK2 also phosphorylates SNAP23 to facilitate SNARE complex formation. Arrows indicate the contributions of these signaling pathways toward mast cell degranulation, arachidonic acid metabolism, and cytokine/chemokine/growth factor production. Note: some arrows do not indicate direct interactions or targets. Bcl10, B cell lymphoma 10; Btk, Bruton's tyrosine kinase; Ca^{2+}, calcium; CaM, calmodulin; CRAC, Ca^{2+} release activated calcium channel; DAG, diacylglycerol; Gab2, Grb2 associated binding protein 2; GADS, Grb2 related adaptor

downstream of Shc; ER, endoplasmic reticulum; Erk, extracellular signal-regulated kinase; I_{CRAC}, Ca^{2+} release activated current; IκB, inhibitor of κB; IKK, IκB kinase; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; ITAM, immunoreceptor tyrosine based activation motif; LAT, linker for activation of T cells; MALT1, mucosa associated lymphoid tissue lymphoma translocation protein 1; NEMO, NFκB essential modulator; NFAT, nuclear factor of activated T cells; NFκB, nuclear factor κB; NTAL, non-T cell activation linker; PI3K, phosphoinositide 3-kinase; $PI(3,4,5)P_3$, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; PL, phospholipase; RasGRP, Ras guanyl nucleotide-releasing protein; S1P, sphingosine 1 phosphate; SK, sphingosine kinase; SLP-76, SH2-domain containing leukocyte protein of 76 kDa; SOS, son of sevenless homolog; Sph, sphingosine; STIM1, stromal interaction molecule 1.

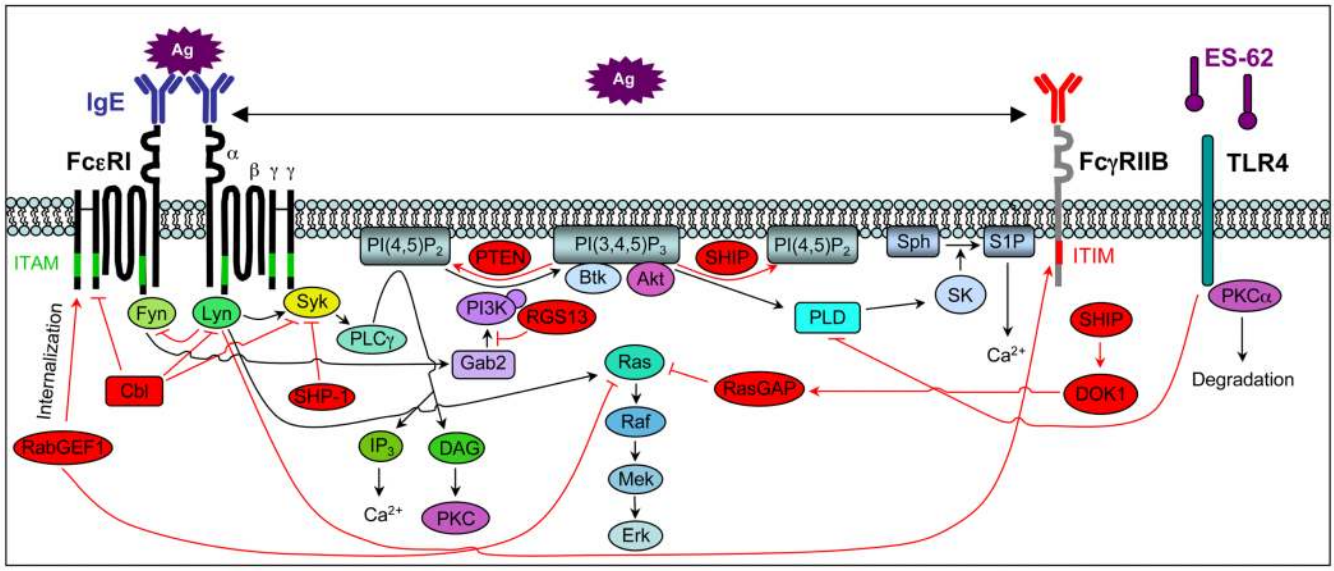

Figure 2.

Negative regulation of FcεRI-mediated signaling events. FcεRI aggregation activates a number of proteins that negatively regulate the positive signaling pathways activated downstream of this receptor. For example, Lyn, which initiates both activating and inhibitory signals, negatively regulates Fyn activity and, thus, Gab2 phosphorylation. Other negative regulators include c-Cbl (which facilitates the ubiquitination of FcεRI, Lyn and Syk), the tyrosinse phosphatase SHP-1 (which dephosphorylates Syk), the lipid phosphatases SHIP (which catalyzes the hydrolysis of $PI(3,4,5)P_3$ to $PI(3,4)P_2$) and PTEN (which catalyzes the hydrolysis of $PI(3,4,5)P_3$ to $PI(4,5)P_2$), RasGAP (which enhances the intrinsic GTPase activity of Ras), RabGEF1 (which enhances FcεRI internalization and can bind to GTP-bound Ras), and RGS13 (which binds to the p85α subunit of PI3K and disrupts its association with Gab2 and Grb2). Ag-induced coaggregation of FcεRI with FcγRIIB inhibits FcεRI-induced signaling events and mast cell activation via Lyn mediated phosphorylation of the FcγRIIB ITIM (red) and the subsequent recruitment of SHIP and DOK1. Finally, ES-62, a glycoprotein secreted by filarial nematodes, forms a complex with TLR4 (which causes the sequesteration and subsequent proteosome-independent degradation of PKCα) to block FcεRI-induced PLD-coupled, SK-mediated Ca^{2+} flux and NFκB activation. DOK1, docking protein 1; Gab2, Grb2 associated binding protein 2; ITAM, immunoreceptor tyrosine based activation motif; ITIM, immunoreceptor tyrosine based inhibititory motif; NFκB, nuclear factor κB; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLD, phospholipase D; PTEN, phosphatase and tensin homolog; RabGEF, Rab5 guanine nucleotide exchange factor; RasGAP, GTPase activating protein; RGS, regulator of G protein signaling; SHIP, Src homology 2 (SH2) domain-containing inositol 5′-phosphatase; SHP-1, SH2 domain-containing tyrosine phosphatase-1; SK, sphingosine kinase; TLR4, toll like receptor 4.

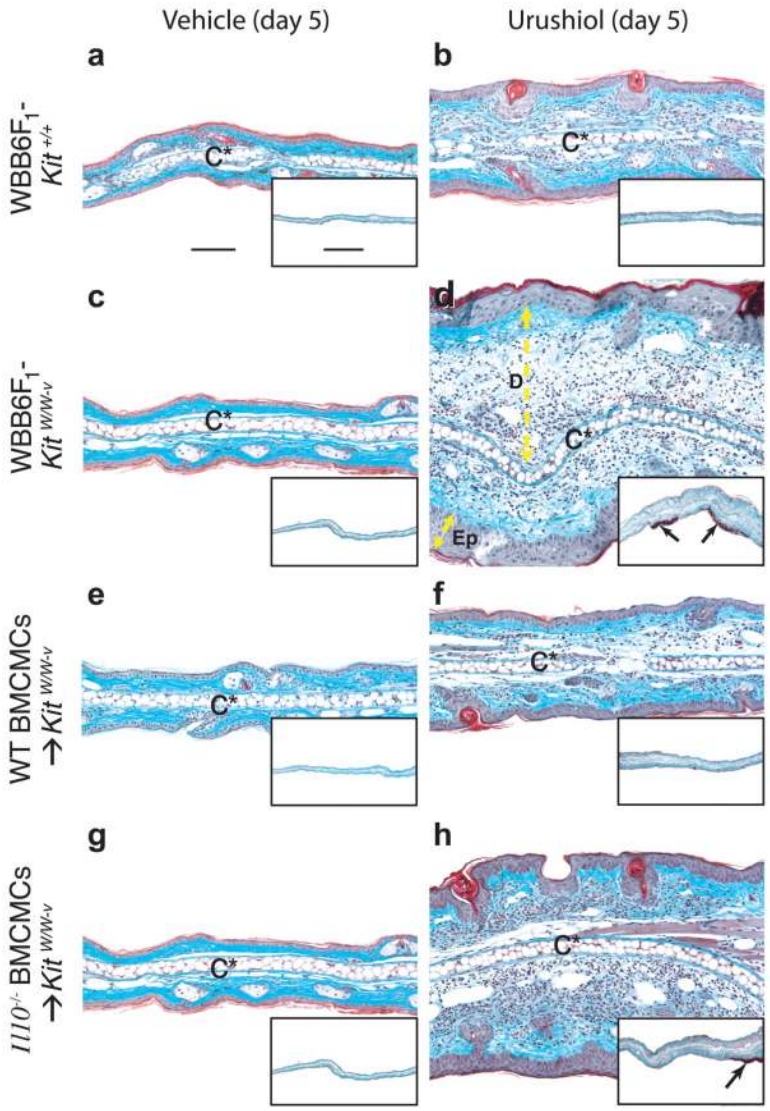

Figure 3.

Mast cell limit the pathology associated with CHS to urushiol. Cross-sections of ears (stained with Masson's Trichrome) from WBB6F$_1$-*Kit*$^{+/+}$ (wild-type) mice (**a,b**), WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice (**c,d**) or WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice engrafted 8 weeks before the experiment with WT BMCMCs (WT BMCMC → *Kit*$^{W/W\text{-}v}$ mice) (**e,f**) or *Il10*$^{-/-}$ BMCMCs (*Il10*$^{-/-}$ BMCMC → *Kit*$^{W/W\text{-}v}$ mice) (**g,h**) were obtained 5 d after challenge with vehicle (100% acetone) only (**a,c,e,g**) or 5 mg/ml of urushiol (**b,d,f,h**). Focal full thickness necrosis of the epidermis and/or ulceration occurred in association with CHS responses to urushiol at 5 d after challenge in 8/10 of the mast cell-deficient WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice and in 8/8 *Il10*$^{-/-}$ BMCMC → *Kit*$^{W/W\text{-}v}$ mice but in none of the 10 wild-type or 7 WT BMCMC → *Kit*$^{W/W\text{-}v}$ mice; $^{*}P < 0.05$ by Chi-square test for all comparisons between rates of epidermal necrosis and ulceration in wild-type (WBB6F1-*Kit*$^{+/+}$) mice or WT BMCMC → Kit$^{W/W\text{-}v}$ mice and the corresponding mast cell-deficient WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice or *Il10*$^{-/-}$ BMCMC → *Kit*$^{W/W\text{-}v}$ mice. Similar findings were observed in association with CHS responses to urushiol in 5 of 7 C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice in response to DNFB. By contrast, epidermal necrosis and ulceration occurred in none of the corresponding congenic wild-type mice or WT BMCMC-engrafted C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (10 or 8 for urushiol and 19 or 16 for

DNFB, respectively); *$P < 0.05$ by Chi-squre test for all comparisons between rates of epidermal ulceration in wild-type or WT BMCMC-engrafted C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice and the corresponding mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. C*: cartilage; double-headed arrows show thickness of dermis (D) or epidermis (Ep); arrows in insets: ulcers with adherent exudates (red). Scale bar in **a** = 100 μm & in inset in **a** = 1000 μm. Photomicrographs are representative of the findings observed in each of the 3 experiments performed (n = 3–7 mice/group per experiment). Taken from[74].

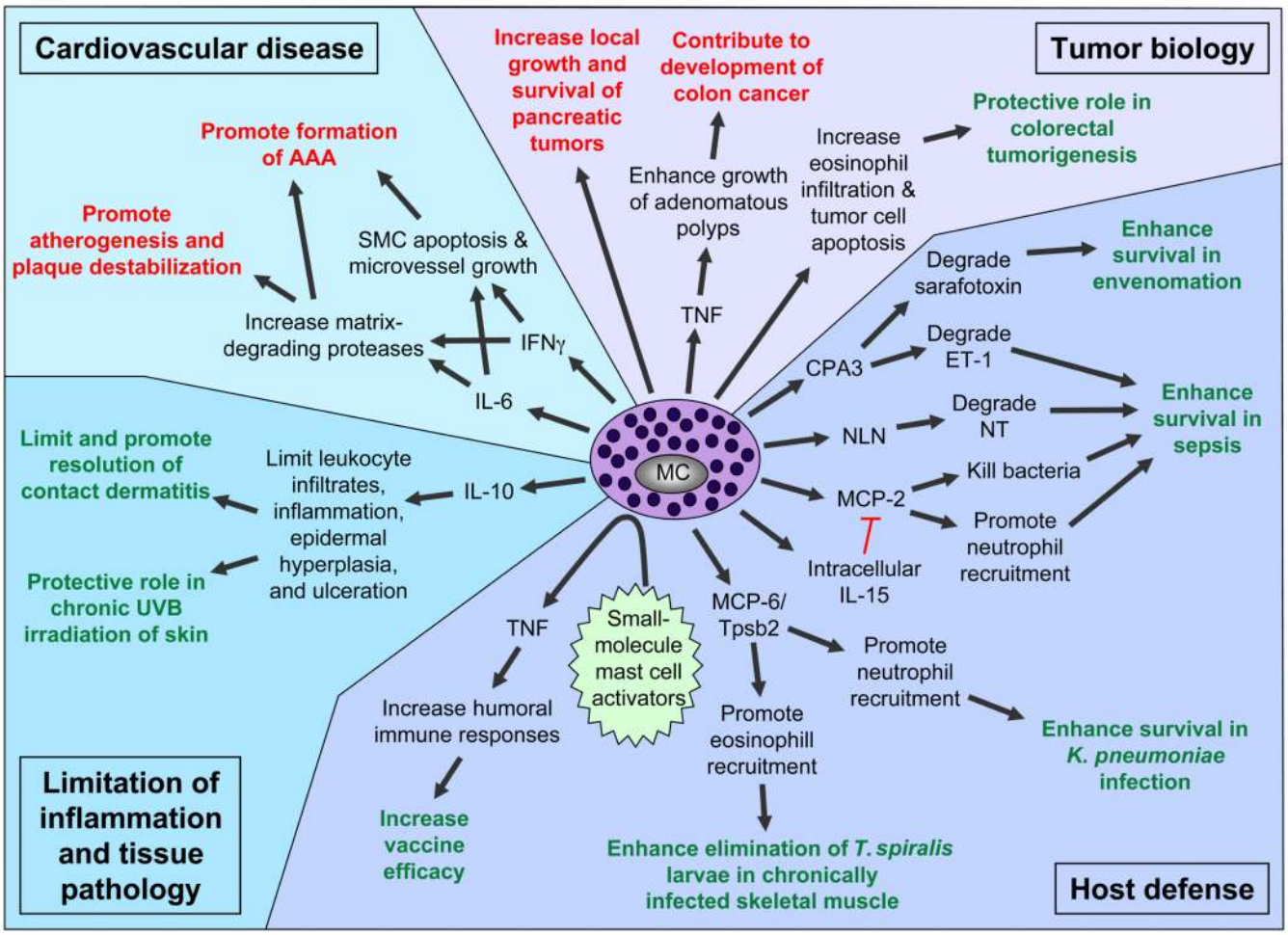

Figure 4.

Newly identified protective (green) or detrimental (red) roles of mast cells and mast cell products in biological responses in mice. AAA, abdominal aortic aneurysm; CPA3, carboxypeptidase A3; ET-1, endothelin-1; IgE, immunoglobulin E; IL, interleukin; MC, mast cell; MCP, mast cell protease; NLN, neurolysin; NT, neurotensin; SMC, smooth muscle cell; TNF, tumor necrosis factor; Tpsb2, tryptase β2.