

# THE CONTROVERSIAL ROLE OF MAST CELLS IN TUMOR GROWTH

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## Abstract

Mast cells (MCs) were first described by Paul Ehrlich (*Beiträge zur Theorie und Praxis der Histologischen Färbung*, Thesis, Leipzig University, 1878). They have long been implicated in the pathogenesis of allergic reactions and protective responses to parasites. However, their functional role has been found to be complex and multifarious. MCs are also involved in various cell-mediated immune reactions and found in tissues from multiple disease sites, and as a component of the host reaction to bacteria, parasite, and even virus infections. They also participate in angiogenic and tissue repair processes after injury. The importance of a possible functional link between chronic inflammation and cancer has long been recognized. As most tumors contain inflammatory cell

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infiltrates, which often include plentiful MCs, a possible contribution of these cells to tumor development has emerged. In this review, general biology of mast cells, their development, anatomical distribution, and phenotype as well as their secretory products will first be discussed. The specific involvement of MCs in tumor biology and tumor fate will then be considered, with particular emphasis on their capacity to stimulate tumor growth by promoting angiogenesis and lymphangiogenesis. Finally, it is suggested that mast cells may serve as a novel therapeutic target for cancer treatment.

**Key Words:** Angiogenesis, Mast cells, Tumor progression. © 2009 Elsevier Inc.

## 1. INTRODUCTION

Mast cells (MCs) are versatile, tissue-homing secretory cells, which were first described by Ehrlich (1878) in his doctoral thesis (Crivellato *et al.*, 2003a). He named these cells Mastzellen because he believed that the intracellular granules, which appeared purple in color when stained with aniline blue dyes, contained phagocytosed materials or nutrients. MCs have long been implicated in pathogenesis of allergic reactions and certain protective responses to parasites. Their functional role, however, has been discovered to be increasingly complex and multifarious. MCs have been implicated in various cell-mediated immune reactions and are found in tissues from multiple disease sites, and as a component of the host reaction to bacteria, parasite, and even virus infections. They have also been shown to participate in angiogenic and tissue repair processes after injury (Galli *et al.*, 2005a,b).

The importance of a possible functional link between chronic inflammation and cancer has long been recognized. As most tumors contain inflammatory cell infiltrates, which often include plentiful MCs, the question as to the possible contribution of MCs to tumor development has emerged progressively. A major point linking MCs to cancer is the well-recognized capacity of these cells to synthesize and release potent angiogenic cytokines. Although some evidence suggests that MCs can promote tumorigenesis and tumor progression, there are some clinical sets as well as experimental tumor models in which MCs seem to have functions that favor the host. Thus, the involvement of MCs in tumor development is complex and far from being settled.

In this review, general biology of these cells, their development, anatomical distribution and phenotype as well as their secretory products will first be discussed. The specific involvement of MCs in tumor biology and tumor fate will then be considered, with particular emphasis on the capacity of these cells to stimulate tumor growth by promoting angiogenesis and lymphangiogenesis. The last section describes drugs that affect the MC number.

## 2. BIOLOGICAL FUNCTIONS OF MAST CELLS

MCs are multi-functional long-lived secretory cells, characterized by the content of numerous cytoplasmic granules storing histamine (also serotonin in rodents), proteoglycans, cytokines, and neutral serine proteases. MCs are phylogenetically old cells, which apparently occur in all species with blood circulation. In histological preparations, MCs usually appear as round or elongated cells with a diameter ranging between 8 and 20  $\mu\text{m}$ , depending on the organ examined. The single nucleus of a MC shows round or oval shape and the cytoplasm contains numerous secretory granules that stain metachromatically with thiazine dyes such as toluidine blue. On electron microscopy, the MC exhibits a nonsegmented monolobed nucleus with peripherally condensed chromatin. The cytoplasm contains a few mitochondria, short profiles of the rough endoplasmic reticulum, free ribosomes, and numerous membrane-bound, moderately electron-dense secretory granules with an average diameter of 1.5  $\mu\text{m}$  (Dvorak, 1991) (Fig. 4.1). Secretory granules may present different substructural patterns, such as

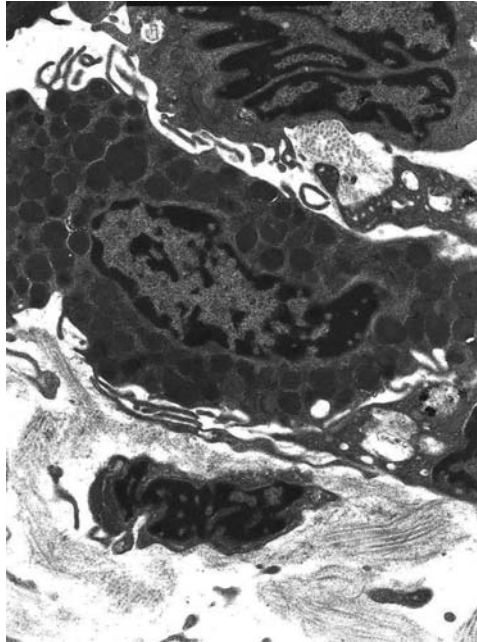


**Figure 4.1** Electron micrograph of a human normal mast cell in the skin. This cell presents an elongated shape and a cytoplasm filled with plentiful electron-dense granules with homogeneous structure. Original magnification:  $\times 6300$ .

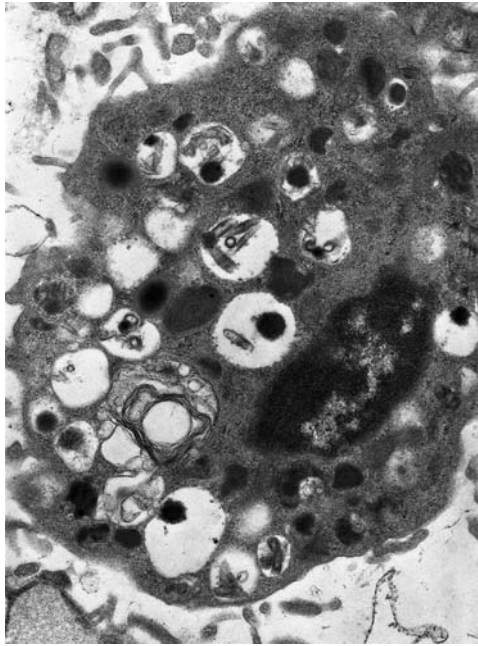


**Figure 4.2** Electron micrograph of human mast cell from the colon mucosa in a patient with inflammatory bowel disease. The cytoplasm contains numerous, partially emptied granules with residues of scroll material. This particulate, nonexocytotic secretory pattern is called piecemeal degranulation. Original magnification:  $\times 10,000$ .

homogeneous, crystalline, scroll, particle or thread-like pattern or a combination of them (Fig. 4.2). Granule ultrastructure has been partly related to their content of serine proteases. Indeed, granules with the chymase protease preferentially exhibit homogeneous or crystalline substructures, whereas granules lacking this protease show mainly a scroll pattern (Schwartz *et al.*, 1987). However, significant granule heterogeneity can be found in any particular tissue and even between granules of a single MC. Of note, MC populations show marked differences in their phenotypic expression in different species, as well as at distinct anatomical sites, a phenomenon called “MC heterogeneity” (Galli, 1990). In addition to the typical secretory granules, human and mouse MCs also contain nonmembrane-bound, highly osmiophilic granules, called lipid bodies (Dvorak, 1991). They are fewer in number and generally larger than secretory granules, and serve as a significant site for arachidonic acid storage and metabolism. Besides cytoplasmic granules, all mammalian MCs express common characteristics, including the stem cell factor (SCF) plasma membrane receptor KIT and the high affinity plasma membrane receptor (Fc $\epsilon$ RI) binding IgE antibodies.

Most of our knowledge on MCs is derived from MC “knock-in” mouse models which have allowed researchers for testing and verifying whether MCs contribute to specific functions. The *Kit*-mutant mice, which are deficient in MCs, have been used in a series of experimental settings (Galli *et al.*, 2005a). The *Kit* gene encodes for the KIT protein (CD117) or SCF receptor on the surface of MCs and other cell types, such as germ cells, melanocytes, and intestinal Cajal cells (Galli *et al.*, 1992; Metcalfe *et al.*, 1997; Nocka *et al.*, 1990). SCF, or KIT receptor ligand, represents the main survival and developmental factor for MCs. Lack of expression of a functional KIT receptor because of spontaneous mutation in both copies of *Kit*, as it occurs in genetically MC-deficient WBB6F1-*Kit<sup>W</sup>-Kit<sup>W-v</sup>* mice (W/W<sup>v</sup> mice), results in a virtual absence of tissue MCs (Kitamura *et al.*, 1978). *Kit<sup>W</sup>* contains a point mutation that encodes a truncated KIT protein, which lacks the transmembrane domain and is therefore not expressed on the cell surface; *Kit<sup>W-v</sup>* 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-*Kit<sup>W-sh</sup>/W-sh* mouse (Grimbaldeston *et al.*, 2005; Zhou *et al.*, 2007). *Kit<sup>W-sh</sup>* contains an inversion mutation of the transcriptional regulatory elements upstream of the *Kit* transcription start site on mouse chromosome 5 (Galli *et al.*, 2005b). Lack of MCs in *Kit*-mutant mice can 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.

The cross-linking of immunoglobulin E (IgE) with bivalent or multivalent antigen results in the aggregation of FcεRI, which is sufficient for initiating down-stream 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). 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, that is immunological and nonimmunological. MCs may also be activated by “alternative,” IgE-independent pathways, such as aggregation of FcγRIII by IgG/antigen complexes, KIT and Toll-like receptor mechanisms, exposure to chemokines, anaphylatoxins C3a and C5a, fragments of fibrinogen, and fibronectin (Gommerman *et al.*, 2000; Johnson *et al.*, 1975; Marshall, 2004; Prodeus *et al.*, 1997; Wojtecka-Lukasik and Maslinski, 1992).

When MCs are activated, they immediately extrude granule-associated substances, such as histamine and proteases. Within minutes, they generate lipid-derived mediators (Brody and Metcalfe, 1998). Then, MC activation is followed also within hours, by the *de novo* synthesis of numerous cytokines and chemokines (Galli *et al.*, 2005a). MCs have long been regarded as key effector cells in IgE-associated immune responses, including allergic disorders and certain protective immune responses to parasites (Galli *et al.*, 2008a; Gurish and Austen, 2001). Consequently, MCs have mainly been considered

in the past for their detrimental role in type I allergic reactions, such as anaphylaxis, hay fever, eczema, or asthma. Several lines of evidence, however, indicate that MCs exert key immunological functions and are critical protagonists in host defence in the context of both innate and adaptive immune responses. In addition, there is growing evidence that MCs exert distinct nonimmunological activities, playing a relevant role in processes like wound healing after injury, tissue homeostasis and remodeling, fibrosis as well as tissue angiogenesis.

## 2.1. Immunological functions

MCs have increasingly been recognized as crucial effectors in both innate and adaptive immune responses. This concept is mostly derived 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). Indeed, recent reports in the literature indicate that MCs can mediate a variety of antimicrobial activities. MCs, for instance, are able to recognize and kill opsonized bacteria. *Salmonella typhimurium* coated with the C3b fragment of complement is recognized through complement receptor 3 (CR3) on the MC membrane (Sher *et al.*, 1979). In addition, MCs express several IgG receptors that are involved in the binding of IgG-coated bacteria to the MC membrane (Talkington and Nickell, 2001). Following their binding to MCs, opsonized bacteria are phagocytosed. MCs are a rich source of early-response cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-4, which are decisive in initiating the immune and inflammatory responses of the host to the invading pathogens (Galli *et al.*, 2005b). MCs release TNF- $\alpha$  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<sup>v</sup> 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<sup>v</sup> 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<sup>v</sup> mice are less protected against experimentally induced lung enterobacterial infections than MC-sufficient or MC-reconstructed W/W<sup>v</sup> 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- $\alpha$  in these mice. Indeed, TNF- $\alpha$ -deficient mice have increased mortality in the “cecal ligation and puncture” model compared with wild-type mice (Maurer *et al.*, 1998). 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). In a mouse model of sepsis, it has been shown that MC may decrease neurotensin-induced hypotension as well as sepsis-related mortality by degrading neurotensin through the protease neurolysin (Piliponsky *et al.*, 2008). Interestingly, other MC-derived products, such as leukotriene B<sub>4</sub> (LTB<sub>4</sub>), human trypsin  $\beta$ I, macrophage inflammatory protein (MIP)-1 $\alpha$  (CCL3), MIP-1 $\beta$ , MIP-2, monocyte chemoattractant protein-1, RANTES (regulated upon activation, normal T cell expressed and secreted) (CCL5), and IL-8 (CXCL8) also appear to contribute to the influx of neutrophils induced by activated MCs (Féger *et al.*, 2002).

By contrast, MCs may have deleterious effects during bacterial infections by excessive or inappropriate release of inflammatory mediators leading to detrimental effects to the host. 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 leukotriene C<sub>4</sub> (LTC<sub>4</sub>), leading to diarrhea and dysentery (Pulimood *et al.*, 1998). MCs may also trigger inflammation in *Helicobacter pylori* infection, and MC accumulation in the mucosa of patients with gastritis and MC degranulation by *H. pylori* products have been described (Masini *et al.*, 1994; Nakajima *et al.*, 1997; Plebani *et al.*, 1994). In addition, although MCs are capable of phagocytosis and kill various opsonized 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 entry of oxygen radicals. The net result is that the bacteria are not killed by MCs but remain in the MC cytoplasm as an intracellular reservoir (Féger *et al.*, 2002).

MCs may also contribute to optimal initiation of acquired immunity by orchestrating migration, maturation, and function of dendritic cells and by interacting with T and B cells (Nakae *et al.*, 2005). MCs promote dendritic cell migration and lymphocyte recruitment mainly through secretion of factors such as histamine, chemokines, LTB<sub>4</sub>, and TNF (Demeure *et al.*, 2005; Jawdat *et al.*, 2006; Maurer *et al.*, 2006). MCs may contribute also to the processing and presentation of bacterial antigens to immune cells. Indeed, MCs express MHC class I and II molecules, and can process and present antigens *in vitro* (Kambayashi *et al.*, 2008). Furthermore, they represent sources of costimulatory activity by expressing molecules of the B7 family, members of the TNF and TNF receptor families, CD28 and CD40 ligand (Nakae *et al.*, 2006). In addition, they may modulate the amplitude of the inflammatory response by secreting anti-inflammatory products, which promote homeostasis, for instance, by limiting endothelin-1-induced toxicity (Maurer *et al.*, 2004). MCs can also limit cutaneous responses to chronic UVB irradiation via IL-10 secretion and enhance

resistance to snake or honeybee venoms possibly by protease degradation (Grimbaldeston *et al.*, 2007; Metz *et al.*, 2006). In humans, MCs have long been recognized as crucial effectors in T helper 2 (Th2) cell-dependent, IgE-associated allergic disorders, such as urticaria, angioedema, allergic rhinitis, atopic dermatitis, bronchospasm, and some food allergies. In this context, activated MCs release Th2 cytokines, namely IL-4, IL-5, IL-9, and IL-13 that polarize the immune reaction and lead to IgE production by B cells.

Several lines of evidence indicate that MCs may play a role in autoimmunity, affecting disorders like arthritis, multiple sclerosis, bullous pemphigoid, and Graves' ophthalmia. They help initiate rheumatoid arthritis (Lee *et al.*, 2002). W/W<sup>v</sup> mice lacking MCs 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 transforming growth factor (TGF)- $\beta$ , in the synovial fluid (Olsson *et al.*, 2001). The invading MCs show ultrastructural signs of cell degranulation and produce several inflammatory mediators; notably TNF- $\alpha$ , IL-1 $\beta$ , and vascular endothelial growth factor (VEGF). TNF- $\alpha$  reportedly plays a pivotal role in the pathogenesis of rheumatoid arthritis, especially in its ability to regulate IL-1 $\beta$  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 (EAE) 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 (Christy and Brown, 2007; Gregory *et al.*, 2006). 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 autoantibodies 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<sup>v</sup> mice does not induce bullous pemphigoid, nor does the injection into wild-type mice pretreated 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 MCs in close proximity to suburothelial nerves is a consistent feature of this yet-to-be-clarified urological pathology (Elbadawi, 1997).

There are also indications that MCs may be implicated in immunological tolerance. MCs indeed serve as enforcers for regulatory T cells, turning down the immune system's reaction to skin allograft possibly by IL-10 secretion (Lu *et al.*, 2006).

## 2.2. Nonimmunological functions

There is now emerging evidence that MCs exert relevant functions in tissue homeostasis, remodeling, repair, and fibrosis (Artuc *et al.*, 1999, 2002; Dvorak and Kissel, 1991; Gruber *et al.*, 1997; Metcalfe *et al.*, 1997; Weller *et al.*, 2006). These functions are accomplished by a direct MC stimulation of specific connective tissue cell types, in particular fibroblasts, and by the release or activation of a series of extracellular matrix (ECM)-degrading enzymes. The presence of MCs in connective tissues has been linked to the development of fibrosis through the production of mediators, cytokines, proteases and growth factors, such as histamine, heparin, tryptase, fibroblast growth factor (FGF)-2, TNF- $\alpha$ , and TGF- $\beta$ , which stimulate the proliferation of myofibroblasts and fibrosis. TGF- $\beta$ , exerts a variety of effects on wound repair including the induction and/or facilitation of directed cell migration, angiogenesis, and granulation tissue formation. In addition, TGF- $\beta$  exerts a potent chemotactic effect on MCs (Gruber *et al.*, 1994). MCs are capable of both responding to and producing TGF- $\beta$ . Moreover, latent TGF- $\beta$  bound to ECM can be released but not activated by MC-derived chymase (Taipale *et al.*, 1995). During the process of wounding, MC granules released into the tissue are phagocytosed by fibroblasts and endothelial cells and may thus contribute to persistently increased tissue histamine levels (Seibold *et al.*, 1990). MCs release platelet-derived growth factor (PDGF) into wounded tissue and thereby influence the healing process from very early stages onward. Tryptases and FGF-2 are potent activators of fibroblast migration and proliferation (Artuc *et al.*, 2002; Rouss *et al.*, 1991). In an *in vivo* model of wound healing, an increased number of MCs, positively stained for FGF-2, were detected during the fibroproliferative stage (Liebler *et al.*, 1997). Of particular interest is the observation that tryptases can stimulate the synthesis and release of collagen from fibroblasts in culture, as well as provoke secretion of collagenase (Cairns and Walls, 1997). Moreover, tryptases cleave fibronectin and type VI collagen. In addition, this class of molecules has the ability to activate the preenzyme forms of some MMP and urinary plasminogen activators (uPA). Indeed, these enzymes have been implied to have a major role in tissue degradation. The ability of tryptases to induce the proliferation of airway smooth muscle cells could be of relevance in conditions such as bronchial asthma, in which smooth-muscle cell hyperplasia is a feature (Thabrew *et al.*, 1996). Tryptases may also have a role in tissue repair processes as a growth factor for epithelial cells. Chymases may contribute to the role of MCs in tissue remodeling by

cleaving type IV collagen and by splitting the dermal–epidermal junction. The production of type VIII collagen by human MCs *in vivo* may influence repair processes as this collagen is believed to facilitate the assembly of endothelial cells and tubes, and its synthesis precedes that of procollagen type I. In addition, MCs contain nerve growth factor (NGF) which actively stimulates neurogenesis after injury. Indeed, in the rat intestinal mucosa, reconstitution of nerve fibers, after experimentally induced inflammation and nerve fiber degeneration, is accompanied by a significant increase in mucosal MC density (Stead *et al.*, 1991). In addition, MCs and MC-derived TNF can promote elongation of cutaneous nerves during contact hypersensitivity in mice (Kakurai *et al.*, 2006).

MC involvement in the pathogenesis of coronary spasm, cardiomyopathy, atherosclerosis, and myocardial ischemia has 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 in leading to the subsequent fibrotic process (Frangogiannis *et al.*, 1998). By using C57BL/6-*Kit*<sup>W-sh/W-sh</sup> mice crossed with atherosclerosis-prone mice deficient in low-density lipoprotein receptor, *in vivo* evidence has been obtained for the implication of MCs in the atherogenic process, as the absence of MCs causes smaller atherosclerotic lesions with fewer inflammatory cell infiltrates (Sun *et al.*, 2007a). MCs may also contribute to the pathogenesis of elastase-induced abdominal aortic aneurysms in mice, as C57BL/6-*Kit*<sup>W-sh/W-sh</sup> mice fail to develop such aneurysms (Sun *et al.*, 2007b).

### 3. MAST CELL DEVELOPMENT, ANATOMICAL DISTRIBUTION, AND PHENOTYPE

MCs develop, like other leukocytes, from hematopoietic stem cells but do not mature before exiting the bone marrow and circulate as committed progenitors (Gurish and Austen, 2001). Kitamura *et al.* (1977) first showed that MCs are derived from bone marrow precursors. Using the abnormal giant cellular granules of beige mice (C57BL-Bg<sup>J</sup>/Bg<sup>J</sup>) as a traceable marker, they found that tissue MCs developed from grafted beige bone marrow in irradiated wild-type recipient mice. In humans, MCs are derived from CD34<sup>+</sup>, CD13<sup>+</sup>, FcεRI<sup>+</sup>, KIT<sup>+</sup> committed progenitors (Kirschenbaum *et al.*, 1991). Committed progenitors, circulating as mononuclear agranular-cells, traverse the vascular space and complete their maturation after moving into diverse peripheral tissues (Rodewald *et al.*, 1996). Here, they

acquire concomitant phenotypic diversity. Recently, a cell population ( $\text{Lin}^- \text{Kit}^+ \text{Fc}\gamma\text{RII/III}^{\text{hi}} \beta 7^{\text{hi}}$ ) 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 (BMCP), can be generated mainly from granulocyte/macrophage progenitors in the bone marrow.

Tissue homing as well as local differentiation and maturation of MC precursors are critically regulated by tissue microenvironmental factors, in particular the SCF secreted by fibroblasts, stromal cells and endothelial cells, which represents the most important cytokine involved in human and rodent MC development (Ashman, 1999). 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.*, 1991). 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 because of spontaneous mutation in both copies of *Kit*, as it occurs in genetically MC-deficient WBB6F1-*Kit*<sup>W</sup>-*Kit*<sup>W<sup>v</sup></sup> mice (W/W<sup>v</sup> mice), results in a virtual absence of tissue MCs (Kitamura *et al.*, 1978). Remarkably, MCs develop in W/W<sup>v</sup> mice if these mice receive bone marrow cells from normal littermates. Integrins are also involved in the process of recruitment of MC precursors into inflamed mucosal tissues. Human MC progenitors express  $\alpha 4 \beta 1$  integrin, which regulates their adhesion to activated endothelial cells (Boyce *et al.*, 2002). Inflammation-induced recruitment of human MC progenitors to the lungs requires both  $\alpha 4 \beta 7$  and  $\alpha 4 \beta 1$  binding to “vascular cell adhesion molecule-1” (VCAM-1), implicating organ-specific control of MC progenitor influx (Gurish and Boyce, 2006). In the mouse, large numbers of MC-committed progenitors are constitutively recruited in the small intestine by a mechanism involving the  $\alpha 4 \beta 7$  integrin (Gurish and Austen, 2001). This integrin expressed on the surface of MC progenitors binds to the “mucosal address in cell adhesion molecule-1” (MAdCAM-1) and to VCAM-1 as endothelial counterligands for this integrin. 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 progenitors derived *in vitro* from cord blood express a set of chemokine receptors including CXCR2, CCR3, CXCR4, and CCR5, and respond to the corresponding ligands *in vitro* (Ochi *et al.*, 1999).

SCF not only drives MC homing, proliferation and differentiation but also MC survival, migration and functional activation. In rodents, IL-3 plays an additional fundamental role in MC development (Lantz *et al.*, 1998). By contrast, the role of IL-3 in the development of human MC is controversial

(Saito, 2005). Other cytokines and growth factors which regulate MC development and differentiation include IL-4, IL-9, IL-10, TGF- $\beta$ , and NGF (Okayama and Kawakami, 2006). 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; Féger *et al.*, 2002).

Thus, MCs acquire their mature phenotype locally in the tissues where they ultimately reside. MCs are found in almost all the major organs and tissues of the body, particularly in association with connective tissue structures such as blood vessels, lymphatic vessels, and nerves, and in proximity to surfaces that interface the external environment such as those of the respiratory, gastrointestinal, and urogenital system, as well as the skin. This selective accumulation at tissue sites where foreign material attempts to invade the host suggests that MCs are among the first cells to initiate defence mechanisms. MCs are not found in avascular tissues such as mineralized bone, cartilage and the cornea. In the human body, MCs 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.

On the basis of their specific staining characteristics, preferential tissue homing, contents of proteases, and reactivity, two morphologically distinct subpopulations of rodent MCs were initially identified as connective tissue MCs CTMCs and mucosal MCs MMCs (Table 4.1) (Enerbäck, 1986). CTMCs can be distinguished from MMCs by staining with safranin because of the presence of large amounts of heparin in the secretory granules. Morphological, biochemical, and functional heterogeneity has been found in human MCs also. Human MCs are conventionally divided into two types depending on the expression of different proteases in their granules and other functional features (Irani *et al.*, 1986). MCs, which contain tryptase only, are designated as MC<sub>T</sub> or “immune cell associated” MCs. They are predominantly located in the respiratory and intestinal mucosa, where they colocalize around T lymphocytes. MCs that contain both tryptase and chymase, along with other proteases such as carboxypeptidase A and cathepsin G, are referred to as MC<sub>TC</sub>. They are 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 differ also in terms of their mediator

**Table 4.1** Characteristics of the two major human mast cell phenotypes

| Mast cells                          | Location                                            | Major enzymes                       | Major arachidonic acid metabolites | Major cytokines                                                                                 |
|-------------------------------------|-----------------------------------------------------|-------------------------------------|------------------------------------|-------------------------------------------------------------------------------------------------|
| Connective tissue mast cells (CTMC) | Submucosa of gastrointestinal tract and skin        | Tryptase, chymase, carboxypeptidase | LTC <sub>4</sub> (↓), PG-D2        | IL-5 (↓), IL-6 (↓), IL-3, IL-4, IL-7, IL-8, IL10, IL-13, IL-16, TNF- $\alpha$ , SCF             |
| Mucosal mast cells (MMC)            | Mucosa of gastrointestinal tract, respiratory tract |                                     | LTC <sub>4</sub> , PG-D2 (↓)       |                                                                                                 |
|                                     | IgE-dependent and independent activation            | Tryptase                            |                                    | IL-4(↓), IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-16, TNF- $\alpha$ , GM-CSF, SCF, TGF- $\beta$ |

content and reactivity. A third type of MC, called MC<sub>C</sub> has been identified. This MC expresses chymase without tryptase and resides mainly in the submucosa and mucosa of the stomach, small intestinal submucosa, and colonic mucosa (Irani and Schwartz, 1994). Interestingly, human MC<sub>T</sub> most closely correspond to murine MMCs, whereas MC<sub>TC</sub> resemble murine CTMCs. MCs from different anatomical sites are able to generate distinct profiles of cytokine expression and different responses to secretagogues. Indeed, MCs from skin seem to be very sensitive to stimulation with substance P, compound 48/80, and morphine, all leading to histamine release, whereas heart and lung MCs only react to compound 48/80 (Fureder *et al.*, 1995). Intestinal MCs do not react to compound 48/80, whereas uterine MCs fail to respond to substance P (Shanahan *et al.*, 1985).



#### 4. MAST CELL-DERIVED SECRETORY PRODUCTS

When MCs are activated, they extrude granule-associated substances, such as histamine, immediately and within minutes, generate lipid-derived mediators (Table 4.2) (Galli *et al.*, 2005a). MC activation is followed also, within hours, by the *de novo* synthesis of numerous cytokines and chemokines (Table 4.2).

Secretory granules of MCs contain crystalline complexes of preformed mediators ionically bound to a matrix of proteoglycans. The metachromatic staining of MC granules probably reflects their content of proteoglycans, such as chondroitin sulfates and heparin. In the mouse, the proteoglycan content of MC granules varies in the different MC subtypes. CTMCs contain heparin that lacks in MMCs. Conversely, MMCs express chondroitin sulfates A and B, which are not found in CTMCs, whereas both MC subtypes store chondroitin sulfate E in their granules (Féger *et al.*, 2002). The dominant proteoglycan in human MCs is heparin, which constitutes

**Table 4.2** Major human mast cell-derived mediators

|                     |                                                                                                                                                                          |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Preformed mediators | Histamine, serotonin, heparin, neutral proteases (tryptase and chymase, carboxypeptidase, cathepsin G), major basic protein, acid hydrolases, peroxidase, phospholypases |
| Lipid mediators     | LTB <sub>4</sub> , LTC <sub>4</sub> , PGE <sub>2</sub> , PGD <sub>2</sub> , PAF                                                                                          |
| Cytokines           | TNF- $\alpha$ , TGF- $\beta$ , IFN- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-5, IL-6, IL-13, IL-16, IL-18                                                            |
| Chemokines          | IL-8, I-309, MCP-1, MIP-1 $\alpha$ S, MIP1 $\beta$ , MCP-3, RANTES, eotaxin                                                                                              |
| Growth factors      | SCF, M-CSF, GM-CSF, FGF-2, VEGF, NGF, PDGF                                                                                                                               |

some 75% of the total, with a mixture of chondroitin sulfates making up the remainder (Church and Levi-Schaffer, 1997). In humans, the heparin content in MC<sub>T</sub> and MC<sub>TC</sub> is roughly the same. Chondroitin sulfate and heparin proteoglycans are thought to bind histamine, neutral proteases, and carboxypeptidases primarily by ionic interactions and therefore contribute to the packaging and storage of these molecules in the granules. Mice that lack the enzyme *N*-deacetylase/*N*-sulfotransferase-2 (NDST-2) and are unable to produce fully sulfated heparin, exhibit severe defects in the granule structure of MCs, with impaired storage of certain proteases and reduced content of histamine (Forsberg *et al.*, 1999; Humphries *et al.*, 1999). During degranulation, the various mediators packaged with proteoglycans dissociate at different rates, histamine very rapidly but tryptase and chymase much more slowly.

Histamine, the first discovered mediator in MCs, is present at a concentration of 1–4 pg/cell in human MCs. Histamine exerts many effects pertinent to the immediate phase of allergic response, including vasodilation, increased vasopermeability, contraction of bronchial and intestinal smooth muscle cells, and increased mucous production. Histamine mediates its action via the histamine receptors 1–3, which can transduce signals leading to the variety of symptoms associated with acute allergic reactions (Galli *et al.*, 2008a,b).

MCs are a rich reservoir of neutral proteases. The major MC protease is tryptase, a 130 kD serine protease, which is stored in a fully active form in the granule. It represents the most abundant constituent of human MCs. Some 10 pg/cell has been detected in MCs in the lung and up to 35 pg/cell in skin MCs (Schwartz *et al.*, 1987). Tryptase cleaves various bronchial and intestinal neuropeptides and matrix components, as well as IgE molecules, thereby possibly down regulating the allergic response (Rauter *et al.*, 2008). In addition, it is emerging as a potent growth factor for fibroblasts, endothelial cells, and muscle cells (Blair *et al.*, 1997; Gruber *et al.*, 1997). Chymase, a 30 kD protease, is present within the granules of the MC<sub>TC</sub> subset of MCs, in an estimated concentration of 4.5 pg/cell (Metcalf *et al.*, 1997; Schwartz *et al.*, 1987). Like tryptase, it degrades some neuropeptides and interleukins, and cleaves collagen and other ECM components (Huang *et al.*, 1998). In the mouse, at least five different granule-associated chymases (mMCP-1, mMCP-2, MMCP-3, MMCP-4, MMCP-5) and three different granule-associated tryptases (mMCP-6, mMCP-7, mMMP-11/transmembrane tryptase [mTMT]) have been described at the protein level (Huang *et al.*, 1998). There appear to be multiple forms of human tryptases as well (tryptases  $\alpha$ , I, II/ $\beta$ , III) (Miller *et al.*, 1989, 1990; Vanderslice *et al.*, 1990). Two other proteases, carboxypeptidase and cathepsin G, have been associated with the MC<sub>TC</sub> subset of human MCs. Interestingly, the specific protease content of individual MCs can vary depending on the MC microenvironment. For example, MMCs in mice express mMCP-1 and mMCP-2, whereas CTMCs express a different pattern of proteases, namely mMCP-3, mMCP-4,

mMCP-5, mMCP-6, mMCP-7, and carboxypeptidase (Miller and Pemberton, 2002; Stevens *et al.*, 1993). MC proteases play an important role in innate host defence. MC tryptase mMCP-6, for instance, has a critical protective function in bacterial and parasite infection. mMCP-6-deficient mice are less able to clear *Klebsiella pneumoniae* injected into their peritoneal cavities, probably because of less recruitment of neutrophils (Thakurda *et al.*, 2007). Delayed expulsion of the adult helminth and increased deposition of larvae in muscles occur in mMCP-1-deficient mice infected with *Trichinella spiralis* (Knight *et al.*, 2000). mMCP-6 as well is important for the clearance of the chronic *T. spiralis* infection (Shin *et al.*, 2008).

Preformed substances stored in the secretory granules can be released by two morphologically distinct secretory pathways, referred to as exocytosis (also called “anaphylactic degranulation”) and piecemeal degranulation (or intragranular activation) (Fig. 4.2) (Dvorak, 2005). Exocytosis consists of a rapid and massive secretory process, which characteristically occurs during IgE-dependent hypersensitivity reactions. In exocytosis, cytoplasmic granule membranes fuse with each other and with the plasma membrane, giving rise to open secretory channels, which allow the release of granule contents into the local extracellular environment. Piecemeal degranulation, conversely, represents a particulate mode of MC secretion, characterized by a slow discharge of granule contents in a “piecemeal” fashion, without membrane fusion events and granule opening to the cell exterior. This degranulation pattern has frequently been observed in MCs infiltrating areas of chronic inflammation or tumors (Dvorak, 1991).

The most important MC-derived lipid mediators are cyclooxygenase and lipoxygenase metabolites of arachidonic acid (Galli *et al.*, 2005a). All these products have potent inflammatory activity and can also modulate the release process. The major cyclooxygenase product of MCs is prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and the major lipoxygenase products derived from them are the sulfidopeptide LTC<sub>4</sub>: LTC<sub>4</sub> and its peptidolytic derivatives LTD<sub>4</sub> and LTE<sub>3</sub>. Human MCs can also produce LTB<sub>4</sub>, although in much smaller quantities than PGD<sub>2</sub> or LTC<sub>4</sub>, and some MC populations represent a source of platelet activating factor (PAF).

More than thirty different cytokines and chemokines have been shown to be produced by human and mouse MCs. MC secretory granules contain pools of stored TNF- $\alpha$  which has pleiotropic proinflammatory effects (Gordon and Galli, 1990, 1991). TNF- $\alpha$  has been implicated in neutrophil recruitment, inducing upregulation of the endothelial-leukocyte adhesion molecule (ELAM-1) (Walsh *et al.*, 1991). TNF- $\alpha$  has also been known to enhance the bactericidal activities of neutrophils (Kenny *et al.*, 1993). Certain MC populations may also have preformed as stores of VEGF (Boesiger *et al.*, 1998). In addition, human MCs have the capacity to generate IL-8, thereby contributing to neutrophil recruitment (Moller *et al.*, 1993). Under allergic conditions MCs produce significant amounts of IL-1 that

may contribute to lymphatic infiltration (Bochner *et al.*, 1990) and IL-4, essential for the triggering of Th2 lymphocytes that themselves produce IL-4 to initiate inflammatory cell accumulation and B lymphocyte immunoglobulin class switching to IgE (Bradding *et al.*, 1993). Other cytokines involved in MCs found in normal and in asthmatic airways are IL-5 and IL-6 which, together with IL-4 and IL-13, would enhance Th2-type immune response and eosinophil chemotaxis, thereby indicating that MCs may play an important role in initiating and maintaining the inflammatory response in asthma (Bradding *et al.*, 1995). Interestingly, a unique profile of cytokines is induced depending upon the nature of the stimulus or type of infection. Human intestinal MCs have been shown to spontaneously produce proinflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-8 at low levels without stimulation of the cell (Lorentz and Bischoff, 2001). Stimulation by IgE receptor cross-linking leads to an enhanced production of proinflammatory cytokines and *de novo* production of Th2 cytokines such as IL-3, IL-5, IL-10, and IL-13. Gram-negative bacteria, in contrast to IgE receptor cross-linking, do not induce the release of Th2 cytokines but enhance that of proinflammatory cytokines. Interestingly, MCs from different anatomical sites are able to generate distinct profiles of cytokine expression. In the tissues of bronchial and nasal mucosae from normal, asthmatic, and allergic rhinitis patients, MC<sub>T</sub> release IL-5, IL-6, and some IL-4, whereas MC<sub>TC</sub> preferentially express IL-4 but little IL-5 and IL-6. A similar predominant IL-4 pattern is recognizable in skin MCs which contain both tryptase and chymase (MC<sub>TC</sub>). Such differences in the distribution of cytokine expression between subsets of MCs suggest a difference in the capacity of MC subsets to produce various cytokines and therefore a difference in their specific roles in allergic inflammation.

MCs have been shown to express an important set of chemokines, which influence recruitment of dendritic cells, lymphocytes, other inflammatory cells, and tissue resident cells at sites of tissue inflammation, as well as migration of dendritic cells to lymph nodes. Cross-linking of Fc $\epsilon$ RI on MCs induces the release of the CCL1 chemokine, which acts to recruit Langerhans-type dendritic cells to sites of atopic skin inflammation (Gombert *et al.*, 2005). MCs also express CCL19, the ligand for CCR7, a chemokine receptor required for dendritic cell migration (Humrich *et al.*, 2006). Other products of MC activation, including CCL5 (RANTES) and TNF- $\alpha$ , can promote dendritic cell (DC) migration (Yamazaki *et al.*, 1998). MCs also appear to orchestrate the migration of T cells. Upon activation, MCs express chemoattractants such as LTB<sub>4</sub>, IL-16, XCL1 (also called lymphotactin), CCL3 (also called MIP-1 $\alpha$ ), CCL2 (mMCP-1), CCL5, CCXCL10, CCL19, and CCL21 (Galli *et al.*, 2005b; Sayed *et al.*, 2008). MC-derived LTB<sub>4</sub> is essential for the recruitment of both CD4<sup>+</sup> and CD8<sup>+</sup> effector cells to sites of inflammation (Ott *et al.*, 2003). CXCL8 (IL-8) is another MC-derived chemokine which exerts basic functions on inflammatory cell recruitment and endothelial cell activation.

## 5. MAST CELLS IN EXPERIMENTAL CARCINOGENESIS

The introduction of novel experimental procedures to induce carcinogenesis in laboratory animals has contributed to increase our understanding of the role of MCs in early tumor growth and angiogenesis. Development of squamous cell carcinoma in a HPV (human papilloma virus) 16-infected transgenic mouse model of epithelial carcinogenesis provided experimental evidence in favor of an early participation of MCs in tumor growth and angiogenesis (Coussens *et al.*, 1999). Accumulation of MCs increased at the hyperplastic and dysplastic stages and in the invasive front of tumor growth, with a typical localization around capillaries and epithelial basement membrane, where MCs degranulated releasing MC-specific proteases tryptase and chymase. On the contrary, MCs were absent within the tumor mass itself. Infiltration of MCs and activation of MMP-9 coincided with the angiogenic switch in premalignant lesions and addition of chymase alone was sufficient to stimulate an angiogenic phenotype when coincubated with a hyperplastic skin sample *in vitro*, while tryptase plays a role in tissue remodeling.

Gounaris *et al.* (2007) developed a transgenic mouse model overexpressing an inducible, stabilized  $\beta$ -catenin in intestinal enterocytes, as well as the APC $\Delta 468$ , as an independent hereditary model of polyposis. In both mice, developing polyps were rapidly infiltrated by CD34<sup>+</sup> MCs and staining confirmed the presence of both connective and mucosal types of MCs in developing tumors. The latter type was more prevalent at the site of newly developing polyps, while the former was more prevalent in the stroma at later stages of tumor development. Treatment with anti-TNF- $\alpha$  antibodies resulted in fewer polyps, reduced polyp growth, and decreased MC infiltration. Reconstitution of W<sup>sh</sup>/W<sup>sh</sup> MC-deficient mice with CD34<sup>-/-</sup> CD43<sup>-/-</sup> bone marrow also resulted in a decrease in polyp growth, as compared to that in mice reconstituted with wild-type bone marrow. Overall, these data suggest that MCs are an essential component for preneoplastic polyp development, as well as a possible target for therapeutic intervention.

In a pancreatic  $\beta$ -cell tumor model, activation of Myc *in vivo* has been shown to induce several chemokines in developing tumors, including CCL2 and CCL5. The latter triggers rapid recruitment of MCs to the tumor site, but not into the islets themselves, required for macroscopic tumor expansion (Soucek *et al.*, 2007). MCs were the only inflammatory cells increased in the vicinity of tumor cells at the early stage of tumor growth and their infiltration correlated with the expansion of islet tumors. Macrophages and neutrophils are also recruited after the onset of tumor angiogenesis. Treatment of established  $\beta$ -cell tumors with disodium cromoglycate (cromolyn), an inhibitor of MC degranulation, rapidly triggers hypoxia, and cell death of

tumor and endothelial cells. The authors conclude that MCs are necessary for tumor angiogenesis and growth and suggest that inhibition of MC function may prove to be therapeutically useful in restraining the growth of pancreatic cancer.

Angiopoietin-1 (Ang-1) secreted by primary murine MCs promotes marked neovascularization in an *in vivo* transplantation assay in nude mice (Nakayama *et al.*, 2004). The MCs studied were bone marrow mast cells (BMMCs) from c57BL/6 mice and two mouse plasmacytoma cell lines. A greater angiogenesis was demonstrated when BMMCs were transplanted into mice together with plasmacytoma cells than when each of the cell types was transplanted alone. The use of Ang-1-neutralizing antibodies reduced significantly the growth of plasmacytoma containing MCs. Overall, these data demonstrated that MC-derived Ang-1 promotes growth of plasmacytomas by stimulating neovascularization.

Huang *et al.* (2008) demonstrated that tumor-derived SCF recruits MCs to the tumor environment and also activates them. Using a SCF-knock-down H22 tumor model, tumor growth was reduced and this correlated with a reduction in MC accumulation in the developing tumor. SCF-stimulated MCs released active MMP-9 into the local environment, disrupting extracellular matrix and releasing further matrix-bound SCF, acting as a positive feed-back loop on MC activation within the tumor. Activation by high levels of SCF *in vitro* led to upregulation of proinflammatory genes, such as IL-6, TNF- $\alpha$ , and VEGF, which could be inhibited by antibodies against the SCF receptor, KIT.

Hart *et al.* (2002), using the W/W<sup>v</sup> mice, demonstrated a direct correlation between MC density in the dermis and susceptibility to ultraviolet-B-induced systemic immunosuppression. These mice which are homozygous for the W (white spotting) mutation, and therefore severely MC deficient, are unresponsive to ultraviolet-induced immunosuppression unless first injected with MC precursors at the irradiated site (Hart *et al.*, 2002). The W locus encoded the KIT tyrosine kinase receptor that binds SCF (Church and Levi-Schaffer, 1997). The MC products involved in ultraviolet-induced immunosuppression are believed to be TNF- $\alpha$  and histamine (Hart *et al.*, 2001).

## 6. MAST CELLS AND TUMOR GROWTH IN HUMANS: PRO AND CONTRA

MCs accumulate at sites of tumor growth in response to numerous chemoattractants (Conti *et al.*, 1997). MCs have a vast array of mediators, some of which have promoting, and others inhibitory, effects on malignancies (Fig. 4.3) (Theoharides and Conti, 2004). The phenotypic expression of MCs is not static, its secretory pattern varies according to the

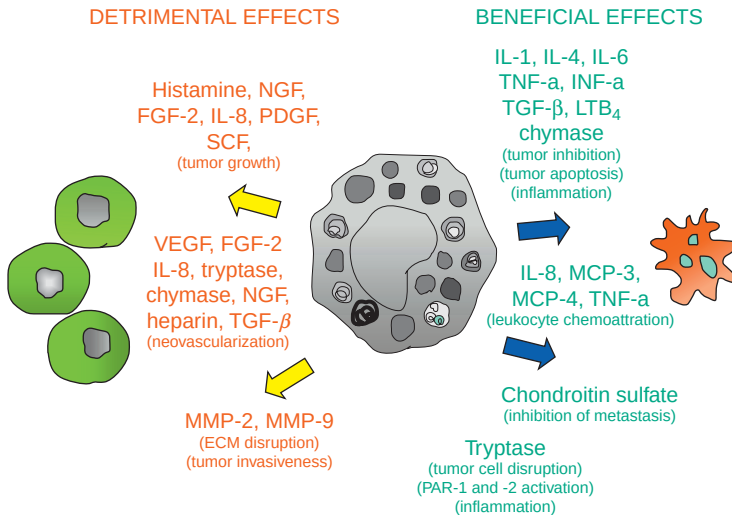


**Figure 4.3** The potential dual role of mast cells in tumor fate. Mast cells may exert detrimental effects to the host by releasing cytokines and growth factors, such as FGF-2, NGF, PDGF, SCF, VEGF and IL-8, which stimulate tumor cell expansion. Mast cells are a major source of histamine, which can induce tumor cell proliferation through H1 receptors, while suppressing the immune system through H2 receptors. Mast cells produce several angiogenic factors, as well as matrix metalloproteinases, which promote tumor vascularization and tumor invasiveness, respectively. By contrast, mast cells may promote inhibition of tumor cell growth, tumor cell apoptosis and inflammation by releasing cytokines such as IL-1, IL-4, IL-6, and TNF- $\alpha$ . TNF- $\alpha$ , in particular, is very effective in leukocyte chemoattraction. Chondroitin sulfate may inhibit tumor cell diffusion and tryptase causes both tumor cell disruption and inflammation through activation of protease-activated receptors (PAR-1 and -2). Abbreviations: FGF-2, fibroblast growth factor-2; NGF, nerve growth factor; PDGF, platelet-derived growth factor; SCF, stem cell factor; TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; VEGF, vascular endothelial growth factor; MMP-2, MMP-9, matrix metalloproteinase-2, -9; IL-1, IL-2, IL-6; IL-8, interleukin-1, -2, -6, -8; ECM, extracellular matrix INF- $\alpha$ , interferon- $\alpha$ ; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; MCP-3, MCP-4, mast cell protease-3, -4.

microenvironment, and MCs have the ability to secrete individual granules or distinct mediators selectively. Secretion of cytokines from MCs could occur without degranulation. This has been termed “differential release,” “intragranular activation,” or “piecemeal degranulation,” and may be associated with the ability of MCs to release some mediators selectively without degranulation (Theoharides *et al.*, 2007).

Dabbous *et al.* (1986) showed that MC degranulation is associated with disruption and lysis of the tumor extracellular matrix, either directly through the action of their enzymes or indirectly through modulation of the collagenolytic activity of fibroblasts, macrophages, and tumor cells (Barom *et al.*, 2001; Stack and Johnson, 1994). Tryptase activates latent MMPs and plasminogen activator, which, in turn, degrade the extracellular matrix (Stack and

Johnson, 1994). MC chymase was demonstrated to cause apoptosis in different target cells (Hara *et al.*, 1999) and to induce the accumulation of tumor associated macrophages, neutrophils, and other inflammatory cells *in vivo* (He and Walls, 1998).

Heparin enhances both the activity and production of collagenase *in vitro* and releases plasminogen activator from endothelial cells (Markwardt and Klocking, 1977; Sakamoto *et al.*, 1973). MCs are the major source of histamine, which mediates many symptoms of allergic reactions. Histamine can induce tumor cell proliferation through H1 receptors identified in human malignant carcinoma, while suppressing the immune system through H2 receptors (Niwa *et al.*, 1991). High histamine concentration inhibits human primary melanoma-cell proliferation, presumably by acting through H1 receptors, an action enhanced by IL-2, whereas low amounts of it through H2 receptors increase proliferation (Lazar-Molnar *et al.*, 2002). It has been reported that the incidence of metastases, as well as the appearance of tumors, correlates inversely with tissue histamine level (Burtin *et al.*, 1985).

MCs exert direct mitogenic effect on tumor cells. For example, FGF-2 and IL-8 are directly mitogenic to melanocytes and melanoma cells (Halaban *et al.*, 1988; Schadendorf *et al.*, 1993). Recently, we have investigated the pattern of distribution of MCs in biopsy samples obtained from four different human tumors, utilizing an image analysis system and a mathematical model to make a quantitative approach to characterizing their spatial distribution (Guidolin *et al.*, 2008). In all tumors, MCs demonstrated a virtually random spatial distribution, albeit with varying densities, suggesting that MC-MC interactions could play a minor role in the formation of the MC pattern in neoplastic tissues. The random distribution of the cells in the tissues could be accounted for by a random walk migration under the influence of cell-matrix interactions or chemotactic fields potentially generated by tumor or endothelial cells.

Tumors of MCs are extremely rare, but certainly not as rare as commonly thought. The most common tumors of MCs are those confined to the skin and manifest in childhood (Lennert and Parwaresch, 1979). The group of the cutaneous mastocytosis consists of two types. The most common is designated urticaria pigmentosa and represents a disseminated involvement of the skin, while the localized type is made of solitary or multiple nodules and is called benign mastocytoma (Lennert and Parwaresch, 1979). The clinical course in cutaneous mastocytosis is benign, in many cases skin lesions disappear during puberty (Caplan, 1963).

Systemic mastocytosis is usually diagnosed in adulthood and is characterized by multiorgan involvement (with or without skin lesions) and disease persistence (Lennert and Parwaresch, 1979). Indolent variants as well as aggressive variants of systemic mastocytosis have been reported (Lennert and Parwaresch, 1979). Patients with systemic mastocytosis may be diagnosed with an associated clonal hematologic non-MC lineage disease

(Travis *et al.*, 1988) and MC-leukemia is a rare subtype of systemic mastocytosis defined by circulating MCs and a rapidly deteriorating clinical course in most cases (Travis *et al.*, 1988).

Increased MC number has been correlated with a poor prognosis in several human tumors such as human melanoma (Ribatti *et al.*, 2003b), oral squamous carcinoma (Iamaroon *et al.*, 2003; Wanachantarak, 2003), and squamous cell carcinoma of the lip (Rojas *et al.*, 2005). Human tumors in which MC infiltration correlates with a poor prognosis include: melanoma, oral squamous carcinoma, Hodgkin's lymphoma, diffuse large B cell lymphoma, angioimmunoblastic T cell lymphoma, Waldenstrom's macroglobulinemia, and prostate cancer.

Sharma *et al.* (1992) reported a higher number of MCs in nodular sclerotic-type Hodgkin's lymphoma (HL) than in other types of HL and the number of MCs was higher in fibrotic areas than in cellular areas. Molin *et al.* (2002, 2004) observed a worse prognosis for a nodular sclerosing HL exhibiting a high MC number. Fukushima *et al.* (2006) demonstrated an increased number of tryptase-positive and chymase-positive MCs in fibrotic areas in diffuse large B cell lymphoma (DLBCL) lymph nodes. The greatest number of MCs among T cell lymphomas was observed in angioimmunoblastic T cell lymphoma (Fukushima *et al.*, 2001). Dave *et al.* (2004) utilized gene array to study the relationship between prognosis and a specific gene expression profile and concluded that the length of survival among patients with follicular lymphoma correlates with the molecular features of nonmalignant immune cells present within the tumor at the time of diagnosis. One of the genes observed to correlate most negatively with survival was that of microphthalmia-associated transcription factor (MIFT), a transcription factor which has been found to be highly expressed in MCs and to play a critical role in the regulation of several key MC-specific genes (Nechushtan and Razin, 2002). Tournilhac *et al.* (2006) demonstrated that MCs may support tumor cell expansion in Waldenstrom's macroglobulinemia through constitutive CD154-CD40 signaling. In detail, MCs expressed CD154, a potent inducer of malignant B cell proliferation, while bone marrow lymphoplasmacytic cells functionally expressed the CD154 receptor, CD40. Moreover, the use of CD154-CD40 signal inhibitor partially inhibited MC-mediated bone marrow lymphoplasmacytic proliferation and/or tumor colony formation.

Nonomura *et al.* (2007) demonstrated that in prostate cancer MC counts were higher around cancer foci in patients with higher Gleason scores than in those with low Gleason scores. The MC number correlated with clinical stage and multivariate analysis revealed that MC count was a significant prognostic factor.

Increased MC number has been correlated also with a good prognosis in several human tumors. Human tumors in which MC infiltration correlates with a good prognosis include: breast cancer, nonsmall-cell lung carcinoma,

and ovarian cancer. [Aaltomaa et al. \(1993\)](#) found a positive correlation between survival and increased MC number in a study of 187 breast cancer biopsies. [Dabiri et al. \(2004\)](#) analyzed the correlation between MC number in breast cancer and patients' prognosis in a study of 438 patients. They found a strong correlation between the presence of MCs and a favorable prognosis. Perivascular tumor-associated MCs in mammary adenocarcinoma could secrete several cytokines and proteolytic enzymes that could be detrimental to the tumor cells. For instance, IL-4, which binds to IL-4 receptors expressed by human breast carcinoma cells, could lead to apoptosis in breast cancer ([Gooch et al., 1998](#)). The histamine content of human breast cancer tissue is much higher than adjacent normal tissue and act as a local immunosuppressant ([Ohno et al., 2002](#)). Moreover, the mean level of serum tryptase in women with breast cancer is three times higher than that in healthy women ([Samoszuck and Corwin, 2003](#)). [Kankkunen et al. \(1997\)](#) observed that significant increase in MC counts in breast carcinoma versus benign lesions is due to tryptase-containing MCs. It was found that in benign lesions, the number of MCs exhibiting tryptase activity was similar to that of chymase-active MCs. However, malignant tumors had two to three times more tryptase-containing than chymase-containing MCs, while the tryptase activity was significantly higher than that in benign lesions. Moreover, in malignant lesions, tryptase-containing MCs were concentrated at the tumor edge, whereas chymase-containing MCs were not increased in this area. Breast cancer patients with metastases in the axillary nodes reveal greater numbers of MCs in all nodes examined compared with patients without metastasis ([Thorensen et al., 1982](#)).

[Welsh et al. \(2005\)](#) analyzed the presence of MCs in the tumor stroma of 175 patients with surgically resected nonsmall-cell lung carcinoma and demonstrated that both macrophage and MC infiltration of the tumor islets was associated with a marked increase in 5-year survival, independently of other favorable prognostic factors including stage. [Chan et al. \(2005\)](#) studied samples of ovarian cancer from 44 patients and demonstrated that tumors with higher microvascular density had a higher mean survival compared with low MC density or low microvessel density.



## 7. MAST CELLS AND TUMOR ANGIOGENESIS AND LYMPHANGIOGENESIS

Inflammatory cells regulate endothelial cell functions related to physiological angiogenesis, as well as inflammatory and tumor-associated angiogenesis. It was Rudolf Virchow in 1863, who critically recognized the presence of inflammatory cells infiltrating neoplastic tissues and first

established a causative connection between the “lymphoreticular infiltrate” at sites of chronic inflammation and cancer.

In neoplastic tissues, inflammatory cells act in concert with tumor cells, stromal cells and endothelial cells to create a microenvironment, which is critical for the survival, development and diffusion of the neoplastic mass. These synergies may represent important mechanisms for tumor development and metastasis by providing efficient vascular supply and easy pathway to escape. Indeed, the most aggressive human cancers, such as malignant melanoma, breast carcinoma, and colorectal adenocarcinoma, are associated with a dramatic host response composed of various inflammatory cells, especially macrophages and MCs.

MCs also promote lymphangiogenesis. Endostatin, a proteolytic fragment of collagen XVIII, is a potent inhibitor of angiogenesis and tumor growth. In transgenic J4 mice, which overexpress endostatin in their keratinocytes, carcinogen-induced skin squamous cell carcinomas were less aggressive and more often well differentiated than those in the control mice, indicating that endostatin regulates the terminal differentiation of keratinocytes (Brideau *et al.*, 2007). Tumor angiogenesis was inhibited by endostatin at an early stage in skin tumor development. Inhibition of angiogenesis was accompanied by significant reduction of lymphatic vessels and lymph node metastasis. Remarkably, accumulation of tumor-infiltrating VEGF-C-positive MCs was markedly decreased in the tumors of the J4 mice. In addition, endostatin inhibited the adhesion and migration of murine MC/9 MCs on fibronectin *in vitro*. These data suggest that endostatin can inhibit tumor lymphangiogenesis by decreasing the VEGF-C levels in the tumors via inhibition of MC migration and adhesion.

### 7.1. Angiogenenic factors stored in mast cells

Angiogenic factors stored in MCs include: heparin, histamine, MMP-2, MMP-9, tryptase, chymase, FGF-2, VEGF, TGF- $\beta$ , TNF- $\alpha$ , IL-8, and NGF.

Heparin, a granule-associated mediator, stimulates endothelial cell proliferation and migration *in vitro* (Alessandri *et al.*, 1984; Thorton *et al.*, 1983). *In vivo*, however, it stimulates (Norrby and Sorbo, 1992; Norrby, 1993; Ribatti *et al.*, 1987), inhibits (Jakobson and Hahnenberger, 1991; Norrby, 1993; Wilks *et al.*, 1991) or has no effect (Castellot *et al.*, 1982; Taylor and Folkman, 1982). These differences seem to be related to its molecular size (high-molecular-weight heparin has been shown to stimulate angiogenesis, whereas low-molecular-weight inhibits it) and degree of sulfation. Heparin acts as a soluble form of the low-affinity FGF-2 receptor (Folkman and Shing, 1992), which displaces FGF-2 in the biologically active-form and allows its rapid interaction with endothelial cells (Yayou *et al.*, 1991).

Histamine has an angiogenic effect through both H1 and H2 receptors (Sorbo *et al.*, 1994). It may also increase the permeability of newly formed

microvessels during tumor angiogenesis, and hence the leakage of plasma proteins and deposition of fibrin. Degradation products of fibrin are angiogenic *in vivo* (Thompson *et al.*, 1995).

Microvascular leakiness in tumors is also a consequence of a defective organization of microvascular endothelium. In fact, tumor endothelial cells do not form a normal monolayer and, therefore, do not have a normal barrier function (McDonald and Boluk, 2002). The cells are disorganized and irregularly shaped, overlap one another, or have luminal projections, and give rise to abluminal sprouts.

MCs synthesize and store large amounts of MMP-2, MMP-9, and serine-proteinases of two subclasses: tryptase and chymase (Di Girolamo and Wakefield, 2000; Fang *et al.*, 1999; Vincent *et al.*, 2000). Given the ability of MMP-2 and MMP-9 to degrade type IV, V, VII, and X collagens, as well as fibronectin, (Raza and Cornelius, 2000), the major components of the interstitial stroma and subendothelial basement membrane, the findings suggest that MCs may contribute to the progression from *in situ* to invasive and metastatic solid tumors, characterized by an enhanced angiogenesis and secretion of proteolytic enzymes (Raza and Cornelius, 2000).

Tryptase and chymase are involved in angiogenesis after their release from activated MC granules (Fig. 4.4). Their proteolytic activities degrade extracellular matrix components or release matrix-associated growth factors (Taipale *et al.*, 1995), and they also act indirectly by activating latent MMP (Gruber *et al.*, 1989) and plasminogen activators (Stack and Johnson, 1994). Blair *et al.* (1997) have demonstrated the angiogenic potential of tryptase *in vitro* and its important role in neovascularization. Tryptase added to microvascular endothelial cells cultured on Matrigel caused a pronounced increase in capillary growth, and this was suppressed by specific tryptase inhibitors. Moreover, tryptase directly induced endothelial cell proliferation in a dose-dependent fashion.

Muramatsu *et al.* (2000a,b) used the hamster sponge-implant model to show that angiogenesis is induced by angiotensin II and inhibited by

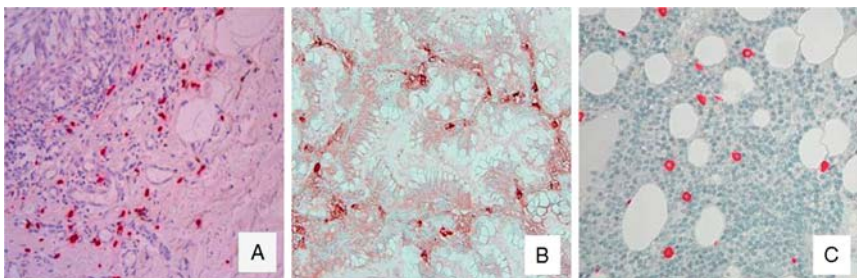


**Figure 4.4** Tryptase-positive mast cells in bioptic specimens of human melanoma (A), nonsmall-cell lung cancer (B) and B cell chronic lymphocytic leukemia (C). Original magnification: (A)–(C),  $\times 250$ .

chymase inhibitors, suggesting that MC-derived chymase is an important mediator of MC-dependent angiogenesis. MCs release several polypeptide growth factors, including FGF-2, VEGF, TGF- $\beta$ , TNF- $\alpha$ , and IL-8. These cytokines are involved both in normal as well as tumor-associated angiogenesis. The spectrum of cytokines expressed appears to vary depending on the maturity state of the MCs and of the tissue of residence. Qu *et al.* (1998) demonstrated that FGF-2 is localized in the cytoplasmic and extruded granules of MCs in several human tissues. Grutzkau *et al.* (1998) demonstrated the expression of VEGF in the human MC line HMC-1 and in human skin MCs. NGF, also contained in MC secretory granules, induces endothelial cell proliferation *in vitro* and angiogenesis *in vivo* in the chick embryo chorioallantoic membrane (CAM) assay (Cantarella *et al.*, 2002; Emanuelli *et al.*, 2002).

## 7.2. Mast cells in experimental tumor angiogenesis

Kessler *et al.* (1976) demonstrated that tumor angiogenesis factor (TAF) elicited a vasoproliferative response when implanted upon the CAM of the chick embryo. This response was first observed stereomicroscopically 2–3 days after implantation and a 40-fold increase in MC density was observed in the vicinity of the implants in 24 h. Poole and Zetter (1983) demonstrated that rat peritoneal MCs migrate in response to conditioned medium from several tumor cell lines. The active chemoattractant(s) in this conditioned medium appeared to be peptide(s) with a molecular weight of 300–1000. They proposed that the chemoattraction of MCs by tumor-derived peptides may be an important, early event in tumor neovascularization.

Starkey *et al.* (1988) investigated the role of host MCs in tumor-associated angiogenesis by comparing the angiogenic response of genetically MC-deficient W/W<sup>v</sup> mice and MC-sufficient +/+ littermates to subcutaneously growing B16-BL6 tumors. The response was slower and initially less intense in W/W<sup>v</sup>. Fewer W/W<sup>v</sup> than +/+ mice developed spontaneous lung metastases. Bone marrow repair of the MC deficiency restored the incidence of hematogeneous metastases to approach that of +/+ mice. These results demonstrate a role for MCs *in vivo* during tumor angiogenesis and also suggest a role for host MCs in hematogeneous metastases.

We have demonstrated that isolated MCs and their secretory granules, but not degranulated MCs, induce an angiogenic response in the CAM assay (Fig. 4.5) (Ribatti *et al.*, 2001). Addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MCs and their secretory granules by 50% and 30%, respectively. These data support the evidence that the angiogenic properties of MCs depend on the angiogenic molecules contained in their secretory granules, and indicate that FGF-2 and VEGF are the angiogenic cytokines primarily and perhaps synergistically responsible for this vasoproliferative activity.

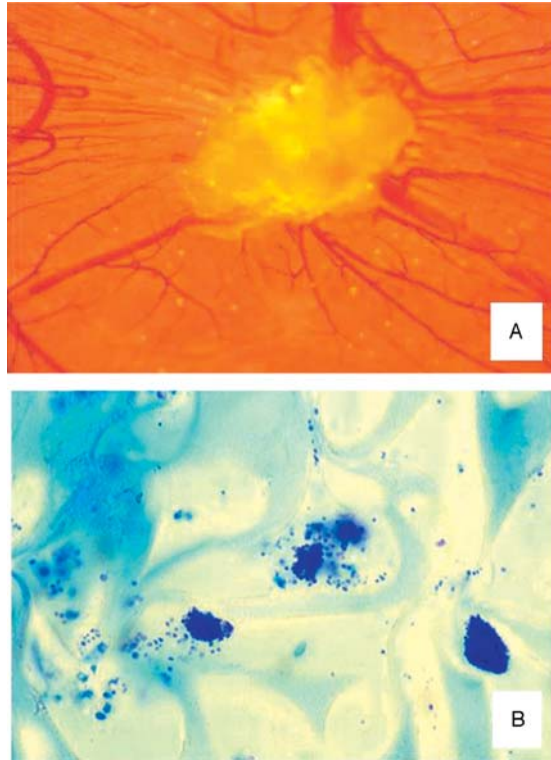


**Figure 4.5** A mast cell suspension has been delivered on the top of the chick embryo chorioallantoic membrane on Day 8 of incubation using a gelatin sponge implant. Macroscopic observation on Day 12 shows the gelatin sponge surrounded by numerous allantoic vessels that develop radially towards the implant in a “spoked-wheel” pattern (A). The histological analysis shows among the sponge trabeculae metachromatic mast cells and their secretory granules (B). Original magnification: (A)  $\times 50$ , (B)  $\times 250$  (adapted with modifications from Ribatti *et al.*, 2001).

### 7.3. Mast cells in human solid and hematological tumor angiogenesis

Solid human tumors, in which MC density correlates with microvascular density, include: human endometrial carcinoma, nonsmall-cell lung cancer, melanoma, squamous cell cancer of the oesophagus, and lymph nodes from patients with breast cancer.

Meanwhile, the following are hematological human tumors in which MC density correlates with microvascular density: B cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma, myelodysplastic syndromes and B cell chronic lymphocytic leukemia.

Tryptase-positive MCs increased in number and vascularization increased in a linear fashion from dysplasia to invasive cancer of the uterine cervix (Benitez-Bribiesca *et al.*, 2001). We have demonstrated that angiogenesis in human endometrial carcinoma is highly correlated with MC tryptase-positive cell counts and that these parameters increase in agreement with tumor progression (Ribatti *et al.*, 2005).

An association of VEGF and MCs with angiogenesis has been demonstrated in laryngeal carcinoma (Sawatsubashi *et al.*, 2000), in nonsmall-cell lung carcinoma, where most intratumoral MCs express VEGF (Imada *et al.*, 2000; Takanami *et al.*, 2000; Tomita *et al.*, 2000), and in melanoma, where MCs express both VEGF (Toth *et al.*, 2000) and FGF-2 (Ribatti *et al.*, 2003a). Lastly, a prognostic significance has been attributed to MCs and microvascular density in squamous cell cancer of the oesophagus (Elpek *et al.*, 2001) and in melanoma (Ribatti *et al.*, 2003b). Imada *et al.* (2000) studied 85 cases of stage I nonsmall-cell lung carcinoma and demonstrated a higher number of tryptase-positive MCs compared to cases of squamous cell carcinoma. A high correlation was observed between intratumoral MC counts and microvessel counts and double staining showed that most intratumoral MCs expressed VEGF.

In melanoma, MC accumulation around the margin of tumors has been observed to peak just as the tumor acquire the angiogenic phenotype and peritumoral MC counts correlated strongly with microvascular density, melanoma progression, and prognosis (Toth *et al.*, 2003a,b; Ribatti *et al.*, 2003a). Furthermore, in melanoma, MCs were closer to one another and to the vessels (Guidolin *et al.*, 2006). This close association between MCs and the endothelium might indicate that MCs are involved in the maintenance reaction necessary for the long lasting functional integrity of the endothelium. We have demonstrated that angiogenesis increases in parallel to the number of tryptase-positive MCs in lymph nodes from patients with breast cancer and that their values are significantly higher in lymph nodes with micrometastases as compared with those without metastasis (Ribatti *et al.*, 2007).

Angiogenesis in benign lymphadenopathies and B cell non-Hodgkin's lymphomas (B-NHL), measured as microvessel counts, is correlated with the total and MC tryptase-positive counts, and both increase in step with the increase in Working Formulation malignancy grades (Ribatti *et al.*, 1998, 2000). In NHL, the cellular expressions of VEGF and FGF-2, as well as MC and vessel counts, were assessed (Fukushima *et al.*, 2001). The number of MCs was greater in T cell lymphomas than in B cell lymphomas and in all NHL a significant correlation was found between vessel count and number of MCs, and between vessel count and number of VEGF-expressing cells. Double fluorescence staining of VEGF mRNA and MC tryptase revealed that MCs expressed VEGF mRNA. These data suggest that MCs intervene in angiogenesis in these lesions by expressing VEGF.

Glumelius *et al.* (2005) evaluated the relationship between the number of MCs and the microvessel count in tissue samples from HL-involved lymph nodes by immunohistochemistry and did not demonstrate any correlation between high microvessel count and the number of MCs.

Bone marrow angiogenesis, evaluated as microvessel area, and MC counts are highly correlated in patients with inactive and active multiple myeloma (MM) and in those with monoclonal gammopathies of undetermined significance (MGUS). Both parameters increase simultaneously in active MM (Ribatti *et al.*, 1999).

In both B-NHL and MM, MCs rest near or around blood or lymphatic capillaries. Their ultrastructural picture includes a typical morphological semilunar feature, or piecemeal partial degranulation of their secretory granules, unlike the IgE-mediated massive degranulation that occurs during immediate hypersensitivity reactions (Crivellato *et al.*, 2002, 2003b; Ribatti *et al.*, 1998, 1999) (Fig. 4.6). This morphology is typical of the slow degranulation that takes place in delayed hypersensitivity reactions and chronic inflammation (Kops *et al.*, 1984). Semilunar appearance may reflect slow but progressive release of angiogenic factors favouring chronic and progressive stimulation of MC degranulation.

Bone marrow samples of patients with myelodysplastic syndromes display a high correlation between microvessel counts and both total and tryptase-positive MCs, and both parameters increase simultaneously with tumor progression (Ribatti *et al.*, 2002). There is also a correlation between

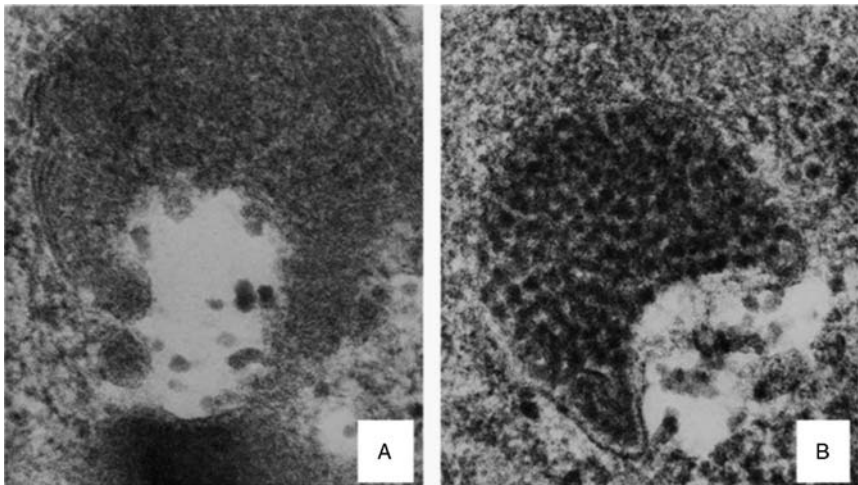


**Figure 4.6** Ultrastructural findings of mast cell granules from high-grade B cell non-Hodgkin's lymphoma. Note in (A) semilunar granular aspect and in (B) a full complement of dense particles recognized in this type of granule. Original magnification: (A, B),  $\times 25,000$  (adapted with modifications from Crivellato *et al.*, 2002).

the extent of angiogenesis and the number of tryptase-positive MCs in patients with early B cell chronic lymphocytic leukemia, and tryptase-positive MCs predict their clinical outcome (Molica *et al.*, 2003; Ribatti *et al.*, 2003c).

## 8. CONCLUDING REMARKS

The roles of MCs in normal physiology and in the pathogenesis of several human diseases in addition to allergic reactions have been a subject of increasing interest. MCs are found in multiple tissues and their presence in human cancer has been established for many years. However, the literature data have described both a positive and a negative correlation between their number and prognosis in various human tumor types. These discrepancies recognized also in experimental studies could be due to differences in the animal model used and the stage of carcinogenesis investigated.

MCs are recruited in tumor development and they play a key role in both angiogenesis and tissue remodeling, promoting both tumor initiation and growth. In addition, as tumor growth progresses, MCs recruit immune cells or alternatively suppress antitumoral responses. Reports addressing MCs specifically as mediators in tumor immunosuppression come from Huang *et al.* (2008), where they mobilize MCs to infiltrate tumors by means of SCF. Tumor-derived, SCF dependent MC infiltration and activation resulted in remodeling of the tumor microenvironment and tumor growth.

MCs may act as a new target for the adjuvant treatment of tumors through the selective inhibition of angiogenesis, tissue remodeling and tumor-promoting molecules, permitting the secretion of cytotoxic cytokines and preventing MC-mediated immune suppression. Moreover, some of the new targeted anticancer therapies have pronounced effects on MCs; in fact, it may be possible that some of their antitumor effect is closely related to their effect on MCs.

Preliminary studies using anti-KIT antibodies (Huang *et al.*, 2008), anti-TNF- $\alpha$  antibodies (Gounaris *et al.*, 2007), or the MC stabilizer disodium cromoglycate (cromolyn) (Soucek *et al.*, 2007) in mouse models demonstrated promising results even if administered after the initiation of tumor development. Treatment of mice bearing mammary adenocarcinoma and pancreatic cancer with cromolyn led to clotting of blood vessels, hypoxia, and tumor cell apoptosis (Samoszuk and Corwin, 2003; Soucek *et al.*, 2007). Unfortunately, cromolyn is a weak inhibitor of human MC secretion and is poorly adsorbed, so it is unlikely to be effective in treating cancer in humans.

The first tyrosine kinase inhibitor introduced into the clinic, STI571 (Imatinib mesylate, Gleevec) has inhibitory activity against the signaling cascade activated by c-kit receptor (CD117) (Heinrich *et al.*, 2000). This inhibitory activity is the basis of the use of this drug against gastrointestinal stromal tumors (GIST), most of which harbor a *kit* mutation (Kitamura and Hirota, 2004). STI571 is also in use for some varieties of mastocytosis, although some c-kit activating mutations involved in mastocytosis are resistant to its inhibitory activity (Akin and Metcalfe, 2004) and cromolyn has been administered with very limited side effects for various allergic inflammatory conditions (Edwards, 1995; Norris, 1996; Pardanani *et al.*, 2003). Ranitidine, a H2 receptor antagonist that is used as adjuvant therapy, prolonged survival of colorectal cancer patients (Nielsen *et al.*, 2002). Another H2 receptor antagonist, famotidine, given preoperatively for 14 days, enhanced tumor infiltrating lymphocytes and increased metastatic lymph node reactive changes in breast cancer in humans (Parshad *et al.*, 2002). Molica *et al.* (2007) demonstrated that the reduction of the extent of bone marrow angiogenesis observed after sequential therapy with low doses of subcutaneous alemtuzumab after a clinical response to fludarabine induction therapy was associated to a reduction in the number of MCs.

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