Chapter 2
The Mast Cell

2.1 A Historical Overview

The controversial, and for long elusive, story of MCs begins on a remote day in the summer 1878, precisely on the 17th June of that year, when a 24-year-old medical student, the future Nobel Prize laureate Paul Ehrlich, discussed his doctoral thesis at the Medical Faculty of Leipzig University (Fig. 2.1). The title of his dissertation was: “Beiträge zur Theorie und Praxis der histologischen Färbung” (“Contribution to the theory and practice of histological dyes”) (Ehrlich 1878). In his presentation, dedicated to the chemical basis of the basic aniline dyes, the young Ehrlich devoted a chapter to the tissue staining properties of the aniline compounds and described for the first time a class of aniline-positive cells of the connective tissues endowed with cytoplasmic metachromatic granules (“granulierte Bindgewebezel- len”) for which the name “Mastzellen” was proposed (Ehrlich 1878). This name, which means “well-fed cells”, was attached to the newly described cell population in the belief that their aniline-positive metachromatic granules might contain deposits of nutrients and might develop as a result of hypernutrition. He was likely to be mistaken about this issue, although it should be noted that recent observations in MC-deficient mice suggest roles for MCs in control of diet-induced obesity (Liu et al. 2009). Remarkably, Ehrlich stressed that the metachromatic staining of “Mastzellen” was due to reactivity of aniline with a “still undetermined chemical substance” stored in the granules (for further historical data, see Crivellato et al. 2003a). In addition, although he recognized that the “Mastzellen” were localized with very high frequency around blood vessels in the loose connective tissues, still these cells in his opinion had not to be regarded as members of a perivascular system. Their functional role, indeed, was mainly related to a “feeding” or “nourishing” activity, hence the term “Mastzellen”. Ehrlich found that aniline-reactive cells had a tendency to collect around developing preformed structures in connective tissues. For instance, he recognized that in certain acinar glands of the goat parotid, the pattern of “Mastzell” accumulation inside the tissue was not determined by the branching of the vascular system but by the ramification of the gland excretory ducts. He argued that “Mastzellen” could also be found around areas of developing
tissues. As to the origin of these cells, he suggested that they might differentiate from fibroblasts.

In successive memories, Ehrlich studied the presence and significance of “Mastzellen” in pathological conditions. He described two situations where connective tissue might be over-nourished, in chronic inflammation, especially when this was aggravated by chronic lymphatic obstruction, and the environs of tumours. Here there existed a lymph stasis, a damming up of tissue fluid rich in nutriment, whereby certain fixed connective tissue cells were stimulated to become mobile, to multiply and to convert some of the abundant extracellular material into specific intracellular granules. According to Ehrlich, the “Mastzellen” were “indices of the nutritional state of the connective tissue” (Ehrlich 1879). They increased during periods of hypernutrition and diminished during periods of relative starvation. Interestingly, Ehrlich found many “Mastzellen” in tumours, especially carcinoma, but it was left to his pupil Westphal (1891) to recognize that the cells tended to accumulate at the periphery of carcinomatous nodules rather than within the substance of the tumour.

Ehrlich studied the staining reactions of blood cells, laying the foundations of modern haematology on the basis of the specific affinities of the leukocytes for various dyes (Ehrlich 1891; Ehrlich and Lazarus 1898). He encountered cells with basophilic, metachromatic granules, and thus came to recognize two types of “Mastzellen”. The first—derived from, and living in the connective tissues (tissue “Mastzell”), the second—the counterpart of the neutrophil polymorph and eosinophil leukocyte—whose origin was in the bone marrow and whose habitat was in the peripheral blood (blood “Mastzell”, basophil or mast leukocyte). Meanwhile Ehrlich (1891) had discovered basophilic granular cells in human blood, though so far only in myeloid leukaemia. Nevertheless, with characteristic insight he at once perceived that the blood MCs in higher vertebrates were true leukocytes stemming from precursors in the bone marrow. By the time that his textbook of 1898 came to be revised (Ehrlich and Lazarus 1909) the evidence for the myeloid origin of the blood MC was complete (Jolly 1900).

Sixteen years after Ehrlich’s first description of Mastzellen, the English histologist and physiologist William Bate Hardy, provided a further contribution to the histochemical and functional definition of MCs. In the beginning, he referred to the
formerly described Ehrlich’s Mastzell with the collective term of “coarsely granular basophile cell” but in two outstanding papers, published in the Journal of Physiology in the years 1894 and 1895 (Kanthack and Hardy 1894; Hardy and Wesbrook 1895), he distinguished two types of granular basophile cells, i.e., the “coarsely granular basophile cells” and the “splanchnic basophile cells”, which both belonged to the population of “wandering cells” (the modern leukocytes). These tissue-homing cells corresponded to the subsets of connective tissue-type and mucosal MCs, respectively, which would be described seventy years later by Enerbäck in rodents (Enerbäck 1966a, b). Among the coarsely granular basophile cells, he also differentiated those cells which populated the serosal cavities—the so-called coelomic coarsely granular basophile cells—from the common coarsely granular basophile cells which were localized in the connective tissues. He stated that the granular basophile cells presented with different morphological and histochemical characteristics in diverse animal species as well as at different anatomical sites, being thus the first scholar to shape the fundamental concept of “MC heterogeneity”. Hardy performed a series of functional experiments on the basophile cells and suggested that different granular basophile cells might express functional specialisations. Hardy’s view of basophile cell function was partly in line with Ehrlich’s concept of a nutritional role for these cells. He believed that these cells might be somehow involved in the up-take and storage of substances as a result of hypernutrition. However, he also explored other experimental areas, such as the potential contribution of granular basophile cells to phagocytosis of pathogens and the participation of these cells to defence mechanisms during infections (for further historical data, see Crivellato and Ribatti 2010a).

For the first 60 years after Ehrlich’s discovery of MCs, the study of these cells was almost entirely histological. MCs were demonstrated in most animal species, although with an irregular, capricious distribution (Michels 1938). In his for long unsurpassed review of the MCs, Michels praised Ehrlich’s pioneering contribution to the study of these cells, in particular his recognition that MC granules were soluble in water. He wrote: “uncounted pages of useless and misleading research have been the result of the failure on the part of many investigators to heed the admonition originally given by Ehrlich and Westphal, that the MC granules are soluble in water and that to preserve them tissues must be fixed in 50% alcohol and stained in alcoholic thionine” (Michels 1938). As for the relation of MCs with basophils, after an initial unitary conception, subsequent studies indicated that at least in higher organisms these two cells differed both in habitat and in parentage, being the derivation of MCs unknown—they were usually interpreted as istiogenic elements—whilst the origin of basophils was from the bone marrow. Michels wrote that “aside from an identical basophilic metachromatic reaction of the granules, the two cell types have nothing in common” (Michels 1938).

At the end of the 1930s, a group of Scandinavian researchers provided fundamental new insight as to MC structural and functional profiles. The mysterious MC component prophesized by Ehrlich as the responsible agent for granule metachromasia was revealed by Jorpes, Holmgren and Wilander (Holmgren and Wilander 1937; Jorpes et al. 1937). Following Jorpes’ discovery that the anticoagulant heparin—a
polysulphuric acid ester, made up of glucuronic acid, glucosamine and sulphuric acid—was subject to stain metachromatically with toluidine blue, Holmgren and Wilander reconsidered Ehrlich’s observation that MC granules stained metachromatically with toluidine blue. These authors were able to set a correlation between the number of toluidine blue-positive MCs in various tissues and their heparin content. Tissues with large amounts of “Ehrlichischen Mastzellen” were particularly rich in heparin and, among MC-rich tissues, the beef liver capsule was described as “a pure culture of MCs” (Holmgren and Wilander 1937; Jorpes et al. 1937). The Swedish investigators formulated the conclusive theory that the task of MCs in the connective tissues was to produce heparin.

The discovery that tissue MCs were the source of heparin was the prelude to the identification of two other crucial substances contained in MCs: histamine and serotonin. A potential correlation between tissue heparin and tissue histamine contents was initially established. Indeed, the release of histamine was shown to be accompanied by a similar release of large amounts of heparin both in vivo and in vitro (Rocha and Silva 1952). In a series of fundamental studies published in the period 1952–1956, the pharmacologists Riley and West in Scotland demonstrated that histamine—the previously identified Lewis’ “H substance” responsible for skin anaphylactic phenomena—was present in MCs. Early studies showed that injection in the rat of histamine liberators, such as stilbamidine and D-tubocurarine, was followed by selective damage to tissue MCs indicating that MCs were the presumptive site of histamine accumulation in the tissues (Riley and West 1952). Further investigations revealed that very high values for heparin and histamine could be found in tissues which were exceptionally rich in MCs, such as the cleaned capsule of normal ox liver, and the sheep and ox pleura (Riley 1955). The loose connective tissues but not the dense connective tissue of the tendons were rich in histamine and MCs as well. A strong positive correlation between histamine tissue contents and the histological demonstration of MCs was also recognized in pathological conditions such as urticaria pigmentosa in man (Riley and West 1953) and MC tumours in dogs (Cass et al. 1954). Further evidence for the presence of histamine in MCs was provided by Fawcett and by Mota and Vugman. Fawcett (1954) demonstrated that the potent histamine liberator, compound 48/80, caused release of MC granules, and that it failed to liberate appreciable amounts of histamine from connective tissue previously depleted of MCs. Mota and Vugman (1956) reported a good correlation between serum histamine levels and disruption of MCs in a guinea pig model of anaphylaxis. On the whole, these data show that by the end of the 1950s experimental studies had delineated a fundamental functional link between MCs, histamine and the allergic and anaphylactic reactions which had been recognized and described long before by Pirquet, who coined the term “allergy” in 1906, and Portier and Richet, who introduced the term “anaphylaxis” in 1902. Thus, MCs could since be defined as the major tissue repository for histamine (“histaminocytes”) and were entitled to play a crucial role in allergic conditions such as hay fever, asthma and anaphylactic shock (for further historical data, see Beaven 2009). It soon appeared, however, that basophils too were rich in histamine and heparin (Graham et al. 1955; Behrens and
Thus, the similarities between MCs and basophils seemed again to outweigh their differences (Riley 1954).

About the same period, Benditt and co-workers demonstrated that 5-hydroxytryptamine (5-HT, serotonin) associated with MCs of the subcutaneous areolar tissue of the rat (Benditt et al. 1955). Serotonin was a vasoconstrictor substance suspected for decades to be contained in platelets. It was isolated and characterized in 1948 by Maurice Rapport and Irvine Page, and discovered to correspond to enteramine by the Italian scientist Erspamer in 1952 (Rapport et al. 1948; Erspamer and Asero 1952). Later work by Parratt and West (1956) revealed that serotonin was concentrated in tissue MCs of the rat and the mouse but not of the guinea-pig, dog, man, rabbit, cow, hamster and cat, and that the skin of the rat contained more than half of the total serotonin of the body. These authors speculated that MC serotonin might be involved in the response of animals to injections of large-molecular-weight substances such as egg-white or dextran. Both histamine and serotonin had potent effects, especially on the vascular system, and their release by MCs, which expressed a preferential perivascular location, could be implicated in the inflammatory reactions occurring in connective tissues (Riley 1963). Remarkably, not only MCs had the ability to synthesize histamine and serotonin but, as demonstrated by Green’s group, both normal and neoplastic MCs were able to take-up exogenous histamine and serotonin (Day and Green 1962; Furano and Green 1964). It was thus made clear that MCs can concentrate biogenic amines.

Hardy’s concept of MC heterogeneity was further developed in the 1960s by Enerbäck. Based on their specific staining characteristics and preferential tissue homing, two morphologically distinct subpopulations of rodent MCs were initially identified and termed connective tissue MCs (CTMCs) and mucosal MCs (MMCs), respectively (Enerbäck 1966a, b, 1986). The former populated the mucosae of the respiratory and gastrointestinal tracts, while the latter homed to the connective tissues and serosae. CTMCs could be distinguished from MMCs by staining in red with safranin due to the presence of large amounts of heparin in their secretory granules. In the mouse, indeed, the proteoglycan content of MC granules varied in the different MC subtypes. CTMCs contained heparin that lacked in MMCs. Conversely, MMCs expressed chondroitin sulphates A and B, which were not found in CTMCs, whereas both MC subtypes stored chondroitin sulphate E in their granules. Thus, in contrast to CTMCs, MMCs were sensitive to routine formalin fixation and could not be identified in standard histological sections. After appropriate fixation and sequential staining with Alcian blue and safranin, the MMCs stained blue, being thus differentiated from CTMCs which stained with safranin and were red. It later appeared that CTMC and MMC subtypes contained distinct classes of proteases and expressed different functional profiles, being activated in part by different stimulators and providing selective secretory responses.

During the 1970s, other crucial aspects of MC involvement in allergic and anaphylactic reactions were recognized. It was not until 1967 that the “reaginic” antibody—the transferable factor responsible for the sensitization phenomenon described by Prausnitz in 1921 (Prausnitz and Küstner 1921)—was eventually identified by Kimishige Ishizaka and Teruko Ishizaka as \( \gamma \)E-antibodies (immunoglobulin
E, IgE), a minor component of the Ig family (Ishizaka and Ishizaka 1967). IgE was shown to be capable to mediate the release of histamine and another mysterious substance called “slow reacting substance of anaphylaxis” (SRS-A) from sensitized tissue MCs (Ishizaka et al. 1970; Orange et al. 1971). The receptor for IgE molecules was later identified at high concentrations on the surface of MCs and was recognized to bind IgE with high affinity and specificity. This was named the “high affinity” receptor for IgE (FcεRI), and it was completely cloned in 1989 (Blank et al. 1989). The cross-linking of IgE with bivalent or multivalent antigen on the surface of MCs resulted in the aggregation of IgE receptors and in the triggering of MC degranulation. SRS-A was initially recognized by Feldberg and Kellaway in 1938 as a spasmogenic substance distinct from histamine (Feldberg and Kellaway 1938). This stuff was capable to increase microvascular permeability and produce long-lasting wheal-and-flare responses in the skin and bronchoconstriction in the lungs during anaphylactic shock (Brocklehurst 1960). It became apparent that SRS-A was a mixture of lipids, collectively named leukotrienes (LTs), which were generated from arachidonic acid through the 5′-lipoxygenase pathway. MCs were recognized to synthesize LTC₄, LTD₄ and LTB₄ as well as other inflammatory lipids (eicosanoids) called prostaglandins (PGDs) (Roberts et al. 1979). Within minutes of MC stimulation with anti-IgE antibodies, which activate the IgE receptor on the cell surface and trigger MC response, MCs were seen to release substantial amounts of PGD₂ (Lewis et al. 1982). Parallel investigations showed that basophils were also endowed with the high-affinity receptor for IgE, a discovery that further linked the two cell lineages in the speculative approach of researchers. In addition, both MCs and eosinophils were recognized to participate to certain protective reactions against parasites (Askenase 1977). Thus, until the mid 1990s, the paradigm prevailed that MCs were to be regarded as tissue elements principally implicated in the pathogenesis of allergic reactions and responsible for defence against certain parasites.

In the 1970s, accurate definition by transmission electron microscopy of a series of MC structural and functional details was provided by several groups. In particular, the group of Ann Dvorak in Boston afforded persuasive demonstration of the heterogeneous structure of MC granules, clarifying the mechanisms of MC degranulation and recovery, and providing new data on the fine, distinctive aspects of MC and basophil ultrastructure. During MC degranulation, cytoplasmic granule membranes were seen to fuse with each other and with the plasma membrane, giving rise to open secretory channels which allowed the release of granule contents into the extracellular environment (Dvorak 1991). This quick, explosive, IgE-mediated process of MC degranulation, characteristic of type I hypersensitivity reactions and categorized as “anaphylactic degranulation” or “compound exocytosis”, was punctually differentiated by a novel pattern of MC secretion Dvorak’s group was able to identify, for which the term “piecemeal degranulation” was coined. In a series of elegant ultrastructural immunochemical experiments, Ann Dvorak and co-workers described the fine aspects of this newly-identified degranulation pathway, which allowed MCs to release subtle amounts of granule-stored material in a prolonged time lapse. There was a slow discharge of granule contents in a “piecemeal” fashion, without membrane fusion events and granule opening to the
cell exterior. Dvorak underlined the concept that “piecemeal degranulation” represented the most common way of MC secretion observed in MCs infiltrating areas of chronic inflammation or tumours whilst the well studied and much more renowned pattern of “anaphylactic degranulation” could rarely be recognized apart from the sites of allergic responses (Dvorak 1991).

The primary involvement of MCs in such so harmful and sometimes life-threatening events as allergic and anaphylactic reactions left researchers somewhat disconcerted as to the real physiological role of MCs. How was it possible that so a ubiquitous and universally distributed cell type might exist only to cause danger to the host? Still in 1975, Harold Dvorak and Ann Dvorak wrote: “much more must be learned before we can confidently describe the role of basophils, or of the closely related MCs, in health or disease. It seems most unlikely that either cell exists for the purpose of destroying the organism in anaphylactic shock. Nonetheless, it is highly probably that basophil/MC function is closely related to the potent chemicals stored within their cytoplasmic granules. One likely possibility holds that small amounts of these chemicals are required for homeostasis (e.g., for regulation of the tone of the microvasculature) and that these cells function by releasing such substances continuously, as they are needed, in small aliquots rather than by explosive discharge” (Dvorak and Dvorak 1975).

By the end of the 1970s, scientists were able to solve the long-lasting enigma of the origin of MCs. Demonstration of MC derivation from bone marrow precursors could be established in 1977 when Kitamura’s group first showed that, using the abnormal giant cellular granules of beige mice (C57BL-Bg/JBg/J) as a traceable marker (Fig. 2.2), tissue MCs were found to develop from grafted beige bone marrow in irradiated wild type recipient mice (Kitamura et al. 1977). This discovery prompted further investigations on the origin of MC lineage leading to the concept that MCs were tissue-homing leukocytes. The paradigm was developed that they arise from pluripotent haematopoietic stem cells, circulate in the blood as agranular progeni-

![Fig. 2.2 Transmission electron microscopy of a mast cell with abnormal giant granules from a beige mouse (C57BL-Bg/JBg/J). The mast cell has been stimulated to exocytose and the granule on the left (arrow) is opening to the cell exterior. Bar: 3 μm. (Reproduced from Crivellato et al. 1999)](image-url)
tors, and then acquire their mature phenotype within tissues. The main growth factor governing tissue MC development and complete differentiation was shown to be the KIT receptor ligand or stem cell factor (SCF). The importance of SCF as a MC growth factor was underlined by the fact that mice with certain loss-of-function mutations affecting either SCF or its receptor KIT were devoid of MCs. Indeed, lack of expression of a functional KIT receptor due to spontaneous mutation in both copies of Kit, as it occurred in genetically MC-deficient WBB6F1-Kit<sup>W</sup>-Kit<sup>W<sub>v</sub></sup> mice (W/W<sub>v</sub> mice), resulted in a virtual absence of tissue MCs (Kitamura et al. 1978). This important finding stimulated further studies on the genetics of the KIT-SCF system. These studies provided a series of MC-lacking mouse strains which revealed extremely useful to study different aspects of MC function. Indeed, lack of MCs in Kit-mutant mice could be selectively repaired by the adoptive transfer of genetically compatible wild-type or mutant MCs derived from in vitro cultures to create the so-called MC “knock-in” mice (Nakano et al. 1985). Most of our current knowledge on MCs is actually derived from MC “knock-in” mouse models which have allowed researchers for testing and verifying whether MCs contribute to specific functions. By the way, the finding that basophils lacked the KIT receptor and were unaffected by the SCF shaped the concept that the developmental pathways of MCs and basophils were different.

In the 1980s, many investigations focussed on the composition of MC granules and the capacity of stimulated MCs to release cytokines, chemokines and growth factors. The concept of MC heterogeneity was further defined at this stage of MC research (Bienenstock et al. 1983). Like rodent MCs, human MCs were also found to express morphological, biochemical and functional heterogeneity. The first evidence that MCs contained proteases was provided by Gomori in 1953 (Gomori 1953). He developed enzyme histochemical techniques for detecting esterase activity inside of cells in sections of fixed tissues and was able to recognize that MCs stained intensely with such procedures. By 1960, two proteases with chymotrypsin- and trypsin-like activity were identified in MCs (Benditt 1956; Benditt and Arase 1959; Glenner and Cohen 1960). Enzyme activity was recognized to localize within intracellular granules. These enzymes were purified in the 1980s and renamed tryptase and chymase (Schwartz et al. 1981; Schechter et al. 1986). It soon appeared that MCs from different anatomical sites contained different profiles of these enzymes as well as of other proteases identified in the meantime. Human MCs were thus divided into two subtypes depending on the expression of different proteases in their granules (and other functional features) (Irani et al. 1986). MCs, which contained tryptase only, were designated as MCs<sub>T</sub> or “immune cell-associated” MCs. They were predominantly located in the respiratory and intestinal mucosa, where they co-localized around T lymphocytes. MCs that contained both tryptase and chymase, along with other proteases such as carboxypeptidase A and cathepsin G, were referred to as MCs<sub>TC</sub>. They were predominantly found in connective tissue areas, such as skin, submucosa of stomach and intestine, breast parenchyma, myocardium, lymph nodes, conjunctiva and synovium. These two subsets of human MCs differed also in terms of their mediator content and reactivity. A third type of MC, called MC<sub>C</sub> was also identified. This MC expressed chymase without tryptase and resided
mainly in the submucosa and mucosa of the stomach, small intestinal submucosa and colonic mucosa (Irani and Schwartz 1994). Interestingly, human MCs\textsubscript{T} were seen to correspond most closely to rodent MMCs, whereas MCs\textsubscript{TC} resembled rodent CTMCs. It was later recognized that the concept of MC heterogeneity was not limited to staining properties but also involved functional characteristics.

Beginning from the end of the 1980s, it progressively emerged that granules in MCs contained a series of highly active biological compounds, such as cytokines, chemokines and growth factors. In 1989, a series of groups investigating on MC responses to various activators reported that stimulated MCs produced and released interleukin (IL)-3, IL-4, IL-5, IL-6 and granulocyte/macrophage-colony stimulating factor (GM-CSF) (Burd et al. 1989; Plaut et al. 1989; Wodnar-Filipowicz et al. 1989) and that this could occur in the absence of MC degranulation. Shortly thereafter, Gordon and Galli (1990) reported that MCs were a biologically relevant source of both preformed and antigen-induced tumour necrosis factor (TNF)-\textalpha. These discoveries were central to set MCs in the midpoint of a complex series of inflammatory and immunological pathways associated to host defensive responses, as revealed by two seminal papers appearing in 1996 which demonstrated that MCs were essential to survival in a mouse model of sepsis (Malaviya et al. 1996; Echternacher et al. 1996). On the basis of these and other succeeding findings, it appeared that MCs were endowed with a large series of preformed and newly-synthesized mediators capable to exert different immunological and non-immunological functions. But this is a much recent story that will be accounted for in the next chapters of this book.

2.2 Biology of Mast Cells

2.2.1 Mast Cell Structure and Ultrastructure

MCs are bone marrow-derived tissue-homing secretory cells, which have been identified in all vertebrate classes. A cell population with the overall characteristics of higher vertebrate MCs is identifiable even in the most evolutionarily advanced fish species (Crivellato and Ribatti 2010b). Overall, MCs present consistent species-specific differences as to shape, dimension and granule staining properties. Besides, MCs coming from different sites in the same species or even the same organism still reveal subtle structural and ultrastructural specializations. We will here briefly sketch the microscopical traits of human and rodent MCs. Viewed by light microscopy, human MCs usually present as round or elongated cells with a diameter ranging between 8 and 20 \(\mu\)m, depending on the organ examined. Their single nucleus shows a round or oval shape and the cytoplasm contains numerous secretory granules that metachromatically stain with thiazine dyes such as toluidine blue (Fig. 2.3). By electron microscopy, these cells exhibit a non-segmented mono-lobed nucleus with peripherally condensed chromatin. The cytoplasm contains a few mitochondria, short profiles of the rough endoplasmic reticulum and numer-
ous free ribosomes. The most characteristic cytoplasmic organelles in human MCs are the membrane-bound, moderately electron-dense secretory granules. Secretory granules are very abundant and correspond to the metachromatic granules seen at the light microscopy level. They have an average diameter of 1.5 μm and present different types of substructural patterns, i.e., homogeneous, crystalline, scroll, particle, thread-like or a combination of them (reviewed by Dvorak 1991) (Figs. 2.4 and 2.5). Granule ultrastructure has been partly related to its content of serine proteases.

Fig. 2.3 Semi-thin section showing three mast cells isolated from the rat peritoneal cavity and stained with toluidine blue. Numerous cytoplasmic metachromatic granules are recognizable. Original magnification ×1,000. (Reproduced from Ribatti et al. 2001b)

Fig. 2.4 Ultrastructural morphology of human mast cell granules. Different patterns of granule texture may be recognized by transmission electron microscopy. In (a) a typical scroll granule from a duodenal mucosa mast cell. In (b) a mixed scroll-homogeneous-crystal granule from a mast cell in the skin. (c) and (f) show two crystal granules with very regular parallel arrays and hexagonal arrays, respectively, from skin mast cells. In (d) a mixed dense-crystal granule from a lung mast cell. In (e) a particle granule from a lymph nodal mast cell. Bars: 0.1 μm
Indeed, granules with the chymase protease preferentially exhibit homogeneous or crystalline substructures whereas granules lacking this protease show mainly a scroll pattern. However, significant granule heterogeneity can be found in any particular tissue and even between granules of a single MC. Aside from the typical secretory granules, human MC also contain non-membrane-bound, highly osmiophilic granules, called lipid bodies (Dvorak 1991). Lipid bodies are prominent in human MCs but less frequent in mouse, rat and guinea pig MCs. They are fewer in number and generally larger than secretory granules, and serve as a significant storage site for arachidonic acid. Recently, both secretory granules and lipid bodies in human MCs have been implicated in RNA metabolism (Dvorak and Morgan 2000; Dvorak et al. 2003).

Rodent MCs may consistently differ from human MCs. In guinea pig MCs, the substructural complexity of granule patterns resembles that of human MCs. Viewed by transmission electron microscopy, granules present either regular crystalline arrays, or arrays of tubules, or irregular thick threads, or finely granular material, or mixtures of these patterns (Dvorak 1991). Conversely, the granules of mouse MCs are filled with homogeneous dense material. Their content does not show the considerable variations in substructure that are seen in both human and guinea pig MCs.

Being MCs highly differentiated cells endowed with plentiful of such secretory organelles, research has much focussed on the mode of granule discharge. Viewed by electron microscopy, MCs present two morphologically distinct patterns of con-

![Fig. 2.5 Examples of mixed granules in a lymph nodal mast cell. In (a) mixed granules mainly contain coarse particles and scrolls. Viewed at higher magnification (b) three types of granules are depicted, a mixed scroll-particle (1), a particle (2) and a homogeneously dense (3) granule. Bars: 0.3 μm in (a), 0.1 μm in (b). (Reproduced from Crivellato et al. 2003c)](image-url)
tent release from granule stores, called: (1) compound exocytosis, also referred to as anaphylactic degranulation, and (2) piecemeal degranulation.

Anaphylactic degranulation was the first studied mechanism of MC secretion. It is characteristically triggered by aggregation of FcεRI due to cross-linking of IgE with bivalent or multivalent antigens. This specific stimulus elicits the explosive release of granules and the liberation of preformed mediators contained inside. The term of anaphylactic degranulation has been attached to this kind of secretory sequence because it is characteristic of the rapid discharge of MC products occurring during anaphylaxis. The distinct phases of this process have been carefully studied by transmission electron microscopy in various experimental conditions (reviewed by Dvorak 1991) (Fig. 2.6). Activated granules initially swell, exhibit matrix dissolution and decreased density. The perigranule membranes of adjacent granules fuse with each other and with the plasma membrane, resulting in the formation of elongated, tortuous chains of interconnected granules, called secretory channels, which eventually open at the surface. These openings allow rapid release of histamine and other low-molecular-weight substances stored in the secretory granules. All these events are very rapid, being ultrastructurally demonstrable as early as 3 minutes after MC stimulation. Degranulating MCs show profound alterations of their plasma membrane profile, being covered with complex elongated folds and surface projections. Later, they exhibit even shedding of cellular membranes. It soon became ap-

Fig. 2.6 Transmission electron microscopy of rat peritoneal mast cells. In (a) the mast cell shows the ultrastructural morphology of a resting cell, with smooth contour and a full complement of cytoplasmic secretory granules. In (b) the mast cell is in a state of active exocytosis and shows elongated surface folds, extruded, membrane-free granules, and cytoplasmic secretory channels filled by swollen granules. Bar equals 3 μm in (a) and 1 μm in (b). (Reproduced from Crivellato et al. 1999, 2002b)
parent, however, that although absolutely typical in its ultrastructural characteristics and highly significant in its dramatic functional consequences, anaphylactic degranulation of human MCs was rarely encountered in \textit{in vivo} conditions. Partial forms of exocytosis, involving release of a limited number of granules, can also be seen.

Besides anaphylactic degranulation, a different type of cell secretion termed piecemeal degranulation was identified in MCs by Ann Dvorak and co-workers in the early 1970s of the last century. This novel form of granule discharge was first recognized in guinea pig and human basophils participating to skin contact allergic reactions or infiltrating tumours but it was soon detected in MCs as well (Dvorak et al. 1973, 1974; Dvorak 2005a, b). When examined by transmission electron microscopy, cells exhibited partially or completely emptied granules in the absence of granule-to-granule or granule-to-plasma membrane fusions (Fig. 2.7). It appeared that piecemeal degranulation was substantially different from anaphylactic degranulation, which effected extrusion of stored material through formation of secretory channels and granule fusion to the cell plasma membrane. The term piecemeal degranulation was coined because cytoplasmic granules showed focal pieces or pack-ets of lost particles leaving characteristic patchy areas of electron-density beside

![Fig. 2.7](image_url) **Fig. 2.7** Transmission electron microscopy of two mast cells exhibiting different stages of piecemeal degranulation. The mast cell in (a), taken from the human intestinal mucosa, is in an active secretory condition and shows a number of expanded, non-fused secretory granules which exhibit characteristic loss of matrix structure. In the mast cell in (b), which is taken from the human skin, the piecemeal degranulation process is in a virtual final stage and the cell cytoplasm is filled by non-fused, almost empty secretory containers. The arrow points to an osmiophilic granule (lipid body). Bars: 1 \(\mu\)m in (a), 1.5 \(\mu\)m in (b). (Reproduced from Crivellato et al. 2003b, 2004)
lucency zones. In addition to granule changes, the cytoplasm of activated MCs presented a large number of smooth, 30–150 nm in diameter, membrane-bound vesicles. Some of these vesicles were filled with particles similar in structure and electron-density to those contained in the granules while others were apparently empty and appeared electron-lucent. Remarkably, vesicles were often seen attached to granules, in a process of budding from or fusing with the perigranule membrane. The piecemeal degranulation phenotype was recognized in MCs localized at sites of chronic inflammatory responses, and a careful scrutiny of MC morphology in different human pathologies led to the conclusion that piecemeal degranulation, not anaphylactic degranulation, was the most common release reaction identified in these cells (reviewed in Dvorak 1991). Dvorak and co-workers provided a theoretical model to explain how granules would empty during this kind of releasing reactions and gave rigorous kinetic bases to the movement of granule content within individual cells. The “shuttling vesicle” hypothesis formulated by Dvorak and Dvorak (1975) postulated a vesicular transport mechanism to effect transfer of granule constituents outside the cells. This hypothesis received substantial experimental confirmations by a series of elegant electron microscopic investigations using ultrastructural tracers and purified cells stimulated in vitro by different secretagogues. According to this model, an outward flow of cytoplasmic vesicles loaded with granule materials effects granule emptying during piecemeal degranulation. Vesicles containing bits of granule contents bud from the perigranule membrane, move through the cytoplasm and fuse with the plasma membrane, leading to content discharge. Endocytic vesicles are retrieved from the plasma membrane, traverse the cytoplasm and fuse with granules in a closely coupled inward flow. If the rate and amount of vesicular traffic are balanced, granule containers empty in a piecemeal fashion but maintain a constant size. If, on the other hand, the inward flow of the endocytic vesicles exceeds the outward flow of the exocytic vesicles, the granule chambers become enlarged. The latter event is what generally occurs during piecemeal degranulation. The electron microscopic changes observable during piecemeal degranulation can be summarized as follows: (1) Being piecemeal degranulation a discrete process affecting single granules in an asynchronic, stepwise progression, what generally results is a unique granule polymorphism, which consists of an admixture of normal resting granules, activated granules with enlarged chambers and diminished constituents, and empty dilated containers. (2) Remarkably, each granule does not fuse with the others or with the cell membrane but maintains its close individual structure during the entire releasing process. (3) The residual secretory material contained in activated granules presents “piecemeal” loss of constituents leading to “semilunar” or “haloed” patterns. (4) A proportion of granules exhibits surface budding projections; these are either apparently empty (thus they appear electron-lucent) or filled by the same electron-dense material that constitutes the granule. (5) Small, smooth, membrane-bound, electron-dense or -lucent vesicles are recognizable attached to the granules or free in the intergranular cytosol or close to the plasma membrane. Thus, identification of piecemeal degranulation relies upon specific ultrastructural criteria, which refer to both granule and cytoplasmic changes.
Functionally speaking, anaphylactic degranulation and piecemeal degranulation are opposite events. On the one hand, anaphylactic degranulation is a rapid and massive process of cell secretion which allows for complete discharge of granule constituents, often even of granule matrices. It does not accommodate the intensity of the releasing process and does not enable the cell to effect a differential discharge of granule-stored products. On the other hand, piecemeal degranulation is a slow, long lasting event, which is likely to permit the cell to single out a definite substance or a limited number of substances from the miscellaneous pool of releasable constituents packed inside secretory organelles. Evidence has been accumulated indicating that piecemeal degranulation in MCs allows for differential release of granule constituents. In 1990, Askenase’s group published an important article that provided indication for a differential release of serotonin without histamine and without anaphylactic degranulation from rat peritoneal MCs pretreated with the tricyclic antidepressant drug amitriptyline and stimulated with compound 48/80 (Kreuter Kops et al. 1990). Viewed at the electron microscope, storage granules lost their homogeneity, exhibited greatly reorganized matrix and were surrounded by clear spaces which were often associated with small (10–100 nm diameter) cytoplasmic vesicles, some of which contained electron-dense material. Secretory granules often had bud-like protrusions. The general pattern of MC secretion corresponded to what had already been known with the term of piecemeal degranulation. As subsequently demonstrated by different groups, piecemeal degranulation can be conceptualized as a tuneable process, which may be triggered and modulated by distinct compounds and which may account for the “surgical” cytokine response exhibited by MCs in different functional settings (Theoharides et al. 2007).

### 2.2.2 Origin, Development and Tissue Homing of Mast Cells

The MC is a type haematopoietic cell which acquires its definite phenotype once entered the homing tissues. These cells originate from progenitor cells in the bone marrow, which move through the circulation and become mature MCs after homing to destination tissues under the influence of the local microenvironment (Gurish and Austen 2001; Kitamura and Ito 2005).

Kitamura et al. (1977) first showed in the mouse that MCs derive from bone marrow precursors. Using the abnormal giant cellular granules of beige mice (C57BL-BgJ/BgJ) as a traceable marker, they found that tissue MCs developed from grafted beige bone marrow in irradiated wild type recipient mice. In humans, MCs derive from CD34+, CD13+, FcεRI−, KIT+ committed progenitors (Kirschenbaum et al. 1991). Committed progenitors, circulating as agranular mononuclear leukocytes, traverse the vascular space and complete their maturation after moving into diverse peripheral tissues (Rodewald et al. 1996). Here, they acquire concomitant phenotypic diversity. Rodewald et al. (1996) first identified the committed progenitors for the MC lineage in mouse fetal blood. This MC progenitor was defined by the
surface phenotype Thy-1\text{low} KIT\text{high}, lacked the expression of FcεRIα transcript and contained cytoplasmic granules. MC colony-forming cells reside within the bone marrow, spleen, peripheral blood, mesenteric lymph nodes and gut mucosa (Crapper and Schrader 1983). MC progenitors, bearing the phenotype Lin^{−} KIT^{+} Sca-1^{-} Ly6c^{−} FcεRIα^{−} CD27^{−} β7^{+} T1/ST2^{−}, were identified in the adult bone marrow (Chen et al. 2005). These cells develop into MCs in culture and reconstitute MC compartment upon their transplantation into MC-deficient mice. Recently, a cell population (Lin^{−} Kit^{+} FcγRII/III^{hi} β7^{hi}) has been identified in the mouse spleen with the characteristics of a bipotent progenitor for the basophil and MC lineages (Arinobu et al. 2005). This cell population, termed basophil/MC common progenitor, can be generated mainly from granulocyte/macrophage progenitors in the bone marrow. The same authors identified MC progenitors (CD45^{−} Lin^{−} CD34^{+} β7^{hi} FcεRIα^{lo}) in the intestine.

The developmental pathway of MCs and the relationship between MCs and other leukocytes are controversial (Arinobu et al. 2009). It has been debated whether MCs are of “myeloid” or “lymphoid” origin or stem from a distinct population of precursor cells. Specific arrays of differentiation factors such as SCF and IL-3 expressed by bone marrow stromal cells promote a distinctive pattern of MC-specific gene expression that includes genes encoding for distinct transcription factors (Winandy and Brown 2007). The main regulatory pathway for normal MC differentiation is not well characterized. Balanced activity of transcription factors PU.1 and GATA is known to be required, along with the transcription factors Mitf and possibly SCL, and the functions of GATA-2 and GATA-1 in this process can now be distinguished (Arinobu et al. 2005; Babina et al. 2005; Nishiyama et al. 2005). Until recently, it has remained obscure whether MCs are “myeloid” or are in a distinct class of their own. They can be derived from precursors separate from most myeloid lineages (Arinobu et al. 2005; Chen et al. 2005). Unlike monocyte and granulocyte lineages, but corresponding to lymphocytes, they develop through a pathway that excludes transcription factor C/EBP-α, which controls monocyte/dendritic cell programs (Iwasaki et al. 2006; Taghon et al. 2007). Recently, results have provided concrete evidence for one specific function, increased GATA-2 or GATA-3 expression, that provides direct access to the MC pathway for an uncommitted but differentiating lymphoid precursor (Taghon et al. 2007).

The zinc finger transcription factor GATA-1 has crucial roles in erythroid, megakaryocytic, and MC differentiation. Remarkably, friend of GATA-1 (FOG-1) is a binding partner of GATA-1 and is indispensable for the function of GATA-1 during erythro/megakaryopoiesis, but FOG-1 is not expressed in MCs (Cantor et al. 2008). A combined experimental system with conditional gene expression and in vitro haematopoietic induction of mouse embryonic stem cells has shown that expression of FOG-1 during the progenitor period inhibits the differentiation of MCs and redirects them into the erythroid, megakaryocytic, and granulocytic lineages (Cantor et al. 2008; Sugiyama et al. 2008). Mutant analysis reveals that this lineage skewing is caused by disrupted binding between GATA-1 and PU.1, a transcription factor that positively or negatively cooperates with GATA-1, which is a prerequisite for MC differentiation (Sugiyama et al. 2008). However, FOG-1 expression in mature
MCs brings approximately a reversible loss of the MC phenotype. Thus, FOG-1 inhibits MC differentiation in a differentiation stage-dependent manner and its down-regulation is a prerequisite for MC development (Cantor et al. 2008; Sugiyama et al. 2008). Although the main function of GATA-3 is to act as a master transcription factor for the differentiation of T helper (Th) 2 cells, new research indicates that GATA-3 too is a crucial factor for MC development. It has been demonstrated that expression of GATA-3 in the absence of Notch-DL1 signalling drives MC development (Taghon et al. 2007). Indeed, overexpression of GATA-3 in thymic progenitor cells promotes MC differentiation. By the way, the thymus has been recognized to be the site of intense MC proliferation during chick embryo development (Crivel- lato et al. 2005a; Fig. 2.8).

Once dismissed from the bone marrow as well as other embryonic sites of haemopoiesis, MC precursors move through the blood circulation to peripheral target sites. Tissue homing of MC precursors is critically regulated by tissue micro-environmental factors. Among these, the SCF secreted by fibroblasts, stromal cells and endothelial cells represents the most important cytokine involved in human and rodent MC development (Ashman 1999). Not only does SCF drive MC homing, proliferation and differentiation but also MC survival, migration and functional activation. Under various experimental conditions, SCF is chemotactic for MCs and their progenitors. For example, local treatment of mice with SCF can induce marked local increase in MC number, reflecting both enhanced recruitment/retention and/or maturation of MC precursors and proliferation of more mature MCs (Tsai et al. 2008).

Fig. 2.8 Transmission electron microscopy of the thymus in a day 16 chicken embryo. In (a) two mast cells are seen in the thymic medulla; in (b) a mast cell in mitosis is depicted. Bars: 2 μm in (a), 3 μm in (b). (Reproduced from Crivelatto et al. 2005a)
The importance of SCF as a MC growth factor is underlined by the fact—as we have said previously—that mice with certain loss-of-function mutations affecting either SCF or its receptor KIT are devoid of MCs. Indeed, lack of expression of a functional KIT receptor due to spontaneous mutation in both copies of Kit, as it occurs in genetically MC-deficient WBB6F1-Kit<sup>W</sup>-<em>Kit<sup>W</sup>-v</em> mice (<em>W</em>/<em>W</em> mice), results in a virtual absence of tissue MCs (Kitamura et al. 1978). <em>Kit<sup>W</sup></em> contains a point mutation that encodes a truncated KIT protein, which lacks the transmembrane domain and is therefore not expressed on the cell surface; <em>Kit<sup>W</sup>-v</em> encodes a mutation in the KIT tyrosine kinase domain that markedly decreases the kinase activity of the receptor. A Kit-mutant mouse has been characterized more recently, the C57BL/6-<em>Kit<sup>W-sh</sup>/Kit<sup>W-sh</sup></em> mice (Grimaldeston et al. 2005; Zhou et al. 2007). <em>Kit<sup>W-sh</sup></em> contains an inversion mutation of the transcriptional regulatory elements upstream of the Kit transcription start site on mouse chromosome 5 (Galli et al. 2005a). Remarkably, MCs develop in <em>W</em>/<em>W</em> mice and in C57BL/6-<em>Kit<sup>W-sh</sup>/Kit<sup>W-sh</sup></em> mice if these mice receive bone marrow cells from normal littermates. The mechanism of SCF in human MCs has basically been identified. Binding of SCF induces autophosphorylation of KIT and subsequent activation of several signalling molecules including PI3K and mitogen-activated protein kinase (Lorentz et al. 2002).

The specific localization of MCs in homing tissues is dependent on their interaction with extracellular matrix proteins. Cell adherence to extracellular matrix proteins is mediated by specific cell adhesion receptors, mainly cell surface receptors of the integrin family. An important integrin expressed by human MC progenitors is the α4β1 integrin, which regulates their adhesion to activated endothelial cells (Boyce et al. 2002). Mucosal MCs also possess β7 integrin, mediating the tissue specific homing of intestinal MC progenitors (Gurish et al. 2001). In the mouse, large numbers of MC-committed precursors are constitutively recruited in the small intestine by a mechanism involving the α4β7 integrin (Gurish and Austen 2001). This integrin expressed on the surface of MC precursors binds to the “mucosal address in cell adhesion molecule-1” (MAdCAM-1) and to “vascular cell adhesion molecule-1” (VCAM-1) as endothelial counterligands for this integrin. Inflammation-induced recruitment of human MC progenitors to the lungs requires both α4β7 and α4β1, implicating organ-specific control of MC progenitors influx (Gurish and Boyce 2006). Remarkably, dendritic cell expression of the transcription factor T-bet, which controls interferon (IFN)-γ production and Th1 cell differentiation from CD4<sup>+</sup> T cells, regulates MC progenitor homing to mucosal tissue. Indeed, homing of MC progenitors to the lung or small intestine in T-bet(-/-) mice is reduced (Alcaide et al. 2007). In addition, chemokine receptors expressed by MC progenitors are most likely involved in directing the progenitors from the circulation into the tissues where they mature. Human MC precursors derived <em>in vitro</em> from cord blood express a set of chemokine receptors including CXCR2, CCR3, CXCR4, and CCR5, and respond to the corresponding ligands <em>in vitro</em> (Ochi et al. 1999). Notch receptor-mediated signalling is involved in MC differentiation and homing. Recently, it has been recognized that Notch2 signalling in MCs is required for proper localization of intestinal MCs, and is critical for MC host-pathogen interface in the small intestine (Sakata-Yanagimoto et al. 2011).
Besides SCF, IL-3 plays in rodents an additional fundamental role in MC development (Lantz et al. 1998). By contrast, the role of IL-3 on the development of human MCs is controversial (Saito 2005). Other cytokines and growth factors which regulate MC development and differentiation include IL-4, IL-9, IL-10, transforming growth factor beta (TGF-β) and nerve growth factor (NGF) (Okayama and Kawakami 2006). IL-4 does not affect MCs by itself, but acts synergistically with SCF in the control of MC survival, proliferation as well as IgE-dependent mediator release (Bischoff et al. 1999). The IL-4 priming of human MCs for enhanced proliferation and mediator release is associated with an increased activity of extracellular signal-regulated kinase (ERK) and c-Fos (Lorentz et al. 2005). Remarkably, IL-4 changes the cytokine profile released by mature MCs by reducing proinflammatory cytokines such as TNF-α and IL-6 and in turn enhancing Th2 cytokines such as IL-5 and IL-13 (Lorentz et al. 2000). IL-9 alone does not have any effect on bone marrow-derived MC (BMMC) proliferation but mouse BMMCs undergo phenotypic changes in the presence of IL-9 in combination with SCF that consist in the acquisition of a mucosal MC phenotype (namely, accumulation of mMCP (monocyte chemotactrant protein)-1 and mMCP-2 transcripts) (Okayama and Kawakami 2006). In human systems, IL-9 and IL-5 stimulate SCF-mediated proliferation of MCs from cord blood cells, bone marrow and peripheral cells (Matsuzawa et al. 2003). IL-10 alone does not support the growth and differentiation of MC progenitors. However, when combined with IL-3 or IL-4, IL-10 enhances their growth. NGF promotes proliferation and differentiation of mouse BMMCs in the presence of IL-3 (Matsuda et al. 1991). NGF does not affect human MC survival but in combination with SCF it synergistically suppresses MC apoptosis (Kanbe et al. 2000).

Committed progenitors are supposed to populate peripheral tissues functioning as a local reservoir. These undifferentiated but committed progenitors do not develop into mature MCs unless adequate inflammatory stimuli ensue. In the adult mouse, for instance, it has been shown that the mucosa of the intestine contains the largest peripheral pool of these committed progenitors (Guy-Grand et al. 1984). Mature MCs can be very long-lived cells, surviving in some cases for years, and can retain their ability to proliferate under certain conditions (Galli and Lantz 1999).

### 2.2.3 Mast Cell Organ and Tissue Distribution

MCs are virtually ubiquitous cells. They reside in almost all of the major organs and tissues of the body. Normally, they localize in proximity to surfaces that interface the external environment, which are common portals for pathogen, allergen and toxin entry. Thus, MCs are strategically located at host/environment interfaces like the skin, airways and gastrointestinal and urogenital tracts. MCs are likely to be among the first inflammatory cells to interact with invading microorganisms and initiate immune responses (Metz et al. 2008). In this perspective, MCs have been shown to increase in inflammatory reactions of the skin as well as the intestinal and respiratory tracts in different species, such as man, rodents, and chickens.
(Rose et al. 1980; Morris et al. 2004). MCs also populate the connective tissues, particularly in association with structures such as blood vessels, lymphatic vessels and nerves, in a position which make them key elements in processes like wound healing, tissue regeneration and remodelling after injury, fibrosis and angiogenesis (Gonzalez et al. 1999; Weller et al. 2006; Grimbaldeston et al. 2007). MCs also reside in proximity to vulnerable spaces such as the peritoneum and the joint cavities. MCs are not found in avascular tissues such as mineralized bone, cartilage and the cornea. In the human body, they collectively comprise a substantial cell population. It has been estimated that if all human tissue MCs were amassed together in a single organ, it would equal the size of a normal spleen (Sayed et al. 2008). This indicates a strong selective pressure in maintaining this cell type throughout the evolutionary scale (Crivellato and Ribatti 2010b).

In humans, MCs express farly different phenotypes according to their homing site. For instance, the vast majority of MCs in the alveolar interstitium contains tryptase and only little chymase (Irani et al. 1989). However, chymase-expressing MCs are a major constituent of MC populations of the pleura and of the airway and vessel wall (Martin et al. 1992; Andersson et al. 2009). A major increase of chymase-positive MCs was noted in the adventitia of small pulmonary arteries in patients who died of asthma (Shiang et al. 2009). In addition, chymase-expressing MCs are also prominent in scarring lung diseases, like interstitial lung disease (Edwards et al. 2005).

In the human intestinal mucosa, MCs consist of approximately 2–3% of the inflammatory cell infiltrate localized in healthy subjects (Bischoff 2009). MCs \( T \) prevail in the lamina propria whilst MCs \( T_C \) are prevalent in the submucosa. These figures change during pathological conditions. In the course of intestinal diseases, such as food allergy and parasitosis, this amount can augment up to tenfold. MCs \( C \) are located mainly in the submucosa of the small intestine and colonic mucosa (Irani and Schwartz 1994). MCs are regarded as key elements of the gut-associated lymphoid tissue (GALT) and participate to several aspects of mucosal defence. In addition, intestinal MCs perform regulatory functions to maintain tissue homeostasis such as the control of the intestinal barrier. MCs density in the intestinal lamina propria has been linked to the maintenance of normal villus architecture. Low mast cell density in the human duodenal mucosa from chronic inflammatory duodenal bowel disorders is associated with defective villous architecture (Crivellato et al. 2003b). A structural interaction among crypt epithelial cells, subepithelial myofibroblasts and pericryptal MCs has been identified in the human small bowel mucosa with potential functional implications in enterocyte proliferation and differentiation (Crivellato et al. 2005b; Fig. 2.9).

In human skin, MCs are preferentially situated in the most superficial layers (more than 80 MCs/mm\(^2\) in the papillary dermis) where up to tenfold more MCs are to be found as compared with the subcutis (Weber et al. 2003). Remarkably, MC numbers are highest at peripheral skin sites, such as the chin and the nose (around 50 MCs/mm\(^2\)), and lowest at central skin sites, such as the abdomen (around 20 MCs/mm\(^2\)). Thus, healthy human skin exhibits a proximal/distal and a central/peripheral MC gradient. Recently, the human skin has been demonstrated to be an
extramedullary reservoir for MC precursors, which reside in the connective tissue sheath of hair follicle (Ito et al. 2010). Remarkably, a local regulatory loop between corticotropin-releasing hormone (CRH) and SCF signalling has been identified which promotes generation of mature MCs from MC precursors in the hair follicle.

The uterus is an important site of MC homing. MCs localize to the endometrium, myometrium and cervix (Cabanillas-Saez et al. 2002). Uterine MCs are morpho-
logically similar to skin and lung MCs (Massey et al. 1991). In the mouse, MCs are implicated in the process of angiogenesis in the cervix during pregnancy (Varayoud et al. 2004). Remarkably, the activity of these MCs as well as their number and histamine content are regulated by female reproductive hormones and increase during the second half of murine pregnancy reaching to maximum by the end of the gestational period (Rudolph et al. 1997). Notably, MC content of histamine comes to normal after delivery. By the end of pregnancy, uterine MCs release histamine, serotonin, prostaglandin D$_2$ (PGD$_2$) and leukotrienes (LTs), which stimulate uterine contraction (Bytautiene et al. 2003). It has been shown, indeed, that uterine MCs play an important role in parturition by effecting uterine contraction induced by estrogen. Drugs such as β-adrenergic agonists or corticosteroids which stabilize MC degranulation prevent preterm labor (Martínez et al. 1999). In human uterine leiomyomas, MCs have been shown to express leptin by immunocytochemistry and leptin was partly confined to tryptase-positive granules (Ribatti et al. 2007a).

MCs are present in normal and in diseased human heart tissue. Within heart tissue, MCs lie between myocytes and in close contact with blood vessels (Patella et al. 1995; Marone et al. 1995). They are also found in the coronary adventitia. Isolated human heart MCs release preformed mediators, such as histamine and tryptase as well as newly generated mediators, like PGD$_2$ and LTC$_4$ after stimulation with immunological or non-immunological stimuli. Human cardiac MCs possess FcεRI and IgE bound to the surface and receptors for the fifth component of complement (C5a), which could explain how cardiac MCs are involved in systemic and cardiac anaphylaxis. Cardiac MCs and those in human coronary arteries also play a role in the early and late stages of atherogenesis and during ischemic myocardial injury.

Recently, the white adipose tissue has gained interest in MC biology. The white adipose tissue is a heterogeneous tissue, found in various locations throughout the body, containing mature adipocytes and a stroma-vascular fraction. The latter component includes a large proportion of immune haematopoietic cells, among which MCs that contribute to diet-induced obesity (Liu et al. 2009). It has been demonstrated that MCs present in mice adipose tissue derive from haematopoietic stem/progenitor cells identified in the tissue (Poglio et al. 2010). Thus, adipose-derived haematopoietic stem/progenitor cells contain a precursor-cell population committed to the MC lineage, and able to efficiently home to peripheral organs such as intestine and skin, where it acquires properties of functional tissue MCs. Additionally, the white adipose tissue contains a significant MC progenitor population, suggesting that the entire MC lineage process take place in the white adipose tissue. Thus, considering the quantitative importance of the white adipose tissue in the adult organism and the increasing roles recently assigned to MCs in physiopathology, this highly specialized tissue may represent an important source of MCs in physiological and pathological situations.

MCs are well represented in the joint cavities. They constitute around 3% of cells in the immediate vicinity of the normal human synovial lining (Castor 1960). They are not observed within the synovial lining layer itself but rather populate the subsynovial loose connective tissue and adipose tissue, where they cluster near blood vessels and nerves (Nigrovic and Lee 2007). They are not seen within the
cartilage and are rare in normal periarticular bone. Synovial MCs mostly belong to the MC\textsubscript{TC} phenotype, although MC staining for tryptase alone (MC\textsubscript{T}) are also observed in varying proportion, usually in MCs found close to the synovial lining (Buckley et al. 1998). In the normal mouse joint, MCs are also localized to the synovial sublining, where they cluster around blood vessels and nerves (Nigrovic and Lee 2007).

MCs have also been identified in the central nervous system. Here, they are most numerous in the leptomeninges, thalamus and hypothalamus and in the dura mater of the spinal cord (Johnson and Krenger 1992). In particular, they have been found in the infundibulum, pineal organ, area postrema, choroid plexuses and in the leptomeninges surrounding the pineal organ and infundibulum (Dropp 1979). Occasional MCs are also seen within the supraoptic crest, the subfornical organ and the ventricles. In addition, they have often been recognized at sites directly adjacent to nerves in different peripheral districts, such as the skin, the intestinal mucosa and the submesothelial lamina of the peritoneum (Stead et al. 1987, 1989; Crivellato et al. 1991).

2.2.4 Mast Cell Receptors

MCs express on their surface a series of important receptors which drive their differentiation and functional activity (Fig. 2.10). One of the most significant is the high affinity receptor for IgE, FcεRI, which mediates interaction with IgE. In general terms, IgE response is accomplished by interaction with two structurally unrelated receptors: the high affinity receptor, FcεRI, which engages IgE with a 1,000-fold higher affinity (K\textsubscript{a} 10\textsuperscript{10} M\textsuperscript{-1}) than does the low affinity receptor FcεRII or CD23 (K\textsubscript{a} 10\textsuperscript{7} M\textsuperscript{-1}) (Sutton and Gould 1993). Mammalian MCs, as well as basophils, express the tetrameric αβγ\textsubscript{2} form of the FcεRI on their surface (Galli et al. 2005a). The cross-linking of IgE with bivalent or multivalent antigen results in the aggregation of FcεRI, which is sufficient for initiating down-stream signal transduction events that activate MC exocytosis as well as the de novo synthesis and secretion of lipid mediators and cytokines (Rivera and Gilfillan 2006).

MCs, but not basophils, express the KIT receptor for SCF which represents a key feature for distinguishing between the two cell types. The expression of the tyrosine kinase KIT receptor on the surface of the MCs is very important for MC functional activity. Indeed it does not only drive terminal differentiation of the MCs but has also other important roles in regulating MC biology, such as survival, activation and degranulation of mature MCs. As we have previously seen, the importance of SCF as a MC growth factor is underlined by the fact that mice with certain loss-of-function mutations affecting either SCF or its receptor KIT are devoid of MCs. Indeed, lack of expression of a functional KIT receptor due to spontaneous mutation in both copies of Kit, as it occurs in genetically MC-deficient WBB6F1-Kit\textsuperscript{W-} mice (W/W\textsuperscript{v} mice), results in a virtual absence of tissue MCs (Kitamura et al. 1978).

Besides the FcεRI and KIT receptors, MCs express a large array of adhesion molecules and chemotactic factor receptors. Adhesion molecules on both progeni-
tors and mature MCs are certainly important factors controlling MC homing within tissues (Vliagolftis and Metcalfe 1997) as well as MC activation. Studies with ex vivo MCs obtained from human tissues demonstrate surface expression of β1 integrins such as VLA-3, VLA-4, VLA-5 and αvβ3 integrin (Valent and Bettelheim 1992; Columbo et al. 1995). The natural ligands of VLA-3, VLA-4 and VLA-5 and αvβ3 integrin are laminin, type I collagen and fibronectin; fibronectin and VCAM-1; fibronectin; and vitronectin, fibronectin, thrombospondin and fibrinogen, respectively. It has been reported that β1 integrins are involved in MC activation, upregulation of cytokine expression and survival (Ra et al. 1994). Studies in humans show that MCs from uterus and lung express the β1 integrins α4β1 and α5β1, known as receptors for fibronectin, and that skin MCs express α3β1 and adhere to fibronectin and laminin (Columbo et al. 1995). Besides these integrins, human intestinal MCs express α2β1 integrin (Lorentz et al. 2002). BMMCs have been shown to express α4, α5 and α6 integrins (Fehlner-Gardiner et al. 1999). As far as
2.2 Biology of Mast Cells

non-integrin adhesion molecules are concerned, human MCs have been reported to express low levels of intracellular adhesion molecules 1 and 3 (ICAM-1, ICAM-3) as well as leukocyte function-associated antigen-1 and 3 (LFA-1, LFA-3) (Bochner and Schleimer 2001). Additional adhesion molecules expressed by MCs are CD44, a hyaluronic acid receptor, and singlec-8, a molecule which binds to sialic acid moieties (Bochner and Schleimer 2001). MCs express surface receptors that depend on their anatomical location and the stage of differentiation and activation. Thyroid MCs, for instance, express thyroid hormone receptors (Catini and Legnaioli 1992) and genital tract MCs are responsive to estrogen and luteinizing hormones (Maurer et al. 2003). Human MCs express androgen receptors but treatment with testosterone exerts no influence on IgE-independent MC degranulation elicited by neuromuscular blocking agents (Chen et al. 2010).

In the resting state, MCs express the activating IgG receptor FcγRIIa (CD32a) and, upon interferon gamma (IFN-γ) activation, the high affinity activating FcγRI (CD64). MCs might also express the complement receptors C3aR and C5aR, receptors for various interleukins, such as IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, IL-15R, growth factors and chemokines, among others the NGF receptor TRKA, GM-CSFR, INF-γR, and the receptors for chemokines CCR3, CCR5, CXCR2, CXCR4 (Nickel et al. 1999; Romagnani et al. 1999). MCs express distinct IL-15Rα isoforms which modulate MC-dependent innate immune response by fine-tuning defined MC protease activities (Orinska et al. 2007). Expression of the receptors for IL-1 family molecules, specifically, IL-1R1, IL-18R and T1/ST2, are detectable intracellularly in human umbilical cord blood-derived MCs by flow cytometry, but is scarcely detectable on the cells’ surface (Iikura et al. 2007). However, IL-1β, IL-18 or IL-33 induce phosphorylation of Erk, p38 and JNK in naïve human umbilical cord blood-derived MCs, and IL-33 or IL-1β, but not IL-18, enhance the survival of naïve human umbilical cord blood-derived MCs and promote their adhesion to fibronectin. IL-33 or IL-1β also induce IL-8 and IL-13 production in naïve human umbilical cord blood-derived MCs, and enhance production of these cytokines in IgE/anti-IgE-stimulated human umbilical cord blood-derived MCs, without enhancing secretion of either PGD₂ or histamine. Moreover, IL-33-mediated IL-8 production by human umbilical cord blood-derived MCs is markedly reduced by the p38 MAPK inhibitor, SB203580. In contrast to findings with mouse MCs, IL-18 neither induces nor enhances secretion of the mediators PGD₂ or histamine by human umbilical cord blood-derived MCs (Iikura et al. 2007).

MCs release histamine but they are also sensible to histamine activity through surface receptors. MCs have been shown to express H2R, H3R and H4R receptors (Jutel et al. 2009). H2R negatively regulates the release of histamine on MCs. The control of MCs by histamine acting on H3R involves neuropeptide-containing nerves and might be related to a local neuron-MC feedback loop controlling neurogenic inflammation (Dimitriadou et al. 1994). H4R has been found on MCs. Histamine released by basophils and MCs themselves may potentiate MCs chemotaxis on the inflammatory setting by interacting with the H4 receptor (Hofstra et al. 2003). In addition, MCs express the CysLTR₁ and CysLTR₂ receptors for leukotrienes. Thus, cysteinyl leukotrienes produced by immunologically activated
MCs may exert through an autocrine loop a variety of responses by activating the receptors CysLTR1 and CysLTR2 (Mellor et al. 2001). IL-4 and LTC4, secreted by MCs and basophils, upregulate the expression of CysLTR1 and stimulate LTC4 and cytokine production by human MCs. MCs express the EP(2) receptor for PGE2. Human MCs are a potent source of vascular endothelial growth factor (VEGF) through activation of the EP(2) receptor (Abdel-Majid et al. 2004). MCs have recently been shown to express a platelet-activating factor (PAF) receptor (Kajiwara et al. 2010). PAF induces histamine release from human lung MCs, a mechanism which provides an amplification loop for MC activation in the generation of anaphylaxis. PAF receptor was not found in skin MCs. This receptor is responsible for a rapid PAF-induced MC degranulation.

MCs also express receptors for the purine nucleoside adenosine. This molecule is produced in high concentration during tumour growth and it has been implicated in promoting angiogenesis. Interestingly, the human MC line HMC-1 expresses A2A, A2B and A3 adenosine receptors (Feoktistov et al. 2003). Selective stimulation of the A2B receptor increases VEGF and IL-8 secretion by HMC-1 MCs whilst the A3 receptor increases the expression of angiopoietin (ang)-2 mRNA. Thus, adenosine acts in a functional fashion to promote tumour angiogenesis by a cooperative paracrine mechanism involving A2B and A3 receptors on infiltrating MCs that, in turn, secrete angiogenic factors. Interestingly, selective release of VEGF by human MCs is regulated by corticotropin releasing hormone (CRH), which implies the presence of a CRH receptor on MC surface (Cao et al. 2006). In addition, PGE2 dose-dependently induces primary MCs to release the proangiogenic chemokine MCP-1 through engagement of EP(1) and EP(3) receptors (Nakayama et al. 2006). Remarkably, MCP-1 is detected by immunoelectron microscopy within MCs at a cytoplasmic location distinct from the secretory granules, which implies an exocytosis-independent mode of molecule extrusion.

As far as CRH receptor is concerned, two CRH receptor subtypes, CRH-R1 and CRH-R2, have actually been detected on subepithelial MCs in the human colon (Wallon and Söderholm 2009) and CRH-R1 has been found in cutaneous MCs from a patient with urticaria pigmentosa (Theoharides et al. 2009). These receptors may explain exacerbations of digestive and cutaneous symptoms in allergic and atopic subjects or may affect the course of ulcerative colitis and irritable bowel syndrome during persistent psychological stress, which implies CRH release from the hypothalamus. Blood-brain-barrier permeability and multiple sclerosis appear to worsen in response to acute stress that leads to the local release of CRH, which activates brain MCs to selectively release IL-6, IL-8 and VEGF. In addition, acute stress shortens the time of onset of experimental allergic encephalomyelitis (EAE) that does not develop in MC-deficient \( W/W^v \) mice or CRH\(-/-\) mice (Theoharides and Konstantinidou 2007).

MCs express adrenoreceptors on their surface. This is most important for the control of asthmatic symptoms. The human lung MC is a crucial effector cell in the mediation of asthma. Activation of MCs by allergens, and other insults, leads to the elaboration of a wide variety of autacoids that cause bronchoconstriction and promote inflammation. Of the drugs that are used to treat asthma, only bron-
chodilator β2-adrenoceptor agonists are effective at inhibiting the elaboration of mediators from MCs. Both short- and long-acting β2-adrenoceptor agonists are effective inhibitors of MCs. Human lung MCs express a homogeneous population of β2-adrenoceptors (Kay and Peachell 2005). The β2-adrenoceptors agonists isoprenaline and salbutamol inhibited anti-IgE-induced release of histamine, PGD$_2$ and LTC$_4$ from human peripheral blood-derived MCs in a dose-dependent manner whilst the selective β3-adrenoceptor agonist BRL-37344 failed to affect anti-IgE-induced histamine release and the β1-adrenoceptor antagonist atenolol did not have any effect (Wang and Lau 2006). B2-adrenoceptors in MCs act via G protein coupled with the receptor. In addition, MCs express cholinergic receptors. For instance, electrical vagal stimulation was observed to induce gastric mucosal MC degranulation in experimental animals. Expression of mRNA of nicotinic acetylcholine receptors-α4, -α7, and -β2 subunits were detected in mucosal-type murine BMMCs (Kageyama-Yahara et al. 2008). IgE-induced degranulation of these cells is negatively regulated via nicotinic acetylcholine receptors, in particular the nicotinic acetylcholine receptors-α7 subunit. Interestingly, MCs in atopic dermatitis but not in healthy skin showed nicotinic acetylcholine receptors-α3 and -α5 subunit immunoreactivity (Kindt et al. 2008). As for muscarinic acetylcholine receptors, a species difference exists in the cholinergic control of histamine release between human and rat airways. In human airways, muscarinic receptors most likely of the M1 subtype are involved in the inhibitory control of MC function, whereas such an inhibitory pathway does not exist in the rat trachea (Reinheimer et al. 2000).

MCs have increasingly been implicated in promoting innate immunity against pathogen invasion. In this setting, MC activation can be elicited by diverse mechanisms that include signalling through Toll-like receptors (TLRs), receptors for complement components and receptors for endogenous peptides. MCs also express adhesion molecules acting as parasite, bacterial and virus receptors (for instance the CD48) (Gilfillan and Tkaczyk 2006). Mammalian TLRs have essential roles in the direct recognition of infectious agents, initiating signalling through NF-kB leading to the initiation of both innate and adaptive immune responses (Leulier and Lemaitre 2008). Human MCs have been shown to express TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9 under certain conditions (Hofmann and Abraham 2009). TLR2, for instance, has been found to recognize and respond to several pathogen-associated molecular patterns, including peptidoglycan, lipoproteins, and lipotheicoic acid (Schwandner et al. 1999). MCs stimulated through TLRs express “surgical” cytokine responses consisting mainly of IL-4, IL-6, IL-8 and TNF-α secretion (Bachelet and Levi-Schaffer 2007). For instance, when TLR2 or TLR4 are activated, human MCs release TNF-α, IL-5, IL-10, and IL-13 which facilitate the recruitment of immune cells to the site of infection (Varadaradjalou et al. 2003). TLR2 and TLR4 activation stimulates production of IL-6 and TNF-α by cultured mouse MCs without affecting degranulation, arachidonic acid production, or calcium mobilization (Quiao et al. 2006).

MCs may also be activated by complement receptor (C3aR, C5aR CR2, CR4, C1qR) and release proinflammatory and chemotactic mediators (Marshall 2004; Gilfillan and Tkaczyk 2006). The complement system is a complex system of
proteins that interact in a proteolytic cascade, leading to pathogen clearance in the serum. It has a more ancient origin than acquired immunity. Besides expressing on their surface major histocompatibility complex (MHC) class I and class II molecules under certain conditions, MCs represent a rich source of co-stimulatory activity by expressing also molecules of the B7 family (ICOS-L, PD-L1, PD-L2, CD80, CD86), members of the TNF/TNF receptor families (OX40L, CD153, Fas, 4-1BB), CD28 and CD40 ligand (Nakae et al. 2006; Hershko and Rivera 2010). Recently, it has been recognized that mouse BMMCs or mouse peritoneal MCs constitutively express Notch1 and Notch2 proteins on the cell surface. It has also been shown that Delta-like 1 (Dll1)/Notch signalling induces the expression of MHC-II and upregulates the expression level of the co-stimulatory molecule OX40L on the surface of the MCs. Dll1/Notch signalling augments FceRI-mediated IL-4, IL-6, IL-13, and TNF production by murine BMMCs. Dll1-stimulated MHC-II+OX40Lhi murine BMMCs promote proliferation of naive CD4+ T cells and their differentiation into Th2 cells producing IL-4, IL-5, IL-10, and IL-13 (Nakano et al. 2009). During the in vitro ingestion of a pathogenic E. coli by human MCs, production of TNF-α, CC chemokine ligands (CCL-1/I-309, CCL-19/MIP (macrophage inflammatory protein)-3β, and CCL-18/MIP-4), the integrin CD49d, and CD80 are upregulated. Remarkably, coincubation of human MCs with E. coli downregulates FceRI expression and FceRI-mediated MC degranulation (Kulka et al. 2004).

MCs also express the urokinase plasminogen activator receptor (uPAR) for the urokinase plasminogen activator (uPA), which may be related to the specific proangiogenic function of MCs, urokinase being an enzyme involved in processes of tissue remodelling such as fibrinolysis, fibroblast and endothelial cell migration and local repair (Sillaber et al. 1997). uPA is a potent chemoattractant for MCs. The finding that angiogenic factors, such as fibroblast growth factor (FGF)-2, VEGF and platelet-derived endothelial cell growth factor (PD-ECGF) stimulate MC migration suggests that MCs would express surface receptors for these proangiogenic cytokines. Indeed, human lung MCs isolated ex vivo have been shown to express mRNA for VEGFR1 and VEGFR2 receptors (Detoraki et al. 2009). These receptors are present on the MC surface. VEGF-A(165), VEGF-B(167), VEGF-C, VEGF-D, and placental growth factor (PIGF) are able to induce MC chemotaxis. These chemoattractant effects are mediated by the activation of both VEGFR-1 and VEGFR-2. It has been recently demonstrated that mouse BMMCs express functional calcitonin gene-related peptide (CGRP)-1 receptors and that their activation causes mobilization of Ca2+ from intracellular stores and piecemeal release of mMCP-1. These findings support the hypothesis that the CGRP signalling from afferent nerves to MMCs in the gastrointestinal wall is receptor-mediated (Rychter et al. 2011).

Human MCs have recently been shown to express the death receptor TRAIL (TNF-related apoptosis inducing ligand), belonging to the TNF receptor superfamily, and the inhibitory receptors CD300a and Siglec-8 (Karra et al. 2009). These receptors might represent interesting selective targets for MC downregulation during allergic diseases, mastocytosis and other inflammatory diseases in which MCs have a central role. TRAIL is the only known functional death receptor on human MCs. Interestingly, its function is upregulated by IgE-dependent MC activation. The in-
hibitory receptors CD300a and Singlec-8 potently downregulate MC activation and survival in vitro and inhibit different IgE-mediated responses in vivo. Remarkably, human MCs express other inhibitory receptors, such as the FcγRIIb receptor which binds IgG complexes and the leukocyte-associated immunoglobulin-like receptor (LAIR)-1 which binds collagen (Lebbink et al. 2006).

2.2.5 Mast Cell Activation

The cross-linking of IgE with bivalent or multivalent antigen results in the aggregation of FcεRI, which is sufficient for initiating downstream signal transduction events that activate cell degranulation as well as the de novo synthesis and secretion of lipid mediators and cytokines (Blank and Rivera 2004). IgE and its receptors are believed to have evolved as a mechanism for protection against parasites. Studies in man demonstrate that the serum presence of antigen-specific IgE correlates with acquired immunity toward certain parasitic helminth infections (Rihet et al. 1991). Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with Schistosoma mansoni (King et al. 1997). So far, IgE as well as IgG have been found exclusively in mammals. Classical IgE-mediated MC activation leads to immediate extrusion of granule-associated substances, such as histamine and proteases. Within minutes, they generate lipid-derived mediators (Brody and Metcalfe 1998). Then, MCs activation is followed also within hours, by the de novo synthesis of numerous cytokines and chemokines (Galli et al. 2005a).

MCs may also be activated by “alternative”, IgE-independent, pathways such as aggregation of FcγRIII by IgG/antigen complexes, KIT and TLR mechanisms, exposure to chemokines, anaphylatoxins C3a and C5a, fragments of fibrinogen and fibronectin (Johnson et al. 1975; Wojtecka-Lukasik and Maslinski 1992; Prodeus et al. 1997; Gommerman et al. 2000; Marshall 2004; Fig. 2.11). Activation of MCs does not necessarily cause cell degranulation. As previously remarked, when cultured mouse MCs are activated by TLR2 and TLR4 stimulation, a production of IL-6 and TNF-α is determined which does not affect MC degranulation, arachidonic acid production, or calcium mobilization (Quiao et al. 2006). Notably, activation of MCs by TLRs leads to “surgical” release of mediators by MCs (McCurdy et al. 2003; Bachelet and Levi-Schaffer 2007). MCs may be activated by complement receptor (C3aR, C5aR CR2, CR4, C1qR) and release proinflammatory and chemo- tactic mediators (Marshall 2004; Gilfillan and Tkaczyk 2006). Of note, MCs can recognize, attach to, and internalize a wide variety of opsonized bacteria (Féger et al. 2002). For example, Salmonella typhimurium coated with the iC3b fragment of complement is recognized through CR3 on the MC membrane (Sher et al. 1979). In the cecal ligation and puncture model, it has been shown that mice deficient for the CR3b (CR3−/−) have impaired MC activation and neutrophil recruitment, associated with reduced bacterial clearance and survival (Rosenkranz et al. 1998). Thus, MCs exploit the CR3 moiety for effecting important activation programs. Human
MCs challenged to IFNγ express FcγRI at sufficient quantity to become activated for mediator release upon FcγRI aggregation (Okayama et al. 2000, 2001). This mechanism could be of relevance for IgE-independent allergic reactions as well as for nonallergic MC activation during type III hypersensitivity reactions or infections (Bischoff 2009). MCs have recently been shown to be activated through PAF receptor (Kajiwara et al. 2010). PAF induces histamine release from human lung MCs and peripheral blood-derived MCs but not skin MCs. Activation of PAF receptor-coupled Gαi leads to degranulation through phospholipase C-γ1 and phospholipase C-β2 activation in human MCs. PAF-induced degranulation is rapid, being maximal at 5 seconds, and is partially dependent on extracellular Ca²⁺. These findings provide a mechanism whereby PAF mediates an amplification loop for MC

Fig. 2.11 Mast cells express on the cell surface a series of receptors which mediate either cell activation (on the right) or cell inhibition (on the left). Activation factors promote stimulation of cell metabolism and secretion from mast cells of pre-stored and/or newly-formed molecules. The FcεRI, which mediates interaction with IgE, and the KIT receptor for stem cell factor are the most important activation receptors. Mast cells activation may be promoted by a number of “alternative” pathways, such as neuropeptide or complement stimulation pathways as well as activation through Toll-like receptor mechanisms. The list of inhibitors includes IgG complexes which bind to the FcγRIIb receptor, collagen which binds to the leukocyte-associated immunoglobulin-like receptor (LAIR)-1, the anti-inflammatory cytokines TGFβ-1 and IL-10, and several other molecules such as retinol, β2-adrenoreceptor agonists, and extracellular matrix proteins binding to CD63. Human mast cells express the death receptor TRAIL and the inhibitory receptors CD300a and Siglec-8. Treg cells directly inhibit the FcεRI-dependent mast cell degranulation through cell-cell contact involving OX40-OX40L interactions.

MCs challenged to IFNγ express FcγRI at sufficient quantity to become activated for mediator release upon FcγRI aggregation (Okayama et al. 2000, 2001). This mechanism could be of relevance for IgE-independent allergic reactions as well as for nonallergic MC activation during type III hypersensitivity reactions or infections (Bischoff 2009). MCs have recently been shown to be activated through PAF receptor (Kajiwara et al. 2010). PAF induces histamine release from human lung MCs and peripheral blood-derived MCs but not skin MCs. Activation of PAF receptor-coupled Gαi leads to degranulation through phospholipase C-γ1 and phospholipase C-β2 activation in human MCs. PAF-induced degranulation is rapid, being maximal at 5 seconds, and is partially dependent on extracellular Ca²⁺. These findings provide a mechanism whereby PAF mediates an amplification loop for MC
activation in the generation of anaphylaxis. Another IgE-independent mode of MC activation is mediated by IL-33. IL-33 is a recently identified member of the IL-1 family of molecules, which also includes IL-1 and IL-18. IL-33 binds to the receptor, T1/ST2/IL-1R4. IL-33, like IL-1β, can induce cytokine production in human MCs even in the absence of stimuli of FcεRI aggregation (Iikura et al. 2007). These findings support the hypothesis that IL-33 may enhance MC function in allergic disorders and other settings, either in the presence or absence of co-stimulation of MCs via IgE/antigen-FcεRI signals. In the mouse, IL-33 induces IL-13 production by MCs independently of IgE-FcεRI signals (Ho et al. 2007).

As previously discussed, preformed substances stored in MC secretory granules can be released by two morphologically distinct secretory pathways, referred to as anaphylactic degranulation and piecemeal degranulation (Dvorak 2005b). Vesicle-mediated degranulation in MCs is effected by IgE-independent activation mechanisms (Theoharides et al. 2007). Instead of utilizing the high-affinity IgE receptor, FcεRI, MCs exploit such receptors as low-affinity IgG receptors FcγRIIa and FcγRIII, complement receptors (C3aR, C5aR CR2, CR4, C1qR), and Toll-like receptors (TLR-2, -3, -4, -7 and -9) (Féger et al. 2002; Marshall 2004; Gilfillan and Tkaczyk 2006). Activated MCs through these receptors selectively release mediators capable of modulating neighbouring cell functions in a manner tailored to the stimulus. It has been recently demonstrated that murine BMMCs express functional CGRP-1 receptors and that their activation causes mobilization of Ca²⁺ from intracellular stores and piecemeal release of mMCP-1. These findings support the hypothesis that the CGRP signalling from afferent nerves to MMCs in the gastrointestinal wall is receptor-mediated (Rychter et al. 2011).

Polyamines mediate numerous cellular and physiological functions and recently these substances have been implicated in MC activation. MCs express antizyme inhibitor 2 (AZIN2), an activator of polyamine biosynthesis (Kanerva et al. 2009). Immunostainings show that AZIN2 is expressed in primary and neoplastic human and rodent MCs. AZIN2 localizes in the Vamp-8 positive, serotonin-containing subset of MC granules, but not in tryptase-containing granules, as revealed by double immunofluorescence stainings. Furthermore, activation of MCs induces rapid up-regulation of AZIN2 expression and its redistribution, suggesting a role for AZIN2 in secretory granule exocytosis. Release of serotonin from activated MCs is polyamine-dependent whereas release of histamine and β-hexosaminidase is not, indicating a granule subtype-specific function for polyamines.

Activation of MCs leads to different patterns of biological response according to the anatomical site and the phenotypic profile of involved MCs. In comparison with MCs isolated from human skin, lung and heart, synovial MCs appear to exhibit particularly brisk responses to the neuropeptide substance P (Kopicky-Burd et al. 1988; de Paulis et al. 1996). Unlike cutaneous MCs, MCs from osteoarthritis synovium do not respond to anaphylatoxin C5a or morphine (Verbsky et al. 1996). In addition to histamine, synovial MCs obtained from osteoarthritis specimens are capable of elaborating approximately equivalent amounts of PGD₂ and LTC₄ when stimulated by IgE cross-linking, unlike MCs in the skin that synthesize PGD₂ preferentially (de
Paulis et al. 1996). Activation of cardiac MCs in vitro with anti-IgE or anti-FcεRI induces the release of preformed mediators, such as histamine, tryptase, chymase, and renin as well as the de novo synthesis of LTC₄ and PGD₂. C5a causes rapid release of histamine and tryptase from human cardiac MCs (Genovese et al. 2010). Remarkably, atrial natriuretic peptide is not a cardiac MC secretagogue whereas it induces significant histamine release from peritoneal MCs (Murray et al. 2007). An important link has been identified in the heart between sensory nerves and renin-containing MCs. Substance P released from sensory nerves plays a significant role in the release of MC renin in ischemia/reperfusion and in the activation of a local cardiac renin-angiotensin system (Morrey et al. 2010). This culminates in angiotensin production, noradrenaline release, and arrhythmic cardiac dysfunction.

During the last few years, in vitro experiments and experiments in animal models have lead to the discovery that there are several inhibitory mechanisms operating in MCs which might counterbalance the effects of activating mediators. The list

---

**Fig. 2.12** The broad spectrum of mast cell activities may roughly be summarized into two main categories, that is immunological and non-immunological functions. Immunological functions entailing substantial mast cell contribution are (1) the innate defence toward bacteria, protozoa and viruses, (2) the initiation and orchestration of acquired immune reactions, (3) polarization toward IgE-mediated responses, (4) the induction of immune tolerance and (5) the crucial support to autoimmunity. Non-immunological functions encompass an even greater spectrum of mast cell specializations, ranging from a substantial contribution to the mechanisms of (1) wound-healing, (2) tissue remodelling, (3) fibrosis and (4) angiogenesis, to the relevant participation to physiological and pathological events, such as (5) tissue development, (6) altered adipose tissue metabolism, (7) atherosclerosis and (8) neuroprotection and neurogenic inflammation.
of inhibitors includes ligands of immunoreceptor tyrosine-based inhibition motif-containing receptors such as FcγRIIB, gp49B1, SIRPa, the human analogs LIR-5, and LILR B4 as well as the anti-inflammatory cytokines TGFβ-1 and IL-10, CD200, intracellular signal molecules like the transmembrane adaptor non-T cell activation linker (NTAL) or RabGEF1, and several other molecules such as retinol, β2-adrenoreceptor agonists, and extracellular matrix proteins binding to CD63 (Bischoff 2007).

Release of preformed and newly formed MC products from activated MCs leads to a series of profound biological effects. For the sake of clearness, the effects of MC activation may be conceptualized into two partly overlapping categories, i.e., immunological and non-immunological functions (Fig. 2.12).

2.2.6 Mast Cell Immunological Functions

MCs have increasingly been recognized as crucial effectors in both innate and adaptive immune responses. They promote immune responses against a large spectrum of pathogens. Their pivotal role in allergic disorders is well-known. In addition, these cells exert a critical role in orchestrating efficient immune responses as well as detrimental immunological functions such as autoimmunity. Thus, they are now considered to be a “linker” between innate and acquired immunity (Galli et al. 2005b).

2.2.6.1 Mast Cells and Innate Immune Responses

MCs are sentinel cells of the innate immunity (Fig. 2.13). This concept mostly derives from studies using MC-deficient mice. In these experimental settings, MCs have been shown to protect against bacteria, fungi, protozoa and perhaps even viruses through the release of proinflammatory and chemotactic mediators (Féger et al. 2002). Thus MCs have come to the forefront as triggers of innate immune responses against pathogens. Their preferential location to the lining surfaces of the body makes these cells an ideal first responder during microbial attack. Several recent reports indicate, indeed, that human and mouse MCs can mediate a variety of antimicrobial effects following activation upon contacts with pathogens. Although the physiological relevance of the phagocytic activity exerted by MCs remains undetermined, human and mouse MCs are capable to eliminate bacteria through an intracellular killing system—partly oxidative, partly nonoxydative—similar to that of professional phagocytes (Féger et al. 2002). MCs possess CR3 and FcyR and therefore have the capacity to recognize pathogens that have been osponized by either complement or IgG. For instance, MCs are able to recognize and kill the bacterium Salmonella typhimurium coated with the C3b fragment of complement and other IgG-coated bacteria (Sher et al. 1979; Talkington and Nickell 2001). These bacteria are then endocytosed via an endosome lysosome pathway. Remark-
ably, MCs have also the innate capacity to recognize pathogens in the absence of opsonins through TLR or mannose receptor (CD48) (Marshall 2004). Recent data suggest that MCs may exert bactericidal activity by an extracellular phagocytosis-independent mechanism similar to the neutrophil extracellular traps, whose major components are tryptase and the cathelicidin antimicrobial peptide LL-37. In addition, mast cells are a rich source of early-response cytokines, such as TNF-α and IL-4, that can rapidly recruit inflammatory cells at the site of pathogen entry. Other mast cell-derived products, such as leukotriene B₄, tryptase and chemokines such as CCL3, CCL5 and CXCL8 contribute to the influx of neutrophils. Mast cells may decrease neurotensin-induced hypotension as well as sepsis-related mortality by degrading neurotensin through the protease neurolysin. Mast cells also release vasoactive mediators, such as histamine, heparin, leukotriene C₄ and chymase, which induce vascular permeability. Epithelium permeability and secretion at the site of bacterial entry is stimulated by mast cell products such as histamine, leukotriene C₄ and prostaglandin D₂.

Fig. 2.13 Mast cells are sentinel cells of the innate immunity. Human and mouse mast cells are capable to eliminate bacteria through an intracellular killing system. Mast cells possess CR3 and FcγR and therefore have the capacity to recognize pathogens that have been opsonized by either complement or IgG. Mast cells have also the innate capacity to recognize pathogens through TLR or mannose receptor (CD48). Mast cells may exert bactericidal activity by an extracellular phagocytosis-independent mechanism similar to the neutrophil extracellular traps, whose major components are tryptase and the cathelicidin antimicrobial peptide LL-37. In addition, mast cells are a rich source of early-response cytokines, such as TNF-α and IL-4, that can rapidly recruit inflammatory cells at the site of pathogen entry. Other mast cell-derived products, such as leukotriene B₄, tryptase and chemokines such as CCL3, CCL5 and CXCL8 contribute to the influx of neutrophils. Mast cells may decrease neurotensin-induced hypotension as well as sepsis-related mortality by degrading neurotensin through the protease neurolysin. Mast cells also release vasoactive mediators, such as histamine, heparin, leukotriene C₄ and chymase, which induce vascular permeability. Epithelium permeability and secretion at the site of bacterial entry is stimulated by mast cell products such as histamine, leukotriene C₄ and prostaglandin D₂.
elicited by skin MCs in the mice has been found to be of critical importance for the induction and development of cutaneous granuloma formation, which is crucial for the successful containment and elimination of many intracellular pathogens (von Stebut et al. 2003).

Besides their potential capacity to clear bacteria through endocytotic pathway, MCs are decisive in initiating the immune and inflammatory responses of the host to the invading pathogens. MCs, indeed, are a rich source of early-response cytokines, such as TNF-α and IL-4, that can rapidly recruit inflammatory cells at the site of pathogen entry (Galli et al. 2005a). TNF-α in particular, is a pivotal molecule in the defensive mechanisms initiated by MCs. MCs release TNF-α stored in secretory granules after incubation with bacteria both in vitro and in vivo (Echternacher et al. 1996; Malaviya et al. 1996). In mutant $W/W^v$ mice, the absence of MCs leads to a defective innate immune response against bacteria. In a model of acute septic peritonitis by cecal ligation and puncture, $W/W^v$ mice exhibited a dramatically increased mortality compared with the wild-type mice (Echternacher et al. 1996). Remarkably, the adoptive transfer of MCs to the peritoneum protected the MC-deficient mice from the lethal effects of cecal ligation and puncture. Similarly, MC-deficient $W/W^v$ mice are less protected against experimentally induced lung enterobacterial infections than MC-sufficient or MC-reconstructed $W/W^v$ mice (Malaviya et al. 1996). The impaired killing of bacteria in MC-deficient mice was directly correlated with reduced neutrophil infiltration in lungs, partly as a result of lower levels of the MC-derived chemotactic TNF-α in these mice. Indeed, TNF-α-deficient mice have increased mortality in the cecal ligation and puncture model compared with wild-type mice (Maurer et al. 1998). Other MC-stored substances are endowed with the capacity to generate important defensive responses. Recent evidence indicate that mMCP-2, a mouse MC serine protease of the chymase type, can contribute to neutrophil recruitment and host survival during cecal ligation and puncture in mice (Orinska et al. 2007). Interestingly, this protease is inhibited by IL-15, which is constitutively expressed and can be induced in MCs themselves (Orinska et al. 2007). In a mouse model of sepsis, it has been shown that MCs may decrease neurotensin-induced hypotension as well as sepsis-related mortality by degrading neurotensin through the protease neurolysin (Piliponsky et al. 2008). Remarkably, other MC-derived products, such as LTβ₄, human tryptase βI, MIP-1α (CCL3), MIP-1β, MIP-2, MCP-1, RANTES (regulated upon activation, normal T-cell expressed and secreted) (CCL5), and IL-8 (CXCL8) appear also to contribute to the influx of neutrophils induced by activated MCs (Yamazaki et al. 1998; Féger et al. 2002).

In some experimental settings, MCs have been shown to exert detrimental effects to the host during bacterial infections by excessive or inappropriate release of inflammatory mediators leading to harmful outcome. For instance, there are indications that Shiga toxin produced by *Shigella dysenteriae* may stimulate intestinal MCs to release excessive amounts of inflammatory mediators derived from arachidonic acid metabolism, in particular LTC₄, leading to deleterious effects such as diarrhea and dysentery (Pulimood et al. 1998). MCs may also trigger inflammation in *Helicobacter pylori* infection, as MC accumulation in the mucosa of patients with
gastritis and MC degranulation by *H. pylori* products have been described (Masini et al. 1994; Plebani et al. 1994; Nakajima et al. 1997). In addition, although MCs are capable to phagocytose and kill various opsonised bacteria, this capacity may be subverted by microbes endocytosed in nonopsonic conditions (Shin et al. 2000). In these cases, the internalized pathogen is sequestered in a MC endosomal compartment that escapes acidification and oxygen radicals entry. The net result is that the bacteria are not killed by MC but remain in the MC cytoplasm as an intracellular reservoir (Féger et al. 2002)

### 2.2.6.2 Mast Cells and Adaptive Immune Responses

In addition to activating the innate immune system during infections, MCs have recently been recognized to exert a profound role in adaptive immunity (Galli et al. 2005b; Sayed and Brown 2007; Frossi et al. 2010) (Fig. 2.14). These cells, indeed, have been shown to be capable to influence the outcome of both physiological and pathological T cell responses. MC involvement in adaptive immune responses is broad: (1) they coordinate adaptive immune responses to pathogens; (2) contribute to the initiation of the primary immune responses to allergens; (3) amplify exacerbations of allergic diseases; (4) exert important role in generating immune tolerance; (5) primarily affect certain autoimmune diseases.

MCs may contribute to optimal initiation of acquired immunity by orchestrating migration, maturation and function of dendritic cells and by interacting with T and B cells (Hershko and Rivera 2010). MCs promote dendritic cell migration mainly by releasing prestored TNF-α at the site of infection. TNF-α, in turn, facilitates migration of dendritic cells to lymph nodes through upregulation of CCR7, a dendritic cell chemokine important for dendritic cell homing to lymph nodes (Yamazaki et al. 1998; Suto et al. 2006). TNF-α also induces maturation of dendritic cells in so far as they are induced to express MHC class II moieties and co-stimulatory molecules thereby becoming an antigen presenting cell for T cells (Ritter et al. 2003). MC-derived TNF-α drained at distance to local lymph nodes also express the capacity to retain lymphocytes circulating from the blood in the nodes (Young et al. 2000). For instance, it has been reported that TNF-α released from MCs contributes substantially to T cell recruitment to the draining lymph nodes in an experimental infection model with *Escherichia coli* (McLachlan et al. 2003). In addition, TNF-α released by MCs upon FcεRI engagement can cause T cell proliferation and cytokine production (Nakae et al. 2005, 2006). MCs may recruit effector T cells also through secretion of histamine, chemokines and LTB₄ (Ott et al. 2003; Demeure et al. 2005; Maurer et al. 2006; Jawdat et al. 2006). MCs may also influence the polarity of T cell responses. Activated MCs release Th2 polarizing cytokines, such as IL-4, IL-10 and IL-13, which induce stimulated naïve CD4⁺ cells to become Th2 cells once activated in the lymph node. These Th2 cells induce strong humoral immune responses that are protective against pathogens (Stelekati et al. 2007; McLachlan et al. 2008). Upon TLR3 engagement, MCs may also express important regula-tory
functions for CD8+ T cell activities both in vivo and in vitro (Orinska et al. 2005). Under certain conditions, MCs can directly activate T cells by functioning as antigen presenting cells (Frandji et al. 1996). MCs express MHC class I and MHC class II in limited circumstances. Malaviya et al. (1996) demonstrated that association of bacterial antigens and MHC class I molecules on the surface of MCs may induce CD8+ T cell responses to pathogens. Recently, Kambayashi et al. (2009) provided evidence for expression of MHC class II on the surface of murine BMDCs as well as murine peritoneal MCs when activated with lipopolysaccharide (LPS) and IFN-γ. Remarkably, MHC class II-expressing MCs seemed to travel from the activation site to regional lymph nodes like dendritic cells. These MCs were also seen to ex-
press CD80 and CD86 co-stimulatory molecules. Such MHC class II-bearing MCs were demonstrated to activate T cells with preferential expansion of antigen-specific regulatory T cells (Tregs) over naïve CD4+ T cells. In addition, Nakano et al. (2009) demonstrated that Notch signalling induce expression of MHC class II along with the co-stimulatory molecule OX40L on the surface of murine BMMCs. These MHC class II, OX40L BMMCs are able to promote proliferation of naïve CD4+ T cells into Th2 cells in vitro. Moreover, treatment of peritoneal cell-derived MCs with INF-γ and IL-4 was shown to induce expression of MHC class II molecules on MCs (Gaudenzio et al. 2009). These MCs were able to present antigen to effector T cells causing their activation, proliferation, and formation of an immunological synapse between the MC and the T cell. Very recently, the antigen-presenting function has been shown to be restricted to a subset of three-week old pure BMMCs expressing both high levels of surface FcεRI and surface MHC class II (Gong et al. 2010).

Collectively, studies indicate that MCs represent a rich source of co-stimulatory activity by expressing also molecules of the B7 family (ICOS-L, PD-L1, PD-L2, CD80, CD86), members of the TNF/TNF receptor families (OX40L, CD153, Fas, 4-1BB), CD28 and CD40 ligand (Nakae et al. 2006; Hershko and Rivera 2010). Evidence for a role of histamine in modulating T cell proliferation via H1 receptor stimulation is suggested by an in vitro study where co-cultures of MCs with helper T cells were seen to cause either increased or decreased T cell proliferation when low or high numbers of MCs, respectively, were challenged with helper T cells (Khan et al. 1986). MCs have recently demonstrated to regulate B cell survival and activation. Coculture assays using mouse splenic B cells and BMMCs revealed that both nonsensitized and activated MCs proved able to induce a significant inhibition of cell death and an increase in proliferation of naïve B cells (Merluzzi et al. 2010). Such proliferation was further enhanced in activated B cells. This effect relied on cell-cell contact and MC-derived IL-6. Activated MCs could regulate CD40 surface expression on unstimulated B cells, and the interaction between CD40 and CD40L on MCs, together with MC-derived cytokines, was involved in the differentiation of B cells into CD138+ plasma cells and in selective IgA secretion. These data were corroborated by in vivo evidence of infiltrating MCs in close contact with IgA-expressing plasma cells within inflamed tissues.

MCs are key effector cells in initiating and/or amplifying IgE-dependent inflammatory reactions, including allergic disorders and certain protective immune responses to parasites (Gurish and Austen 2001; Galli et al. 2005a, 2008b). As a result, they have mainly been regarded in the past for their detrimental role in type I allergic reactions, such as anaphylaxis, hay fever, eczema or asthma. In addition, they also express immunoregulatory functions in the same settings (Gribaldeston et al. 2006; Galli et al. 2008a; Rauter et al. 2008). MCs are activated during IgE-associated anaphylaxis. Anaphylaxis is an acute-onset, potentially fatal systemic allergic reaction that can be triggered by immunological or non-immunological mechanisms (Estelle et al. 2008). IgE play a crucial role in the immediate hypersensitivity response through binding with the high-affinity receptor FcεRI but other IgE-independent mechanisms, such as G protein-coupled receptor and TLR activation processes may intervene (Marshall 2004; Vines and Prossnitz 2004). Activated
MCs, along with basophils, release Th2 cytokines (IL-4, IL-5, IL-9 and IL-13) that polarize the immune reaction, and produce various bioactive chemical mediators, such as histamine and lipid metabolites, that provide vasoactive, chemotactic and immunoregulatory functions. In addition to their roles in classic acute IgE-associated immediate hypersensitivity responses, several lines of evidence indicate that MCs can also contribute to late-phase and chronic allergic reactions (Holgate 2002; Galli et al. 2008b). MCs have been shown to change their degranulation pattern from acute to chronic allergic responses (Theoharides et al. 2007). Anaphylactic degranulation is triggered by IgE-dependent and neuropeptide activation mainly during the early phase of allergic reactions. Differential release without degranulation is activated by mediators such as IL-1 (in humans), SCF (in mice), LPS (in rats) and CRH (in humans), and classically occurs during chronic inflammatory diseases (Cao et al. 2005; Theoharides et al. 2007). Many clinical symptoms of IgE-dependent late-phase reactions, both in the respiratory tract, gastrostintestinal tract and the skin, reflect the actions of the leukocytes recruited to these sites by MCs. Cytokines (TNF-α, IL-6, IL-8) and neutral proteases, as well as histamine and lipid mediators, may contribute to MC-dependent leukocyte recruitment—in particular eosinophil recruitment—in such settings (Puxeddu et al. 2005). Leukocytes, in turn, expand the inflammatory reaction by providing additional pro-inflammatory mediators and cytokines (“MC-leukocyte cytokine cascade”). MC cytokines, such as TNF-α, VEGF, FGF-2 and TGF-β, contribute to chronic allergic inflammation through effects on fibroblasts, vascular endothelial cells, and other cells resident at the sites of these reactions (Galli et al. 2008b). Persistent chronic allergic inflammation can result in remodelling of the affected tissues and these structural changes are often associated with activation of the angiogenic process.

Very recently, MCs have been associated with a new type of immune function that is the induction of immune tolerance. Participation of MCs to this kind of activity explains certain aspects of MC involvement in the dynamics of tumour development and progression. Although immune surveillance works at an early stage of tumorigenesis, the established tumours primarily induce immune tolerance, by creating sites of immune privilege and by inducing the shift of the immune balance from activation to tolerance (Pardoll 2003; Munn and Mellor 2006). In this perspective, MCs have recently been proposed to be mechanistically involved in the negative modulation of immune surveillance in the tumour microenvironment. MCs, indeed, can promote suppression of immune reactions not only by producing inhibitory cytokines, such as IL-10, but also by promoting the immune tolerance mediated by Treg cells (Ullrich et al. 2007; Grimbaldeston et al. 2007). Indeed, MCs serve as enforcers for Treg cells, turning down the immune system’s reaction to skin allograft possibly by IL-10 secretion (Lu et al. 2006; Grimbaldeston et al. 2007). Ultraviolet B irradiation, which represents the most important skin immunosuppressor and initiator of cutaneous malignancies, activates MCs (Kripke 1984; Ch’ng et al. 2006). Upon irradiation of the skin, trans-urocanic acid in the epidermis isomerizes to cis-urocanic acid, which stimulates substance P and CGRP release from neural C-fibres. These neuropeptides, in turn, trigger secretion of histamine, TNF-α and other mediators from MCs, leading to suppression of the cellular immune system.
Using the MC-deficient W/W<sup>v</sup> mice, a direct correlation was demonstrated between MC density in the dermis and susceptibility to ultraviolet-B-induced systemic immunosuppression (Hart et al. 2002). In a skin transplantation model of allograft tolerance in the mouse, MCs were crucial for graft acceptance as MC-deficient C57BL/6-Kit<sup>W<sub>sh</sub></sup>/W<sup>−</sup>sh mice showed inability to induce tolerance (Lu et al. 2006). Activated Treg cells in the tolerant tissue produced high levels of IL-9, a cytokine which seems important in MC recruitment, growth and activation. This cytokine appears a crucial factor in mediating regional tolerance because neutralization of IL-9 greatly accelerated allograft rejection in tolerant mice (Lu et al. 2006). In a mouse model of tumorigenesis, SCF-activated MCs exacerbated the immunosuppression in the tumour microenvironment (Huang et al. 2008). MCs were shown to promote the decrease of the immune activatory factor IL-2 mRNA in the tumour whereas inducing the increase of immune suppressory factors such as IL-10, TGF-β, and Foxp3 RNAs. A recent study suggests that MCs and Treg cells may cooperate with each other in hepatocellular carcinoma determining a poorer tumour evolution (Ju et al. 2009). Remarkably, the percentage of Treg cells was shown to increase because CD4<sup>+</sup>CD25<sup>+</sup> T cells can be converted into CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells by TGF-β expression of transcription factor Foxp3 (Chen et al. 2003). Indeed, in an ovalbumin peptide TCR transgenic adoptive transfer model, TGF-β-converted transgenic CD4<sup>+</sup>CD25<sup>+</sup> suppressor cells proliferated in response to immunization and inhibited antigen-specific naive CD4<sup>+</sup> T cell expansion in vivo. In addition, in a murine asthma model, coadministration of these TGF-β-induced suppressor T cells has been shown to prevent house dust mite-induced allergic pathogenesis in lungs (Chen et al. 2003). Another mechanism of potential physiological and pathological significance in MC-driven Treg cell expansion might be linked to the LPS- and IFN-γ-induced expression of MHC class II on MCs. As previously reported, the expression of MHC class II grants MCs the ability to process and present antigens directly to T cells. Remarkably, MCs preferentially expand antigen-specific Treg cells over naïve T cells, a fact that may explain one of the mechanisms that governs allograft tolerance induction by MCs (Kambayashi et al. 2009). MCs, are poor stimulators of naïve T cells in vitro, however, the role for MHC class II expression on MCs may be to activate regulatory Treg cells and dampen the immune response or avoid self-reactivity. Activation of Treg cells by MCs may contribute to the protective effect of MCs on skin allografts (Lu et al. 2006; Kambayashi et al. 2009). Conversely, MCs are also able to counteract Treg-mediated suppression through IL-6 secretion and OX40/OX40L interaction (Piconese et al. 2009). This reversal Treg suppression leads to the establishment of Th17-mediated inflammatory responses. The cross-talk between Treg cells and MCs is bidirectional. Treg cells, indeed, have been shown to influence the immediate hypersensitivity response MCs. Treg cells directly inhibit the FcεRI-dependent MC degranulation through cell-cell contact involving OX40-OX40L interactions between Treg cells and MCs, respectively (Gri et al. 2008).

Finally, some words should be devoted to the role of MCs in autoimmunity. Several lines of evidence indicate that MC may play a role in autoimmunity, affecting disorders like arthritis, multiple sclerosis, bullous pemphigoid and Graves’ ophthalmia. MCs help initiate rheumatoid arthritis (Lee et al. 2002). W/W<sup>v</sup> mice lacking
MC do not develop the rodent equivalent of this debilitating condition. In humans, an increased number of MCs are found in the synovial tissues and fluids of patients with rheumatoid arthritis and at the site of cartilage erosion, reflecting the presence of MC chemotactic or survival factors, such as SCF and TGF-β, in the synovial fluid (Olsson et al. 2001). The invading MCs show ultrastructural signs of cell degranulation and produce several inflammatory mediators, notably TNF-α, IL-1β and VEGF. TNF-α reportedly plays a pivotal role in the pathogenesis of rheumatoid arthritis, especially in its ability to regulate IL-1β expression, this being important for the induction of prostanoid and matrix metalloproteinase (MMP) production by synovial fibroblasts and chondrocytes. In addition, MCs promote leakage of fluids into the joints, which in turn allows penetration of self-targeted antibodies that might lead to tissue damage by activating the complement cascade (Nigrovic and Lee 2007).

Growing evidence suggests that MCs play a crucial role in the inflammatory process and subsequent demyelination observed in patients suffering from multiple sclerosis. Indeed, recent results from animal models with experimental autoimmune encephalomyelitis clearly indicate that these cells act at multiple levels to influence both the induction and the severity of the disease, possibly by enhancing Th1 cell response through secretion of IL-4 (Gregory et al. 2006; Christy and Brown 2007). Bullous pemphigoid is another human disease whereby MCs have been proposed to exert a relevant pathogenic role. This autoimmune skin disease is characterized by subepidermal blisters resulting from auto-antibodies against two hemidesmosomal antigens, BP230 and BP180. Intradermal injection of antibodies against BP180 into neonatal mice causes a blistering disease mimicking bullous pemphigoid. Injection of antibodies against BP180 into MC-lacking \( W/W^v \) mice does not induce bullous pemphigoid, nor does the injection into wild-type mice pre-treated with the MC stabilizer cromolyn sodium induce it (Chen et al. 2002). Interstitial cystitis has gained increasing attention for an involvement of MC in its pathogenesis. Indeed, the presence of activated MC in close proximity to suburothelial nerves is a consistent feature of this yet-to-be-clarified urological pathology (Elbadawi 1997).

In conclusion, the picture sketched in this paragraph underlines the pivotal role played by MCs in different immunological contexts and experimental settings. These cells are increasingly being recognized as key elements in orchestrating complex defensive and immune reactions which usually protect the host but, sometimes, may turn out to be dangerous or even lethal. In this perspective, the importance of MCs as initiators and effectors of both innate and adaptive immunity in healthy individuals has recently been appreciated insofar as MC activation can be used as an adjuvant to promote Ag-specific humoral immune responses upon vaccination (Fang et al. 2010).

### 2.2.7 Mast Cell Non-immunological Functions

MCs play an important role not only in immediate hypersensitivity and late phase inflammation but also in tissue remodelling in different organs. Evidence indeed has been accumulated that MCs may exert a series of relevant non-immunological
functions, which couple in a strict temporal sequence inflammatory and healing processes such as tissue homeostasis, repair, remodelling, and fibrosis (Fig. 2.15). The prototype of these in vivo experimental models is skin wound healing. Cutaneous wound healing is characterized by three sequential phases: (1) inflammation, as a direct consequence of wounding, (2) proliferation and (3) remodelling. Involvement of MCs in the various steps of cutaneous wound healing has long been recognized (Dvorak and Kissel 1991; Gruber et al. 1997; Metcalfe et al. 1997; Artuc et al. 1999, 2002). During the early phase of wound healing, MCs contribute to local coagulation, extravasation and leukocyte recruitment through secretion of histamine, TNF-α and other mediators. Proliferation of fibroblasts, endothelial cells and keratinocytes is sustained by MC products such as FGF-2, VEGF, TGF-β, histamine and tryptase. In particular, MCs can promote the conversion of fibroblasts into myofibroblasts, which facilitates wound contraction. The final step, tissue remodelling,
is assisted by proteolytic, extracellular matrix-degrading enzymes such as MMPs produced by MCs. Formal demonstration of the essential role of MCs in the process of wound healing has been provided only recently using MC-deficient mice (Weller et al. 2006). In this study, experimentally induced skin wounds showed impaired closure in MC-deficient W/Wv mice. In addition, W/Wv mice showed diminished extravasation and recruitment of neutrophils to the wound areas. All these parameters were restored in MC-reconstituted W/Wv mice. Remarkably, secretion of histamine, through H1 receptors, and TGF-α is essential for MC-mediated effective wound healing. Recent reports point to a protective role of MCs in murine anti-glomerular basement membrane glomerulonephritis (Hochegger et al. 2005; Kanamaru et al. 2006). These findings have been explained either in the light of the ability of MCs to engender repair mechanisms or by an immunomodulatory effect of MCs in the inflammatory setting. Although the interpretation of involved mechanisms may be different, the net result can be reconciled with the crucial role of MCs in maintaining tissue homeostasis. Conversely, MCs play a relevant role in airway remodelling and in the maintenance of airway hyperresponsiveness in asthmatic subjects. The extent of airway remodelling correlates with severity of asthma. The infiltration of the bronchial wall by MCs is associated with the disordered airway function. The increase in airway smooth muscle mass is recognized as one of the most important factors related to persistent airway hyperresponsiveness and to the severity of asthma. MC mediators such as tryptase, chymase, activin A, TNF-α, PDGF, TGF-β, plasminogen activator inhibitor (PAI) and amphiregulin can contribute to smooth muscle cell and goblet cell hyperplasia, thickening of the reticular basement membrane and angiogenesis (Okayama et al. 2007). MCs are found to contribute to the development of multiple features of chronic asthma in MC-deficient mice.

MCs have been closely linked with the development of fibrosis in several organ sites. Intestinal strictures associated with Crohn’s disease have been shown to be densely populated by chymase-positive MCs. MC-derived chymase has been suggested to play a crucial role in fibrosis production by interfering with the local angiotensin II system (Suekane et al. 2010). In addition, chymase-expressing MCs are also prominent in scarring lung diseases, like interstitial lung disease (Edwards et al. 2005). Another mechanism of fibrosis induction may be mediated by the PDGF-osteopontin axis. Expression of osteopontin in wound fibroblasts is elicited by PDGF secreted by MCs and macrophages (Mori et al. 2008). Heparin and related glycosaminoglycans secreted by MCs differentially regulate PDGF-induced lung fibroblast proliferation, chemotaxis and MMP activity (Sasaki et al. 2000). These effects, in turn, may have a key role in extracellular matrix remodelling in inflammatory lung disease.

MCs may intervene to stimulate tissue growth and differentiation in health subjects. A role for MCs in promoting development of parenchymal tissues has been proposed since early Ehrlich’s dissertation. In the last decade, MCs have increasingly been conceived as “feeding” and “nourishing” cells—a concept postulated by Paul Ehrlich—in a series of developmental settings. For instance, postnatal mammary gland branching morphogenesis in mice has been
recognized to depend on MC functional integrity (Lilla and Werb 2010). Genetic and pharmacological disruption of MC function in the mammary gland reveals that MCs are involved in rapid proliferation and normal duct branching during puberty. Remarkably, MC degranulation and serine protease activation are required for normal mammary gland development. It is known that tissue-type MC proteases are implicated in angiogenesis and extracellular matrix protein degradation either directly or by activation of other proteases. The normal complement of active granule proteases is a critical factor in sustaining the growth of mammary gland ductal epithelium indicating that MCs contribute to the complex regulation of cell proliferation in the growing mammary gland. MCs are found throughout the mammary stroma as well as concentrated around the invading terminal end buds, a distribution pattern already recognized by Ehrlich in the developing goat parotid. Indeed, he wrote: “In certain acinar glands (goat parotid), the pattern of MC accumulation [inside the organ] is not determined by the branching of the vascular system but the ramification of the gland excretory ducts” (Ehrlich 1878). As normal development and tumorigenesis are closely linked processes, the potential role of MCs in facilitating mammary gland neoplasia has recently been investigated. It has been found that plasma kallikrein, a potent activator of the plasminogen cascade of serine proteases, is localized to CTMCs in the mouse mammary gland (Lilla et al. 2009). Active plasma kallikrein regulated ductal epithelial cell apoptosis, adipocyte differentiation and stromal remodelling during mammary gland involution. As plasminogen cascade directs tumorigenesis in the mammary gland, an active participation of local MCs in the neoplastic development may be suggested.

A contribution of MCs to diet-induced obesity and diabetes has recently been suggested. It has been shown that white adipose tissue from obese humans and mice contains more MCs than white adipose tissue from their lean counterparts (Liu et al. 2009). In addition, MC-deficient mice or mice treated with MC stabilizers show reduced body weight gain, reduced levels of inflammatory cytokines, chemokines and proteases, such as IL-6, TNF-α, IFN-γ, MCP-1, MMP-9 and cathepsin S in serum and white adipose tissue along with improved glucose homeostasis and energy expenditure. Thus, MCs are believed to contribute to diet-induced obesity and glucose intolerance by promoting adipose tissue protease expression and angiogenesis, which may favour adipose tissue growth.

MC involvement in the pathogenesis of coronary spasm, cardiomyopathy, atherosclerosis and myocardial ischemia has recently been suggested. It has been shown that chymase cleaves angiotensin I to angiotensin II more effectively than the angiotensin-converting enzyme (Church and Levi-Schaffer 1997). Studies in the canine model of myocardial ischemia and reperfusion indicate a role for MC mediators in initiating the cytokine cascade which is ultimately responsible for neutrophil accumulation in the ischemic area. In addition, MCs have been claimed to play a crucial role for leading to the subsequent fibrotic process (Frangogiannis et al. 1998). By using C57BL/6-Kit-W-sh/W-sh mice crossed with atherosclerosis-prone mice deficient in low-density lipoprotein receptor, in vivo evidence has been obtained that MCs is implicated in the atherogenic process, as MC absence causes smaller atherosclerotic lesions with fewer inflammatory cell infiltrates (Sun et al. 2007a).
MC may also contribute to the pathogenesis of elastase-induced abdominal aortic aneurysms in mice, as C57BL/6-Kit\(^{W-sh/W-sh}\) mice fail to develop such aneurysms (Sun et al. 2007b).

It has been reported that MCs intervene in the mechanisms controlling intestinal permeability. In particular, it has recently been discovered that CRH acts on intestinal MCs causing an increase of mucosal permeability to horseradish peroxidase (Wallon et al. 2008). The increased permeability to horseradish peroxidase was abolished by the MC stabilizer, lodoxamide. In this study, electron microscopy showed transcellular passage of horseradish peroxidase through colonocytes. CRH receptor subtypes R1 and R2 were detected in the HMC-1 cell line and in lamina propria MCs in human colon, suggesting that CRH mediates transcellular uptake of horseradish peroxidase in human colonic mucosa via CRH receptor subtypes R1 and R2 on subepithelial MCs. CRH-induced macromolecular uptake in human colon mucosa may have implications for stress-related intestinal disorders.

MCs have long been identified in the central nervous system, whereby they have been implicated in the regulation of permeability of the blood-brain-barrier (Esposito et al. 2002). In addition, it has been suggested that MCs in the central nervous system may function as a gate to the hypothalamic-pituitary-adrenal axis, thus participating in the counter-regulation of inflammatory immune responses (Matsu- moto et al. 2001). Blood-brain-barrier permeability and multiple sclerosis appear to worsen in response to acute stress that leads to the local release of CRH, which activates brain MCs to selectively release IL-6, IL-8 and VEGF (Theoharides and Konstantinidou 2007). Recently, participation of MCs to blood vessel growth and differentiation in the rat nervous system during postnatal development has been proposed (Khalil et al. 2007). Here, MCs occur in two locations, namely the pia mater and the brain parenchyma. MC population in the pia reaches a maximum at postnatal day 11, and declines rapidly thereafter. In contrast, the thalamic MC population expands from postnatal day 8 to reach adult levels at postnatal day 30. Stereological analysis demonstrates that MCs home to blood vessels. Indeed, more than 96% of MCs are inside the blood-brain barrier, with ~90% contacting the blood vessel wall or its extracellular matrix. MCs express \(\alpha_4\) integrins which represents a potential mechanism for adhesion to the vascular wall. At all ages studied, MCs are preferentially located on large diameter vessels (>16 \(\mu\)m; possibly arteries), and contact only those maturing blood vessels that are ensheathed by astroglial processes. MCs not only home to large vessels but also maintain a preferential position at branch points, sites of vessel growth. This observation presents the possibility that MCs participate in and/or regulate vasculature growth or differentiation.

Numerous microanatomical and ultrastructural investigations over the past years have demonstrated the presence of close nerve-MC contacts in different organs (skin, intestine, peritoneum) of various species, including man (Stead et al. 1987, 1989; Crivellato et al. 1991). It has also been reported that electrical stimulation of nerve fibers causes degranulation of tissue MCs and that these effects are inhibited by atropine or prior treatment with capsaicin (Javed et al. 1992). For instance, electrical vagal stimulation was observed to induce gastric mucosal MC degranulation. These findings have prompted further studies on a possible func-
tional interaction between the peripheral nerve system and the tissue MC populations. It has subsequently been shown that several neurotransmitters and neuropeptides are able to modulate MC function and to induce granule release (Foreman and Jordan 1980). MCs in turn are able to stimulate nerve fibers through histamine release, thus amplifying the nerve-MC loop, or conversely decrease the local effects of nerve mediators by releasing neuropeptide-degrading proteases such as tryptase and chymase. In the skin, MCs have been found in close anatomical and functional association with sensory nerves, a condition which may explain neurogenic inflammation. During psychological stress the neuroendocrine system and peripheral sensory nerves are activated leading to release of mediators, such as neuropeptides, neuropeptides, CRH and α-melanocyte-stimulating hormone, which are capable of activating MCs (Harvima et al. 2010). On the other hand, MC mediators released, e.g. histamine, tryptase and NGF, can in turn excite and stimulate surrounding neuropeptide-containing C-fibers possibly resulting in feed-forward loop and potentiation of neurogenic inflammation. In these mechanisms, proinflammatory cytokines and chemokines are released from MCs. In chronic skin diseases, such as psoriasis, atopic dermatitis and palmoplantar pustulosis, the contacts between tryptase-positive MCs and sensory nerves are increased in number, which provides the morphological basis for increased MC-sensory nerve interaction in chronically inflamed skin. A key link between the neural tissue and MCs is NGF. The first evidence that MCs are receptive to NGF showed that exogenous administration of NGF in newborn rats induced a marked increase in the number and size of MCs in peripheral tissues (Aloe and Levi-Montalcini 1977). The effect of NGF on MCs includes stimulation of proliferation, differentiation, survival and mediator secretion. Furthermore, proliferation and differentiation of murine CTMCs has been shown to be dependent on the MC degranulation property of NGF (Matsuda et al. 1991). It has been ascertained that MCs, in turn, synthesize and release NGF (Nilsson et al. 1997). NGF appears to be increased in the circulation in a variety of inflammatory and autoimmune conditions; it most consistently appears to be elevated in the circulation of patients with multiple allergic diseases, including asthma (Bonini et al. 1996).

Very recently, a role of MCs in the bone metabolism has been suggested. In particular, MCs seem to be mechanistically involved in the formation of skeletal defects observable during chronic hyperparathyroidism, which is a common cause of metabolic bone disease. Bone biopsies from hyperparathyroid patients revealed an association between parathyroid bone disease and increased numbers of bone marrow MCs. In addition, mature MCs in rats were preferentially located at sites undergoing bone turnover, and the number of MCs at the bone-bone marrow interface was greatly increased following treatment with parathyroid hormone (Turner et al. 2010). Time-course studies revealed that mature MC redistribution from bone marrow to bone surfaces precedes and is associated with osteitis fibrosa, a hallmark of parathyroid bone disease. Importantly, mature MCs were not observed in the bone marrow of mice, a species that is resistant to the development of parathyroid hormone-induced bone marrow fibrosis. These findings suggest that the MCs may play a crucial role in metabolic bone disease.
Mast Cells and Tumours
from Biology to Clinic
Ribatti, D.; Crivellato, E.
2011, VIII, 142 p. 28 illus., 18 in color., Hardcover
ISBN: 978-94-007-1468-7