Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data

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Abstract | The versatile role of mast cells in allergy, in innate immune responses and in the regulation of tissue homeostasis is well recognized. However, it is often not made clear that most mast-cell data derive solely from experiments in mice or rats, species that obviously never suffer from allergic and most other mast-cell-associated human diseases. Data on human mast cells are limited, and the mast-cell source and species from which findings derive are frequently not indicated in the titles and summaries of research publications. This Review summarizes recent data on human mast cells, discusses differences with murine mast cells, and describes new tools to study this increasingly meaningful cell type in humans.

For several decades, human mast cells have been established as the key effector cells of allergic inflammation[1–4]. They are located at strategic sites such as the skin, and the vascular and mucosal barriers; they bind IgE on their surface by expressing the high-affinity Fc receptor for IgE (FcεRI); and they release histamine and other mediators after crosslinking of surface-bound IgE by allergen. Mast-cell research defines to a large extent our current understanding of the pathophysiology of allergic diseases and supports the conclusion that human mast cells function as cellular mediators of allergy. Such research efforts have also increased our understanding of mast-cell biology in general, and have raised new ideas about potential functions of mast cells that are unrelated to allergy.

As a result, mast cells have been recognized in the past decade as cells that not only regulate allergy, but also many tissue functions, such as blood flow and coagulation, smooth-muscle contraction and peristalsis of the intestine, mucosal secretion, wound healing, regulation of innate and adaptive immune responses and, most recently, peripheral tolerance[5–8] (FIG. 1). This explains why mast cells have been found to be involved in so many different types of human disease in addition to allergic disorders, including inflammatory diseases, neurological diseases and functional diseases such as irritable bowel syndrome, functional dyspepsia and fibromyalgia[9–11]. Recent studies in mice and rats, and to some extent also in humans, have shown that mast cells have a central role in host defence against bacteria and parasites, through the release of cytokines and other mediators that recruit neutrophils, eosinophils and T helper 2 (T_H2) cells to the site of infection[12,13]. These findings indicate another key function of mast cells in innate immunity and fit with the long-standing observation that this cell type is typically located at strategically important body barriers.

Mast cells are now viewed similarly to lymphocytes and other major immune cells involved in host defence and homeostasis[7,8,12]. Indeed, mast cells have versatile physiological functions[14–16] and they should no longer be considered simply as 'allergy cells' (FIG. 1). The focus of this Review is on human mast-cell biology and function in health and disease, including allergic, non-allergic and non-inflammatory conditions. The emphasis is on the functional differences between human and murine mast cells, such as receptor expression, responsiveness to cytokines and mediator expression, which have sometimes caused confusion in the past.

Laboratory tools to study human mast cells

A particular problem of human mast-cell research is the difficulty in obtaining human cell material for *in vitro* studies. Therefore, most *in vitro* mast-cell experiments have been carried out either with human cell materials that might have limited functional significance, such as transformed cell lines or partially immature mast cells, or with murine primary mast cells that can be easily obtained, such as peritoneal mast cells (TABLE 1). Murine peritoneal mast cells have been widely used, as a single peritoneal lavage yields large numbers of mast cells that can be easily purified[17]. However, this mast-cell

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doi:10.1038/nri2018

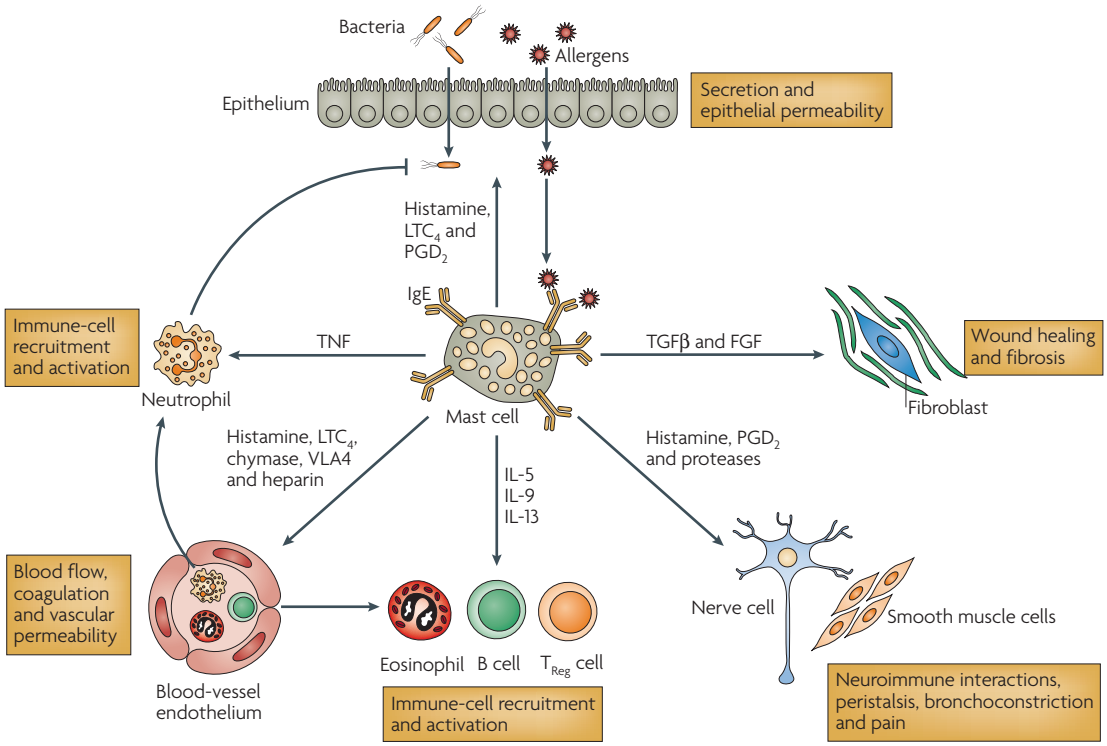

Figure 1 | **Proposed functions of human mast cells under normal conditions.** Physiological mast-cell functions include the regulation of epithelial functions (secretion and epithelial permeability), smooth-muscle functions (peristalsis and bronchoconstriction), endothelial functions (blood flow, coagulation and vascular permeability), immune functions (recruitment and activation of neutrophils, eosinophils and lymphocytes), neuronal functions (neuroimmune interactions, peristalsis and pain) and other tissue functions (wound healing and fibrosis). The physiological triggers are poorly defined and might include growth and other tissue factors, infectious agents, neuropeptides, protein antigens and physiochemical conditions, such as a change in pH or in osmolarity. FGF, fibroblast growth factor; IL, interleukin; LTC_4, leukotriene C_4; PGD_2, prostaglandin D_2; TGFβ, transforming growth factor-β; TNF, tumour-necrosis factor; T_{Reg} cell, $CD4^+CD25^+$ regulatory T cell; VLA4, very late antigen 4. Figure adapted with permission from REF. 14 © (2003) Blackwell Publishing.

subtype probably does not exist in humans, whose peritoneal cavity is normally free of mast cells. It is important to note that the biology of other murine mast-cell subtypes, such as mucosal, skin or vascular-associated mast cells, is less well known. This is because the amount of tissue that can be obtained is small (particularly in mice) and because the mast-cell density in such murine tissues is low (estimated to be one-fifth to one-tenth of the densities in humans, depending on the animal strains), which makes it difficult to establish primary cultures for routine work[18–21] (S.C.B., unpublished observations). Therefore, techniques for generating mast cells from progenitor cells such as bone-marrow cells are preferred among murine mast-cell researchers. Similar approaches have been established for humans using cord blood or peripheral blood as a progenitor source. However, all of these tools are limited by the fact that mast-cell maturation cannot be fully achieved by the different culture protocols, as indicated by functional studies and analyses of surface antigen expression in human mast cells[21,22]. Although mast-cell maturation is not well defined, morphological and functional properties such as the expression of FcεRI and the response to FcεRI crosslinking or the response to interleukin-4 (IL-4) are assumed to be related to maturation status[23,24].

To avoid such limitations, primary cultures of human mast cells are desirable. Human mast cells can be isolated from solid tissues and purified by complicated selection means and long-term cultures. Interestingly, contrary to previous assumptions, recent studies have shown that the long-term *in vitro* culture of human mast-cell suspensions is not necessarily a disadvantage as it might imitate the *in vivo* tissue environment rather than change the 'natural' *in vivo* characteristics of human mast cells. Functional studies from various groups have shown that the culture of isolated murine and human tissue mast cells for 2–3 weeks results in a restoration of normal cell functions that were temporarily disrupted by the isolation procedure[25–28]. However, an important limitation of this method is the dependency on fresh human tissue and the limited yield of purified mast cells. Therefore, a human mast-cell line is required that is not transformed as is the currently available HMC-1 cell line. Progress in this regard has been made by establishing the leukaemia-derived LAD-1 and LAD-2 cell lines, which in contrast to HMC-1 require stem-cell factor (SCF; also known as KIT ligand) for survival and therefore are more similar to primary cultures of human mast cells[29].

'Wheal and flare' reaction
The acute response of the skin to allergen in a skin-prick test. The wheal refers to the swelling, the flare refers to the reddening of the skin over a wider area that is induced by vasodilation, local oedema and neuronal mechanisms.

The advantage of using murine mast cells is the availability of multiple animal models, including transgene, transfer and knockout models, which allow the study of mast-cell function in complex *in vivo* settings that cannot be carried out in humans. Nevertheless, considering the marked functional differences that exist between mast cells derived from different species and different anatomical sites, and between those of different maturation states, there is a strong requirement for the confirmation of findings from murine studies by experiments with human cells. At the very least, mast-cell sources need to be clearly indicated in experimental studies, and data derived from a particular mast-cell source should not be generalized to mast cells as a whole.

Origin of human mast cells

Human mast cells develop from myeloid-cell progenitors under the influence of particular growth factors such as SCF and IL-4, cytokines that also regulate the development of mast-cell subtypes[30–32]. The relationship between human mast cells and other leukocyte lineages is not yet clear. Human mast cells have been described as the tissue equivalents of basophil granulocytes, because both cells contain basophilic plasma granules, release histamine and express FcεRI; however, the definition of a cellular relationship on the basis of growth-factor responsiveness during development or cellular markers is limited[33–37]. Morphological and functional analyses, which are probably more relevant, have indicated that human mast cells are more closely related to monocytes and macrophages, whereas basophils share properties mainly with eosinophils (FIG. 2). Gene-expression and mutation studies have shown that cultures of murine mast cells can still have monocytic features[38], and that human mast cells and basophils do not derive from a common bilineage-restricted committed progenitor[39]. This is supported by the observation that although both human mast cells and basophils express functional SCF receptors, the gene encoding this receptor is mutated only in mast cells in patients with mastocytosis[40]. By contrast, murine mast cells have some functional properties in common with human basophils, whereas human mast cells seem to form a separate cell type lacking a full equivalent in rodents. For example, some murine mast-cell populations, and human basophils, respond well to IL-3, whereas human mast cells either lack the IL-3 receptor or hardly respond to IL-3 (REFS 41–43). This is true not only for cell development but also for the regulation of mature mast cells by cytokines[44].

Human mast-cell mediators

Human and murine mast cells exert their biological functions almost exclusively by humoral immune mechanisms. There are a few reports of mast-cell phagocytosis and other non-humoral functions in mice and rats, but typically, mast-cell functions are restricted to the release of mediators. The array of mediators released by human mast cells is enormous and explains how mast cells can be involved in so many different physiological and pathophysiological functions. Of particular relevance in the pathogenesis of allergic inflammation are histamine and leukotriene C_4 (LTC_4), which induce 'wheal and flare' reactions, mucus

Table 1 | **Laboratory tools to study human and murine mast cells**

Tool	Human	Murine	Limitations
Transformed mast-cell lines	• Leukaemia-derived human mast-cell lines (HMC-1, LAD-1 and LAD-2)	• Rat basophilic leukaemia cells • Other murine cell lines (IL-3 dependent and IL-3 independent)	• Transformation substantially alters normal cell functions (for example, KIT is permanently phosphorylated in HMC-1 cells, which therefore become independent of SCF; by contrast, LAD cells require SCF for survival and therefore might be more appropriate as a human mast-cell model)
Primary cultures of mast cells from progenitor cells	• Cord-blood-derived mast cells • Peripheral-blood-derived mast cells	• Mouse bone-marrow-derived mast cells • Others (such as fetal skin-derived mast cells, fetal liver-derived mast cells and spleen-derived mast cells)	• Long-lasting process; development of mast cells requires progenitor cell cultures for 6–12 weeks, and addition of a cocktail of cytokines • It is currently questionable whether full mast-cell maturation can be achieved by this means
Primary cultures of tissue mast cells	• Human skin mast cells, human mucosal mast cells (lung/intestine) • Human mast cells of other origin (heart, uterus and kidney)	• Peritoneal mast cells • Isolated mucosal or skin mast cells are more difficult to obtain (small amounts of tissue and low mast-cell densities)	• Murine peritoneal mast cells might differ from tissue mast cells in functional terms and they do not exist in the peritoneal cavity in humans under normal conditions • Murine mucosal mast cells occur only at small numbers in normal mucosa (mouse < rat < human) • Human tissue mast cells: dependency on fresh tissue specimen, limited cell yield (~10^4–10^5 mast cells per gram of tissue), cumbersome isolation procedures and purification techniques
In vivo examination of mast cells	• Histology (Carnoy fixation, trypan blue staining) • Immunohistochemistry (anti-tryptase staining) • Mast-cell mediator measurement (tryptase or methyl-histamine in urine)	• Mast-cell-deficient mice and rats ($Kit^{W/W-v}$, $Kit^{W-f/W-f}$ and $Kit^{W-sh/W-sh}$ mice, and $Kit^{W-s/W-s}$ rats)	• In humans, there are limited *in vivo* approaches • In rats and mice, versatile options to study mast-cell functions relevant to human disease exist; however, there are limitations depending on the use of mast cells generated from progenitor cells (see above) or the extent of repopulation of particular tissue sites

HMC, human mast cell; IL, interleukin; LAD, leukocyte-adhesion deficiency; SCF, stem-cell factor.

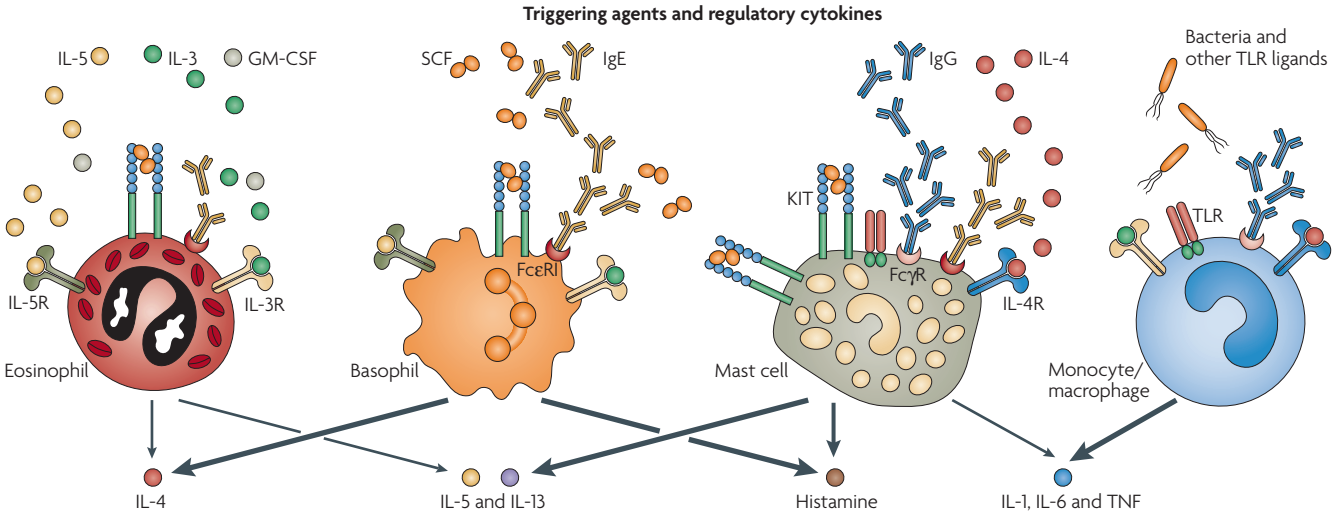

Figure 2 | **Comparison between human mast cells and related bone-marrow-derived cells.** Mast cells have similarities and differences both with eosinophil and basophil granulocytes, and with monocytes and macrophages. Mast cells share expression of the high-affinity Fc receptor for IgE (FcεRI) and histamine release with basophils. Mast cells share responsiveness to interleukin-4 (IL-4) and bacterial products, as well as nuclear morphology, with monocytes. However, mast cells generally do not express CD14 like monocytes, or IL-3 receptor (IL-3R) or IL-5R like basophils or eosinophils. Mast cells express almost exclusively the stem-cell factor (SCF) receptor KIT. Triggering agents and regulatory cytokines of the four cell types are shown above; mediators released from the cells after stimulation are shown below. GM-CSF, granulocyte/macrophage colony-stimulating factor; TLR, Toll-like receptor; TNF, tumour-necrosis factor.

and electrolyte secretion, smooth-muscle constriction and nerve-cell activation; LTB_4, which targets neutrophils and mast-cell progenitors; prostaglandin D_2 (PGD_2), which functions as a pro-inflammatory and T_H2-cell-regulatory lipid mediator; and particular cytokines, such as IL-3 (basophil recruitment and activation), IL-5 (eosinophil recruitment and activation) and IL-13 (induction of IgE synthesis by B cells)[45–48]. Another important group of mast-cell mediators are the proteases, which have been used for the classification of mast-cell subtypes[49–51]. Several tryptases with different specificities have been characterized in humans, but only a single chymase has been characterized[49,50]. By contrast, in murine mast cells, a large number of chymases with distinct and narrow proteolytic specificities have been found[51]. Expression of chymases and tryptases by mast cells varies between tissues. Human pulmonary and intestinal mast cells express chymase at lower levels than tryptases, whereas murine mucosal mast cells express mucosa-specific chymases[51]. However, mast-cell classifications based on protease content are ambiguous, because protease expression can change depending on the tissue environment and cytokine culture conditions. In mature human mast cells, IL-4 preferentially increases the expression of tryptases, whereas culture of human mast cells with human endothelial cells promotes chymase expression[52,53]. In mice, transforming growth factor-β (TGFβ) regulates the expression of the mucosa-specific chymase mast-cell protease 1 (MCP1)[51]. Tryptases and chymases contribute to inflammation and tissue remodelling through the selective proteolysis of matrix proteins and the activation of protease-activated receptors and matrix metalloproteinases[49–51].

Recently, gene-expression studies have shown that in both resting and activated cells stimulated by FcεRI aggregation, only some of the genes that are expressed correspond in murine and human mast cells[54]. For example, human mucosal mast cells and murine mast cells obtained from bone marrow differ in their ability to produce IL-4, IL-5 and tumour-necrosis factor (TNF) (TABLE 2). Therefore, results from studies carried out in the mouse system are not easily transferable to the human situation. This holds true not only for mast-cell biology and function, but also for diseases associated with mast cells, such as allergy and asthma. Indeed, although murine disease models have been used as valuable tools to study aspects of mast-cell function that would not be possible in humans, the relevance of such findings for clinical disease is not always evident[55,56]. For example, an IL-5-specific antibody completely blocked airway hypersensitivity in experimental animal models of asthma[57], but did not do so in humans[58]. An online database is now available providing information about the mast-cell genes that are upregulated in human mast cells compared with mouse mast cells after FcεRI stimulation[59] (see Mast-cell microarray data, details in Online links box).

This new transcriptome approach confirms and extends previous findings derived from classical cell biology. Most human mast cells respond poorly to IL-3, and human lung mast cells do not express IL-3 receptors[42,43]; by contrast, IL-3 is an important growth factor and priming cytokine for mouse mast cells and human basophils[33,60,61]. Mouse mast cells and human basophils produce IL-4, both spontaneously and to a

Table 2 | **Major functional differences between murine and human mast cells**

Feature	Murine mast cells	Human mast cells	References
Protease content	Several tryptases and chymases with different specificities (such as mouse MCP1–MCP14) and β-hexosaminidase	Three tryptases (α, β and γ); one chymase	42–44
Functional IL-3 receptor expression	+++	+/–	34–36
IL-4 production	+++	–	41,53,56
IL-5 production*	+ ?	+++	40,41
TNF production	+++	+	58,59
Constitutive FcγRI expression	+++	+/–	78
CD14 expression	++	–	12, unpub.‡
Functional TLR expression§	+++	+/–	12, unpub.‡

*Murine mast cells express IL-5 but IL-5 protein synthesis and release from murine mast cells has not been studied quantitatively. ‡S.C.B., unpublished observations. §See text for details. FcγRI, high-affinity Fc receptor for IgG; IL, interleukin; MCP, mast-cell protease; TLR, Toll-like receptor; TNF, tumour-necrosis factor.

greater extent after stimulation by FcεRI aggregation[62–64], whereas human mast cells do not produce IL-4 under normal conditions[48,52]. Initial data indicating IL-4 production by human mast cells could not be confirmed or were restricted to the mast cells of allergic individuals[65]. Moreover, murine mast cells are an important source of TNF, whereas human mast cells of the mucosal subtype produce only small amounts of TNF compared with murine mast cells or human monocytes[66–68]. By contrast, human mucosal mast cells produce large amounts of IL-5, which explains how mast cells contribute to eosinophil recruitment at sites of allergic inflammation[47].

Regulation of human mast cells

Through Fc receptors for IgE. The regulation of mediator release by mast cells is complex, because a large number of agonists and antagonists, and also differences between mast-cell subtypes and species, have to be considered under physiological and pathophysiological conditions. The classical, and possibly most effective, human mast-cell stimulus is the crosslinking of cell-surface-bound IgE by allergen in sensitized individuals. This mechanism of mast-cell activation through FcεRI is a crucial event in type I hypersensitivity reactions, but is probably of little or no importance in healthy individuals of industrialized countries. During the past decade, great progress has been made in understanding the detailed mechanism of IgE-dependent mast-cell activation. High-affinity and low-affinity IgE receptors have been cloned and sequenced, and several interesting details of FcεR signal transduction, mostly derived from studies using mouse mast-cell lines, have been unravelled and recently summarized elsewhere[69–71]. Many, but not all, of these findings can be transferred to the human system. For example, we found that, in contrast to previous findings in mice and rats, phosphatidylinositol 3-kinase (PI3K) is not required for FcεRI-mediated TNF synthesis by human intestinal mast cells, and that the induction of IL-5 expression depends on mitogen-activated protein kinases (MAPKs) rather than on nuclear factor of activated T cells (NFAT)[72]. By contrast, many findings, such as activation of the extracellular-signal-regulated kinase (ERK) branch of the MAPK pathway after crosslinking of FcεRI, could be confirmed both in the mouse and the human system.

Interestingly, IgE not only mediates mast-cell activation when crosslinked by allergen, but also regulates mast-cell functions by itself. Exposure of murine mast cells to high levels of IgE in the absence of specific antigen resulted in increased surface expression of FcεRI and, subsequently, increased mediator release after crosslinking of surface-bound IgE by allergen[73]. Moreover, monomeric IgE can render mast cells resistant to apoptosis induced by growth-factor deprivation *in vitro*, and can induce cytokine production without inducing degranulation or leukotriene release[74]. These findings in mice have been partially confirmed in human mast cells, although results from two publications are conflicting. In one study, monomeric IgE induced cytokine production but no histamine release, LTC_4 production or mast-cell survival after SCF removal[75]; in the other study, monomeric IgE induced a long-lasting, dose-dependent histamine release, LTC_4 production and IL-8 synthesis[76]. The discrepancy could be related to the different mast-cell sources (human lung and cord-blood-derived mast cells, respectively) used in the two studies. More recently, a novel mechanism of antigen-dependent mast-cell activation by immunoglobulin-free light chains has been described in mice[77]. Although the underlying mechanism is unclear at present, it seems that this pathway could be of relevance for both allergic and T-cell-mediated responses[78]; however, this requires confirmation in the human system.

By growth factors and cytokines. Particular growth factors and cytokines promote human mast-cell development from progenitor states and/or function as regulators of mediator release. The most relevant and still unique mast-cell growth factor is SCF, which is the ligand of KIT, a receptor with tyrosine-kinase activity that is expressed on the surface of all human and murine mast cells[31]. SCF, either membrane bound or in its soluble form, promotes both mast-cell development and the survival of mature mast cells and adhesion to

Type I hypersensitivity
Immunological hypersensitivity reactions have been classified by Coombs and Gell into four types depending on the antigen-recognizing molecule. Type I hypersensitivity is defined as an IgE-mediated hypersensitivity reaction, also known as an 'anaphylaxis reaction', consisting of an early phase (wheal and flare reaction) and a facultative late-phase reaction.

extracellular matrix (ECM) proteins[25,53,79]. In addition, SCF can regulate mediator release by human mast cells by either enhancing IgE-dependent mediator release or directly inducing mediator release by mast cells kept in an SCF-deprived milieu[80,81]. The mechanisms of the effects of SCF on human mast cells have been identified to a large extent, and are summarized elsewhere[71].

More recently, IL-4 has been described as a novel human mast-cell regulator. In contrast to SCF, IL-4 does not affect mast cells by itself, but functions synergistically with SCF on mast-cell survival, proliferation and IgE-dependent mediator release[52,75,82]. Moreover, it changes the cytokine profile released by mast cells by decreasing the production of pro-inflammatory cytokines such as TNF and IL-6, and increasing the production of T_H2 cytokines such as IL-5 and IL-13 (REFS 48,83). The IL-4-mediated priming of human mast cells for increased proliferation and mediator release is associated with increased activity of ERK and FOS, which is the downstream target of ERK and a component of the transcription factor AP1 (activator protein 1)[83]. Interestingly, the enhancing effects of IL-4 are reversible, and are restricted to mature human mast cells, whereas IL-4 exerts opposite effects, such as the inhibition of proliferation, on immature human mast cells[32,82,83]. Considering that IL-4 also induces the development of T_H2 cells and a switch to IgE production by B cells, this cytokine is a key mediator in the pathogenesis of allergic inflammation. It is unclear at present whether the effects of IL-4 on mature mast cells are unique to human mucosal mast cells or also occur in murine mucosal mast cells, which have not yet been examined in this respect.

In addition to SCF and IL-4, other cytokines such as IL-3 (which has similar effects to IL-4, but is less efficacious) and IL-9 (which is required for mast-cell-dependent immune suppression, as shown in mice) have been shown to regulate mast-cell functions[8,43].

By FcγR, TLRs and other IgE-independent triggers. Our knowledge of IgE-independent triggers other than cytokines that might regulate human mast cells under physiological conditions is still limited[6,14]. The list of IgE-independent mast-cell agonists varies between human and murine mast cells, and also between human mast cells from different body sites. Human mucosal mast cells challenged with interferon-γ (IFNγ) express FcγRI at a sufficient level to become activated for mediator release after FcγRI aggregation[84], whereas murine mast cells express this and other FcγRs constitutively (TABLE 2). This mechanism could be of relevance for the otherwise poorly understood IgE-independent allergic reactions, and for non-allergic mast-cell activation during type III hypersensitivity reactions or infections.

In mast cells from human skin, which resemble murine peritoneal mast cells in some respects, further mediators, such as the anaphylatoxins C3a and C5a of the complement system, substance P (also known as neurokinin 1) and other neuropeptides, function as triggers for mediator release[85,86]. By contrast, human mucosal mast cells, such as lung or intestinal mast cells, do not respond to such triggers. However, if they are primed by cytokines such as SCF and IL-4, human mucosal mast cells start to express the neurokinin 1 receptor and can respond to substance P with mediator release[87,88]. Recently, CC-chemokine ligand 3 (CCL3; also known as MIP1α) has been identified as an amplifier of IgE-dependent mediator release by murine mast cells[89]. It is not yet clear if this finding is also true for human mast cells, and if priming agents, such as IL-4 and CCL3, can render mast cells responsive to otherwise ineffective IgE-independent triggering agents such as neuropeptides or anaphylatoxins. Nevertheless, these reports show that mast cells, similar to T cells, require the cooperation of two signals for optimal activation: an antigen-dependent signal such as IgE bound to the cell surface (or T-cell receptor in the case of T cells), and a co-stimulatory molecule, such as SCF and/or IL-4 (or CD80 for T cells).

In recent years, it has become evident from *in vivo* and *in vitro* mouse studies that mast cells interact with bacteria and, most interestingly, can contribute to the host defence against bacterial infection by releasing TNF and other mediators required for the recruitment of neutrophils and other immune cells[12]. These findings have led to a new area of mast-cell research — the expression of Toll-like receptors (TLRs) by mast cells and the effects of TLR ligands on mast cells. Clearly, TLRs are not the only means by which mast cells might be stimulated by bacteria, but this topic is highlighted by recent interesting studies. Depending on the cell source and the culture conditions, human mast cells can express TLR1, TLR2, TLR3, TLR4, TLR6, TLR7 and TLR9, but not CD14 or functional CD48 (REF. 12). In contrast to murine mast cells, the expression levels are often low and the function of these receptors remains unclear in human mast cells (TABLE 2). Whereas murine mast cells, which express CD14, can be easily activated by lipopolysaccharide (LPS), this is not always possible for human mast cells, which might require soluble CD14 to respond to LPS[90]. One should consider that mast-cell–bacteria interactions in humans have been studied mostly in cord-blood-derived mast cells, which are thought to differ from primary isolates of human mast cells in terms of maturity, FcεR α-chain expression and the expression of other genes[21,91,92]. Therefore, it might not be possible to generalize such data to all types of human mast cell. Indeed, we have found that human mast cells from the intestine, similar to intestinal macrophages[68], lose expression of CD14 and functional TLRs once they have entered the tissue, and fail to respond to LPS and other TLR ligands, possibly because of desensitization following permanent exposure to the bacterial flora (S. Krämer and S.C.B., unpublished observations). In addition, if mast cells generated from peripheral blood or cord blood are used, contamination with other blood cells such as dendritic cells (DCs) or B cells has to be considered. It is known, for example, that TLR9 is almost exclusively expressed by these two cell types, meaning that even a low level of contamination by such cells could produce a false-positive result[59]. Finally, according to the mast-cell expression databases, human mast cells express only TLR2, TLR4 and TLR6 consistently[59],

Type III hypersensitivity
An immune-complex-mediated hypersensitivity reaction; the immune complexes consist of exogenous or endogenous antigens and IgG.

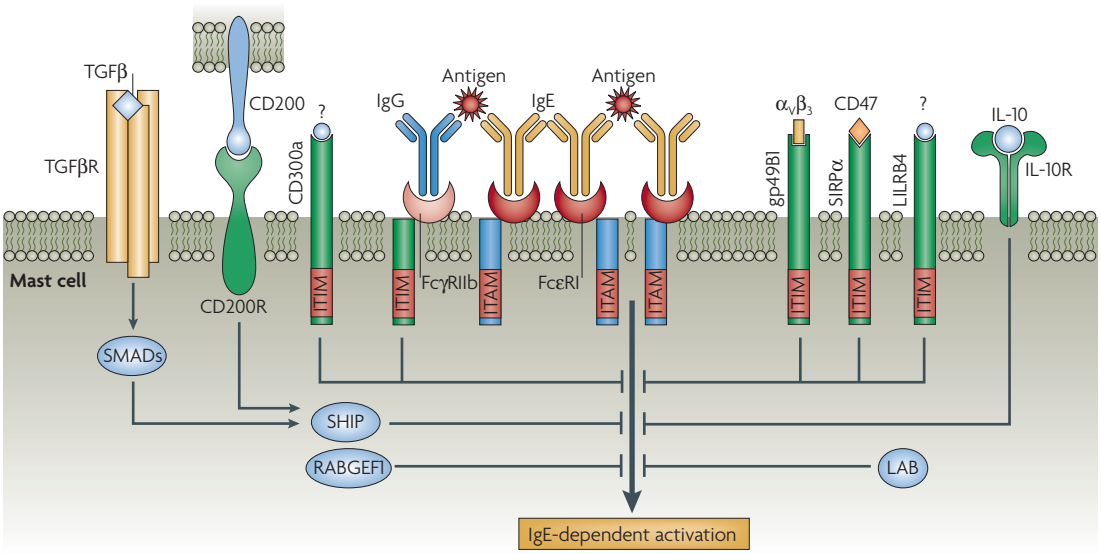

Figure 3 | **Inhibitory signals for mast-cell mediator release induced by FcεRI aggregation.** The inhibitors include ligands of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors, such as FcγRIIb, gp49B1, signal regulatory protein-α (SIRPα), human leukocyte immunoglobulin-like receptor, subfamily B, member 4 (LILRB4) and CD300a, the anti-inflammatory cytokines transforming growth factor-β (TGFβ) and interleukin-10 (IL-10), CD200 and the intracellular signalling molecules, such as LAB (linker for activation of B cells; also known as non-T-cell activation linker, NTAL) or RABGEF1 (RAB guanine nucleotide exchange factor 1). Most of the data derive from *in vitro* experiments or from animal models. ITAM, immunoreceptor tyrosine-based activation motif; R, receptor; SHIP, SH2-domain-containing inositol polyphosphate 5′ phosphatase; SMAD, mothers against decapentaplegic homologue.

in contrast to earlier reports proposing the expression of additional TLRs[12]. As other cell types, such as monocytes and neutrophils, abundantly express almost all TLRs at high levels, the biological significance of the rather weak expression of TLRs by human mast cells, and in particular by human intestinal mast cells, should be considered.

By mast-cell inhibitors. During the past few years, important progress has been made in understanding mast-cell regulation by the discovery of several inhibitory mechanisms that might balance the agonistic activities of the mediators discussed previously. The inhibitors include ligands of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors (such as FcγRIIb and CD300a)[93–98], the anti-inflammatory cytokines IL-10 and TGFβ (REFS 99,100), CD200 (REFS 101,102), and intracellular signalling molecules[70,96,103,104] that modulate FcεRI-mediated mast-cell activation (FIG. 3). Additional molecules such as retinol, β2-adrenoceptor agonists and ECM proteins binding to CD63 have been reported to inhibit mast-cell proliferation and functions[105–108]. The inhibition data derive mostly from experiments in the murine system; therefore, the *in vivo* relevance of such findings for humans in health and disease cannot be determined at present. However, it seems possible that some of the findings could be extended to the human system — for example, CD200R and CD300a are expressed by, and functional in, human cord-blood-derived mast cells[96,101]. Furthermore, TGFβ was found to inhibit the SCF-dependent growth of human intestinal mast cells and to change the mediator profile released after FcεRI aggregation by decreasing histamine, leukotriene and TNF release while selectively increasing PGD_2 production[99]. Also, mast-cell inhibition by IL-10 and β2-adrenoceptor agonists has been shown in human mast cells[100].

Most interestingly, the concept of blockade of FcεRI-mediated mast-cell activation by co-aggregation of FcεRI and FcγRIIb, which was previously shown in the murine system, was confirmed recently in a clinical trial using blood basophils from donors allergic to cats or sensitized cord-blood-derived mast cells, and a chimeric human–cat fusion protein composed of an Fc fragment of human IgG and the main cat antigen Fel d1. This protein binds to surface IgE specific for Fel d1 and to FcγRIIb. The resulting aggregation of FcγRIIb and FcεRI inhibits FcεRI-mediated signals. This concept provides a new therapeutic platform for immune-based therapy of allergic disease[109]. This strategy, which is not restricted to particular antigens, would possibly become even more effective if it was also used to ablate IgE-producing B cells[110]. However, the possibility that such fusion proteins could also activate mast cells through FcγRIIa, which is known to be expressed by human mast cells[93], has to be considered.

Another approach in mast-cell inhibition is to ablate the whole cell by targeting SCF, its receptor KIT or the downstream signalling cascade of KIT. Indeed, drugs targeting the SCF receptor have been developed, such as STI571 (Imatinib, Glivec), which inhibits tyrosine kinases such as KIT, PDGFR (platelet-derived growth factor receptor) activation pathways and arginine kinase[111]. The drug is effective for the treatment of

Immunoreceptor tyrosine-based inhibitory motif (ITIM). A structural motif containing tyrosine residues that is found in the cytoplasmic tails of several inhibitory receptors, such as FcγRIIb. The prototype six-amino-acid ITIM sequence is (Ile/Val/Leu/Ser)-Xaa-Tyr-Xaa-Xaa-(Leu/Val). Ligand-induced clustering of these inhibitory receptors results in tyrosine phosphorylation, often by SRC-family tyrosine kinases, which provides a docking site for the recruitment of cytoplasmic phosphatases that have an SH2 domain.

patients with gastrointestinal stromal tumours and a few particular forms of mastocytosis; however, its use for the treatment of mast-cell-associated diseases such as allergy is unclear. Animal studies have shown that STI571 might ameliorate signs of allergic asthma and delayed-type hypersensitivity reactions[112,113], but proof of this concept in humans is lacking so far. Possibly, KIT tyrosine-kinase inhibitors other than STI571 or KIT-independent mast-cell inhibitors, such as inhibitors targeting the IL-4-dependent proliferation and hyper-responsiveness of human mast cells, need to be developed for a more successful mast-cell-specific therapy in allergy.

Interestingly, most anti-allergy drugs do not target mast cells directly but rather target receptors of mast-cell mediators such as histamine, sulphidoleukotrienes or proteases. One exception is sodium cromoglicate, which is thought to function as a mast-cell stabilizer by modulating cell-membrane properties and thereby reducing mast-cell mediator release. The compound was found to be effective for the treatment of allergic diseases such as allergic rhinitis, conjunctivitis and gastroenteritis in about 50% of individuals, when administered for several weeks. This drug is almost free of adverse effects, which indicates that it specifically affects mast cells for unknown reasons[114,115].

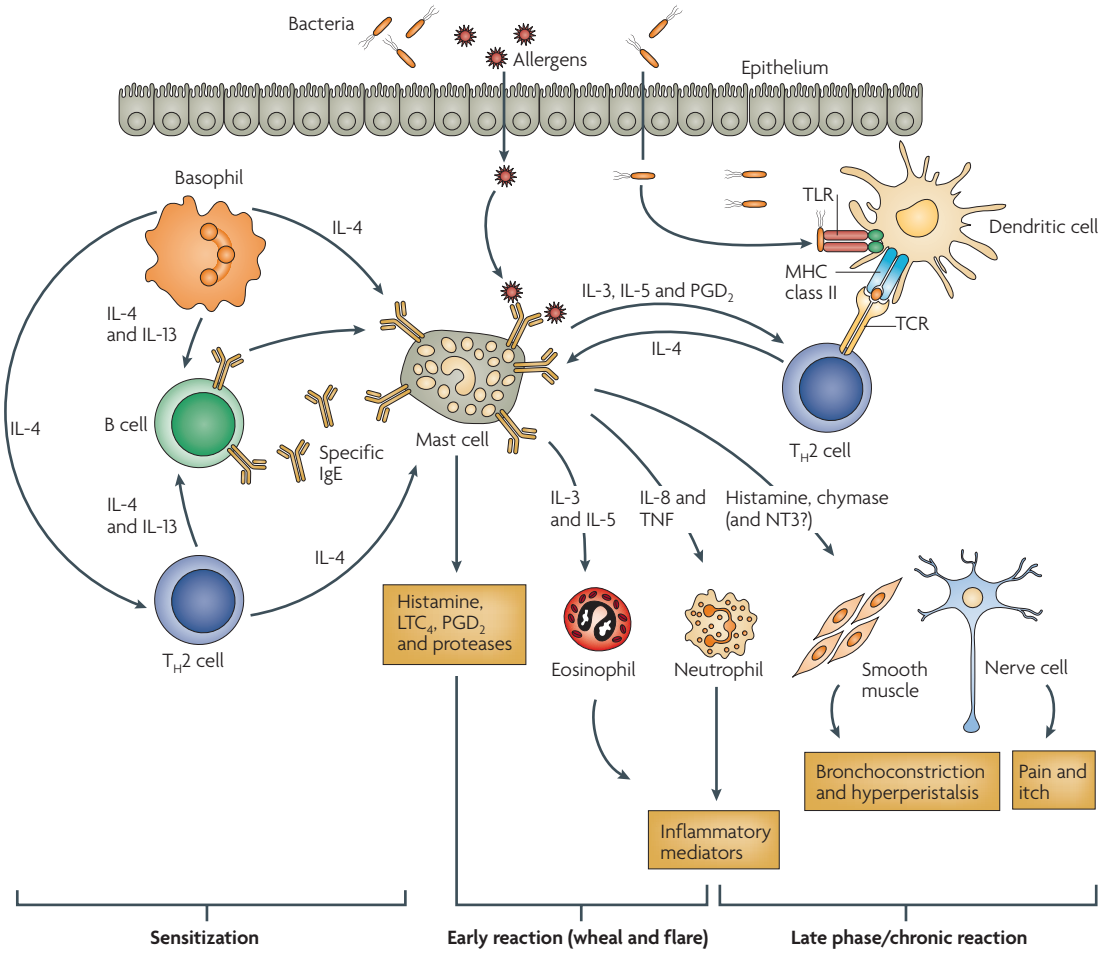

Figure 4 | **Role of mast cells in allergic inflammation.** Mast-cell activation by IgE crosslinking with allergen requires access of allergen into the tissue (for example, by impairment of the mucosal barrier because of nonspecific inflammation by bacterial pathogens or loss of defence mechanisms such as defensins) and input from the adaptive immune system to be effective. Mast-cell activation requires not only the synthesis of specific IgE by B cells (regulated by interleukin-4 (IL-4) and IL-13 derived from T helper 2 (T_H2) cells and basophils), but also mast-cell priming by IL-4 for enhanced mediator release. The subsequent release of mast-cell mediators such as histamine, leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2) leads to an early reaction, consisting classically of a 'wheal and flare' reaction of the skin or the mucosa. These mediators affect the mucosa, the blood vessels and sensory nerves (pain). Other mast-cell mediators, such as IL-3, IL-5, IL-8, tumour-necrosis factor (TNF), neurotrophin 3 (NT3) and proteases contribute to the initiation of a facultative late-phase reaction by recruiting and activating eosinophils, neutrophils and T_H2 cells, and by interaction with tissue cells such as nerve cells, smooth-muscle cells, endothelial cells and the epithelium. Ongoing dysregulation of such cell types not only causes symptoms of allergy, but also organ dysfunction, including loss of barrier function and, subsequently, increased bacterial translocation. This enables non-specific triggers to access mast cells, dendritic cells and other cells. Triggers such as bacterial products, or immunoglobulin (fragments) such as monomeric IgE and light chains might perpetuate the inflammatory process, even in the absence of allergen. TCR, T-cell receptor; TLR, Toll-like receptor.

Mast cells in allergic inflammation

It is well established that human mast cells mediate the early phase of type I hypersensitivity reactions by releasing histamine, LTC_4 and other mediators after crosslinking of surface-bound IgE by allergen in sensitized individuals[2–4,116–118]. However, allergic diseases do not consist only of 'early responses', but also of subsequent events summarized as late-phase reactions that occur facultatively following early-phase reactions, and that are thought to cause the recurrent and chronic symptoms of allergic individuals[119]. The role of mast cells in these clinically more important late-phase reactions (FIG. 4), as well as in hypersensitivity reactions other than type I reactions, such as type IV hypersensitivity reactions, has been addressed in several recent studies summarized elsewhere[4]. Most importantly, human mast cells induce the recruitment and local activation of eosinophils by expressing factors such as IL-5 after IgE-dependent activation, as described previously for T_H2 cells[47], and induce the recruitment of neutrophils by releasing IL-8 and TNF[48,66]. Neutrophil recruitment has been shown *in vitro* for both human and murine mast cells, as well as in murine disease models[120–122]. In contrast to mouse mast cells, however, the amount of TNF produced by human mast cells on a per-cell basis is small, compared with monocytes, and the portion that is preformed and stored in granules is even smaller, although it is consistently detectable[66,68]. Nevertheless, human mast cells, by releasing even small quantities of preformed TNF, might be responsible for the discrete neutrophil infiltration typically seen at sites of allergic inflammation.

In vitro studies indicate that human mast cells also participate in regulating lymphocyte functions in the course of allergic inflammation. After IgE crosslinking, mast cells produce IL-13, a cytokine that supports the production of allergen-specific IgE by B cells. The release of IL-13 can be further increased by the presence of IL-4, which is known to shift the cytokine profile produced by human mast cells away from pro-inflammatory cytokines such as TNF, IL-1 and IL-6, to T_H2 cytokines including IL-13 (REF. 48). Human mast cells can also regulate T-cell functions, for example through PGD_2, which almost exclusively derives from activated mast cells and is released during allergic reactions[123]. Recently, exciting new functions of PGD_2 have been identified that indicate a particular role for PGD_2 at the onset and for the perpetuation of asthma in young adults. The lipid mediator evokes airway hypersensitivity and chemotaxis of T cells, basophils and eosinophils through interaction with two receptors, the prostaglandin D_2 receptor (PTGDR) on granulocytes and smooth muscle cells, and CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) on T_H2 cells[124,125]. Furthermore, gene-mutation analyses have identified *PTGDR* as an asthma-susceptibility gene[125]. In addition to PGD_2, other human mast-cell mediators such as LTB_4, CCL3 and CCL4, OX40 ligand (also known as CD134) and TNF are involved in recruiting T cells and triggering T-cell-mediated adaptive immune responses, including memory induction, which enhance and perpetuate allergic reactions[67,126,127].

However, at least under normal conditions, mast cells are not a relevant source of IL-4. It has been repeatedly claimed that mast cells, in addition to T_H2 cells, produce IL-4; however, thorough *in vitro* studies using mature human mast cells from non-allergic individuals, as well as mouse *in vivo* studies, did not confirm such findings[48,52,64]. Instead, T_H2 cells and basophils seem to be the relevant sources of IL-4 in humans, whereas mast cells, if at all, might contribute to local IL-4 production under allergic conditions[65]. This is in accordance with the recent *in vivo* finding in mice that basophils are crucial for the induction of IgE-mediated chronic allergic inflammation, for which T cells and even mast cells are dispensable[128].

The important role of human mast cells in allergic disease and asthma has been further emphasized by the recent observation that SCF, which is produced by alveolar macrophages, and its receptor KIT, are strongly upregulated in asthmatic airways[129]. In the intestine, activated mast cells have been proposed to induce the inflammation, tissue transformation and fibrosis observed in both allergic and non-allergic processes, such as Crohn's disease[4,130]. More recently, it became evident that mast cells stimulated by IgE crosslinking also trigger local nerve responses resulting in pain and diarrhoea[11,118]. The molecular basis of mast-cell–nerve interactions has now been clarified to a large extent; however, the *in vivo* importance of such findings for allergy, in particular with respect to new treatment options, needs to be precisely defined[131].

Mast cells in non-allergic diseases

The biological significance of mast-cell activation by FcεRI aggregation in the absence of allergy has been repeatedly questioned and a definitive answer is still lacking. The most intriguing hypothesis in this respect is possibly the anti-parasite hypothesis, which proposes that FcεRI aggregation through crosslinking of parasite-specific IgE is a mechanism for parasite recognition, initiating an anti-parasite immune reaction, and that mast-cell products including T_H2 cytokines are key mediators required for host defence against parasite infection. This hypothesis is confirmed by murine disease models, which have shown that parasite-specific IgE is generated following a parasite infection, and that blocking mast-cell mediators or mast cells leads to an impairment of the host defence against parasites[132]. Among the mast-cell mediators, IL-5 (for eosinophil recruitment) and IL-13 (for B-cell and T_H2-cell immunity) seem to be of particular importance. Similar mechanisms have been anticipated in humans, although direct proof of their existence is lacking. As human mast cells are not a relevant source of IL-4 (REFS 48,52), the IL-4 that is produced and required in the early phases of parasite infection in humans probably derives from basophils, which can be activated for IL-4 release by worm antigens, and later from T_H2 cells[63,64,133].

Recently, *in vivo* studies using the *Trichinella spiralis* infection model in mice showed that antagonizing the IL-4-driven T_H2 milieu by IL-18 promotes intestinal parasite survival, whereas IL-18-deficient animals are highly resistant to *T. spiralis* infection[134]. These findings further

Late-phase reaction
IgE-mediated allergic reactions occurring within a few minutes (early-phase reaction) can be followed by a facultative secondary response phase starting typically 2–4 hours after allergen challenge. This late-phase anaphylaxis, in contrast to the early phase, is characterized by a pronounced cellular infiltration at the site of allergen challenge, which might lead to long-lasting inflammation and tissue dysfunction.

Type IV hypersensitivity
T-cell-mediated hypersensitivity reactions, also known as delayed-type hypersensitivity reactions. Despite some similarities to the late-phase reactions in the course of type I hypersensitivity reactions, they are separated because they are not preceded by an IgE-dependent early-phase reaction.

argue for a protective role of mast cells and T_H2 immune responses against parasites not only in the murine, but also in the human, intestine. However, a cytokine milieu shifted too far towards T_H2 cytokines could allow otherwise harmless infections that require T_H1 immune responses for defence to initiate severe inflammatory reactions[135].

More recently, evidence has accumulated showing that human mast cells might be involved in host defence against viruses that directly infect human mast cells, such as HIV, and also against double-stranded RNA viruses recognized by TLR3. In mice, mast cells express TLR3, and the stimulation of mast cells through TLR3 using polyI:C (polyinosinic–polycytidylic acid) leads to the recruitment of $CD8^+$ T cells[136]. Human mast cells also express TLR3, and *in vitro* studies have shown that TLR3 stimulation causes decreased adhesion of the cells to ECM proteins and decreased mediator release in response to antigen–IgE complexes, but causes an increase in IFNα production. Thereby, human mast cells might have a protective role in host defence against viral infections[137].

Apart from their role in allergy and host defence against microorganisms, it has been proposed that mast cells are involved in several other pathologies, such as proliferative diseases, autoimmune diseases, vascular diseases and diseases of the central nervous system[138–143]. The experimental evidence for mast-cell involvement in such diseases is based mostly on murine studies and at best only partially confirmed for the human system; therefore, the details are not discussed here.

Future directions

Mast cells have now been established as important immune cells connecting innate and adaptive immune responses, as well as connecting the immune system with the nervous system. The data on which such notions are based, however, are limited by the fact that different mast-cell sources and disease models have been used that do not always reflect the human situation. Several functional differences between human and murine mast cells have become obvious during the past few years; however, the main difference so far is the large amount of information that we have on murine mast cells compared with our limited knowledge of human mast cells. Therefore, an important goal in mast-cell research must be to establish further appropriate human *in vitro* and *in vivo* models for mast cells and mast-cell-associated diseases, as well as new research tools applicable to the human system such as *in vivo* cell imaging, mediator and cell tracking, and mast-cell knockout by specific and safe drugs.

The recently established human mast-cell gene databases have initiated new hypotheses on mast-cell biology and function. They are valuable tools and should next be supplemented by proteomic analyses and by functional *in vitro* studies with whole cells. Of particular interest in this context is the application of recent advances in systems biology at the single-cell level to human mast cells. The number of receptors expressed by mast cells and the number of mediators produced on activation are already confusing, and the intracellular signalling pathways are just starting to be unravelled for human cells. Therefore, mathematical modelling of signal input, processing and output could be fascinating, in particular if results could be confirmed at the cellular level. Promising approaches have been made recently for cell types other than mast cells[144,145]. Such approaches could support the development of new mast-cell-specific drugs, whereby in addition to the classical pharmacological approach, nutrients such as retinol that might modulate mast-cell functions should also be considered[106,146].

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Acknowledgements
I thank all previous and current fellows in my former laboratory in Hannover (1992–2004) and my current laboratory in Stuttgart (since 2005) for their engagement in mast-cell research. In particular, I thank C. Dahinden and J. Bienenstock for continuous discussions.

Competing interests statement
The author declares no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
FcεRI | FcγRI | FcγRIIB | IL-3 | IL-4 | IL-5 | IL-8 | IL-10 | IL-13 | KIT | SCF | TGFβ | TNF

FURTHER INFORMATION
Mast-cell microarray data: http://www.nch.go.jp/imal/GeneChip/public.htm
Stephan Bischoff's homepage: http://www.uni-hohenheim.de/ernaehrungsmed/
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