Meeting report

What is new in systemic vasculitis?

A report from the 9th International Vasculitis/ANCA Workshop

From 12 to 15 April 2000 the 9th International Vasculitis/ANCA Workshop was held at the University Hospital Groningen, The Netherlands.¹ This series of workshops started as an informal ANCA directed workshop in 1989 in Copenhagen and has grown into an international meeting dealing with the whole spectrum of primary systemic vasculitides, including ANCA associated vasculitis. The present workshop was attended by more than 300 delegates from 19 countries.

Endothelium-leucocyte interactions

The first part of the workshop dealt with endothelium-leucocyte interaction and the role of autoantibodies in this process. Mayet (Mainz, Germany) reviewed the studies of his group showing that endothelial cells, but also other cells like tubular epithelial cells and lung epithelium, express proteinase 3 (PR3), the main target antigen of antineutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis. Expression was shown not only at the protein level but also at the mRNA level. Stimulation with proinflammatory cytokines resulted in a strong expression of PR3 at the surface of endothelial cells, making PR3 accessible for circulating PR3-ANCA. Indeed, PR3-ANCA were able to upregulate the expression of adhesion molecules and the production of interleukin 8 (IL8) by endothelial cells that were prestimulated with proinflammatory cytokines such as tumour necrosis factor α . Daha *et al* (Leiden, The Netherlands) were not able to confirm PR3 expression by endothelial cells. In contrast, they demonstrated a PR3-binding molecule of 111 kDa on the membrane of endothelial cells. Adding PR3, both in its enzymatically active and inactive form, to endothelial cells resulted in enhanced production of IL8, a strong chemoattractant for neutrophils, and of MCP-1, which is chemotactic for monocytes and T cells. In addition, incubation of endothelial cells with PR3 resulted in upregulation of endothelial leucocyte adhesion molecules. Also, dose dependent apoptosis and cytolysis of endothelial cells was seen in vitro after addition of PR3.

Pendergraft and Yang (Chapel Hill, NC, USA) showed, in concordance with the data of Daha, that the proteolytically inactive C terminal part of PR3 can induce apoptosis of endothelial cells and that internalisation of PR3 (and myeloperoxidase (MPO)) results in endothelial damage. Thus PR3 released from neutrophils, may directly activate or damage endothelial cells by interacting with specific (?) receptors. Whether PR3 is expressed by endothelial cells, is still a matter of debate.

How do autoantibodies affect neutrophil-endothelium interaction in systemic vasculitis? Meroni (Milan, Italy) discussed the role of anti-endothelial antibodies. The antigenic targets of those antibodies are, as yet, poorly characterised. Nevertheless, in vitro studies have shown their potential to activate endothelial cells to the production of cytokines and the expression of adhesion molecules and their ability to induce apoptosis of endothelial cells. Savage (Birmingham, UK) presented new evidence to explain why neutrophils are retained within the microvasculature and damage the endothelium in ANCA associated small vessel

vasculitis. She showed that ANCA can induce stationary adhesion of neutrophils and stimulate those neutrophils to secrete IL8, which inhibits transendothelial migration of those cells. The interaction of ANCA with apoptotic neutrophils was another subject that drew much attention. As shown by Savage, ANCA may induce accelerated apoptosis of neutrophils and decreased clearance of those neutrophils, resulting in secondary necrosis of the cells, a highly phlogistic event. Otherwise, ANCA may bind to their target antigens expressed on the surface of apoptotic neutrophils. Those opsonised neutrophils, next, will be cleared by macrophages through Fc receptors, which will activate the macrophages as shown by Csernok (Bad Bramstedt, Germany) and Harper (Birmingham, UK). This process will amplify the inflammatory reaction.

Pathogenesis of systemic vasculitis

In the second session the pathogenesis of systemic vasculitis was discussed. Cohen Tervaert (Maastricht, The Netherlands) pointed to the role of *Staphylococcus aureus* in Wegener's granulomatosis. Chronic nasal carriage of *Staphylococcus aureus* has been established as an important risk factor in this disease. Several hypotheses have been tested to explain this association. Firstly, superantigens derived from *Staphylococcus aureus* may activate T cell populations with particular V β molecules including autoreactive clones. Indeed, within the group of patients with Wegener's granulomatosis those carrying the superantigen producing *Staphylococcus aureus* had a far higher relapse rate and showed some skewing of their T cell V β distribution. Secondly, preliminary data were presented suggesting that a cationic protein from *Staphylococcus aureus*—that is, staphylococcal acid phosphatase, can act as a planted antigen on (glomerular) endothelium, inducing glomerulonephritis. In the presence of ANCA, focal glomerulonephritis may develop into necrotising glomerulonephritis owing to the neutrophil activating ability of ANCA. This concept is presently being tested in animal models.

The significance of animal models for our understanding of ANCA associated vasculitis was discussed by Heeringa (Chapel Hill, USA). Although various models, particularly for anti-MPO associated vasculitis/glomerulonephritis, are available, none of these models is fully satisfactory. Generally, those models show that the autoimmune response to MPO is in itself not sufficient to induce disease but certainly can amplify inflammatory events in vivo. Better models, particularly for anti-PR3 associated vasculitis, are clearly needed.

Interestingly, Gilbred *et al* (Tel Hashomer, Israel) showed that injection of apoptotic neutrophils in mice induced anti-lactoferrin antibodies in those mice.

Besides the autoantibodies, autoreactive T cells may have a role in the pathogenesis of ANCA associated vasculitis. Trabandt *et al* (Bad Bramstedt, Germany) presented data suggesting that Th1-like immune responses are predominant in granulomatous nasal tissue in Wegener's granulomatosis. Mayet *et al* (Mainz, Germany), however, were able to generate *Staphylococcus aureus*-specific T cell

clones from nasal biopsy specimens of patients with Wegener's granulomatosis that showed a Th2 cytokine profile and provided B cell help to ANCA production of autologous B cells.

Anti-PR3 and anti-MPO antibodies

Currently, many target antigens for ANCA have been identified in a wide spectrum of (inflammatory) diseases. In the third session Wiik (Copenhagen, Denmark) reconfirmed that only anti-PR3 and anti-MPO antibodies are relevant in the diagnostic work up of patients suspected of vasculitis. He emphasised the importance of combining the indirect immunofluorescence technique with antigen-specific tests for PR3 and MPO to achieve a methodology that shares a high diagnostic specificity with an acceptable sensitivity for systemic vasculitis. Standardising antigen-specific tests is of utmost importance and the availability of recombinant antigens in those tests would be particularly helpful. Specks (Rochester, USA) mentioned that anti-PR3 and anti-MPO antibodies in patients, generally recognise conformational epitopes that are, in many cases, not fully expressed by recombinant proteins. For PR3, some recombinant proteins expressed in human cell lines or in eukaryotic systems seem promising as substrates for antibody testing in the near future. A more precise definition of antigenic targets of anti-PR3/anti-MPO epitope mapping is now being studied by different groups.

Cause and genetic base of the systemic vasculitides

The fourth session dealt with the cause and genetic base of the systemic vasculitides. Hoffman (Cleveland, USA) discussed the role of infectious agents in inducing vasculitis. Many microbial agents may precipitate vasculitis in susceptible hosts as shown both by clinical observations and experimental models. In the latter models herpes viruses can induce different phenotypes of large vessel vasculitis in mice with genetically determined deficiencies, such as interferon γ and interferon γ receptor deficiency. Besides microbial factors, environmental factors such as silica exposure may play a part in the expression of vasculitis. Elseviers *et al* (Antwerp, Belgium), indeed, showed that patients with ANCA positive glomerulonephritis had an increased risk for occupational exposure to a particular form of silica. No correlation was found by Van Gorp *et al* (Amersfoort, The Netherlands) between hydrocarbon exposure and the development of ANCA associated vasculitis. Several drugs have been associated with ANCA positive systemic vasculitis, including propylthiouracil and hydralazine. The mechanisms involved have not been elucidated, but Zhou *et al* (Sherbrooke, Canada) showed that the autoantibody response in propylthiouracil associated vasculitis is driven by many antigens, includes elastase, and

is clearly different from the monospecific responses to either PR3 or MPO that are seen in the idiopathic vasculitides.

Prognosis and treatment

The last session dealt with prognosis and treatment. Falk *et al* (Chapel Hill, USA) showed that serum creatinine at the start of treatment is the most important predictive indicator of progressive renal insufficiency, whereas the most important long term prognostic factor of death is the presence of pulmonary haemorrhage. Relapses occur more frequently in patients positive for PR3-ANCA than in those positive for MPO-ANCA. Both in the USA and Europe collaborative networks have been established for the study of systemic vasculitis. As a result of these efforts standardised treatment regimens for the induction and maintenance of remission of the systemic vasculitides are now widely used. In addition, multicentre studies on new treatments are continuing, both in the USA and in Europe. Jayne (London, UK) presented the results of one of those studies, in which the possible benefit of using azathioprine instead of cyclophosphamide for maintaining remission in ANCA associated vasculitis was evaluated. Azathioprine proved as effective and save for maintaining remission as cyclophosphamide. During the remission phase an equal number of relapses in each arm of the study was seen, but there was a trend towards more severe adverse events in the cyclophosphamide group (n=13) than in the azathioprine group (n=7). Thus azathioprine can be advocated as first choice of treatment for maintenance of remission. Other trials are underway.

At the end of the workshop Sir Keith Peters (Cambridge, UK) presented the Martin Lockwood Memorial Lecture dedicated to the legacy of Martin Lockwood who died last year. Peters reviewed the tremendous contribution of Martin Lockwood to the study of the ANCA associated vasculitides.

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