

Eleventh International Vasculitis and ANCA Workshop, October 2–5, 2003, Prague, Czech Republic

Antineutrophil cytoplasmic antibodies (ANCA) detected by indirect immunofluorescence technique (IIF) were first described in 1982 by Davies, *et al*¹. Six years later, at the 1st International Workshop on ANCA, held in Copenhagen, Falk, *et al* reported on myeloperoxidase (MPO) being the major antigen specificity for the perinuclear immunofluorescence staining pattern (p-ANCA) found in patients with microscopic polyangiitis (MPA) and its renal variant, pauci-immune necrotizing glomerulonephritis². A year later, at the 2nd International Workshop on ANCA, in Leiden, Ludemann, *et al* reported that proteinase 3 (PR3) was the target antigen for diffuse granular cytoplasmic immunofluorescence staining (c-ANCA) seen in patients with Wegener's granulomatosis (WG)³. These landmark discoveries have had a major impact on the diagnosis and classification of small vessel vasculitis. The role of ANCA in the pathogenesis and especially in the monitoring of disease activity remains more controversial, and was one of the main areas of discussion at the 11th International Vasculitis and ANCA Workshop, held in Prague in October 2003. New data on the epidemiology, clinical manifestations, and management of vasculitides were also presented. The abstracts presented at the Workshop can be found in *Kidney & Blood Pressure Research* 2003;26:249-302.

Epidemiology

R. Watts, Norwich, UK, reported that the incidence of WG appears to be increasing, but it is unclear if this simply represents better case recognition. Geographical differences in the incidence of WG and MPA in Europe have recently been described, with WG being more common in Northern Norway (10.5/million) than in Spain (4.9/million), while MPA is more common in Spain (11.6/million) than in Norway (2.7/million). The reasons for these differences are unclear. Churg-Strauss syndrome remains the rarest of the ANCA related vasculitides (0.5–3.1/million).

L.F. Flores-Suarez, Mexico City, Mexico, presented data confirming the previously reported association between occupational and toxic-related exposure, including organic and inorganic silica, insecticides, oil, and fertilizers, and the development of ANCA positive vasculitis. A group from the Czech Republic reported an association between exposure to

asbestos and ANCA positivity (20.3% vs 6.9%) but not with vasculitis.

Pathogenesis

In a landmark article published last year in *The Journal of Clinical Immunology*, H. Xiao and her colleagues from Chapel Hill, USA, reported definitive experimental animal evidence that MPO-ANCA are pathogenic in the development of vasculitis and not just an epiphenomenon⁴. Using MPO knockout mice immunized with murine MPO, they transferred the splenocytes from these mice or from control mice to recombina-activating gene-2-deficient (RAG2^{-/-}) mice that lack functioning B and T lymphocytes. All mice that received anti-MPO splenocytes, but none who received the control mouse splenocytes, developed necrotizing and crescentic glomerulonephritis. Some of the mice also developed systemic vasculitis, including hemorrhagic pulmonary capillaritis and granulomatous inflammation. The transfer of purified anti-MPO antibodies alone resulted in similar, although milder, findings.

The Chapel Hill group presented further evidence at the Workshop of the pathogenic role of anti-MPO antibodies. Transferring a preparation of purified T lymphocytes from the MPO knockout mice into RAG2^{-/-} mice did not induce renal lesions, suggesting that the pathogenic process is dependent on B cells rather than T cells. H. Xiao presented very convincing evidence that neutrophils are essential for the induction of necrotizing glomerulonephritis. C57BL/6j mice that were depleted of neutrophils and control mice were injected with anti-mouse MPO IgG. Six days later, the neutrophil depleted mice had normal urine and no histologic lesions. In contrast, all mice from the control group had developed hematuria, proteinuria, and histologic evidence of focal necrotizing glomerulonephritis. Administering tumor necrosis factor- α (TNF) or lipopolysaccharide (LPS) to the C57BL/6j control mice resulted in a dose-dependent augmentation of the pathogenicity of the antibodies, suggesting that priming of circulating neutrophils by TNF or LPS makes them more receptive to activation by anti-MPO IgG.

R. Falk, Chapel Hill, presented intriguing data supportive of a new mechanism for the development of autoimmunity. Patients with PR3-ANCA related vasculitis not only harbor

antibodies against PR3, but they also produce antibodies against a peptide translated from the antisense DNA strand of PR3. These complementary antibodies (cPR3) can then induce anti-idiotypic antibodies that will cross-react with PR3 causing significant inhibition of the proteolytic activity of PR3. What triggers the production of cPR3 is unclear, but it is interesting that cPR3 is homologous to a number of microbial proteins.

C. Kallenberg, Groningen, The Netherlands, reviewed the potential role of T cells in the pathogenesis of ANCA-associated vasculitis. Although the cellular infiltrates present in the kidneys, lungs, and nasal tissue of patients with WG consist mainly of macrophages and T and B cells, the antigenic specificity of the infiltrating T cells so far has not been identified. A shift in T cell response appears to occur as patients with localized disease exhibit predominantly a Th1 response, in contrast to patients with generalized disease, who have a Th0/Th2 pattern. What triggers this shift in Th response is presently unclear.

ANCA Testing, Its Use in Diagnostics and Monitoring of Disease Activity

IIF technique and direct ELISA assays remain the most commonly used tests to detect ANCA. The detection of c-ANCA with clear PR3 activity or of p-ANCA with clear MPO reactivity is 99% specific for necrotizing small vessel vasculitis. In contrast, p-ANCA with negative or low positive MPO reactivity are not associated with vasculitis. A. Wiik, Copenhagen, Denmark, suggested that the latter be renamed neutrophil-specific autoantibodies rather than ANCA in order to avoid misinterpretation of results.

Other techniques, including radioimmunoassays, capture ELISA assays that use a monoclonal antibody for anchoring PR3 to the solid support, and new automated fluorescence immunoassays (ELIA MPO and PR3) may be more sensitive than direct ELISA. However, studies in large cohorts of patients will be required before the use of either of these techniques is recommended.

Are ANCA titers helpful in predicting vasculitis relapses? According to C. Stegeman, Maastricht, The Netherlands, they are. Studies (reviewed in⁵) on the relation between increases in ANCA levels and disease relapse found widely different results (positive predictive value varying between 24% and 100%). However, most of these studies were retrospective and included small numbers of patients and relapses. The interval between sequential ANCA measurements and the interval between increases in ANCA levels were not standardized, nor were relapses uniformly diagnosed. If one restricts the analysis to the 3 studies that did not have these limitations, predictive values $\geq 50\%$ for a relapse within 6 or 12 months were found for a 4-fold rise in ANCA titer by IIF or $> 75\%$ increase of PR3-ANCA level measured by direct ELISA. The situation for MPO-ANCA is less well studied.

J.S.F. Saunders, Groningen, The Netherlands, reported that

a positive c-ANCA titer at 3 and 6 months after diagnosis of a PR3-ANCA associated vasculitis is associated with an increased relapse rate [at 3 months, relative risk (RR) 2.2, 95% confidence interval (CI) 1.29–4.95; at 6 months RR 2.6, 95% CI 1.41–7.17]. The role of preemptive treatment was studied in a multicenter trial in which 40 patients with WG in remission, but who had an increase in their PR3-ANCA of 75% compared to their previous level, were randomized to start azathioprine (75 mg daily, tapered in 9 mo) and prednisolone (30 mg daily tapered in 4.5 mo) or to receive no treatment. During followup, 5 of the 20 (25%) patients receiving preemptive treatment relapsed, compared to 12 of 20 (60%) patients receiving no treatment, after a median period of 11.7 months (range 0.2 to 29) after randomization. The favorable results observed with preemptive treatment did not last, as late relapses occurred after cessation of preemptive treatment. The decision to maintain a patient on longterm maintenance therapy should be individualized and balanced with the risk of toxicity from treatment.

Clinical Manifestations and Therapy

P. Merkel, Boston, USA, speaking for the Wegener's Granulomatosis Etanercept Trial (WGET) Research Group, reported that 16 of 228 patients entered in WGET developed a well documented venous thrombotic event (VTE) while in the trial. None of these patients had a previous history of VTE, and there was no difference in the rate of VTE between the 2 treatment groups. This incidence of 7.0 per 100 person-years in patients with WG (CI 0.63–77.9) is much higher than the incidence of VTE in the general population (0.3 per 100 person-years; RR 23) and also higher than the incidence in patients with SLE (0.98 per 100 person-years; CI 0.66–1.78, RR 7.1).

Interestingly, D. Albert, Philadelphia, USA, reported a cluster of 6 patients living in a small community in Pennsylvania who developed WG within an 18 month period. Five of these 6 patients had a VTE. These observations suggest that the vasculitic process in WG could affect the venous system, or alternatively that the disease could be associated with a hypercoagulable state. Further work will be needed to elucidate the underlying pathogenesis of these VTE; in the meantime, clinicians need to be aware of this association in their management of patients with WG.

C. Pagnoux, Bobigny, France, representing the French Vasculitis Study Group, presented results of a randomized controlled trial in 41 patients with severe Churg-Strauss syndrome (≥ 1 poor prognostic factor according to the 5-factor score) comparing 6 or 12 cyclophosphamide pulses (0.6 g/m² on days 0, 15, and 30 and then monthly) combined with corticosteroids (CS). For comparable tolerance, the 12 pulses were more effective in preventing both major and minor disease relapses (7/17 patients in the 12-pulse group relapsed vs 15/16 patients in the 6-pulse group; $p = 0.005$).

In 3 separate open-label studies, mycophenolate mofetil

(2000 mg/day; 20 patients), infliximab (5 mg/kg; 30 patients), and rituximab (375 mg/m² weekly × 4; 11 patients) combined with CS were effective in inducing remission in patients with active, and in some cases, refractory, ANCA-associated vasculitis. Further investigation of these agents in formal clinical trials appears justified. G. Hoffman also reported favorable results with the use of anti-TNF agents (etanercept in 7 and infliximab in 11) in 15 patients with active, relapsing Takayasu's arteritis.

The workshop ended this year with a full-day postgraduate review course on vasculitis organized in collaboration with the European League Against Rheumatism and the International Society of Nephrology. The 12th International Vasculitis and ANCA Workshop will be held in Heidelberg, Germany, June 1-4, 2005.

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