Activation, apoptosis and clearance of neutrophils in Wegener's granulomatosis
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 1

Introduction to the thesis

André P. van Rossum
INTRODUCTION

Wegener’s granulomatosis

Wegener’s granulomatosis (WG) is an autoimmune inflammatory disease affecting mainly arterioles, venules, and capillaries in conjunction with extravascular necrotizing granulomatous inflammation. The disease was first described by the German pathologist Friedrich Wegener in 1936. Patients had a very poor prognosis with a median survival of five months. In the early 1970s, Fauci and Wolff introduced treatment with cyclophosphamide and prednisone as a therapeutic strategy for patients with WG, which resulted in a nearly complete and long-lasting remission of the disease. This implicated that the pathophysiology of WG is strongly mediated via immunological mechanisms. In general, vasculitis and glomerulonephritis is frequently associated with immune complex deposition. However, no depositions of immune complexes could be found at the affected sites in WG, suggesting that the vascular injury is of a cellular nature and not mediated by type III hypersensitivity reactions. Diagnosis was suspected on clinical features only, including complications of the upper and lower respiratory tract, renal disease, and variable manifestations of disseminated vasculitis, and confirmed by tissue biopsies.

Autoantibodies in WG

A major breakthrough was made by Van der Woude et al in 1985 who reported an autoantibody sensitive and specific for the disease. These autoantibodies reacted with the cytoplasm of ethanol-fixed neutrophils, and monocytes, and were called anti-neutrophil cytoplasmic autoantibodies (ANCA). Five years later, three independent groups showed that the azurophilic granule enzyme proteinase 3 was the target autoantigen recognized by ANCA (PR3-ANCA). Next to proteinase 3, another granule protein, myeloperoxidase (MPO) was identified as target autoantigen of ANCA as well (named MPO-ANCA). However, PR3-ANCA are most frequently (80-90%) found in patients with WG. Further investigations showing that PR3-ANCA are not only disease specific but also highly sensitive for active WG, and that changes in antibody levels are closely associated with disease activity, strengthened the idea that WG is an autoimmune disease probably mediated by pathogenic ANCA. More supporting proof concerning the pathogenicity of these autoantibodies originated from in vitro studies as well as from experimental animal studies. In vitro experiments showed that these ANCA are capable to activate neutrophils. When neutrophils become primed with low dose of pro-inflammatory cytokines, translocation of PR3 to the membrane via exocytosis of the PR3 storing- granules and vesicles can occur, resulting in membrane-bound proteinase 3 expression (mPR3). Expression of PR3 on the cell surface of neutrophils makes the autoantigen accessible for interaction with ANCA, resulting in further activation of these neutrophils leading to degranulation of lytic enzymes and the production of reactive oxygen species. When this process is taking place on (activated) endothelium, the endothelium will become damaged, finally ending up in vasculitis. These pathogenic consequences of ANCA were demonstrated in experimental animal models showing that MPO-ANCA alone can cause necrotizing and crescentic glomerulonephritis.
Proteinase 3 expression on neutrophils

Activated neutrophils are supposed to play a central role in the pathophysiology of WG. In biopsies from WG patients, neutrophils are present at sites of injury. Histological studies have shown that large numbers of activated neutrophils are present at affected sites in vasculitis. In addition, numbers of activated neutrophils in renal biopsies correlate with renal damage. As said, activation of neutrophils is supposed to occur via interaction with PR3-ANCA. In this activation process, F(ab’)2 fragments of PR3-ANCA bind to their autoantigen PR3 whereas the Fe parts of PR3-ANCA interact with neutrophil Fcγ-receptors resulting in full activation although F(ab’)2 fragments of ANCA alone have been shown to induce minor activation as well. A prerequisite for this activation is the translocation of proteinase 3 to the membrane by stimulation, i.e. priming, with low dose of pro-inflammatory cytokines such as TNF-α. Cytokine levels of TNF-α, among other pro-inflammatory cytokines, are elevated in vasculitis. However, not all neutrophils are able to translocate proteinase 3 upon priming with TNF-α, which is independent from their priming state. Based on the ability to express membrane bound proteinase 3 (mPR3), two subsets of neutrophils can be defined: neutrophils that hardly express proteinase 3 (mPR3- neutrophils) and neutrophils that do express proteinase 3 substantially (mPR3+ neutrophils). Percentages of mPR3+ neutrophils range from 0 to 100% of the total population of neutrophils within individuals. Furthermore, the level of membrane mPR3 expression on the mPR3+ positive subpopulation of neutrophils can differ between individuals. Interestingly, in Wegener’s granulomatosis an increased percentage of mPR3+ neutrophils is found. Moreover, increased expression of mPR3 in WG patients is associated with an increased incidence and rate of relapse.

Neutrophil apoptosis and clearance in WG

Next to activated neutrophils, also large numbers of apoptotic and necrotic neutrophils are found in vasculitis. Following activation, neutrophils become apoptotic with further membrane expression of PR3. In WG, opsonisation of PR3-expressing apoptotic neutrophils with PR3-ANCA may not only facilitate their uptake, but may also perpetuate inflammation by release of pro-inflammatory cytokines during clearance. However, also disturbances in the clearance of apoptotic neutrophils of WG patients are found, as shown by the occurrence of leukocytoclastic vasculitis in the skin. One of the key features of leukocytoclastic vasculitis is the accumulation of unsavaged apoptotic or necrotic neutrophils and fragmented nuclei of neutrophils in the tissue around vessels. The phenomenon of leukocytoclasia is interesting, since normally dying cells are quickly engulfed by phagocytes, particularly at sites of inflammation. Furthermore, accumulation of dying cells and the presence of nuclear debris in these lesions suggest that the removal of dead (apoptotic or necrotic) neutrophils is incomplete. The factor(s) influencing normal clearance of dead neutrophils in these lesions are yet unknown. Complement components and short pentraxins can facilitate clearance of apoptotic cells. In contrast to short pentraxins, the long pentraxin PTX3 was shown to bind to Jurkat cells and, subsequently, inhibit clearance of apoptotic Jurkat cells by dendritic cells. PTX3 is locally produced by endothelial cells, but also by cells of the monocytic lineage after stimulation with pro-inflammatory stimuli. Interestingly, PTX3 was shown to bind to late apoptotic...
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neutrophils as well. Furthermore, levels of the pentraxin PTX3 proved to parallel disease activity in patients with WG and other small vessel vasculitides. These observations may be relevant in view of the persistence of the leukocyte remnants in the vessel wall, found in leukocytoclasia.

**Aim of the thesis**

So, neutrophils, in their activated or apoptotic state, play an important role in the pathophysiological phenomena seen in WG. Therefore, in this thesis the role of neutrophils in WG was further investigated during the process of activation, apoptosis and clearance. In **part I** of this thesis, the pathogenic role of neutrophils in WG is explored, in particular in relation to membrane proteinase 3 expression. This was done by studying the occurrence, functionality, and regulation of membrane-bound PR3 expression in relation to the pathophysiology of WG. **Part II** of this thesis concerns studies on the clearance of apoptotic neutrophils in relation to the presence of the vasculitis-related pentraxin PTX3 in order to understand the accumulation of unscavenged dying neutrophils found in leukocytoclastic lesions.

As an overview of both parts, **chapter 2** reviews current knowledge on activation, apoptosis and clearance of neutrophils in the context of WG. Then a detailed literature review on membrane expression of PR3 as a pathogenic determinant in the pathophysiology WG is presented (**chapter 3**). In **chapter 4**, the requirement of priming for the assessment of mPR3 expression on neutrophils was studied. Furthermore, a comparison was made between differential mPR3 expression with membrane expression of activation markers. Finally, mPR3 expression on neutrophils in WG and other chronic inflammatory diseases was assessed after priming with TNF-α. **Chapter 5** describes a fundamental issue in the pathophysiology of WG, that is, binding of PR3-ANCA to mPR3 expressing neutrophils. Due to a challenging report suggesting that ANCA actually do not bind to neutrophils in blood, a study was undertaken to explore binding of ANCA to neutrophils. In **chapter 6**, functional consequences of mPR3 expression after stimulation with PR3-ANCA are studied. **Chapter 7** describes important mediators in regulatory pathways involved in mPR3 expression on neutrophils.

**Part II** concerns the study of PTX3 in relation to leukocytoclastic phenomena in WG. Therefore **chapter 8** starts with an *in vitro* study exploring the role of PTX3 in the clearance of late apoptotic neutrophils by macrophages. In **chapter 9**, an *in vivo* study was undertaken to demonstrate presence of PTX3 at sites of leukocytoclastic lesions in skin of patients with WG. In **chapter 10**, the observations described in this thesis are summarized and discussed.

**REFERENCES**

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