Expression of tenascin-C in aseptic loosening of total hip replacement

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Abstract

Objective—To assess if the bonding interlayer between the implant and bone in aseptic loosening of total hip replacement (THR) is qualitatively deteriorated by excessive accumulation of anti-adhesive glycoprotein, tenascin-C.

Methods—Alkaline phosphatase-anti-alkaline phosphatase (APAAP) method was used for immunohistochemical staining of tenascin-C in interface tissue and control synovial tissue.

Results—Tenascin-C was found to be a major component of the extracellular matrix at a hitherto unrecognised site, namely the synovial membrane-like interface tissue between implant and bone in aseptic loosening of THR. The overall tenascin-C staining (median score 4.0) was greatly increased in aseptic loosening compared with synovial membrane (median score 2.0; $p < 0.001$) and fibrous capsule (median score 2.0; $p < 0.001$) from primary THR operations. Topological analysis disclosed that tenascin-C was also found at the critical implant-interface and interface-bone surfaces.

Conclusion—Local tenascin-C expression is increased as a result of a chronic foreign body type reaction associated with excessive cytokine production and tissue injury mediated by microtrauma and neutral endoproteases. This qualitative and topological deterioration of the bonding interlayer by an increase of anti-adhesive tenascin-C expression may inadvertently contribute to loosening.

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The extracellular matrix (ECM) is no longer viewed as a passive structure that only provides physical strength and elasticity.¹ New observations indicate a dynamic interplay between cells and the surrounding matrix. Thus, the ECM plays a profound part in events such as development, tissue injury, healing, and diseases.^{2–3} Some of the matrix components are constitutively expressed, whereas others demonstrate a surprising temporo-spatial restriction in their expression. Tenascin-C, a highly regulated glycoprotein component of the ECM, was originally found in myotendinous junctions and has been reported to be abundant in embryonic tissues.⁴ Expression of tenascin-C in adult tissues is normally restricted, but is upregulated in inflammation and tissue repair.^{5–9} For example, tenascin-C expression is increased in wound healing and

can typically be found in the matrix of the granulation tissue and under the proliferating and migrating epidermal layer. After healing, however, tenascin-C expression returns to normal.¹⁰

Total hip replacement (THR) as a surgical procedure has a very good cost-benefit ratio. Today, cost of revision surgery dominates overall cost and is more important than the differential cost of the implant.¹¹ In 1992, 118 000 inhabitants of the United States received THR—that is, 1 per 2118. The projected incidence of THR for the year 2010 is 157 000 and for the year 2030 is 219 000.¹² Aseptic loosening of initially well inserted implants in the long term is its major complication.^{13–14} Tenascin-C may play a part in the delicate balance between osseointegration versus aseptic loosening of the implant (vide infra). Implantation with the prosthetic devices leaves an open wound inside the body. In contrast with a regular wound, this type of wound is not replaced by scar tissue, because of the continuous movement of the artificial joint. Presence of tenascin-C in periprosthetic tissue could indicate a continuously ongoing wound healing process. Secondly, aseptic loosening has been shown to be associated with a chronic foreign body type inflammation, which leads to activation of the local inflammatory cells, in particular those of the monocyte/macrophage lineage. Many of the cytokines known to upregulate tenascin-C gene transcription have been described to be increased in the synovial membrane-like interface tissue, which regularly is found between the loose implant and surrounding bone. Therefore, the local conditions might favour increased local tenascin-C expression, although this has not been studied yet.

Based on the fact that tenascin-C expression is upregulated in wound healing and synovial inflammation, we speculate that the expression of tenascin-C is increased in aseptic loosening of THR. This study aimed to show the possible presence, extent, and localisation of tenascin-C in the synovial membrane-like interface tissue. Synovial and capsular samples from patients undergoing primary THR were used as controls.

Methods

PATIENTS AND CONTROLS

Ten samples of synovial membrane-like interface tissue from the proximal stem around loose hip prostheses were collected at revision operations performed for aseptic loosening of THR at the Orthopaedic Hospital of the Invalid Foundation, Helsinki, Finland,

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Table 1 Total hip replacement (THR) patients analysed in the present work

Case no	Diagnosis	Age (y)	Sex	Time to revision (y)	Revised site	Type of prosthesis	Type of alloy	Type of fixation
1	OA	79	M	8	B	Mathys cup/Bimetric stem	CoCrMo	Cementless
2	OA	72	M	4	A	Biomet Head-Neck	TiVAl	Cementless
3	OA	65	F	9	B	Lord	CoCrMo	Cementless
4	OA	74	F	1	C	Wagner	CoCrMo	Cementless
5	OA	70	M	7	C	Müller	CoCrMo	Cemented
6	OA	74	F	1	C	Wagner	CoCrMo	Cementless
7	OA	66	M	6	B	Biomet	TiVAl	Cementless
8	OA	63	M	3	C	Lubinus	CoCrMo	Cemented
9	RA	78	F	20	A	Brunswik	CoCrMo	Cemented
10	CDH	37	F	4	C	Biomet cdh	TiVAl	Cementless

OA = primary osteoarthritis; CDH = secondary osteoarthritis resulting from congenital dislocation of the hip, RA = rheumatoid arthritis. A = both components; B = only acetabular cup; C = only prosthesis stem. CoCrMo = cobalt-chromium-molybdenum-alloy; TiVAl = titanium-vanadium-aluminum-alloy.

between February and May 1995. Of the 10 patients, five were women and five were men, mean age 67.8 years (range 37 to 79 years). Eight THRs had originally been performed to treat primary osteoarthritis (OA), one for secondary OA caused by congenital dislocation of the hip joint, and one for rheumatoid arthritis (RA) but with low disease activity at the time of primary and revision operation. Mean time from primary THR to revision was 6.3 years (1 to 20 years). Table 1 shows the clinical and demographic data for patients.

For comparison, synovial tissue and fibrous capsular tissue samples were collected from 10 patients undergoing primary THR. Of these 10 patients, seven were women and three were men, mean age 70.1 (range 40 to 83 years). Nine THRs were performed to treat primary OA, one for aseptic necrosis of the femoral caput. These samples were embedded and frozen in OCT compound (Lab-Tek Products, Division of Miles Laboratories, Elkhart, IN, USA) and kept at -70°C until used for immunohistochemistry.

IMMUNOHISTOCHEMISTRY

Monoclonal 143DB7 IgG_{2a} antibodies were produced in the authors' laboratory against human embryonal fibroblast tenascin-C affinity purified with Mab 100EB2 antibodies coupled to CNBr-activated Sepharose 4B. Hybridomas were initiated by standard techniques and screened by enzyme linked immunoassay. Specificity of the antibody was also demonstrated in immunoprecipitation, in immunoblotting, and in enzyme immunoassays by use of bacterial fusion proteins.^{15 16}

Six μm cryostat sections were fixed in acetone for five minutes at $+4^{\circ}\text{C}$. The sections were incubated with (1) mouse antihuman

tenascin-C antibody (dilution 1:10) for 60 minutes at $+22^{\circ}\text{C}$; (2) rabbit antimouse immunoglobulin (dilution 1:25, DAKO, Glostrup, Denmark) for 30 minutes at $+22^{\circ}\text{C}$; (3) APAAP solution (dilution 1:25, DAKO, Glostrup, Denmark) for 30 minutes at $+22^{\circ}\text{C}$. Finally, 5% new fuchsin (NF)/8.3% naphthol AS-BI-phosphate (NABP) in alkaline phosphatase buffer for 15 minutes in a dark room, and the reaction was stopped with 20 mM EDTA in 20 mM TRIS-HCl, 150 mM NaCl, pH 7.4 (TBS), for five minutes at $+22^{\circ}\text{C}$. For the staining control, the specific primary IgG_{2a} antibody was replaced with monoclonal IgG (DAKO) with an irrelevant specificity (Aspergillus niger glucose oxidase), but of the same subtype and concentration as the specific primary antibody. Between one step and the next, the sections were washed three times in TBS. All sections were counterstained with Harris haematoxylin. The counterstained sections were dehydrated, cleared, and mounted.

For histological examination, 8 μm cryostat sections were cut from the aforementioned samples and stained with haematoxylin and eosin.

STATISTICAL ANALYSIS

Intensity and extent of tenascin-C staining were divided into five categories. The intensity scores were designated values as following: no staining (0 point), very weak staining (1 point), weak staining (2 points), moderate staining (3 points), and strong staining (4 points). The extent of the staining was measured using Leitz Diaplan lens system (Wetzlar, Germany) and the scores were designated as follows: no staining (0 point), staining of less than 10% of the area (1 point), staining of 10–30% of the area (2 points), staining of 31–50% of the area (3 point), staining of more than 50% of the area (4 points). The final staining scores were obtained by adding the scores of intensity and extent in one specimen and dividing by 2. The values are given as median and quartiles (Q1 and Q3). The Mann-Whitney test was used for pairwise comparison and the Kruskal-Wallis one way analysis of variance for comparison between multiple groups.

Results

TENASCIN-C IMMUNOREACTIVITY IN SYNOVIAL MEMBRANE-LIKE INTERFACE TISSUE

All cases studied showed immunoreactive tenascin-C with staining controls confirming the specificity of the staining. Overall, two of

Table 2 Overall staining scores of tenascin-C in the interface tissue from aseptic loosening of total hip replacement (THR); comparison of interface tissue (median score 4.0, quartiles 3.8 and 4.0) to control synovial tissue (median score 2.0, quartiles 2.0 and 2.0) and fibrous capsule (median score 2.0, quartiles 1.0 and 2.3) from primary total hip replacement (THR)

Revision THR case no	Interface tissue staining scores	Primary THR case no	Synovial tissue staining scores	Fibrous tissue staining scores
1	3	1	2	1
2	4	2	2	2
3	3	3	2	3
4	4	4	2	1
5	4	5	2	2
6	4	6	2	3
7	4	7	2	2
8	4	8	2	2
9	4	9	1	1
10	4	10	2	1

Figure 1 Immunoreactivity for tenascin-C in the synovial membrane-like interface tissue from aseptic loosening of THR and control synovial samples (APAAP staining, original magnification $\times 250$). (A) Immunoreactivity for tenascin-C in the synovial membrane-like interface tissue. Tenascin-C staining was very strong in the synovial lining-like layer (arrows) of interface tissue. This sample/section also contains intervening matrix, which does not stain for tenascin. (B) Tenascin-C was detected in all fields/extracellular matrix of the synovial membrane-like interface tissue in this revision THR patient. Particularly strong staining was found in the synovial lining-like layer (larger arrows). Heavy deposits of debris (see (C) for verification) were found embedded in the tissue (small arrows). (C) Same section as in (B) photographed with polarised light shows that there were many birefringent polyethylene particles embedded in the tissue (some are marked with arrows). (D) Fibroblasts and their pericellular matrix (arrows) showed usually intense tenascin expression in the synovial membrane-like interface tissue samples, whereas the extracellular collagenous matrix did not stain as strongly (the white intervening areas). Note, that fibroblasts send long and slender extensions, which pass between the collagenous fibres of the connective tissue and would be difficult to identify without tenascin-C staining. (E) Same section as in (D) photographed with polarised light shows polyethylene deposits (some are marked with small arrows). (F) Very weak tenascin-C staining in the control synovial sample from osteoarthritis. (G) For staining control the specific primary IgG_{2a} antibody were replaced with monoclonal IgG with an irrelevant specificity (*Aspergillus niger* glucose oxidase), but of the same subtype and concentration as the specific primary antibody. Comparison with the (A) confirms the specificity of the staining.

Figure 2 Histological examination of the interface tissue samples with different staining scores (haematoxylin and eosin staining, original magnification $\times 250$). (A) Macrophage-like cells accumulation in a synovial membrane-like interface tissue sample with high staining score (staining score = 4). (B) Matrix fibrosis in the interface tissue sample with lower staining score (staining score = 3).

the 10 patient samples showed staining of some areas of the matrix with tenascin-C negative intervening areas (fig 1A), whereas the eight remaining cases showed strong staining of all of the matrix (fig 1B, table 2). Tenascin-C staining was particularly intensive in areas facing the implant (for example, the synovial lining-like layer) and the bone. However, the intensity of

matrix staining varied in different regions of interface tissue: pericellular fibroblast matrix (fig 1D), perivascular regions (figure not shown), and the superficial sublining/lining-like layer (fig 1A and 1B) invariably showed stronger immunoreactivity than was visible in other areas, including interstitial fibrous tissue. All tissues contained much debris in the extracellular matrix (fig 1C and 1E).

TENASCIN-C IMMUNOREACTIVITY IN CONTROL SYNOVIAL MEMBRANE AND FIBROUS CAPSULAR TISSUE

In synovial membrane, tenascin-C staining was always very weak (fig 1F, table 2). In the fibrous capsular tissue, staining was moderate in two samples, weak in four samples, and very weak in four samples (table 2). Expression in the synovial membrane was restricted to blood vessels and perivascular regions and to the border between the synovial lining cell layer and the loose connective tissue below it. In fibrous tissue, with the exception of one sample, which showed weak staining of some areas of the matrix, tenascin-C expression was found only in the perivascular regions (figure not shown). The median tenascin-C staining score was 4.0 (3.8, 4.0) in the synovial membrane-like interface tissue compared with 2.0 (2.0, 2.0) in the control synovial membrane ($p < 0.001$). A similar difference was noted between the synovial membrane-like interface tissue and fibrous capsular tissue (median score 2.0, quartiles 1.0 and 2.3; $p < 0.001$). Multiple comparison with the Kruskal-Wallis one way analysis of variance gave similar results for group differences ($p < 0.001$). Staining controls with monoclonal mouse IgG_{2a} with an irrelevant specificity and used at the same concentration as the primary antibodies, were negative (fig 1G).

Histological examination of the specimens demonstrated that there were morphological differences between interface specimens with different immunohistochemical staining scores. The accumulation of macrophage-like cells in the sublining area and deep stroma was the dominant feature of the specimens with higher staining score (staining score = 4, fig 2A), while the two specimens with lower staining score (staining score = 3) were characterised by the fibrosis of matrix (fig 2B).

Discussion

Because aseptic loosening is one of the commonest complications of THR, mechanical and biological factors that may contribute to loosening have been extensively studied. However, little attention has been paid to the compositions of the ECM in the synovial membrane-like interface tissue that is often found in aseptic loosening. Several conclusions can be drawn from the present findings: firstly, tenascin-C expression is usually highly regulated and has a typical spatially and temporally restricted pattern of expression.^{17 18} In the musculoskeletal system in healthy adult tissues, tenascin-C is found in only small amounts in the perivascular regions, at the interface between the synovial lining and sublining,^{19 20} in the superficial zone of the articular

cartilage,⁸ and in periosteum and endosteum.²¹ In contrast, tenascin-C expression can be prominent in inflammatory conditions such as rheumatoid synovitis and pannus tissue.^{22–23}

Based on certain similarities in histopathology and in tenascin-C expression of rheumatoid synovitis tissue and of the synovial membrane-like interface tissues from THR revision patients, it is of interest to note that both conditions also have considerable tissue destructive potential. This tissue destruction involves bone: in RA in the form of erosions and in aseptic loosening in the form of periprosthetic osteolysis. Interestingly, aseptic loosening is characterised by a chronic, foreign body type inflammation with activated, debris containing monocyte/macrophages embedded in a vascularised connective tissue stroma, but relatively few cells of lymphocyte lineage.^{14–24} Also in RA the tissue destructive potential is mainly attributable to a mechanism known as mesenchymoid transformation of the synovial tissue rather than to T lymphocytes in themselves.^{25–26}

Aseptic loosening is usually a long term complication of THR. Although in some cases of the present series the time interval from primary operation to revision was short, we found no other factors that may have contributed to loosening, for example, mechanical failure or bacterial infection. The diagnosis of aseptic loosening in these cases was well established based on the clinical data and typical periprosthetic osteolysis. There was no correlation between tenascin-C staining scores and the time interval from primary operation to revision. However, there was a difference between samples with different histopathological features. Both of the interface tissue specimens with low staining score displayed hypocellular fibrotic features.

Ten different soluble factors are at present known for their capacity to upregulate tenascin-C expression. These factors include tumour necrosis factor α (TNF α),²⁷ interleukin 1 (IL1),²⁷ platelet derived growth factor (PDGF),²⁸ basic fibroblast growth factor (bFGF),²⁹ and transforming growth factor β (TGF β),³⁰ all of which have been shown to be increased in the periprosthetic tissue membrane from loose THR compared with the normal or nearly normal control membrane.^{31–36} Increased expression of tenascin-C in the synovial membrane-like interface tissue may represent an *in vivo* equivalent to the *in vitro* findings on the tenascin-C gene transcription activating properties of the aforementioned cytokines.

Tenascin-C expression is increased in healing wounds and can typically be found in the matrix of the granulation tissue and under the proliferating and migrating epidermal layer. After healing, however, tenascin-C expression returns to normal.¹⁰ Such changes may relate to the function of tenascin-C, which is known for its anti-adhesive properties.^{37–38} It may thus mediate cell migration, which is a crucial event in wound healing.³⁹ High tenascin-C expression in the periprosthetic tissue suggests that this tissue is actively involved in a somewhat

similar process. Injury in the periprosthetic tissues in the loosening of THR might result from an excessive accumulation and activation of macrophages and other cells to produce various neutral endoproteases, including fibroblast type collagenase 1 (MMP1), stromelysin (MMP3), and gelatinases.^{40–44} Enzymatically mediated and mechanically accentuated disruption of the collagen fibres may represent the nidus for reparative processes leading to increased local expression of tenascin-C.

Increased expression of tenascin-C thus seems to imply a regenerative process driven by microinjuries and by cytokines, both rising as a result of a chronic foreign body inflammation. It would accordingly indicate an actively ongoing remodelling/inflammatory/tissue destructive process. The role of tenascin-C in the aseptic loosening may extend further than that. Good osseointegration in well fixed prosthesis is probably mediated by adhesive macromolecules of the ECM, which at the critical implant to bone interface mediate attachment of the implant to the surrounding bone. One result of the above mentioned processes associated with aseptic loosening is increased production of tenascin-C. Under normal circumstances this increased tenascin-C production is transient and seems to serve a useful purpose. When excessive and long lasting, as seems to be the case in aseptic loosening, tenascin-C may inadvertently contribute to loosening. Because of its anti-adhesive properties and by interfering with the action of adhesive macromolecules such as fibronectin,¹⁰ increased tenascin-C expression may be equivalent with impaired osseointegration, which would contribute to loosening. Usually aseptic loosening has been attributed to cyclic mechanical loading and destruction of the periprosthetic bone. A third factor in the biological equation of loosening is comprised by the adhesive properties of the interface between the implant and periprosthetic tissues. Based on the present results, it seems that microtrauma and inflammation lead to changes in the composition of the ECM, more specifically to an abnormally high expression of tenascin-C, which may impair the adhesive properties of the “molecular bonding interlayer” at the implant to bone interface. Because of the topology of tenascin-C expression, this would also seem to involve the implant-interface and interface-bone surfaces. Therefore, more detailed studies on the functions of tenascin-C in general and its role in aseptic loosening in particular seem to deserve attention in the future.

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