M1 and M2 Macrophage Polarization in Pseudomembranes from Total Joint Replacement

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Statement of Purpose: 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. The wear produces particles which have been most commonly identified as ultra high molecular weight polyethylene, bone cement, metallic and ceramic debris (Ma and Goodman, 2010). Chronic and persistent debris production can lead to chronic inflammation which can lead to periprosthetic bone loss, or osteolysis, with adverse clinical implications for patients. Particles produced from wear are phagocytosed and processed by monocyte/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 macrophage expression 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 was collected from 10 patients undergoing primary TJR and 10 patients undergoing revision surgery for loosening with/without osteolysis. Samples were embedded in OCT compound (Sakura Finetek) and fresh frozen in liquid nitrogen and stored at −80 °C for immunohistological analysis. Serial sections were cut with a cryostat and mounted on positively charged microscope slides. Sections were double stained for presence of mouse anti-humanCD68, a general macrophage marker, and either mouse anti-human HLA-DR, an M1 marker, or mouse anti-human CD163, an M2 marker. Primary antibodies were tagged with secondary antibodies AlexaFluor rat anti-mouse 488 for CD68, and AlexaFluor rat anti-mouse 594 for either HLA-DR or CD 163. Imaging was performed using a Leica confocal microscope. Positive HLA-DR and CD163 fluorescence was calculated based on intensity, using negative slides from each sample to establish baseline threshold. Threshold for positive slides were adjusted to exclude obvious background and non-specific staining.

Results: M1 and M2 macrophages in human synovial tissue were compared to revision pseudomembranes. Primary synovium showed a greater number of M2 macrophages (Figure 1), whereas a greater proportion of M1 macrophages were found in revision pseudomembranes (Figure 2). This finding is consistent with the premise that M1 macrophages promote an inflammatory response, associated with periprosthetic bone loss (osteolysis) and the need for revision surgery.

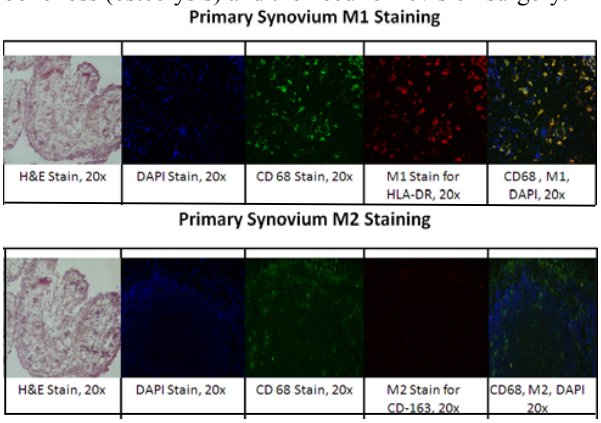

Figure 1. M1 and M2 staining in primary synovium

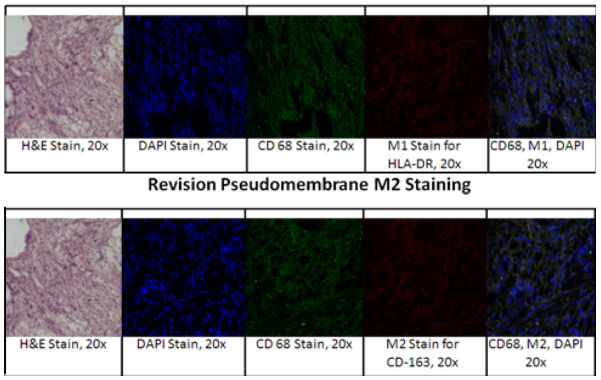

Figure 2.M1 and M2 staining in revision pseudomembrane

Conclusions: 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.

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