学 位 論 文 の 要 旨

氏名　　錦織　頼子

学　位　論　文　名　　Pathophysiological Role of Skin Mast Cells in Scald Wound Healing in Mice

発　表　雑　誌　名
（巻，初頁～終頁，年）
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　　Refer to the attached document

論 文 内 容 の 要 旨

INTRODUCTION

Wound healing is a complex but systematic process which can be divided into three overlapping phases: acute inflammation, proliferation, and remodeling. Acute inflammation phase: inflammatory cells infiltrate into injured area to remove necrotic tissues. Proliferation phase: regeneration of granular tissues, re-epithelialization, and angiogenesis occur. Remodeling phase: reorganization of extracellular matrix (ECM) and tissue remodeling proceed, eventually resulting in wound retraction.

Mast cells (MCs) reside in the skin connective tissue and liberate a variety of growth factors and proteases. Reportedly, MCs accelerate the development of skin fibrosis in Tight Skin mice; MCs accumulate in hypertrophic scars in humans. These facts suggest that MC activation may be involved in the dermal proliferative response. Roles of MCs in wound healing process have also been studied with simple skin incision or excision model of rodent, but failed to establish the importance of MCs without any controversy. In this point, it should be noted that the major mechanism of wound closure in rodents is contraction, which leads to rapid closure of skin defect before MCs begin to proliferate to form granulation tissues. Instead, the healing process after scald injury is much more complicated and prolonged than that after skin incision or excision, and is not accomplished without proliferative response where MCs are supposed to be potentially involved.

Therefore, we first established murine scald injury model, wherein the MCs role was examined (study 1: reference 1). Then, to further investigate potential involvement of MCs in

wound healing process after scald injury, we compared the process between MC-deficient mice and normal mice (study 2: reference 2).

MATERIALS AND METHODS

Study 1: Skin MC promotion of matrix remodeling in burn wound healing in mice: relevance of chymase

Deep-dermal second-degree scald injury was induced on the back of ICR mice by applying a heated (100°C) cylindrical lead block for 5 seconds. The size of scald injury, density of capillaries, collagen accumulation, number of MCs, and chymase (a major protease of MCs) activity were measured before and at 1, 3, 7, and 14 days after inducing scald injury.

Study 2: Pathophysiological role of skin MCs in wound healing after scald injury: study with MC-deficient W/W^v mice

The same procedure as Study 1 was induced on the back of male MC-deficient mice (W/W^v) and their congenic normal (+/+) littermates. The size of scald injury, thickness of dermis, collagen accumulation, numbers of MCs and microvessels, and chymase activity were measured before and at 1, 3, 7, 14, and 21 days after inducing scald injury. Immunohistochemical analysis was performed to investigate the localization of chymase, FGF2, TGF-β1, and VEGF.

RESULTS AND DISCUSSION

Our Study 1 illustrated that MC number and chymase activity both were increased markedly in the scald-injured tissues, concurrently with the accelerated granular tissue formation and angiogenesis in the proliferation phase. The results suggest that our scald injury model is more appropriate to investigate the MC role in wound healing than previous murine skin incision or excision model which failed to show clear increment of MC number in the healing process. MCs produce a variety of angiogenic factors and growth factors; chymase is one of the most important MC-derived proteases to regulate ECM formation and fibroblast proliferation. In contrast, in the acute inflammation phase, both MC number and chymase activity were decreased. These findings suggest that MCs may play dominant role in the wound healing process especially in the proliferation phase. Noteworthy is that the numbers of MCs and of microvessels in injured tissues were continuously increased up to the end of experiment at day 14. This finding raised the possibility that MCs also participate in the early and presumably the late remodeling phases.

Thus, to further investigate potential involvement of MCs in the wound healing process, we compared the process between MC-deficient W/W^v mice and normal +/+ mice, from acute

inflammation phase throughout late remodeling phase (day 21) after inducing scald injury. Our Study 2 clearly showed that fibrous proliferation and angiogenesis were weaker in W/W^v mice than in +/+ mice in the proliferation phase, although re-epithelialization occurred at apparently the same speed in both +/+ and W/W^v mice. Importantly, vascular regression already took place in +/+ mice in the late remodeling phase at day 21, but was not observed at all in W/W^v mice, indicating a substantial delay in completion of wound healing process in W/W^v mice. While MC number was very low in the injured tissues of W/W^v mice, MCs highly accumulated in close proximity to the granulation tissues of +/+ mice at days 14 and 21. These findings confirmed that MCs accelerate fibrous formation and angiogenesis at proliferation phase, and induce vascular regression at late remodeling phase.

Furthermore, MCs which accumulated in the injured tissues of +/+ mice produced and liberated chymase, FGF2, TGF-β1, and VEGF. Chymase activity was once decreased at the early phase, but recovered to pre-injured control level at day 14 and remained increased up to day 21 in +/+ mice. On the contrary, chymase activity in $W\text{-}W^v$ mice remained very low throughout the experiment. MCs produce latent TGF-β1, which is immediately converted by co-secreted chymase into active TGF-β1 that is a well known potent pro-fibrotic growth factor. Because MCs highly accumulated in the injured tissue of +/+ mice, the local concentration of active TGF-β1 in the injured tissue may be higher in +/+ mice than in $W\text{-}W^v$ mice, particularly in the later phases. In addition, MCs also produce several potent angiogenic factors such as FGF2 and VEGF. These MC-derived angiogenic factors supposedly promote angiogenesis in the proliferation phase. Interestingly, TGF-β1 stimulates endothelial cell function and neovascularization at its low concentration, whereas it inhibits angiogenesis at relatively high concentration. The delay in vascular regression observed in W/W^v mice at the late remodeling phase may be ascribed to the low concentration of local TGF-β1. Furthermore, MCs are potent regulator of matrix remodeling; both the composition and microenvironment of ECM are crucial factors that regulate angiogenesis. Taken together, MCs may play an important role in the fibroproliferative response, and affect the delicate balance between pro- and anti-angiogenic factors throughout both proliferation and remodeling phases of the wound healing.

CONCLUSION

MCs may play an important role to determine the general quality of the healed tissues after scald injury. In other words, MCs are supposed essential to promote the sound healing of the wound.

別紙

氏　名　錦　織　頼　子

論 文 名

1. Skin Mast Cell Promotion of Matrix Remodeling in Burn Wound Healing in Mice: Relevance of Chymase

2. Pathophysiological Role of Skin Mast Cells in Wound Healing After Scald Injury: Study With Mast Cell-Deficient W/W^V Mice

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