Platelet-monocyte complexes in touch with the endothelium

van Gils, J.M.

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Preface

Introduction
The capacity of leukocytes to leave the blood stream and migrate into tissues is a critical feature of the immune system and essential in the defence against pathogens. The adhesion to and migration across an endothelial monolayer into the vessel wall are tightly regulated processes. The endothelial cells form a vital barrier between the blood and underlying tissue, and play a major role in the regulation of the endothelial permeability for plasma constituents and in leukocyte adhesion and transmigration. Upon inflammation, the endothelial cells produce cytokines and express adhesion molecules on their surface to activate and bind leukocytes. This initiates the multiple-step transmigration process (Figure 1), starting with rolling, which triggers expression and activation of leukocyte integrins. Next, these integrins mediate firm adhesion, followed by transmigration across the endothelium. The transmigration of the leukocytes across the endothelial monolayer requires a collaboration of both cell types to preserve the barrier function of the endothelium. When leukocyte adhesion and transmigration are repressed, inflammation is not resolved, but extensive efflux of inflammatory cells will lead to chronic inflammation, and diseases such as atherosclerosis or rheumatoid arthritis may develop.

Figure 1: Multi-step model of leukocyte (-platelet complex) extravasation. In the circulation platelet-leukocyte complexes can be formed upon platelet activation. At sites of inflammation, leukocytes are tethered to the vascular endothelial cells. The leukocytes then roll over the vessel wall, until they arrest and firmly adhere. Finally they spread and migrate between endothelial cells into the surrounding tissues, leaving the platelet on the endothelium.
The main function of blood platelets is primary haemostatic by facilitating coagulation upon vascular damage. However, platelets also play an important role in inflammation, by binding to and activating the endothelium, and forming a powerful substrate for leukocyte adhesion and activation\(^3\). Activated platelets may also occur in the circulation, which can bind to circulating leukocytes and form platelet-leukocyte complexes (Figure 1)\(^4\). Interaction of activated platelets with leukocytes leads to activation of these leukocytes\(^5\), resulting in increased adhesion capacities, as compared to platelet-free leukocytes\(^6\). Mainly in patients with coronary artery diseases an increased number of these platelets-leukocyte complexes are observed\(^7,8\). Altogether, it is hypothesized that the platelet-leukocyte complexes and especially platelet-monocyte complexes (PMC) are pro-atherogenic. In particular, monocytes are very important in the development and progression of atherosclerosis. The differentiation of monocytes to macrophages upon transendothelial migration and the uptake of oxidized low-density lipoproteins in the vascular wall lead ultimately to foam-cell formation, a critical cell type in atherosclerosis progression\(^9\). In conclusion, the functionalities of platelets, monocytes and, endothelial cells are interdependent and should be considered in concert to understand inflammation and related pathologies such as atherosclerosis.

**Scope of the thesis**

This thesis aims to gain more insight in leukocyte transmigration and endothelial barrier function influenced by platelet interactions. **Chapter 1** presents an overview of the molecular mechanisms involved in the interactions between monocytes, platelets and endothelial cells. In this review we also address the potential consequences of these interactions, especially of the complex formation between monocytes and platelets, for the development of cardiovascular diseases.

In **Chapter 2** monocyte adhesion and transmigration towards the cytokine MCP-1 is studied, in particular concerning the role of the guanine exchange factor Epac in this process, since Epac has been reported to play a role in lymphocyte adhesion and motility\(^10\). We measured the expression of the Epac1 protein in different subsets of leukocytes and demonstrate a function of the Epac1-Rap1 signalling pathway in monocyte adhesion and transmigration.

**Chapter 3** reports the influence of platelets on the monocyte adhesion and transmigration capacity. The binding of activated platelets to monocytes depends on the interaction between P-selectin, expressed on activated platelets, and its counter-receptor P-selectin glycoprotein ligand-1 (PSGL-1), constitutively expressed on monocytes\(^4\). This binding results in increased monocyte adhesion to endothelial cells\(^6\). To better explain this increased adhesion, we studied the changes in integrin functionality upon platelet binding to monocytes, specifically via PSGL-1. The platelet-monocyte complexes (PMC) not only adhere better, but also have an increased transmigration capacity. This was confirmed in **Chapter 4**, in which we continued to study the PMC transmigration. We focused on the fate of the platelet within these complexes, since platelets can have profound pro-inflammatory effects on the endothelium and the monocytes. The PMC dissociate upon transmigration of the monocytes and the platelets remain on the endothelium (Figure 1). In Chapter 4 the mechanism of this dissociation is unravelled. Next, in **Chapter 5** we studied the potential consequences on the endothelial phenotype of platelet-endothelial cell interactions via endothelial PSGL-1, because this protein, which is expressed on leukocytes, was recently found also to be expressed on endothelial cells\(^11,12\). PSGL-1 mediates
cell-cell interactions by binding to P-selectin (expressed on endothelial cells and platelets), endothelial-expressed E-selectin or leukocyte-expressed L-selectin. However, it is unknown whether ligation of endothelial PSGL-1 induces signalling, as has been reported for leukocyte-expressed PSGL-1. In Chapter 5, we show that PSGL-1 ligation, in contrast to its effect on leukocytes, seems to induce an anti-inflammatory phenotype in the endothelial cells.

In Chapter 6 we investigated whether maternal human platelet-antigen alloantibodies (HPA-1a), which induce neonatal alloimmune thrombocytopenia and bleedings, affect the endothelium barrier function. HPA-1a alloantibodies can bind to β3-integrin (CD61) on endothelial cells. We show that HPA-1a alloantibodies have a direct effect on endothelial cell spreading and monolayer integrity. This mechanism may contribute to the increased bleeding tendency in children with neonatal alloimmune thrombocytopenia.

Finally in Chapter 7, the findings and conclusions of this thesis are summarized and discussed in the context of the current knowledge and possible interesting future research directions.

References
