

## Theme Issue Editorial

## Frontiers of vascular biology: Mechanisms of inflammation and immunoregulation during arterial remodelling

Christian Weber

Institute for Molecular Cardiovascular Research (IMCAR), RWTH Aachen University, Aachen, Germany

Cardiovascular disease is responsible for almost half of the morbidity and mortality world-wide (with some 7 million fatalities per year in the Western world alone) and develops mostly from atherosclerosis and subsequent obstruction or thrombosis of large arteries. The widely accepted inflammatory paradigm of atherogenesis postulates that this multifactorial process follows metabolic dysregulation, such as diabetes and hyperlipidemia in conjunction with other prominent risk factors, such as arterial hypertension and smoking, leading to an activation of endothelial cells and dysfunction and entailing mononuclear cell infiltration into the neointima. This cellular response of the arterial vessel wall is crucial for the initiation of atherosclerotic lesion development, for the progression, the destabilisation and eventually the rupture of plaques with fatal consequences such as myocardial infarction or stroke. By analogy to leukocyte recruitment in other chronic inflammatory diseases, the atherogenic recruitment involves sequential actions of adhesion molecules and small chemotactic cytokines termed chemokines, mediating the adhesion and emigration of mononuclear cells. Specifically, a delicately controlled balance of T-helper cell-specific index cytokines and anti-inflammatory mechanisms governed by regulatory T-cells, as well as contributions of different monocyte and granulocyte subsets have been crucially implicated in atherogenesis (1).

The GfMVB society (Gesellschaft für Mikrozirkulation und Vaskuläre Biologie) is dedicated to the interdisciplinary investigation, translational research study and communication of various aspects of microcirculation and vascular biology. These include for instance endothelial function and rheology, signalling and cell trafficking in the vascular wall, angiogenesis and angioadaptation. Given the strong emphasis on atherosclerosis research and the locally active international graduate school EuCAR on Arterial Remodeling at the Universities of Aachen and Maastricht, the Annual Meeting 2008 in Aachen was consequently and distinctively focused on the mechanisms of immunoregulation and inflammation affecting vascular biology and remodelling. The meeting further embedded a Symposium on chemokines and adhesion molecules in cardiovascular pathogenesis funded by the Deutsche Forschungsgemeinschaft

(DFG), a Symposium on the immunopathogenesis of atherosclerosis endorsed by the European Vascular Genomic Network and the 2<sup>nd</sup> Euregio Symposium on plaque instability. In fact, the city of Aachen as the residence of great European Charlemagne created the appropriate stage and atmosphere (see Fig. 1) for a European meeting and the local scientific context provided by the Euregio (the triangle at the border of Belgium, The Netherlands and Germany) fostered a unique focus on atherosclerosis and arterial remodelling. Accordingly, the series of articles in this theme issue of *Thrombosis and Haemostasis* represents a collection of contributions derived from invited lectures of this Annual GfMVB Meeting to cover this topic.

## Initial mechanisms of inflammatory recruitment

Like for many other chronic inflammatory and autoimmune diseases or the host response to infectious agents, the recruitment of immune cells is also the central step in the pathogenesis of atherosclerosis (1). The first article by Chavakis et al. sets out to provide an excellent overview on novel aspects in the regulation of the multistep model of leukocyte adhesion (2). The process of leukocyte extravasation from the circulation into sites of inflammation requires a complex cascade of adhesive events between the leukocytes and the endothelium including leukocyte rolling, adhesion and transmigration. The interactions of leukocytes with the activated endothelium are mediated by binding between adhesion receptors on both cell types. Namely, this is controlled by leukocyte integrins and their endothelial counter-receptors of the immunoglobulin superfamily, which also encompass recently identified anti-adhesive interaction partners, modulating the leukocyte adhesion cascade.

Recent evidence has emerged to demonstrate an important role of polymorphonuclear leukocytes (neutrophils) in early phases of atherosclerosis (1). During their extravasation, neutrophils can release a panoply of granule proteins, which may promote and launch the subsequent influx of monocytes. Various models and concepts, which define how neutrophil granule proteins may regulate this process, are reviewed in an article by Soehnlein et al. (3). For instance, granule components deposited

Correspondence to:  
Christian Weber, MD  
Universitätsklinikum Aachen – Kardiovaskuläre Molekularbiologie  
Pauwelsstr. 30, 52072 Aachen, Germany  
Tel.: +49 241 808 8692, Fax: +49 241 808 2703  
E-mail: cweber@ukaachen.de

Received: June 16, 2009  
Accepted: June 16, 2009

Prepublished online: July 3, 2009  
doi:10.1160/TH09-06-0371

on the endothelium can induce endothelial adhesion molecular expression and can directly trigger monocyte adhesion and activation. In addition, granule proteins can sculpt the local chemokine environment through proteolytic modification or enhanced local synthesis, or auxiliary direct chemotactic effects. Thus, multiple neutrophil- and granule protein-related mechanisms contribute to the early recruitment cascade of inflammatory monocytes with possible relevance to atherosclerosis.

## Immunoregulation of atherosclerotic plaque formation

The cellular and humoral immune response with an activation of antigen-presenting cells and subsequent T-cell priming and B-cell isotype switching is also an essential component of the atherogenic process and can be accomplished and instructed by co-stimulatory pathways, e.g. the CD40-CD40L dyad. The disruption of the CD40-CD40L axis constitutes an attractive therapeutic target to reduce atherosclerosis and to induce a stable plaque phenotype, thus limiting its clinical complications. This concept is introduced in the article by Lutgens et al. (4), which also elaborates on proatherogenic effects of CD40 and CD40L on plaque macrophages, neutrophils, endothelial cells, vascular smooth muscle cells and platelets, respectively. It is further proposed that unraveling the cell type-specific CD40-CD40L interactions and the specific signaling components of CD40 that do not compromise host defense will enable therapeutic applicability of these multifunctional pathways.

The family of interleukin-6 (IL-6) cytokines and their signal transduction have been reported to contribute to atherosclerotic plaque development and destabilisation through a multitude of mechanisms that are summarised in a review article by Schütt et al. (5). These involve the release of other inflammatory cytokines and prothrombotic mediators, lipoprotein oxidation by phospholipases, stimulation of acute phase proteins and the activation of matrix metalloproteinases. Conversely, the formation of reactive oxygen species by vascular enzyme systems participates in the regulation of IL-6. The article further discusses the mechanisms underlying a regulatory role of IL-6 trans-signalling via its soluble receptor in atherosclerosis.

Among the mononuclear cell subsets less well studied as to their involvement in the atherogenic process, natural killer T (NKT) cells have attracted recent interest as an unusual subset of innate immune cells because of their potential to regulate immune responses to a variety of pathogens (1). Like cells of the adaptive immune system, NKT cells express a highly restricted repertoire of T-cell receptors generated by somatic DNA arrangement that recognize glycolipid antigens bound with the antigen-presenting molecule CD1d and produce substantial amounts of immunomodulatory cytokines upon antigenic stimulation. Since apolipoprotein E (apoE) is involved in the presentation of lipids, findings that NKT cell activation can accelerate atherosclerosis in apoE<sup>-/-</sup> mice may be misleading. The study by Kuiper et al. (6) demonstrates that treatment with  $\alpha$ -galactosylceramide, a synthetic glycolipid agonist of NKT cells, resulted in a profound reduction of diet-induced and/or collar-mediated atherosclerosis in LDL-receptor-deficient mice but not in apoE



**Figure 1:** Aachen cathedral viewed from Inconoration Hall on the night of the GfMVB 2008 dinner.

<sup>-/-</sup> mice. This was associated with a stronger proliferation and IL-10 production in Ldlr<sup>-/-</sup> mice, which may be attributable to a defective lipid presentation on CD1d in the absence of apoE, and provides the first evidence for an atheroprotective function of NKT cells.

## Fatal consequences: plaque rupture and myocardial infarction

Upon continued progression and sustained inflammation, plaque growth eventually enters the stage of a vulnerable phenotype, meaning that the lesions become unstable and prone to rupture, imminently predisposing for life-threatening events such as acute coronary syndrome or stroke. The article by Daemen et al. (7) is dedicated to current developments in biomarker research aiming to identify individuals at risk, as well as vulnerable plaques. A shift from classical population-based risk factors has been stipulated by research into the predictive value of novel, e.g. inflammatory, surrogate markers and systemic biomarkers. For instance, antibody profiling with a phage-display cDNA library derived from ruptured plaques identified a serum response against two products strongly associated with the presence of ruptured lesions and may thereby constitute a promising approach for differential diagnosis of atherosclerotic lesions. This is paralleled by the quest for non-invasive imaging modalities to detect and characterize the vulnerability of a plaque. Upon appropriate validation, improved individualised diagnosis and prediction will help to limit the fatalities of atherosclerosis.

Once plaque rupture in the coronary arteries has occurred, acute myocardial infarction causes major morbidity and mortality in affected individuals and requires immediate revascularisation of the target artery. However, the restoration of blood flow can give rise to severe damage of the ischaemic myocardial tissue, which is termed reperfusion injury. The article by Mach et al. (8) highlights the inflammatory response and its crucial mediators, namely oxidative stress and leukocyte infiltration, as underlying mechanisms of cardiac damage in the reperfusion phase. This sets the stage to discuss novel therapeutic ap-

proaches, e.g. modulation of chemokine secretion and activity, to tame the deleterious inflammatory processes promoting myocardial ischaemia-reperfusion injury.

## Future perspectives

In keeping with the aphorism ascribed to the physics Nobel laureate Niels Bohr, it remains true that prediction is very difficult, especially about the future. Given that platelets are not only essential mediators of haemostasis, e.g. at sites of vascular injury, but can also promote and modulate inflammation and tissue repair by interacting with endothelial and mononuclear cells, they may represent excellent target hubs to therapeutically interfere with all of the above processes. The article by Lisa Jennings (9) introduces an interesting concept, according to which novel anti-platelet therapies may confer a strong interference with platelet activation without increasing bleeding risk to further decrease the risk for ischaemic events. Inhibition of platelet activation stimulated by thrombin through a protease-activated receptor (PAR)-1 pathway is proposed as an emerging approach to

achieve comprehensive inhibition of platelet activation in combination with current oral antiplatelet agents without increasing bleeding risk or not affecting haemostasis.

A new dimension to the functional plasticity and fine-tuning of chemokine activities in vascular biology has been recently established by the paradigm of heteromeric interactions between platelet chemokines (10). Sophisticated biophysical and molecular dynamics studies were instrumental in obtaining a structural model of the prototypic chemokine heteromer CXCL4-CCL5 and in allowing the synthesis of a stable cyclic peptide, which selectively disrupts this heteromer. The application of this peptide enabled a striking and specific reduction of atherosclerosis without side-effects on the immune system and provided the first evidence that heteromer formation of platelet chemokines is functionally relevant *in vivo*, allowing for their selective therapeutic targeting. It can be predicted that the combinatorial diversity in the 'chemokine interactome' may open additional avenues in detecting and targeting the functions of other chemokine heteromers in vascular biology and disease.

## References

1. Weber C, Zernecke A, Libby P. The multi-faceted contributions of leukocyte subsets to atherosclerosis. *Nat Rev Immunol* 2008; 8: 802–815.
2. Chavakis E, Choi EY, Chavakis T. Novel aspects in the regulation of the leukocyte adhesion cascade. *Thromb Haemost* 2009; 102: 191–197.
3. Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release of granule proteins. *Thromb Haemost* 2009; 102: 198–205.
4. Lievens D, Wouter J, Eijgelaar WJ, et al. The multifunctionality of CD40L and its receptor CD40 in atherosclerosis. *Thromb Haemost* 2009; 102: 206–214.
5. Schuett H, Luchtefeld M, Grothusen C, et al. How much is too much? Interleukin-6 and its signalling in atherosclerosis. *Thromb Haemost* 2009; 102: 215–222.
6. van Puijvelde GHM, van Wanrooij EJA, Hauer AD, et al. Effect of natural killer T cell activation on initiation of atherosclerosis. *Thromb Haemost* 2009; 102: 223–230.
7. Eijgelaar WJ, Heeneman S, Daemen MJAP. The vulnerable patient: 1 Refocusing on the plaque? *Thromb Haemost* 2009; 102: 231–239.
8. Steffens S, Montecucco F, Mach F. The inflammatory response as a target to reduce myocardial ischaemia and reperfusion injury. *Thromb Haemost* 2009; 102: 240–247.
9. Jennings L. Mechanisms of platelet activation: Need for new strategies to protect against platelet-mediated atherothrombosis. *Thromb Haemost* 2009; 102: 248–257.
10. Koenen RR, von Hundelshausen P, Nesmelova IV, et al. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. *Nat Med* 2009; 15: 97–103.