Mast Cells and Wound Healing

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Background: Mast cells (MC) are ubiquitous resident cells, traditionally viewed as effector cells of allergic reactions that can store and synthesize *de novo* many mediators upon activation by a variety of stimuli. Exciting new insights are unveiling MC involvement in the pathogenesis of connective tissue disorders including wound healing and fibrosis.
The Problem: Abnormal wound repair is associated with an increased number of MC strategically located around blood vessels. Therapeutic local manipulation of MC population and reactivity may help improve and even prevent impaired repair processes for which there is no cure.
Basic/Clinical Science Advances: Chymase, a MC-restricted protease, is prestored in MC cytoplasmic granules with other mediators. The development of a highly specific inhibitor targeting chymase established its pivotal effect on fibrosis pathogenesis in a mouse model of silica-induced fibrosis. This novel finding evokes the potential therapeutic relevance of chymase inhibition to prevent aberrant wound healing.
Clinical Care Relevance: MC are increased in number in a variety of fibrotic diseases, compared to normal scars. Chymase has become a rising target prompting the development of chymase-specific inhibitors to be used as prophylactic or therapeutic agents. Another emerging strategy may consist in evaluating the efficacy of mast cell stabilizing drugs such as cromolyn in abnormal wound healing—drugs which are already approved for human use in other MC-driven disorders.
Conclusion: Limited treatment success of dysregulated wound healing underscores the need for novel targets be considered such as MC and/or MC-derived mediators and the necessity to design new therapeutic strategies for wounds that remain difficult to treat.

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Abbreviations and Acronyms

ECM = extracellular matrix
MC = mast cell
mMCP = mouse mast cell protease
MMP = matrix metalloproteinase
TGF-β = transforming growth factor-beta
tPA = tissue plasminogen activator

BACKGROUND

Mast cells (MC) are ubiquitous resident cells, traditionally viewed as effector cells of allergic reactions and parasitic diseases.[1] Their ability to store and synthesize, *de novo*, an armamentarium of mediators upon activation by a composite range of stimuli allow these cells to potentially control not only many facets of inflammatory processes and immune responses but also tissue homeostasis.[2] Exciting new insights are unveiling MC involvement in the pathogenesis of a wide range of diseases such as bacterial infections, cancer to connective tissue disorders including fibrosis, and wound healing.[3]

Wound repair is conceptually divided into three phases—inflammation, proliferation, and remodeling—tied together as a dynamic sequence of cellular events triggered by an initial injury.[4] Tissue trauma initiates signals to recruit specialized cells to the

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injury site, to induce proliferation of specific cells involved in the repair process and to control the structural remodeling and reconstitution of the extracellular matrix (ECM). The occurrence of abnormal or impaired wound healing, *e.g.* following surgical procedures, in diabetic patients or in burn victims, remains a challenging clinical issue, which could result in resistance to treatment.[5,6] This review will explore the importance of MC in regulating healing processes and the potential usefulness of MC-directed therapies in tissue repair.

TARGET ARTICLES

1. Shih B, Garside E, McGrouther DA, and Bayat A: Molecular dissection of abnormal wound healing processes resulting in keloid disease. Wound Repair Regen 2010; **18:**139.

2. Younan G, Suber F, Xing W, Shi T, Kunori Y, Abrink M, Pejler G, Schlenner SM, Rodewald HR, Moore FD Jr, Stevens RL, Adachi R, Austen KF, and Gurish MF: The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5. J Immunol 2010; **185:**7681.

3. Takato H, Yasui M, Ichikawa Y, Waseda Y, Inuzuka K, Nishizawa Y, Tagami A, Fujimura M, and Nakao S: The specific chymase inhibitor TY-51469 suppresses the accumulation of neutrophils in the lung and reduces silica-induced pulmonary fibrosis in mice. Exp Lung Res 2011; **37:**101.

CLINICAL PROBLEM ADDRESSED

Inflammatory response is necessary to the resolution of wound repair subsequent to various traumatic conditions, including epidermal burn,[7] diabetes complications,[6] bone fractures, surgical incisions,[8] and gastric ulcers.[6] But an excessive response can lead to chronic inflammatory situations such as hypertrophic scars, keloids, and fibrosis.[4] Regardless of the etiology, abnormal wound repair is associated with increased number of MC strategically located around blood vessels.[3] Their beneficial or deleterious influence on local or systemic cellular responses still remain to be fully elucidated. Thus, therapeutic local manipulation of MC population and reactivity (*i.e.*, sensitivity to stimuli) may pave new avenues to improve abnormal repair processes.

RELEVANT BASIC SCIENCE CONTEXT

Wound healing consists in temporally overlapping phases: hemostasis, inflammatory, proliferative, and maturation phases. Critical processes in these phases evoke MC as pivotal effector cells: hemostasis, inflammation, angiogenesis, fibroblast proliferation, wound contraction, and remodeling,[4,6] as shown in the summary illustration. In plasma from patients with multiple tissue injuries, the anaphylatoxin C5a was found very early, and its appearance correlated with the level of coagulation and complement activation.[9] This report suggests that coagulation/fibrinolysis proteases may act as natural C3 and C5 convertases, generating biologically active anaphylatoxins C3a and C5a, both potent chemoattractants and stimuli for MC,[10] linking both cascades and initiating the inflammatory phase. Chemokines and cytokines are released by resident MC and recruit neutrophils and monocytes that become activated macrophages (all also cytokine producers). They act as scavengers of cell debris and, in conjunction with MC, help to generate new tissue and transition to the proliferative phase of fibroblasts, keratinocytes, and endothelial cells through growth factor and cytokine production.[4] Proliferative cells can also produce these factors. Neovascularization accompanies proliferation, helping tissue formation by insuring nutrients and oxygen supply. Proliferative fibroblasts generate a new extracellular matrix (ECM) to replace the fibrin clot. As healing progresses, a portion of the wound fibroblast population differentiates into myofibroblasts, and their contraction helps the wound to become smaller. Remodeling of ECM by constant enzymatic turnover is key to scarring. Tissue plasminogen activator (tPA) is one of the main components of the plasminogen/fibrinolytic system involved in fibrin homeostasis (*i.e.*, blood clot resorption) and tissue remodeling through the activation of matrix metalloproteinases and degradation of ECM.[11] Perivascular MC constitutively release tPA in its free and enzymatically active form;[12] however, with exposure to components of the complement system, such as C5a, MC can also produce plasminogen activator inhibitor-1, which antagonizes tPA activity,[13] therefore greatly influencing blood clotting and wound healing at all steps.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

Mast cells have only recently entered the arena of wound healing. To date, no human disease has been described due to a deficiency of MC, thus

suggesting that they may not be essential to maintain homeostasis.[14] However, there are MC-deficient mice due to naturally occurring mutations.[15] First depicted as a deficiency in melanocytes and gametes, scientists later discovered these animals were almost entirely devoid of MC. MC are tissue-dwelling cells that do not circulate in the blood as fully differentiated cells in normal conditions as do other leukocytes, nor are they found mature in the bone marrow.[15] These circumstances made it difficult to expand them *in vitro*. MC are found close to connective tissue vessels of skin and mucosa, making them sentries and first responders in case of trauma. Their ability to store and produce many mediators and their strategic locations make MC ideal candidates to control wound healing but their pleiotropic nature makes them more difficult to study and draw definite preclinical conclusions. However, MC-deficient mice can be reconstituted by adaptive transfer of MC developed *in vitro* from animals knocked out for a gene to assess the importance of this MC-derived mediator in the diseases processes and wound healing.[16]

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The critical role for MC in tissue homeostasis and repair is now increasingly recognized.[2,16] MC-derived multifunctional cytokines/mediators fine-tune many aspects of wound healing with contrasting actions depending on timing and amount released. Thus, histamine can inhibit or stimulate collagen synthesis as a function of its local concentration. Chymase, an MC-derived chymotrypsin-like serine protease, is mitogenic for fibroblasts but has minimal effects on keratinocytes, suggesting its primary function in connective repair. Chymase is stored in MC granules with other proteases and mediators. Injection of human chymase induces accumulation of neutrophils and eosinophils in guinea pig skin and mouse peritoneum. Whether it is a direct effect of chymase is still unknown. The use of chemical inhibitors is at risk for potential off-target effects, since the development of highly specific inhibitors is quite challenging. Takato *et al.* developed such an inhibitory compound, TY-51469, with sole actions on human chymase and on its functional equivalent mouse mast cell protease (mMCP)-4.[17] Expression of mMCP-4, though only analyzed at the messenger RNA level, is increased in this mouse model of silicosis (or silica-induced pulmonary fibrosis) induced by intratracheal injection of silica. The authors show the suppressive effects of TY-51469 on fibrosis and chemokine expression driving neutrophil and monocyte recruitment to lungs. Silicosis is associated with increased number of MC, whereas MC-deficient mice display impaired signs of the disease. Increased chymase expression correlates with the development of fibrosis. The rapid increase of transforming growth factor-β (TGF-β) observed after chymase injection is also mitigated by TY-51469. Since TGF-β is associated with fibrosis, early inhibition of chymase after silica exposure may prevent the subsequent development of lung fibrosis.

A second-degree epidermal burn injury also elicits an inflammatory response resulting in ulceration and scarring. Younan *et al.* found that burn injury is associated with early MC degranulation/activation and is absent in MC-deficient mice.[7] This article describes a protective effect against a second-degree scald burn in animals lacking chymase (mMCP-4) or elastase (mMCP-5), highly homologous serine proteases displaying distinct substrate specificities. Animals deficient in other MC-derived proteases showed no protection. Furthermore, topical application of mMCP-5 to the scalded area early after the injury increases epidermal injury in mMCP-5-deficient mice. Topical application of human chymase to mMCP-4- but not mMCP-5-deficient mice also restored burn injury to mMCP-4-deficient mice exposed to epidermal burn. These results evoke the non-redundant MC protease dependency of epidermal injury, as an essential contributor to ulceration and remodeling of the burn site. The availability of mouse strains genetically deficient for these different proteases was a real asset for this study to dissect their functions.

Keloids are pathologic aberrant scars that may occur consequently to burns and trauma with abnormal continuation of wound healing process. The etiology is complex and includes genetic predisposition. High MC numbers are found in the keloid. Dysregulated cytokine levels are noticed, including elevated levels of TGF-β. TGF-β overexpression stimulates collagen transcription and decreases its degradation leading to excess deposition. However, the lack of an animal model impairs the elucidation of keloid pathogenesis.[4] A better understanding of keloids is needed for improved management of clinical complications by devising better therapeutic strategies. Even though MC constitute the perfect culprits for abnormal healing and keloids, more knowledge is necessary pertaining to their activation and migration in this context since there is no cure for keloids but surgical resection.

Current models for evaluating MC functions involve *in vitro* investigation with questionable relevance.[14] Primary mouse MC are often difficult

to expand in numbers and approximation to human MC is inadequate. Cultured bone marrow-derived mast cells are considered immature. Optimal *ex vivo* expansion of human MC is achievable but results often into mixed human MC phenotypes. None of these methodologies can substitute for in vivo clinical studies. Novel methods are developed in mice and nonhuman primates to quantify MC population dynamics in wound healing[18] using flow cytometry to track MC and report the potential usefulness of MC-directed therapies through cluster of differentiation 117 (CD117, or cKit; the receptor for stem cell/growth factor for MC) antagonism. MC-stabilizing drugs may also help producing finer scars, less prone to aberrant healing. Nonetheless, MC are required for normal skin healing in mice.[19,20] A clear picture of their exact functional significance has yet to emerge.

INNOVATION

MC-restricted chymase is primarily an angiotensin II-forming enzyme, which acts on vasoconstriction and vascular proliferation. Angiotensin II promotes VEGF expression, which in turn can stimulate matrix metalloproteinase (MMP)-9 expression. Chymase also converts precursors of TGF-β and MMP-9 to their active form both leading to ECM degradation and tissue remodeling. Little information was available on these fibrotic functions of chymase. Takato *et al*. reported the anti-fibrotic effects of a newly developed highly specific chymase inhibitor, compound TY-51469, on lung fibrosis subsequent to silica exposure in a mouse preclinical model. Mice genetically invalidated for MC-restricted mMCPs are partially protected against burn injuries, conversely to mice deficient for other MC proteases. Resident MC could also be the missing causative cells for keloid or aggressive scar pathogenesis.

TAKE-HOME MESSAGE

Basic science advances

- MC mediators and functions can orchestrate each step of the wound healing process. Already present in tissues and strategically located close to nerve endings and blood vessels, MC are first responders to local injury. They could potentially initiate healing processes and drive excessive fibrosis and scarring in aberrant repair. Aggravated scars consecutive to trauma are absent in the absence of MC.
- Further studies are warranted, however, to clarify the importance of MC involvement in repair processes since different animal models lead to contrasted observations.
- MC-restricted chymase (mMCP-4) has been under further scrutiny for its function in overzealous healing resulting in fibrotic deterioration, as it is overexpressed in fibrosis in correlation with MC number. The development of a highly specific inhibitor for this particular protease sets the stage for a better understanding of its function in wound healing, as its inhibition prevents fibrosis. This was confirmed using a genetic approach of an epidermal scald model on mice lacking mMCP-4 or mMCP-5 (elastase).

Clinical science advance

- Silencing/prevention of MC activation is routine strategy in the field of asthma and allergic reactions. Mediator antagonists such as antihistamines and MC stabilizers such as cromolyn alleviate symptoms associated with MC activation. Animal models evoked a beneficial effect of MC stabilization during the early steps of healing to prevent aberrant healing and excessive scarring. These drugs are already approved for human use, which constitutes a tremendous advantage. The relevance of animal models to human disease remains a major issue, however, given the diverse site- and species-specific phenotypes of MC pertaining to their stimulation and susceptibility to treatment.

Relevance to clinical care

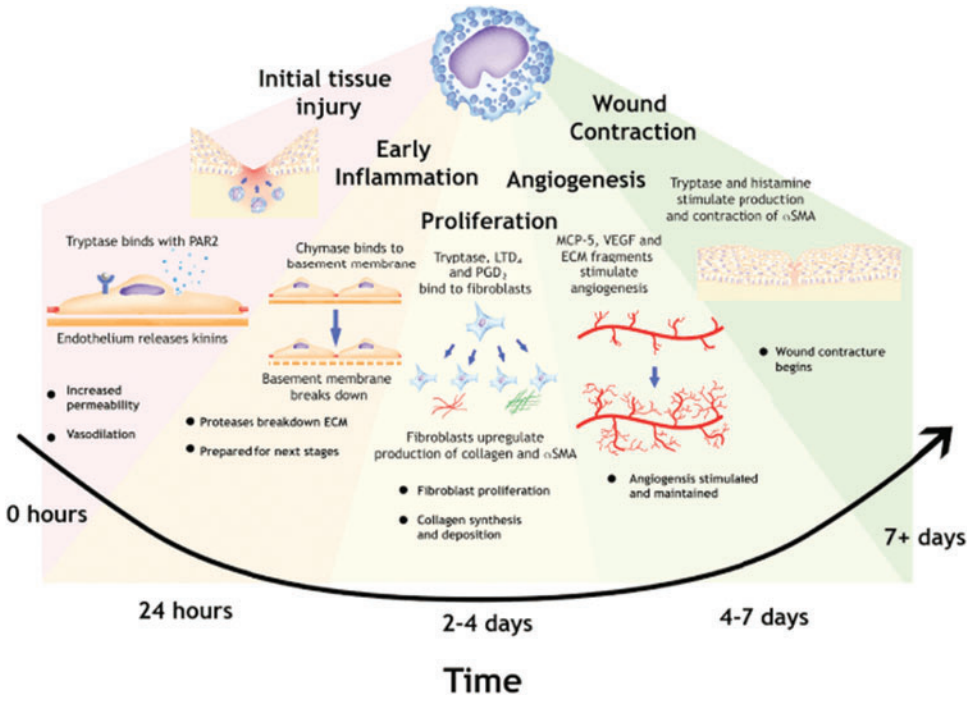
- Studies show increased numbers of MC in keloids compared to normal scars, consistent with observations in other fibrotic diseases such as lung fibrosis and sclerodermatous skin lesions. Because the causative cells are unclear, resident MC may constitute the perfect culprit considering their strategic locations in tissues and their idiosyncratic ability to store preformed mediators and synthesize a wide range of others *de novo*.
- MC-restricted chymase is coincidentally increased with MC hyperplasia and has become a rising target involved in fibrotic diseases at large, such as abdominal aortic aneurism (a widespread destruction of ECM with inflammation in the vascular wall); abnormal diabetic wound healing; cardiomyopathy (myocardial fibrosis); and chronic fibrotic liver disease in the metabolic syndrome comprising obesity, diabetes, and hypertension. Therefore, highly specific chymase inhibitors may be promising prophylactic agents against tissue fibrosis.

SUMMARY ILLUSTRATION

Mast cells in the different stages of wound healing. PAR, protease activated receptor; LT, leukotriene; PG, prostaglandin; VEGF, vascular endothelial growth factor; SMA, smooth muscle actin. Reprinted by permission from Michael FY Ng.[14]

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

The exciting diversity of MC populations observed in different species and tissues is precisely why it is so difficult to draw a definitive conclusion regarding their involvement in normal and pathological wound healing. Contrasting findings



stemmed from preclinical studies using animal models, likely related to MC pleiotropism. This makes any of these results more difficult to translate to human diseases. However, these studies, confined to limited treatment success of dysregulated wound healing (for example, in diabetic patients), stimulated the assessment of MC in these processes only to recognize their relevance to the field. As much as MC aficionados believe that MC are the grand coordinators of health and disease, complex pathological processes are likely to be controlled by more than one cell type just like they are the result of sequential intervention of multiple mediators. Nonetheless, despite multifaceted approaches developed to prevent aberrant wound healing, keloid disease remains a challenging issue for post-surgical issue patients and patients with metabolic syndrome, underscoring the need for new players such as MC to be considered and for the use of human MC models to evaluate the relevance of animal studies.

FUTURE DEVELOPMENT OF INTEREST

Improper healing processes can result in a variety of aberrances, from aesthetically unpleasant scars to post-surgical complications. Abnormal repair may also occur upon tissue/tumor resection, *e.g.*, in patients with cancer. MC could exert beneficial or deleterious functions of the healing process depending on the circumstances. They are necessary to proper healing but too many MC can lead to aberrant scarring. Thus, MC degranulation and mediator release promotes anastomotic healing and leak prevention in case of colonic resection by increasing local blood supply. Conversely, increased number of MC correlates with keloids or pathological scars evoking their participation in excessive treatment-resistant scarring following various conditions, such as minimal trauma after surgery, at the site of healing bone fractures, perivascular fibrosis after aortic constriction or in diseased kidneys of diabetic patients. The development of appropriate animal models relevant to study the exact involvement of MC in human wound healing is necessary to design novel therapeutic interventions in the field.

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The author has no competing interests. This article was written by its author.

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