Modulatory effect of mast cells in the pathology of peri-prosthetic fibrosis around a retrieved knee implant

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Summary

This case study evaluates the tissue around retrieved titanium knee implants, with emphasis on mast cells and localization of their profibrotic mediators -tryptase and interleukin-4(IL-4). Periarticular tissues were analyzed for elemental titanium content, fibrous capsule thickness, types of wear debris, and their immune response, with the aim of identifying an association between mast cells and collagen deposition. Heavy infiltration of inflammatory cells along with metal, polymer, and cement debris was observed. Positive staining for mast cell tryptase and IL-4 confirmed their activated state, whereas toluidine blue staining identified numerous degranulated mast cells as well. Histomorphometrical analysis revealed a thicker collagen capsule, along with increased mast cell density, in the articulating zone as compared to the non-articulating zones. Results obtained from this study substantiate our hypothesis that particulate debris from implants surge the mast cell density which potentially results in an increased fibrillar collagen deposition (i.e. profibrotic response).

KEY WORDS: aseptic loosening; peri prosthesis fibrosis; mast cells; metallosis; wear debris.

Introduction

Custom made or modular megaprostheses is a common method of limb reconstruction after surgical resection of bone tumors; but the long-term failure of these prosthesis has been reported. Some patients require revision surgery and one of the prime etiological factors of implant failure is aseptic loosening (1). Aseptic loosening is an adverse inflammatory response to the particulate debris resulting from wear and tear of implant components such as metal, ceramic, polymer or cement (2). Release of particulate matter into the peri-implant tissue initiates the infiltration of macrophages, lymphocytes, fibroblasts and foreign body type of giant cells and triggers release of their cytokines. The role of mast cells in mediating periprosthetic osteolysis has been suggested (3); however their role in fibrosis development in peri-implant tissue has not been extensively studied.

Case report

This study was approved by the Institutional Ethics committee at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCT/IEC/330/MARCH-2011). In 2007, a male osteosarcoma patient who underwent surgical reconstruction for both limbs was fitted with custom titanium megaprostheses (CMP). Figure 1 A, B shows the X-rays of the implanted CMP. The custom made titanium (Ti) implant had an Ultra High Molecular Weight Polyethylene (UHMWPE) polymer lining on the articulating surface, and was anchored into the tibia and femur with a cemented intramedullary stem. Due to local reoccurrence of osteosarcoma after two years, the patient underwent amputation of the limbs and two total knee joint prostheses were retrieved for pathological evaluation. We investigated the peri-prosthetic tissue for possible case of metallosis and aseptic loosening of the CMP. Gross pathological observations for the explanted CMPs (right and left knees) were performed. Further, tissue specimens were collected for elemental quantification of titanium, assessment of mast cell infiltration, presence of wear debris (titanium, cement, or UHMWPE). We hypothesize that the mast cells are activated by the wear debris, and these activated cells release the serine protease, tryptase and cytokine IL-4 which mediate implant debris induced fibrosis.

Gross observations

In addition to the femoral and tibial components of the CMP, patient’s femur, tibia and fibula up to the ankles were also included. Majority of the superficial tissues (including skin and other musculature) were removed, and numerous areas of black discoloration suggestive of excessive metallosis were observed in the skeletal muscle as well as in the...
μg/g); whereas zone I and zone VI were below elemental titanium detection threshold of the ICP-AES. The mean total concentration of titanium element in the retrieved tissues were 12.33 μg/g of dry tissue.

**Histopathological analysis**

Metachromatic staining of periprosthetic tissue using toluidine blue revealed the presence of numerous mast cells as identified by their characteristic extensive cytoplasm with deep blue purple granules sections (Figure 2 A). Morphologically most cells were rounded, but some were oval and spindle in shape. Extensively degranulated mast cells were seen at sites adjacent to the implant whereas most of the cells were located perivascularly (Figure 2 B). Titanium particle were found scattered throughout the peri-prosthetic tissues appearing as predominantly black,
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Irrespective of its composition, particulate debris (metal, polymer, or cement) induces an inevitable influx of polymorphonuclear leukocytes and macrophages in the peri-prosthetic tissue. In many cases, a non-specific cellular reaction leading to extensive foreign body response [composed of macrophages, foreign body giant cells (FBGC), fibroblasts, and occasional lymphocytes and small caliber blood vessels], and ultimately, chronic inflammatory reaction (4) is elicited (Figure 3). Connective tissue deposition; primarily collagen has always been sequelae to infiltration of immune mediators such as leukocytes, cytokines, etc. (5-7) (Figure 2 G, J). As witnessed in this case study, the presence of a fibrotic collagen capsule is a common observation in retrieved orthopedic implants explanted due to aseptic loosening (6-8). The collagen capsule (thickness ranging from 700μm to 4200μm) was observed in both articular and non-articulating zones of the knee megaprosthesis. Characteristically, loose connective tissues and synovial membrane formation are the chief histological features of retrieved articulating metal-on-polymer implants (8). Nevertheless, the collagen deposits due to particulates in larger volume leads to generation of a thicker collagen capsule around the implant; this coupled with increased osteoclastic activity reduces the ability of the bone to anchor to the implant resulting in the loosening of the prosthesis (9).

Due to the presence of released particulate debris, namely titanium, cement, and UHMWPE, the routine immune response is biochemically altered. In this study, we found evidence of particulate trifecta (titanium, cement, and UHMWPE) with each particle eliciting a different type of inflammatory response leading to failure of the implant. However, contributions of each individual particulate or the respective amalgamation of two or more particles have not been delineated.

High concentration of titanium particles in the peri-prosthetic region observed in our study could potentially induce a cell-mediated type IV immune reaction; and the induced chemokines and inflammatory cells could spread into the “effective joint space” (4). This spreading phenomenon is an innate part of “debris removal” process initiated by the non-specific cellular reaction. As a result, the implant debris, mostly metallic, is capsulated in villous structures or capsular lesions around the peri-prosthetic area. Similar

Discussion and conclusion

Irrespective of its composition, particulate debris (metal, polymer, or cement) induces an inevitable influx of polymorphonuclear leukocytes and macrophages in the peri-prosthetic tissue. In many cases, a non-specific cellular reaction leading to extensive foreign body response [composed of macrophages, foreign body giant cells (FBGC), fibroblasts, and occasional lymphocytes and small caliber blood vessels], and ultimately, chronic inflammatory reaction (4) is elicited (Figure 3). Connective tissue deposition; primarily collagen has always been sequelae to infiltration of immune mediators such as leukocytes, cytokines, etc. (5-7) (Figure 2 G, J). As witnessed in this case study, the presence of a fibrotic collagen capsule is a common observation in retrieved orthopedic implants explanted due to aseptic loosening (6-8). The collagen capsule (thickness ranging from 700μm to 4200μm) was observed in both articular and non-articulating zones of the knee megaprosthesis. Characteristically, loose connective tissues and synovial membrane formation are the chief histological features of retrieved articulating metal-on-polymer implants (8). Nevertheless, the collagen deposits due to particulates in larger volume leads to generation of a thicker collagen capsule around the implant; this coupled with increased osteoclastic activity reduces the ability of the bone to anchor to the implant resulting in the loosening of the prosthesis (9).

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to our study, Santavirta et al. also reported granulomatous lesions and villous structures at multiple locations around retrieved orthopedic implants (7). In addition, this spreading and debris removal behavior of inflammatory cells in the peri-prosthetic region could explain the tissue discoloration and possibly explain the observed metallosis phenomenon in our study. Bone cement, usually polymethylmethacrylate, particulates have a direct role in activation of granulocyte-macrophage colony-stimulating factors (10) which is a known mediator of bone resorption. A recent study from our group was suggested that nickel and chromium ions from stainless steel implants have led to induction of hypoxic environment thereby leading to elevated TLR4 expression (activates inflammatory cytokines) (11). Likewise, in our study, the implant particulates induced an inflammatory response leading to an increase in cellular density of monocytes and other inflammatory cells. This increased presence of activated mast cells and their released cytokine, IL-4 could potentially lead to increased profibrotic response.

Cell-mediated immune responses due to UHMWPE (in MP implants) leading to aseptic loosening has not been clearly established (8); however, the presence of degranulated mast cells and mast cell mediated hypersensitivity in the peri-implant region of orthopedic implants have been documented (12). Furthermore, it has been reported that the polymeric particulate could coalesce with titanium to develop radiodense masses, fluid filled mass of titanium debris or titanium cysts (13). Often these masses are accompanied with a synovium-like membrane that is capable for initiating collagenase, interleukin 1, and tumor necrosis factor (14), which could induce extra cellular matrix (ECM) remodeling and bone resorption leading to failure of the implant.

An important contribution of this study is the elucidation of the exceptional involvement of mast cells in the aseptic loosening of the knee implant. A recent article by Gallo et al. highlighted that macrophages and T-cells were the most investigated etiological factors whereas natural killer cells and mast cells were the least studied etiological factors for loosening and osteolysis of orthopedic implants (8). Only a handful of studies has reported on the contribution of mast cell in bone-implant tissue interface biology (3, 12, 15). Similar to Solovieva et al. (12), influx of both activated and degranulated mast cells was a common observation in the bulk of the retrieved tissue pathology (Figure 2 A, B). It is possible that the particulate debris may provide stimulus for mast cell activation, which further leads to release of interleukins including IL-4 and tryptase (Figure 2 G, H). It has been noted that both activated and degranulated mast cells...
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cells can act as growth factors for fibrotic collagen deposition (5). We postulated that the mast cell cascade, and contribution from other inflammatory cells could have resulted in a profibrotic response resulting in the fibrotic collagen capsule formation (a detailed explanation is shown in Figure 3). Further, this collagenous capsule also has the ability to either induce ECM remodeling enzymes (such as collagenase) and other biochemical factors that could lead to osteolysis (3) and thus culminates in the aseptic loosening of the implant.

This study reports that the aseptic loosening of the knee implant is significantly enhanced by the cellular processes advent from the released implant debris (2), and thereby leading to a profibrotic collagen deposition. It is, however, unclear if the presence of some remnant cancerous cells and cancer-based chemokines in the patient’s body were responsible for this accelerated fibrosis and/or bone resorption, resulting in the aseptic loosening of the CMP. Since collagen deposition is a cumulative outcome of many different mediators, further in depth study is warranted at the molecular level, the results of which will aid in the identification of potential target molecules that can prevent this excessive collagen deposition.

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Disclosure

The Authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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