

Widespread Immunological Functions of Mast Cells: Fact or Fiction?

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Immunological functions of mast cells are currently considered to be much broader than the original role of mast cells in IgE-driven allergic disease. The spectrum of proposed mast cell functions includes areas as diverse as the regulation of innate and adaptive immune responses, protective immunity against viral, microbial, and parasitic pathogens, autoimmunity, tolerance to graft rejection, promotion of or protection from cancer, wound healing, angiogenesis, cardiovascular diseases, diabetes, obesity, and others. The vast majority of *in vivo* mast cell data have been based on mast cell-deficient *Kit* mutant mice. However, work in new mouse mutants with unperturbed *Kit* function, which have a surprisingly normal immune system, has failed to corroborate some key immunological aspects, formerly attributed to mast cells. Here, we consider the implications of these recent developments for the state of the field as well as for future work, aiming at deciphering the physiological functions of mast cells.

Introduction

The original recognition of immunological hyperreactivity and allergic inflammation dates back more than 100 years. Paul Ehrlich, referring to self-reactive antibodies, coined the term “horror auto-toxicus” (the fear of being toxic to oneself) but rejected the idea that immunological autoaggression would exist or make sense (Ehrlich, 1901). Clemens von Pirquet conceived the concept of allergy as immunological hyperreactivity (von Pirquet, 1906). The mast cell, discovered (Ehrlich, 1877) well before allergy was recognized as a regular inducible immunological phenomenon, happens to be at the center of anaphylaxis and allergic inflammation (Mota and Vugman, 1956), with IgE (Ishizaka and Ishizaka, 1966) providing the link between antigen (allergen) and the high-affinity IgE receptor (FcεRI) (Blank et al., 1989) expressed on mast cells. Mast cells can be viewed as the immune system’s loaded gun. There is arguably no second cell type in the immune system as powerfully equipped with a large array of chemically diverse and highly potent compounds. Mast cell products cover a wide range of biological activities from the promotion of local inflammation to the regulation of systemic blood pressure and vessel permeability. Upon stimulation, mast cells rapidly release by degranulation, among other products, histamine, heparin and other proteoglycans, granule-associated proteases, and they produce leukotrienes and prostaglandins. Mast cell products can kill humans and animals within minutes in what is known as anaphylaxis (Portier and Richet, 1902). In addition, mast cells also produce chemokines and cytokines (reviewed in Galli et al., 2008), notably IL-4 and IL-13, key factors initiating or augmenting type 2 immunity (Liang et al., 2012).

To this date, the “positive” counterpart to the mast cell’s “negative” role in the allergic arm of immunity remains puzzling. Although exposure to and protective immunity against pathogens has been found inversely correlated with the emergence of allergic disease (Ege et al., 2011), the phenomenon of allergy per se, i.e., hyperreactivity upon repeated encounter

with innocuous substances, remains a paradox of the immune system. As with other weapons, the terms of usage (i.e., licensed or not) and the targets (e.g., self-tissues or pathogens) determine harm (as in allergy) or protection (as in resolving and lasting immunity). The notion of a primary function of mast cells in the promotion of allergic inflammation is difficult to accept. Yet, exactly this undesired and in extreme cases devastating role of mast cells in allergic disease remains the best-understood and -documented function. The essential role of mast cells in this type of immune reaction is underscored by the fact that mast cell-deficient mice are unable to mount IgE-mediated local and systemic anaphylaxis (Feyerabend et al., 2011; Lilla et al., 2011; Sawaguchi et al., 2012; Wershil et al., 1987). In *Kit^{W/W^v}* (Wershil et al., 1987) and in *Cpa3^{Cre}* (Feyerabend et al., 2011) mice, the defect in the response was restored upon mast cell transfers.

For a long time, this culprit role of mast cells in allergic disease, and the concomitant lack of known functions in protective immunity, hindered mast cells from entering immunology’s center stage. This situation has changed dramatically over the past years, as manifold functions for mast cells have been reported. Roles for mast cells have been reported in the regulation of innate and adaptive immune responses, including tolerance to skin graft rejection (de Vries and Noelle, 2010; Lu et al., 2006), in settings of T cell and antibody mediated autoimmunity (Sayed et al., 2008), in protective immunity against viral (Wang et al., 2012) and microbial (Chan et al., 2012) pathogens, in tissue remodeling, wound healing (Gilfillan and Beaven, 2011) and angiogenesis (Coussens et al., 1999), in cancer promotion or protection from cancer by participation in tumor stroma (Tlsty and Coussens, 2006), and in immune-metabolic syndromes of diabetes and obesity (Liu et al., 2009). Beyond these immune functions, mast cells have been proposed to be involved in diverse areas including vascular diseases (Bot and Biessen, 2011), anxiety behavior (Nautiyal et al., 2008), male (Haidl et al., 2011) and female (Menzies et al., 2011) fertility, and

mammary gland development (Coussens and Pollard, 2011). It is evident from this all but complete list that, if indeed true, mast cells have a huge spectrum of functions. Outside observers might not be the only ones concerned that this list could be an “immunological bubble.” If not, mast cells were indeed among the cells with the most pleiotropic functions in the immune system, and beyond. It is interesting to note that many authors of primary and reviewing literature emphasize the versatility of mast cell functions, meaning that mast cells’ behavior, e.g., as suppressors or enhancers of inflammation, may vary depending on the context. It is often ambiguous whether “context” refers to experimental conditions or to physiological or pathological states. Although this idea may appropriately describe mast cell functions, it can also accommodate conflicting data, and a degree of vagueness remains inherent to this concept. Many of the reported mast cell functions listed above have remained controversial or, at least, have not been independently verified in different experimental settings, such as in independent mouse models. In conclusion, mast cells and the mast cell enigma have kept their fascination, and, in our view, many physiological functions of mast cells beyond allergic disease remain to be unequivocally demonstrated.

In this article, we refrain from an in-depth review of the most prominent and recent mast cell literature along the lines of the listed functions stated above. Instead, our goal is to provide a framework in which to consider past and discuss future experimental settings and their implications to test relevant mast cell functions under physiological and pathological conditions. With this in mind, key topics to be covered are, in brief, the role of Kit in mast cell research, the use of cultured mast cells as in vivo tools, and the quest for Kit-independent mast cell deficiency models, followed by a brief overview of proven and more speculative roles of mast cell functions. We have no answer to the title of this review yet, but we hope to stimulate discussions and, most of all, new experiments that may eventually provide the answer in the future.

The Receptor Tyrosine Kinase Kit and Mast Cells: A Close Liaison in Its 35th Year

Mast cells are strictly tissue-localized cells, and under normal healthy conditions, they are undetectable in bone marrow and peripheral blood. Bone marrow transplantation (Kitamura et al., 1977) and mast cell progenitor identification (Rodewald et al., 1996) established that mast cells belong developmentally to the hematopoietic lineage. The mutation underlying the phenotype of dominant white spotting (*W*) mouse strains (Russell, 1979) was linked to mast cells by showing that *W* mouse mutants are mast cell-deficient (Kitamura et al., 1978). The reconstitution of donor mast cells in recipient *W* mice by wild-type bone marrow transplantation marked the beginning of the era in which *W* mice served as in vivo models to investigate mast cell functions. White spotted mice exist in variable phenotypic penetrations, depending on the different naturally occurring alleles, termed *Kit^W*, *Kit^{W^v}*, and *Kit^{W-sh}* (Nocka et al., 1990), to name those most relevant for the mast cell field. The receptor tyrosine kinase Kit maps to the mouse *W* locus, and *Kit* mutations are causal for the phenotypes of *W* mice, including their mast cell deficiency. Kit ligand (Kitl) is a microenvironmental stromal cell factor encoded at the Steel

(*Sl*) locus. *Sl* mutants also lack mast cells (Galli and Kitamura, 1987).

The vast majority of the existing in vivo mast cell literature is based on the viable compound mutant *Kit^{W^vW^v}* mouse. The *Kit^W* allele-encoded protein cannot be expressed on the cell surface, while the kinase activity is impaired in the *Kit^{W^v}*-encoded protein (Nocka et al., 1990). *Kit^{W^vW^v}* mice are severely affected by their Kit deficiency in several tissues. In the context of the long-standing use of these mutants as mast cell deficiency models, it is worth to consider some key aspects of Kit biology. Kit is expressed inside and outside of the hematopoietic system in cell types of diverse developmental origin. Within the immune system, Kit is an important hematopoietic stem and progenitor cell marker, which is expressed in these cell types at high levels. In most lineages, Kit expression is lost with differentiation. The notable exception are mast cells in which Kit expression remains very strong throughout their development. Kitl is a key growth factor for mast cells in vivo. The block in mast cell development in *Kit* mutant mice is, however, not absolute. In vitro, interleukin-3 (IL-3) can drive the development of bone marrow-derived mast cell cultures (BMMCs) from *Kit* mutant bone marrow cells. In contrast to the strict requirement for Kit signaling in mast cells in vivo, IL-3 is sufficient and the most potent mast cell growth factor in vitro (Nabel et al., 1981; Yokota et al., 1984; Yung and Moore, 1982), where Kitl synergizes with IL-3 for optimal mast cell growth. Surprisingly, steady state mast cell development was normal in *Il3*-deficient mice (Lantz et al., 1998), underscoring fundamentally different growth requirements in vivo and in vitro. The mast cell deficiency of *Kit^{W^vW^v}* mice can be partially overcome by chronic inflammatory stimuli in vivo (Gordon and Galli, 1990; Waskow et al., 2007), which may limit the use of these mice under certain conditions of chronic inflammation.

In addition to their mast cell defect, *Kit^{W^vW^v}* mice have multiple hematopoietic abnormalities that include compromised fitness of hematopoietic stem and progenitor cells (Russell, 1979), severe macrocytic anemia (Waskow et al., 2004), impaired T development in the thymus (Waskow et al., 2002), a shift in intraepithelial T cells in the gut in favor of TCR $\alpha\beta^+$ and against TCR $\gamma\delta^+$ cells (Puddington et al., 1994). Importantly, this *Kit* mutant is neutropenic, which may be a major factor affecting immune responses in this strain (Zhou et al., 2007).

It has been reiterated (Galli and Kitamura, 1987; Grimbaldes-ton et al., 2005) that numbers of basophils are not affected in *Kit^{W^vW^v}* mice. This claim appears to rest on an older study based on the morphological identification of basophils in peripheral blood (Jacoby et al., 1984). Morphology and cell surface markers of mouse basophils have been controversial (Lee and McGarry, 2007). Based on flow cytometric phenotype, cytokine (IL-4) production, and other functions, the current consensus on mouse basophils is that these cells can be identified by their DX5⁺Kit⁺Fc ϵ RI⁺ phenotype (Karasuyama et al., 2011; Siracusa et al., 2011 and references therein). Using this definition, two recent reports found 75%–90% reduced basophil numbers in the peripheral blood (Mancardi et al., 2011) and spleen (Feyerebend et al., 2011) of *Kit^{W^vW^v}* mice. This paucity of basophils may be a contributing factor in phenotypes of *Kit^{W^vW^v}* mice that have been attributed to the lack of mast cells.

More recent publications have often used *Kit^{W-sh/W-sh}* mice for mast cell studies because these mice have fewer defects

compared to *Kit^{W/W^v}* mice (Grimbaldeston et al., 2005). In contrast to *Kit^{W/W^v}* mice, *Kit^{W-sh/W-sh}* mice are fertile and have normal red blood cell numbers. Initially, *Kit^{W/W^v}/Kit^{W-sh/W-sh}* mice were thought to have normal levels of all major classes of hematopoietic cells including leukocytes (Grimbaldeston et al., 2005 and references therein). However, a study found neutrophilia, megakaryocytosis, and thrombocytosis in *Kit^{W-sh/W-sh}* mice, which were associated with splenomegaly and histological aberrations of the spleen (Nigrovic et al., 2008). The *Kit^{W-sh}* allele is characterized by a large genomic inversion on chromosome 5, the 3' end of which is located ~75 kb upstream of the *Kit* locus (Berrozpe et al., 1999; Nigrovic et al., 2008). This genomic rearrangement does not only deregulate the temporal and tissue-specific expression of *Kit* itself, which can explain the mast cell deficiency (Berrozpe et al., 2006), but also potentially leads to the disruption or deregulation of the 27 genes located in the inverted genomic region, including disruption of *Corin*, which causes cardiac hypertrophy (Nigrovic et al., 2008). In summary, *Kit* is an essential receptor driving mast cell development in vivo, and mice carrying inactivating mutations in *Kit* have remained the main models in studies of mast cell functions. However, in addition to their mast cell deficiency, *Kit* mutants suffer from further defects in the hematopoietic system.

Roles of Kit Outside of the Immune System

Kit functions are important in germ cells and melanocytes (Bessmer et al., 1993), intestinal pacemaker cells (Sergeant et al., 2002), neuronal cells (Milenkovic et al., 2007), and liver metabolism (Magnol et al., 2007). *Kit*-related phenotypes in these tissues may indirectly perturb immunological experiments locally or systemically, e.g., an altered threshold of nociception may influence inflammatory responses. Roles of mast cells have often been studied in organs that are intrinsically affected by *Kit* mutations. In *Kit* mutant mice, intestinal pacemaker cells of Cajal fail to develop, which results in insufficient intestinal peristaltic movement (Huizinga et al., 1995; Maeda et al., 1992), gut dilatation, and delayed intestinal passage times (Snoek et al., 2011). These pathophysiological parameters can influence the composition of intestinal microbiota, or intestinal pathology. *Kit^{W/W^v}* mice are more susceptible to enterobacterial sepsis in the caecal ligation and puncture (CLP) model (Echtenacher et al., 1996). This higher vulnerability of *Kit^{W/W^v}* mice has been explained by their mast cell deficiency, leading to insufficient production of protective TNF- α . However, the situation in the CLP model is complex because TNF- α has also been implicated in a worse outcome in a severe version of the model (Piliponsky et al., 2010). In addition, an involvement of the *Kit* mutations in CLP experiments in *Kit^{W/W^v}* mice cannot be excluded because the cecum, which is the target organ in this model, is highly dilated in *Kit^{W/W^v}* mice, and this intestinal phenotype on its own may impair resolution of the puncture. Collectively, the involvement of *Kit* signaling in the development or function of cells outside of the immune system may have an impact on mast cell research in *Kit* mutants.

Mast Cell Reconstitution of *Kit* Mutant Mice: A Gold Standard Less Golden?

It is obvious that the multitude of non-mast cell-related deficiencies in *Kit* mutants may influence the outcome of experi-

ments addressing in vivo mast cell functions. Bone marrow transplantation can reconstitute wild-type hematopoietic cells in *Kit^{W/W^v}* mice, and it cures their mast cell deficiency and, for example, their anemia, but their mast cell defect is not selectively corrected. In contrast, reconstitution of *Kit* mutant mice only with BMMCs without hematopoietic stem cells appeared to offer a solution for this problem (Nakano et al., 1985; Tsai et al., 2005). The rationale appears compelling: If mast cell-reconstituted *Kit* mutant mice show a reversal of the presumed mast cell-dependent phenotype, e.g., if wild-type mice are resistant to sepsis, *Kit^{W/W^v}* mice are susceptible, and mast cell-repleted *Kit^{W/W^v}* mice are resistant, the conclusion seemed warranted that the mast cell deficiency of *Kit^{W/W^v}* mice was the responsible cellular defect underlying susceptibility (Echtenacher et al., 1996). Effectively, the mast cell reconstitution system has been, by and large, the mainstay to test mast cell functions in vivo, and this approach has been considered a pillar of immunology (Kawakami, 2009).

Does mast cell reconstitution lead to physiological mast cell populations? Although BMMCs can adopt the phenotype of normal tissue mast cells after in vivo transfer (Nakano et al., 1985), numbers, distribution, and functional responses of reconstituted mast cells may not be physiological (Ebmeier et al., 2010; Grimbaldeston et al., 2005; Nakano et al., 1985). The distribution is a worthwhile consideration because mast cell numbers may not only be lower than normal in one tissue but also higher than normal in another. The route of transfer (local versus systemic) matters, and the reconstitution can vary between individual mice. Mast cell reconstitution has regularly been demonstrated histologically, e.g., acquisition of a connective tissue mast cell phenotype by safranin staining, but it is difficult to go further and prove that engrafted mast cells behave immunologically like normal endogenously developed mast cells.

Recent experiments have uncovered that BMMC reconstitution can erroneously lead to the reversal of a phenotype in *Kit* mutants. Disturbingly, experiments comparing conventional *Kit* mutant mast cell-deficient mice and mast cell reconstituted *Kit* mutant mice versus selectively mast cell-deficient mice wild-type for *Kit* (Dudeck et al., 2011; Feyerabend et al., 2011, discussed in Katz and Austen, 2011) led to fundamentally different results. Roers and colleagues studied contact hypersensitivity in the skin. These authors found that mast cell-specific deletion of *Il10* in *Mcpt5-Cre* mice, a Cre transgene that deletes LoxP-site flanked gene segments in connective tissue mast cells, failed to reveal a role for mast cell-derived IL-10 in the regulation of contact hypersensitivity (Dudeck et al., 2011). Earlier studies using the mast cell reconstitution approach by transfer of *Il10*-deficient BMMCs into *Kit* mutants concluded that mast cell-derived IL-10 is important (Grimbaldeston et al., 2007). Further conflicting results comparing *Kit* mutant mast cell-deficient and *Kit*-independent mast cell-deficient mice were found in experiments on the roles of mast cells in autoimmunity (discussed in Brown et al., 2012; Rodewald, 2012). On the basis of work in *Kit^{W/W^v}* mice, key roles for mast cells had been reported in the K/BxN autoantibody-driven arthritis models (Lee et al., 2002) and in myelin oligodendrocyte glycoprotein (MOG)_{35–55} peptide immunization-driven experimental autoimmune encephalomyelitis (EAE) (Secor et al., 2000, reviewed in Benoist and Mathis, 2002; Sayed et al., 2008). In both cases, the mast

cell involvement was demonstrated by reconstitution of *Kit*^{W/W^v} mice with BMMCs. Surprisingly, Kit-independent mast cell-deficient *Cpa3*^{Cre/+} mice were fully susceptible to the K/BxN arthritis and to EAE (Feyerabend et al., 2011). In these studies, *Kit*^{W/W^v} mice were included in the experiments as controls. For the K/BxN arthritis, the susceptibility of *Kit*^{W/W^v} mice was confirmed, but notably not for EAE (Feyerabend et al., 2011). Questions about the importance of mast cells in arthritis had been raised earlier. Whereas *Kit*^{W/W^v} mice were not only protected in the K/BxN model but also in anti-collagen antibody-induced arthritis, *Kit*^{W-sh/W-sh} mice were susceptible in both models (Mancardi et al., 2011; Zhou et al., 2007).

Inevitably, these examples strongly suggest that *Kit* mutations can contribute to the phenotype of mast cell experiments, and that mast cell-reconstituted *Kit* mutants can behave differently from normal mice with and without mast cells, a divergence that remains to be understood. These examples of fundamental discrepancies between *Kit* mutant mast cell-deficient mice and mast cell-deficient mice wild-type for *Kit* may remain the exception. We believe, however, that the robustness of the *Kit*-based system, including mast cell reconstitution, is doubtful as soon as a single example has been found in which the system did not report with fidelity on in vivo mast cell functions. Although cumbersome and possibly of little reward, it may be prudent to readdress key mast cell findings based on *Kit* mutant mice also in *Kit*-independent models.

Kit-Independent Mast Cell-Deficient Mice: Problems, Solutions, and Limitations

The quest to develop Kit-independent mast cell-deficient mice was aided by advances in gene targeting and transgenesis. The identification of genes expressed specifically in mast cells helped to identify “driver loci” that are instrumental for genetic manipulation of mast cells in vivo. Some years ago, several groups reported the generation of mice expressing Cre recombinase under the control of mast cell protease genes (Feyerabend et al., 2009; Müsch et al., 2008; Scholten et al., 2008), and in 2011, four laboratories used their mice or additionally generated lines to obtain Kit-independent mast cell-deficient mouse strains (Dudeck et al., 2011; Feyerabend et al., 2011; Lilla et al., 2011; Otsuka et al., 2011). The strains differ in the selected gene loci, the methods to drive ectopic gene expression in mast cells (targeted knock-in or transgenic overexpression), and the depletion mechanisms. In brief, Dudeck et al. (2011) and Otsuka et al. (2011) used the diphtheria toxin (DT) system for depletion of the mast cell lineage, Feyerabend et al. (2011) ablated mast cells constitutively by exploiting the genotoxicity of Cre-recombinase, and Lilla et al. (2011) depleted mast cells by Cre-mediated deletion of an apoptosis suppressor gene. Strain-specific characteristics are summarized in Table 1.

Dudeck et al. (2011) expressed Cre recombinase from the first exon of mouse mast cell protease 5 (*Mcpt5*) in a bacterial artificial chromosome (BAC) transgene harboring the *Mcpt5* locus (Scholten et al., 2008) and used *Mcpt5*-Cre to activate a Cre-dependent expression cassette in the ROSA26 locus of the mast cell lineage. Two different versions of mice have been presented. Depending on the ROSA26 allele, expression of the catalytically active diphtheria toxin A subunit (R-DTA) or of the human diphtheria toxin receptor (iDTR) was induced. Therefore,

Mcpt5-Cre x R-DTA mice represent a model of constitutive mast cell deficiency, while in *Mcpt5*-Cre x iDTR mice mast cell ablation can be induced by repetitive diphtheria toxin injections. Given that expression of the *Mcpt5* transgene is restricted to connective tissue mast cells (CTMCs), CTMCs can be targeted, whereas mucosal mast cell (MMC) compartments remain unaffected. Deletion was most complete in peritoneal mast cells. Remaining numbers of skin mast cells varied from 3.5% (ear skin) to 11% (abdominal and back skin) in R-DTA mice. In iDTR mice, mast cells were reduced to 2.5% (ear skin) after DT injections. Long-term experiments in *Mcpt5*-Cre x iDTR mice required continuous DT injections because, within 3 weeks after the last DT injection, ~10% of normal mast cell numbers reappeared. A major advantage of the *Mcpt5*-Cre mice is that these mice can also be used to delete floxed genes in CTMCs (Dudeck et al., 2011) and that numbers of basophils are, at least in the bone marrow, not impaired. Possible disadvantages are potential side effects of toxin applications (Lahl and Sparwasser, 2011), the mast cell rebound after ablation, which complicates long-term experiments, and the exclusion of MMCs.

The mast cell-deficient mouse reported by Otsuka et al. (2011) and Sawaguchi et al. (2012) is an inducible model based on DTR expression. In mast cell-specific enhancer-mediated toxin receptor-mediated conditional cell knockout (Mas-TRECK) mice, DTR expression is driven as a transgene by an *Il4* intronic enhancer element that normally drives IL-4 expression in mast cells. As in the case of *Mcpt5*-Cre x iDTR mice, repetitive toxin injections are required for mast cell depletion. The authors achieved full depletion of mast cell compartments in peritoneal cavity, ear, and back skin, stomach, and mesentery, which included CTMCs and MMCs. It remains to be tested whether under certain inflammatory conditions mast cells can overcome the depleting effect of DT. Of note, Mas-TRECK mice are fully deficient for both mast cells and basophils. As a result, Mas-TRECK mice are fully protected (Otsuka et al., 2011; Sawaguchi et al., 2012) from mast cell-dependent IgE-mediated local and systemic anaphylactic responses as well as basophil-dependent IgE-mediated chronic allergic inflammation (Karasuyama et al., 2011). An additional mouse strain selectively lacking basophils but not mast cells has been generated by a similar strategy (Bas-TRECK) (Otsuka et al., 2011; Sawaguchi et al., 2012), and the authors propose to analyze mast cell functions by comparison of Mas-TRECK and Bas-TRECK mice, or to make use of a “window,” in which basophils, but not mast cells, have rebound from the bone marrow after depletion.

Cre recombinase is widely used to delete and recombine DNA fragments within the mouse genome between introduced loxP sites, but Cre can also be toxic independent of loxP target sites when its expression is very strong or long lasting (Schmidt-Suppran and Rajewsky, 2007). In the mast cell-deficient mouse made by Feyerabend et al. (*Cpa3*^{Cre/+}), the genotoxic effect of Cre constitutively deleted the entire mast cell lineage. An expression construct, containing a codon-improved cDNA of Cre (iCre), preceded by an untranslated exon and an intron for RNA stability (references in Feyerabend et al., 2011), was introduced into the first exon of the *Cpa3* locus by homologous recombination in embryonic stem cells. The *Cpa3* locus is very strongly expressed in mast cells, as highlighted in a reporter knockin mouse (unpublished data). Cre expression from the *Cpa3*

Table 1. Overview of Kit-Independent Mast Cell Deficiency Mouse Models

	Mcpt5-Cre	Mas-TRECK	Cre-Master	Cpa3-Cre
Official Nomenclature	Tg(Cma1-cre) ARoer		Cpa3tm3(icre)Hrr	Tg(Cpa3-cre)3Glli
Reference	Dudeck et al., 2011	Otsuka et al., 2011; Sawaguchi et al., 2012	Feyerabend et al., 2011	Lilla et al., 2011
Mast Cell Specificity	Mcpt5 BAC transgene (67 kb 5' and 46 kb 3')	IL4 intronic enhancer (4.5 kb) plus IL4 promoter (0.76 kb) transgene	targeted knockin into the endogenous Cpa3 locus	Cpa3 promoter (0.78 kb) transgene
Induced or Depleted	R-DTA	iDTR	not required	Mcl-1 ^{fl/fl}
Additional Loci				
Depletion Mechanism	constitutive: Cre-mediated induction of DTA expression from the ROSA26 locus	inducible: Cre-mediated induction of the DT-receptor + repetitive DT injections	inducible: DTR expression + repetitive DT injections	constitutive: Cre-mediated genotoxicity
				constitutive: conditional knockout of the intracellular anti-apoptotic factor Mcl-1
Mast Cell Depletion	up to 97% of CTMCs, depending on the DT injection protocol; MMCs are not depleted	deficient for CTMCs and MMCs (steady state)	deficient for CTMCs and MMCs (steady state and upon inflammation)	92%–100% including CTMCs and MMCs
Basophils	not affected	entirely depleted	reduced to 38%	reduced to 22%–42%
Significant Alterations in Other Blood Cells	not detected	not detected	not detected	macrocytic anemia, neutrophilia
IgE-Mediated Anaphylactic Response	not analyzed	absent	absent	reduced
MC Recurrence	steady state: MCs back to 10% at 1–3 weeks after last DT injection	steady state: MC deficiency lasts >18 days after last DT injection; blood basophil numbers are normal again by day 12	steady state: no inflammation or parasite infections: no	not analyzed

This table summarizes genetic and functional properties of recently developed Kit-independent mast cell deficiency models. Official nomenclature refers to the entry at the Mouse Genome Informatics (MGI) Database, The Jackson Laboratories.

locus leads to a p53-dependent loss of mast cells, probably due to chromosomal lesions brought about by Cre action on endogenous pseudo-loxP sites in the mouse genome (discussed in Feyerabend et al., 2011). Cre-mediated mast cell eradication (Cre-Master; *Cpa3*^{Cre/+}) mice were entirely mast cell-deficient for both CTMCs and MMCs in peritoneal cavity, ear skin, and intestinal mucosa, and they were protected from passive local or systemic anaphylaxis. Chronic inflammatory conditions did not overcome the mast cell deficiency. This holds true for CTMCs, as demonstrated in PMA-induced dermatitis, and for MMCs in the intestine upon nematode infections. Detailed analyses of hematopoietic cell subsets and blood parameters revealed a normal steady-state immune system in Cre-Master mice. Even though *Cpa3* expression is not absolutely restricted to the mast cell lineage, expression levels in other lineages are too weak to render Cre recombinase toxic, except for a reduction of basophils to ~40% of wild-type mice. Further work will be required to analyze whether the residual basophils are functionally impaired due to Cre expression in this lineage.

Lilla et al. developed a *Cpa3-Cre* transgenic mouse strain, carrying a 780 bp region of the *Cpa3* promoter in front of the

Cre recombinase cDNA. This mouse was crossed to mice with floxed myeloid cell leukemia sequence 1 (*Mcl1*) alleles to specifically delete the *Mcl1* gene in mast cells. Mcl1 is an intracellular antiapoptotic factor and conditional deletion of its gene in *Lck-Cre* or *CD19-Cre* mice has previously been shown to induce depletion of T and B cell lineages. Lilla et al. analyzed a comprehensive set of organs, including connective and mucosal tissues, for the presence or absence of mast cells, and reported 92%–100% mast cell depletion. The relatively largest residual mast cell numbers were found in back skin and the peritoneal cavity. In addition, basophil numbers were reduced by 58%–78% in spleen and bone marrow, respectively. These mice also showed a 56% increase in splenic neutrophils and macrocytic anemia. The reasons for these hematological abnormalities are unclear, but it is possible that *Cpa3* expression outside of the mast cell lineage, e.g., in other myeloid progenitors and in the majority of progenitor T cells (Feyerabend et al., 2009), contributes to some of these alterations. In any case, it is unlikely that these defects are related to the mast cell deficiency because other mice selectively lacking mast cells did not show deviations in neutrophils or erythrocytes.

Collectively, it is likely that these strains are useful in mast cell research. These mice shall directly allow assessments on the roles of mast cells independent of Kit defects. By comparison to *Kit* mutants, they will also shed light on the roles that Kit played in particular physiological or pathological questions that were studied in the context of mast cell functions. Further advantages of these strains are their ease of breeding, which will facilitate the generation of mast cell-deficient mice on immunologically interesting genetic backgrounds, which critically effect immunological responsiveness, like T helper cell skewing and tissue transplantation, to name a few. Likewise, it will be easier to study the role of mast cells in complex immunological diseases like diabetes (NOD) and rheumatoid arthritis, or to combine mast cell deficiency with transgenic models. Finally, the possibility to conditionally delete genes in mast cells will be crucial for rigorous evaluation of specific gene functions in mast cells.

Does Mast Cell Deficiency Exist in Humans?

Genetic analysis of human patients is an important tool for the discovery of cellular and molecular mechanisms underlying immunity and for the identification of mutations associated with clinical disease. To our knowledge, humans lacking mast cells have not been reported. A possible but far-fetched explanation could be lethality prior to birth caused by lack of mast cells. Alternatively, lack of mast cells does not cause immunodeficiency or immune deviation, in which case individuals are clinically inconspicuous. If mast cell deficiency in humans, as in mice, were neither lethal nor disease-causing, individuals lacking mast cells might be “hidden” in the nonallergic population. It shall be important to gain insights into the “positive” immunological roles of mast cells also in humans before global interference with mast cells or their functions can be considered a therapeutic goal in the treatment or prevention of allergic disease. In selectively mast cell-deficient mice, steady state immune parameters (numbers and proportions of large sets of lymphocyte and myeloid subpopulations; serum Ig titers; hematopoietic stem and progenitors in bone marrow; T helper cell-2-dependent IgG1 antibody response including class switching and somatic hypermutation) were all unaffected by the lack of mast cells (Feyerabend et al., 2011). A teleological argument, that mast cells must be important because they exist, is not helpful in deciphering the biological functions of mast cells. The hurdle in screening for those myeloid cell types that cannot readily be identified automatically in peripheral blood, and the unexpectedly restricted spectrum of immunodeficiency in humans devoid of dendritic cells has recently been discussed (Collin et al., 2011). Although analogous data for mast cells are lacking, similar considerations for the identification of mast cell deficiency should apply. In brief, while human mast cell deficiency might have tremendous implications for mast cell biology, the search for such individuals remains complex.

Experimental Basis for Assumed Mast Cell Functions in the Immune System

A critical assessment of immunological and nonimmunological roles of mast cells requires considerations of the underlying experimental evidence. We consider mast cell functions here in the context of innate immunity, allergy, adaptive immunity, auto-

immunity, and vascular diseases and in association with tumors, knowing that this list is not exhaustive. We deem it useful to operationally distinguish protective, harmful, and currently ill-defined roles of mast cells (Figure 1). In the latter, we refer to reports on mast cell functions that have either been shown in the context of Kit deficiency only or that have not been confirmed when tested in Kit-independent models (e.g., deviated Th2 response, antibody-driven arthritis, EAE, or the role of IL-10 in contact hypersensitivity) (Dudeck et al., 2011; Feyerabend et al., 2011; Katz and Austen, 2011; Knight et al., 2000) or in cases of conflicting data between *Kit* mutants (e.g., in collagen-induced arthritis (Zhou et al., 2007), EAE (Bennett et al., 2009; Li et al., 2011; Piconese et al., 2011; Secor et al., 2000), or tumor growth (Gounaris et al., 2007; Sinnamon et al., 2008).

Protective Roles of Mast Cells

Mast cells are able to degrade the snake venom toxin sarafotoxin 6b (S6b) (Metz et al., 2006), and the responsible enzyme for this protective C-terminal cleavage is Cpa3 (Schneider et al., 2007). Analysis of a panel of now available mouse mast cell protease mutants revealed that mouse mast cell protease-4 (Mcp4), a chymase, can degrade helodermin, a toxic component of Gila monster venom, and reduce the morbidity and mortality induced by venoms from scorpion species (Akahoshi et al., 2011). Although the underlying data on toxin degradation are compelling, and such mechanisms of toxin inactivation may be vital for mice in the wild, more or broader functions of mast cell proteases in immunity may still be uncovered. It seems to confound immunological wisdom of receptor diversity and antigen recognition that mast cells use individual proteases to degrade single or small groups of toxins.

Endothelin 1 (ET-1), the most potent blood pressure regulating endogenous peptide, is highly homologous in peptide sequence to S6b. ET-1 can activate mast cells by binding to endothelin receptors (ET-A) on the surface of mast cells. In response, mast cells degranulate and release mediators including IL-6 and TNF- α (Matsushima et al., 2004). Mast cell proteases degrade ET-1, and studies using pharmacological inhibitors initially concluded that a chymase was the responsible enzyme (Maurer et al., 2004). However, mice expressing an inactive form of Cpa3 with no other defects in mast cell proteases failed to degrade ET-1, demonstrating that Cpa3, and not chymase activity, is essential (Schneider et al., 2007). The physiological role of endothelin degradation by mast cells remains enigmatic. The test for mouse survival upon intraperitoneal injection of ET-1 (Maurer et al., 2004; Schneider et al., 2007) is an artificial system, and the physiological or pathological implications, possibly involving the local regulation of blood pressure, of this response loop (activation by and degradation of ET-1 by mast cells) are elusive.

Mast cells have been reported to play important roles in resistance to bacterial and parasitic infections which has been comprehensively reviewed (Abraham and St John, 2010). In addition to the CLP experiments referred to above (Echtenacher et al., 1996), the role of mast cells in bacterial resistance has originally been studied by subjecting *Kit^{W/W^v}* mice to intraperitoneal injection of *Klebsiella pneumonia* (Malaviya et al., 1996). The impaired clearance of this infection by *Kit* mutant mice suggested that mast cells can promote survival during a bacterial

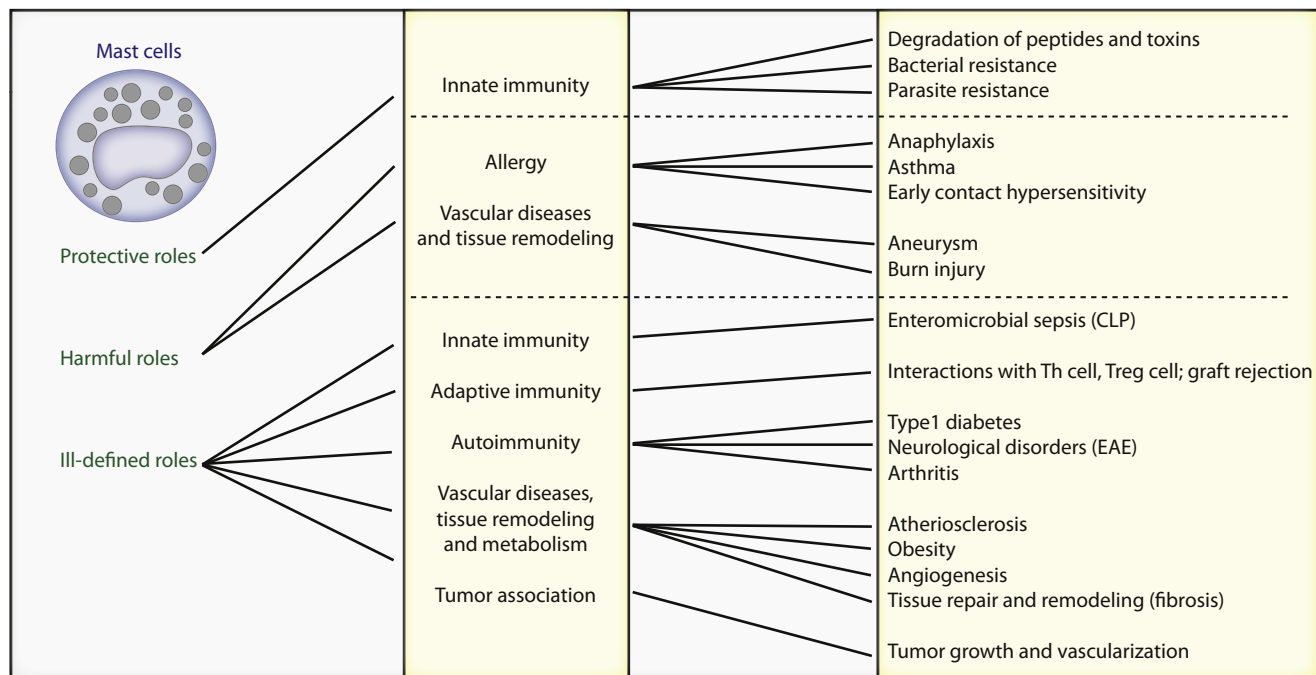


Figure 1. Diversity and Experimental Basis of Assumed Functions of Mast Cell in the Immune System and Beyond

Mast cell functions are classified into “protective,” “harmful,” and “ill-defined.” The latter category comprises studies that were based on analyses of *Kit* mutants only, that gave conflicting data in different *Kit* mutants, or that have not been confirmed when tested in *Kit*-independent models. These categories are further structured for immunological functions, e.g., innate and adaptive immunity, allergy, or autoimmunity, and for diseases, e.g., infections, anaphylaxis, or arthritis. The experimental bases of the mast cell analyses are discussed and referenced in the text.

infection by promoting neutrophil recruitment, and evidence pointed at an important role of TNF- α in this process. These findings have been substantiated independently of a *Kit* mutant mouse by a report showing that *Mcpt6*^{-/-} mice cannot efficiently clear *Klebsiella pneumoniae* from the peritoneal cavity (Thakurdas et al., 2007). *Mcpt6*^{-/-} mice also have phenotypes different from wild-type mice in *Trichinella* (*T.*) *spiralis* infection (Shin et al., 2008), in the K/BxN serum transfer arthritis (Shin et al., 2009), in allograft tolerance (de Vries et al., 2010), in experimental colitis (Hamilton et al., 2011), and in aortic aneurysm formation (Zhang et al., 2011). It is intriguing that a single tryptase seems to be directly or indirectly involved in the regulation of such diverse pathologies. However, *Mcpt6* mRNA expression, though strongest in mast cells, is not absolutely restricted to mast cells but expression is also found in subsets of macrophages and dendritic cells, and in other tissues (www.immgen.org). Hence, although the experiments listed above invoke mast cells as the only source of *Mcpt6*, formal proof of an involvement of mast cells in these models would require deletion of *Mcpt6* specifically in mast cells by conditional gene targeting.

Immunity against parasites is among the most widely believed mast cell functions. However, evidence from specific host-pathogen interactions is limited. The expulsion of *T. spiralis* was delayed in *Kit*^{W^WV} mice (Ha et al., 1983). Compound *Kit*^{W^WV} *IL-3*^{-/-} mice displayed delayed expulsion of the nematode *Strongyloides venezuelensis* but not of *Nippostrongylus* (*N.*) *brasiliensis* (Lantz et al., 1998), suggesting roles for IL-3 or Kit or mast cells in immunity against some parasites. More specifi-

cally, *Mcpt1* null mice showed a substantially delayed expulsion of *T. spiralis* and increased deposition of muscle larvae. However, in *N. brasiliensis*-infected *Mcpt1*^{-/-} mice, worm expulsion was not impaired (Knight et al., 2000). Interestingly, a comparison of *T. spiralis* infections in *Kit*^{W^WV} and *Mcpt1*^{-/-} mice revealed comparably delayed parasite elimination and ameliorated infection-associated enteropathy in both strains of mice, while the Th2 responses were significantly reduced only in *Kit*^{W^WV} but not in *Mcpt1*^{-/-} mice (Lawrence et al., 2004). This is another example of dissociated phenotypes comparing *Kit* mutants and specific mast cell mutants. Finally, immunity to *N. brasiliensis* and to *H. polygyrus* was unimpaired in mast cell-deficient *Cpa3*^{Cre/+} mice (unpublished data). In conclusion, the involvement of mast cells or their products in nematode expulsion appears to be a selective process, and more work will be required to understand the mechanisms underlying this parasite specificity.

From the data summarized here, a picture emerges in which mast cells may contribute to resistance against certain pathogens but one cannot assume that mast cells are broadly involved in immunity against bacteria, viruses, and parasites. More work will be required to understand the potential selectivity of protective mast cell responses.

Harmful Roles of Mast Cells

Roles of mast cells in anaphylaxis have been documented in several independent systems. *Kit* mutants are defective in IgE-driven local and systemic anaphylaxis (Becker et al., 2011; Finkelman, 2007; Martin et al., 1989; Zhou et al., 2007). Several

Kit-independent mast cell deficiency models have been tested for susceptibility to passive IgE-mediated local and systemic type I hypersensitivity (Feyerabend et al., 2011; Lilla et al., 2011; Sawaguchi et al., 2012). Depending on the extent of mast cell deficiency, anaphylaxis was abrogated or strongly ameliorated. A key pathophysiological parameter in anaphylaxis-associated shock, the drop in body temperature, requires histamine synthesis, which is ablated in mice deficient for histidine decarboxylase (Makabe-Kobayashi et al., 2002). Hence, there is now definitive evidence for the essential role of mast cells in the initiation of IgE-mediated allergic and anaphylactic reactions.

In mouse models of asthma, animals undergo repetitive immunization and challenge with Ova, and airway hyperresponsiveness (AHR) is measured upon methacholine inhalation. Although it is generally accepted that IL-4, which could be provided by mast cells and basophils, is a central player in development of AHR (Maes et al., 2012), the extent of mast cell contribution in this model of mouse asthma seems to be highly dependent on the immunization protocol (Williams and Galli, 2000) and on the genetic background of the mice (Becker et al., 2011). Combined studies on Mas-TRECK and Bas-TRECK mice demonstrated the involvement of mast cells in the effector phase of AHR (Sawaguchi et al., 2012).

Reports on the role of mast cells in contact hypersensitivity (CHS) have been controversial (Biedermann et al., 2000; Grimbaldston et al., 2007; discussed in Dudeck et al., 2011), perhaps partially because of different skin reactions that are summarized as "CHS." The classical assay is done by sensitization of mice with small haptens or irritants like DNFB, TNCB, or oxazolone on the shaved trunk skin, followed by challenge on the ear skin a few days later, leading to ear swelling and inflammation. This response is regarded as a prototype of T cell-mediated delayed type hypersensitivity (DTH). However, several phases of ear swelling can be observed during the whole time course, which probably depend on different mechanisms and effector cell types. Dudeck et al. demonstrated that DNFB or FITC cause ear swelling already within 2 hr and that this response is mast cell dependent and sensitization independent. Ear swelling 24–48 hr after challenge and migration of DCs to the draining lymph nodes is also facilitated by mast cell activation in CHS (Dudeck et al., 2011; Otsuka et al., 2011). Interestingly, *Kit*^{W-sh/W-sh} and *Kit*^{W/Wv} mice, but not mast cell-deficient mice wild-type for *Kit*, showed exaggerated and prolonged CHS responses (ear swelling 4–6 days after challenge) (Dudeck et al., 2011; Grimbaldston et al., 2007). IL-10 production might here be inhibitory but it is not mast cell-derived IL-10 in the model examined by Dudeck et al. (2011).

Mast cells have been associated with vascular diseases and tissue remodeling in humans. Several mast cell protease knockout mice have been used in different disease models. In experimental abdominal aortic aneurysm, tryptase (*Mcpt6*) and chymase (*Mcpt4*)-deficient mice, but not *Mcpt5*^{-/-} mice, had significantly reduced inflammatory cell infiltrations and showed less aortic expansion and lesions (Sun et al., 2009; Zhang et al., 2011). Experiments on epidermal burn injury showed that *Mcpt4*^{-/-} or *Mcpt5*^{-/-} mice, but not mice lacking *Mcpt6* or *Mcpt7* proteins or mice lacking *Cpa3* activity, were protected from scald injury. Topical application of the respective enzymes restored susceptibility (Younan et al., 2010).

In sum, harmful roles of mast cells are among the best-characterized consequences of mast cell activation.

Ambiguous Roles of Mast Cells

The conclusion that mast cells have a critical protective role in resistance to enteromicrobial sepsis in the CLP model was initially based on experiments in *Kit*^{W/Wv} mice (Echtenacher et al., 1996). However, a recent study using *Kit*^{W/Wv} and *Kit*^{W-sh/W-sh} revealed a more complicated picture: depending on the mouse strain background, the nature of the mutation resulting in a mast cell deficiency, and type and severity of infection, mast cells can have no effect, promote survival, or increase mortality (Piliponsky et al., 2010). To our knowledge, the role of mast cells in survival of CLP has not been confirmed in Kit-independent mast cell-deficient mice, or in knockout mice specifically and only lacking mast cell products, and hence it cannot be excluded that Kit defects contribute to the variability of the various strains in CLP sepsis experiments.

Mast cells have also been linked to adaptive immunity, tolerance, and graft rejection. A link between mast cells and regulatory T (Treg) cells has been suggested. The Treg cell frequency is reduced in lymphoid organs of *Kit*^{W-sh/W-sh} mice (Picconese et al., 2011). It is currently unknown whether this phenotype is due to the absence of mast cells or not. In a model of tolerance induction toward allogeneic skin grafts, mediated by allogeneic splenocyte transplantation plus anti-CD154 (CD40 ligand), Noelle et al. reported an essential role of mast cells in Treg cell-dependent peripheral tolerance (Lu et al., 2006). Allogeneic F1 C57BL/6 (B6) × BALB/c skin was accepted in tolerized B6 recipients but rejected in tolerized *Kit*^{W-sh/W-sh} mice. However, in this setting of transplantation greater than 50% of *Kit*^{W-sh/W-sh} mice even rejected B6 skin, albeit with delayed kinetics. This rejection seems surprising given that *Kit*^{W-sh/W-sh} mice were considered to be on the C57BL/6 background. Minor histocompatibility differences or lack of tolerance to antigens lacking in *Kit*^{W-sh/W-sh} mice may account for this phenomenon. Hence, either donor and host were not syngeneic or mast cell- and melanocyte-bearing tissues induce an immune response in mice lacking these cells in the first place. It remains to be determined whether these histoincompatibilities between donor tissues and *Kit*^{W-sh/W-sh} hosts, or only the mast cell deficiency of *Kit*^{W-sh/W-sh} mice, contributed to the failure of the tolerogenization protocol in *Kit*^{W-sh/W-sh} mice. In any case, the possibility of an important mast cell role in the establishment of tolerance to allogeneic grafts is intriguing, and future work using inbred strains lacking mast cells may further illuminate this area.

On the basis of studies in *Kit* mutant mice, major contributions of mast cells in autoimmune disease models EAE, arthritis, and type 1 diabetes have been postulated. Considerations on the role of mast cells in EAE and the K/BxN arthritis model have been presented above. Regarding diabetes, there has been a notion that introduction of *Kit*^{W/Wv} mutations into nonobese diabetic (NOD) mice protected from type 1 diabetes development (Hatfield et al., 2010, J. Immunol., abstract). A role for mast cells in diet-induced obesity (DIO) and diabetes-associated parameters has been analyzed in *Kit*^{W-sh/W-sh} mice and in mice treated with pharmacological mast cell stabilizers. Absence of mast cells, or inhibition of mast cell degranulation, both reduced

body weight gain in DIO and improved glucose regulation (Liu et al., 2009), as well as prolonged disease-free survival in diabetes-prone rats (Geoffrey et al., 2006). Another study, however, reported delayed onset of diabetes in NOD mice treated with anti-FcεRI antibody that activated basophils and mast cells (Hübner et al., 2011; reviewed in Shi and Shi, 2012).

Finally, roles of mast cells have been described in the areas of arteriosclerosis, angiogenesis, tissue repair, and tumor growth. Mast cells are equipped with potent vasoactive molecules, including Vegf and famous heparin that can regulate vascular growth and permeability (Oschatz et al., 2011; reviewed in Kunder et al., 2011). Moreover, mast cells could modulate local and systemic blood flow via the aforementioned Cpa3-mediated degradation of endothelin 1. The spectrum of mast cell products that have the potential to regulate vascular functions and frequent blood vessel association of mast cells suggest that mast cells could participate in cardiovascular pathology. Experiments addressing the possible contributions of mast cells to aortic aneurysm have been stated above. Potential roles of mast cells in arteriosclerosis have also been suggested (reviewed in Bot and Biessen, 2011). Indicators of arteriosclerosis, including atherogenic lipid profile, vascular inflammation, and plaque dimensions, were ameliorated in *Ldlr*^{-/-} *Kit*^{W-sh/W-sh} mice (Heikkilä et al., 2010; Sun et al., 2007), and the IgE-FcεRI axis seems to contribute to atherogenesis (Wang et al., 2011). However, the link between mast cells, diet-induced blood lipids, and arteriosclerosis remains to be understood more closely. Given that Kit is expressed in hepatocytes, and that Kit signaling has been linked to liver and lipid metabolism (Magnol et al., 2007), mutations in *Kit* may not be neutral in arteriosclerosis experiments.

Within the immune system, the impact of mast cell-derived products on blood vessels may be a key driving force in the initiation, enhancement, or maintenance of acute and chronic inflammation. However, in these and further interesting aspects of mast cell biology, including angiogenesis, tissue remodeling, wound healing (Gilfillan and Beaven, 2011), and promotion of or protection from cancer by participation as element of tumor stroma (Tlsty and Coussens, 2006), the decisive question is whether or not mast cells are functionally involved. In other words, although there is ample structural evidence that mast cells bear and release a host of tissue-modifying substances, we still know too little about the functional meaning of these data. Neither the mere presence of mast cells nor of their products are proof of functional importance, leaving the question unsolved as to whether mast cells are bystanders or culprits under any particular condition. Collectively, it is evident from this discussion and the complexity of the underlying experiments that many roles of mast cells in the immune system and beyond remain presently ambiguous.

Concluding Remarks

Mast cells remain fascinating cells, yet many facets of their physiological role in the immune system still need to be determined and understood. *Kit* mutant mice have served for decades as standard models to test in vivo mast cell functions. Because Kit is critically involved in the development and function of many stem and mature cells inside and outside of the immune system, phenotypes unrelated to the mast cell deficiency of *Kit*

mutants may contribute to the experimental outcome of experiments targeted to reveal only mast cell functions. The reconstitution of *Kit* mutants with cultured mast cells has been thought to reliably indicate whether an observed defect is due to the lack of mast cells. Recently, the first *Kit*-independent mast cell-deficient mice have been reported. In these mice, some key data obtained earlier in the *Kit* mutant mast cell reconstitution system have not been confirmed. Of course, this does not discredit all of the available mast cell literature but cautions that conclusions based on *Kit* mutations may not in every case hold true when tested independently. Comfortingly, experiments in *Kit*-independent models have confirmed some mast cell functions, most of all the essential role of mast cells in mediating allergic disease and anaphylaxis. Beyond this expected finding, selectively mast cell-deficient mice have a surprisingly normal immune system. In these mice as well as in mutants with specific loss of mast cell products, mast cells have been shown to play a role in the context of asthma, contact hypersensitivity, infections with selective bacterial and parasitic pathogens, degradation of toxins and endothelin, graft rejection, burn injury, and some vascular pathologies. In these experiments, mast cells either contributed to protection (as in toxin degradation or bacterial resistance) or were harmful (as in anaphylaxis or inflammatory exaggeration of burn injuries). By contrast, widespread functions of mast cells in innate and adaptive immunity, as well as in autoimmunity, immune metabolic diseases, and in many other areas (Figure 1), remain currently ambiguous. The new mouse mutants, and probably tools to come, shall provide a broader basis for conclusive experiments that will separate pivotal from less important functions of mast cells in immunity.

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