The plasminogen activation system is upregulated in loosening of total hip prostheses

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Interface tissues and pseudocapsules from loose total hip replacements were removed during revision of 11 cases and were investigated for the plasminogen activation system and IL-1ß. Control samples of synovium were taken during knee arthroscopy (n 8), and from the hip joint during primary total hip replacement (n 5). The concentrations of urokinase plasminogen activator (uPA), tissue type plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) and interleukin 1ß were all found to be significantly different in interfaces and in pseudocapsules, compared to controls. Immunohistochemistry disclosed localization in periprosthetic tissues of uPA, uPA-receptor and tPA in macrophages with phagocytosed metal, polyethylene, cement particles or accompanying pieces of necrotic bone. PAI-1 staining was present in the neighboring areas that stained for uPA or tPA, but PAI-1 staining was also found overlapping and outside these areas.

These findings suggest a role for the uPA/uPA-receptor and PAI-1 in activation and focalization of extracellular matrix degradation in periprosthetic tissues. The expression of the plasminogen activation system by macrophages containing phagocytosed material suggests undegradable microdebris as a possible initiating and perpetuating stimulus for a proteolytic activation cascade, which may contribute to loosening of the prosthesis.

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Proteinases, including the matrix metalloproteinases (MMP-1, 2, 9) and cathepsin-G and -B (Goldring et al. 1983, Schüller et al. 1993, Takagi et al. 1994a, 1994b), have been localized in the membrane formed around loose hip implants. Interstitial type collagenase and gelatinases from the periprosthetic tissues are secreted in an inactive zymogen form and need activation to degrade substrate (Goldring et al. 1983, Alexander and Werb 1992, Kim et al. 1993). The activation mechanism most often implicated for MMP-1 is the plasminogen activation system (Werb et al. 1977, Leloup et al. 1991). The urokinase and tissue-type plasminogen activators (uPA and tPA), the uPA receptor (uPAR) and PA inhibitors (PAI-1 and PAI-2) constitute the main system regulating and focalizing extracellular matrix degradation in vivo through regulation of collagenase activation (He et al. 1989, Thomson et al. 1989, Vassalli et al. 1991, Pelletier et al. 1992). Interleukin-1ß (IL-1ß) is probably the main cytokine for upregulation of both the plasminogen activation system and the collagenases (Michel and Quertermous 1989, Pelletier et al. 1990).

We investigated the presence, localization and antigen levels of the various components of the plasminogen activation system and the antigen levels of IL-1ß in interface and pseudocapsular tissues from loose total hip replacements.

Material and methods

Patients and tissue preparation

Samples were collected at revision of painful loose total hip replacements (THR) in 11 patients (Table 1). Interface tissues between the bone and loose acetabular cups and samples from the pseudocapsules were removed during revision. None of the loosenings showed radiographic signs of localized osteolysis. Cultures of joint fluid and the tissues were negative for aerobic and anerobic bacteria.

Control samples were taken from the synovium during knee arthroscopy in 8 patients (age 20–46 years, 6 meniscal tears, 2 negative findings), and from the synovium during primary total hip replacements for primary arthrosis in 5 patients (age 44–74 years). The control samples were used for tissue extract analysis and, since no differences in antigen levels were found, the controls were pooled.

Table 1. Characteristics of the patients and the prostheses at revision

Case no.	Diagnosis	Age	Sex	Years to revision	Previous THR	Prosthesis removed	Revised component	Cement	Metal	Metallosis at revision
1	A	47	M	3.5	–	Biomet	C	–	Titanium	+
2	A	76	M	5	–	Lord	C	–	CrCoMb	–
3	A	68	F	2	2	Biomet	C	–	Titanium	+
4	A	53	M	4.5	–	Lubinus	C	+	CrCoMb	–
5	A	72	F	1	2	Müller	C	+	CrCoMb	–
6	A	81	M	5	3	Biomet	C	–	Titanium	+
7	A	71	F	5	2	PCA	C	–	CrCoMb	–
8	CDH	54	M	8	–	Lord	C	–	CrCoMb	–
9	CDH	62	F	9	–	Lubinus	C	+	CrCoMb	–
10	CDH	59	M	7	1	Lord	C	–	CrCoMb	–
11	CDH	46	F	1.7	–	Biomet	C	–	Titanium	+

A arthrosis, CDH congenital dislocation of the hip

Tissue extracts

All tissue extraction procedures were done at +4 °C. Samples were rinsed in TBS (20mM Tris-HCl, 150 mM NaCl, pH 7.5), and minced into small pieces and homogenized with Ultra-Turrax T25 (Janke & Kunkel GmbH & Co. IKA Laboratory Technology, Staufen, Germany) in an ice bath after addition of neutral salt extraction buffer (50 mM Tris-HCL, 10 mM CaCl2, 2 M KCl, pH 7.5, 1 mL buffer/1 g tissue). After incubation for 1 hour, homogenates were centrifuged at 100×10^4 g for 60 min. Dialysis of supernatants was performed with 0.5 M acetate buffer (pH 5.3) for 24 hours.

Antigen level analysis

The antigen levels of uPA, tPA, PAI-1 and PAI-2 (TintElize™, Biopool, Umeå, Sweden) and IL-1ß (Quantikine™, R&D systems, Minneapolis, MN, USA) were determined by enzyme immunoassay (EIA).

Immunohistochemistry

Interface tissues and pseudocapsules from 6 randomly selected patients were analyzed by immunohistochemistry (Table 3). The tissue samples were embedded and frozen in OCT (Lab-Tek Products, Division of Miles Laboratories, Elkhart, IN, USA) immediately after the operation, and stored at –70 °C. 6 µm sections were cut on a microtome at –20 °C (Reichert-Jung Cryostat 1800, Leica Instruments GmbH, Nussloch, Germany) and collected directly onto chrome-alum gelatin-coated slides. After 2 hours of air drying the sections were fixed in acetone at 4 °C for 5 min. One section from each sample was stained with hematoxylin and eosin for histological examination. Cryostat sections were stained by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique previously described by Buø et al. (1993). Monoclonal mouse anti-human antibodies were applied for 60 min in the following concentrations: 50 µg/mL of anti-uPA (U 101-6, Monozyme, Virum, Denmark), anti-t-PA (PAM 4, Biopool), anti-PAI-1 (MAI-11, Biopool), anti-PAI-2 (MAI-21, Biopool), and anti-uPAR (no. 3936 American Diagnostica Inc., Greenwich, CT, USA), 7.6 µg/mL of anti-CD68 (activated macrophages KP1, M814, Dako, Copenhagen, Denmark) and 1.7 µg/mL of anti-CD3 (T-lymphocytes, Leu-4, Becton Dickinson, Mt. View, CA, USA). The second and third reagents were rabbit anti-mouse IgG and APAAP complexes (Dako), diluted 1:40 (50 µg/mL and 4.25 µg/mL, respectively). A red color reaction was developed with alkaline phosphatase substrate [10 mg naphthol AS-MX phosphate (Sigma, St. Louis, MO, USA) in 1 mL dimethylformamide (Sigma) and 49 mL Tris buffer (0.1 M, pH 8.2) and 50 µl 1 M levamisole (Sigma L9756), with 50 mg Fast Red TR (Sigma) added immediately before use]. Incubations were performed in a moist chamber at room temperature, with intervening washes in phosphate-buffered saline. The sections were counterstained with hematoxylin, mounted in polyvinyl alcohol (pH 7.4) and examined by light microscopy. Omission of the primary antibody was used as a negative control. Results were arbitrarily scored as negative (–), occasional positive cells (–/+), positive cells present in several (+), in most (++) or in all (+++) areas (Santavirta et al. 1992). The immunohistochemistry evaluation was done blindly by two independent observers. The sections were examined under incident and polarized light.

Statistics

Mean and standard error of the mean were calculated for the tissue extracts. Differences between control and loose THR tissues were analyzed by the Mann-Whitney U-test. Differences between interface and

Table 2. Antigen levels for urokinase (uPA) and tissue (tPA) plasminogen activator, plasminogen activator inhibitor (PAI-1 and PAI-2) in tissue extracts from interface tissues and pseudocapsules of loose total hip prostheses (n 11), and from control knee synovium removed during arthroscopy (n 8) and hip joint synovium (n 5) in patients undergoing primary total hip replacement for primary arthrosis. Mean *SEM*

Sample	uPA (ng/mL)		tPA (ng/mL)		PAI-1 (ng/mL)		PAI-2 (ng/mL)	
Interface	18.0	*1.8*[a]	22.2	*3.9*[c]	78	*14*[a]	4.4	*1.3*[e]
Pseudocapsule	15.3	*2.2*[b]	28.7	*10.6*[d]	81	*17*[a]	4.7	*1.1*[f]
Control	4.6	*0.8*	76.0	*9.7*	4.3	*2.1*	2.3	*1.8*

[a] P 0.0001, [b] P 0.0003, [c] P 0.0002, [d] P 0.0005, [e] P 0.04, [f] P 0.02

Table 3. Immunohistochemical evaluation of staining for the compounds of the plasminogen activation system in interface membranes (IN) and pseudocapsules (CA) from 6 patients with loose total hip prostheses

Case	uPA		tPA		uPAR		PAI-1		PAI-2		CD68		CD3	
	IN	CA	IN	CA	IN	CA	IN	CA	IN	CA	IN	CA	IN	CA
3	++	–/+	–	–/+	+	–/+	++	+	–	–	+++	+++	–	–/+
4	+	–	+	+	+	+	++	+	+	–	+++	+++	–/+	+
6	+	+	+	++	–	+	+	+	–	–	+++	+++	+	+
7	+	–	++	–/+	+	+	+++	++	–/+	–/+	+++	+++	–/+	+
9	+	–/+	–/+	+	+	+	++	+	–/+	–	+++	++	–	–/+
10	–/+	–/+	–/+	+	+	+	++	+	–	–	+++	+	–/+	–

uPA urokinase plasminogen activator, tPA tissue plasminogen activator, uPAR urokinase plasminogen activator receptor, PAI plasminogen activator inhibitor, CD68 macrophage marker, CD3 T-lymphocyte marker.
– negative, –/+ occasional, + present in several areas, ++ present in most areas, +++ present in all areas.

pseudocapsular tissues were analyzed by the Wilcoxon signed rank test for paired groups.

Results

Tissue extracts

IL-1ß was 193 *72* (mean *SEM*) ng/mL in the interfaces and 114 *57* ng/mL in the pseudocapsules, while it was not detected in the controls.

uPA was present in extracts from all samples (Table 2). The mean antigen concentrations in the interface tissues and the pseudocapsules were 4 times higher than in the control samples. tPA concentrations in the interface tissues were 22.2 *3.9* ng/mL and in the pseudocapsules 28.7 *10.6* ng/mL, compared to 76 *9.7* ng/mL in the control tissues.

PAI-1 was found in much higher concentrations than PAI-2 in the tissue extracts from the loose prostheses, but both they were higher than in the controls (Table 2).

Morphology and immunohistochemistry

The interface and the pseudocapsular tissues consisted of scattered cells in a fibrous stroma with aggregations of cell-rich areas, consistent with previous findings in loose THRs (Goldring et al. 1983). Both in the interface tissues and in the pseudocapsules, the cell aggregates mainly contained CD68 positive macrophages, but with fewer such areas in the pseudocapsules. Some giant cells were found, but only a few granulomas. Only scattered CD3 positive T-lymphocytes were observed in interface tissues, while more cells were stained in the pseudocapsules (Table 3). Two membranes and one capsule were totally negative for CD3 staining.

Plasminogen activators-uPA and tPA

All 6 interface tissue samples revealed positive staining for uPA (Figure 1), whereas only 4 pseudocapsules contained uPA positive cells, and in smaller areas (Table 3). This difference was confirmed by comparing the tissue extract uPA concentrations in these 6 immunohistochemistry cases: 19.9 0.7 ng/mL in the interface tissues versus 13.9 1.5 ng/mL in the pseudocapsules (P 0.04). The staining varied between and within the samples, from small areas staining moderately positive to larger areas heavily stained. 5 interface tissues and all pseudocapsules were positive for tPA. Most cells staining for uPA or tPA were heavily loaded with metal, polyethylene or cement particles (Figure 1). In addition, large bone pieces

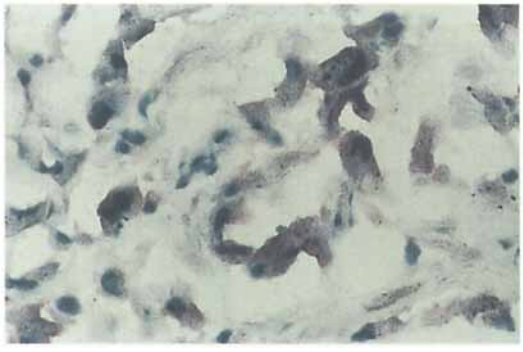


Figure 1. Frozen section of interface membrane (case 10) stained for uPA. Macrophage-like cells with phagocytosed metal particles show strong staining, ×900.

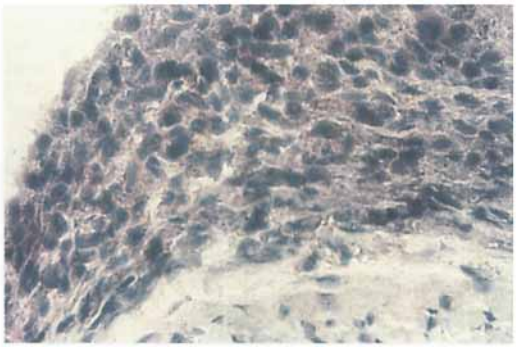
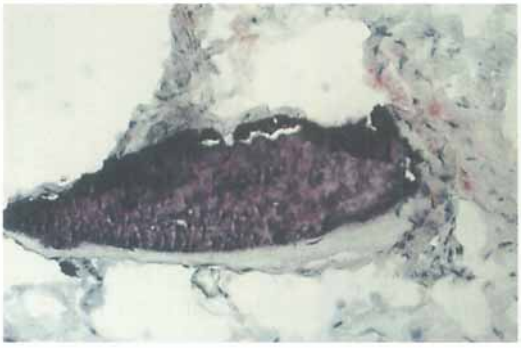
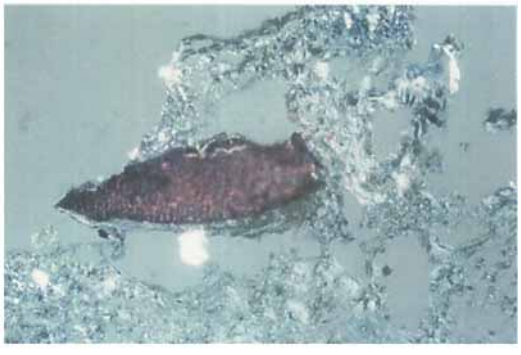


Figure 3. Frozen section of pseudocapsule (case 9) stained for uPAR. Both slender, elongated fibroblast-like cells and macrophage-like cells stain moderately-to-strongly positive. Note the absence of staining in the fibrous tissue under the cell-rich area, ×400.

Figure 2. Frozen section of interface membrane (case 9) stained for uPA.



Cells associated with the piece of dead bone show moderate-to-strong staining, ×400.



In polarized light, the cells are seen to be heavily loaded with polarizing polyethylene particles, ×200.

were surrounded with CD68 positive cells staining intensely for uPA in cases 4 and 9. In polarized light, numerous polyethylene particles were revealed intracellularly in the cells around the bone pieces (Figure 2).

Plasminogen activator inhibitors-PAI-1 and PAI-2

All interface tissues and pseudocapsules stained for PAI-1. Compared to staining for uPA and tPA, larger areas were stained for PAI-1, especially in the interface tissues (Table 3). PAI-2 staining was not so intense or extensive as for PAI-1, with only 1 pseudocapsule and 3 interface tissues showing slightly positive staining. Comparison of consecutive slides stained for uPA and PAI-1 revealed neighboring areas as being positive for inhibitor and activator, but also with areas that were positive for both.

Urokinase plasminogen activator receptor-uPAR

uPAR positive cells were observed in 5 of 6 interface tissues and in all pseudocapsules. In the pseudocapsules, uPAR positive staining cells were confined to the cell-rich areas underneath the synovial-like lining cells (Figure 3), while they were more evenly distributed in the interface tissues. Macrophages and fibroblast-like cells stained positive for uPAR.

Discussion

A key event in the loosening process of total hip prostheses is thought to be the activation of collagenases that can either destroy the interface tissues and/or remove osteoid and thereby promote osteolysis by providing a surface for osteoclastic bone resorption (Thomson et al. 1989). Previous studies have shown that several proteinases are present in periprosthetic tis-

sues (Goldring et al. 1983, Takagi et al. 1994b). In the present study, we report the presence of the plasminogen activation system, which is capable of activating collagenases and inducing tissue destruction, including bone resorption (Thomson et al. 1989, Vassalli et al. 1991).

The antigen levels in the periprosthetic tissues with high uPA and PAI-1, together with low tPA compared to the control synovium, are similar to the differences reported for malignant and normal colon mucosa and breast tissue (Jänicke et al. 1991, Sier et al. 1991). The resemblance of the findings to those in malignant tumors shows that the tissues around loose THRs may have a high destructive capacity. It is interesting that, although the PAI-1 antigen concentration was higher than the activators, activation may still have taken place on the cell surface in smaller compartments secluded from the inhibitor (Saksela and Rifkin 1988). Similar findings have been reported in invasive cancer, and they are correlated with a poor prognosis (Schmitt et al. 1991). This suggests that PAI-1 is an integral part of the aggression of the plasminogen activation system. PAs may also be produced by osteoblasts and osteoclasts as a part of the periprosthetic bone resorption (Thomson et al. 1989, Grills et al. 1990, Leloup et al. 1991). PAI-1 could thus represent a form of self-defense against proteolysis, as seen in invading carcinomas, which express PAs at the invasion zone and PAIs in the tumor tissue (Buø et al. 1993). Immunohistochemistry of serial sections of periprosthetic tissues disclosed that many cells/areas expressing PAs and PAI-1 did not overlap. In addition, it was impossible in individual cells to ascertain whether the cells were polarized for, e.g., uPA/uPAR versus PAI-1 expression. Expression of the proteinase and its inhibitor by the same cell is common (Alexander and Werb 1992), and is probably a mean for targeted matrix degradation.

In the present study PAs seemed to be expressed almost exclusively by CD68 positive macrophages with intracellular particles, without any obvious difference between cells having phagocytosed metal, cement or polyethylene. Macrophages phagocytosing particulate debris also produce IL-1 (Glant et al. 1993). Our finding that IL-1ß was high in the periprosthetic tissues corroborates these in vitro results, and IL-1ß could thus have been at least one of the cytokines mediating the increased uPA and PAI-1 production observed in the present material (Michel and Quertermous 1989, Pelletier et al. 1990).

Macrophages were the dominating cell type in the cell-rich aggregates of the samples studied and, in the fibrous areas, CD68 positive cells were as numerous as the fibroblasts. CD3 positive T-lymphocytes were rare or absent in 5 of the 6 interface membranes, and a little more frequent in the pseudocapsules. The cell distribution, the content of PA/PAI in tissue extracts, and the immunohistochemistry of pseudocapsules and interface membranes were, in general, not very different. This is in accordance with findings by Goldring et al. (1983). In the present study, the amount of particles was high also in the pseudocapsules, and the results point to the role of the pseudocapsules in the loosening process. Particles will have easy access to the pseudocapsules and, vice versa, proteinases and cytokines produced by the pseudocapsules can reach the bone through the pseudocapsular fluid (Glant et al. 1993, Ohlin and Lerner 1993).

As the findings were made in already loose prostheses, cellular mechanisms at the initiation of loosening may be different. Tissue samples from human prostheses at the initiation of loosening were not accessible but, because the plasminogen activation system has been widely implicated in tissue degradation, we believe that its presence in already loose prostheses indicates that it is of importance also at the start of the loosening. Pharmacological manipulation of the plasminogen activation system has shown promising results in the spread of cancer and in rheumatoid arthritis, and it may become possible to stop or retard the loosening process by specific inhibition of this system (Pelletier et al. 1992, Ellis and Danø 1993).

In conclusion, our study showed the presence of all components of the plasminogen activation system and high levels of IL-1ß in the periprosthetic tissues. This system generates the focalized activation of plasmin, which may be the starting point of a cascade activating collagenases and thereby tissue destruction and loosening of the prostheses. IL-1ß may be an important regulatory cytokine in this process.

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