Abstract. Synovial tissues in joints with prostheses display characteristic morphological changes in cases with aseptic failure, particularly macrophage infiltration. Since proliferation of the synovial lining cell layer represents a feature characteristic of autoimmune joint diseases, the possibility of morphological changes of the synovial lining cell layer in periprosthetic tissues was investigated. Synovial biopsies from five groups of morphologically well-defined lesions (osteoarthritis, rheumatoid arthritis, aseptic loosened metal-on-polyethylene and metal-on-metal arthroplasty and suggested metal hypersensitivity) were compared using a conventional staining method and immunohistochemistry. The synovial lining cell layer was substantially enlarged in both rheumatoid arthritis and cases suggestive of metal hypersensitivity. Macrophage infiltrates were apparent in rheumatoid arthritis and all specimens from retrieved hip arthroplasties. Although both synovial and subsynovial macrophages were positive for CD163 (indicating synovial M2 macrophages), the remaining fibroblast-like synoviocytes and scattered stromal fibroblasts showed a positive reaction with the D2-40 antibody (indicating fibroblast-like synoviocytes). Furthermore, in contrast to CD163-positive macrophages, the enlarged D2-40-positive fibroblast-like synoviocytes displayed cytoplasmatic tubular projections. Proliferation of the periprosthetic synovial lining cell layer occurred in cases with unexplained groin pain following metal-on-metal hip resurfacing arthroplasty, suggestive of hypersensitivity. Despite some important study limitations, the present observation adds to the evidence that metal hypersensitivity shares characteristic morphological features with autoimmune diseases of the joints.

Primary total hip arthroplasty is a growing procedure worldwide. Recent improvements in manufacturing processes have led to an important decrease in catastrophic component failures caused by corrosive and non-corrosive wear adverse reactions. Indeed, metal-on-metal technology is now used in over one-third of all hip arthroplasties performed in the United States of America (1). At the same time, there is increasing awareness of potential biological consequences unique to the metal-on-metal bearing couples (2). The metal-on-metal bearing surface is made from high-carbon, cobalt-chromium-molybdenum alloy. Despite a successful decrease in volumetric wear after the implementation of new modern materials and designs, there is growing evidence that the prostheses might evoke specific reactions in bone (3, 4) and the periprosthetic soft tissues (3, 5-12), resulting in the need for revision of prosthesis.

Similar to other inflammatory conditions that take place in the native synovium, both the periprosthetic wear particle-induced reactions and the suggested delayed-type hypersensitivity reactions are associated with cellular infiltration of periarticular tissues due to lymphocytes and macrophages and their interactions with the host. Although most investigators have focused on the presence of necrotic changes, as well as the level and distribution of the lymphocyte infiltration (13-15), within the periprosthetic soft tissue associated with possible delayed-type hypersensitivity reactions in metal-on-metal arthroplasty, other authors considered them nonspecific for metal bearings (16, 17). In our previous study on retrieved hip resurfacing arthroplasty, both excessive osseous T-lymphocyte infiltration and proliferative desquamative synovitis were observed in cases with unexplained groin pain following metal-on-metal hip resurfacing arthroplasty, while lymphocyte infiltration was

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quite variable (3). Although a number of distinct variables can be evaluated in inflammatory joint diseases and several two- to seven-tied (18-22) scoring systems are used worldwide for the classification of synovial pathological changes, the proliferation of the synovial lining cell layer represents a generally accepted feature characteristic of autoimmune joint diseases, such as rheumatoid arthritis.

The current retrospective observational morphological study was conducted to investigate the phenotype of the synoviocytes proliferated in a setting of suggested delayed-type hypersensitivity reaction following metal-on-metal hip resurfacing arthroplasty and to compare them with the synovial membrane pattern in other well-defined arthroplasty complications and in the native synovial membrane in osteoarthritis and rheumatoid arthritis.

Materials and Methods

Selected synovial biopsies from the archive of the Institute of Pathology of the University Medical Center Hamburg-Eppendorf obtained from 2006 to 2009 were retrospectively analyzed in order to characterize phenotype of the synovial lining cells. Five distinct clinically and morphologically well-defined groups were chosen (2 groups with native joint biopsies and 3 groups with synovial biopsies obtained at revision surgery following aseptic failure of hip arthroplasty) for the further investigation. A comparative morphological study was performed on simultaneously stained slides using both conventional staining and immunohistochemical reactions against two established routine markers. All the patients were women between 46 and 68 years of age suffering from hip diseases. The study was performed according to the Declaration of Helsinki.

Histopathological criteria. Low-grade synovitis was diagnosed according to the classification schema proposed by Krenn et al. (18-19) in native synovial specimens showing minimal hyperplasia of both the synovial lining cells and stromal mesenchymal cells, along with inconspicuous inflammatory infiltration of stroma. High-grade synovitis was characterized by hyperplastic synovial lining cell layer (23, 24), proliferative changes of the stroma and lymphocyte infiltration, as described in the classification’s coring system proposed by Krenn et al. (18, 19). Polyethylene wear particle-induced synovitis was defined by a dense macrophage infiltration within the synovium in the presence of polyethylene wear particles recognized under polarized light. The excision of the biopsy had been performed during revision surgery for aseptic loosening of metal-on-polyethylene total hip arthroplasty. All the patients in this group were treated for primary osteoarthritis and had not complained about metal allergy (such as itchy disorders, erythema, papules and vesicles). The cases with macroscopic metallosis of the articular tissues following the metal-on-metal hip resurfacing arthroplasty were characterized by the presence of metallic-colored foreign material within the femoral head and neck tissues and/or within the synovial tissue when investigated with the naked eye. These hips were revised for the malpositioning of the metal-on-metal hip resurfacing arthroplasty leading to groin pain associated with femoral and/or the acetabular component loosening. Proliferative desquamative synovitis was diagnosed in the synovial specimens from retrieved metal-on-metal hip resurfacing arthroplasty that were revised for unexplained persistent groin pain and were histopathologically characterized by conspicuous proliferation and desquamation of the synoviocytes along with variable lymphocyte infiltrate within the synovial membrane (3).

Immunohistochemical analysis. Histological slides from the archive of the Institute of Pathology of University Medical Center Hamburg-Eppendorf stained with hematoxylin-eosin and Giemsa stains were re-reviewed in order to select representative tissue for further analyses. One paraffin block was selected from each case for further immunohistochemical investigation. Immunohistochemical reactions with CD163 for synovial M2 macrophages (clone: 10D6; Novocastra Laboratories, Newcastle upon Tyne, UK; dilution: 1:100) and podoplanin for fibroblast-like synoviocytes (clone: D2-40; Signet Laboratories, Inc., Dedham, MA, U.S.A.; dilution: 1:40) were performed simultaneously on the freshly cut paraffin-embedded archival tissues from each case. Both staining intensity (scored as negative, weak, moderate or strong) and distribution (cytoplasmatic or membranous) were recorded, along with the relative proportion of immunohistochemically positive cells.

Lymphocyte transformation test (LTT). LTT was performed using blood samples from two female patients obtained at the follow-up control investigation. Peripheral blood mononuclear cells were obtained from the heparinized blood of both patients by density centrifugation on Ficoll-Hypaque solution (PAA Laboratories GmbH, Pasching, Austria). The cells were cultured in RPMI-1640 medium (PAA Laboratories) supplemented with heat-inactivated 10% human AB serum, glutamine, antibiotic-

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Figure 1. Synovial lining cell layer phenotypes. A: Synovial surface covered by a single layer of synoviocytes. Vascularized loose to collagen-rich fibrous subsynovial tissue. B: Scattered macrophages both within the synovial lining cell layer and in the subsynovial stroma. C: D2-40 expression in several cells within the synovial lining cell layer. D: Synovial lining cell layer containing more than 5 cell layers and enlarged synoviocytes. E: CD163 strongly positive in both synovial and subsynovial macrophages. F: Fibroblast-like synoviocytes and a few subsynovial fibroblastic cell elements showing positive reactions towards the D2-40 antibody. G: Inconspicuous synovial lining cell layer covering the subsynovial stroma filled with dense histiocytic infiltrate. H: Synovial macrophages showing strong positivity for CD163 and subsynovial macrophages with ingested polyethylene wear particles displaying moderate positive reaction. I: Remaining fibroblast-like synoviocytes and subsynovial lymphatic vessel walls strongly D2-40-positive. Wear particle-phagocytozing macrophages show only a faint nonspecific reaction. J: Superficial zones of the synovial membrane paucicellular in metal wear-induced synovitis, deeper subsynovial soft tissue densely infiltrated by histiocytic cells. K: The majority of CD163-expressing macrophages located in the subsynovial stroma. L: Scattered D2-40-positive stromal cells and fibroblast-like synoviocytes. M: Synovial lining cell layer containing several cell layers of enlarged synoviocytes. Variable number of macrophages and lymphocytes within the subsynovial tissue. N: CD163-expressing macrophages within both the synovial lining cell layer and the subsynovial stroma. O: Similar distribution of enlarged synoviocytes and stromal fibroblasts using D2-40 antibody. HE: Hematoxylin-eosin; CD163 and D2-40 immunohistochemical stain. Original magnification: ×100.
antimycotic solution and nonessential amino acids. All the cultures were performed in quadruplicate in 96-well plates (Nunc, Roskilde, Denmark). The stimuli were: the pan T-cell mitogen phytohaemagglutinin 2.4 μg/ml, tetanus toxoid 5 μg/ml, NiSO4, CrCl3, CoCl2, MnCl2, and Na2MoO4 and culture medium alone as control. After 5 days, the cells were pulsed with 3H thymidine overnight and proliferation was assessed by measuring the incorporated radioactivity. The stimulation index (SI) was calculated by the ratio of the mean count per minute (cpm) of the stimulated to unstimulated cultures. An SI greater than 3 was considered positive for hypersensitivity.

Results

The low-grade synovitis group consisted of five patients with primary osteoarthritis and the histological finding of low-grade synovitis without previous implantation surgery. The superficial layer of the synovial membrane consisted of one layer of flat or oval cells (Figure 1A). Immunohistochemically, a few scattered CD163-positive macrophages were detected within the synovial intima and in the subsynovial fibrous stroma (Figure 1B). Similarly, several D2-40-positive cells were apparent both within the synovial intima and in the subsynovial stroma (Figure 1C).

The high-grade synovitis group contained biopsies from five patients with newly diagnosed seropositive rheumatoid arthritis without any previous anti-inflammatory treatment or implantation surgery. Microscopically, the synovial tissues displayed increased cellularity with 5 and more cell layers thick synovial intima and dense lymphocytic and plasma cell infiltration of synovial fibrous stroma, although it is not possible to differentiate between distinct populations of synoviocytes by conventional histology (Figure 1D and Figure 2A). Both the synovial intima and the stroma contained multiple round to oval CD163-positive macrophages (Figure 1E and Figure 2B) and D2-40-positive fibroblast-like synoviocytes (Figure 1F and Figure 2C). The latter were characterized by the formation of tubular projections and elongated or centrally, somewhat stellate, cell forms. Interestingly, multinucleated synoviocytes also showed appositive reaction with CD163.

The polyethylene wear particle-induced synovitis group, with five total hip arthroplasty patients, was characterized by dense macrophage infiltrations of the synovial stroma in cases with aseptic loosening caused by massive polyethylene wear particles. Under polarized light, multiple bi-refrangent particles were present within the macrophage infiltration (not shown). The synovial lining cell layer consisted of one to two cell layers of round synoviocytes (Figure 1G). The majority of the synovial lining cells had strong reaction with the CD163 antibody. Similarly, the dense subsynovial macrophage infiltration showed also moderate positive reaction with CD163 antibody (Figure 1H). Although scattered synovial lining cells and synovial lymphatic vessels were strongly positive for D2-40 (Figure 1I), the subsynovial stroma contained virtually no D2-40-positive cells.

Two patients with failed metal-on-metal hip resurfacing arthroplasty showed massive metallosis (metal wear particle-induced synovitis and intraosseous macrophage infiltration leading to their metallic color) of both the soft and bone tissues. The first specimen was revised for the loosening of the acetabular component. The second patient (Figure 1J-L and Figure 3A/B) had a pathological fracture due to severe metallosis of the femoral remnant. Macroscopically, both specimens showed metallic coloring of both the synovial membrane and bone tissue of the femoral remnant. Histologically, however, the synovial membrane displayed a paucicellular synovial surface covered focally by a fibrinous exudate. Subsynovial fibrous tissue was infiltrated by macrophages ingesting the metal wear particles. It is noteworthy that despite the massive wear, the LTT performed on the blood of the patient with the pathological fracture revealed negative results (SI<1.8).
The proliferative desquamative synovitis group consisted of five cases with hip resurfacing arthroplasty which became clinically symptomatic due to unexplained groin pain (Figure 3C/D). Histologically, each specimen was characterized by proliferating synovial lining cells and subsynovial infiltration of macrophages and lymphocytes of variable cellular density (Figure 1M). CD163 macrophages were present both within the superficial layer and subsynovial stroma (Figure 1N), but fewer than seen on cases with metallosis. Furthermore, D2-40-positive enlarged fibroblast-like synoviocytes with tubular projections were detected immunohistochemically (Figure 3).
10). Due to the retrospective nature of the study material, it was impossible to perform additional tests such as patch tests. Similarly, LTT were not performed except for one patient from this group. The results were negative for all tested metals (SI<1.9), except for molybdenum (Na2MoO4; SI>3.1).

**Discussion**

Synovial tissue is built from several distinct cell types, such as synoviocytes, stromal fibroblasts and cellular elements of the blood vessel walls, and in pathological conditions, also inflammatory infiltrates. The expression of CD163 has been shown to be largely restricted to monocytes and tissue macrophages (25-28). CD163 staining has been helpful in distinguishing synovial macrophages from fibroblast-like synoviocytes in the setting of rheumatoid arthritis, where its specificity for macrophages was found to be superior to that of CD68, which does not discriminate between these types (29). D2-40 is a monoclonal antibody to M2A antigen, a 40-kDa O-linked sialoglycoprotein (30). Besides the high specificity of D2-40 for normal lymphatic endothelium, it is also expressed in other normal and tumoural non-endothelial cells (31-37). Podoplanin expression was specifically linked with the formation of pseudopodia (38) and cell migration (39, 40). In the present study, scattered round D2-40-positive fibroblast-like synoviocytes were observed in low-grade synovitis and polyethylene wear-induced synovitis, while these cells were highly proliferated in high-grade synovitis and in proliferative desquamative synovitis (associated with suggested metal hypersensitivity). Furthermore, the size and form of the cells expressing D2-40 changed substantially, becoming longitudinal or of stellate shape and forming tubular projections that were focally connected with the synovial surface. In the cases with metallosis, the number of D2-40-expressing cells seemed to be somewhat increased within the subsynovial stroma, with some D2-40-positive synoviocytes appearing enlarged. However, they did not reach the degree of proliferation seen in high-grade synovitis or in the proliferative desquamative synovitis.

Characteristic enlargement of the synovial lining cell layer was observed in both the high-grade synovitis (native joints of patients with rheumatoid arthritis) and proliferative desquamative synovitis (associated with suggested metal hypersensitivity). Both these lesions were characterized by the presence of stellate to elongated D2-40 positive fibroblast-like synoviocytes that formed tubular projections. Furthermore, the number of CD163-positive macrophages was substantially increased both within the synovial lining cell layer and in the subsynovial stroma in both groups. The number of CD163-expressing macrophages, however, was even higher in the cases with typical polyethylene and metal wear-induced synovitis, which were also characterized by minimal changes of the cellular content within the synovial lining cell layer. Interestingly, the LTT in the patient with severe metallosis of bone and soft tissue gave negative results.

The positive results of the LTT for molybdenum in the patient with proliferative desquamative synovitis (without metallosis) further supported the suggestion that this morphological lesion might be a characteristic associated with periprosthetic metal hypersensitivity. Based on the present findings, it seems possible that synovial effusion linked with hypersensitivity might be a characteristic associated with proliferative desquamative synovitis. Although laboratory tests for periprosthetic hypersensitivity are considered nonspecific, Thomas et al. (9) reported enhanced proliferation in the LTT to molybdenum in 5 out of 16 patients with suggested hypersensitivity. As varying densities of lymphocyte infiltration of the synovium were observed in cases with suggested hypersensitivity in our previous study (3), it seems possible that the morphological changes of the synovial lining cell layer represent an even more characteristic feature of periprosthetic metal hypersensitivity than T-lymphocyte infiltration. In fact, recent studies have demonstrated that lymphocytic infiltration within periprosthetic tissues is not specific for metal-on-metal hip implants (16, 17). While some other investigators (6, 8, 12) focused on analysing necrotic periprosthetic soft tissues, the current study investigated viable synovial tissues adherent to the femoral neck. The observed necrotizing granulomatous reactions, however, preferentially developed anterior to the femoral neck (10), indicating other mechanistic factors might have played a substantial role in their development.

Several important limitations in the current study are recognized. Firstly, although the study and control groups were quite small, every group was defined by standard histopathological criteria. Secondly, it must be noted that specific laboratory tests for hypersensitivity reaction related to arthroplasty are currently unavailable. Nonetheless, both cases with LTT results available represented characteristic examples of metallosis and proliferative desquamative synovitis.

To summarize, substantial morphological similarities were observed between proliferative desquamative synovitis in hips with suggested metal hypersensitivity and high-grade synovitis in native joints suffering from rheumatoid arthritis. Although synovial changes induced by wear particles are typically associated with macrophage infiltration, along with an inconspicuous synovial lining cell layer, suggested metal hypersensitivity is characterized by a considerable proliferation of the synovial lining cell layer similar to that observed in rheumatoid arthritis.
References


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