

Mast cell activity in the healing wound: more than meets the eye?

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Abstract: Mast cells (MCs) are an important part of the innate immune system and are abundant in barrier organs such as the skin. They are known primarily for initiating allergic reactions, but many other biological functions have now been described for these cells. Studies have indicated that during wound repair, MCs enhance acute inflammation, stimulate reepithelialization and angiogenesis and promote scarring. MCs have also been linked to abnormal healing, with high numbers of MCs observed in chronic wounds, hypertrophic scars and keloids. Although MCs have gained attention in the wound healing field, several unique

features of MCs have yet to be examined in the context of cutaneous repair. These include the ability of MCs to: (i) produce anti-inflammatory mediators; (ii) release mediators without degranulating; and (iii) change their phenotype. Recent findings highlight the complexity of MCs and suggest that more information is needed to understand their complete range of activities during repair.

Key words: inflammation – mast cell – repair – scar – wound

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Introduction

Wound healing issues affect millions of people worldwide and pose significant health and financial concerns (1). Chronic, non-healing wounds can be painful and leave patients at risk of potentially deadly infections. Even when wounds close in a timely manner, scar tissue deposition can impair normal skin function and visible scars can have a psychological impact on patients (2). Great strides have been made to define the molecular and cellular events required for healing, but effective therapies to optimize repair and limit scar formation are still lacking.

Wound healing is a complicated, multistep process that can be divided into three major phases: inflammation, proliferation and scar formation/remodelling. The compartmentalization of this process into discrete stages gives the illusion of simplicity, but in reality, it is much more complicated. For efficient healing to occur, complex interactions between multiple cell types, soluble factors and extracellular matrix components are required to rebuild the tissue (3). These interactions are not static but rather are in a state of constant flux, resulting in a microenvironment that is continually evolving as the wound moves through the repair process. Mast cells are one cell type involved in multiple stages of healing (Fig. 1a) (4). This review will outline the established roles for MCs in repair, highlighting important findings in this area. Possible alternative roles for MCs in wounds will also be discussed, drawing from newly described MC features which have not yet been evaluated in wound healing, but are likely to be important.

MCs enhance acute inflammation

As an innate immune cell abundant in the dermis, it is not surprising that MCs regulate acute wound inflammation. Activated MCs are notoriously involved in allergic responses, but MC activation is also prominent early after injury (Fig. 1b). While the precise pathways leading to MC activation in skin wounds have not been well described, pathogens, pathogen products and various cytokines are likely involved (5,6). MCs are also sensitive to mechanical stimulation, and mechanical alterations were recently shown to enhance MC degranulation in murine wounds (7).

Injury activates MCs to release many different mediators (Supplemental Table 1) that are either preformed and stored in granules or synthesized *de novo* (8). Many of these mediators are pro-inflammatory, causing hallmarks of inflammation like vasodilation, vascular permeability and activation/recruitment of circulating immune cells. Immediately after injury, MCs stimulate vascular permeability by secreting mediators like histamine and vascular endothelial growth factor (VEGF) (9). In fact, studies in MC-deficient mice indicate that the initial increase in vascular permeability after injury is MC-dependent (10). Mast cells are also important for recruiting circulating inflammatory cells to the wound site, especially neutrophils. Reduced neutrophil recruitment has been reported in excisional wounds of MC-deficient mice by several groups (10,11). Studies in other injury models have suggested that MC protease-4 (chymase) is important for neutrophil recruitment (12–14). Mast cells can also contribute to inflammation indirectly as some MC mediators, including histamine, stimulate pro-inflammatory mediator production by keratinocytes (15–17).

Although MCs are visibly active early in the inflammatory phase, surprisingly, the importance of MCs in fighting wound infection has not been examined. However, MCs are known to contribute to the innate immune response to infection (18–20), and MCs can limit bacterial and viral infections in the skin by releasing antimicrobial peptides and recruiting neutrophils (21–23). These studies support the idea that robust MC activation ultimately serves to bolster the immune response and help prevent wound infection.

MCs stimulate the proliferative phase of healing

Activated MCs produce cytokines and growth factors that support the proliferation and migration of several cell types in the skin and promote the proliferative phase of healing. Mast cell mediators can stimulate keratinocytes, aiding in the restoration of the epidermal barrier during reepithelialization. They can also affect angiogenesis by activating endothelial cells and activate fibroblasts.

MCs produce several mediators that stimulate keratinocytes, including keratinocyte growth factor and epidermal growth factor (Supplemental Table 1). Several studies performed in animals

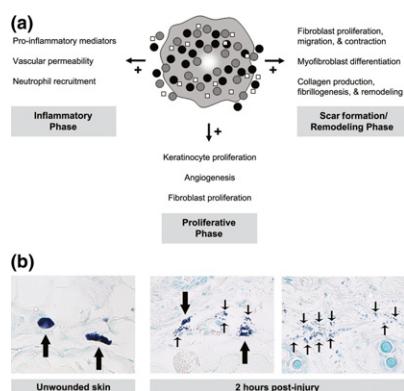


Figure 1. Mast cell activities during wound healing. (a) MCs secrete an array of mediators that affect multiple phases of wound healing. MCs stimulate inflammation by releasing pro-inflammatory mediators that induce vascular permeability and recruit neutrophils (left). Mast cell-derived cytokines and growth factors stimulate keratinocytes, endothelial cells and fibroblasts, leading to reepithelialization and angiogenesis during the proliferative phase (middle). Mast cells also influence the scar formation/remodelling phase by secreting proteases that cleave extracellular matrix components and by producing a variety of factors that stimulate fibroblasts (right). (b) Many MC mediators are released through the process of degranulation, during which mediator-rich intracellular granules are released into the extracellular space. Toluidine blue-stained tissue sections, which stain MC granules dark blue, show that granules are confined to the MCs in uninjured skin (left). Immediately after injury, MCs begin to release granules (middle) and in some areas just adjacent to the wound bed, degranulation is so extensive that individual MCs are no longer visible and only the released granules can be seen (right). Large arrows are pointing to MCs; small arrows indicate MC granules.

lacking MCs or treated with drugs that prevent degranulation have not observed significant differences in wound closure rates of excisional or burn wounds (11,24–26), but other studies have reported delayed wound closure in MC-deficient mice (7,10). In one of these studies, MC-derived histamine was identified as an important factor for epidermal closure based on its ability to promote keratinocyte proliferation (10).

Mast cells also produce factors that stimulate endothelial cells, such as VEGF, platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2) (26,27), suggesting that MCs contribute to angiogenesis. As with wound closure data, results on vascularity have varied in MC-deficient mice. Some groups have not observed differences in blood vessel density in the absence of MCs in standard excisional wound models (11), while others have described reduced angiogenesis in other models (7,26).

It is clear that MCs are capable of releasing mediators that activate keratinocytes and endothelial cells, but not all studies examining MC function have reported measurable effects on wound closure or angiogenesis. This could be partially due to inconsistencies in the experimental wound models used in these studies. Wound repair varies depending on the type of wound generated (excisional or open wound, incision, burn, etc.) and the size of the initial wound (28,29), so MCs might not function identically in all wound models.

MCs augment scar formation

There is strong experimental evidence that MCs affect the activity of fibroblasts, the cells responsible for collagen deposition and remodelling during the proliferative and scar formation/remodelling phases of repair. Several studies have shown that MC numbers or the extent of MC activation is lower in tissues that heal with minimal scarring compared with those which heal with fibrotic scars. For example, oral mucosal wounds heal scarlessly and

contain fewer MCs than scar-forming cutaneous wounds (30). Furthermore, foetal skin, which undergoes regenerative, scarless healing at early stages of development, contains low numbers of dermal MCs that do not degranulate after injury (31). In contrast, fibrotic wounds created in more developed fetuses contain more MCs that degranulate extensively in response to damage (31). Furthermore, wounds in MC-deficient mice heal with reduced scar tissue compared with wild-type mice (26,31). Some studies have also suggested that MCs contribute to scar formation *in vivo* by affecting collagen maturation and remodelling (7,11,25), rather than simply increasing collagen production. High numbers of MCs have also been described in both hypertrophic scars and keloids in human samples and in animal models (24,32–36). These results are in line with studies linking MCs to fibrotic diseases of the skin and other organs (37–44). Together, these findings suggest that altering MC number or activity in wounds could be useful clinically to diminish scarring. Studies in animal models have shown that preventing MC degranulation or blocking MC protease activity can reduce scar tissue production (13,24,41,45,46). Additionally, tyrosine kinase inhibitors targeting c-Kit signalling such as imatinib can reduce MC numbers in experimental wounds (47) and may represent another way to manipulate MCs during repair.

There are several possible mechanisms by which MCs could stimulate fibroblasts leading to scar formation/fibrosis. Activated MCs release several profibrotic mediators such as transforming growth factor- β (TGF- β), and PDGF, as well as many others that can affect fibroblasts (48). Histamine can stimulate fibroblast migration, proliferation and differentiation into contractile myofibroblasts (49–51). Tryptase activity is high in human scars (52), and this enzyme promotes fibroblast proliferation and chemotaxis and also stimulates collagen synthesis, contraction and differentiation of fibroblasts into myofibroblasts (49,53,54). Additionally, chymase can directly cleave procollagen type I and promote collagen fibril formation (55). Recent studies have also shown that MCs and fibroblasts can form heterocellular gap junctions, allowing for direct intercellular communication. Gap junction formation has been shown to stimulate fibroblast proliferation, myofibroblast differentiation and contraction (56–59). While direct MC–fibroblast interactions have been demonstrated experimentally, the clinical implications of this type of direct contact are not yet known. It is also important to note that a reciprocal relationship may exist, as fibroblast-derived mediators can affect MCs (60,61). Overall, it is likely that MCs encourage fibrosis through both direct fibroblast interactions and the release of fibroblast-stimulating paracrine mediators.

Major open questions

Progress has been made over the last decade in defining the actions of MCs during normal repair. However, several important MC features have been described that suggest potential alternative roles for MCs in wound healing which have not yet been evaluated. Several questions need to be answered to more accurately define the function of MCs during repair:

1. *Can MCs help resolve inflammation?* Consistent with their multifunctional nature, MCs produce a diverse set of mediators upon activation. Many of these mediators are pro-inflammatory, which is one reason MCs are often associated with inflammation. However, MCs also produce anti-inflammatory and immunosuppressive cytokines, such as interleukin (IL)-4, IL-10 and TGF- β (62–65), indicating that these

cells have the capacity to both stimulate and suppress the immune system (66,67) (Fig. 2a). Several studies have now described immunosuppressive activities for dermal MCs (63,68–70). For example, MCs are important for maintaining skin grafts (70), and they mediate ultraviolet light-induced immunosuppression through the release of histamine and IL-10 (63,68). Together, these experiments and the fact that MCs provide a source of anti-inflammatory/immunosuppressive mediators suggest that MCs may not only stimulate inflammation during the earliest stages of repair, but also aid in shutting down the inflammatory response at later stages of healing. Resolution is now recognized as an important, active process necessary for effectively terminating the inflammatory response (71). The possibility that MCs are involved in resolution during wound repair has not been examined, but could be important because prolonged inflammation and improper resolution contribute to chronic wounds (72,73). Interestingly, cytokines like SCF and IL-33 that normally stimulate cytokine production by MCs (74–76) can instead reduce MC activation and induce hyporesponsiveness after long-term exposure (77,78). The reduced response to these cytokines over time may act to suppress MC activation at later time points, aiding in resolution.

2. *How important is alternative mediator release?* MC activity depends heavily on the production of mediators that affect surrounding cells. Mediator release accompanies the robust degranulation evident early after injury, but MCs can also release mediators without undergoing complete degranulation (6,79). In response to some stimuli, MCs can either secrete individual granules, secrete contents of granules without degranulating, or release certain mediators selectively through secretory vesicles (Fig. 2b). For example, IL-1 can induce IL-6 secretion without histamine release in human cord blood–derived MCs, and IL-33 can stimulate leukotriene, TNF and IL-6 production in bone marrow–derived murine MCs without histamine release (80,81). In addition, toll-like receptor ligands and cytokines like SCF and IL-33, which do not cause MC degranulation alone, can amplify cytokine production in response to other stimuli (74,75,82). Because these alternative methods of mediator release cannot be readily assessed in whole tissue, it is possible that MCs actively secrete mediators at times when overt degranulation cannot be detected. Therefore, the proportion of mediators assumed to originate from MCs at later stages of healing could be severely underestimated.
3. *Do MCs change their phenotype during healing?* MCs are a heterogeneous group of cells with different characteristics in different locations within the body. Rodent MCs are often categorized as either mucosal mast cells (MMC) or connective tissue mast cells (CTMCs) depending on the tissue of origin (5). These cells can vary in the types or amount of mediators stored within granules, how responsive they are to external stimuli, and what mediators they synthesize upon activation. Mast cells can also alter their characteristics over the progression of a biological response or after exposure to certain factors (83). For example, exposure to IL-4 or IL-10 changes the protease content within granules of human cord blood–derived MCs (84,85). Mast cells also exhibit a certain

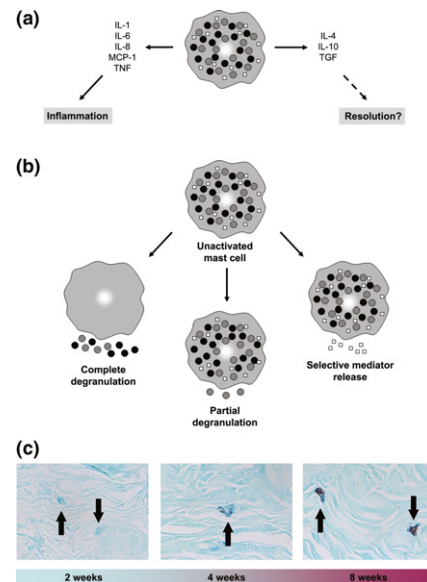


Figure 2. Potential alternative roles for mast cells in wound healing. Mast cells are now beginning to be appreciated as complex cells with diverse actions. While MCs have defined roles in many aspects of repair, there are several features of MCs that have yet to be examined in wound healing. (a) MCs release many pro-inflammatory mediators in response to injury. However, MCs are also capable of producing anti-inflammatory molecules, and recent studies suggest that MCs can limit inflammation or have immunosuppressive effects in the skin. This raises the possibility that MCs could play a role in the resolution of inflammation, which is needed for proper wound healing. (b) MCs are activated very quickly in response to injury and undergo robust degranulation, resulting in the release of preformed mediators into the surrounding tissue (left). In addition to complete degranulation, other methods of mediator release have been described. Mast cells can release a subset of granules, undergoing partial degranulation rather than completely releasing all granules (middle) or they can selectively release certain mediators without degranulating (right). Because these alternative mechanisms of mediator release cannot be easily detected *in vivo*, MCs could be silently influencing healing at times during which degranulation is not typically observed. (c) Finally, the plasticity of MCs has not been assessed during the repair process, despite the fact that these cells can alter their phenotype and behaviour as a result of changes in the microenvironment. Alcian blue-safranin staining, which stains MC granules differently depending on the polysaccharide content, is shown to illustrate how bone marrow–derived MCs change over time when injected intradermally into MC-deficient *Kit^{W/W^v}* mice. Two weeks after injection, the MC granules are blue, indicative of an immature phenotype. At 4 weeks, cells with blue and red granules can be seen. By 8 weeks, mature connective tissue MCs can be seen, which contain mostly red granules. The ability of MCs to their phenotype in the skin over time suggests that MCs may alter their function during the course of wound repair as the microenvironment within the wound changes.

amount of plasticity and are able to change their phenotype as a result of microenvironmental cues. For example, murine bone marrow–derived MCs, which are similar to MMCs, can take on characteristics of CTMCs when injected into the skin (86) (Fig. 2c). Additionally, murine CTMCs from the peritoneum undergo a phenotypic change and begin to resemble MMCs when cultured *in vitro* (87). Interestingly, fibroblasts have also been reported to influence MC phenotype in co-culture systems (60,61). The plasticity of MCs suggests that the phenotype of these cells likely changes over time, which could allow them to change their function as a wound heals and could help explain the diversity of their actions during repair. Additionally, because the wound microenvironment is heterogeneous, it is possible that MCs located at the wound margin (in the structurally normal skin adjacent to the wound) behave differently than MCs within the wound bed itself, although this has not been examined in detail.

Conclusions

Mast cells play a role in many events associated with wound repair. From our point of view, the most convincing evidence points to MCs being important for the early inflammatory response when mast cell degranulation is evident and for promoting scar formation by stimulating fibroblasts. It should be noted that many studies examining the function of MCs in repair have relied on commercially available strains containing *Kit* mutations, which do have several limitations (Supplemental Data S1). Despite the increasing number of studies examining MCs in normal repair processes, more work must be done to understand the degree to which MCs are involved in chronic wounds (88–90) (Supplemental Data S2) and to determine the utility of modulating MCs to reduce scarring or improve other aspects of healing. New information about basic MC

biology leads us to speculate that these cells may carry out functions during repair beyond those which are traditionally attributed to these cells by producing anti-inflammatory mediators, releasing mediators independently of degranulation and altering their phenotype throughout the healing process. Overall, it is clear that a simplistic view of how MCs operate during wound healing is no longer accurate and that more work is needed to fully understand the complex functions of these cells during repair.

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Conflict of interests

BCW and TAW have no conflicts of interest to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. MC-deficient mouse models.

Data S2. MCs in chronic wounds.

Table S1. Prominent mast cell mediators involved in repair.

Supplemental Information 1

MC-deficient mouse models

MC-deficient mouse strains are a commonly used tool to study the function of MCs *in vivo* in addition to mast cell stabilizers such as cromolyn and ketotifen. Two widely used, commercially available strains of MC-deficient mice harbor mutations in the *Kit* gene, *Kit*^{W/W-v} mice and *Kit*^{W-sh/W-sh} mice. The *Kit* gene encodes the receptor for stem cell factor (SCF), which is required for the development and maturation of mast cells (1); therefore a deficiency in MCs is observed in mice with *Kit* mutations. To date, *Kit*^{W/W-v} mice have been used almost exclusively in wound healing studies (2-8). It is important to note that because of the *Kit* mutations, macrocytic anemia, neutropenia and other abnormalities have been described in *Kit*^{W/W-v} and *Kit*^{W-sh/W-sh} mice that can affect the outcome of MC studies (1, 9-11). This could impact the usefulness of the wound healing data generated from these strains. To get around these problems, some investigators compare *Kit*^{W/W-v} mice to *Kit*^{W/W-v} mice that have been reconstituted with MCs from wild type mice, which are sometimes referred to as “MC knock-in” mice (12). However, only one wound healing study has used this approach to verify reepithelialization data in *Kit*^{W/W-v} mice (4).

Recently, two additional MC-deficient mouse strains have been described (13, 14). These mice do not have *Kit* mutations and therefore lack the confounding issues associated with *Kit*^{W/W-v} and *Kit*^{W-sh/W-sh} strains. In the future, these new and improved mouse strains might offer more useful information about the role of MCs in wound healing.

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Supplemental Information 2

MCs in chronic wounds

Although most wounds heal successfully, millions of patients worldwide develop chronic wounds (1). Common types of chronic wounds include pressure ulcers, venous ulcers, and diabetic ulcers. Healing in these wounds is delayed for months or even years, and infections that develop in these open wounds have the potential to be deadly. Compared to normal, actively healing wounds, the wound environment differs greatly in chronic wounds. Chronic wounds contain abnormally high levels of matrix metalloproteinases (MMPs) and other proteases (2-4) which contribute to delayed healing by damaging the extracellular matrix and degrading critical pro-healing growth factors. These wounds are also characterized by overactive inflammation (2, 5). New evidence suggests that a defect in the resolution of inflammation may be to blame for the extended inflammation in chronic wounds, in part due to a reduction in the function of pro-resolving macrophages (6).

Studies examining MCs in chronic wounds is limited, but the presence of MCs have been described in multiple types of chronic wounds (7). Abundant tryptase-positive MCs have been reported in the skin adjacent to chronic venous leg ulcers compared to normal human skin or normally healing wounds (8, 9). An overabundance of active MCs could heighten the production of MC-derived proteases. Abnormally high levels of MC proteases could cause degradation of the extracellular matrix which may reduce keratinocyte adherence and impede reepithelialization in chronic ulcers (10). Additionally, large numbers of pro-inflammatory MCs may contribute to the persistent inflammation that is believed to contribute to the chronicity of these wounds.

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Supplemental Table 1. Prominent mast cell mediators involved in repair.^{1,2, 3}

Chemokines/

Cytokines:

CCL2/Monocyte chemotactic protein-1 (MCP-1) (1, 2)
CCL3/Macrophage inflammatory protein-1 α (MIP-1 α) (2-4)
Granulocyte macrophage-colony stimulating factor (GM-CSF) (5, 6)
Interleukin-1 α / β (IL-1 α / β) (7-9)
Interleukin-4 (IL-4) (10, 11)
Interleukin-6 (IL-6) (12-14)
Interleukin-10 (IL-10) (15-17)
Transforming growth factor- β 1 (TGF- β 1) (18, 19)
Tumor necrosis factor- α (TNF- α) (20, 21)

Growth Factors:

Epidermal growth factor (EGF) (22, 23)
Fibroblast growth factor-2 (FGF-2) (24, 25)
Keratinocyte growth factor (KGF) (26, 27)
Nerve growth factor (NGF) (28, 29)
Platelet-derived growth factor (PDGF)(30)
Transforming growth factor- β 1 (TGF- β 1) (18, 19)
Vascular endothelial growth factor (VEGF) (31-33)

Lipids:

Leukotrienes (LTB₄, LTC₄) (34)
Platelet activating factor (PAF) (35, 36)
Prostaglandins (PGD₂, PGE₂) (34, 37, 38)

Proteases:

Chymase (39)
Trypsin (40, 41)

Other mediators:

Heparin (42, 43)
Histamine (42, 44)
Substance P (45, 46)

¹ Citations included in the table demonstrate the activity of each mediator in wound healing.

² The following references demonstrate that the mediators included in this table are produced by mast cells: (47-61).

³ This table includes a subset of mast cell mediators that are important for wound healing, and is not a comprehensive list of all mast cell mediators. Please refer to review articles for more detailed lists of mast cell mediators (62-65).

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