Extracellular matrix metalloproteinases around loose total hip prostheses

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We have explored the tissue localization of extracellular matrix metalloproteinases MMP-1 (fibroblast collagenase), MMP-2 (72-kDa gelatinase/Type IV collagenase), MMP-3 (stromelysin), MMP-8 (polymorphonuclear leukocyte collagenase) and MMP-9 (92-kDa gelatinase/Type IV collagenase) in the tissues around loose hip prostheses. The findings were compared with those in synovial tissues obtained from patients with a fractured femoral neck. MMP-type specific antisera were applied in the sensitive avidin-biotin-peroxidase complex methods.

MMP-1 was found in monocyte/macrophages, fibroblasts, and vascular endothelial cells in both interface tissues between bone and acetabular components and the pseudocapsular tissues obtained from loosening of hip prostheses. In these tissues, MMP-8 was occasionally found, but only in polymorphonuclear leukocytes. Cells showing immunoreactivity to 72- and 92-kDa gelatinase/Type IV collagenase, MMP-2 and MMP-9, respectively, and stromelysin, MMP-3, were abundant in both interface and pseudocapsular tissues in loose hip prostheses. In contrast, in hip fractures, immunoreactivity to MMP-1, 2, 3, and 9 was weak and only observed in synovial tissues. Immunoreactivity to MMP-8 was confined to polymorphonuclear leukocytes attached to the synovial membrane or in the infiltrate around blood vessels in the subsynovial connective tissues.

The finding of MMP-1, 2, 3, and 9 in the tissues around loose hip prostheses suggests that they play a role in the weakening of connective tissues, and this leads to loosening.

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Although it is reasonable to assume that loosening of hip prostheses is caused by cyclic mechanical loading combined with cellular host reaction to implants, molecular mechanisms of the biological response to implants are still unknown as also is the mechanical triggering of such mechanisms. This study aims to demonstrate the presence of MMPs in periprosthetic tissues and determine their role in periprosthetic loosening. Of importance here is that these enzymes play a major role in connective tissue remodeling, and are capable of degrading the collagenous proteins which constitute the major extracellular matrix macromolecules of periprosthetic tissues.

Extracellular matrix degradation in vivo occurs in the extracellular space at neutral pH. Therefore, the extracellular matrix metalloproteinases (MMPs), which are active at physiological pH, are considered important in the cascades of connective tissue degradation in both normal tissues and pathological conditions. These MMPs comprise a gene family (Muller et al. 1988) which includes interstitial fibroblast collagenase, MMP-1 (Goldberg et al. 1986) and polymorphonuclear leukocyte collagenase, MMP-8 (Hasty et al. 1990), which mainly degrade Type I, II and III collagens, the major components of the extracellular matrix. After the initial enzymatic cleavage, interstitial collagens are denatured into gelatins at body temperature and then further digested by enzymes, probably by the gelatinase/Type IV collagenase, MMP-2 (Collier et al. 1988) and MMP-9 (Wilhelm et al. 1989). These proteinases can also degrade Type IV collagen, one of the major compo-

Table 1. Data for 6 hip revision patients

Case	Diagnosis[a]	Age	Sex	Years from index operation	Prosthesis revised	Revised component[b]	Cement fixation	Type of alloy[c]	Metalosis
1	OA	47	M	3.5	BIOMET	C	–	TiVAl	+
2	OA	76	M	5.0	LORD	C	–	CoCrMb	–
3	OA	66	F	9.0	LUBINUS	C, S	+	CoCrMb	–
4	OA	54	M	5.0	LUBINUS	C, S	+	CoCrMb	–
5	CDH	54	M	8.0	LORD	C	–	CoCrMb	–
6	CDH	46	F	1.5	BIOMET	C	–	TiVAl	+

[a] OA primary arthrosis, CDH arthrosis secondary to congenital dislocation
[b] C acetabular cup, S femoral stem
[c] TiVAl titanium-vanadium-aluminum alloy, CoCrMb cobalt-chromium-molybdenum alloy.

nents of basement membrane. Stromelysin, MMP-3 (Wilhelm et al. 1987), degrades proteoglycan, Types III, IV and VII collagens, laminin, fibronectin and gelatin, and it also activates other MMPs (Ito et al. 1988, He et al. 1989, Werb 1989, Goldberg et al. 1992, Ogata et al. 1992).

In this study, the immunohistochemical localization of MMPs 1, 2, 3, 8, and 9 was examined in the interface tissues between bone and implants and in the pseudocapsular tissues in loosening of hip prostheses. This enabled us to determine which of these proteinases were associated with prosthetic loosening and to compare their localization with that of synovial tissues obtained from patients with a fractured femoral neck.

Material and methods

Patients and samples

6 samples of the interface tissues around loose acetabular components and the pseudocapsular tissues were obtained in total hip revisions performed for aseptic loosening (Table 1). 6 samples of synovial capsular tissues were obtained at operations for intracapsular femoral neck fracture in 5 women and 1 man aged 76 (63–85) years with mean time of 19 (8–24) hours from accident to operation. The tissue samples, 5–10 mm in diameter, were embedded and frozen in Tissue-Tek® (Lab-Tek Products, Division of Miles Laboratories, Elkhart, IN, U.S.A.) and kept at –20 °C until used for immunohistochemistry.

Immunohistochemistry

6 µm-cryostat sections were fixed in acetone for 5 min at +4 °C, and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. The sections were then treated serially with normal horse serum (dilution 1:60, Vector Laboratory, Burlingame, CA, U.S.A.) for 20 min and with anti-rabbit MMP-1 (1:7 dilution, Goldberg et al. 1986), MMP-3 (1:11 dilution, Wilhelm et al. 1987), MMP-8 (1:350 dilution, Michaelis et al. 1990), MMP-9 (1:500 dilution, Karelina et al. 1993) and anti-chicken MMP-2 antibody (1:100 dilution; prepared and characterized as described by Collier et al. 1988 for anti-rabbit MMP-2 antibody) for 60 min at room temperature.

Each section was then exposed to biotinylated anti-rabbit or anti-chicken IgG for 30 min and to avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, U.S.A.) for 30 min at room temperature. Finally, the sites of peroxidase binding were revealed with a combination of 3,3'-diaminobenzidine tetrahydrochloride (Wako Junyaku Co. Osaka, Japan) and hydrogen peroxide (Hsu et al. 1981). All the sections were counterstained with hematoxylin. Between each step, the slides were washed twice with TBS (20 mM Tris-HCl, 150 mM NaCl, pH 7.4). Omission of primary antibodies in the staining sequence and incubation with non-immune rabbit or chicken immunoglobulin-G were used as controls. The number of immunoreactive cells to each antiserum was counted. Cell-counting areas in this study were the adjacent area to interface between tissue and implant, the pseudosynovial area in pseudocapsular tissues, and capsular tissue, including synovial membrane in fracture samples. The average numbers of positive cells in 5 different high-power fields (×250) were calculated and expressed as positive cells/high power field (HPF).

Statistics

Mean and standard error of mean (*SEM*) were calculated for the numbers of positively staining cells for MMP- 1, 2, 3, 8, and 9. The groups were compared by analysis of variance (ANOVA), with Scheffe *F*-test for detecting between-group differences. Statistical significance was set at $P < 0.05$.

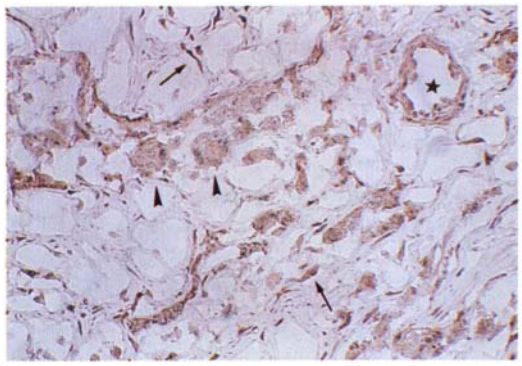

Figure 1. Immunostaining of MMP-1 in the interface tissues of loose hip prostheses. Monocyte/macrophages (arrow heads), fibroblasts (arrows) and vascular endothelial cells (asterisk: vascular lumen) showed marked immunoreactivity to MMP-1. ×120.

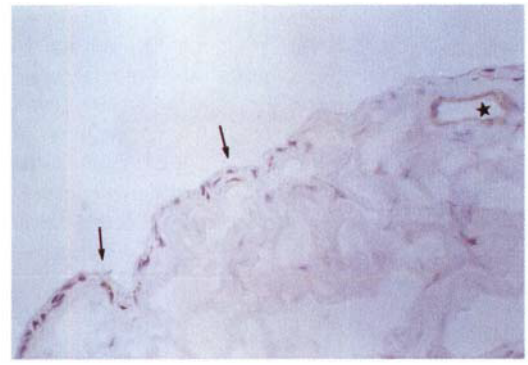

Figure 2. Immunostaining of MMP-1 in fractured hip. Synovial lining cells revealed slight-to-moderate immunoreaction (arrows) and subsynovial fibroblasts and vascular endothelial cells (asterisk: vascular lumen) showed slight immunoreactivity. ×120.

Table 2. The number of MMP-immunoreactive cells. Values are mean *SEM*

Type of MMPs	Tissue examined	Total cells/HPF (n 6)	Type of fixation: Cemented (n 2)	Type of fixation: Cementless (n 4)	Type of alloy [a]: TiVAl (n 4)	Type of alloy [a]: CoCrMb (n 2)
Interstitial collagenase						
MMP-1	Interface	[d]218 *20*	209 *6*	223 *28*	212 *54*	221 *7*
	Pseudocapsule	[d]196 *16*	209 *23*	190 *18*	213 *25*	188 *17*
	Fracture [b]	54 *13*				
MMP-8	Interface	5 *2*	2 *1*	7 *2*	8 *3*	3 *1*
	Pseudocapsule	4 *1*	2 *1*	5 *1*	6 *1*	3 *1*
	Fracture	9 *2*				
Gelatinase/IV collagenase						
MMP-2	Interface	[c]114 *12*	120 *22*	111 *11*	121 *21*	111 *12*
	Pseudocapsule	[c]133 *24*	90 *2*	155 *28*	169 *36*	115 *23*
	Fracture	17 *4*				
MMP-9	Interface	[d]180 *12*	175 *19*	183 *14*	193 *21*	174 *12*
	Pseudocapsule	[d]164 *9*	184 *5*	154 *8*	145 *1*	174 *9*
	Fracture	22 *6*				
Stromelysin						
MMP-3	Interface	[d]184 *20*	163 *14*	195 *25*	231 *11*	161 *18*
	Pseudocapsule	[d]172 *17*	190 *23*	163 *19*	169 *34*	174 *16*
	Fracture	45 *7*				

[a] TiVAl titanium-vanadium-aluminum alloy, CoCrMb cobalt-chromium-molybdenum alloy.
[b] Synovial capsular tissue of fractured hip.
[c] P 0.002, [d] P 0.001, interface and pseudocapsule versus fracture by ANOVA and Scheffe F-test.

Results

Interstitial collagenase, MMP-1 and MMP-8

MMP-1 was observed in both interface and pseudocapsular tissues in loose hip prostheses. Monocyte/macrophages, fibroblasts and vascular endothelial cells revealed marked intracellular immunoreactivity to MMP-1 (Figure 1). In synovial capsular tissues obtained from the fractured hips, the synovial lining cell layer had slight-to-moderate MMP-1 immunoreactivity. Only occasional stromal fibroblasts and vascular endothelial cells showed slight positive immunostaining for MMP-1 (Figure 2). The number of MMP-1 positive cells was 218 *20* cells/HPF in the interface tissues and 196 *16* cells/HPF in the pseudocapsular tissues in loose prostheses. These cell numbers were higher than those found in the synovial capsular tissues of the fractures (54 *13* cells/HPF, P 0.001, Table 2).

Immunoreactivity to MMP-8 was only observed in scattered polymorphonuclear leukocytes in both interface and pseudocapsular tissues of loose pros-

theses. In contrast, marked MMP-8 immunoreactivity was observed in polymorphonuclear leukocytes associated with or close to the synovial membrane and in or around the vessels of subsynovial connective tissues of fractured hips. The numbers of MMP-8 positive cells were 5 *2* cells/HPF in the interface tissues, 4 *1* cells/HPF in the pseudocapsular tissues in loose prostheses, and 9 *2* cells/HPF in the fracture tissues (P 0.1, Table 2).

Gelatinase/Type IV collagenase, MMP-2 and MMP-9

In both interface and pseudocapsular tissues, fibroblasts stained strongly positive for MMP-2 whereas monocyte/macrophages showed moderate immunostaining for MMP-2. Some of the synovial lining cells and stromal fibroblasts of fracture samples showed slight MMP-2 immunoreactivity. The number of MMP-2 positive cells was 114 *12* cells/HPF in the interface tissues and 133 *24* cells/HPF in the pseudocapsular tissues, respectively, and thus higher than that in the synovial capsular tissues of fractured hips (17 *4* cells/HPF, P 0.0002; Table 2).

In both interface and pseudocapsular tissues around loose prostheses, monocyte/macrophages revealed marked intracytoplasmic MMP-9 immunoreactivitiy, and fibroblasts and vascular endothelial cells of these tissues showed moderate immunostaining for MMP-9. MMP-9 positive polymorphonuclear leukocytes were rarely observed. In the synovial capsular tissues of fractured hip, slight MMP-9 immunostaining was observed in the synovial lining cell layer, stromal fibroblasts and polymorphonuclear leukocytes. The number of MMP-9 positive cells was 180 *12* cells/HPF in the interface tissues and 164 *9* cells/HPF in the pseudocapsular tissues in loose prostheses, and thus much higher than that in the synovial capsular tissues of fractured hips (22 *6* cells/HPF, P 0.0001, Table 2).

Stromelysin, MMP-3

Monocyte/macrophages, fibroblasts and vascular endothelial cells in both interface and pseudocapsular tissues in loose endoprostheses revealed moderate to markedly positive immunoreactivity to MMP-3. In the fracture samples of hip, staining of synovial lining cells, stromal fibroblasts, and vascular endothelial cells was weaker. The numbers of MMP-3 positive cells were 184 *20* cells/HPF and 172 *17* cells/HPF in the interface tissues and in the pseudocapsular tissues in loose prostheses, respectively. They were higher than that in the synovial capsular tissues obtained from hip fractures (45 *7* cells/HPF, P 0.0001; Table 2).

Although the number of cases examined was too small for statistical analysis, all the numbers of MMP-1, 2, 3, and 9 immunoreactive cells were higher in each case of both interface tissues and pseudocapsular tissues obtained from different types of fixation (cemented versus cementless) and types of alloy (Cobalt-Chromium-Molybdenum versus Titanium-Vanadium-Aluminum) of loose prostheses other than those in the synovial capsular tissue obtained from patients with hip fractures (Table 1).

Discussion

Due to their unique ability to cleave specifically the collagen triple helix, mammalian interstitial collagenases, MMP-1 and MMP-8, are considered as key initiators of collagen degradation. Subsequent structural degradation of extracellular matrix macromolecules is mediated by other MMPs—e.g., MMP-2, 3, and 9—and perhaps by other proteinases (Werb 1989).

The results presented here demonstrate that certain MMPs are induced in the interface tissues of loose hip prostheses. In particular, the number of cells in the interface tissues which stained positive for MMP-1, 2, 3, and 9 was higher than in the synovial capsular tissues obtained from fractured hips, even though the fracture samples do not represent normal tissue due to the presence of an influx of polymorphonuclear leukocytes associated with the acute inflammatory changes induced by trauma (Takagi et al. 1993). It is noteworthy that normal cells do not actively synthesize MMPs, at least to the extent that they can be demonstrated by immunohistochemical means. Although hip fracture capsular tissue does not represent normal capsular tissue (which for ethical and practical reasons was not available for purposes of comparison), there was a significant difference between the periprosthetic and fracture tissues as to the staining intensity and the relative proportion of the cells induced to produce MMPs (i.e., the number of immunoreactive cells/HPF). This MMP profile would be compatible to a sequence of events in which interstitial MMP-1, but not MMP-8, cleaves, in particular, Type I and III collagens (Werb 1989, Birkedal-Hansen et al. 1993) which are denatured into gelatins at body temperature and further degraded by gelatinase/Type IV collagenases (MMP-2 and 9). Stromelysin (MMP- 3) may assist in the degradation of other collagen types and proteoglycans.

Not only monocyte/macrophages but also fibroblasts and vascular endothelial cells synthesized

MMPs in both interface and pseudocapsular tissues. In contrast to polymorphonuclear leukocytes which synthesize and store MMP-8 and MMP-9 in their specific (or secondary) and c-type granules, respectively, all MMPs produced by mononuclear cells and fibroblasts are secreted into the extracellular space soon after being synthesized (Birkedal-Hansen et al. 1993). These findings indicate that periprosthetic tissue represents activated connective tissue involved in pathological matrix degradation, resulting in prosthesis loosening, and tissue remodeling. It was of particular interest to observe that induction of MMPs was not confined to the interface tissues interposed between bone and implants but was also seen in the pseudocapsular tissue around prostheses. The relative contributions of the interface tissues, which would be able to directly weaken periprosthetic tissues, and of the pseudocapsular tissues, which could contribute via production of MMPs into the synovial fluid, are presently unknown.

In this study, we have shown that MMPs were induced in cells in periprosthetic tissues. Both interface tissues between bone and implants, and pseudocapsular tissues around loose endoprostheses contained a larger number of MMP-immunoreactive cells than were found in the synovial capsular tissues obtained from fractured hips. The number of different MMPs found suggests that local conditions induce a wide spectrum of MMPs capable of degrading extracellular matrix macromolecules and mediating prosthetic loosening. A deeper understanding of the proteolytic cascade in loosening may in future lead to prevention of prosthetic loosening, because MMPs can be chemically inhibited (Wewers et al. 1987, Suomalainen et al. 1992, Lauhio et al. 1993, Sorsa et al. 1993) and it may become possible to modulate MMPs by synthetic and recombinant reagents in pathological conditions.

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