Dense Innervation in Pseudocapsular Tissue Compared to Aneural Interface Tissue in Loose Totally Replaced Hips

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ABSTRACT. ***Background.*** The function of many inflammatory cells is in part regulated by neuronal cells, which may lead to so-called neurogenic inflammation. Sensory nerves also mediate the pain sensation.

Methods. This immunohistochemical study focused on visualization of C-sensory and sympathetic innervation in the synovial membrane-like interface and pseudocapsular tissue around loosened total hip replacement.

Results. The synovial membrane-like interface did not contain C-sensory peptidergic or sympathetic neural structures. Only limited attempts to neural regeneration were detected. In contrast, pseudocapsule expressed dense innervation with strong CPON-ir sympathetic innervation and osteoarthritis also had C-sensory fibers. Intense neural regeneration was seen in these synovial membranes. Surprisingly, stellate and/or highly dendritic fibroblast-like cells in the fibrotic areas in the interface tissue expressed strong immunoreactivity to the neural marker PGP 9.5, ubiquitin carboxyterminal hydrolase.

Conclusion. Pain related to aseptic loosening cannot arise in the aneural interface membrane. Inflammation in interface/aseptic loosening seems to be driven by non-neurogenic factors, such as foreign bodies and micromovement. Insufficient lysosomal degradation of denatured proteins causes accumulation of ubiquitinated conjugates and enzymes involved in the process. This leads to insufficient degradation of platelet derived growth factor (PDGF)-receptor complex and can contribute to the accumulation of connective tissue in the interface. Failure in ubiquitin mediated proteolysis might support overgrowth of interface tissue and aseptic loosening. (J Rheumatol 2002;29:796-803)

Key Indexing Terms:
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Aseptic loosening is the most important complication of the total hip replacement (THR). Mechanical cyclic loading[1,2], together with adverse host reaction to methylmetacrylate cement, ultra-high molecular weight polyethylene and other foreign wear particles[3,4] have been proposed as the main reasons for aseptic implant loosening. It is evident that several inflammatory cells activated by foreign body reaction and their proinflammatory mediators like cytokines[5], and degradative enzymes[2,4,6] contribute to the pathogenesis of chronic aseptic inflammation and implant loosening. The functions of the cells involved, namely macrophages[3,7-9], lymphocytes[10], fibroblast-like cells[11], osteoblasts[7], and osteoclasts[12] are at least in part neurally regulated and modulated and many of them act as amplifiers of neuropeptide-mediated effects[13,14].

Neuropeptides have trophic effects on healthy tissues and contribute to bone remodeling[13,15]. They act as growth factors in inflamed tissues[16] and are involved in wound healing[17], and in healing of fractures[18]. Imbalanced attempts at tissue regeneration may lead to tissue fibrosis[11], increased osteoclast function and bone resorption[13,19]. Nerve fibers are injured and degenerate as a result of inflammation and tissue trauma such as in implantation of joint implant or in arthritis[20,21]. Aseptic loosening of hip is histologically characterized by an ongoing granulomatous inflammation, progressive tissue damage, degradation of bone, and over-

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growth of synovial membrane-like interface tissue (IF). Knowledge of neural factors and nerve cells in these processes is limited[22].

Based on pain associated with implant loosening and demonstration of neurogenic inflammation in synovitis/arthritis[23], our aim was to compare peripheral nerves and neuropeptides in THR interface tissue and pseudocapsular tissue around loosened implants.

MATERIALS AND METHODS

Patients. Synovial membranes and synovial membrane-like interface tissue samples were collected at the University Hospital of Helsinki, Finland, and at the University Hospital of Yamagata, Japan. Ten samples were collected from the interface between cement and bone in the osteolysis areas from patients undergoing revision THR due to aseptic loosening of cemented prostheses. The mean interval from primary THR to revision was 13 years (range, 5-22). Pseudocapsular sample was obtained in 4 cases (range 8-10 yrs).

Eleven patients who underwent THR for osteoarthritis and one case of necrosis of the femoral head served as controls. In each case a synovial sample was obtained. For demographic data see Table 1.

Immunohistochemistry. Antisera to protein gene product 9.5 (PGP 9.5), substance-P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and C-flanking peptide of neuropeptide Y (CPON), raised in rabbits, were purchased from Cambridge Research Biochemicals (Cambridge, UK). Their specificity was confirmed as reported[24,25-26]. Antibodies to growth associated protein (GAP-43/B-50) (Chemicon, Temecula, CA) were monoclonal mouse-anti human antibodies of IgG class[27-28].

Samples for immunohistochemistry were fixed in Zamboni's solution followed by washes in 15% sucrose solution. These samples were then snap frozen and stored at –70˚C. Cryostat sections (20 μm thick) were mounted on 3-aminopropyltriethoxysilane (Sigma Chemical Co., St Louis, MO, USA) -coated slides and post-fixed in Zamboni's solution for 10 minutes at 22˚C. Endogenous peroxidase was inhibited by soaking the sections in 0.3% H_2O_2 in methanol for 30 minutes at 22˚C. The sections were incubated sequentially in a humid chamber with (1) primary antibodies (PGP 9.5 1:6000, CGRP 1:4000, SP 1:4000, VIP 1:10,000, CPON 1:4000 and GAP-43/B50 1:1000) diluted in 0.1 M phosphate-buffered saline (PBS) at pH 7.2 with 0.1% w/v bovine serum albumin (BSA; Sigma) overnight at 4˚C; (2) biotinylated goat anti-rabbit IgG for rabbit antibodies and goat anti-mouse IgG for mouse antibodies (1:100) in PBS with 0.1% w/v BSA for 60 minutes at 22˚C; and (3) avidin-biotin-peroxidase complex (ABC; Vector Laboratories, Burlingame, CA, USA; diluted in 1:200 PBS) for 60 min at 22˚C. After each step the sections were rinsed 3 times in PBS for 5 minutes. Staining was amplified using the glucose oxidase - 3,3-diaminobenzidine (DAB) - nickel method[29]. Briefly, after 2 washes in 0.1 M acetic buffer, pH 6, for 10 min, the sections were incubated for 4-5 min in a chromogen solution of DAB containing beta-D-glucose, glucose oxidase (Sigma), and ammonium nickel sulfate (Fluka Chemicals, Neu-Ulm, Switzerland). Sections were washed twice in acetic buffer and in running tap water for 5 min. Counterstaining was performed with methyl green and toluidine blue. The slides were dehydrated in alcohol and mounted in Permout (Fisher Chemicals, New Jersey, USA).

Microscopic evaluation. The Leica DMLB microscope and computer supported digital camera system (AC200, Leica, Germany) were used for evaluation and photographic documentation.

RESULTS

Periprosthetic interface tissue in loosened THR. Prominent granulomatotic giant-cell rich foreign body inflammation and fibrotic tissue areas characterized the periprosthetic interface tissue around loosened THR stems. Membranes were in part synovial-like and revealed hyperplastic growth.

Table 1. Clinical data: Patients 1–10 had revision for loosened totally replaced hips. Patients 11–21 had primary totally replaced hips

Patient/gender	Nationality	Primary Diagnosis	Age (yrs)	Time from Primary THR (yrs)	Type of Failed Prothesis	Loose Components	Fixation of Loose THR
1/F	Japanese	Osteoarthritis	58	22	Charnley	C,S	Cemented
2/F	Japanese	Osteoarthritis	66	7	YU	C,S	Cemented
3/F	Japanese	Osteoarthritis	68	14	Charnley	C,S	Cemented
4/F	Finnish	Osteoarthritis	80	13	Lubinus	C,S	Cemented
5/F	Finnish	Osteoarthritis	82	18	Christiansen	C,S	Cemented
6/M	Finnish	Posttraumatic Osteoarthritis	75	6	Mathys	C,S	Cementless stem, Cemented cup
7/M	Finnish	Osteoarthritis	66	5	ABG	C,S	Cemented
8/F	Japanese	Osteoarthritis	67	7	YU	S	Cemented
9/F	Japanese	Osteoarthritis	73	20	T-28	C,S	Cemented
10/F	Japanese	Osteoarthritis	72	22	Charnley	C,S	Cemented
11/F	Finnish	Posttraumatic Osteoarthritis	88	—	—	—	—
12/F	Japanese	Osteoarthritis	58	—	—	—	—
13/F	Japanese	Osteoarthritis	50	—	—	—	—
14/F	Japanese	Osteoarthritis	54	—	—	—	—
15/F	Japanese	Osteoarthritis	60	—	—	—	—
16/F	Finnish	Osteoarthritis	69	—	—	—	—
17/F	Finnish	Osteoarthritis	69	—	—	—	—
18/M	Finnish	Necrosis of femoral head	37	—	—	—	—
19/F	Finnish	Osteoarthritis	71	—	—	—	—
20/M	Finnish	Osteoarthritis	66	—	—	—	—
21/F	Japanese	Osteoarthritis	59	—	—	—	—

YU: Yamagata University THA system (Ti-Al-V), C: Cup; S: Stem.

The peripheral nerves were visualized using the general neural marker PGP 9.5 and were easily discernible as varicose profiles, in pseudocapsule and osteoarthritic synovial membranes. In contrast, interface tissue contained no nerve trunks, perivascular nerves, or free nerve endings. Except for very limited areas, no neural structures were found in any of the interface tissue samples (Table 2, Figure 1A).

Surprisingly, analysis of the periprosthetic interface tissue revealed strong PGP 9.5 staining in fibroblast-like cells in areas of granulomatotic inflammation (Figures 1B and E), stellate and/or highly dendritic cells in adjacent fibrotic connective tissue (Figure 1C), and to some extent in the surface layers of interface membrane (Figure 1D).

Neural regeneration visualized with GAP 43/B-50 was restricted to very limited areas of interface membrane surface, and half of the samples lacked any neural regeneration as visualized with GAP-43 (Table 2, Figure 1F).

No SP-, CGRP- or VIP-immunoreactive nerve fibers were detected in the periprosthetic interface tissue (Table 2). Similarly, interface tissues contained no CPON-immunoreactive postganglionic sympathetic nerves. The sparsity of mast cells in granulomatous inflammation infiltrates was a constant finding. Only one interface sample (Case 6) contained a few mast cells in the fibrotic connective tissue.

Pseudocapsular tissue. In contrast to interface membranes, pseudocapsular tissues in loosened THR expressed prominent PGP 9.5-immunoreactive innervation with perivascular nerve fibers, nerve trunks, and free nerve fibers and endings (Table 2, Figure 2B). A prominent CPON-immunoreactive sympathetic innervation was also a constant finding (Figure 2D). Expression of SP and CGRP was relatively weak compared with CPON-immunoreactive innervation. VIP-ir innervation was not detected. Pseudocapsules contained PGP 9.5 immunoreactive peripheral nerves, but not any fibroblast-like or stellate and/or highly dendritic cells (Photoplate 2, Figures 2B and D).

GAP 43/B50-immunoreactive innervation was detected in perivascular fibers and in nerve trunks (Figure 2F). Pseudocapsule synovial membranes expressed GAP-positive fibers and thickenings, identical to those seen in interface and osteoarthritis.

Osteoarthritis (OA). Synovial and capsular tissues of osteoarthritic hips that were obtained in primary THR were without exception highly vascularized, and most of the samples included adipose tissue. Also very dense connective tissue and pieces of cartilage were seen. All samples contained many mast cells, with the exception of areas with lymphocyte infiltrates. All samples of OA expressed prominent perivascular PGP 9.5-immunoreactive innervation.

Table 2. Neural immunoreactivity in interface tissue of loose hip (IF), pseudocapsular tissue (PC) and osteoarthritis (OA).

Patient	PGP 9.5	GAP	CPON	SP	CGRP	VIP
1. IF	FC+	F, S+	—	—	—	—
2. IF	FC+	F,S+	—	—	—	—
3. IF	FC+	F+	—	—	—	—
4. IF	FC+	F,S+	—	—	—	—
5. IF	FC+	—	—	—	—	—
6. IF	FC+	—	—	—	—	—
7. IF	FC+	—	—	—	—	—
8. IF	FC+	—	—	—	—	—
9. IF	FC+	—	—	—	—	—
10. IF	FC+	F,S+	—	—	—	—
8. PC	PV,F,T+	F,S+	PV, F,T+	F+	PV, F,T+	—
9. PC	PV,F,T+	F,T+	PV, F,T+	—	PV,F,T+	—
10. PC	PV,F+	F,S+	PV+	—	—	—
4. PC	PV,F+	F+	PV+	—	PV, F+	—
11. OA	F,T+	F+	PV,F+	—	—	—
12. OA	F+	F+	—	—	F+	—
13. OA	F,S+	PV,S+	F+	F+	F+	—
14. OA	PV, F,S+	—	PV,F+	F+	PV,F+	—
15. OA	PV,F,S+	—	—	F+	—	—
16. OA	PV,F,T+	T+	PV,F+	F+	F+	—
17. OA	PV,F+	F+	PV,F+	—	—	—
18. OA	PV,F,S+	PV,S+	PV,F,T+	PV+	—	—
19. OA	F,S+	—	PV,F+	—	—	—
20. OA	PV,F,T+	PV,F+	PV,F+	—	—	—
21. OA	PV,F,T+	PV,F,S+	PV,F+	PV,F+	PV,FT+	F+

PGP 9.5: protein gene produet 9.5. GAP: growth associated protein; CPON: C- flanking peptide of neuropeptideY; SP: substance-P; CGRP: calcitonin gene related peptide; VIP: vasoactive intestinal polypeptide. +: Immunoreactivity present; — : Immunoreactivity absent. F: Nerve fiber; T: Nerve trunk; FC: Fibroblast-like cells; S: Synovial membrane; PV: Perivascular nerve fibers.

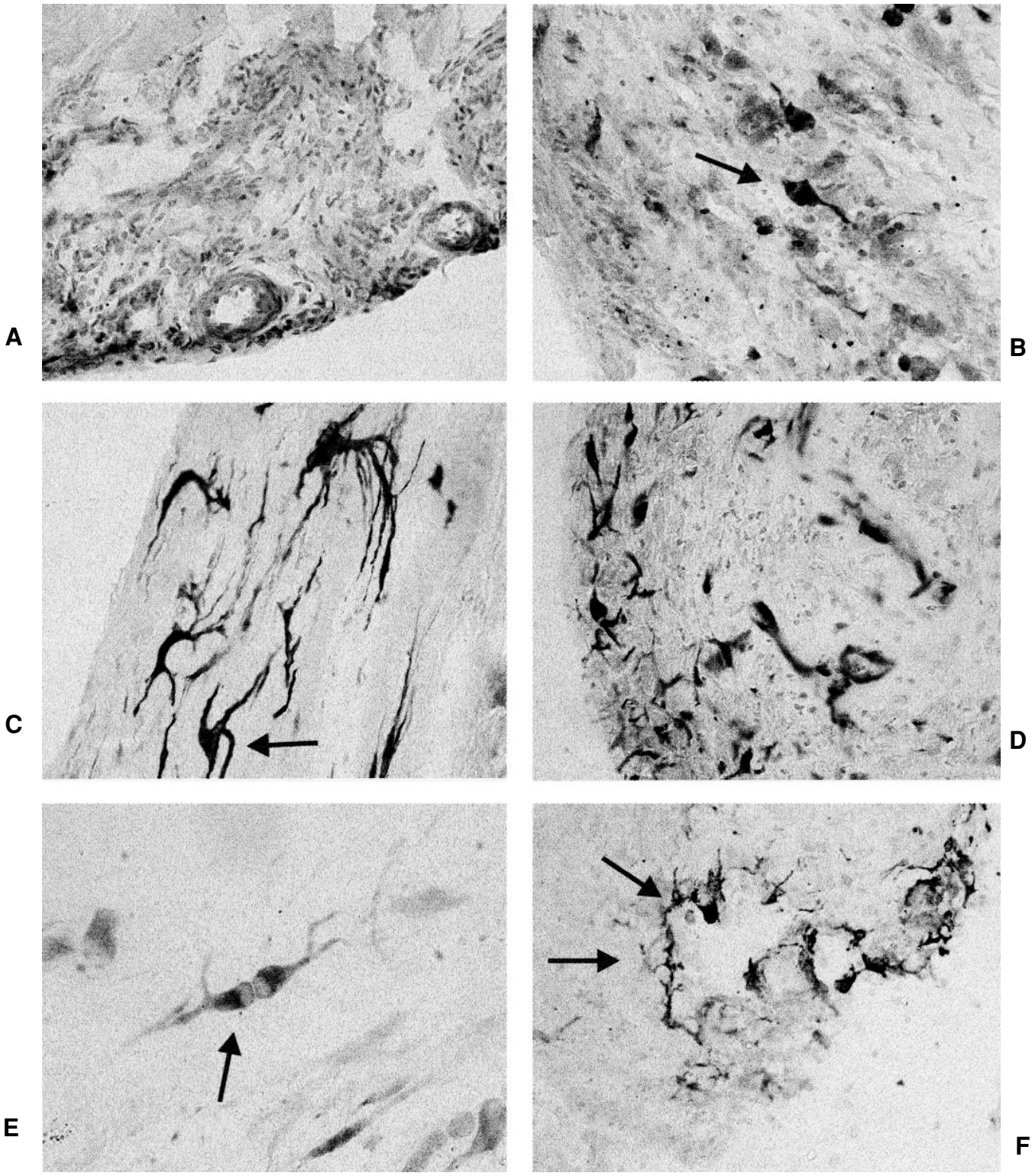

Figure 1. Interface tissue. (A) Overall view of interface tissue with granulomatous inflammation with absence of nerve fibers, staining with PGP 9.5. (Patient 3, original magnification × 47). (B) PGP 9.5 positive cells in granulomatous giant-cell rich inflammation in area of wear debris (arrow). No neural structures. (Patient 6, original magnification × 94). (C) PGP 9.5 immunoreactive fibroblast-like stellate cells in highly fibrotic connective tissue area of IF. Flattened cell bodies can be recognized (arrow). (Patient 6, original magnification × 94). (D) Interface membrane surface with PGP 9.5 immunoreactive fibroblast-like cells. (Patient 7, original magnification × 94). (E) High magnification of PGP 9.5 immunoreactive cells with cell bodies and fibroblast-like cytoplasmic processes in IF tissue (arrow). (Patient 5, original magnification × 94, zooming double size). (F) Interface membrane with GAP 43/B-50 immunoreactive fibers and cells with finger-like extensions in surface area (arrows). (Patient 4, original magnification × 47, zooming double size).

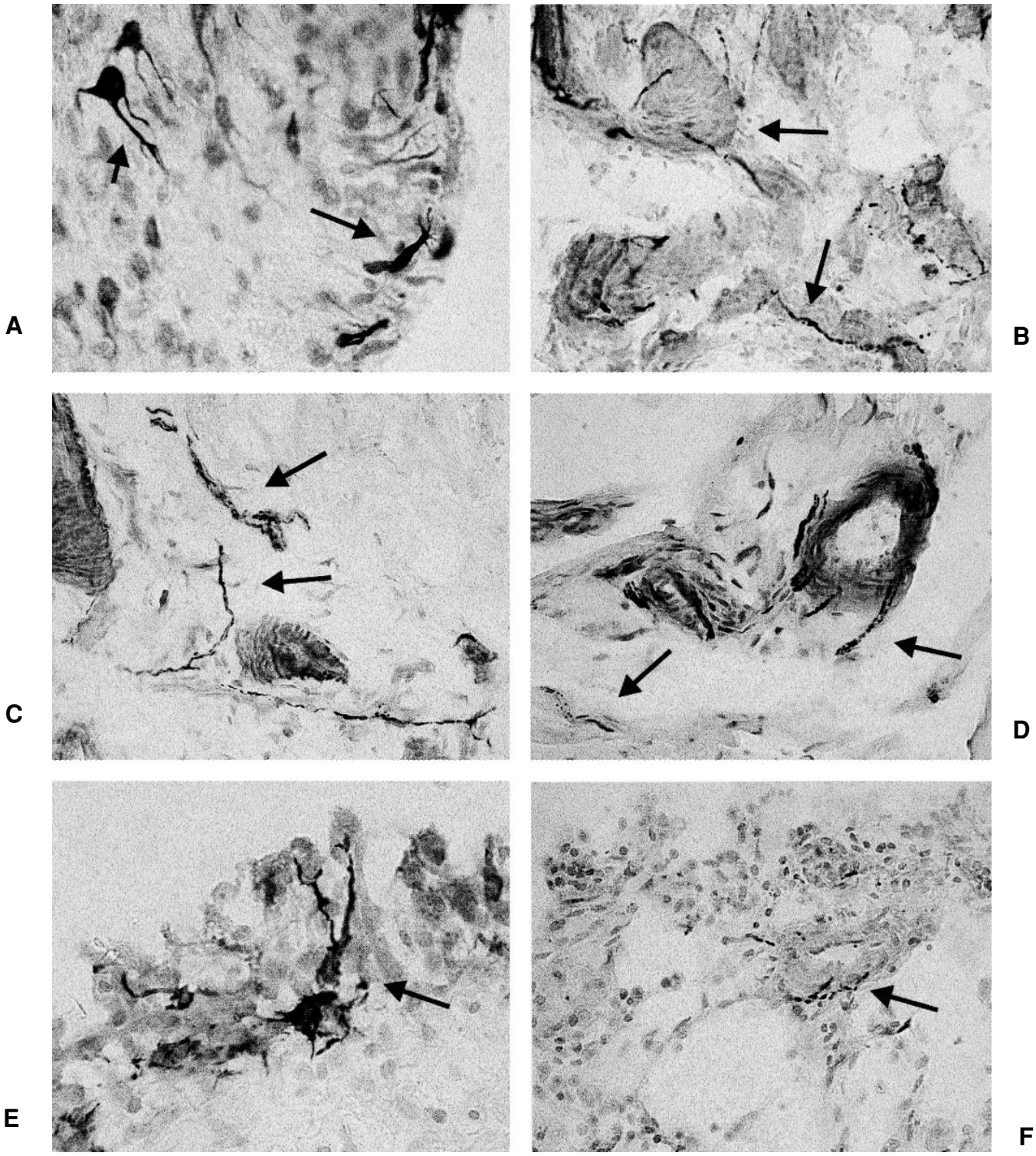

Figure 2. Osteoarthritis and pseudocapsule. (A) Osteoarthritis: PGP 9.5 immunoreactive synoviocyte-B-like cells in synovial membrane (arrow). Fibroblast like PGP 9.5 positive cells with long cytoplasmic processes and cell bodies (short arrow). Nerve fibers are not present in this view. (Patient 14, original magnification × 94). (B) Pseudocapsule: Perivascular innervation and nerve fibers visualized by PGP 9.5 in fragile pseudocapsular tissue (arrows). (Patient 8, original magnification × 94). (C) Osteoarthritis: Overall view on CPON-immunoreactive sympathetic innervation (arrows). (Patient 20, original magnification × 47). (D) Pseudocapsule: Prominent perivascular sympathetic innervation visualized by CPON. (Patient 8, original magnification × 94). (E) Osteoarthritis: GAP 43/B-50 immunoreactive fibers and accumulation in synovial membrane surface in osteoarthritis (arrow). (Patient 13, original magnification × 40, zooming double size). (F) Pseudocapsule: GAP 43/B-50 immunoreactive perivascular fibers in pseudocapsular tissue (arrow). (Patient 8, original magnification × 94).

Free nerve fibers and large nerve trunks were also seen. Tissue areas close to synovial lining were poorly innervated but in areas of hyperplastic synovial membrane and inflammation some superficial nerve fibers were detected (Table 2).

All cases of OA contained some slightly dendritic fibroblast-like cells, especially in areas of inflammation close to the hyperplastic synovial lining (Figure 2A). PGP 9.5 also stained some synoviocytes, which looked like type B synoviocytes with degenerated villous process (Figure 2A).

CPON, a marker for sympathetic postganglionic peripheral nerves was expressed in most cases strongly in perivascular nerve networks, but also in apparently free sympathetic nerve fibers (Figure 2C).

Expression of GAP-43 was prominent in the synovial membrane surface, so that GAP-43/B50-positive staining covered practically all of the synovial surface area (Figure 2E).

DISCUSSION

Our results on innervation of the synovial membrane of the osteoarthritic hip confirm previous histological findings on innervation in OA[30,31]. In contrast to previous reports describing sparse C-sensory innervation in interface tissue[22], no varicose SP and/or CGRP-immunoreactive neural structures were detected. Instead, prominent PGP 9.5-immunoreactive staining of fibroblast-like cells with nerve fiber-like dendritic processes was constantly seen in the periprosthetic interface tissue. Similar PGP 9.5-immunoreactive type B synoviocytes have recently been reported in horse synovial membrane[32]. OA is clinically characterized by inflammatory pain and functional disability of the affected joint. Nociceptive information is conveyed to the central nervous system by myelinated A-delta and unmyelinated, C-afferent type nociceptive nerve fibers and sympathetic afferent innervation. A close functional coupling exists between the sensory peptidergic and sympathetic nervous systems[33,34]. Trophic changes after nerve injury, combined with regeneration and sprouting of nerve fibers, may lead to impaired C-sensory and sympathetic function and an ongoing inflammatory process[35,36] with sympathetically mediated neuropathic pain responses[23]. In the periprosthetic interface tissue, absence of PGP 9.5-immunoreactive innervation, both C-sensory and sympathetic, and very limited attempts at neural regeneration suggest that inflammation in the interface is not of neurogenic origin. The character of inflammation seen in interface tissue supports this conclusion. Lymphocytes and mast cells function as effector cells in neurogenic inflammation, whereas histological features of interface tissue include giant cell-rich inflammation with macrophages and fibroblasts, but lymphocyte infiltrates and mast cells are rare. Reactive overgrowth of the synovial-like interface membrane is probably maintained by factors other than innervation, such as foreign bodies, activated macrophages and fibroblasts and their metabolites. In general, innervation contributes to the local production of proinflammatory factors after tissue trauma[37]; and after inflammation induced retraction and degeneration of nerve fibers[23,38,39], local tissue factors continue to maintain chronic inflammatory process in the absence of neural factors.

Pseudocapsular tissues are richly innervated and in contact with the interface tissues via the fluid phase. They play a role in supporting the growth of interface tissue and may contribute to pain responses in loose THR, because pain associated with aseptic loosening cannot be elicited in the synovial membrane-like interface tissue.

The osteolytic process is in part regulated by neuropeptides of C-sensory and sympathetic nerves[13]. Studies on a neonatal rat model indicate that loss of sympathetic neural control after sympathectomy leads to the activation of osteoclasts and coincident acceleration of bone resorption[19]. It is possible that impaired sympathetic innervation in interface tissue promotes osteoclastic activity and may support bone destruction in aseptic loosening of THR.

PGDF-α and -ß, produced by fibroblasts and macrophages, have been demonstrated both in interface tissue and pseudocapsular tissue in failed hip prostheses[40]. PDGF stimulates protein synthesis in fibroblasts resulting in accumulation of connective tissue matrix in various pathological states including rheumatoid arthritis[41]. Ubiquitin-related proteolysis regulates matrix formation by targeting membrane bound, active PDGF-receptor complex for degradation[42]. In this study, "neural marker" PGP 9.5 was also found in dendritic fibroblast-like cells. Interestingly, PGP 9.5 is a member of the family of ubiquitin carboxyl-terminal hydrolases[43,44] corresponding to the ubiquitin carboxyterminal hydrolase isoenzyme L1 (UCH-L1) in the ubiquitin-related proteolytic pathway[45]. UCH-L1 targets short lived signaling proteins, such as PDGF-α, PDGF-ß and fibroblast growth factor for selective degradation[42]. Proteins, damaged by heat denaturation or exposure to chemical/toxic materials, are degraded by the ubiquitin-related proteolytic pathway[45]. Defective proteolytic function leads to accumulation of ubiquitinated protein conjugates in non-neuronal cells, such as cultured mouse fibroblasts[46,47]. Proliferating cultured human fibroblasts express high levels of neuronal protein gene product 9.5 (PGP 9.5)[48]. Strong PGP 9.5-immunoreactive staining has been demonstrated in non-neuronal cells in granulation tissue of non-healing human ulcers[49] and synovial cells in horses[32]. Accumulation of PGP 9.5-positive inclusion bodies was originally demonstrated in neurodegenerative diseases, such as Alzheimer's disease and Parkinson disease[43].

Our present findings on cellular expression of PGP 9.5 in fibroblast-like cells reflect a derangement in ubiquitin-mediated proteolytic function, which leads to connective tissue overgrowth and accumulation of ubiquitinated protein conjugates. Periprosthetic tissues are exposed to both

mechanical stress/mechanical load, and chemical stress/foreign wear particles and formation of toxic corrosion products of heavy metals. If the chaperone function of heat shock proteins fails, harmful proteins are guided to ubiquitin-mediated degradation. Insufficient proteolytic function leads to increased accumulation of ubiquitinated protein conjugates in lysosomes of fibroblasts and downregulates lysosomal proteolysis[47].

Mechanisms described above might explain our findings of the strong accumulation of PGP 9.5 immunoreactive fibroblast-like cells in interface tissue, in OA and to a smaller degree, in pseudocapsular tissue. Elevated fibroblast activity may contribute to the overgrowth of interface membrane, aggressive periprosthetic granulomatosis, and loosening[50].

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