The Importance of Mast Cells in Dermal Scarring

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Significance: Mast cells are resident inflammatory cells present in high numbers in the skin. They are one of the first cell types to respond to damage and they do so by quickly releasing a variety of preformed mediators that are stored within mast cell granules. Mast cells are not only active early on, where they help induce inflammation, but they also stimulate the proliferation of several important cell types and influence the production and remodeling of collagen.

Recent Advances: Recent studies have highlighted the importance of mast cells in determining the amount of scar tissue that forms as a result of the repair process. Mast cells are found in low numbers and in a less activated state in scarless wounds, whereas high numbers of activated mast cells are associated with scarring and fibrosis. Furthermore, animals that lack mast cells or have been treated with degranulation inhibitors or drugs that block the activity of mast cell proteases have been shown to heal with reduced scar tissue.

Critical Issues: Despite evidence suggesting that mast cells regulate scar tissue development, the entire range of mast cell activities during wound repair and scar formation has not been completely characterized. In addition, the potential therapeutic benefits of targeting mast cells clinically have yet to be fully explored.

Future Directions: More studies are needed to determine whether inhibiting mast cell activation and blocking the function of mast cell mediators are viable options to prevent or reduce the appearance of scars.

SCOPE AND SIGNIFICANCE

Efficient wound repair requires the coordinated effort of many different cell types. A healing wound typically goes through phases of inflammation, proliferation, and scar formation/remodeling. The magnitude of the first of these phases, inflammation, is important for determining how much scar tissue will be produced at the conclusion of the healing process. One cell type that helps regulate the inflammatory response after injury is the mast cell. These cells are resident inflammatory cells, and as normal constituents of the skin they are in an optimal position to respond to skin damage. When the skin is injured, mast cells become activated, degranulate, and release a large number of mediators that stimulate the recruitment of circulating inflammatory cells to the site of injury. In addition to enhancing inflammation, which can indirectly promote scar tissue production by fibroblasts, mast cells also produce a number of profibrotic mediators and can interact directly with fibroblasts to influence the quality of the healed wound. This review will discuss the role of mast cells in wound repair, focusing on the ability of mast cells...
to affect the outcome of healing by determining whether scarless or fibrotic healing will take place.

**TRANSLATIONAL RELEVANCE**

Mast cells produce a large number of mediators in response to injury that have a wide range of biological activities. As a result, multiple roles for mast cells in wound healing have been described. These cells can help initiate inflammation, promote re-epithelialization, and simulate angiogenesis. In addition, both direct and indirect interactions between mast cells and fibroblasts are believed to impact scar formation. Despite the knowledge that mast cells are involved in many aspects of healing, there is still much that we do not understand about how these cells function in vivo. More information is needed about how mast cells contribute to wound healing so that we might find ways to enhance the beneficial functions, while limiting any potentially harmful effects of these cells.

**CLINICAL RELEVANCE**

Billions of health care dollars are spent each year on wound-healing complications just in the United States. Aside from chronic, nonhealing wounds, another important issue with the repair process that affects patients is the replacement of injured skin with scar tissue. This is particularly significant in the case of abnormal scars, which include hypertrophic scars, keloids, and scar contractures. In addition to being weaker than normal skin, scars can cause many problems such as limited joint mobility, growth impairment, and loss of normal skin function. Scars are also cosmetically undesirable and can negatively impact a patient's quality of life. A large number of patients are impacted by scars, which is highlighted by the fact that over 70 million in-patient and out-patient surgeries are performed in the U.S. each year. With effective therapies lacking, novel antiscarring targets are needed. Inhibiting mast cell function and neutralization of mast cell mediators show promise in animal models as unique approaches to limit scar formation.

**DISCUSSION**

**Mast cell biology**

Mast cells, which are characterized by abundant intracellular granules, were first described in detail by Paul Ehrlich in the late 1800’s. These bone marrow-derived cells are not typically found in the bloodstream, but instead circulate as immature hematopoietic progenitors and mature locally after reaching resident tissues. One important factor for the growth, maturation, and survival of mast cells is stem cell factor (SCF), which signals through the c-kit receptor. While c-kit is widely expressed by hematopoietic stem cells, a limited number of cells retain c-kit expression after they differentiate. Differentiated cells that express c-kit include NK cells and dendritic cells, as well as melanocytes and mast cells. The importance of c-kit to mast cells is highlighted by mouse strains harboring c-kit mutations, which are mast cell deficient. Mast cells are long-lived cells that can proliferate locally when stimulated appropriately. Mature mast cells are distributed throughout the body and their characteristics differ depending on their location. Different subpopulations of mast cells are often described depending on whether they reside in mucosal or connective tissues. Mast cells originating from different tissues vary in the types or amount of stored mediators present within granules, how responsive they are to external stimuli, and what mediators they produce upon activation. Mast cells are often found near blood vessels and nerves, and are prominent in organs that interact routinely with the environment like the respiratory tract, gastrointestinal tract, and the skin. The location of mast cells in the dermal layer of the skin is ideal for quickly responding to injury.

**Mast cell activation.** Mast cells rapidly release the contents of their granules upon activation (Fig. 1). The most well-documented mechanism of mast cell activation is mediated by IgE during allergic reactions. However, pathogens (bacteria, viruses, and parasites) or pathogen products, chemicals, neuropeptides, and cytokines can also activate mast cells. Physical stimuli such as heat or mechanical injury cause mast cells to become activated as well, and prominent mast cell activation is seen at early stages of wound healing.

Classically, mast cells release mediators through degranulation. Here, a large bolus of preformed mast cell mediators is released into the tissue through the extrusion of cytoplasmic granules. This type of degranulation is often so extensive that only phantom mast cells containing no granules are present in the tissue. These phantom cells cannot be detected using standard histological stains, which bind to mast cell granules. Because a strong mast cell degranulation response occurs in the skin following injury, a reduction or disappearance of mast cells is often described in wounds stained with granule-specific dyes.
In addition to releasing mediators through degranulation, recent studies have demonstrated that mast cells are also capable of releasing mediators without undergoing complete degranulation. Mast cells can secrete individual granules or a subset of granules. In response to some stimuli, mast cells can also release certain mediators selectively through secretory vesicles without releasing granules. These alternative types of mediator release are difficult to detect in vivo; however, it is likely that mast cells release mediators throughout the wound-healing process using these other mechanisms and not just at early stages when mast cell degranulation is obvious.

Mast cell mediators. The mediators released by activated mast cells have a diverse range of activities (Fig. 2), which may explain why multiple functions have been described for these cells during wound healing. Many preformed mediators, such as histamine, proteases, and proinflammatory cytokines like tumor necrosis factor alpha (TNF-α), are stored in cytoplasmic granules and can be released quickly through degranulation. Mast cells also synthesize mediators de novo upon activation. Arachidonic acid can be quickly converted to proinflammatory lipid mediators like prostaglandins and leukotrienes. Over a longer period of time, mast cells also synthesize and release a number of different cytokines and growth factors. Many of these mast cell mediators can affect inflammation, re-epithelialization, and angiogenesis. Additionally, mast cells produce mediators with documented profibrotic activity, including histamine, proteases like tryptase and chymase, and growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor

![Figure 1. Schematic representation of mast cell degranulation. Mast cells are resident inflammatory cells found in the dermal layer of the skin. In uninjured tissue, mature mast cells contain abundant intracellular granules (black and gray circles), which are used to store a variety of preformed mediators. Immediately after injury, mast cells begin to secrete these granules. Eventually, many of the mast cells will fully degranulate or release all of their granules into the extracellular space.](image1)

![Figure 2. Mast cell mediators. Mast cells are capable of secreting a diverse set of mediators upon activation. Mast cell mediators can be released from granules (black and gray circles) or from secretory vesicles (white squares). A list containing some of the prominent mast cell mediators are shown, which include cytokines and chemokines, lipid mediators, proteases, vasoactive amines, and growth factors. This is not a complete list of all mast cell mediators. For a more comprehensive list, please see Galli et al. GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MIP-1α, macrophage inflammatory protein 1-alpha; TNF-α, tumor necrosis factor alpha; LTB4 and LTC4, leukotrienes B4 and C4; PAF, platelet activating factor; PGD2 and PGE2, prostaglandins D2 and E2; EGF, epidermal growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor.](image2)
Overview of mast cells in wound healing

Diverse actions have been described for mast cells during wound healing, and mast cell involvement has been documented for each of the three main phases of repair: inflammation, proliferation, and scar formation/remodeling (Fig. 3). Mast cell activation begins immediately after injury, which is evident based on reduced numbers of fully granulated mast cells and the presence of extra-cellular granules in the tissue.²⁰–²²

Not surprisingly, given that they are resident inflammatory cells, mast cells play an important part in the inflammatory phase of healing. Several mediators stored in mast cell granules, like histamine and TNF-α, stimulate inflammation. Mast cells also synthesize a number of proinflammatory lipid mediators and cytokines after activation, such as prostaglandins, interleukin (IL)-1, and IL-6. Together, the preformed and newly synthesized mast cell mediators induce vascular permeability and adhesion molecule expression on endothelial cells, and stimulate the recruitment of circulating inflammatory cells to the wound site. Many studies examining mast cell function in wound healing have taken advantage of the mast cell-deficient KitW/W-/- mouse strain. These mice lack mast cells due to a spontaneous mutation in the c-kit gene, which encodes the receptor for an important mast cell growth factor, SCF. Using these mice, studies have shown that mast cells are especially important for the infiltration of neutrophils into the wound.²⁷,²⁸

Mast cells also produce many cytokines and growth factors that affect keratinocyte and endothelial cell function, which is important during the proliferative phase of healing. Mast cells generate epidermal growth factor, keratinocyte growth factor, and other mediators known to stimulate keratinocytes.²⁹ One study reported a delay in re-epithelialization in large excisional wounds from mast cell-deficient mice and suggested that this effect is dependent on mast cell-derived histamine.²⁸ Mast cells also produce several important proangiogenic molecules, including VEGF, PDGF, and fibroblast growth factor-2 (FGF-2), and in some studies reduced wound angiogenesis has been described in mast cell-deficient mice.³⁰,³¹ Mast cells can also affect fibroblasts,²⁵,²⁶ which are active during the proliferative phase and deposit/remodel collagen to repair the dermal layer in wounded skin. Consequently, mast cells are thought to regulate the scar formation/remodeling phase.

Mast cells and scar tissue

Multiple lines of evidence suggest that mast cells influence scar formation and fibrosis in the skin as well as other organs. Analysis of samples from both animal models and human tissues has shown that mast cell numbers and/or the levels of mast cell activation increase as the extent of scarring and fibrosis increase, ranging from low mast cell activity in models of scarless healing to abnormally high mast cell activity in atypical scars and fibrotic conditions.

Mast cells in models of scarless healing

Fetal wounds. Many studies have now demonstrated that fetal skin is capable of healing wounds in a unique way. At early stages of development (first and second trimesters), fetal skin heals very rapidly, with little or no inflammation, and without forming scar tissue.³²–³⁴ This regenerative process leads to the renewal of skin appendages, such as hair follicles and sebaceous

Figure 3. Mast cell functions during wound healing. Multiple roles for mast cells in wound repair have been described. Activated mast cells produce a large number of proinflammatory mediators that stimulate the recruitment and activation of additional inflammatory cells. Mast cells contribute to the proliferative phase of wound healing by releasing mediators that stimulate keratinocytes and endothelial cells, leading to re-epithelialization and angiogenesis, respectively. During the proliferative and scar formation/remodeling phases, mast cells also activate fibroblasts through multiple mechanisms, ultimately promoting scar formation.
glands, and restoration of normal dermal collagen instead of scar tissue. However, the ability to undergo scarless healing is heavily dependent on the gestational age of the fetus because in the late stages of development (third trimester) the skin loses its ability to regenerate. Instead, the skin begins to heal with fibrotic scars, similar to what occurs in postnatal skin. In addition to healing with significant scarring, late gestation fetal wounds heal more slowly and with a strong inflammatory response.

Given that mast cells are important for inducing inflammation and that the magnitude of the inflammatory response determines whether a fetal wound will heal with or without a scar, we recently examined mast cells in a mouse model of fetal wound healing. The findings are summarized in Fig. 4. In this model, full-thickness incisional wounds were created in fetal skin at embryonic day 15 (E15) or embryonic day 18 (E18) to generate scarless or fibrotic fetal wounds, respectively. Before injury, there were significantly more dermal mast cells in E18 skin compared to E15 skin. In addition, the mast cells in E18 skin were larger, contained more granules, and were more mature based on histological staining. After injury, mast cells in E18 wounds were clearly activated, and a high number of extracellular granules that had been released by mast cells could be detected. In contrast, almost no mast cell degranulation was observed in E15 wounds. These results suggested a positive correlation between mast cell number/activity and scar formation. Additionally, lysates from cultured E18 mast cells inhibited the regenerative capacity of E15 wounds, and E18 wounds created in mast cell-deficient fetuses healed with reduced scarring compared to E18 wounds from wild-type fetuses. Together, these data support an important role for mast cells in determining whether fetal skin wounds will heal with or without scars.

Oral mucosal wounds. Like fetal skin, unique healing properties have been described for the oral mucosa. Clinical observations and animal studies have suggested that mucosal wounds in the oral cavity heal more quickly, with less inflammation, and with reduced scarring compared to mature skin wounds. One study in mice reported a reduction in mast cell degranulation in oral mouse wounds compared to cutaneous wounds. In another study, fewer mast cells were found at late time points in oral pig wounds compared to skin. Thus, similarly to scarless fetal skin wounds, the mast cell response appears to be diminished in oral mucosal wounds which heal with minimal scarring.

The role of mast cells in scarring and fibrosis in mature tissues

While scarless wounds contain low numbers of activated mast cells, studies have shown that higher mast cell numbers or mast cell activity is associated with scarring and fibrosis in mature tissues. Several studies have shown collagen alterations in wounds from mast cell-deficient mice. One group reported less fibrosis at the edges of scald wounds in mast cell-deficient mice compared to wild-type mice. Other studies have suggested that mast cells may affect collagen maturation and remodeling more than collagen production. Mast cells have also been linked to the formation of abnormal scars, such as hypertrophic scars and keloids. With the exception of one report, studies have shown that compared to normal skin or normal scars, mast cell numbers are significantly higher in both hypertrophic scars (from humans and animal models) and human keloids. One study showed a functional role for mast cells by examining the effects of a mast cell stabilizer ketotifen on hypertrophic scar formation in red Duroc pigs. Reduced wound contraction and scar formation were reported in ketotifen-treated animals. Scars from pigs receiving ketotifen also displayed thinner and less dense collagen fibers and reduced numbers of alpha-smooth muscle actin-positive myofibroblasts.

Multiple studies also support a role for mast cells in fibrotic conditions of the skin and other organs. Scleroderma patients have high levels of the mast cell growth factor SCF, and changes in the number of tryptase-positive mast cells as well as increases in mast cell activation have been

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Figure 4. Summary of data on mast cells in fetal wound healing. Recent studies showed that mast cells correlate with scar formation in a mouse model of fetal wound healing, in which wounds created at E15 heal scarlessly and wounds created at E18 heal with a fibrotic scar. Before injury, normal unwounded E15 skin contained fewer mast cells and mast cells that were less mature compared to those in normal E18 skin. After injury, significantly less mast cell activation was observed in E15 scarless wounds compared to E18 fibrotic wounds. E15 and E18 are used to designate embryonic days 15 and 18, respectively.
described in cutaneous scleroderma lesions. High numbers of mast cells and degranulated mast cells have been described in the tight skin (TSK) mouse model of scleroderma. Furthermore, preventing mast cell degranulation or inhibiting chymase reduces fibrosis and mast cell-deficient TSK mice develop less fibrosis than their mast cell-containing counterparts. Similar findings have also been observed for fibrosis in other organs. Mast cells are abundant in Crohn’s disease, where patients often develop intestinal fibrosis and form strictures. In interstitial renal fibrosis, higher numbers of tryptase-positive mast cells are associated with increased fibrosis. The degree of fibrosis and cirrhosis in a model of carbon tetrachloride-induced liver fibrosis has been shown to correlate closely with mast cell numbers. Additionally, mast cell-deficient mice showed reduced bleomycin-induced fibrosis in the lung.

Mechanisms of mast cells in scar formation

Several mechanisms have been proposed to describe how mast cells increase scarring and fibrosis, including paracrine, indirect, and direct effects of mast cells on fibroblasts (Fig. 5). Activated mast cells release a variety of known profibrotic molecules and other mediators that stimulate fibroblasts in a paracrine manner. Mast cells are capable of producing TGF-β1, one of the most potent and well-recognized proscarring molecules. Histamine, an abundant mast cell mediator, has been shown to stimulate fibroblast migration, proliferation, and differentiation. Mast cell proteases have also been shown to promote fibrotic responses in fibroblasts. Tryptase has chemotactic and mitogenic effects on fibroblasts and also stimulates collagen synthesis, contraction, and differentiation into myofibroblasts. Part of these effects may be due to the ability of mast cell histamine and tryptase to stimulate the production of FGF-2 and FGF-7 by fibroblasts. Another mast cell protease, chymase, has been shown in vitro to cleave procollagen type I and promote collagen fibril formation directly. Other mediators produced by mast cells, such as PDGF, prostaglandin E2, and VEGF have also been shown to promote fibrosis in fetal wounds.

Mast cells can also stimulate scar formation indirectly by stimulating inflammation. Activated mast cells increase the magnitude of inflammation.
in wounds by enhancing vascular permeability, stimulating endothelial cell adhesion molecule expression, and producing chemokines that attract circulating leukocytes to the wound. Studies have shown that inflammation contributes to scar formation in adult skin. Furthermore, fetal wound-healing studies have demonstrated the absence of inflammation in scarless wounds and the presence of inflammation in fibrotic wounds, and that artificially inducing inflammation in early gestation fetal wounds causes these wounds to heal with a scar, even at a gestational age that normally corresponds to scarless healing.

Recent studies have now shown that mast cells can also interact directly with fibroblasts. Mast cells and fibroblasts can form gap junctions, allowing direct communication between the two cell types. Heterocellular gap junction formation between mast cells and fibroblasts involves the gap junction protein connexin-43, and stimulates fibroblast proliferation, myofibroblast differentiation, and collagen lattice contraction. Although direct mast cell–fibroblast interactions have been demonstrated in experimental systems, their involvement in the development of fibrosis in vivo is not yet known.

Targeting mast cells to reduce scarring

The evidence supporting a role for mast cells in stimulating scar formation and fibrosis suggests that blocking mast cell function could be used to prevent or minimize scarring. Multiple approaches could potentially be used to target mast cells (Fig. 6). There is a class of drugs known as mast cell stabilizers, which prevent mast cell degranulation. These include drugs like cromolyn (disodium cromoglycate) and ketotifen, which have shown promise in animal models. However, while these drugs are able to prevent mast cell degranulation, they may also affect other cell types and it is unclear whether the release of mast cell mediators via secretory vesicles is affected by

Figure 6. Methods to target mast cells. There are multiple ways in which drugs could be used to limit the activity of mast cells in the skin. Mast cell stabilizers, such as cromolyn and ketotifen, prevent mast cell degranulation and the release of prestored mediators (top). Small molecule tyrosine kinase inhibitors with activity against c-kit, a receptor for the mast cell growth factor stem cell factor, have been shown to reduce mast cell numbers in wounds (middle). Another strategy that has been used is to block the activity of mast cell mediators (bottom). For example, multiple drugs that neutralize the activity of the mast cell protease chymase have been successfully used in animal models of fibrosis.
these drugs. Another potential way to reduce the effects of mast cells is to limit the number of mast cells in the skin after injury by inhibiting the activity of the SCF receptor c-Kit. For example, several c-Kit-blocking antibodies and small molecule tyrosine kinase inhibitors that block the activity of c-Kit are used for cancer treatment. Anti-c-Kit antibodies and imatinib, which blocks c-Kit signaling, were recently shown to reduce mast cell numbers during cutaneous wound healing.\(^8\) Finally, drugs could be used to block the downstream activity of mast cell mediators. This approach has been used with chymase, a mast cell protease. Several drugs that neutralize chymase activity have been shown to reduce fibrosis in animal models.\(^6\),\(^8\)

CONCLUSIONS

Although mast cells produce several factors that may aid in the repair process, there is strong evidence linking mast cells to excessive scarring and fibrosis. Mast cells stimulate inflammation, which indirectly promotes scar formation. In addition, mast cells produce a number of profibrotic mediators and they can also interact directly with fibroblasts through the formation of gap junctions. By affecting fibroblasts in these ways, mast cells can stimulate fibroblast proliferation, migration, and contraction, enhance the conversion of normal fibroblasts into contractile myofibroblasts, and augment collagen production. As a result, mast cells contribute to the ultimate outcome of the wound-healing process by influencing how much scar tissue will be produced by fibroblasts. Additional studies will be needed to determine whether mast cells are a viable target to reduce scar formation and fibrosis in humans.

ACKNOWLEDGMENTS

AND FUNDING SOURCES

The authors receive support from the National Institutes of Health grants CA127109 and ES020462 (T.W.).

TAKE-HOME MESSAGES

- Mast cells are resident inflammatory cells present in high numbers within the dermal layer of the skin. These cells become activated quickly after injury, which causes them to release the contents of their granules into the extracellular space. Mast cells release a variety of mediators that affect wound healing, including preformed proteases and cytokines stored in granules, as well as lipids, cytokines, and growth factors that are produced de novo.
- Several different functions have been described for mast cells during wound repair, including initiating the inflammatory response, stimulating re-epithelialization, and promoting angiogenesis. In addition, there is increasing evidence that mast cells promote scar tissue production both during normal wound healing and in fibrotic diseases.
- High mast cell numbers or levels of mast cell activation have been linked to scar tissue formation in a variety of animal models and in human samples. Mast cells are found in lower numbers and do not become activated in response to injury in scarless wound-healing models. In contrast, higher numbers of mast cells or activated mast cells are associated with scarring and fibrosis in the skin and other organs. Studies have also demonstrated that drugs which inhibit mast cell activity reduce the extent of scar formation and fibrosis, further supporting a role for mast cells in the regulation of scar tissue formation.
- It is likely that mast cells enhance the production of scar tissue through multiple mechanisms, such as releasing mediators that augment inflammation, producing profibrotic growth factors that stimulate fibroblasts, and directly interacting with fibroblasts through gap junctions.
- Additional studies are needed to determine whether drugs that target mast cells or mast cell mediators can be used clinically to prevent or reduce scarring.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors declare that they have no competing financial interests to disclose. The content of this article was expressly written by the authors listed and no ghostwriters were used.

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Traci Wilgus, PhD, is an Assistant Professor and Brian Wulff, PhD, is a postdoc in the Department of Pathology at The Ohio State University. They are interested in understanding scarless healing in fetal skin and how the inflammatory response affects scar formation.
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