Increased expression of EMMPRIN in the tissue around loosened hip prostheses

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Matrix metalloproteinases (MMPs) have been shown to play a role in aseptic loosening of total hip replacement (THR). Extracellular matrix metalloproteinase inducer (EMMPRIN) can upregulate expression of several MMPs but has little effect on their tissue inhibitor (TIMP). Using the avidin-biotin-peroxidase complex immunostaining method, we detected strong immunoreactivity of EMMPRIN in the lining-like layers, sublining area and vascular endothelium of synovial membrane-like interface tissue around loosened prostheses. In contrast, EMMPRIN staining was very weak in the synovial samples from patients with hip arthrosis. Double immunofluorescence labeling revealed EMMPRIN/MMP-1 double-positive cells in lining-like layers and the sublining area of interface tissue.

Our findings indicate that EMMPRIN expression is upregulated in interface tissue, and that locally accumulated EMMPRIN may modulate MMP-1 expression. An imbalance in the activity of MMPs and TIMP may lead to tissue destruction and periprosthetic osteolysis. These biological responses, combined with mechanical stress caused by micromotion and oscillating fluid pressure, may eventually cause aseptic loosening of THR.

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Biological and mechanical factors may play important roles in aseptic loosening of THR (Aspenberg et al. 1992, Aspenberg and van der Vis 1998). The formation of a synovial membrane-like interface tissue between prosthesis/cement and bone is a common pathologic finding. Matrix metalloproteinases (MMPs) produced by the different cells of the interface tissue play an important role in the loosening process (Santavirta et al. 1993, Takagi et al. 1998a). However, the regulating mechanism of local MMP production is unclear.

EMMPRIN (extracellular matrix metalloproteinase inducer) is a M_r ~58,000 glycoprotein belonging to the immunoglobulin superfamily (Biswas et al. 1995). It may regulate degradation of collagenous and noncollagenous components of the extracellular matrix (ECM) by increasing the production of several MMPs (Guo et al. 1997). EMMPRIN is widely expressed in tumor cells and is also found in some normal human cells, such as monocytes (Kasinrerk et al. 1992, Kataoka et al. 1993, van de Oord et al. 1997). This is the first report about the expression of EMMPRIN in the interface tissue from aseptic loosening of THR.

Patients and methods

Patients and samples

Between February 1996 and July 1997, 10 samples of synovial membrane-like tissue were collected from the cement-bone interface of patients undergoing revision operations because of aseptic loosening of cemented THRs at the Helsinki University Central Hospital. Their mean age was 70 (37–85)

years, 7 were women. The reason for THR was arthrosis. The mean interval from the primary THR to revision was 8 (4–20) years. For comparison, 10 samples of synovial membrane were collected from patients undergoing primary THR due to arthrosis. Their mean age was 70 (40–83) years and 6 were women. These samples were embedded in Tissue-Tek OCT compound (embedding medium for frozen tissue specimens) and frozen in isopentane precooled with dry ice and kept at -70 °C until use.

Immunohistochemical staining

6 μm-thick cryostat sections were fixed in cold acetone for 15 minutes at 20 °C. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in absolute methanol for 30 minutes at +22 °C. The sections were then treated as follows: 1) Incubation with normal horse serum (Vector Laboratory, Burlingame, CA, dilution 1:50 in TBS containing 0.1 %BSA) for 20 minutes at +22 °C. 2) After blocking of the excess serum, the sections were incubated with mouse Mab (1G6.2) against human EMMPRIN (Chemicon, Temecula, CA, diluted 1:50 in TBS containing 1% BSA) overnight at +4 °C. 3) Incubation with biotinylated horse anti-mouse IgG (Vector Laboratories, diluted 1:100 in TBS containing 0.1% BSA) for 30 minutes at +22 °C. 4) Incubation with avidin-biotin-peroxidase complex (ABC, Vector Laboratories, diluted 1:100 in TBS) for 30 minutes at +22 °C. 5) Incubation with a combination of 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, 35 mg dissolved in 150 mL TBS) and 0.006% hydrogen peroxide for 5 minutes at +22 °C. Between 2 steps, the sections were washed for 3 × 5 minutes in TBS. Finally, the slides were counterstained with hematoxylin, dehydrated in a graded ethanol series, cleared in xylene and mounted using Diatex. Normal mouse IgG1 with irrelevant specificity was used at the same concentration as and instead of the primary antibody as a negative staining control. For statistical analysis, EMMPRIN staining was scored by determining the mean value reported by 3 researchers: no staining (0 point), weak staining (1 point), moderate staining (2 points), strong staining (3 points) and very strong staining (4 points). The Wilcoxon rank sum test was used for the statistical analysis.

Double immunofluorescence labeling

After fixation in cold acetone for 20 minutes at –20 °C, the sections were incubated with normal goat serum (Vector Laboratories, diluted 1:20 in PBS containing 1.25% BSA) for 30 minutes at +22 °C, followed by blotting of the excess normal serum. Then the sections were incubated with the following primary and conjugated antibodies: 1) mouse Mab (1G6.2) against human EMMPRIN IgG1 (diluted 1:50 in PBS containing 1.25% BSA) for 60 min at +22 °C; 2) FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, diluted 1:100 in PBS containing 12.5% BSA) for 30 min at +22 °C in the dark; 3) polyclonal rabbit anti-human MMP-1 IgG (Chemicon, diluted 1:1000 in PBS containing 1.25% BSA) for 60 min at +22 °C; 4) TRITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, diluted 1:100 in PBS containing 12.5% BSA) for 30 min at +22 °C in the dark. Human IgG (Sigma, St Louis, MO, 0.8 g/L) was added to the conjugated antibody solutions to reduce nonspecific staining. The sections were washed with PBS for 3 × 5 minutes between 2 steps. The slides were air-dried and mounted, using Vectashield (Vector Laboratories), and kept in the dark at +4 °C. We used mouse IgG1 and rabbit IgG as the negative staining control instead of first and second primary antibodies, respectively. The specimens were examined by fluorescence microscopy.

Results

Histological evaluation of interface tissue

The synovial lining-like layer of the interface samples was usually 1–3 layers thick. The stroma of the interface tissue was characterized by a foreign body reaction. Wear particles were present in all samples. The stroma was divided into two types: a cell-rich area containing macrophage-like cells and multinucleated giant cells, and a fibrotic area consisting of collagenous fibers and elongated fibroblasts.

Expression of EMMPRIN

All samples of the interface tissues showed immunoreactive EMMPRIN. The staining intensity var-

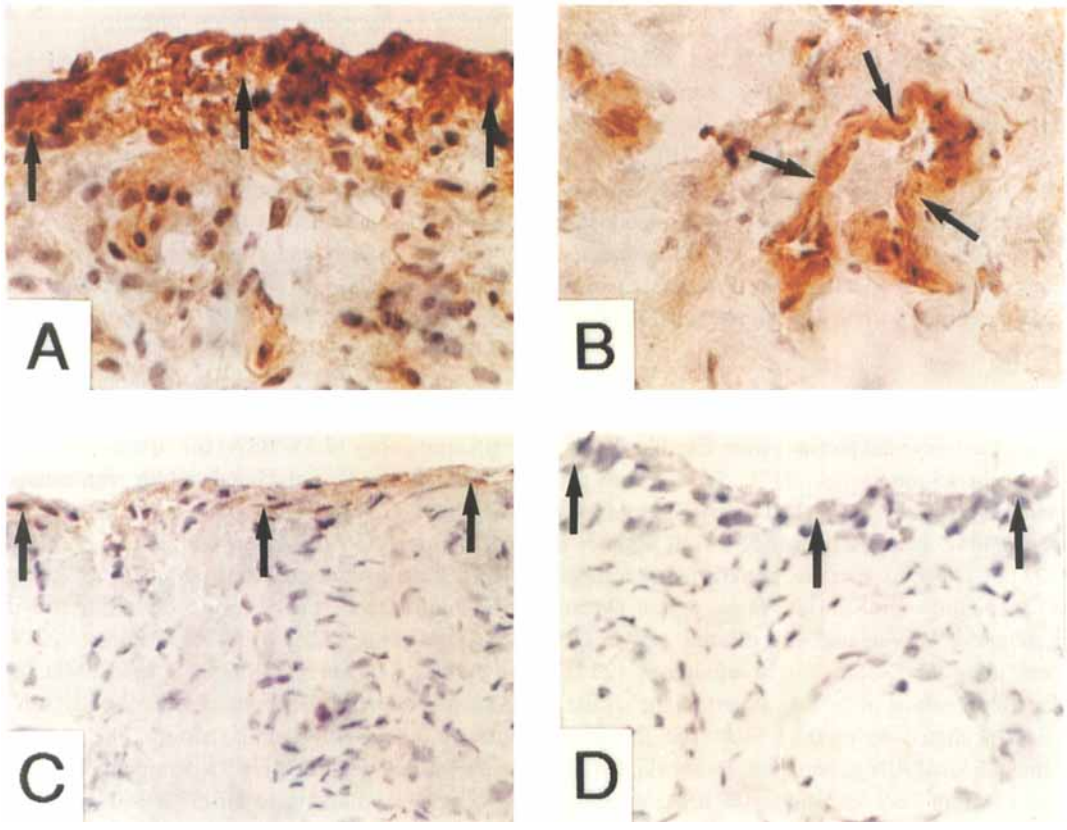

Figure 1. Expression of EMMPRIN in synovial membrane-like interface tissue and control synovial membrane (Avidin-biotin-peroxidase complex method, counterstained with hematoxylin).
A. Strong immunoreactivity of EMMPRIN in lining-like layers (arrows) of interface tissue (×250).
B. Strong staining in vascular endothelium (arrows) in stroma of interface tissue (×400).
C. Very weak staining in the lining layer of control synovial membrane (arrows). Note there was no staining in the deep stroma (×250).
D. Negative control staining with irrelevant monoclonal mouse IgG1 demonstrated the specificity of the staining method (×250).

ied in different samples and different regions. The areas facing the implant/cement (i.e., the synovial lining-like layers) and bone invariably showed strong immunoreactivity, while in the deep stroma, the staining was relatively weak and irregular. EMMPRIN staining was occasionally found in a pericellular pattern around macrophage-like cells in cell-rich areas. No positive immunoreactivity was found in the fibrotic areas. Staining was stronger in the walls of blood vessels in the deep stroma than in the surrounding matrix (Figure 1).

In control synovial samples, EMMPRIN staining was usually very weak. Immunoreactivity was restricted to the lining layer or to individual cells in the sublining tissue. No positive staining was detected in the stroma of the synovial tissue. Staining controls with monoclonal mouse IgG1, having an irrelevant specificity and used in the same concentration as EMMPRIN, were negative (Figure 1). The staining scores differed ($p = 0.001$) between the synovial membrane-like interface tissue and the control synovial samples (Table).

Colocalization of EMMPRIN with MMP-1

Double immunofluorescence labeling, using FITC and TRITC conjugated antibodies, revealed that, in lining-like layers and sublining areas of synovial-like membrane there were many cells labeled both by mouse Mab against human EMMPRIN and polyclonal rabbit antiserum against human MMP-1. Colocalization of EMMPRIN and

EMMPRIN staining scores (p = 0.001)

Samples [a]	Scores	Controls [b]	Scores
1	2	1	1
2	3	2	1
3	3	3	2
4	1	4	2
5	4	5	1
6	3	6	1
7	4	7	1
8	2	8	3
9	3	9	1
10	3	10	2

[a] Synovial membrane-like interface tissue from loosened THR
[b] Synovial samples from patients with hip arthrosis

MMP-1 was occasionally detected in vascular endothelial cells and macrophage-like cells in the deep stroma. Single EMMPRIN or MMP-1 positive cells were also found in the sublining area. No EMMPRIN labeling was found in some MMP-1 positive fibroblasts (Figure 2).

Discussion

The mechanism of aseptic loosening of THR is not understood. It is well established that wear particles may induce macrophage activation. Activated macrophages produce a series of bioactive substances that regulate osteoclastic bone resorption (Lassus et al. 1998). In a recent study, van der Vis et al. (1998) reported that short periods of oscillating fluid pressure at the implant/bone interface induced bone lysis. Cyclic loading may change the phenotype and function of the involved cells via matrix-integrin-cytoskeleton interaction. The combined effects of biological and mechanical factors finally cause aseptic loosening of prosthetic components after THR.

In this study, we have demonstrated the presence of EMMPRIN in the extracellular space of lining-like layers, and in vascular endothelial cells, lining cells, and macrophage-like cells in the interface tissue. EMMPRIN was previously referred to as tumor-cell-derived collagenase stimulatory factor (TCSF). It is identical with the M6 leukocyte activation antigen, and highly homologous to rat OX-47 or CE9, mouse basigin or gp42, and chicken HT-7 or neurothelin molecules (Kasinrerk et al. 1992). Two forms of EMMPRIN, soluble and membrane-bound, have been found in various tissues. The soluble form of EMMPRIN can form a complex with integrin α3ß1 and α6ß1, but not with α2ß1 or α5ß1 (Berditcheviski et al. 1997). Physical association of EMMPRIN and integrin stimulates the local production of MMPs, whereas it has little effect on TIMPs expression (Guo et al. 1997, Polette et al. 1997). EMMPRIN can increase the production of MMP-1 and MMP-

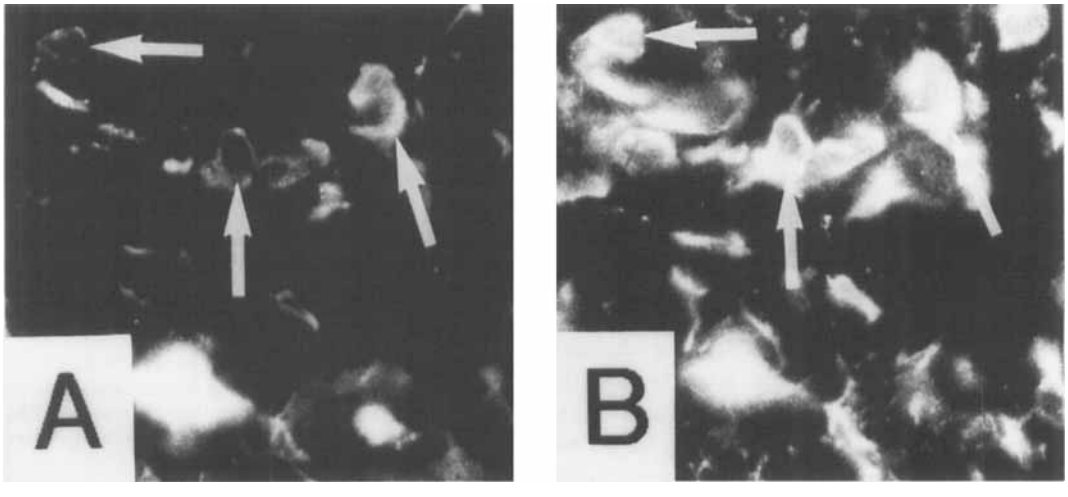

Figure 2. Double immunofluorescence labeling, using FITC and TRITC conjugated antibodies.
A. EMMPRIN positive cells in the lining-like layers and sublining area of synovial membrane-like interface tissue (arrows)
B. MMP-1 positive cells in the same area (arrows). Note that there were some EMMPRIN/MMP-1 double-positive cells (×920).

3 3- to 10-fold, and MMP-2 about 2-fold (Berditcheviski et al. 1997, Guo et al. 1997). Induction of MMP-1 mRNA by EMMPRIN occurs in a concentration-dependent manner. Tyrosine kinase activity is required for the process (Lim et al. 1998). In this study, we found many EMMPRIN/MMP-1 double-positive cells in the lining-like layers and sublining area of synovial membrane-like interface tissue, which further added to the evidence of the regulatory role of EMMPRIN in local MMP production.

EMMPRIN is widely distributed in the developing embryo. In contrast, most normal adult tissues express very low levels of EMMPRIN (Biswas et al. 1995). It is often found on the surface of tumor cells, but not on adjacent fibroblasts. In addition, it is a novel human leukocyte activation-associated cell surface glycoprotein. Peripheral granulocytes from patients with rheumatoid arthritis express a higher level of M6 (EMMPRIN) compared to granulocytes from healthy subjects. Monocytes constitutively express EMMPRIN in the resting stage. Granulocyte-macrophage colony stimulating factor (GM-CSF) may stimulate its production in monocytes, while tumor necrosis factor-alpha (TNF-α) has no effect on its expression (Kasinrerk et al. 1992, Schuster et al. 1996). GM-CSF has been shown to be upregulated in aseptic loosening of THR (Xu et al. 1996), which may in part explain the increased expression of EMMPRIN in the interface tissue.

MMPs are a major group of enzymes able to cleave both ECM constituents and nonmatrix proteins (Takagi et al. 1998a). Bone resorption involves the degradation of ECM. Whereas several proteases are implicated in this process, it becomes increasingly clear that MMPs play an essential role. MMPs can function as coupling factors, allowing osteoblasts to initiate bone resorption by generating collagen fragments that activate osteoclasts (Holliday et al. 1997). Degradation of the osteoid layer by MMPs is a necessary process before osteolysis can occur because the osteoid layer hinders osteoclasts from adhering to bone. In addition, MMPs are necessary for the migration of preosteoclasts from other areas to the resorption sites (Blavier et al. 1995, Sato et al. 1998). After attachment of osteoclasts to the mineralized surface, an area with a low pH is created, which results in dissolution of the mineral. Cysteine proteinases, active at such a low pH, then digest part of the bone matrix, and finally, when the pH has increased, MMPs become active (Everts et al. 1998).

Fibroblasts, macrophages and endothelial cells in synovial membrane-like interface tissue from aseptic loosening of THR can produce various MMPs (Santavirta et al. 1993, Takagi et al. 1994, Takagi et al. 1998b). However, the regulatory mechanism underlying MMP production is not yet clear. In this study, we found EMMPRIN positive lining-like cells, vascular endothelial cells and macrophage-like cells in the interface tissue. EMMPRIN may upregulate the expressions of MMP-1, MMP-2, MMP-3 and MT-MMP-1. In contrast, it has little effect on TIMP production (Polette et al. 1997). An imbalance in the activities of MMPs and TIMPs may cause uncontrolled ECM degradation and periprosthetic osteolysis. This may then contribute to the development of aseptic loosening of prosthetic components after THR. Pharmaceutical intervention in local EMMPRIN production may arrest or, at least, delay the loosening process.

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