Parsing Inflammatory Cues in Angiogenesis

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Statement of Purpose: Biomedical implants that facilitate communication and interaction with the surrounding tissue and circulatory system are rendered ineffective by the huge diffusion barrier and increased electrical resistance presented by the fibrous capsule. For successful implants, it is ideal to have the device surrounded and penetrated by highly vascularized tissue. Both angiogenesis and inflammation are inescapable *in vivo* responses to all biomaterial implants. There is emerging evidence that inflammatory cells regulate the functions of endothelial cells related to angiogenesis(*1, 2*). However, the signals initiating angiogenesis in inflammation are complex and difficult to define. The goal of this study is to addresses the hypothesis: the biomaterial-induced inflammatory response may be critical to control angiogenesis.

Methods: In order to address the hypothesis, we have fabricated porous hydrogels using polyethylene glycol (PEG)-cross-linked tyrosine-derived polycarbonate[3]. The hydrogel scaffolds have been made more bioactive via hybridization of pro/anti anigiogenic and/or anti-inflammatory synthetic peptides with extracellular matrix (ECM) materials (e.g., collagen or fibrin gel). The release of peptides from hydrogels has been measured. This system has been utilized for an *in vitro* culture of endothelial and inflammatory cells, their co-culture, and for *in vivo* implants. Using a series of *in vitro* and *in vivo* assays to investigate the effects of the degraded products on angiogenesis and inflammatory responses, we have studied endothelial cell function and behavior modulated by the interactions of inflammatory cells with the bioactive peptide-modified hydrogels.

Results: Peptides were mixed in ECM materials (e.g., collagen or fibrin gel) and stably hybridized to hydrogels. Peptides were released more slowly from collagen-hydrogels, compared to fibrin-hydrogels. Laminin-derived pro-/anti-angiogenic peptides (C16 and C16Y, respectively) regulated *in vitro* migration and tubulogenesis of human microvascular endothelial cells. Thymosin β4-derived anti-inflammatory Ac-SDKP peptides efficiently reduced *in vitro* phagocytosis of human blood-derived macrophages. *In vivo* experiments showed that anti-inflammatory Ac-SDKP not only decreased phagocytosis but also caused disconnected and poorly branched vasculature architecture. When pro-angiogenic C16 was included in the scaffold, a highly interconnected and branched vasculature was formed, but it was surrounded by a dense population of phagocytic cells. In co-culture with a combination of peptides, the activation of macrophages in the hydrogel scaffolds was quantified using Vybrant® Phagocytosis assay kit (Molecular Probes, Eugene, OR), resulting in green color

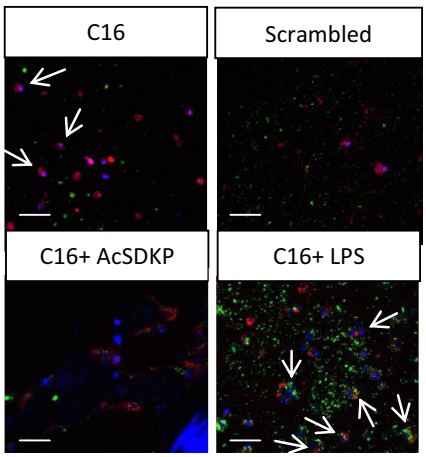

Figure 1. *In vitro* co-culture of human monocytes and human umbilical vein endothelial cells (HUVECs) with combinations of pro-angiogenic C16, anti-inflammatory AcSDKP, and pro-inflammatory lipopolysaccharide (LPS). Red=VCAM1 expressing endothelial cell, blue=Hoechst nuclear stain, green=phagocytic macrophage. White arrows indicate tubulogenesis. Scale bar =50μm.

in Figure 1, and endothelial cells were labeled with an APC conjugated antibody for VCAM1/CD106 (BioLegend San Diego, CA), shown in red in Figure 1. Nuclei were counter stained with Hoechst (blue in Figure 1). Alone C16 increased tubulogenesis, without a significant increase in phagocytosis, as compared to scrambled peptide. The combination of C16 and AcSDKP decreased both tubulogenesis and inflammation. While C16 in combination with pro-inflammatory lipopolysaccharide (LPS) increased both tubulogenesis and phagocytosis as compared to C16 alone, indicating the interplay between inflammation and angiogenesis.

Conclusions: These results suggest that the inflammatory cell response aids in the formation of functional blood vessels, and that angiogenesis and inflammation influence each other in the hydrogel implants. Elucidating a clear mechanism for angiogenesis in biomaterial-induced inflammation through further studies will provide new paradigms of design for the next generation of biomaterials.

Grant support: R21 HL091465 NIH / NHLBI

References:

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