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The relevance of von Willebrand factor platelet mediated activation and transmigration of monocytes in the context of vascular remodeling processes

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Cardiovascular diseases comprise a heterogeneous group of illnesses including coronary heart disease and pulmonary embolism, which are closely linked by the fact that they all share a critical feature in their pathogenesis, i.e. the development of atherosclerosis. Atherosclerotic lesions are the result of a chronically dysfunctional endothelium, leading to an increase in endothelial cell permeability, which facilitates migration of monocytes into the lesion. Monocytes adapt in response to the various stimuli they encounter in their microenvironment, resulting in alterations in phenotype and function. In addition to many other factors, the interaction of CD40 and its ligand (CD40L) have previously been identified as playing an important role in the pathogenesis of atherosclerosis.
To investigate monocyte transmigration under shear stress conditions upon stimulation of CD40, an in vitro model of a vessel was developed. The level and orientation of shear stress was calculated using numerical and analytical methods. Upon application of a certain level of shear stress, the transmigration of monocytes was almost completely enabled, but was able to be enhanced several fold by means of stimulation with CD40L and amplified by the addition of activated platelets, leading to soluble, CD40L-induced, ultra-large von Willebrand factor-platelet string formation. Applying a CD40L antibody resulted in an impaired release of von Willebrand fractor multimers, indicating the specific role of CD40-CD40L signaling. Thus, for the first time, we demonstrated CD40L-induced, von Willebrand factor/platelet-mediated transmigration of monocytes through an endothelial cell monolayer and its significance in the presence of shear stress. Subsequently, the influence of monocyte transmigration on their state of activation and differentiation was determined. PCR analysis revealed that typical M1 markers are upregulated after simple transmigration, rise further upon stimulation with CD40L, and increase even more upon stimulation with histamine. However, regulation of the M2 markers upon transmigration was not as distinct because they responded diversely. FACS analysis, in contrast, revealed a distinct upregulation of all M1 and M2 markers upon migration, but a significant further increase after stimulation was absent. Answering the question of whether transmigration leads to a pro- or an anti-inflammatory phenotype has thus to be further investigated.
In addition to the above mentioned system, a spheroid assay was developed as a new model for monocyte transmigration. Imaging using a 2-photon-microscope demonstrated massive monocyte adhesion and directed migration through the endothelial cells towards the core of the spheroid. Taken together, these findings contribute to the understanding of how monocytes may be capable of overcoming high rates of shear stress that occur within arterial vessels and are therefore still able to migrate into an atherosclerotic lesion. This process occurs through CD40L-induced release and deposition of ultra-large von Willebrand factor multimers on the luminal endothelial cell surface. Moreover, the in vitro models that were developed allow for the selective assessment of monocyte transmigration and differentiation in the context of CD40-CD40L signaling. Additionally these models can be applied to future questions concerning the transmigration of monocytes in chronic inflammation, thus surpassing the scope of our specific question.