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Review

Contributions of human tissue analysis to understanding the mechanisms of loosening and osteolysis in total hip replacement

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ABSTRACT

Aseptic loosening and osteolysis are the most frequent late complications of total hip arthroplasty (THA) leading to revision of the prosthesis. This review aims to demonstrate how histopathological studies contribute to our understanding of the mechanisms of aseptic loosening/osteolysis development. Only studies analysing periprosthetic tissues retrieved from failed implants in humans were included. Data from 101 studies (5532 patients with failure of THA implants) published in English or German between 1974 and 2013 were included. "Control" samples were reported in 45 of the 101 studies. The most frequently examined tissues were the bone–implant interface membrane and pseudosynovial tissues. Histopathological studies contribute importantly to determination of key cell populations underlying the biological mechanisms of aseptic loosening and osteolysis. The studies demonstrated the key molecules of the host response at the protein level (chemokines, cytokines, nitric oxide metabolites, metalloproteinases). However, these studies also have important limitations. Tissues harvested at revision surgery reflect specifically end-stage failure and may not adequately reveal the evolution of pathophysiological events that lead to prosthetic loosening and osteolysis. One possible solution is to examine tissues harvested from stable total hip arthroplasties that have been revised at various time periods due to dislocation or periprosthetic fracture in multicenter studies.

1. Introduction

It is estimated that about two million total hip arthroplasties (THAs) are performed worldwide each year and projections of rising demand are reported at least for the USA [1]. However, some THAs fail during the period of service and require revision surgery, which is more expensive than the primary operation and brings less satisfactory outcomes and increased risk for complications [2,3]. This causes a significant economic impact on the health care system. Therefore, understanding current failure mechanisms of primary THAs and especially the potential for prevention are crucial.

Although instability, infection, pain and periprosthetic bone fractures prevail as reasons for reoperation in the first five years after an index surgery, the most frequent cause of late failure is aseptic loosening accompanied by osteolysis [4]. Since the pioneering work of Willert et al. [5,6], there has been a tendency to associate these late complications with a local tissue response to large numbers of tiny particles generated from bone cement and articulating/non-articulating surfaces of THA. Small particles are phagocytosed by macrophages or stimulate cells in a non-phagocytic manner. These cells then release pro-inflammatory molecules that trigger pathways influencing the osteoclast–osteoblast coupling in bone multicellular units [7,8]. Particle-associated dysregulation of osteoclast–osteoblast coupling in favor of osteoclasts over-weight leads eventually to net bone resorption at the bone–implant interface. In support of this concept, studies have demonstrated inflammatory and osteolytic responses after cell/organ culture stimulation by polymethylmethacrylate, polyethylene and titanium particles [9–15].

Immediately after the surgery, mechanical factors influence the development of the bone–implant interface. These are associated with intermittent loading of the artificial hip during daily living

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activities, and later with hydrodynamics of the artificial joint fluid creating significant pressures in the adjacent tissues. From some time postoperatively, biological and mechanical pathways interact together, creating conditions appropriate to periprosthetic osteolysis and aseptic loosening. We have previously described these processes in detail [16,17]. Here, we summarize current evidence derived from analyses of tissues retrieved during the reoperation of THA performed due to aseptic loosening and periprosthetic osteolysis.

2. Search strategy and rules for evaluation

We included all research studies that examined human periprosthetic tissues retrieved either during the THA surgery or post-mortem using histopathological examination and immunostaining. Further, we used articles and resources focusing on this issue. One of the authors (J.V.) searched for potentially relevant studies in the PubMed database. Articles published between January 1974 and June 2013 were identified with the keywords and medical subject heading terms "aseptic loosening" and "periprosthetic osteolysis" and "total hip arthroplasty" and "bone loss" or "immunohistochemistry" or "cytokines" or "RANKL" or "hypersensitivity" or "apoptosis" or "interleukin" or "infection". We reviewed all of the retrieved articles and extracted relevant data, which we incorporated in tables in the Excel 2010 software package (Microsoft). Although 223 articles were identified, 82 were excluded (Table 1, Fig. 1) because they lacked data about histopathological examination of retrieved tissues. In agreement with recent requirements for research in biomedicine, data should be considered preliminary until replicated by a different center. Therefore, all molecules, pathways and cell groups reported only in an initial histopathological study were stated as being preliminary, while those also featuring in a replication study were considered as proven.

3. Results

Data from 101 studies (5532 patients with failure of THA implants) published between 1974 and 2013 were included. "Control" samples were reported in 45 of the 101 studies.

3.1. Methods used for investigation of periprosthetic tissues

The retrieved tissues (or derived cell/organ cultures) were studied histologically and histochemically, especially using immunostaining or molecular biology methods. Another set of methods aimed to detect the prosthetic particles. The aims of all of these investigations were: (i) to distinguish between septic and aseptic THA failures; (ii) to detect prosthetic by-products in periprosthetic tissues; (iii) to analyze cell/tissue structure; and (iv) to detect the signaling proteins and proteolytic enzymes in the periprosthetic tissues.

Table 1
The sample size of studies.

No. of patients	No. of studies
⩽20	55
21–50	20
51–100	9
101–150	5
151–200	5
201–300	2
301–551	3

3.2. Protocols for tissue processing

Some authors examined results from cell/organ cultures derived from periprosthetic tissues [18–22], but their results were excluded because we focused on direct histopathologic examinations of the periprosthetic tissues. These were processed post-operatively using approximately 5–6 μm thick frozen tissue sections (41 of 101 studies) or 3–10 μm thick paraffin-embedded tissue sections (45/101), or both methods (10/101). Methylmethacrylate-embedded sections were used in seven studies [5,23–28], and a further two studies did not report the methodology [29,30]. Mostly, the sections were cut from periprosthetic tissues fixed immediately after harvesting in 10% buffered formalin and then embedded in paraffin (formalin fixed, paraffin embedded; FFPE), or were prepared as freshly frozen sections in the cryostat. The sections were stained for comprehensive microscopic evaluation after hematoxylin–eosin staining. For precise demonstration of the specific molecular components within the cells and in the proper tissue context, special histological stains (Table 2) and immunostains were used (Figs. 2 and 3).

Different staining methods and molecular and cellular biology techniques were used. The most frequently used methods capable of identifying specific RNA or DNA molecules are polymerase chain reaction (PCR) and in situ hybridization (ISH). Some authors also used PCR to detect bacterial DNA, or inflammatory cytokines and other mediators. In most cases, the results from the molecular biology methods were compared to immunostaining of the same tissue samples [31,32].

Immunostaining can specifically identify categories of cell lineage and their regulatory molecules (proteins), and detect the presence of specific antigens in cells with high sensitivity. The immunostains used were based on the reaction between antigen and primary and secondary antibodies, with one of them being labeled with an enzyme (horseradish peroxidase, alkaline phosphatase, biotin), the fluorophore fluorescein isothiocyanate [33,34] or tetramethylrhodamine isothiocyanate [34].

Immunoenzyme protocols with many different principles were applied for antibody-aided detection, including: (i) the avidin–biotin complex method [19,25,35–51]; (ii) the labeled streptavidin–biotin method [32,33,52–59]; and (iii) the polymer-based detection method [60–67]. The presence of antigen was most often visualized by chromogen 3,3′-diaminobenzidine tetrachloride, which produces a brown reaction that can be seen with a light microscope. Occasionally, a fast red TR salt [35], aminoethylcarbazole [38] or fuchsine [39,52], giving a red stain, or chromogen, with the blue-colored precipitate nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate [41], was used. Finally, sections were counterstained with hematoxylin prior to mounting.

In some cases, double immunostains were used, which can identify two sets of antigens in the same section when the antibodies are applied in sequence or at the same time. Some authors followed an immunofluorescence staining protocol [38,40,50,61,68], while others preferred antigens labeled by enzymes [38,48,50,61,68,69].

Histological analysis was mostly carried out at 2–500× magnification, using a light or fluorescence microscope. Polarized light, electron or transmission electron microscopy was used to identify various sizes of wear particles and the intracellular pathology induced by prosthetic by-products [70]. Polyethylene wear debris is strongly birefringent in polarized light, unlike ceramic or metal particles.

3.3. Distinction between aseptic and septic failure

This analysis is based on the detection of the number of polymorphonuclear neutrophil leukocytes (PMNs) in the examined