Neutrophil-endothelial interaction in ANCA associated vasculitis

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Chapter

1

Introduction to the thesis

ANCA-associated systemic vasculitides

Antineutrophil cytoplasmic autoantibodies (ANCA) associated vasculitides comprise three disease phenotypes: granulomatosis with polyangiitis (GPA), formerly Wegener's Granulomatosis, microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS).[1,2] ANCA-associated systemic vasculitis (AAV) is a relatively rare disease, with an annual incidence of approximately 20/million in Europe. There seems to be an upward trend of incidence during the last decades, which, however, is perhaps due to an increase of disease awareness and improved diagnosis amongst physicians.[3] A central feature of AAV is fibrinoid necrosis affecting small- to medium-sized vessels with little or no deposition of immunoglobulins or complement, in a so-called pauci-immune pattern.[4] By electron microscopy, subendothelial edema, microthrombosis and degranulation of infiltrated neutrophils are observed.[5] Vascular lesions may be limited to a single organ, but commonly affect multiple organ systems. When lungs or kidneys are involved, often presenting with aggressive renal failure or pulmonary haemorrhage, the disease becomes life-threatening.[6] AAV has a universally poor prognosis with mortality approaching 100% within 5 years when left untreated.[7] The current standard treatment of AAV is based on cyclophosphamide and glucocorticoids, which is effective in 70~90% of patients and transforms AAV from a rapidly fatal disease to a state of chronic relapsing disease. Also due to the broad and severe side effects of these regiments, AAV is still in need of improvements in therapeutic strategies.[8,9]

Pathogenesis of AAV

The pathogenesis of AAV has not been fully understood. A widely accepted paradigm holds ANCA, primed neutrophils and activated vascular endothelium as key players.

During infection, proinflammatory cytokines up-regulate expression of adhesion molecules on endothelial cells (ECs), transforming ECs into a pro-adhesive status. Meanwhile, upon stimulation of neutrophils by these cytokines, proteinase 3 (PR3) or myeloperoxidase (MPO), both of which are stored in neutrophil granules, are translocated to the cell surface as part of priming of neutrophils. Adhesion molecules on neutrophils are also upregulated in this process. When ANCA are present, they bind to autoantigens on primed neutrophils, converting slow rolling into firm adhesion of neutrophils and leading to neutrophil degranulation and respiratory burst. Released proteolytic enzymes and reactive oxygen species (ROS) may cause necrosis, apoptosis and detachment of adjacent endothelial cells.

Hence, ANCA-mediated activation of adherent neutrophils may directly attack the vessel wall.[10]

In addition, granuloma formation, particularly occurring in PR3-ANCA AAV, suggests involvement of cellular immunity. Increased numbers of effector memory T cells have been observed in peripheral blood in AAV patients during remission and have been detected in urine during active disease, suggesting a pathogenic role in AAV.[11,12] Furthermore, it has been shown in a mouse model of MPO-ANCA associated vasculitis that complement activation via the alternative pathway is also required for AAV development.[13]

Interaction of neutrophils and endothelial cells in AAV

It is intriguing that ANCA-mediated vascular lesions preferentially occur in the microvasculature, the loci where neutrophil trafficking takes place. This would suggest that close interaction of neutrophils and endothelial cells is important for disease development. Indeed, it has been shown that infiltrated neutrophils during acute vasculitis are at or within glomerular capillary loops with rather poor penetration into the interstitial tissue or peri-tubular region where their chemoattractants are detectable, suggesting that neutrophils are retained within the microvascular compartment by certain mechanisms.[14] To gain insight into the interaction between neutrophils and endothelial cells, mouse cremasteric microvasculature was studied via intravital microscopy. In the presence of local inflammatory stimuli, anti-MPO antibodies were found to reduce neutrophil rolling while increasing firm adhesion of leukocytes.[15] Also, ANCA-mediated neutrophil activation requires an adhesive state of neutrophils, suggesting that ANCA not only induce close interaction of neutrophils and endothelial cells, but also that ANCA-associated events are dependent on this close contact.[16]

Aim and outline of the thesis

In this thesis, the mechanisms underlying retaining of neutrophils within the vascular wall and the ensuing vascular damage were investigated. Secondly, up-regulated expression of ANCA antigens, such as membrane-bound proteinase 3 (mPR3), is an important event for ANCA-induced neutrophil activation. The mechanisms of PR3 expression on the neutrophil membrane were, therefore, also studied and presented in this thesis.[17]

In **Chapter 2**, an overview is given on the mechanisms regulating neutrophil-endothelial interaction during inflammation and specifically in AAV. Current knowledge on the effector mechanisms taking place in this process, which induce

endothelial damage in AAV, is summarized. As mentioned before, persistent inflammation within the vessel wall suggests perturbed neutrophil trafficking of activated neutrophils through the endothelium. CXCR1 and CXCR2, being major chemokine receptors on neutrophils, are largely responsible for neutrophil recruitment, and are, therefore, studied in **Chapter 3**. In this chapter, we tested the hypothesis that down-regulated expression of CXCR1/2 retains neutrophils within the vessel wall and, consequently, leads to persistence of neutrophils in the microvasculature. Anti-endothelial cell autoantibodies (AECA) have been described in AAV as one of the effector mechanisms causing vascular damage. The presence of AECA in patients with AAV has been reported by several groups with conflicting data on prevalence ranging from 8% to 100%. Types of substrate cells used for AECA testing partially explain this variation. In **Chapter 4**, we investigated AECA prevalence in AAV using a human glomerular endothelial cell line in comparison with primary human umbilical vein endothelial cells, which have frequently been used for AECA detection.

Membrane expression of the ANCA-antigens, such as PR3, allows ANCA binding and is a crucial step of ANCA-mediated neutrophil activation. Indeed, up-regulation of PR3 on the neutrophil membrane has been shown during neutrophil adhesion. Since the PR3 molecule does not contain a transmembrane domain in its sequence, the mechanisms of membrane expression of PR3 and the proposed signal transduction, therefore, become interesting and are reviewed in **Chapter 5**. CD177 glycoprotein has been demonstrated as a receptor of PR3 on the neutrophil membrane. The expression profile of CD177 and its role in PR3-ANCA-mediated neutrophil activation is studied in **Chapter 6.** We found an enlarged neutrophil subset with CD177 expression in patients with AAV. The molecular function of CD177 is largely unknown, so the functional differences between $CD177^{+}$ and $CD177^{-}$ neutrophils cannot be easily deduced. Therefore, in **Chapter 7**, we performed a gene microarray-based study to investigate differences between $CD177^{+}$ and $CD177^{-}$ neutrophils, which may help to clarify the pathophysiologic significance of the enlarged $CD177^{+}/mPR3^{high}$ neutrophil subset in order to better understand the role of neutrophils in the pathogenesis of AAV.

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