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Development of Mast Cells and Importance of Their Tryptase and Chymase Serine Proteases in Inflammation and Wound Healing

Jeffrey Douaiher[*], **Julien Succar**[†], **Luca Lancerotto**[*], **Michael F. Gurish**[‡], **Dennis P. Orgill**[*], **Matthew J. Hamilton**[§], **Steven A. Krilis**[¶], and **Richard L. Stevens**[‡,1]

[*]Division of Plastic Surgery, Department of Surgery, Brigham and Women's Hospital, Boston, Massachusetts, USA

[†]Division of General Surgery, Department of Surgery, University of Kentucky, Lexington, Kentucky, USA

[‡]Division of Rheumatology, Immunology, and Allergy, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA

[§]Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA

[¶]Department of Infectious Disease, Immunology, and Sexual Health, The St. George Hospital, University of New South Wales, Kogarah, New South Wales, Australia

Abstract

Mast cells (MCs) are active participants in blood coagulation and innate and acquired immunity. This review focuses on the development of mouse and human MCs, as well as the involvement of their granule serine proteases in inflammation and the connective tissue remodeling that occurs during the different phases of the healing process of wounded skin and other organs. The accumulated data suggest that MCs, their tryptases, and their chymases play important roles in tissue repair. While MCs initially promote healing, they can be detrimental if they are chronically stimulated or if too many MCs become activated at the same time. The possibility that MCs and their granule serine proteases contribute to the formation of keloid and hypertrophic scars makes them potential targets for therapeutic intervention in the repair of damaged skin.

1. INTRODUCTION

Although mast cells (MCs) were discovered more than a century ago by Nobel Laureate Paul Ehrlich (Ehrlich, 1878), the importance of these immune cells in homeostasis and pathogen defense was appreciated only recently. MCs are not abundant in any tissue (Metcalfe, Baram, & Mekori, 1997), and they complete their development only after their poorly granulated progenitors home to tissues (Fig. 6.1). Thus, the inability to obtain sufficient numbers of *in vivo*-differentiated mature MCs for study greatly hindered our

[1]Corresponding author: rstevens@rics.bwh.harvard.edu.

understanding of the importance of these cells and why they had been conserved for more than 500 million years of evolution. A contributing factor that prevented the identification of MC-restricted genes and transcripts was the observation that mature, *in vivo*-differentiated MCs contained very little mRNA relative to the contaminating cells in varied tissue preparations.

The discovery in the 1980s that the T-cell-derived factor interleukin (IL)-3 selectively promoted the viability, proliferation, and differentiation of a pluripotent population of mouse MCs from their hematopoietic progenitors that were free of contaminating cells finally facilitated the generation of large numbers of MCs for study (Razin, Cordon-Cardo, & Good, 1981; Razin, Ihle, et al., 1984; Schrader, Lewis, Clark-Lewis, & Culvenor, 1981). These *in vitro*-generated mouse bone marrow-derived MCs (mBMMCs) were less mature than those in the jejunum, skin, and other connective tissues. Nevertheless, the ability to generate nontransformed MCs *in vitro* from wild-type (WT) and transgenic mice on different genetic backgrounds allowed detailed studies on the developmental control and functions of these cells at the molecular level. The resulting data led to a better understanding of the importance of mouse MCs and their human equivalents in acquired and innate immunity, inflammation, and blood coagulation. The observation that IL-3-developed mBMMCs contained more mRNA on a per cell basis than mature *in vivo*-differentiated MCs enabled the identification and cloning of many of the MC's mediators (e.g., mouse MC protease (mMCP)-5 (McNeil, Austen, Somerville, Gurish, & Stevens, 1991)), receptors (e.g., gp49B1/Lilrb4; Katz et al., 1996), and intracellular signaling proteins (e.g., RasGRP4; Yang et al., 2002). IL-3-developed mBMMCs have been used in nearly 1000 peer-reviewed publications. Since it was subsequently found that similar populations of MCs could be generated from fetal liver (Razin, Stevens, et al., 1984) and even embryonic stem cells (Tsai, Tam, Wedemeyer, & Galli, 2002), IL-3-dependent mouse MCs were particularly valuable for evaluating at the molecular level the functions of ubiquitously expressed proteins like Rac2 (Gu et al., 2002) and other intracellular signaling proteins that are critical for embryonic development. Thus, the *in vitro* method developed by Razin, Ihle, et al. (1984) for generating IL-3-dependent mouse MCs was a major technological advance.

The identification of "reaginic" immunoglobulin by the Ishizakas in the 1960s (Ishizaka, Ishizaka, & Hornbrook, 1966) led to the discovery that the IgE-dependent activation of MCs can result in life-threatening systemic anaphylaxis. The generation of mBMMCs and numerous variants (McGivney, Crews, Hirata, Axelrod, & Siraganian, 1981) of the transformed RBL-1 rat MC line (Eccleston, Leonard, Lowe, & Welford, 1973) allowed investigators to deduce the mechanisms at the molecular level by which these cells participate in IgE-dependent reactions. More recent studies revealed that MCs are involved in many non-IgE-dependent processes. In that regard, some populations of mouse and human MCs can be induced to degranulate by thrombin via protease-activated receptor-1 (Par-1) (Razin & Marx, 1984; Vliagoftis, 2002), by IgG complexes via FcγRIIa or FcγRIIIa (Malbec & Daeron, 2007), by ATP via P2X, P2Y, and adenosine receptors (Forsythe & Ennis, 1999; Kurashima et al., 2012; Sudo et al., 1996), and by complement-derived anaphylatoxins via the C3a and C5a receptors (el Lati, Dahinden, & Church, 1994; Erdei & Pecht, 1996) (Fig. 6.2). MCs express numerous Toll-like receptors (TLRs). While some

populations of mouse and human MCs that have been examined so far do not degranulate when exposed to the TLR ligand lipopolysaccharide (LPS), the treated cells release numerous proinflammatory cytokines and chemokines (Matsushima, Yamada, Matsue, & Shimada, 2004; McCurdy, Olynych, Maher, & Marshall, 2003). Whether or not MCs are active participants in the inflammation, proliferation, and/or remodeling stages of wound healing remains an area of investigation. In this review, we present recent literature that details the diverse functions of MCs and their protease mediators that help orchestrate this complex process.

2. DEVELOPMENT OF MCs

MCs originate from the $CD34^+$ pluripotent stem cells in the bone marrow and fetal liver (Arinobu et al., 2005; Kirshenbaum, Kessler, Goff, & Metcalfe, 1991; Kitamura, Shimada, & Go, 1979; Kitamura, Shimada, Hatanaka, & Miyano, 1977) (Fig. 6.1A). After exiting those compartments, the committed progenitors home to virtually every organ in the body (Fig. 6.1B). The number of MC-committed progenitors in the mouse is highest in the gut mucosa (Crapper & Schrader, 1983), presumably so that the mouse can quickly expand the number of MCs in the jejunum to combat helminth and bacterial infections. In support of this conclusion, the ability to expel the nematode *Trichinella spiralis* from the jejunum is delayed in infected mice lacking the chymase mMCP-1 (Knight, Wright, Lawrence, Paterson, & Miller, 2000).

In vivo-differentiated MCs are heterogeneous, and several polarized subsets have been identified in rodents and humans. These subsets were initially distinguished in rats histochemically (Enerback, 1966), ultrastructurally (Enerback & Lundin, 1974; Friend et al., 1996), and biochemically, based on their expression of different protease–serglycin proteoglycan (SGPG) complexes in their granules (Le Trong, Parmelee, Walsh, Neurath, & Woodbury, 1987; Stevens et al., 1986; Woodbury et al., 1978; Yurt, Leid, & Austen, 1977), as well as by their ability to generate varied types of eicosanoids (Heavey et al., 1988; Lewis et al., 1982), cytokines, and chemokines (Fig. 6.2).

MCs undergo the final stages of differentiation and maturation in their target tissues (Fig. 6.1B). These processes are controlled in tissue-specific manners, resulting in distinct phenotypes in different tissues (Friend et al., 1998; Gurish et al., 1995; Nakano et al., 1985; Xing, Austen, Gurish, & Jones, 2011). The greatest numbers of mature MCs are present in the tongue, skin, and respiratory and gastrointestinal tracts (Kube, Audige, Kuther, & Welle, 1998), which are at the interfaces of the host and external environments. The MCs in mice, rats, and humans are heterogeneous cells, and the varied tissue microenvironments where they reside ultimately determine the phenotypes, and thereby functions, of these cells during their life spans (Gurish et al., 1995; Kanakura et al., 1988; Nakano, Kanakura, Asai, & Kitamura, 1987; Nakano et al., 1985; Otsu et al., 1987; Sonoda et al., 1986; Xing et al., 2011).

Although constitutive $heparin^+$ MCs are long-lived cells in tissues (Padawer, 1974), they and other populations of *in vivo*- and *in vitro*-differentiated MCs can reversibly alter their phenotypes, especially which types of SGPGs and enzymatically active proteases they store

in their secretory granules (Friend et al., 1996, 1998; Ghildyal, Friend, Nicodemus, Austen, & Stevens, 1993; Gurish et al., 1995; Sonoda et al., 1986; Stevens & Austen, 1982). The mature MCs in the rat peritoneum contain SGPGs that preferentially have heparin glycosaminoglycans (GAGs) (Yurt et al., 1977), whereas the mature MCs in the jejunum of helminth-infected rats have SGPGs that preferentially have chondroitin sulfate-diB/E GAGs (Enerback, Kolset, Kusche, Hjerpe, & Lindahl, 1985; Stevens et al., 1986). The first demonstration that a mature MC could reversibly alter what types of mediators it stores in its secretory granules occurred in 1982 when Stevens and Austen showed that purified rat peritoneal MCs could be induced to rapidly switch their biosynthesis of heparin to chondroitin sulfate E when exposed to *p*-nitrophenyl-β-D-xyloside (Stevens & Austen, 1982).

Polarized subsets of MCs exist in mice, rats, and humans, but *in situ* hybridization studies revealed that even a histochemically identical population of MCs can differ substantially at the individual cell level in terms of what cytokine transcripts they express when activated via their high-affinity IgE receptors (Gurish et al., 1991). Because of the substantial plasticity in their development and the heterogeneous nature of mediator generation when activated via different mechanisms, it is possible that no two MCs in a mouse or human are identical.

In normal skin, fully developed MCs are preferentially found in the dermis and hypodermis. Maturation of MCs and their ability to exocytose their varied mediators depend on the cell's expression of numerous receptors which can respond to the diverse range of factors and pathogens that these cells encounter (Moon et al., 2010). IL-1β, IL-3, IL-4, IL-6, IL-9, IL-10, IL-33, nerve growth factor, substance P, transforming growth factor-β (TGF-β), kit ligand (Kitlg)/stem cell factor, glucocorticoids, and interferons are some of the many endogenous factors that regulate the development and function of mouse and human MCs (Fig. 6.1B).

In regard to the trafficking and homing of MC-committed progenitors into various tissues, Gurish and colleagues demonstrated that the presence of these precursor cells in the intestine of the mouse was dependent on the expression of the α4β7 integrin and the chemokine receptor Cxcr2 on the surface of the MC progenitor (Abonia et al., 2005; Gurish et al., 2001). The expression of Madcam1 and Vcam1 on the intestinal endothelium also was critical for cellular recruitment. In contrast, the inflammation-induced trafficking of MC-committed progenitors into the lung required the expression of the α4β7 and α4β1 integrins, as well as the activation of Ccr2/Ccl2 signaling pathways (Abonia et al., 2006; Collington et al., 2010; Hallgren et al., 2007). MCs and their progenitors also use the α4β1, α5β1, and α6β1 integrins to adhere to laminin- and fibronectin-rich extracellular matrices (ECMs) (Fehlner-Gardiner, Uniyal, von Ballestrem, & Chan, 1996; Fehlner-Gardiner, Uniyal, von Ballestrem, Dougherty, & Chan, 1996). Aside from those in the intestine and lung, other cytokines (e.g., TGF-β) and chemokines (e.g., Rantes/Ccl5 (Fig. 6.1B) and eotaxin/Ccl11) were found to be important in the recruitment and accumulation of MC-committed progenitors in the skin and other tissues. The movement of senescent mature MCs from a tissue, as occurs in the jejunum in the recovery phase of a helminth infection (Friend, Gurish, Austen, Hunt, & Stevens, 2000), to the draining lymph nodes and then the spleen is

likely to be dependent on some of these same chemokines and their receptors. Senescent MCs undergo nuclear segmentation during their translocation via the blood to the spleen when they have outlived their usefulness, as occurs in the recovery phase of a helminth infection (Friend et al., 2000). Because normal peripheral blood basophils are histamine$^+$/ FcεRI$^+$ granulocytes that have a segmented nucleus, senescent MCs sometimes have been mistaken as basophils.

Besides integrins, cell adhesion molecule-1 (Cadm1)/SgIGSF is a plasma membrane protein that MCs and their progenitors also use to adhere to fibroblasts and other mesenchymal cells (Ito et al., 2003). Of equal importance is the tyrosine kinase receptor Kit/CD117 on the surface of MCs (Geissler, Ryan, & Housman, 1988) which recognizes Kitlg on the plasma membrane of fibroblasts and other mesenchymal cells (Flanagan & Leder, 1990; Martin et al., 1990). Ligand binding to Kit leads to receptor dimerization and autophosphorylation which, in turn, leads to the activation of micropthalmia transcription factor (Mitf) and other downstream intracellular proteins that are necessary for maintaining the viability, development, proliferation, and function of MCs (Galli, Zsebo, & Geissler, 1994; Ronnstrand, 2004; Tsai et al., 1991). Mitf is particularly important in the transcription of the genes that encode a number of the serine proteases stored in the secretory granules of MCs (Funaba et al., 2003; Jippo et al., 1999; Morii et al., 1996, 1997; Murakami, Ikeda, Ogawa, & Funaba, 2003), as are PU.1, c-jun, and the GATA family of transcription factors (Kim & Lee, 2004; Walsh et al., 2002; Zon et al., 1991). Thus, Kitlg-defective WCB6F$_1$/J-*Kit*^{Sl}/ *Kit*$^{Sl\text{-}d}$ and Kit-defective WBB6F$_1$-*Kit*^{W}/*Kit*$^{W\text{-}v}$ (*Kit*$^{W/W\text{-}v}$) and C57BL/6-*Kit*$^{W\text{-}sh}$ mice constitutively have markedly reduced numbers of protease-rich MCs in their tissues (Kitamura, Go, & Hatanaka, 1978; Tsai, Grimbaldeston, & Galli, 2011; Wolters et al., 2005), as do *mi/mi* mice that produce an abnormal isoform of Mitf (Ebi et al., 1990). The demonstration that the MC deficiency in the stomach (Sonoda et al., 1986) and peritoneal cavity (Nakano et al., 1985, 1987; Otsu et al., 1987) of the adult Kit$^{W/W\text{-}v}$ mouse could be corrected, in part, by the adoptive transfer of *in vitro*-differentiated mBMMCs developed from histocompatible Kit$^+$mice allowed investigators new opportunities to better understand the importance of protease-rich MCs in a living animal. Numerous transformed mouse-, rat-, and human-cultured MC lines were found to have an activating gain-of-function mutation in a portion of the *Kit* gene that encodes the intracellular domain of this tyrosine kinase receptor (Tsujimura et al., 1995). The significance of this cytokine receptor in the development of human MCs was conclusively shown in the 1990s when it was discovered that most patients with systemic mastocytosis have a comparable mutation in their *KIT* gene (Longley et al., 1996; Nagata, Okada, Worobec, Semere, & Metcalfe, 1997).

Although Kitlg/Kit/Mitf-signaling pathways play vital roles in the survival and expansion of mouse MCs and their progenitors in tissues and in the expression of their granule proteases, other surface receptors are required for MCs to develop into their varied polarized subsets. For example, Kit$^{W/W\text{-}v}$ mice have increased numbers of MCs in their tissues when the T-cell cytokine IL-3 is present in abundance, as occurs in idiopathic chronic dermatitis (Galli, Arizono, Murakami, Dvorak, & Fox, 1987), due to the fact that IL-3/ IL-3Rα- and Kitlg/Kit-dependent signaling pathways in MC-committed progenitors are similar (Welham & Schrader, 1992). Immature mBMMCs also undergo further differentiation and granule

maturation when they encounter IL-9 (Eklund, Ghildyal, Austen, & Stevens, 1993), IL-10 (Ghildyal et al., 1993), or fibroblasts (Levi-Schaffer, Austen, Gravallese, & Stevens, 1986; Levi-Schaffer et al., 1987). Initially, it was thought that the ability of fibroblasts to induce granule maturation was solely due to its expression of Kitlg. Kitlg is required for the early stages of MC differentiation, and this cytokine is needed to maintain the viability of mBMMCs in culture medium lacking IL-3 (Galli et al., 1994). Nevertheless, exposure of IL-3-developed mBMMCs to recombinant Kitlg alone did not lead to granule maturation *in vitro* (Gurish et al., 1992). The key fibroblast-derived factor required for that process in the mouse is IL-33 which recognizes the receptor ST-2/IL1RL1 on the surface of the immature MC (Kaieda et al., 2010) (Fig. 6.1B).

MCs also can respond to inhibitory signals that are controlled by CD200 (Zhang, Cherwinski, Sedgwick, & Phillips, 2004; Zhang & Phillips, 2006), Lilrb4/gp49B1 (Katz et al., 1996), FcγRIIb (Malbec, Fridman, & Daeron, 1999), and IRp60/CD300a (Bachelet, Munitz, Moretta, Moretta, & Levi-Schaffer, 2005). Hence, the development and functional responses of a protease-rich MC in any particular situation, such as a wound, are regulated in complex ways which must be evaluated in the context of both the stimulatory and inhibitory factors present in the tissue at that time.

3. SECRETORY GRANULE PROTEASES OF HUMAN AND MOUSE MCs

The neutral proteases that reside in the secretory granules constitute ~50% of the total protein content of a mature, *in vivo*-differentiated connective tissue MC in all examined species. Mouse MCs express different combinations of mMCP-1 to -11, transmembrane tryptase/tryptase-γ/protease serine member S (Prss) 31, cathepsin G, granzyme B, neuropsin/Prss19, and carboxypeptidase A3 (Cpa3) (Table 6.1) (Chu, Johnson, & Musich, 1992; Hunt et al., 1997; Lützelschwab, Huang, Kullberg, Aveskogh, & Hellman, 1998; McNeil et al., 1991, 1992; Reynolds, Gurley, Austen, & Serafin, 1991; Reynolds, Stevens, et al., 1989; Serafin et al., 1990, 1991; Trong et al., 1989; Wong et al., 1999; Wong, Yang, Yasuda, Li, & Stevens, 2003; Wong, Yasuda, Morokawa, Li, & Stevens, 2004). Most, if not all, of these 16 neutral proteases are packaged in the cell's acidic granules ionically bound to heparin- (Forsberg et al., 1999; Humphries et al., 1999) or chondroitin sulfate E/diB- (Enerback et al., 1985; Razin, Stevens, Akiyama, Schmid, & Austen, 1982; Stevens et al., 1986) bearing SGPGs (Abrink, Grujic, & Pejler, 2004; Stevens, Nicodemus, & Avraham, 1990).

When MCs are activated, the majority of their exocytosed protease–SGPG macromolecular complexes remain intact in the ECM for hours (Ghildyal et al., 1996) (Fig. 6.3). Due to their large size, most of these macromolecular complexes are retained near the degranulated MC, thereby limiting their biological effects to the local microenvironment. The exceptions in the mouse are the chymase mMCP-1 (Wastling, Scudamore, Thornton, Newlands, & Miller, 1997) and the tryptase mMCP-7 (Ghildyal et al., 1996). Because the binding of these two granule proteases to SGPGs is weak at neutral pH, small amounts of these proteases can reach the circulation when they are exocytosed from activated tissue MCs. Prss31 (Caughey et al., 2000; Wong et al., 1999) is another exception due to its novel C-terminal membrane-spanning domain. This tryptase is preferentially retained at the outer leaflet of the plasma

membrane of the degranulated MCs, thereby allowing Prss31 to regulate those cell types which MCs physically contact and interact (Wong et al., 2002).

The hTryptase-β family of tetramer-forming serine proteases, which are derived from the homologous *TPSAB1* and *TPSB2* genes on human chromosome 16p13.3 (Table 6.1), are the major neutral proteases present in human MCs (Miller, Moxley, & Schwartz, 1990; Pallaoro, Fejzo, Shayesteh, Blount, & Caughey, 1999; Schwartz, Lewis, & Austen, 1981; Vanderslice et al., 1990). While the homologous *TPSD1* gene is also present on human chromosome 16p13.3 (Pallaoro et al., 1999), the last exon of this gene has a premature translation–termination codon which causes the expression of a truncated tryptase that has greatly reduced enzymatic activity. Numerous point mutations in the human *TPSAB1* and *TPSB2* genes have been identified in recent years by the *Human Genome Consortium* that result in the expression of >25 different protein isoforms of each tetramer-forming enzyme. Because the functional significance of these allelic isoforms has not been deduced experimentally, investigators generally refer to the enzymatically active products of the human *TPSAB1* and *TPSB2* genes collectively as hTryptase-β. Complicating the situation in humans, novel isoforms of hTryptase-β have been identified that are the result of differential splicing of their precursor transcripts (Jackson et al., 2008). Studies carried out in the 1980s and 1990s used native hTryptase-β purified from pooled skin and/or lung biopsies. It is now known that those preparations contained numerous functionally distinct enzymes derived from polymorphic genes and differentially spliced transcripts. It is therefore difficult to interpret the data reported in many of those earlier studies, even if one assumes that the preparations were not contaminated with other biologically active factors (e.g., the tryptases Prss22 (Wong et al., 2001) and Prss31 (Caughey et al., 2000; Wong et al., 2002)).

However, human MCs also store other mediators in their secretory granules ionically bound to SGPGs. As occurs in mouse MCs, the presence of these mediators can differ considerably. For example, the hTryptase-β^+ MCs in human skin often contain substantial amounts of PRSS31 (Caughey et al., 2000; Wong et al., 1999), CPA3 (Reynolds, Gurley, et al., 1989), and the chromosome 14q11.2 family members cathepsin G (Schechter et al., 1990), granzyme B (Strik et al., 2007), and chymase-1 (CMA1) (Caughey, Zerweck, & Vanderslice, 1991; Urata et al., 1991). As occurs in mice, the neutral proteases present in human MCs are highly restricted, and only 15 and 4 of the >8.7 million ESTs in the current database originated from the respective PRSS31 (Caughey et al., 2000; Wong et al., 1999) and CMA1 (Caughey et al., 1991) genes (see GenBank UniGene Hs.592076 and Hs. 135626). In support of these EST data, the levels of the PRSS31 and CMA1 transcripts are below detection in normal peripheral blood histamine$^+$/FcεRI$^+$ human basophils (see microarray data at http://www.nch.go.jp/imal/GeneChip/public.htm).

The primordial gene that gave rise to the human CMA1 gene expanded in mice to give rise to the eight genes on mouse chromosome 14C3 that encode mMCP-1 (Trong et al., 1989), mMCP-2 (Serafin et al., 1990), mMCP-3/L (Serafin et al., 1991), mMCP-4 (Serafin et al., 1991), mMCP-5 (McNeil et al., 1991), mMCP-8 (Lützelschwab et al., 1998), mMCP-9 (Hunt et al., 1997), and mMCP-10/Cma2 (Chu et al., 1992). Because a similar situation occurs in rats (Lützelschwab, Pejler, Aveskogh, & Hellman, 1997), the expansion of the serine protease gene complex on chromosome 14C3 took place prior to the evolutionary

divergence of rats and mice. The *Human* and *Mouse Genome Consortiums* concluded that the mouse ortholog of human CMA1 is mMCP-5 due to the highest degrees of similarities of their genes and translated products. In support of that conclusion, CMA1 and CPA3 are almost always coexpressed in human MCs, as occurs for mMCP-5 and Cpa3 in mouse MCs. Moreover, targeted inactivation of the *mMCP-5* gene adversely impacts the storage of Cpa3 protein in mouse MCs and vice versa. Nevertheless, the substrate preference of mMCP-4 is more similar to that of human CMA1 (Andersson, Karlson, & Hellman, 2008; Kunori et al., 2002). Thus, most studies carried out on mice that have attempted to obtain insight into the function of human CMA1 in inflammation and wound healing have used transgenic animals that lack mMCP-4 or mMCP-5.

The biologically active proteins that are most critical to our existence are regulated at multiple levels. There are no proteins in the body that are more tightly regulated than the MC's serine proteases. Numerous studies revealed that epigenetic, transcriptional, posttranscriptional, translational, and post-translational mechanisms are used to control the expression, activation, and granule storage of the MC's proteases in defined molar amounts. For example, even though the genes that encode mMCP-1, mMCP-2, and mMCP-4 are transcribed in IL-3-developed mBMMCs, the levels of their transcripts are below detection by blot analysis because of a cytokine- and glucocorticoid-regulated posttranscriptional mechanism that dominantly controls their stability (Eklund et al., 1997; Xia, Ghildyal, Austen, & Stevens, 1996). Outside of the activated MC, the levels of the cell's exocytosed proteases and their enzymatic activities are controlled by protease inhibitors (Itoh, Ide, Ishikawa, & Nawa, 1994; Pemberton, Huntley, & Miller, 1998; Pemberton, Wright, Knight, & Miller, 2006; Wong et al., 2002) and the ability of bystander macrophages, fibroblasts, and endothelial cells in tissues to endocytose the MC-derived protease–heparin SGPG complexes and destroy them in their primary lysosomes (Atkins, Friedman, & Metcalfe, 1985; Atkins & Metcalfe, 1983; Fabian, Bleiberg, & Aronson, 1978). Thus, varied mechanisms are used in the different tissue environments to control the half-lives and tissue retention of the exocytosed protease–SGPG complexes.

Human MCs were initially classified as MC_T or MC_{TC} by Schwartz and his coworkers (Irani, Schechter, Craig, DeBlois, & Schwartz, 1986) based on whether or not they contained detectable amounts of CMA1 in their secretory granules. This simplistic subset classification is rarely used today because it led to the erroneous conclusion that there were only two subsets of MCs in humans and that these phenotypically different populations of MCs originated from distinct progenitors. For example, it is now known that some heparin$^+$/tryptase$^+$/CMA1$^+$ human MCs store substantial amounts of CPA3 in their granules (Irani, Goldstein, Wintroub, Bradford, & Schwartz, 1991), whereas others do not (Abonia et al., 2010), even though all MCs originate from a common progenitor. We now appreciate that, like what occurs in the mouse, different populations of human MCs can vary considerably in terms of the number and types of mediators they produce when activated. Thus, one of the major challenges is to understand how tissues and cells orchestrate appropriate biological responses to so many MC-derived biologically active factors.

MCs use an extensive array of surface and intracellular receptors to react to changes in their tissue microenvironments. Triggering of these receptors often leads to degranulation, which

is characterized by the extrusion of the contents of the cell's cytoplasmic protease-rich granules (Rivera & Gilfillan, 2006) (Figs. 6.2 and 6.3) which can have both beneficial and adverse consequences. FcεRI is the main receptor on the surface of MCs that binds IgE (Kinet, 1999; Metzger, Goetze, Kanellopoulos, Holowka, & Fewtrell, 1982). Antigen crosslinking of this receptor-bound immunoglobulin leads to activation of signaling pathways that are the primary cause of MC-dependent hypersensitivity reactions *in vivo* (Kraft & Kinet, 2007), including life-threatening systemic anaphylaxis. MCs also can be activated via non-FcεRI mechanisms. In those situations, the exocytosed mediators promote tumorigenesis (Chang et al., 2011; Sinnamon et al., 2008), arthritis (Lee et al., 2002; McNeil et al., 2008; Pimentel, Sampaio, D'Acquisto, Perretti, & Oliani, 2011; Shin et al., 2009), colitis/inflammatory bowel disease (Hamilton et al., 2011), autoimmune disease (Christy & Brown, 2007), heart disease (Kovanen, 2007; Sun et al., 2007, 2009; Zhang et al., 2011), and even cigarette smoke-induced chronic obstructive pulmonary disease (COPD; Beckett et al., 2013).

Despite their adverse roles in many inflammatory diseases, MCs and their exocytosed proteases have beneficial roles in bacterial (Huang et al., 2001; Thakurdas et al., 2007), helminth (Knight et al., 2000; Pennock & Grencis, 2006; Shin et al., 2008), and pneumovirus infections (Ptaschinski and Foster, unpublished findings). Human MCs and their progenitors express the HIV-1 coreceptors CD4, CCR3, CCR5, and CXCL-4, and therefore are susceptible to M-tropic strains of the retrovirus (Bannert et al., 2001; Li et al., 2001; Taub et al., 2004). Considering the importance of the tetramer-forming tryptases in innate and acquired immunity, the HIV-1 infection of MCs and their progenitors is now believed to be a contributing factor in the inability of AIDS patients to combat opportunistic infections efficiently.

4. MC INVOLVEMENT IN WOUND HEALING

Wound healing conventionally has been divided into three stages that have been designated as the inflammation phase, proliferation phase, and maturation/remodeling phases (Schilling, 1976) (Fig. 6.4). Growing evidence has implicated MCs and their protease mediators in all three aspects of the wound-repair process (Ng, 2010; Nishikori et al., 1998; Noli & Miolo, 2001, 2010; Younan et al., 2010).

4.1. Inflammation phase

Two to eight percent of the cells in healthy skin are mature MCs. They are located in both the hypodermis and the dermis in the vicinity of nerves, hair follicles, and blood vessels (Weber, Knop, & Maurer, 2003) (Fig. 6.3), and are activated following tissue injury (el Sayed & Dyson, 1993). The exocytosis of their granule mediators is an early step of the wound-induced inflammatory cascade, as evidenced by the decreased staining intensity of the protease–SGPG complexes in the cell's secretory granules 24 h after wound initiation. Those MCs in close proximity to the site of the tissue injury underwent more extensive degranulation (Weller, Foitzik, Paus, Syska, & Maurer, 2006) (Fig. 6.3).

Activated MCs release mediators that can induce bystander cells to increase their expression of Kitlg, IL-3, IL-33, and other factors which then regulate MCs by feedback-loop

mechanisms (Fig 6.1B). This situation ultimately affects the viability and function of MCs and their progenitors at the site of injury (Baghestanian et al., 1997; Metcalfe et al., 1997). The increased numbers of MCs in and around a wound could be due, in part, to the local differentiation of nongranulated progenitors. However, studies suggest that the increase in the number of MCs at the site of a wound is primarily due to the migration of mature MCs from nearby connective tissues. Not only do these MCs lack the Mki67 antigen that is expressed in proliferating cells (Trautmann, Toksoy, Engelhardt, Brocker, & Gillitzer, 2000), they are fully granulated (Ghildyal et al., 1993). In support of this conclusion, injection of the chemokine Ccl5 into the muscle of a WT mouse (but not an MC-deficient $Kit^{W/W\text{-}v}$ mouse) resulted in the rapid accumulation of mature MCs at the site of instillation (Conti, Reale, Barbacane, Letourneau, & Theoharides, 1998).

The thrombin-dependent conversion of fibrinogen to fibrin is a crucial initial aspect of wound healing following tissue injury. Immediately after a wound has been created, the body tries to minimize bleeding and the entry of pathogens by constricting blood vessels at the site of injury and initiating the coagulation cascade. Once that occurs, there is an increase in the permeability of blood vessels surrounding the injured area to allow the perfusion of the damaged tissue with beneficial plasma-derived factors, as well as to facilitate the accumulation of effector cells like neutrophils which are needed to hinder the local growth of bacteria and other pathogens (Broughton, Janis, & Attinger, 2006). MCs were shown to be involved in both responses due, in part, to the complement-derived anaphylatoxins C3a and C5a which can induce MC degranulation (Johnson, Hugli, & Muller-Eberhard, 1975) and chemotaxis (Hartmann et al., 1997). The inflammatory response that occurs after a burn to the epidermis is dependent on the binding of natural IgM autoantibodies to newly expressed epitopes in the damaged connective tissue which then leads to complement activation (Suber, Carroll, & Moore, 2007). It therefore is likely that natural IgM autoantibodies also participate in the healing process of surgically wounded skin.

Degranulated MCs rapidly induce vascular permeability and subsequently the accumulation of plasma-derived fibrinogen into the wounded tissue, which then leads to its thrombin-dependent conversion to fibrin (Mekori & Galli, 1990) and clotting. Histamine and eicosanoids released from activated MCs are major factors affecting vascular permeability (Maekawa, Austen, & Kanaoka, 2002). However, other MC-derived factors also have been implicated. For example, microvascular leakage occurred by an unknown mechanism when an MC tetramer-forming tryptase was injected into the skin of a guinea pig (He & Walls, 1997).

Once a clot is created at the wound as an external barrier, it is more important to prevent further accumulation of fibrin in order to avoid life-threatening thrombus formation. To that end, MCs prolong bleeding time (Kauhanen, Kovanen, Reunala, & Lassila, 1998) and prevent the excess accumulation of fibrin and fibrin–platelet clots in the damaged skin by exocytosing their enzymatically active tryptase–heparin complexes which degrade fibrinogen before substantial amounts of this plasma protein can be converted to fibrin by thrombin (Huang et al., 1997; Prieto-Garcia et al., 2012; Thomas, Wheeless, Stack, & Johnson, 1998). Heparin also contributes to the anticoagulation cascade by catalyzing the

antithrombin-III-dependent inactivation of thrombin (Rosenberg, 1978). Mouse MCs express Par-1 (Razin & Marx, 1984; Vliagoftis, 2002), and use this thrombin receptor to exocytose their fibrinogen-destroying tryptase–heparin complexes in situations where the local concentration of thrombin is unusually high. In support of these mouse data, some pediatric mastocytosis patients who have an excess of hTryptase-β^+/heparin$^+$ MCs in their tissues have excessive bleeding of their skin and gastrointestinal tract (Kettelhut & Metcalfe, 1991). Moreover, MC tryptase-null mice contained more fibrin deposits in their skin relative to WT mice 6 h after the two groups of IgE-sensitized animals were given antigen in a passive cutaneous anaphylaxis reaction (Prieto-Garcia et al., 2012).

Several lines of evidence suggest that the release of MC protease mediators during an inflammatory stimulus promotes the rapid accumulation of neutrophils. This was observed in the joints of mice during experimental arthritis (Lee et al., 2002; Pimentel et al., 2011) and in the skin during urticarial reactions (Nakamura et al., 2009). Although MC-deficient *Kit*$^{W/W\text{-}v}$ mice have fewer neutrophils in their injured skin relative to their Kit$^+$ littermates (Egozi, Ferreira, Burns, Gamelli, & Dipietro, 2003; Weller et al., 2006), these mice constitutively have decreased numbers of the circulating granulocytes (Nigrovic et al., 2008; Zhou, Xing, Friend, Austen, & Katz, 2007), thereby complicating data interpretation. Nevertheless, the roles of tryptase–SGPG complexes released from activated MCs dictating the accumulation of neutrophils in the tissues of Kit-sufficient mice have been firmly established. For example, the injection of nMol amounts of LPS-free mMCP-6– or hTryptase-β–heparin complexes into different tissue sites of a naïve WT or *Kit*$^{W/W\text{-}v}$ mouse results in the rapid recruitment of large numbers of neutrophils (Huang et al., 1998, 2001). Furthermore, the pharmacologic inactivation of mMCP-6 resulted in diminished numbers of granulocytes in the antigen-treated airways of WT mice (Oh et al., 2002).

A major problem encountered with studies carried out on MC-deficient mice (including *CPA*$^{Cre/+}$ mice; Feyerabend et al., 2011) is that these immune cells produce numerous factors that have contrasting bioactivities (e.g., anti-inflammatory prostaglandin D_2 and IL-10 versus proinflammatory mMCP-6/hTryptase-β and tumor necrosis factor-α). In addition, the release of these factors from a MC is controlled by a delicate balance between stimulation of the cell's activating receptors (e.g., FcεRI, FcγRIIa, FcγRIIIa, Par-1, and C5aR1/CD88) versus stimulation of the cell's inhibitory receptors (e.g., Lilrb4, FcγRIIb, CD200, and CD300a). Such counterbalancing factors and mechanisms cannot be appreciated when MC-deficient mice are used in studies. To address these deficiencies, a number of inbred C57BL/6 mouse lines have been created using homologous recombination approaches in which a single gene was disrupted in most instances. Confirming the importance of MC tryptases in immunity and inflammation, transgenic C57BL/6 mice that lack the tetramer-forming tryptases mMCP-6 and mMCP-7 had a diminished ability to recruit neutrophils into their bacteria-infected peritoneal cavities (Thakurdas et al., 2007), arthritic joints (McNeil et al., 2008; Shin et al., 2009), and inflamed colons (Hamilton et al., 2011) and lungs (Beckett et al., 2013) relative to WT mice.

The fact that mice and human MCs express three tryptases that have overlapping substrate specificities contributed to the lack of appreciation of these neutral proteases in different diseases. Protease redundancy is the likely reason why the adjacent *TPSAB1*, *TPSB2*, and

PRSS31/TPSG1 genes on human chromosome 16p13.3 were not identified in numerous quantitative trait locus and genomewide association studies carried out on humans with different inflammatory diseases. The mechanisms by which MC tryptases regulate granulocyte and/or macrophage accumulation in tissues remain to be determined at the molecular level but cultured endothelial cells, synovial fibroblasts, and bone marrow-derived macrophages markedly increased their expression of numerous chemokines when they briefly encountered tryptase–heparin complexes (Beckett et al., 2013; Compton, Cairns, Holgate, & Walls, 1998; Huang et al., 1998; Shin et al., 2009). In support of these *in vitro* data, the levels of a number of cytokines, chemokines, and matrix metalloproteinases (MMPs) were markedly reduced in the colons and lungs of mMCP-6$^{-/-}$/mMCP-7$^{-/-}$ C57BL/6 mice subjected to experimental colitis (Hamilton et al., 2011) or COPD (Beckett et al., 2013), respectively.

In the early inflammatory phase of a skin wound, the ECM proteins fibronectin and type-III collagen accumulate only to then decrease in the late inflammatory phase (Broughton et al., 2006). MC-derived chymases and tetramer-forming tryptases can degrade ECMs in indirect and direct manners in preparation for the proliferation phase of wound healing (Ng, 2010). In this regard, one of the targets of hTryptase-β and its related tryptases mMCP-6 and mMCP-7 is the C terminus of the α chain of fibrinogen (Huang et al., 1997; Prieto-Garcia et al., 2012; Thomas et al., 1998).

DuBuske, Austen, Czop, and Stevens (1984) showed that fibronectin is a preferential target of an undefined serine protease present in the secretory granules of IL-3-developed mMCP-4^{-}/mMCP-5^{+}/mMCP-6^{+}/mMCP-7^{+}/Prss31^{-} BALB/c mBMMCs. Because mBMMCs generated from mMCP-5-null C57BL/6 mice could not degrade fibronectin in this *in vitro* system, the relevant enzyme is mMCP-5 (Stevens, unpublished data). Fibronectin is also susceptible to degradation by mMCP-4 (Tchougounova, Pejler, & Abrink, 2003). The fact that the MCs that are constitutively present in normal human skin have substantial amounts of CMA1 (Schechter et al., 1994) raises the possibility that this serine protease proteolytically destroys fibronectin in the wounded skin when cutaneous MCs in humans degranulate, particularly in the late inflammation/early proliferation phase. These data suggest that some of the proteases exocytosed from activated cutaneous MCs alter integrin-dependent signaling pathways in tissues.

Finally, MC proteases can contribute to the inflammation phase by activating tissue-resident macrophages (Chen et al., 2002; Rodgers & Xiong, 1997). In this regard, *in vitro*-differentiated mouse macrophages markedly increased their expression of Cxcl1, IL-1β, and numerous other biologically active factors when briefly exposed to tryptase–heparin complexes (Beckett et al., 2013). In an experimental COPD model, mMCP-6 and mMCP-7 played prominent roles in the accumulation of macrophages, which is the major destructive cell in the disease. The importance of Prss31 in inflammation and wound-repair mechanisms has not been investigated in depth but the cigarette smoke-induced accumulation of macrophages in the diseased lungs also was significantly reduced in Prss31-null C57BL/6 mice (Hansbro and Stevens, unpublished observation). Prss31 is highly susceptible to inactivation by α1-antitrypsin (A1AT)/SERPINA1 (Wong et al., 2002). In support of the finding that Prss31 participates in experimental COPD in mice, humans that are deficient in

A1AT are at an increased risk of developing emphysema (Cohen et al., 1977). The latter data emphasize the importance of obtaining a better understanding of the factors and mechanisms that result in the inactivation and clearance of the serine proteases exocytosed from activated MCs in the lung, skin, and other tissues.

4.2. Proliferation phase

This aspect of wound healing is distinguished by the formation of granulation tissue, which involves fibroblast proliferation, angiogenesis, re-epithelialization, and increased deposition of type-I collagen in the ECM (Broughton et al., 2006). Here, MCs likely play critical roles by promoting the migration and proliferation of fibroblasts (Fig 6.1B), as was shown in an *in vitro* model (Kupietzky & Levi-Schaffer, 1996; Levi-Schaffer & Kupietzky, 1990). In this regard, exposure of cultured fibroblasts to increasing amounts of a MC chymase led to a dose-dependent increase in cellular proliferation (Dong, Chen, Zhang, & Cen, 2012). Similarly, treating dermal fibroblasts with a hTryptase-β–heparin complex isolated from human lung led to a dose-dependent increase in the number of fibroblasts, as well as the accumulation of type-1 collagen (Abe, Kurosawa, Ishikawa, Miyachi, & Kido, 1998). Similar results were obtained when fibroblasts encountered histamine (Hatamochi, Fujiwara, & Ueki, 1985; Hatamochi, Ueki, Mauch, & Krieg, 1991; Russel, Russell, & Trupin, 1977). Activated MCs also release basic fibroblast growth factor (Qu et al., 1998) which is a cytokine that promotes the proliferation of fibroblasts. Thus, MCs release numerous factors that synergistically affect the fibroblasts and other cell types in the wound to ultimately promote tissue repair.

The proximity between MCs and endothelial cells that line blood vessels facilitated the interactions between the two cell types and their production of various mediators. The impact of MCs on the vasculature supports a prominent role for these granulocytes. Angiogenesis is increased in mice that have been given the MC-degranulating agent compound 48/80 (Jakobsson, 1994; Norrby, Jakobsson, & Sorbo, 1986), and several of the mediators exocytosed from activated MCs promote angiogenesis. While heparin is required for the accumulation of many of the proteases in the MC's granules (Forsberg et al., 1999; Humphries et al., 1999), this GAG also promotes the migration of endothelial cells (Azizkhan, Azizkhan, Zetter, & Folkman, 1980) and vascular growth (Norrby & Sorbo, 1992), which can be reversed by the administration of the heparin antagonist protamine sulfate (Jakobsson, Sorbo, & Norrby, 1990). A similar vascular response was demonstrated with a MC chymase in a granulation-tissue model carried out on hamsters (Muramatsu, Katada, Hattori, Hayashi, & Majima, 2000). MC-derived proteases also contribute to angiogenesis by proteolytically destroying the ECM.

MCs have been associated with re-epithelialization and keratinization in several models. For example, they are modulators of hair-follicle cycling (Maurer et al., 1997). Consistent with this observation, $Kit^{W/W\text{-}v}$ mice have deficiencies in their hair-growth cycles (Maurer et al., 1997). Hair follicles are keratinocytic structures that are a part of the epithelial barrier. They are rich in stem cells, and they play significant roles in re-epithelialization during wound healing because they are a source of epithelial cells. Some of the mediators released from activated MCs can induce keratinocyte proliferation in the wounded skin, thereby inducing

re-epithelialization that begins at the edges of the wound. Histamine, IL-1α, -1β, -6, and tryptase–heparin complexes are MC-derived mediators that can affect epithelial cells, including their proliferation (Cairns & Walls, 1996; Gschwandtner et al., 2008; Katayama, Yokozeki, & Nishioka, 1992).

4.3. Maturation/remodeling phase

At the end of the proliferation phase and at the start of the maturation/remodeling phase, fibroblasts differentiate into myofibroblasts which are distinguished by the expression of α-smooth muscle actin (Moulin et al., 1998). In normal wound healing, this is usually a transient process important in the contraction and closure of the wound. MCs have been implicated in this process as well. For example, exposure of fibroblasts to the HMC-1 MC line resulted in increased expression of α-smooth muscle actin and increased fibroblast-driven contraction of a collagen gel (Gailit, Marchese, Kew, & Gruber, 2001). Histamine and tryptase partly mediate the latter process, as their addition to fibroblasts cultured in a collagen matrix also led to contraction. Yamamoto, Hartmann, Eckes, and Krieg (2000) concluded that the interaction of Kitlg on the surface of fibroblasts with Kit on the surface of MCs is needed for optimal gel contraction, as the addition of antibodies against either protein resulted in an ~70% reduction of this process. Moyer, Saggers, and Ehrlich (2004) reported the existence of a heterotypic gap junction between the two cell types, and these investigators found that exposure of the cocultured cells to a fatty acid amide hydrolase inhibitor led to diminished lattice contraction. Although these studies differ in explaining the primary mechanism as to how MCs and fibroblasts interact in wounded skin, they agree that both cell types must be present and in direct contact in order for collagen contraction to take place efficiently.

For proper remodeling of damaged connective tissue (e.g., surgically wounded skin) to occur, a delicate balance between the degradation, synthesis, and maturation of the ECM needs to be orchestrated so that the repair of damaged skin takes place in a timely manner with minimal scarring. MC proteases can promote the degradation of ECMs and disrupt the epidermal–dermal junction (Briggaman, Schechter, Fraki, & Lazarus, 1984) by both direct and indirect manners. In regard to the former, the hemidesmosomal transmembrane protein BP180/type XVII collagen (Lin et al., 2011) and the tight-junction protein claudin-4 (L.G. Bankova & M.F. Gurish, unpublished data) are targets of mMCP-4. Moreover, the rat mucosal MC chymase rMCP-II alters epithelial cell monolayer permeability in association with altered distribution of the tight junction proteins ZO-1 and occludin (Scudamore, Jepson, Hirst, & Miller, 1998). In support of these rat and mouse data, patients with bullous mastocytosis have increased numbers of degranulated MCs at the base of their subepidermal blisters (Golitz, Weston, & Lane, 1984; Kirshenbaum, Kettelhut, Metcalfe, & Garriga, 1989).

A number of the MC's serine proteases also can activate numerous metalloproteinase (MMP) zymogens (Lees, Taylor, & Woolley, 1994; Lin et al., 2011; Magarinos et al., 2013). Tetramer-forming MC tryptases, for example, can activate pro-stromelysin/MMP3 which, in turn, can activate latent procollagenase (Gruber et al., 1989) to ultimately cause connective tissue remodeling. The finding that hTryptase-β– and mMCP-6–heparin complexes can

activate pro-MMP3 and pro-MMP13 constitutively present in femoral head cartilage explants raises the possibility that these tryptases activate other members of the “typical” family of released MMPs (namely MMP-1, -3, -8, -10, -12, -13, -18, -19, -20, -22, and/or -27). The substrate preference of mMCP-4 is similar to that of human CMA1, and mMCP-4 has been implicated in the activation of pro-MMP2 and pro-MMP9 (Lin et al., 2011; Tchougounova et al., 2005). Similarly, human CMA1 can degrade ECMs indirectly by activating interstitial procollagenase/MMP1 (Saarinen, Kalkkinen, Welgus, & Kovanen, 1994). At least one MC chymase can activate latent TGF-β (Taipale, Lohi, Saarinen, Kovanen, & Keski-Oja, 1995; Zhao et al., 2008), which is a cytokine that counteracts the MMP-dependent degradation of the ECM. Thus, the proteases exocytosed from activated MCs in connective tissue remodeling can affect wounds in complex ways. Moreover, the complexity and redundancy outlined here further explains why the importance of MCs and their granule serine proteases in wound healing and varied inflammatory disorders were missed by quantitative trait locus and genomewide association studies.

Another event that takes place in the maturation/remodeling phase of wound healing is the crosslinking of the newly synthesized collagen that is deposited in the ECM of the repaired wound. These posttranslational modification events of collagen are the result of the lysyl oxidase-dependent modification of the protein’s lysine and hydroxylysine residues. The modification of hydroxylysine results in the formation of pyridinoline, and the levels of pyridinoline are markedly increased in hypertrophic scars (Moriguchi & Fujimoto, 1979). While the importance of MCs and their mediators in collagen maturation has not been investigated in depth, a proteomic study revealed that the levels of proly-4-hydroxylase in mouse 3T3 fibroblasts differed considerably when these mesenchymal cells were cocultured with mBMMCs generated from WT, mMCP-6-null, and mMCP-5-null C57BL/6 mice (R.L. Stevens, unpublished data). These data raise the possibility that MC granule proteases somehow influence the type and/or extent of collagen crosslinking in wounded skin in the maturation/remodeling phase of wound healing by regulating the expression of key biosynthetic enzymes in that process. In support of this conclusion, collagen accumulation in the airways of cigarette smoke-treated Prss31-null C57BL/6 mice was markedly reduced relative to that in the airways of similarly treated WT C57BL/6 mice (Hansbro, Hamilton, and Stevens, unpublished observation).

4.4. Temporal aspects of MC activation

Although the above evidence highlights the involvement of MCs and their protease mediators throughout the different phases of wound healing, their indispensability to the process remains a matter of debate due to the fact that most of these early studies were carried out on problematic $Kit^{W/W\text{-}v}$ mice. Comparing the effects of skin injury in MC-deficient $Kit^{W/W\text{-}v}$ mice and MC-sufficient Kit^{+} mice, Weller and coworkers noted that the absence of protease-rich MCs led to delayed closure of 6-mm wounds of the skin (Weller et al., 2006). Even though both groups of mice had complete wound closure by day 10, the wounds of $Kit^{W/W\text{-}v}$ mice only started to decrease in size 12 h after injury. In a study of microdeformation wound healing in Kit^{+} versus $Kit^{W/W\text{-}v}$ mice, the presence of MCs was associated with increased wound tissue granulation, cell proliferation, blood vessel sprouting, and collagen maturation (Younan et al., 2011).

In contrast, other investigators were unable to detect a significant difference in wound closure between Kit^{+} and $Kit^{W/W\text{-}v}$ mice when the rate of wound closure in punch biopsies was evaluated by histomorphometric analysis for re-epithelialization 3 days postinjury (Egozi et al., 2003). These contrasting findings highlight the temporal contribution of MCs in the repair mechanism, which is most noticeable in the acute aftermath of injury. As wound closure eventually occurred in both models, it is thought that mice have MC-independent fallback mechanisms to repair damaged connective tissue. Nevertheless, it is worthwhile to note that histological differences were found even though wound closure eventually occurred in both of the above models. In that regard, Iba and coworkers demonstrated that collagen aggregation at the wound edges was tighter and less interwoven in $Kit^{W/W\text{-}v}$ mice, as opposed to Kit^{+} mice, 20 days after wounding (Iba, Shibata, Kato, & Masukawa, 2004). It is anticipated that a better understanding of the importance of MCs and their specific mediators in the repair process will be obtained once follow-up studies are carried out on recently created transgenic mouse lines that lack specific MC mediators.

4.5. MCs and pathologic healing

Although MCs contribute to the different stages of wound healing in beneficial ways, they also can have detrimental roles, especially when they are chronically activated. In the skin, MCs have been studied in fibrotic processes that leads to scarring and the hypertrophy of scars (Kischer, Bunce, & Shetlah, 1978). Younan and coworkers noted pronounced differences in the wound healing patterns between MC-deficient $Kit^{W/W\text{-}v}$ and MC-sufficient Kit^{+} mice when these animals received a scald burn (Younan et al., 2010). The treated $Kit^{W/W\text{-}v}$ mice showed significantly less scarring and a reappearance of more hair follicles at day 13 relative to the treated Kit^{+} mice. Using transgenic mice, these differences were shown to be primarily due to mMCP-4 and mMCP-5.

Wulff and coworkers investigated wound healing in fetal mice (Wulff et al., 2012). They discovered that mice wounded at embryonic day 15 (E15) healed without scars. In contrast, those animals wounded at embryonic day 18 (E18) healed with scars. Interestingly, histologic analysis revealed fewer MCs in the dermis of the E15 mice. In support of an adverse role for an undefined preformed MC-derived mediator in the scarring process, the injection of a lysate of MCs into the E15 embryos led to scarring similar to that observed in wounded E18 mice. MC-deficient $Kit^{W/W\text{-}v}$ embryos wounded at E18 also showed significantly less scarring than MC-sufficient Kit^{+} embryos wounded at that time point.

Keloids are benign tumor-like scars with exuberant growth; they are *in vivo* models of abnormal healing of wounded skin. MCs have been implicated in this process, as histology revealed increased numbers of MCs in keloids (Shaker, Ayuob, & Hajrah, 2011). When keloids were treated with intralesional cryotherapy, improvement of the lesions was correlated with decreased numbers of MCs, as assessed histochemically (Har-Shai et al., 2011). Histamine levels were elevated in keloid lesions which may lead to the abnormal collagen crosslinking seen in such pathology (Placik & Lewis, 1992). Nevertheless, the primary mediators exocytosed from cutaneous MCs that promote keloid development have not been identified.

Coneely and coworkers evaluated MC degranulation in rats that had undergone surgical colon anastomosis (Coneely, Kennelly, Bouchier-Hayes, & Winter, 2010). These investigators found that intraperitoneal administration of compound 48/80 led to an increase in bursting pressure and hydroxyproline content of the hypoperfused bowel area, as well as stronger anastomosis. In a pathology-directed approach, Gallant-Behm and coworkers evaluated the effects of administering the MC stabilizer ketotifen on the contraction and fibrosis of wounded skin in two pig models (Gallant-Behm, Hildebrand, & Hart, 2008). The Yorkshire pig was studied because it is an animal whose wounded skin heals in a manner similar to that of normal humans. The red Duroc pig was studied because this animal heals with some features of human hypertrophic scars, including the pathologic contraction of skin. Gallant-Behm and coworkers found that ketotifen treatment reduced collagen deposition and wound contraction in Duroc pigs but not in Yorkshire pigs. It therefore was concluded that preventing the release of undefined mediators from activated MCs could help in the prevention of pathologic development of wounds, while maintaining physiologic healing.

5. CONCLUSIONS AND THERAPEUTIC DIRECTIONS

The involvement of MCs and their serine proteases in the physiology and pathology of inflammation and wound repair identifies these immune cells and their granule constituents as intriguing targets for therapy. MCs have the ability to respond to a variety of stimuli that are important for the different phases of wound healing. Notably, the beneficial roles of MCs are lost in some conditions (e.g., chronic stimulation). Detrimental effects can occur, and this is often dependent on inappropriate cellular activation during the varied phases of tissue repair. These data suggest that the beneficial roles of MCs in tissue repair are highly dependent on the proper regulation of cellular activation, what granule proteases they express, and how much and when these proteases are released. Nevertheless, we do not yet fully understood at the molecular level how MCs and their proteases promote wound healing and hinder scar formation. Thus, further studies employing transgenic mice that lack MC-specific mediators will be invaluable in dissecting the role of the cell in the wound-repair processes. Furthermore, identifying the MC-dependent pathways that regulate the processes associated with tissue repair has substantial therapeutic implications.

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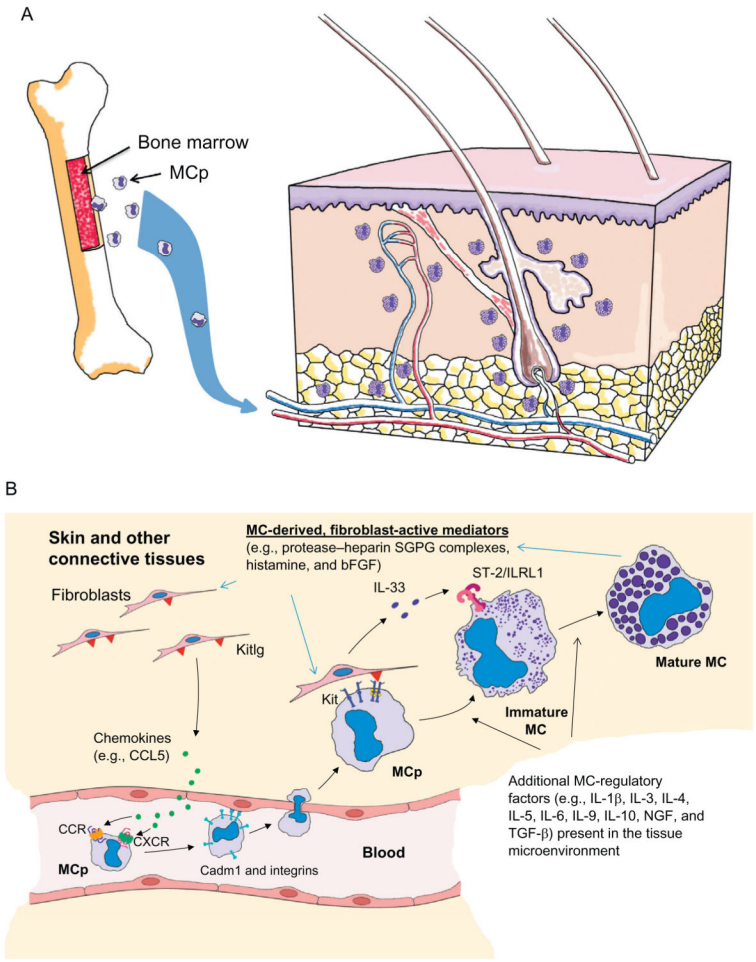

Figure 6.1.

Development of cutaneous MCs from their hematopoietic progenitors. The bone marrow (A) constitutively releases MC-committed progenitors (MCp) into the blood. These circulating, nongranulated $CD34^+$ progenitors enter the skin and other organs when they encounter the appropriate combination of chemokines (e.g., Ccl5) and other recruitment factors (B). The retention of these progenitors in tissues also is highly dependent on Kitlg (▼), Kit, Cadm1, and varied integrins (I). Once in the skin, the progenitors undergo their final stages of differentiation and maturation. This includes their expression and granule accumulation of different combinations of protease–SGPG complexes. The phenotype of an MC at any stage of its life span is highly dependent on the factors the mature cell and its progenitor encounter in the tissue microenvironments. A mature MC can even reversibility alter what genes and proteins it expresses. Although fibroblast-derived Kitlg and IL-33 are essential in the development of cutaneous MCs, other cytokines (e.g., TGF-β and IL-9) play important roles in determining what families of mediators are expressed.

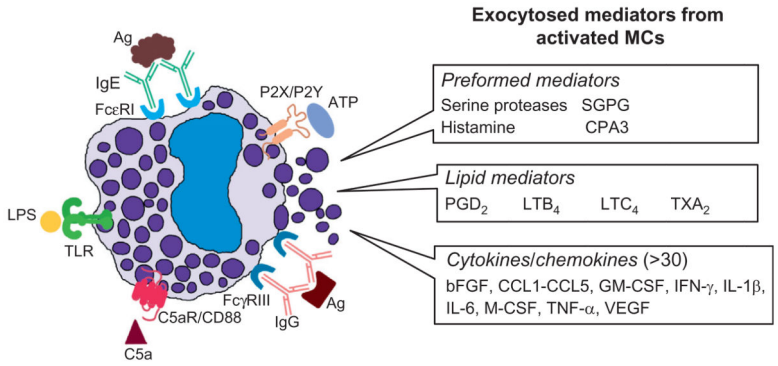

Figure 6.2.

Exocytosed mediators from activated MCs. The MCs that reside in the skin and other connective tissues contain numerous receptors on their plasma membranes that they use in innate (e.g., complement and TLRs) and acquired (e.g., FcεRI and FcγRIII) immunity. MCs also can recognize and respond to numerous endogenous factors like ATP that are often increased in wounded skin, as well as epitopes recognized by naturally occurring IgM autoantibodies. In the early phase, activated MCs rapidly exocytose the contents of their secretory granules and increase their expression of varied lipid mediators. In the late phase, the activated cell markedly increases the expression of numerous cytokines and chemokines. MCs also have inhibitory receptors that dampen cellular activation.

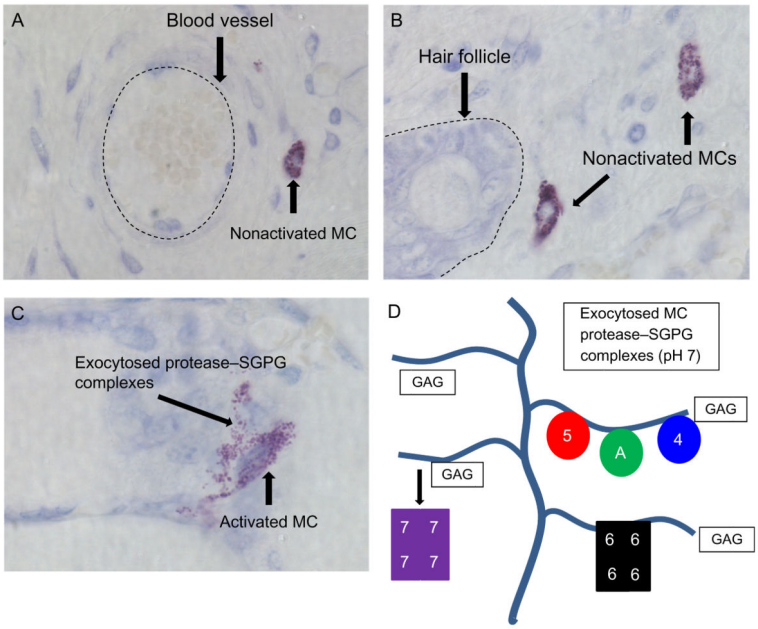

Figure 6.3.

Histochemistry of nonactivated and activated cutaneous MCs. Mature MCs are recognized in the skin and other tissues histochemically by the ability of their SGPGs to bind to toluidine blue and other cationic dyes. Cutaneous MCs tend to reside near blood vessels (A), hair follicles (B), and nerves (data not shown). When activated (C), cutaneous MCs quickly exocytose the content of their secretory granules which consist primarily of histamine and varied protease–SGPG complexes. Most of the cell's positively charged granule proteases (D) (e.g., mMCP-5 (5), Cpa3 (A), mMCP-4 (4), and mMCP-6 (6)) are ionically bound so tightly to the negatively charged glycosaminoglycans (GAGs) of SGPGs that the exocytosed macromolecular complexes remain intact for hours in the ECM. An exception is mMCP-7 (7), due to its less positively charged SGPG-binding domain. The large size of these protease–SGPG complexes minimize their diffusion and ability to enter the circulation. Instead, most of them are eventually endocytosed by other cell types in the inflammatory site where they are destroyed in primary lysosomes. The depicted images in panels (A)–(C) were from surgically wounded mouse skin.

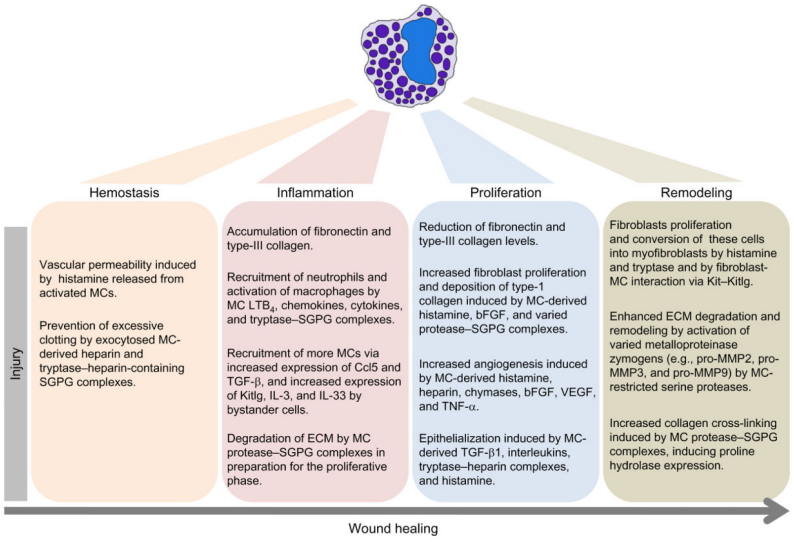

Figure 6.4.

Roles of MCs in different stages of wound repair. MCs and their mediators play important roles in hemostasis and in the inflammation, proliferation, and remodeling phases of wound healing.

Table 6.1

Prominent granule protease of mouse and human MCs

Mouse MC granule proteases			Human MC granule proteases		
Protease	**Gene symbol**	**GenBank GeneID**	**Protease**	**Gene symbol**	**GenBank GeneID**
mMCP-1[a]	*Mcpt1*	17224	–	–	–
mMCP-2[a]	*Mcpt2*	17225	–	–	–
mMCP-3/ mMCP-L[a]	*Mcptl*	17233	–	–	–
mMCP-4[a]	*Mcpt4*	17227	–	–	–
mMCP-5	*Cma1*	17228	Chymase-1	*CMA1*	1215
mMCP-6[b]	*Tpsb2*	17229	hTryptase-β[b]	*TPSB2*	64499
mMCP-7[b]	*Tpsab1*	100503895	hTryptase-β[b]	*TPSAB1*	7177
–	–	–	hTryptase-δ[b]	*TPSD1*	23430
mMCP-8[a]	*Mcpt8*	17231	–	–	–
mMCP-9[a]	*Mcpt9*	17232	–	–	–
mMCP-10[a]	*Cma2*	545055	–	–	–
Prss31	*Tpsg1*	26945	PRSS31	*TPSG1*	25823
Carboxypeptidase A3	*Cpa3*	12873	Carboxypeptidase A3	*CPA3*	1359
Cathepsin G	*Ctsg*	13035	Cathepsin G	*CTSG*	1511
Granzyme B	*Gzmb*	14939	Granzyme B	*GZMB*	3002
Neuropsin/ Prss19[c]	*Klk8*	259277	Kallikrein-related protease-8[c]	*KLK8*	11202

[a]Mouse MCs store varied combinations of 16 proteases in their granules, some of which do not have human orthologs (namely the genes that encode mMCP-1, mMCP-2, mMCP-3/L, mMCP-8, mMCP-9, and mMCP-10). The heparin^{+} MCs that reside in the mouse's skin and other connective tissues express both mMCP-4 and mMCP-5. Although the *Human* and *Mouse Genome Consortiums* concluded that mMCP-5 is the mouse ortholog of CMA1, mMCP-4 has a more similar substrate preference in terms of its ability to cleave low molecular weight peptide substrates. Thus, there is some debate as to whether mMCP-4 or mMCP-5 is the true ortholog of human CMA1.

[b]Mouse MCs store two tetramer-forming tryptases in their granules that originate from the *mMCP-6/ Tpsb2* and *mMCP-7/Tpsab1* genes. It was initially thought that human MCs have only one gene that encodes functional tetramer-forming tryptases. It is now know that the corresponding *TPSB2* and *TPSAB1* genes in the human genome give rise to similar enzymes that regrettably have been called hTryptase-β even though the translated proteins originate from two genes. Complicating the situation, the transcripts that originate from the *TPSB2* and *TPSAB1* genes can give rise to functionally different proteases due to variable splicing of the precursor transcripts. Thus, studies carried out in the 1980s and 1990s using "hTryptase-β" preparations purified from pooled human lung or skin biopsies actually were a complex mixture of enzymes, some of which likely differed in their substrate preferences. Human MCs also express hTryptase-δ whose *TPSD1* gene is closely related to the human *TPSAB1* and

TPSB2 genes. However, this tryptase has reduced enzymatic activity due to a premature translation–termination codon that causes loss of one of the seven loops that form the enzyme's substrate-binding site.

[c] Some mouse MCs express neuropsin/Prss18/Klk8. Although there is a corresponding *KLK8* gene in the human genome, it remains to be shown that this kallikrein is expressed in any population of human MCs.

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