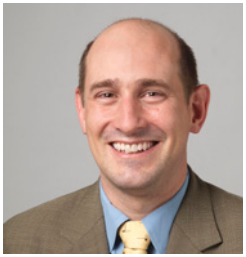

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dsRNA Stimulates Toll-like Receptor-3 and Increases Endothelin-1 Production by Pulmonary Artery Endothelial Cells

We propose, in this work, that dsRNA stimulates toll-like receptor (TLR)3 on endothelial cells, causing endothelin-1 (ET-1)-mediated fibrosis, vasoconstriction, and inflammation, as a mechanism of relevance in immune-mediated causes of pulmonary arterial hypertension (PAH). Research will focus on investigating how the immune system causes endothelial cell dysfunction, a hallmark of systemic sclerosis (SSc), and how these events may lead to PAH, a frequent cause of mortality in SSc. We have shown that interferon (IFN)-regulated genes are expressed highly in SSc patients' peripheral blood mononuclear cells and also have demonstrated that this can be replicated by nucleic acid-containing immune complexes stimulating TLRs. We have recently extended this finding to endothelial cells and hypothesize that the phenotype of SSc, particularly the vasculopathy, may be induced through extracellular dsRNA binding to a specific TLR on endothelial cells, called TLR3. TLR3 is unique among the family of TLRs in that it signals using a different adaptor protein, and also has both endosomal and cell surface expression in endothelial cells. Stimulation of endothelial cell TLR3 has been shown to be pro-inflammatory and inhibit blood vessel growth (antiangiogenesis). Our preliminary data demonstrate that dsRNA stimulates endothelial cells via a TLR3-dependent

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mechanism to produce the vasoactive and pro-fibrotic peptide, ET-1, a molecule strongly implicated in SSc pathogenesis. We also show that continuous infusion of dsRNA into mouse dermis leads to inflammation, marked up-regulation of IFN-regulated genes, increased ET-1 in the skin, lung, and serum, and increased dermal fibrosis. All of these effects are abrogated in mice deficient in TLR3 signaling. Therefore, we hypothesize that dsRNA, possibly contained in circulating immune complexes or in cellular debris, triggers TLR3 on endothelial cells, resulting in inflammation, endothelial cell dysfunction, and vasculopathy. To test our hypothesis, we will: 1) examine the role of TLR3 stimulation in pulmonary artery endothelial cell (PAEC) dysfunction and apoptosis; and 2) investigate the effects of prolonged infusion of dsRNA into mice and quantify endothelial cell activation, ET-1 production, and histologic changes.