M1 and M2 Macrophage Polarization, Wear Particles and Joint Replacement

Abstract Introduction: Total joint replacement (TJR) is a very successful operation for patients suffering from disabling arthritis. However, wear of joint replacements is inevitable with usage of the joint and often requires revision surgery. Wear particles include ultra high molecular weight polyethylene, bone cement, metallic and ceramic debris. Wear debris induces chronic inflammation which can lead to periprosthetic bone loss, or osteolysis, that can undermine the prosthesis bone bed. Particles produced from wear are phagocytosed by macrophages, leading to their proliferation, differentiation, and activation. A current hypothesis suggests that macrophage activation in osteolysis may be polarized, possibly with M1 macrophages promoting an inflammatory response early on following debris formation, and M2 macrophages acting later in an anti-inflammatory response to promote bone healing, debris scavenging, wound healing, and angiogenesis. The focus of this research is to investigate the differential expression of M1 and M2 macrophages in periprosthetic tissues undergoing revision total joint replacement surgery. Our hypothesis is that there is a higher ratio of M1/M2 macrophages immediately prior to revision joint replacement surgery, leading to bone degeneration and inflammation.

Methods: This study has been approved by the IRB. Tissue samples were collected from 10 patients undergoing primary TJR (synovium) and 10 patients (tissue harvested from the bone-implant interface) undergoing revision surgery for loosening with/without osteolysis. Immunohistochemistry

Serial sections were cut with a cryostat and double stained for presence of CD68, a general macrophage marker, and either HLA-DR, an M1 marker, or CD163, an M2 marker. Primary antibodies were tagged with secondary antibodies AlexaFluor 488 for CD68, and AlexaFluor 594 for either HLA-DR or CD163. Imaging was performed using a Leica confocal microscope.

Western Blots

Protein was extracted from the synovium of osteoarthritic patients undergoing primary TJR from bone-implant interface and from the bone-implant interface of patients undergoing revision TJR who have periprosthetic osteolysis, without evidence of infection. The protein was then cleaned and purified for analysis by Western Blotting using an SDS Tris-Glycine system. Detection of CD68, HLA-DR, and CD163 was performed and visualized using an ECL detection system and hyperfilm chemiluminescence.

Results: Primary synovium showed a greater number of M2 macrophages (Figure 1), whereas a greater proportion of M1 macrophages were found in revision tissues (Figure 2). Western blotting confirmed this finding (Figure 3), which is consistent with the premise that M1 macrophages promote an inflammatory response, associated with periprosthetic bone loss (osteolysis).

Discussion: Particle production due to wear of prosthetic joints leads to macrophage polarization into M1 and M2 macrophages. M1 macrophages are differentially expressed as a result of particle production, as evidenced by the increased M1/M2 ratio in pseudomembranes taken during revision surgery. Preventing M1 macrophages polarization may therefore be a future target for therapy to prevent inflammation and periprosthetic bone loss, potentially reducing the necessity for revision surgery.

Significance: Revision joint replacement surgery is more complex than primary replacement and results in more complications; therefore it is desirable to develop a non-surgical, pharmacologic approach to treat osteolysis. The manipulation of macrophage populations to promote bone healing, revascularization and anti-inflammatory responses may potentially extend the lifetime of a joint replacement even with the continued production of wear particles.