Abstract: P551

Effects of interferon gamma on endothelial barrier function: differential role of classical and non-classical pathways

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Background: Increased vascular permeability is a surrogate marker for the development of atherosclerotic lesions. Loss of EC barrier integrity exposes the underlying interstitium to a variety of cytokines present in the blood creating a local inflammatory microenvironment which harbours more inflammatory cells. During the progression of atherosclerosis the expression of several cytokines including interferon gamma (IFN-g) is upregulated. The role of IFN-g in the development and progression of atherosclerosis is increasingly debated due to the presence of evidence conveying both pro- and anti-atherogenic actions of IFN-g. While EC activation and loss of their integrity is one of the major factors contributing towards progression of atherosclerosis, little is known about its effects on EC barrier function and related signalling.

Methods: The study was carried out on cultured human umbilical vein endothelial cells (ECs) and rat coronary microvascular ECs. Endothelial barrier function was analysed by measuring the flux of albumen through EC monolayers cultured on filter membranes. Gene expression was analysed by qPCR-based assays.

Results: Quantitative PCR analysis showed that ECs express both IFNGR1 and IFNGR2. Chronic treatment of confluent EC monolayers with IFN-g (40 ng/mL) for 48h attenuated thrombin-induced EC hyper-permeability, actin cytoskeleton remodelling, and loss of cell-cell junctions (n=5; p<0.05). Thrombin-induced activation of RhoA, Rho kinase, and phosphorylation of myosin light chain (MLC; a marker of EC contractile activation) which was significantly attenuated in EC monolayers pre-treated with IFN-g. Likewise, adhesion of freshly isolated human monocytes was significantly reduced on EC monolayers pre-treated with IFN-g. In next step, ECs were primed towards classical IFN-g-stat1 signalling pathway by short term pre-treatment with PDGF (10 ng/mL; 3h). Surprisingly, priming of ECs towards classical IFN-g signalling resulted in loss all IFN-g-mediated protective effects on endothelial barrier function, contractile activation and cell-cell junction. Likewise, IFN-g transformed the PDGF-primed endothelial monolayers towards inflamed condition resulting in massive adhesion of non-activated monocytes. Conclusion: The data of the present study demonstrate that the effects of IFN-g on endothelial monolayers is dependent upon the basal state of ECs. In non-primed ECs IFN-g exerts endothelial barrier protective effect while in ECs primed for classical pathway, IFN-g has barrier disruptive and pro-inflammatory effects.