THE EFFECTS OF FLOW ON ADHESION MOLECULE EXPRESSION AND MONOCYTE-ENDOTHELIAL INTERACTIONS *IN VITRO*

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Abstract

Atherosclerosis is a cardiovascular disease that can have fatal complications, such as a heart attack or stroke. A key event in early lesion development is monocyte adhesion and transmigration through the endothelium. Lesion localization suggests that local hemodynamics plays an important role during atherogenesis. Lesion-prone regions are characterized by disturbed flow, which exhibits low wall shear stress, flow recirculation and flow reattachment. In this study, we hypothesize that local hemodynamics plays a significant role in monocyte-endothelial cell interactions during early atherosclerosis by (1) increasing the adhesiveness of the endothelium by the expression of selective adhesion molecules and (2) regulating the transport of monocytes, adhesion and subsequent transmigration by local remodeling of intercellular junctional complexes. In this thesis, we investigated monocyte-endothelial interactions in an *in vitro* model of disturbed flow that resembles the local hemodynamics of athero-prone regions. Endothelial cell activation through the use of cytokines is a currently accepted model to observe early atherosclerosis events *in vitro*. A key element in our experimental model is that flow is used as the only stimulus to activate endothelial cells. No cytokines were used to ensure that the observed monocyte-endothelial interactions are the sole results of the local hemodynamic environment.

A parallel-plate flow chamber is used to expose human umbilical vein endothelial cells (HUVEC) to physiologically relevant shear stresses under laminar or disturbed flows, which represent lesion-resistant and lesion-prone regions, respectively. The expression of the adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were evaluated. Functional

monocyte adhesion and transmigration assays were conducted and remodeling of the junctional protein JAM-A during transmigration was assessed. It was determined that E-selectin expression at the protein and mRNA level is regulated by flow. Specifically, fluorescent *in situ* hybridization (FISH) demonstrated: a) mRNA upregulation under laminar flow in a time-dependent fashion with peak expression occurring after 4 hours and reset to control levels by 24 hours and b) mRNA upregulation in the disturbed flow region is sustained over 24 hours while the adjacent regions in fully recovered laminar flow has reset to control levels. E-selectin protein expression was similarly upregulated, with transient expression under laminar flow and sustained expression only in the disturbed flow region. Protein immunocytochemistry of the adhesion molecules ICAM-1 and VCAM-1 demonstrated that ICAM-1 remained at baseline values under both types of flow while VCAM-1 was upregulated only as a result of disturbed flow. Monocytes perfused over the flow-conditioned endothelial cell layer resulted in localized monocyte adhesion and transmigration corresponding to sites of E-selectin and VCAM-1 protein expression. Visualization of the JAM-A-RFP construct revealed a redistribution of cellular JAM-A to sites of activated monocytes and its active participation in the transmigration process.

In summary, our studies showed that exposure to shear stress alone upregulated relevant surface and junctional adhesion molecules, which contributed to functional monocyte adhesion and transendothelial migration. In addition, these changes in expression and resulting monocyte-endothelial interactions were only sustained in areas exposed to disturbed flows, which simulate lesion-prone regions *in vivo*. The findings of

this thesis work support a regulatory role for hemodynamics during early atherosclerotic lesion development.