M1 and M2 Macrophage Polarization in vitro with exposure to LPS and IL-4

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Abstract Introduction:
Replacement of arthritic joints with artificial materials is a frequently performed surgical procedure. The articulating surfaces wear with usage generating particulate debris, which is usually processed and phagocytosed by monocyte/macrophages leading to their proliferation and activation. Once activated, macrophages produce pro-inflammatory cytokines leading to periprosthetic bone loss, or osteolysis. A current hypothesis suggests that macrophage activation in osteolysis may be polarized. M1 macrophages may promote an inflammatory response early with production of mediators such as IL-1, IL-6, IL-8, MMPs, and TNF-α. In contrast, M2 macrophages may act later in an anti-inflammatory response to promote bone healing, debris scavenging, wound healing, and angiogenesis by utilizing mediators such as IL-4, IL-10, and IL-13. In this study, we investigate the differential expression of M1 and M2 murine macrophages when stimulated by lipopolysaccharide (LPS). The use of LPS stimulates endotoxemia, which has been reported to be present on some revised implants and byproducts of wear of joint replacements, possibly contributing to periprosthetic osteolysis. Our hypothesis is that there is a higher ratio of M1/M2 macrophages in response to activation with LPS, and that the administration of IL-4 can drive these M1 macrophages to M2 macrophages in vitro.

Methods:
This protocol is IRB and EHS approved.

Cell Culture
We harvested bone marrow aspirates from femurs of 8- to 12-week old C57BL mice. We suspended the bone marrow aspirates in 40 mL RPMI buffer + 25% LCM + 10% FBS + 1% Pen/Strep and allowed cells to adhere overnight at 37°C in a tissue culture dish. At day 7, we added 100mg/ml of LPS, 0.25 mg/ml IL-4, and used leukocyte-conditioned media (LCM) as a control. At day 10, we added IL-4 to one dish of LPS stimulated cells. The media was collected and saved from all dishes for analysis by ELISAs. Cell smears were created and immunohistochemical analysis was then performed using primary antibodies directed against CD68 (recognizing macrophages), HLA-DR and iNOS (recognizing M1 macrophages), and CD163 antigen and Arg1 (recognizing M2 macrophages). Double staining was performed of CD68 and CD163/Arg1 or CD68 and HLA-DR/iNOS, using AlexaFluor488 to recognize CD68 and AlexaFluor594 to recognize M1 or M2.

ELISA
ELISA cytokine assays (R&D Systems) were run on the culture media collected from the cell culture of murine macrophages with LCM, LCM+IL-4, and LCM+IL-4+LPS. Results are shown as concentration derived from a standard curve.

Results:
Immunohistological staining showed that un-induced macrophages had a low M1 and M2 profile. However, there was a higher M1/M2 ratio following administration of LPS, which supports the hypothesis that LPS may preferentially polarize macrophages to M1 pro-inflammatory macrophages (Figure 1). Following administration of IL-4 alone, there was a moderate decrease in the M1/M2 ratio, however IL-4 administration after LPS induction produced the largest decrease in the M1/M2 ratio (Figure 2). This suggests that macrophage polarization may be preferentially shifted from M1 to M2 using IL-4 administration. Using ELISA, TNF-α levels decreased after administration of IL-4 to LPS-stimulated macrophages. IL-10 levels increased after IL-4 administration alone than after LPS and IL-4 administration (Figure 3).

Discussion:
Administration of LPS and IL-4 to in vitro cultures of murine macrophages leads to polarization into M1 and M2 macrophages respectively. M1 macrophages are differentially expressed after the administration of LPS, however, the administration of IL-4 was able to polarize this response preferentially to M2 macrophages. This suggests that IL-4 may be able to induce an anti-inflammatory state in the joint space even in the presence and continued production of wear particles. 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 it is desirable to develop non-surgical, pharmacologic approaches to treatment of osteolysis. One strategy is the manipulation of macrophage populations present in the joint following total joint replacement surgery to medically promote bone healing and anti-inflammatory responses.

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