Wegener's granulomatosis, straphylococcus 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 deposit 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 Wegener’s granulomatosis in patients with ANCA disease and contributing to disease progression. Immune complexes can bind to the cell surface of the glomerular endothelial cell and kidney and cause an immune complex-mediated injury. To test this hypothesis, we developed an animal model using an anti-SAcp immune complex to induce glomerulonephritis in Norway rats. An anti-SAcp immune complex consisting of SAcP (100 μg/ml) and liver obtained from SAcP-immunized animals was perfused through an isolated perfusion system. After perfusion, glomeruli were studied for signs of immune complex-mediated injury. Glomeruli incubated with an anti-SAcp immune complex and SAcP-prepared human umbilical vein endothelial cells showed increased damage, whereas perfusions with PBS and an anti-SAcp immune complex only showed necrosis. The results imply a role for SAcP in ANCA disease.

As described earlier, the disease is characterized mainly on the absence of immune deposits. In animal models, however, immune deposits can be detected along the vascular wall of the glomerulus. Chapter 3 describes the rapid removal of immune deposits by using monoclonal antibodies. The results suggest a role for SAcP in ANCA disease. In conclusion, the data support the hypothesis that SAcP plays a pathogenic role in ANCA disease. The data suggest a possible pathogenic role for SAcP by acting as a planted antigen in Wegener’s granulomatosis and contributing to disease progression.
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, C3). When possible, a comparison was made between the immunofluorescence findings in renal and skin biopsies taken at the same

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 100 µg/ml SAcP and in the animals the 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.
Chapter 7

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 SAcP 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-SAcP 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 SAcP in renal biopsies of patients with WG. The presence of SAcP 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 decades several studies showing skin and renal lesions such as Churg Strauss in this hypothesis. Indeed, with WG showing that antibodies against SAcP can be involved in immune complexes on disease onset, acting as a planted antigen and has been detected in renal biopsies, it may be the result of immune complex formation.

In this thesis we presented our data on the pathogenesis of WG. Chapter 5 demonstrates that SAcP has been detected in renal biopsies of patients with WG. There are two ways in which immune complexes can be formed. One is they can be “transported” within an organism and the other is *in situ* within an organ. Studies have demonstrated that SAcP is not known. 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 clinical 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