

Matrix Metalloproteinases and Their Inhibitors in Normal and Aberrant Wound Repair

Expression patterns of collagenases-1 and -3, stromelysins-1 and -2, matrilysin, metalloelastase and TIMPs-1, -2, -3, and -4 in healing cutaneous wounds and in chronic ulcers of the skin and the intestine

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Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes, that are collectively able to degrade most, if not all, components of the extracellular matrix (ECM). This capacity is needed in conditions with active remodeling of the connective tissue, such as fetal development, cancer invasion and metastasis, as well as wound healing. In this study, the expression patterns of MMPs and tissue inhibitors of metalloproteinases (TIMPs) were investigated in normally healing and chronic cutaneous wounds, and in chronic intestinal ulcerations. The principal methods used were *in situ* hybridization and immunohistochemistry.

In normally healing cutaneous wounds, the migrating front of keratinocytes expressed collagenase-1 and stromelysin-2. These enzymes were induced within 1–3 days after wounding, and the expression was turned off after complete re-epithelialization.



Dr. Maarit Vaalamo (centre) defended her thesis in Helsinki, February 4th, 2000. Faculty Opponent Professor Jyrki Heino, Department of Biological and Environmental Sciences, University of Jyväskylä (left) and Chairperson, Professor Annamari Ranki, Department of Dermatology and Venereology, University of Helsinki (right).

Stromelysin-1 was expressed by proliferative keratinocytes on a newly formed basement membrane. Collagenase-1 and stromelysins-1 and -2 were expressed in the same spatial pattern also in the epithelium of chronic ulcers. In addition to epithelial events, MMPs were involved in stromal remodeling. In both normally healing and chronic wounds, the expression of collagenase-1 by stromal cells was a constant finding. Contrasting this, collagenase-3 was only expressed in fibrotic areas of chronic ulcers, but not in acute wounds. Stromelysin-1 mRNA was detected in the stroma in both acute and chronic wound samples.

TIMPs-1 and -3 were expressed by basal, proliferating keratinocytes in normally healing, but not in chronic cutaneous wounds, suggesting imbalance of the MMPs and their inhibitors in chronic ulcers. TIMPs-1 and

-3 mRNAs were also expressed by stromal cells in both wound types. TIMP-2 protein was detected in wound stroma, and particularly in acute wounds, surrounding the migrating front of keratinocytes. TIMP-4 protein was only found in few stromal cells in chronic ulcers.

No collagenase-1 nor stromelysin-1 mRNAs were detected in intestinal epithelium. Instead, matrilysin and stromelysin-2 were expressed by migrating intestinal epithelial cells. Collagenase-1, collagenase-3 and stromelysin-1 were abundantly expressed by activated fibroblast-like cells beneath erosions or ulcerations in inflammatory bowel disease (IBD). Macrophage metalloelastase mRNA was detected in macrophages within the inflammatory infiltrate, and underneath the shedding epithelium. TIMPs-1 and -3 mRNAs were detected in the stroma of IBD lesions as well,

but, as in chronic cutaneous wounds, the epithelium remained negative.

In conclusion, successful wound healing is accompanied by tightly scheduled expression of metalloproteinases and their inhibitors. Their imbalance may delay wound healing and result in chronic ulcers. MMPs and TIMPs are also involved in both tissue destruction and mucosal reparative processes during the course of inflammatory bowel diseases.

Original publications

- I Saarialho-Kere U, Vaalamo M, Puolakkainen P, Airola K, Parks WC, Karjalainen-Lindsberg M-L. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. *Am J Pathol* 1996; 148: 519-526.
- II Vaalamo M, Weckroth M, Puolakkainen P, Saarinen P, Kere J, Lauharanta J, Saarialho-Kere U. Patterns of matrix metalloproteinase and TIMP-1 expression in chronic and normally healing cutaneous wounds. *Br J Dermatol* 1996; 135: 52-59.
- III Vaalamo M, Mattila L, Johansson N, Karjalainen-Lindsberg M-L, Kariniemi A-L, Kähäri V-M, Saarialho-Kere U. Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. *J Invest Dermatol* 1997; 109: 96-101.
- IV Vaalamo M, Karjalainen-Lindsberg M-L, Puolakkainen P, Kere J, Saarialho-Kere U. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12) and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. *Am J Pathol* 1998; 152: 1005-1014.
- V Vaalamo M, Leivo T, Saarialho-Kere U. Differential expression of tissue inhibitors of metalloproteinases (TIMP-1, -2, -3 and -4) in normal and aberrant wound healing. *Human Pathol* 1999; 30: 795-802.

Predictive Testing for Contact Allergy. Comparison of Some Guinea Pig and Mouse Protocols Including Dose-Response Designs

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Contact allergy (delayed hypersensitivity) may develop as a result of skin exposure to contact allergens (haptens) and can lead to allergic contact dermatitis. The purpose of this study was to evaluate some predictive animal test methods for contact allergens. It was done with the aim that the test methods giving the clinically most relevant results should be used in risk assessment of chemicals and in research.



Helen Wahlkvist defended her thesis on January 14, 2000 at the Unit of Dermatology and Venereology, Department of Medicine, Karolinska Institute. Faculty Opponent was Professor Klaus E. Andersen, Odense University Hospital, Odense, Denmark and Chairman was Associate Professor Carola Lidén, Occupational and Environmental Dermatology, Stockholm County Council, Stockholm, Sweden.

A slightly modified multi-dose-response induction protocol was evaluated with two model contact allergens when applied to three guinea pig predictive test methods.

The protocol was easily applied to the cumulative contact enhancement test (CCET) and the Freund's complete adjuvant test (FCAT), which have only one induction route. However, for the