**VEGF INCREASE IN PERIPROSTHETIC OSTEOLYSIS IS SECONDARY TO INCREASED NUMBERS OF MACROPHAGES AT THE BONE-IMPLANT INTERFACE**

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**Introduction:**
The pro-angiogenic cytokine VEGF has been implicated in periprosthetic osteolysis (OL) and subsequent aseptic loosening of implants following total hip arthroplasty (THA). We hypothesized that macrophages are the major source of VEGF production at the bone-implant interface and that increased levels of VEGF are secondary to a greater number of VEGF-producing cells. We used double-immunofluorescent staining to assess the quantity and location of VEGF and VEGF-producing cells—including macrophages, fibroblasts, and endothelial cells—in periprosthetic OL tissue and osteoarthritis (control) synovium.

**Materials and Methods:**
Periprosthetic tissue was obtained at the location of osteolysis from 4 patients undergoing revision THA for aseptic loosening. For comparison, synovial tissue was obtained from 4 patients with osteoarthritis (OA) undergoing primary THA. Consent was obtained from all patients; this research was approved by the Stanford University School of Medicine’s Administrative Panels on Human Subjects in Medical Research.

All samples were embedded in OCT compound (SakuraFinetek) and flash frozen in liquid nitrogen. Serial sections (6 μm thick) were double-immunofluorescent stained using rabbit anti-human VEGF antibody (Santa Cruz Biotechnology) as the first primary antibody and Rhodamine-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratory) as the first secondary antibody. Second primary antibodies to cell markers were as follows: mononuclear mouse anti-CD68 (DakoCytomation) for macrophages; monoclonal mouse anti-CD11b (DakoCytomation) for macrophages; mononuclear mouse anti-5b5 (DakoCytomation) for fibroblasts; monoclonal mouse anti-CD31 (DakoCytomation) for endothelial cells; mononuclear mouse anti-CD90/Thy-1 (Oncogene Research Products) for fibroblasts and activated endothelium. We used FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratory) as the second secondary antibody. All samples were coverslipped with a DAPI anti-fade mounting medium (Chemicon International) both to preserve samples and to fluorescently stain nuclei. Imaging was performed on a Carl Zeiss AxioCam fluorescence microscope (Carl Zeiss, Figure 1). Immunofluorescent images were analyzed with Metamorph 6.2 (Universal Imaging). Positive FITC and Rhodamine fluorescence was calculated based on intensity above thresholds based on negative samples.

**Figure 1:** Double-immunofluorescent staining of CD68+ macrophages (green) and VEGF (red). Co-localized signal is yellow.

OL tissue  | OA tissue

**Results:**
Comparison of DAPI-positive nuclei per high powered field (HPF) in OL and OA groups showed greater than a 350% increase in cellularity of OL versus OA tissue (766 vs 216 nuclei per HPF, p<0.05, Figure 2A). Tissue samples from the OL group exhibited both higher overall VEGF expression (p<0.05, Figure 2B), and a significant increase in macrophages (p<0.05, Figure 2C) compared to OA tissue samples. However, there was no significant increase in fibroblasts or endothelial cells in the OL group compared to the OA group (Figure 2C). The co-localization of CD68 and CD11b signal with VEGF signal—as calculated by positive-signal overlap in image analysis—was significantly higher in the OL group than in the OA group (p<0.05, Figure 2D), although some degree of co-localization was demonstrated in all samples.

**Discussion:**
Macrophages are recognized as important mediators of osteolysis, and increased numbers of macrophages are directly related to the amount of particulate debris in retrieved membranes from loose prostheses. VEGF has been implicated as a contributor to bone resorption and increased levels of VEGF have been found both in the synovial fluid and periprosthetic membranes of patients with osteolysis. VEGF is both a product of activated macrophages and can serve as a chemoattractant for monocytes and osteoclast precursor cells through the VEGF receptor Flt-1. Our results demonstrate that CD68+/CD11b+ VEGF-producing macrophages are the primary cells recruited to the bone-implant interface. Endothelial cells and fibroblasts—acknowledged producers of VEGF—are not found in higher numbers in OL tissue, nor do they appear to contribute significantly to the amount of VEGF in OL tissue. Though VEGF is primarily a pro-angiogenic cytokine and mitogen, its role as a chemoattractant for monocytes, i.e., as a pro-inflammatory cytokine exacerbating the chronic inflammatory process, may be more important in the development of osteolysis. As such, VEGF may be a legitimate target for therapeutic intervention in the amelioration of osteolysis.

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