Arch Orthop Trauma Surg (1996) 115:286–289

CLINICAL AND EXPERIMENTAL FORUM

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Periprosthetic microvasculature in loosening of total hip replacement

Received: 3 April 1994

Abstract This study was performed to quantitate vascularity in periprosthetic tissues of loose total hip replacements (THRs), because most likely revascularization and endothelial cells are important for implant osseointegration and loosening. Interface and pseudocapsular tissue samples obtained from loose THRs were stained with an immunohistochemical labelling (ABC technique) for von Willebrand factor. Non-inflammatory synovial samples served as controls. The results were quantitated by morphometry using the Kontron image analysis system. Evaluation of the mean endothelial index (EI; positively stained area $\mu m^2/mm^2$ of tissue) revealed that in the control samples synovium was better vascularized than was the case in the cellular areas of the periprosthetic pseudocapsule ($P = 0.0008$) and interface ($P = 0.0004$) of loose THRs. There was no significant difference between mean EI of cellular areas in the interface and that of the pseudocapsule ($P = 0.24$). In the interface the vascularity was irregular. Vascular injury and decreased blood supply seem to occur at the implant-host interface, which may be one of the reasons for insufficient implant osseointegration and loosening.

Introduction

Cellular events influenced by mechanical factors and wear debris [4] in the implant-host interface play an important role in total hip replacement (THR) loosening [3, 6, 7]. The lytic periprosthetic reaction associated with loosening varies from aggressive granulomatosis to poorly cellular fibrotic forms. In previous immunohistochemical studies the vascular endothelium in aggressive granulomas expressed more marked immunoreactivity for IL-6 and for matrix metalloproteinase (MMPs) than the interface in the common type of loosening without marked osteolysis [9].

Because local vascularity is important in wound healing [1] and most probably also in osseointegration of implants, we assessed von Willebrand factor (vWf; Factor VIII related antigen) as an immunohistochemical marker of endothelial cells [8] to quantitate endothelial and vascular meshwork changes at the interface and in the newly formed pseudocapsule surrounding loose THRs.

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Materials and methods

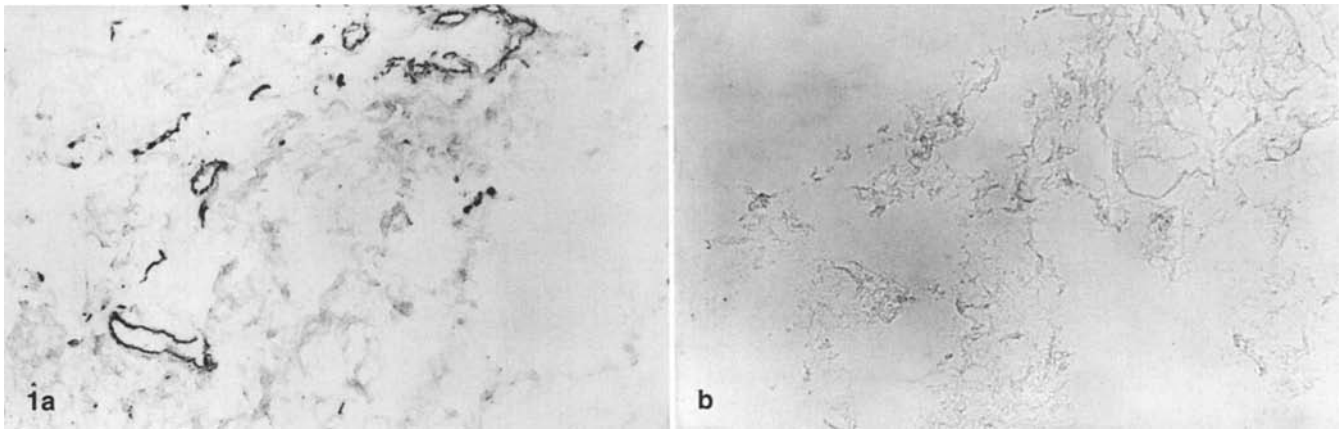
Samples from periprosthetic interfaces and pseudocapsules of 6 patients (Table 1), 4 men and 2 women (age ranged from 53 to 81 years, mean 66.3 years), were obtained at revision procedures performed because of aseptic loosening of THR 3–9 years after the primary operation. Three prostheses were cemented and three cementless. For controls 6 synovium samples were obtained from the suprapatellar recessus during an arthroscopic procedure. Arthroscopy was performed to examine/treat minor meniscal lesions, and the knees were regarded as non-inflammatory.

Immunohistochemistry

Samples were frozen, embedded in OCT compound (Lab-Tek Products, Division of Miles Laboratories, Elkhart, Id., USA) and kept at –20° C until staining. Immunohistochemistry was performed on 6-μm cryostat sections. The sections were fixed in cold acetone for 5 min at +4° C. Endogenous peroxidase activity was inhibited with 0.3% H_2O_2 in methanol for 30 min. Then the sections were incubated serially at room temperature in: (1) normal goat serum (dilution 1:60, Vector Laboratories, Burlingame, Calif. USA) for 30 min: (2) polyclonal rabbit anti-human vWf (dilution 1:1500, Dakopatts A/S, Glostrup, Denmark) for 45 min; (3) biotinylated goat anti-rabbit immunoglobulin (in dilution 1:130, Vector Laboratories), for 30 min; (4) avidin-biotin-peroxidase complex (Vector Laboratories) for 30 min; and (5) 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St Louis, Mo., USA) and 0.006% H_2O_2 in 0.05 M trishydroxymethylaminomethane-buf-

Table 1 Clinical characteristics of patients (*F* female, *M* male, *OA* primary osteoarthritis, *CD* secondary osteoarthritis due to congenital dislocation of the hip joint, *TOA* post-traumatic osteoarthritis, *CoCrMo* cobalt-chromium-molybdenum alloy, *TiAlV* titanium-aluminium-vanadium alloy, *S* stem (femoral component), *A* acetabular component)

Case no.	Sex	Age (years)	Diagnosis	Type of prostheses	Fixation	Time to revision (years)	Revised side
1	F	66	OA	CoCrMo Lubinus	Cemented	9	S
2	M	59	CD	CoCrMo Lord	Cementless	7	A
3	F	68	TOA	TiAlV Biomed	Cementless	3	A
4	M	53	OA	CoCrMo Lubinus	Cemented	4	A, S
5	M	81	OA	CoCrMo Müller	Cemented	3	A
6	M	71	OA	CoCrMo PCA	Cementless	4	A, S



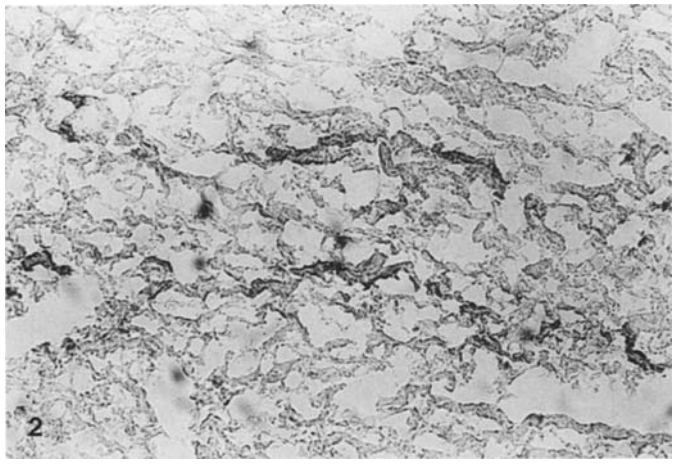
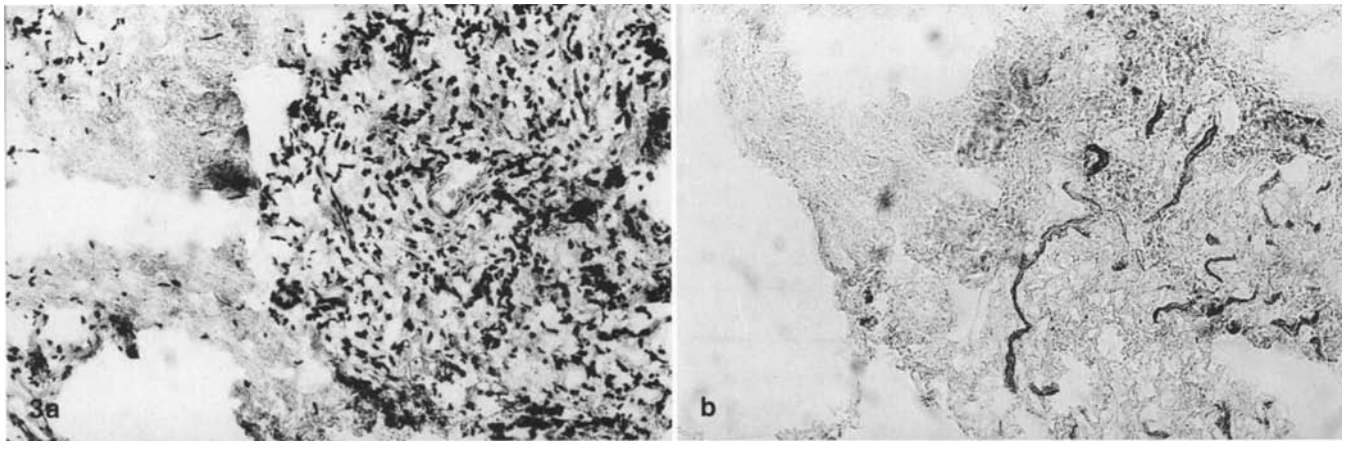


Fig. 1 Staining of frozen sections of control non-inflammatory synovium with rabbit anti-human von Willebrand factor antibodies did not reveal prominent changes in the vascular walls: **a** well-vascularized control knee synovium (× 250), **b** negative control of the staining, where TBS was used instead of primary antibodies (× 250)

Fig. 2 Staining of cryostat sections with rabbit anti-human von Willebrand factor antibodies indicates damage of vascular walls in the bone-prosthesis interface of loose total hip replacement (THR): weak and diffuse staining is seen in the subendothelial connective tissue in the interface (× 250)

Fig. 3 Increased endothelial cell index (EI = positive staining in μm^2 per mm^2 of tissue analysed) was recorded in highly cellular areas in interface tissue around loose THR compared with fibrous areas in the same specimen. Staining of frozen sections with rabbit anti-human von Willebrand factor antibodies: **a** strong and clearly confined positive staining of vascular endothelium in a cell-rich area to the right surrounded by avascular fibrous tissue to the left (× 250, haematoxylin counterstaining); **b** the same area in a consecutive section without counterstaining (× 250)



fered 0.15 M NaCl (TBS), pH 7.40. Consecutive samples were prepared with and without counterstaining with haematoxylin and washed with tap water, dehydrated in alcohol series, cleared in xylene and mounted in synthetic mounting medium (Diatex, Becker Industrifärg AB, Märsta, Sweden). Between each step slides were washed 3 times for 5 min in TBS. Dilutions of serum and antibodies were made using 0.1% solution of bovine serum albumin in TBS. Omission of the primary antibody was used as a negative staining control.

Microscopic and morphometric evaluation

Leitz Diaplan lens system was used to evaluate tissue cellularity and nature of vascular profiles. For morphometry semiautomatic Kontron image analysis and processing systems (Kontron Bildanalyse, Munich, Germany) equipped with VIDAS 2.1 programme (Kontron Elektronik, Munich, Germany) were used. Specimens were analyzed under × 250 magnification by consecutive fields. The positively stained area in relation to the reference tissue area was determined using two grey thresholds with cell-rich and fibrous areas being separately quantitated. Results were expressed as endothelial index (EI), i.e. positively staining area (μ^2) per mm^2 of tissue.

Statistics

Statistical software of BMDP-PC 7.01 was used to calculate the mean EI and standard error of mean (± SEM) as well as P value of pooled t between grouped data.

Results

Light microscopy

Histopathologically, the three types of tissues analysed in the present study were different, as seen in samples counterstained with haematoxylin. Interface tissue samples contained clearly defined, highly cellular zones with rich vascular meshwork and fibrous tissue. Pseudocapsular tissue samples contained fewer cellular areas. Control knee synovium was without any signs of inflammation and was supplied with apparently larger blood vessels, which had a clearly defined lumen, in contrast to periprosthetic tissues, in which small vessels were more predominant. Metallosis was found in the interface and pseudocapsule from a titanium-alloy prosthesis.

Immunohistochemistry

Staining with rabbit anti-human vWf antibodies revealed well-defined positive staining of vascular endothelial cells in the control knee synovium (Fig. 1). Also, staining of the endothelial cells in the pseudocapsule was well-defined except in cases 3 and 4, which showed diffuse staining of the immediate perivascular space of some blood vessels (not shown). In contrast, all samples of the interface tissue contained vascular profiles with irregular and diffuse positive staining (Fig. 2), with other interface areas showing more normal-looking endothelial cells with staining confined to the endothelial cell cytoplasm. Highly cellular areas of interface revealed an abundance of positively stained blood vessels compared with less vascularized fibrotic areas (Fig. 3).

Table 2 Endothelial index in the periprosthetic tissues of loose THR. Magnitudes are expressed in μm^2 positively stained area for von Willebrand factor/mm^2 of tissue (*A* cell-rich areas, *B* fibrous areas, * inadequate sample, ** no such area for present)

Case no.	Interface		Pseudocapsule		Control
	A	B	A	B	
1	*	**	14691	7172	46415
2	7942	2111	**	2757	23507
3	2692	2071	9455	2812	28459
4	13206	5246	14118	289	44515
5	19013	10527	21374	1772	47646
6	4913	2552	11014	662	38833
Mean ± SEM	9553 ± 2950	4502 ± 1617	14110 ± 2054	2577 ± 1012	38230 ± 4114

[a] Standard error of mean: $P < 0.001$ for comparison with control; interface A vs B $P = 0.17$ and pseudocapsule A vs B P 0.0005; interface A vs pseudocapsule A $P = 0.24$ and interface B vs pseudocapsule B $P = 0.32$; interface A vs pseudocapsule B $P = 0.04$ and interface B vs pseudocapsule A $P = 0.01$

Endothelial index

Control knee synovium had a higher mean EI (Table 2) than the cell-rich areas of the pseudocapsule ($P = 0.0008$) or interface ($P = 0.0004$). Mean EI of the cell-rich tissue areas did not differ from fibrous tissue in the interface ($P = 0.17$) but was higher in the pseudocapsule ($P = 0.0005$). Mean EI of cell-rich areas in the interface and pseudocapsule did not differ from each other ($P = 0.24$).

Discussion

Vascular endothelial cells play an important role in the vitality of tissues and participate in the production of extracellular matrix (ECM) components. Distinct functions are attributed to stimulated endothelium in local tissue reaction. After autocrine and paracrine induction by cytokines (in particular, tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) endothelial cells express adhesion molecules and recruit inflammatory cells, synthesize prostanoids, reactive oxygen species (ROS) (including nitric oxide, endothelium-derived relaxing factor) and proteinases all of which are implicated in weakening of the periprosthetic connective tissue bed and bone loss and could support fibroblasts for induction of fibrotic scar formation in the so-called common type of THR loosening [2, 3, 5, 9].

Blood vessels have an exclusive ability to sprout (angiogenesis), which is under direct or indirect control of fibrin degradation products, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), TNF-α,

platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), prostaglandin E_2 (PGE_2), IL-1, IL-6, IL-8 and substance P [1]. Reparative processes after surgery ensure feasible osseointegration or formation of a fibrous implant bed, which is formed by the ingrowth of small blood vessels and fibroblasts [7]. In patients, micromotion associated with cyclic loading is thought to lead to the formation of a synovial-like interface [2]. Histopathological examination at various time points after implantation demonstrates extravasation of fibrin, signs of fibrinoid necrosis [2] or granulation tissue at the bone surface suggesting tissue healing. Fibrin deposition activates fibrinolysis, platelet-mediated teromboxane (TxA_2) secretion, recruitment of inflammatory cells and endothelial cell migration, necessary for reparative angiogenesis. According to the present study the periprosthetic tissues are not as well vascularized as the normal synovial tissue. The most important vascular parameters which govern the efficacy of the oxygen delivery are capillary density, capillary spatial distribution and blood flow. Therefore, a decreased degree of vascularization may lead to weakening of the periprosthetic anchoring tissues.

vWf is a secretory product of endothelial cells and megakaryocytes. Weibel-Palade bodies are the main reservoir of vWf within the endothelium. Upon release, some of the vWf can be found in serum, and accordingly, serum vWf is a useful marker of endothelial cell damage. The vWf is also bound to the subendothelium (basement membrane, adjacent ECM), where after vascular injury it assures platelet adhesion [10]. The present findings on extracellular vWf in the perivascular connective tissue matrix suggest vascular damage, particularly in the interface tissue. Such endothelial cell damage followed by vWf release and platelet adherence probably leads to a decreased blood flow, oedema and focal hypoxia in excess of that already caused by decreased vascularity. Micromotion is likely to cause alterations of microvasculature in periprosthetic tissue [4] and also secretory products of activated macrophages and mast cell, ROS and particulate debris play a role in this context.

In conclusion, the present findings suggest that vascular damage and reduced blood flow associated with secretory-proliferative responses of endothelial cells are integrated processes associated with impaired osseointegration in THR loosening.

Acknowledgements This study was supported by the Finnish Academy, Siviä Kosti Foundation, Paulo Foundation and Center for International Mobility (CIMO), Finland; Grant-in-Aid for Scientific Research, the Ministry of Education, Science and Culture, Grant-in-Aid for JSPS Fellows, Japan; European League Against Rheumatism (EULAR), Switzerland; the Nordic Research Academy (NorFA), Norway.

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