Wegener's granulomatosis

Stegeman, Coen Albert

INTRODUCTION

Wegener's granulomatosis in its classic form is characterized by necrotizing granulomatous inflammation of especially nasal and paranasal structures and lungs in combination with pauci-immune necrotizing glomerulonephritis and small vessel vasculitis (1,2). Although a clinical presentation in its classical form is not uncommon, a patient with Wegener's granulomatosis may present with a wide variety of clinical problems, even without some or even all classic features (3). In patients with a less typical presentation the physician can easily be mislead and a long diagnostic delay can occur (4,5). In addition, Wegener's granulomatosis is a rare disease with a yearly incidence of only a few new cases per million people (6). Therefore, most physicians will not encounter more than a few patients in their medical career. The discovery of anti-neutrophil cytoplasmic antibodies (ANCA) which proved both sensitive and specific for this disease has provided the clinician with a helpful tool in the diagnostic work-up of patients with systemic vasculitis (7). Moreover, the discovery of these antibodies has probably increased the awareness of Wegener's granulomatosis and related disorders among physicians (6) and has boosted research into both the pathophysiology and the treatment of these diseases.

For the studies presented in this thesis patients with Wegener's granulomatosis were studied in an attempt to elucidate immunological and other mechanisms that play a role in the development of disease activity of this relapsing disease. In addition to a search for pathophysiological phenomena per se, these phenomena are assessed in terms of monitoring or predicting disease activity or influencing the course of the disease. As an introduction to the specific items explored in the subsequent chapters, ***chapter 1*** gives an outline of the several proposed pathophysiological mechanisms that may play a role in ANCA-associated vasculitis. The hypothesis put forward to explain the development of disease activity in ANCA-associated vasculitis is that different mechanisms involving ANCA, T-cell reactivity, and microbial agents, either alone or in combination, under certain conditions can cause a vicious circle leading to ongoing neutrophil activation with extensive tissue destruction. Central issues in this hypothesis are that ANCA can stimulate neutrophils and can influence the function of its target antigen.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA): A CLASS OF DIFFERENT ANTIBODIES

In the 10 years since the discovery of the relation between active Wegener's granulomatosis and antibodies producing a characteristic fine granular cytoplasmic staining pattern by indirect immunofluorescence on ethanol fixed granulocytes, knowledge on these autoantibodies has expanded enormously. We now know that the originally described ANCA, called cANCA, represent antibodies directed against proteinase 3, a serine protease from the azurophilic granules of neutrophils. It has also been established that ANCA are found in patients with a variety of vasculitic and other autoimmune diseases directed towards other antigens present in neutrophils. In ***chapter 2*** a review is given of the current knowledge on ANCA with respect to target antigens, detection procedures, and value and limitations of these antibodies in clinical practise. Antibodies directed against proteinase 3 (cANCA) have been found highly specific and sensitive for Wegener's granulomatosis or closely related disorders and are a valuable aid in the diagnostic process. These antibodies decline or disappear during treatment and tend to reappear or increase in the months preceding a relapse of disease activity. Although the value of the presence of these antibodies during follow-up in monitoring disease activity is accepted by most, it is disputed by some authors. Antibodies against myeloperoxidase, another enzyme present in the azurophilic granules of neutrophils, are found in sera of patients with idiopathic necrotizing crescentic glomerulonephritis and different forms of small vessel vasculitis. These latter antibodies generally produce a perinuclear fluorescence pattern by indirect immunofluorescence (pANCA). Since not all sera that produce a pANCA pattern by indirect immunofluorescence contain anti-myeloperoxidase antibodies, it is a dangerous misconception to interpret pANCA as equivalent to anti-myeloperoxidase antibodies. ANCA with other antigenic specificities producing a perinuclear or atypical cytoplasmic pattern by indirect immunofluorescence have been identified in patients with non-vasculitic diseases. It is therefore mandatory to confirm or exclude the presence of specific autoantibodies in sera producing a pANCA or atypical cytoplasmic pattern by using antigen-specific assays. Given the wide range of different antigens that have been identified, and the lack of specificity of the pANCA or atypical ANCA pattern for a particular antigen, it is wise to abandon the terms cANCA or pANCA. Instead, the names indicating the antigenic specificities of the antibodies should be used such as anti-proteinase 3 antibodies or PR3-ANCA, and anti-myeloperoxidase antibodies or MPO-ANCA.

ANTI-PROTEINASE 3 ANTIBODIES IN RELATION TO DISEASE ACTIVITY

Several longitudinal studies have shown that anti-proteinase 3 antibodies measured as cANCA titer by indirect immunofluorescence often rise preceding a relapse of Wegener's granulomatosis (4,7-12). The correlation between cANCA titer and disease activity of Wegener's granulomatosis is, however, not perfect. The interval between rises in cANCA titer and the occurrence of a clinical relapse can vary from a few days to more than one year (12). In addition, not all rises in cANCA titer are followed by a relapse, nor are all relapses preceded by a rise in cANCA titer (4,12-14). Therefore, it is conceivable that not only quantitative changes but also qualitative changes in anti-proteinase 3 antibodies are related to disease activity in Wegener's granulomatosis.
In ***chapter 3*** a study is presented on the relation between cANCA titer, IgG subclass distribution of these anti-proteinase 3 antibodies, and disease activity at diagnosis and during follow-up in a cohort of 38 patients with Wegener's granulomatosis followed for at least 24 months. At the time of diagnosis, cANCA titers, IgG3- and IgG4-anti-proteinase 3 antibody levels were higher in patients with more extensive disease activity. The response on treatment of the cANCA titer was shown to have prognostic value for future relapses. Patients still positive for cANCA at 12 months after diagnosis showed a significantly increased risk for experiencing a relapse within the ensuing 4 years as compared to patients who were cANCA negative at 12 months (relative risk 4.37, 95% confidence interval 1.49 to 12.77). During follow-up 18 relapses occurred in 15 patients. A significant rise of cANCA titer consisting of at least two titer step (fourfold) on two consecutive monthly blood samples was found in 10 episodes (56%) in the 6 month period before relapse. All 10 episodes showed a concomitant doubling of IgG3-anti-proteinase 3 antibody levels at the moment of cANCA rise. Out of the remaining 8 relapses not preceded by a significant rise in cANCA titer, 6 were preceded by a 100% increase in IgG3-anti-proteinase 3 antibody levels in the 6 months prior to relapse. In all, 16 of the 18 relapses (89%) were preceded by a 100% increase in IgG3-anti-proteinase 3 antibody levels. In this group of 38 patients 13 episodes of a significant rise in cANCA titer within one month were found which were not followed by a relapse within 6 months. IgG3-anti-proteinase 3 levels doubled in only 1 of these 13 episodes. A significant rise in cANCA titer combined with a concomitant doubling of IgG3- anti-proteinase 3 levels thus had a positive predictive value of 0.91 (95% confidence interval, 0.59 to 1.00) for a relapse within 6 months compared to a value of 0.43 (95%

confidence interval, 0.23 to 0.65) for a rise of cANCA titer alone. None of the episodes of a rise in cANCA titer without concomitant doubling of IgG3-anti-proteinase 3 levels was followed by a relapse within 6 months. From these data it is concluded that levels of IgG3-anti-proteinase 3 antibodies and changes in these levels are closely related to disease activity in Wegener's granulomatosis.

In vitro studies have shown that binding of anti-proteinase 3 antibodies to proteinase 3 inhibits the irreversible inactivation of the enzyme by α_1-antitrypsin, its natural inhibitor (15). In a longitudinal study in 8 patients (***chapter 4***) it was shown that the *in vitro* inhibitory activity of the serum on proteinase 3-α_1-antitrypsin complexation increased preceding a relapse of Wegener's granulomatosis, while the inhibitory capacity at the moment of relapse was clearly elevated compared to 8 control patients in remission with comparable cANCA titers. Moreover, the inhibitory capacity rather than the cANCA titer at the moment of relapse correlated with disease activity. This suggests that the functional capacity of anti-proteinase 3 antibodies to prevent the enzyme from being inactivated may contribute to the inflammatory process. Although most anti-proteinase 3 positive sera not only inhibit inactivation of proteinase 3 but also, at least partly, its enzymatic activity (15), the reversible antigen-antibody binding may be dissolved at the site of inflammation allowing the enzyme to display lytic activity. Indirect support for the view that enzymatic activity of proteinase 3 plays a role in the pathogenesis of Wegener's granulomatosis is rendered by the observation of an increased prevalence of phenotypes partly or severely deficient for α_1-antitrypsin in patients with Wegener's granulomatosis (16,17).

Neutrophil activation is thought to play an essential role in the pathogenesis of Wegener's granulomatosis (18-20). *In vitro* ANCA both with specificity for proteinase 3 and myeloperoxidase are capable of activating primed neutrophils (21-24). The data presented in ***chapter 5*** show that IgG isolated from sera from 17 patients with active Wegener's granulomatosis have a higher capacity for stimulating primed neutrophils than 17 sera taken during disease remission. Moreover, IgG from patients with extended disease activity (n=10) produced higher neutrophil stimulation than IgG from patients with limited (locoregional) Wegener's granulomatosis (n=7). The neutrophil activating capacity of sera correlated with the cANCA titer at the moment of active disease, but not during remission. Furthermore, a significant correlation was found between changes in the relative amount of anti-proteinase 3 antibodies of the IgG3 subclass and changes in neutrophil activating capacity of paired sera taken during active disease and subsequent remission in the same patient. These data suggest that the

capacity of anti-proteinase 3 antibodies to stimulate neutrophils is related to disease activity of Wegener's granulomatosis, and that especially IgG3-anti-proteinase 3 antibodies may be involved.

NON-ANCA MEDIATED IMMUNE MECHANISMS INVOLVED IN DISEASE ACTIVATION

T cell reactivity in Wegener's granulomatosis

A role for T cell involvement in Wegener's granulomatosis is suggested by large mononuclear infiltrates containing activated T cells in active lesions (25-28). The predominance of CD4 positive T cells in these lesions and the formation of granulomas suggest a delayed type hypersensitivity reaction to as yet unidentified antigen(s). Autoreactive T cells responding *in vitro* to proteinase 3 have been found in the peripheral blood of patients with Wegener's granulomatosis, but whether these T cells play a role in disease activation is unclear (29). Data in an animal model of necrotizing glomerulonephritis associated with anti-myeloperoxidase antibodies fail to show an important role for T cell mediated immunity (30,31).

In an attempt to elucidate a possible role of T cell activation in the development of disease activity in Wegener's granulomatosis, we measured soluble IL-2 receptor, soluble CD4, and soluble CD8 in serial samples of 18 patients developing a relapse of Wegener's granulomatosis. These soluble antigens are released upon T cell activation and serum levels of these antigens are thought to reflect overall T cell activation. At the time of relapse serum levels of soluble IL-2 receptor were elevated in patients with a major relapse (n=8), and correlated both with disease activity as assessed by disease activity score and with C-reactive protein levels in the whole group (***chapter 6***). In only 9 of the 18 patients an increase of at least 25% in serum levels of soluble IL-2 receptor levels was found prior to (n=4) or at the time of relapse (n=5). In none of the patients did a rise in soluble IL-2 receptor level precede a rise in cANCA titer. However, 16 of the 18 patients had soluble IL-2 receptor levels above the normal range during the whole period of 6 months preceding a relapse. Levels of soluble CD4 and soluble CD8 did not change preceding or at the time of relapse. These data suggest a low level of persistent T cell activation even without clinical disease activity in most patients with Wegener's granulomatosis as has also been demonstrated in patients with systemic lupus erythematosus (32,33). Although a relation with disease activity at the time of relapse is found these data fail to

support a major role for T cell activation in the development of disease activity given the late occurrence of a discernible rise in soluble IL-2 receptor level occurring in only half of the patients. These facts also severely limit the clinical application of measurement of soluble IL-2 receptor in the follow-up of patients with Wegener's granulomatosis.

The role of adhesion molecules in vasculitic inflammation

Inflammation involves the recruitment and transmigration of cells such as granulocytes, monocytes, and lymphocytes to sites of injury or infection. This process of adherence and transmigration is governed by the interactions of specific adhesion molecules on both endothelial cells and circulating immunocompetent cells (34-36). The initial step in neutrophil adherence to the endothelial surface is an interaction between leukocyte L-selectin and carbohydrate ligands with endothelial P-selectin and E-selectin; both endothelial selectins are expressed upon stimulation with cytokines such as tumor necrosis factor α and interleukin-1ß. Once activated, neutrophils express $ß_2$-integrins (CD11a, CD11b, CD11c, CD18) which are ligands to intercellular adhesion molecule (ICAM) 1, 2, and 3 expressed on endothelial cells. The importance of these interactions is underscored by the severe immunodeficiency demonstrated by patients who have an inherited defect in their $ß_2$-integrin (37). Monocytes, which are often present in lesions in patients with Wegener's granulomatosis (25-27), adhere to vascular cell adhesion molecule 1 (VCAM-1) expressed on activated endothelium.

In renal biopsy specimens from patients with Wegener's granulomatosis and from rats with anti-myeloperoxidase antibody associated necrotizing glomerulonephritis an association between the expression of ICAM-1 and the number of infiltrating neutrophils has been found (38,39). Furthermore, *in vitro* data suggest an important role for adhesion molecules in endothelial cell lysis mediated by ANCA-activated neutrophils (40,41). Blocking of CD18 on the neutrophils by monoclonal antibodies completely prevents lysis of endothelial cells in this setting (41). Expression and upregulation of some of the adhesion molecules can not only be studied by immunohistological techniques, but is also possible by measuring soluble forms of these adhesion molecules in serum, since ICAM-1, VCAM-1, and E-selectin are shed from the membrane after expression (42). In the study presented in ***chapter 7*** measurements of serum levels of soluble ICAM-1, soluble VCAM-1, and soluble E-selectin were performed in patients with Wegener's granulomatosis, both at the time of diagnosis and during follow-up.

The goals of the study were to evaluate whether levels of these soluble adhesion molecules reflect endothelial activation or damage during active vasculitis and whether they can be used for evaluation of disease activity during follow-up. At the time of diagnosis in 22 patients with Wegener's granulomatosis, soluble ICAM-1 and soluble VCAM-1 levels were significantly elevated compared with controls and correlated with disease activity. In 12 patients studied serially a significant increase in all 3 soluble adhesion molecules was found at the time of relapse compared with levels at 6 months prior to relapse, but only soluble VCAM-1 levels were significantly elevated compared with those in controls. Again, correlations were found between disease activity and levels of soluble ICAM-1 and E-selectin. Levels of soluble adhesion molecules at the time of relapse did not differ from those measured in 18 patients during an upper airway infection without disease activity of Wegener's granulomatosis. From these data it can be concluded that ICAM-1, VCAM-1 and probably E-selectin are involved in active Wegener's granulomatosis. However, their expression, as assessed by measurement of soluble levels of these adhesion molecules, occurs only at the time of active disease. The clinical relevance for assessing disease activity during follow-up by measuring levels of these soluble adhesion molecules is further limited by the lack of specificity for disease activity of Wegener's granulomatosis.

INFECTIOUS AGENTS AND DISEASE ACTIVATION IN WEGENER'S GRANULOMATOSIS

In many patients infections, especially of the upper respiratory tract, precede the development of disease activity in Wegener's granulomatosis (12,43,44). Infections are thought to trigger one or more mechanisms involved in active Wegener's granulomatosis. It is at present unclear which mechanisms are involved, or whether disease activity is provoked by certain specific microorganisms. A role for infectious agents in the induction of disease activity in this disease is also suggested by reported beneficial effects of trimethoprim-sulfamethoxazole in the treatment of Wegener's granulomatosis (45-47).
In a considerable number of patients recurrent infections of the paranasal sinuses and other paranasal structures occur during follow-up, predominantly caused by *Staphylococcus aureus* (3,5). In order to evaluate the possible relation between upper airway infections and the role of *S. aureus* in the development of disease activity of Wegener's granulomatosis, we studied a cohort of 57 patients with Wegener's granulomatosis with respect to the occurrence of infections and nasal

carriage of *S. aureus* (***chapter 8***). The majority of patients (63%) was found to be chronic nasal carrier of *S. aureus*. During a follow-up ranging from 12 to 42 months 23 patients (40%) developed a relapse of Wegener's granulomatosis. Only 9 of the 23 patients (39%) had a proven or clinically suspected episode of upper airway infection during the 6 months preceding the relapse. However, 21 of 36 patients (58%) who had chronic nasal carriage of *S. aureus* relapsed as compared with only 2 of 21 patients (10%) with nasal cultures negative for *S. aureus* ($P<0.001$). Not surprisingly, chronic nasal carriage of *S. aureus* was associated with persistent or intermittent cANCA positivity during follow-up. Other factors found to influence the risk for relapse of Wegener's granulomatosis were renal function and a history of previous relapse of Wegener's granulomatosis. Proportional-hazards regression analysis including these factors revealed an adjusted relative risk of 7.16 (95% confidence interval, 1.63 to 31.50) for chronic nasal carriage of *S. aureus*. These data suggest that, even without apparent infection, the presence of *S. aureus* in the upper airways is associated with disease activity in Wegener's granulomatosis. As discussed in chapter 8 this microorganism possesses some features with respect to activation of B and T lymphocytes and neutrophils that may explain a more causal role in initiating disease activity in Wegener's granulomatosis, a role that is still entirely speculative.

Uncontrolled data have suggested that monotherapy with co-trimoxazole (trimethoprim-sulfamethoxazole) is effective in the treatment of active locoregional Wegener's granulomatosis confined to the airways and maintains remission (45-47). However, no prospective studies on the efficacy of co-trimoxazole treatment in Wegener's granulomatosis are available. Given the toxicity of conventional treatment with cyclophosphamide and corticosteroids, a non-toxic treatment able to reduce the number of relapses of Wegener's granulomatosis would be welcomed (5,48). In order to assess the efficacy of prophylactic treatment with co-trimoxazole, we conducted a randomized placebo controlled multicenter study in 81 patients with Wegener's granulomatosis (***chapter 9***). Co-trimoxazole treatment during 24 months significantly reduced the number of relapses of Wegener's granulomatosis as compared with patients receiving placebo. This reduction was especially evident in the number of relapses of Wegener's granulomatosis involving the upper airway region. The relative risk for relapse of Wegener's granulomatosis, adjusted for cANCA positivity at the start of the study, was 0.32 (95% confidence interval, 0.13 to 0.79) for patients with co-trimoxazole treatment as compared with placebo. Treatment with co-trimoxazole also significantly reduced the number of

respiratory and non-respiratory infections. However, comparable with the results from a previous study (chapter 8), only about 50% of the patients experienced a respiratory infection in the 6 months preceding the relapse, and no correlation between the number of infections and the occurrence of relapse was found. Treatment with co-trimoxazole did not result in a discernible decrease in cANCA titer, while reducing the number of relapses. How co-trimoxazole exerts its beneficial effect on the course of Wegener's granulomatosis is at present unclear. It has been suggested that co-trimoxazole exhibits anti-inflammatory effects through interference with the formation of specific oxygen derived radicals by activated neutrophils similar to diafenylsulfon and sulfapyridine (49). On the other hand, given the association found between nasal carriage of *S. aureus* and the increased risk for relapse of Wegener's granulomatosis, it is tempting to speculate on the possibility that co-trimoxazole is effective through eliminating or suppressing the presence of *S. aureus* in the upper airways. However, no data on the nasal *S. aureus* status are available in these patients. A hypothesis explaining the relation between *S. aureus* and disease activation of Wegener's granulomatosis is that the presence of or a low grade infection with this microorganism in the upper airways induces so called "priming" of neutrophils, which identifies astate of pre-activation not yet resulting in degranulation. These partially activated neutrophils have been shown to express proteinase 3 on their membrane (21,50). Anti-proteinase 3 antibodies (cANCA) have been shown *in vivo* to be able to further activate these primed neutrophils and to induce degranulation (21-24). Release of proteinase 3 by degranulation could lead to stimulation of specific B- and possibly T-lymphocytes (Chapter 1, figure 1, page 7). By merely stimulating neutrophils, even without apparent infection, *S. aureus* could trigger the development of a vicious circle in the presence of cANCA. The hypothesis could explain why co-trimoxazole is effective as single therapy in locoregional Wegener's granulomatosis and why there is a noticeable absence as compared with the placebo group of disease activity especially in the upper airways in patients treated with co-trimoxazole.

CONCLUSIONS

In the present thesis mechanisms possibly involved in the disease activation of Wegener's granulomatosis have been explored. Although we are still a long way from understanding the pathophysiology of Wegener's granulomatosis, some pieces of the puzzle become visible.
First of all, not only the amount of anti-proteinase 3 antibodies (cANCA), but

also qualitative or functional aspects of these antibodies as assessed *in vitro* appear to be related to disease activity in Wegener's granulomatosis. Disease activity is particularly related to the presence of anti-proteinase 3 antibodies of the IgG3 subclass (chapter 3). Functionally, anti-proteinase 3 antibodies of the IgG3 subclass seem to determine the capacity to stimulate neutrophils *in vitro*, which was also found to be related to disease activity (chapter 5). Another aspect of anti-proteinase 3 antibodies related to disease activity is the capacity of these antibodies to interfere with the irreversible complexation and inhibition of the enzymatic activity of proteinase 3 with α_1-antitrypsin (chapter 4).
Secondly, the data on soluble markers of T cell activation (chapter 6) do not support an initiating role of T cells in the development of disease activity of Wegener's granulomatosis, although the antigen-specific ANCA response by B lymphocytes is probably T cell dependent. In addition, no discernible rise in soluble adhesion molecules was found preceding a relapse (chapter 7). These 2 mechanisms are, however, clearly involved in the active phase of the disease as shown by the correlation of both the levels of soluble IL2-receptor and of soluble ICAM-1, VCAM-1, and E-selectin with clinical disease activity.
Last but not least, a role for microbial agents in the development of disease activity in Wegener's granulomatosis is supported by the data presented in chapter 8 and 9. Although, in contrast to previous findings, no relation between clinically apparent infections and relapses were found, a strong association between chronic nasal carriage of *S. aureus* and an increased risk for relapses of Wegener's granulomatosis was established (chapter 8). At present one can only speculate on the possible causal role of *S. aureus* in the development of disease activity in Wegener's granulomatosis. Prolonged therapy with co-trimoxazole is able to reduce the number of relapses of Wegener's granulomatosis. Since there are no firm data supporting an immunosuppressive or anti-inflammatory effect of co-trimoxazole in humans, the efficacy of this antimicrobial agent further suggests a role for microbial agents in the development of active Wegener's granulomatosis.

FUTURE PERSPECTIVES

Pathophysiology of ANCA-associated vasculitis
An aspect not addressed in this thesis is the induction of antibodies directed against neutrophil antigens such as proteinase 3 and myeloperoxidase. Why do some patients develop these autoantibodies? Although in the past weak associations of Wegener's granulomatosis and ANCA-associated vasculitis with

HLA class II DR2 and DQ7 antigens have been described (51,52), a recent Dutch collaborative study involving over 200 patients with Wegener's granulomatosis and other ANCA-associated vasculitides failed to show an association with specific HLA class I and II antigens (manuscript submitted). Studies on possible exogenous antigens that show epitopes with cross reactivity to proteinase 3 or myeloperoxidase are not available. Given the association found between cANCA, active Wegener's granulomatosis, and *S. aureus*, antigens of this microorganism are obvious candidates to look for (53). The fact that co-trimoxazole treatment reduces the number of relapses but does not seem to lower cANCA titers argues somewhat against this possibility. Other possibilities are the polyclonal stimulation of B or T cells by exogenous bacterial superantigens (54) or disturbances in B and T cell regulation caused by viral infections such as Parvo B19 virus (55).
At present evidence for a direct pathogenetic role of ANCA is only circumstantial. As long as we do not have an experimental model for ANCA mediated vasculitis in which single items can be manipulated to address their significance, progress will be slow. The absence of such a model will not only limit the possibilities to study specific functional aspects of ANCA in the pathogenesis of vasculitis, but also of other mechanisms such as T cell activation, interactions between endothelium and neutrophils, and microbial agents.

Monitoring of disease activity in ANCA-associated vasculitis
The discovery of ANCA has provided the clinician with a specific and sensitive aid in the diagnostic process of certain forms of primary vasculitis. However, during follow-up of patients with ANCA-associated vasculitis, even in Wegener's granulomatosis, the relation between changes in ANCA levels and the development of disease activity is at present not firm enough to enable pre-emptive immunosuppressive treatment on changes in ANCA titer routinely (12,13). Increasing the predictive value for disease activity by monitoring certain qualitative aspects of these antibodies as suggested in this thesis, can possibly overcome this problem. The techniques as used for assessing certain functional aspects of ANCA in this thesis are not suited for routine use. Measurement of IgG subclasses of ANCA could be a possibility. In addition, a recent study demonstrated a restricted number of epitopes on proteinase 3 to which anti-proteinase 3 antibodies were directed (56). ELISA systems using small peptide fragments of proteinase 3 confined to one or a few epitopes may replace the more cumbersome assays analyzing a certain functional aspect of ANCA. Prospective studies addressing these issue are clearly warranted.

As long as the pathophysiology of ANCA-associated vasculitis is unclear, specific treatment will remain an illusion. On more empirical or theoretical grounds some new therapeutic strategies are or will be evaluated in controlled multicenter trials in a collaborative effort (57):

1. Removal of ANCA: given the suspected role of ANCA in the pathophysiology of vasculitis removal by plasmapheresis or extracorporal immuneadsorption may be an option. Results in patients with pauci-immune necrotizing glomerulonephritis do suggest some improvement with the addition of plasmapheresis to immunosuppressive therapy with cyclophosphamide and prednisolone (58).
2. Inhibition and/or down-regulation of ANCA: an uncontrolled and small study with intravenous gammaglobulin (0.4 g/kg/day for 5 days) has shown beneficial results in patients with ANCA-associated vasculitis (59). Preparations of pooled gammaglobulin have been shown to contain anti-idiotypic antibodies directed to ANCA (60).
3. Suppression of T lymphocytes: therapy with cyclosporin A, anti-thymocyte globulin, or with monoclonal antibodies directed to CD4 have been used in a small number of patients (57). As yet, no data are available to assess the efficacy of these forms of treatment.
4. Antimicrobial treatment: prolonged treatment with co-trimoxazole has been effective in reducing the number of relapses of Wegener's granulomatosis in patients treated with cyclophosphamide and prednisolone. The efficacy of co-trimoxazole monotherapy in active locoregional Wegener's granulomatosis of the airways is, however, not established. Furthermore, the question remains whether the efficacy of co-trimoxazole in reducing the number of relapses is due to some as yet unknown immunosuppressive or anti-inflammatory effect or due to its antimicrobial activity. In the latter case, other antimicrobial agents, probably as long as they are effective against *S. aureus*, may be effective as well.

REFERENCES

1. Wegener F. Über generalisierte, septische Gefäßerkrankungen. *Verh Dtsch Pathol Gesellsch.* 1936;26:202-10.
2. Wegener F. Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des