Wegener's granulomatosis, staphylococcus aureus and immune complexes; Clinical and Experimental studies.
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Chapter 7

SUMMARY AND GENERAL DISCUSSION

I would like to take the opportunity to summarize all findings and put these into perspective. In this thesis, we have studied the possible role of *Staphylococcus aureus* in the pathophysiology of Wegener’s granulomatosis (WG). As described in Chapter 1, Friedrich Wegener was the first to suggest an infectious etiology for WG. The pathophysiology of WG is still unknown today. Evidence shows that the majority of patients with WG are chronic nasal carriers of *S. aureus*, a serious risk factor for developing a relapse of the disease. Due to chronic *S. aureus* carriage/subclinical infections, cationic antigens such as SACP could enter the bloodstream and bind to negatively charged structures on endothelial cells. Antibodies directed against SACP, present in the plasma of patients with WG, may bind to SACP resulting in the formation of immune complexes. SACP could also be responsible for the formation of circulating immune complexes once it enters the bloodstream and comes in contact with anti-SACP antibodies. These circulating immune complexes can subsequently deposits in organs resulting in inflammation. Should SACP be involved in the pathogenesis of WG this disease might not be as “pauci-immune” as is currently believed. In this thesis we have tried to supply evidence that *S. aureus* play an important role in the pathophysiology of WG and that it might not be a true “pauci-immune” ANCA-associated vasculitis. We focussed our efforts on a cationic protein of *S. aureus*, staphylococcal acid phosphatase (SACP) as cationic proteins have been found in renal biopsies of patients suffering from acute post-streptococcal glomerulonephritis.

To test the hypothesis that SACP can act as a planted antigen in WG, we studied the ability of SACP to bind to human umbilical vein endothelial cells (HUVEC) and human glomerular endothelial cells. Chapter 2 describes that SACP can act as a planted antigen by binding to both types of endothelial cells in a concentration-dependent manner. Binding of concentrations as low as 4 μg/ml can be detected on HUVEC within 5 minutes following incubation. Binding of SACP to endothelial cells was charge-dependent and did not activate endothelial cells. This seems to rule out an interaction with a receptor. Finally, endothelial cell-bound SACP was recognized by antibodies present in sera of patients with WG. The data suggest a possible pathogenic role for SACP by acting as a planted antigen in WG. Immune complexes can deposit in the kidney and cause an immune-mediated damage. Norway rats were immunized and perfused with SACP but perfusions with SACP-immunized animals did not induce glomerulonephritis whereas perfusions of non-immunized animals did (IgG and C3). Finding increased damage in glomerulonephritis. As described earlier, mainly on the absence of disease. In animal models, immune deposits (IgG and C3) can be detected along the capillary loop development. If ANCA is present, rapid removal of immune deposits (IgG and C3) in skin biopsies from patients with WG. Chapter 3. Using direct immunofluorescence, immune deposits (IgG and C3) between the immunofluorescence.
role for SAcP by acting as a planted antigen thereby initiating glomerulonephritis and vasculitis in patients with WG. This would imply that immune complexes could play a role in the pathogenesis of WG.

Immune complexes can either be deposited from the circulation or formed in situ in the kidney and cause an immune complex-mediated glomerulonephritis. In Chapter 3 we developed an animal model for S. aureus-associated glomerulonephritis. Brown Norway rats were immunized with SAcP or control-immunized with phosphate-buffered saline. Fourteen days later, glomerulonephritis was evoked by unilateral perfusion of the left kidney using different concentrations of SAcP (0, 10, 25, 50 and 100 µg/ml). After 10 days, the rats were sacrificed and renal sections were stained for the presence of inflammatory cells and immune deposits. In animals that were sham-immunized and perfused with SAcP and in the animals that were immunized with SACP but perfused with PBS no abnormalities were observed in the kidney. SAcP-immunized animals perfused with SAcP developed proteinuria due to crescentic glomerulonephritis with influx of neutrophils and monocytes and immune deposits (IgG and C3). Findings were dose-related as increases in SAcP concentration resulted in increased damage. Perfusions of 10 µg/ml SAcP induced mild glomerulonephritis whereas perfusions of ≥ 25 µg/ml SAcP were accompanied by severe glomerulonephritis. In conclusion, this animal model for S. aureus-associated immune complex-mediated glomerulonephritis could be useful for further understanding of this form of infection-related glomerulonephritis.

As described earlier, WG is considered a pauci-immune systemic vasculitis based mainly on the absence of immune deposits in renal biopsies of patients with active disease. In animal models of ANCA-associated glomerulonephritis immune deposits can be detected along the glomerular capillary wall at early stages of lesion development. If ANCA-induced neutrophil activation is indeed responsible for the rapid removal of immune complexes, these may still be detected in very early lesions in patients with WG. Chapter 4 details, therefore, our efforts to detect immune deposits in skin biopsies from patients with WG, taken within 48 hrs of lesion development. Using direct immunofluorescence, 32 skin biopsies were examined for the presence of immune deposits (IgG, IgA, IgM, C3c). When possible, a comparison was made between the immunofluorescence findings in renal and skin biopsies taken at the same
time. Four out of 11 biopsies taken at initial presentation of the disease and 4 out of 21 biopsies taken at the onset of a relapse of WG showed IgG and/or IgA-containing immune deposits in sub-epidermal blood vessels. All renal biopsies taken at the same time showed pauci-immune glomerulonephritis irrespective of the presence (n=5) or absence (n=4) of immune deposits in the skin biopsy. Hence, a substantial number of skin biopsies showed immune deposits during active disease. This is compatible with a role for immune complexes in lesion development.

These results, however, do not confirm that S. aureus is involved in these immune deposits. In order to prove that S. aureus is involved in immune complex formation in patients with WG, antibodies to its antigens need to be present. Chapter 5 describes our experiments on detection of antibodies to SAaP in blood from patients with WG and detection of S. aureus antigens in renal biopsies of patients with WG. Sixty-one plasma samples of WG patients were examined for the presence of antibodies directed against staphylococcal acid phosphatase (α-SAcP). Anti-SAaP antibodies were elevated at the time patients show signs of the disease for the first time (initial disease manifestation). During a relapse or remission these antibodies are not elevated anymore, possibly due to antibiotic treatment or the fact that physicians are quicker to recognize a relapse and respond accordingly. We also studied the presence of SAaP in renal biopsies of patients with WG. The presence of SAaP was detected in 3 out of 19 renal biopsies taken from patients with WG but not in 24 renal biopsies taken from disease controls. Hence, glomerulonephritis may start as an immune complex mediated disease involving staphylococcal antigens in at least a subset of patients with WG. In Chapter 6, we review current literature with respect to the hypothesis that ANCA-associated vasculitides, particularly WG, may start as an immune complex mediated disease. We hypothesize that the paucity of immune deposits in lesions of these patients is because these are rapidly cleared due to the presence of ANCA. Neutrophils are attracted to and activated by the immune deposits thereby releasing their reactive oxygen species (ROS) and lytic enzymes such as myeloperoxidase (MPO), proteinase 3 (PR3) and human leukocyte elastase (HLE) all of which are anti-neutrophil cytoplasmic antibody (ANCA) antigens. ANCA binds to these ANCA antigens, thus exaggerating the neutrophil response, resulting in increased release of ROS and lytic enzymes. This leads to rapid removal of immune deposits, ultimately resulting in a clinical picture that is consistent with several studies decades several studies decades ago showing the presence of immune complexes in skin and renal lesions of patients with WG such as Churg Strauss.

In this thesis we present our investigations on the pathogenesis of WG. Our findings are consistent with WG showing that antibodies to S. aureus antigens against SAaP can be induced in patients with WG, indicating that immune complexes containing S. aureus antigens might be acting as a planted antigen in the skin of patients with WG. We also found that the presence of immune deposits in skin biopsies may be the result of immune complex mediated disease involving staphylococcal antigens in patients with WG.

There are two ways in which immune complexes can be formed. They can be formed in place and in situ within an organ by immune complexes being “transported” to this organ. In a pilot study using neutrophils of healthy donors (unpublished data) we demonstrated that loaded neutrophils in patients with WG. In a pilot study using neutrophils of healthy donors (unpublished data) we demonstrated that loaded neutrophils in patients with WG can lead to the formation of immune complexes in tissues. Studies in patients with WG [1-5]. Since these studies, the presence of circulating immune complexes in patients. We also found that immune complexes (unpublished data). It remains to be further investigated.
In this thesis we presented the hypothesis that *S. aureus* plays a role in the pathogenesis of WG. The possible formation of immune complexes plays a major part in this hypothesis. Indeed, immune deposits can be detected in skin lesion of patients with WG showing that WG is not as pauci-immune as is believed. Antibodies directed against SAcP can be found in plasma of patients raising the possibility that circulating immune complexes could be formed. SAcP can bind to endothelial cells potentially acting as a planted antigen and causing *in situ* immune complex formation. SAcP itself has been detected in renal biopsies of patients, indicating that glomerulonephritis could be the result of immune complex formation in the kidney. And finally, the animal model demonstrates that SAcP is capable of inducing an immune complex-mediated crescentic glomerulonephritis.

There are two ways in which immune complexes can be formed; circulating immune complexes can be formed in the blood circulation, or immune complexes can be formed *in situ* within an organ. The presence of SAcP in renal biopsies shows that SAcP can be "transported" to this organ. The way in which this "transport" occurs is not known. In a pilot study using FACS we demonstrated that exogenous addition of SAcP to neutrophils of healthy donors, resulted in the binding of SAcP to neutrophils (unpublished data). We were unable to demonstrate the presence of circulating SAcP-loaded neutrophils in blood obtained from patients with WG. The question remains whether these immune complexes are deposited from the circulation or are formed *in situ* in tissues. Studies have described the presence of circulating immune complexes in serum of untreated, newly diagnosed WG patients. We also found that out of 9 sera tested, 5 had elevated levels of circulating immune complexes (unpublished data). The involvement of SAcP in these complexes is not known.

It remains to be further evaluated which role ANCA plays in the rapid removal of

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Summary and discussion

clausal picture that is consistent with a pauci-immune vasculitis. Indeed, in the past decades several studies have described the presence of immune deposits in pulmonary, skin and renal lesions of patients with WG and other ANCA-associated vasculitides, such as Churg Strauss syndrome.
immune complexes. Our animal model, described in chapter 3, is not a true model for WG since immune deposits can be detected in renal lesions. In chapter 1 we proposed a mechanism for the rapid removal of immune complexes in WG. Due to the presence of ANCA, immune deposits can be removed quickly (fig. 1 of the introduction). We have, therefore, tried to develop the same model using rats which were not only immunized with SAcP but also with human myeloperoxidase (hMPO). Heeringa et al. immunized rats with hMPO and showed that antibodies were formed after 14 days that cross-reacted with rat MPO (rMPO) [6]. Unfortunately, we were unable to reproduce these results. All rats developed antibodies directed against hMPO but these antibodies did not cross-react with rMPO. We were, therefore, unable to confirm the proposed hypothesis describing that the presence of ANCA increases the speed in which immune complexes are removed.

In conclusion, this study questions the paucity of immunoglobulin deposits in early lesions of patients with WG. *S. aureus*, and in particular its cationic protein SAcP, could be a causal agent in the development of immune complexes in this disease. However, at present, the data supporting this claim is insufficient to prove a causal relationship.

**References**