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ANCA-associated renal vasculitis

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CASE PRESENTATION

A 62-year-old white woman presented to another hospital with a five-week history of profound malaise and small joint arthralgia. Initially she had noticed a mild sore throat that settled spontaneously. She had transient redness of her left conjunctiva. One week after the onset of symptoms, she had a single episode of macroscopic hematuria for which she received antibiotics from her family doctor. She denied dysuria or urinary frequency. For one week prior to admission, she had suffered nausea and vomiting without abdominal pain or bowel disturbance. She had lost one stone (14 pounds) in weight. She had been hypertensive five years previously, but she had had no other antecedent illnesses. There was no family history of renal disease, hypertension, or rheumatic complaints. She had four adult children, all of whom were well. Medication on admission comprised a beta blocker (atenolol, 100 mg daily) for hypertension. She did not smoke cigarettes and drank less than three units (24 g) of alcohol per week. She was a poultry farmer and lived in a rural setting.

Clinical examination revealed that she was pale and dehydrated. She had no rash, no active synovitis, and no lymphadenopathy. Her temperature was 37.2°C; pulse, 70 beats/min; and blood pressure, 160/90 mm Hg lying and 145/80 mm Hg standing. Her jugular venous pressure was not elevated and she had no sacral or ankle edema. The heart sounds were normal with no added sounds. The peripheral pulses were present, equal, and symmetrical. No abnormalities were found in the respiratory, abdominal, or nervous systems. She had no retinopathy and ophthalmoscopy revealed normal fundi. Urinalysis disclosed 3+ blood and 1+ protein.

Initial laboratory investigations demonstrated a serum creatinine of 513 μmol/L (5.8 mg/dL); hemoglobin, 9.2 g/dL; and normal white blood cell and platelet levels. Renal ultrasound revealed two normal-sized kidneys. She was rehydrated with normal saline, given a one-unit blood transfusion, and transferred to the Queen Elizabeth Hospital in Birmingham after 48 hours. At that point she was passing 1.0 to 1.5 liters urine/24 h and her blood pressure was 170/100 mm Hg. Further laboratory investigations demonstrated a serum creatinine of 317 μmol/L (3.6 mg/dL); BUN, 23 mmol/L; and serum albumin, 2.9 g/dL. The hemoglobin level was 8.8 g/dL with normal indices. The erythrocyte sedimentation rate was 70 mm/hr. C-reactive protein was 30 mg/L (normal <10 mg/L), and she had a positive antineutrophil cytoplasmic antibody (ANCA) test to a titer of >1:400 serum dilution by indirect immunofluorescence with a perinuclear pattern. Subsequent antigen-specific ELISA confirmed reactivity to myeloperoxidase (MPO), denoting MPO-ANCA (Fig. 1). The ANA, anti-DNA, and anti-GBM antibody tests were negative. Complement C3 and C4 levels were normal, and cryoglobulins were not detectable. Hepatitis B and C serologies were negative. After control of blood pressure, a renal biopsy was performed; the tissue contained eight glomeruli. Of these, one was globally sclerosed, three were normal, and four contained acute segmental lesions of various sizes with thrombosis, tuft disruption, and a few cells in Bowman's space (Fig. 2). Tubules were acutely damaged, with blood in a few. A patchy infiltrate of chronic inflammatory cells was present. Small arteries and arterioles appeared virtually normal. Immunoperoxidase study revealed no significant immunoprotein deposition within glomeruli.

The clinical history, renal biopsy findings, and blood serology were consistent with an acute vasculitic (pauci-immune) glomerulonephritis of the microscopic polyangiitis type. An assessment of vasculitic activity showed a Birmingham Vasculitis Activity Score (BVAS) of 13 and a Vasculitis Damage Index (VDI) score of 0. Therapy with prednisolone, 1 mg/kg/day, and oral cyclophosphamide, 2 mg/kg/day, was begun. Following 3 months of therapy, her serum creatinine was 95 μmol/L (1.1 mg/dL) with a creatinine clearance of 87 mL/min. ANCA were no longer detectable (Fig. 1). The BVAS score was 0, indicating complete remission, and the VDI was 2, indicating a mild to moderate degree of damage. The prednisolone dosage had been gradually reduced according to our local guidelines, and at 3 months she was receiving 15 mg/day. The cyclophospha-

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Key words: microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome, crescentic glomerulonephritis

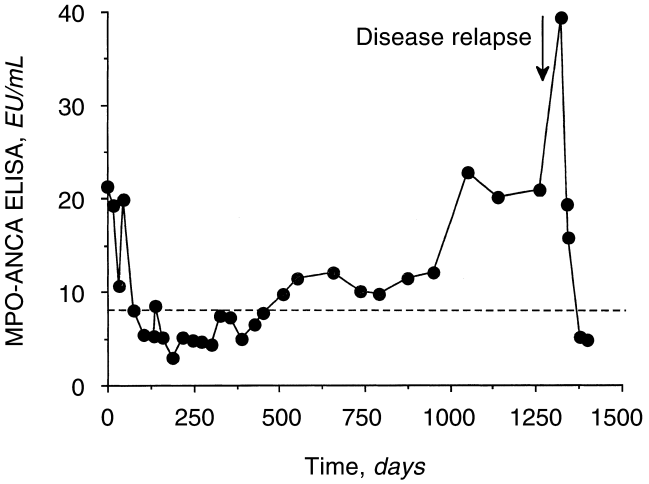

Fig. 1. ELISA for MPO-ANCA showing serial antibody titers (EU/mL) over time.

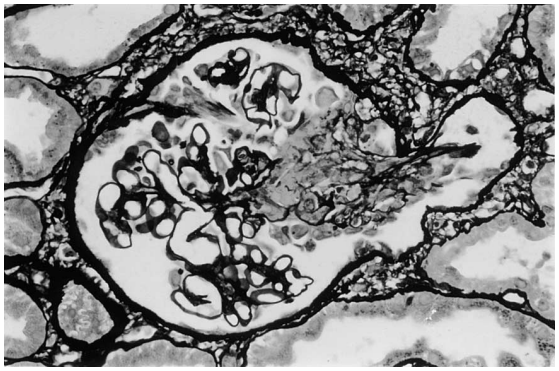

Fig. 2. Glomerulus in the patient's first renal biopsy showing an acute segmental lesion with thrombosis, disruption of capillary loops and adhesion of the tuft to Bowman's capsule. Periodic acid-methenamine silver (PA silver) ×250.

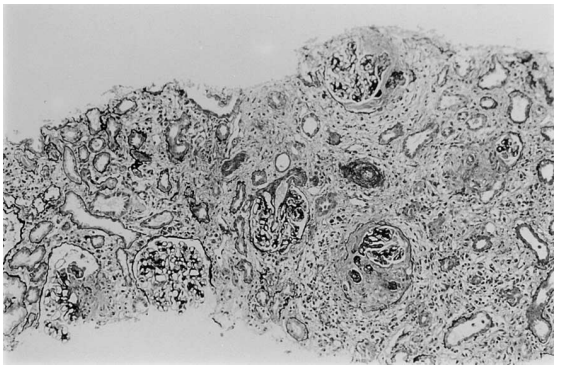

Fig. 3. Cortex in the patient's second renal biopsy showing acute segmental lesions in several glomeruli. PA silver ×50.

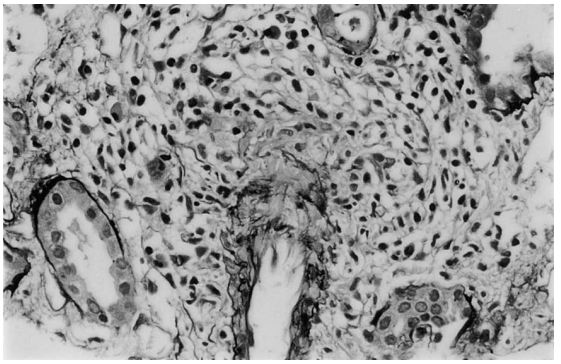

Fig. 4. Small artery in the patient's second renal biopsy showing localized destruction of the wall with acute inflammation. PA silver × 250.

mide dose had been maintained throughout, as there had been no episodes of leukopenia (white blood cell count $<4.0 \times 10^9$/L). As she was in remission, cyclophosphamide was discontinued and azathioprine was commenced at 2 mg/kg/day. She remained well with no clinical evidence of vasculitic activity, and 18 months from diagnosis (15 months from the time of remission) her therapy was prednisolone, 5 mg/day, and azathioprine, 100 mg/day. In addition, she was receiving three agents for control of hypertension, calcium and vitamin D tablets (later changed to Didrone® PMO), and an H_2 receptor antagonist. At 17 months (~500 days), MPO-ANCA again was detectable in the serum (Fig. 1). Therapy was not altered because there was no clinical evidence of vasculitis, urinalysis showed 1+ protein, renal function was stable, and the C-reactive protein level was <10 mg/L. She remained under review in the clinic.

She was well until 44 months following her acute illness, when she presented to the renal vasculitis outpatient clinic with a 4-week history of fatigue, upper respiratory tract symptoms of coryza and a sore throat, and new episcleritis. The BVAS score had risen to 8. Urinalysis revealed 3+ blood and 1+ protein. The serum creatinine had risen to 125 μmol/L (1.4 mg/dL). The MPO-ANCA titers had risen further (Fig. 1; ~1320 days), and the C-reactive protein level was 37 mg/L. A nasal swab did not reveal carriage of *Staphylococcus aureus*, and although 6 of 6 nasal swabs taken during the first 6 months of illness had been positive for this organism, later swabs had been negative. A relapse of microscopic polyangiitis was suspected. She was admitted to the hospital and a second renal biopsy was performed. The specimen contained 15 glomeruli, one globally sclerosed, one with a small old area of capsular adhesion, 5 normal, and 8 with segmental lesions of various sizes showing thrombosis, tuft disruption, and a few cells in Bowman's space (Fig. 3). The tubules appeared acutely damaged, with blood in a few and some patchy atrophy. Interstitial tissues were edematous and contained a patchy inflammatory infiltrate. Small arteries and arterioles had chronic intimal thickening. One small artery contained a patch of fibrinoid necrosis (Fig. 4). Immunoperoxidase staining showed no significant deposition of immunoproteins in the glomeruli. Features were those of reactivation of severe renal vasculitis, both acute vasculitic glomerulonephritis and an acute arteritis. Daily oral cyclophosphamide and prednisolone were begun as previously. Within 10 weeks, the BVAS score was 0, urinalysis was negative for blood and protein, serum creatinine had returned to 96 μmol/L (1.1 mg/dL), C-reactive protein was <10 mg/L, and ANCA were no longer detectable in the serum.

DISCUSSION

Dr. Caroline O. S. Savage (*Professor of Nephrology, MRC Centre for Immune Regulation, and Division of Med-*

ical Sciences, The Medical School, The University of Birmingham, United Kingdom): The patient today is typical of many with ANCA-associated renal vasculitis of the microscopic polyangiitis type. The presenting signs of systemic disease were subtle, but the marked fatigue, arthralgia, and episcleritis attest to the systemic nature of microscopic polyangiitis. In fact, the presentation of this patient is almost identical to that of Patient 1 presented by Dr. Ronald Falk in the last Nephrology Forum to consider vasculitis, 11 years ago [1]. That patient had cANCA with reactivity towards proteinase 3 by ELISA, denoting PR3-ANCA. At the time there was uncertainty as to the spectrum of ANCA-associated vasculitides, and three types were proposed. Patient 1 was given the diagnostic label "polyarteritis nodosa"; the other two suggested types of ANCA-associated vasculitis were Wegener's granulomatosis and "idiopathic" crescentic glomerulonephritis. The question of specificity of cANCA for Wegener's granulomatosis was raised, given that Patient 1 did not fulfill the diagnostic criteria developed by Godman and Churg in the 1950s [2]. In the intervening 11 years since Falk's Forum, diagnostic criteria have been put forward by the Chapel Hill International Consensus Conference [3], large multinational studies have been undertaken to examine the sensitivity and specificity of ANCA for vasculitis, and three ANCA-associated vasculitides have become widely recognized, namely Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome. Nowadays Patient 1 would be considered typical of microscopic polyangiitis. A variety of descriptive labels have been applied to this vasculitis limited to the kidney, including idiopathic, crescentic, and pauci-immune, and the disorder is now recognized for its propensity to progress to systemic microscopic polyangiitis or Wegener's granulomatosis if untreated [4].

Defining ANCA-associated vasculitis

The International Consensus Conference in 1994 made an important attempt to clarify the nomenclature for vasculitis, attaching definitions to the major recognized syndromes to facilitate international understanding and awareness [3]. Definitions were provided for giant cell arteritis, Takayasu arteritis, polyarteritis nodosa, Kawasaki disease, Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, Henoch-Schönlein purpura, cryoglobulinemic vasculitis, and cutaneous leukocytoclastic angiitis. Three diseases, Wegener's granulomatosis, microscopic polyangiitis (acquiring clear distinction from polyarteritis nodosa and with the preferred term "microscopic polyangiitis" over "microscopic polyarteritis"), and Churg-Strauss syndrome, were acknowledged to be "commonly associated with ANCA." The association of Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome with ANCA has been backed up by several large, including multinational, studies which I will discuss later. The definitions for these three entities appear in Table 1.

A vascular pathology is shared among these three disorders. Focal necrotizing lesions are the common vascular pathology that characterizes these three disorders. These lesions can affect many different vessels and lead to a variety of symptoms and signs. For example, involvement of glomerular capillaries causes nephritis, of alveolar capillaries causes lung hemorrhage, of epineural arteries causes mononeuritis multiplex, and of dermal venules causes purpura. In the kidney, early glomerular lesions have focal segmental necrosis of capillary loops with some thrombosis and neutrophil infiltration; with progression, mononuclear cells are recruited, and breaks in the glomerular basement membrane quickly lead to crescent formation [4, 5]. The acute lesions evolve into sclerotic lesions. Immunohistology shows little deposition of immune reactants; this feature distinguishes lesions due to ANCA-associated vasculitis from those of antiglomerular basement membrane disease, IgA nephropathy, and lupus nephritis. Patients with Wegener's granulomatosis and Churg-Strauss syndrome have additional granulomatous necrotizing lesions, with areas of necrosis surrounded by mixed infiltrates of neutrophils, lymphocytes, monocytes, macrophages, and scattered multinucleate giant cells. These necrotic areas are found most often in the respiratory tract and are separate from "granuloma-like" structures that can develop in the kidney from periglomerular infiltrates located around involved glomeruli. Eosinophils are very conspicuous in the lesions of Churg-Strauss syndrome.

The relative rarity of vasculitis, the lack of clear definitions, and the overlap among syndromes have made the collection of epidemiologic data difficult [reviewed in 6]. In the 1970s, the overall annual incidence for all forms of systemic vasculitis occurring in the west of England was 10/million. Data collected in Leicester, United Kingdom in 1980 to 1986 and 1987 to 1989 suggested the combined annual incidence of Wegener's granulomatosis and microscopic polyangiitis to be 1.5/million and 6.1/million, respectively. In the United States over a similar period (1979 to 1988), the prevalence of Wegener's granulomatosis was approximately 30/million. During 1988 to 1998, the overall incidence of ANCA-associated vasculitides was 21.5/million within the Norwich area in east England. During 1988 to 1992 and 1993 to 1998, the incidence was 17.4/million and 23.8/million, respectively; this rate suggests an increased incidence of ANCA-associated vasculitis. Studies from Norway suggest a doubling incidence of Wegener's granulomatosis from 1992-1994 to 1995–1998. These figures probably reflect a real increase in incidence as well as improved awareness and diagnosis. A very high incidence of microscopic polyangiitis (24/million) is seen in Kuwait.

Vasculitides must be recognized early if successful

Table 1. Diseases commonly associated with ANCA

Wegener's granulomatosis	Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small- to medium-sized vessels (e.g., capillaries, venules, arterioles, and arteries). Necrotizing glomerulonephritis is common.
Microscopic polyangiitis	Necrotizing vasculitis with few or no immune deposits affecting small vessels (i.e., capillaries, venules, or arterioles). Necrotizing arteritis involving small and medium-sized arteries can be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs.
Churg-Strauss syndrome	Eosinophil-rich and granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small- to medium-sized vessels; associated with blood eosinophilia and usually asthma or other form of atopy.

Modified with permission from Jennette et al [3].

treatment is to be implemented. ANCA-associated vasculitides can occur at any age, including in children and the elderly; the peak occurrence is in the 55- to 70-year age group [6]. Early diagnosis is important for reducing the ability of acute vasculitis to cause death from major organ failure, for example, respiratory failure from pulmonary hemorrhage, and to reduce major long-term morbidity, for example, end-stage renal failure. As in our patient at the time of her relapse, severe, biopsy-proven disease can be accompanied by apparently mild clinical organ involvement. Our patient's serum creatinine was 1.4 mg/dL.

Causes

Although the causes of ANCA-associated vasculitides are unknown, the presence of ANCA suggests an autoimmune basis. Witebsky and colleagues used modifications of Koch's postulates, which had been developed to establish the pathogenicity of a transmissible agent, to define an autoimmune etiology for a disease [7]. Thus, an autoantibody or cell-mediated immune response is required, the corresponding antigen must be identified, an analogous autoimmune response must be induced in experimental animals, and the immunized animal must develop similar disease. Rose and Bona identified direct, indirect, and circumstantial evidence that supports an autoimmune cause for ANCA-associated vasculitis [8].

Direct evidence includes transfer of patient serum, purified immunoglobulin, or autoantigen-specific T-cells to experimental animals, or transplacental transfer of pathogenic IgG. For ANCA-associated vasculitides, direct evidence of autoimmunity is lacking. Retinal vasculitis has been reported in a single individual after intravenous human immunoglobulin containing ANCA was used for treatment of a nonvasculitic condition [9], but in other studies intravenous immunoglobulin has been used successfully to treat ANCA-associated vasculitis [10]. There has been no documentation of transferred ANCA inducing disease in experimental animals, analogous to the induction of glomerulonephritis in squirrel monkeys after injection of antiglomerular basement membrane antibodies eluted from the kidneys of patients who had died from Goodpasture's disease. About 20 cases of Wegener's granulomatosis have been reported in pregnant women. The pregnancies were successful and little evidence indicates transfer of disease to the fetus [reviewed in 11]. The cause of fetal death in two reported spontaneous abortions was not provided.

Indirect evidence includes presence of autoantibodies or self-reactive T-cells in the target organs or lesions of the disease, or reproduction of autoimmune disease in experimental animal models. ANCA-associated vasculitides have long been associated with a lack of immunoglobulin deposition in lesions [4]. CD4+ and CD8+ T-cells, present in lesions, are predominantly of the CD45RO memory phenotype, but their antigen specificity is unknown [12]. Antigen-specific, PR3- or MPO-reactive T-cells have been detected in the peripheral blood of patients with ANCA-positive vasculitis [13, 14]. A common dominating T-cell receptor BV8-F/L-G-G-A/Q-G-J2S3 β chain sequence was found in CD4+ T-cells from four unrelated patients with vasculitis, all of whom were HLA-DRB1*0401 allele positive; this finding suggested that all four patients had been exposed to, and elicited a cell-mediated immune response against, a common antigen [15].

Attempts have been made to reproduce vasculitis in experimental animals [reviewed in 16]. Brown-Norway rats immunized with human MPO develop antihuman MPO antibodies that cross-react with rat MPO, but vasculitis does not develop. However, rats subsequently subjected to other manipulations such as renal perfusion of neutrophil lysosomal extract (consisting primarily of MPO) followed by either H_2O_2 perfusion or clamp ischemia, develop a crescentic necrotizing glomerulonephritis. Attempts at reproducing this animal model have resulted in significant immune complex deposition, but the original observations, together with an ability of circulating anti-MPO antibodies to aggravate subnephritogenic anti-glomerular basement membrane (GBM) disease in rats, suggest that anti-MPO antibodies could have pathogenic co-factor potential. That MPO-ANCA alone are insufficient to induce vasculitis is also suggested by studies with SCG/Kj mice derived from BXSBx MRL/Mp-*lpr/lpr* F1 hybrid mice. These mice develop a crescentic glomerulonephritis together with anti-MPO antibodies

that can bind to murine neutrophils. However, transfer of MPO-specific hybridomas induces proteinuria but no vasculitis or nephritis. Thus, even if MPO and neutrophils are necessary for development of vasculitis, they are not sufficient to induce it. Other experimental models such as mercuric chloride ($HgCl_2$)-induced vasculitis or spontaneous vasculitis in autoimmune MRL/*lpr* mice do not truly replicate human ANCA-associated vasculitides, either in terms of clinical course or pathology, whereas the ANCA develop as part of a polyclonal antibody response. Attempts at developing PR3-ANCA-associated vasculitis have largely been unsuccessful, mainly because of the poor homology between rodent and human PR3. Altogether, perhaps the most important observation from the animal models is that ANCA alone are not pathogenic. Additional factors inducing priming or activation of neutrophils, monocytes, or vascular endothelial cells are required, after which the inflammatory process can be exacerbated by ANCA-neutrophil-endothelial cell interactions, and perpetuated by T-cells and monocytes.

Circumstantial evidence includes association of autoimmune mediators such as autoantibody with disease activity, association with other autoimmune diseases, lymphocytic infiltration in target organs, major histocompatibility complex class-II associations, and response to immunosuppression. Considerable circumstantial evidence suggests an autoimmune cause in the three ANCA-associated vasculitides.

A strong association exists between vasculitis and the presence of ANCA. Recent large multicenter studies combining immunofluorescence and enzyme immunoassays have demonstrated a specificity approaching 99% for Wegener's granulomatosis, microscopic polyangiitis, or their renal-limited variant [17, 18]. In a single-center study of 123 patients with Wegener's granulomatosis or microscopic polyangiitis, ANCA positivity was 97% according to indirect immunofluorescence and ELISA, the newer capture-ELISA technique [19]. In a meta-analysis of cANCA testing in Wegener's granulomatosis, the pooled sensitivity was 91% (CI, 87% to 95%) and the pooled specificity was 99% (CI, 97% to 99.9%) for the subset of patients with active disease, compared with 63% (CI, 57% to 69%) and 99.5% (CI, 99.1% to 99.7%) for those with inactive disease, where confidence intervals are given [20]. Methodologic discrepancies and flaws might have diluted the association between ANCA and its associated vasculitides in earlier studies.

The correlation of ANCA titers with clinical disease activity supports an autoimmune cause and direct pathogenicity of ANCA [21, 22]. Further, in one study, disease relapse could be predicted and prevented by intensifying immunosuppression in response to rising titers [23]. Persistent or intermittent ANCA positivity is an independent risk factor for relapse [24, 25].

Disease response to immunosuppression also supports an autoimmune process. Untreated, vasculitis carries a very poor prognosis. In early studies, Wegener's granulomatosis carried a 90% two-year mortality rate [26]. Mortality data for untreated microscopic polyangiitis are not available, because early studies contained heterogeneous vasculitides including polyarteritis nodosa. Cytotoxic therapy using cyclophosphamide together with corticosteroids induces remission in more than 90% of patients [27]. In addition, a role for cell-mediated immunity in systemic vasculitis is suggested by beneficial responses of some patients to treatment with monoclonal anti-T-cell antibodies (CD4, CD52) [28].

Clustering of autoimmune diseases provides further support for the autoimmune basis of each individual disorder. Concurrence of anti-GBM disease and ANCA-associated vasculitis is recognized [29]. In one study, ANCA occurred in 38 of 100 patients with anti-GBM antibodies [30]. At present this clustering cannot be explained through major histocompatibility complex (MHC) associations. Anti-GBM disease is strongly associated with HLA-DRB1*1501 (a DR2 allele), and this association lends strong support to the occurrence of cell-mediated immune responses, but MHC associations have been more difficult to discern for the ANCA-associated vasculitides. The literature contains conflicting reports of positive associations with, among others, HLA-B8, -DR2, and -DQw7; negative associations with DR13DR6; or a lack of association [reviewed in 31].

In short, good circumstantial evidence supports the autoimmune nature of systemic vasculitis associated with ANCA. A small proportion of patients remain ANCA-negative. Some of these patients might not have true vasculitis. For example, TAP deficiency with an associated reduced MHC class-I expression on leukocytes has been described recently in a group of patients with a Wegener-like condition [32].

An association has been noted between the antithyroid drug propylthiouracil and the development of ANCA-associated vasculitis [reviewed in 33]. This correlation has led to the intriguing suggestion that MPO, which is involved in the formation of reactive metabolites from propylthiouracil, binds covalently to one of the metabolites to form an immune complex. If the metabolite then acted as a hapten, ANCA formation might be induced in genetically susceptible individuals. Thus, autoimmunity to ANCA antigens could result from modified self. Other drugs associated with ANCA-positive vasculitis include hydralazine and penicillamine. A number of cases of Churg-Strauss syndrome have been reported following the introduction of leukotriene receptor antagonists. It remains unclear whether vasculitis is secondary to the drug or whether the drug permits a reduction of steroid dose and the manifestation of latent Churg-Strauss syndrome [34]. Each view has its protagonists. Case-controlled studies are lacking.

Infectious agents have long been suspected of playing a role in the development of the vasculitides. Strong evidence indicates a hepatitis B viral cause in some cases of polyarteritis nodosa and for hepatitis C in cryoglobulemia. In ANCA-associated vasculitis, evidence conflicts concerning the seasonal incidence; some reports suggest that the onset of symptoms is more common in winter. Chronic nasal carriage of *Staphylococcus aureus* appears a significant risk factor for disease relapse in Wegener's granulomatosis [25], and results of a European Vasculitis Study group (EUVAS) trial to confirm or refute this association are awaited. That patients carrying superantigen-positive staphylococcal strains seem at particular risk suggests a role for staphylococcal enterotoxins as superantigens in disease induction [35]. Cotrimoxazole therapy might have beneficial effects on control of disease activity and possibly reduces relapse rates [36], but whether this occurs via effects on *S. aureus* or via other immunomodulatory activities remains to be determined.

An association between ANCA-associated vasculitis with renal involvement and silica exposure has been reported in three small case-control studies, with odds ratios between 6 and 12 [reviewed in 37]. However, a more recent case control study of renal and nonrenal Wegener's granulomatosis did not confirm this association [38]. Further studies are required to confirm or refute this association and also to determine the role, if any, of hydrocarbon exposure.

Are ANCA pathogenic?

A number of careful clinical studies have suggested, as I already mentioned, that ANCA contribute to the pathogenesis of vasculitis. At the last Nephrology Forum on vasculitis, in 1990, Dr. Falk noted that ANCA could induce live neutrophils to degranulate and undergo a respiratory burst [1]. At the time, evidence suggested that cytokine priming of neutrophils allowed externalization of the target antigens to the cell surface to facilitate this process. Ten years on, the premise that ANCA activate cytokine-primed neutrophils has stood the test of time. Our understanding of how neutrophils are activated has advanced, the idea has emerged that neutrophils are not only activated but that function is dysregulated, and ANCA have been found to alter the apoptotic rate of primed neutrophils and to interact with neutrophils already undergoing apoptosis. An attempt has been made to understand how these interactions between ANCA and neutrophils contribute to the development of the earliest lesions of vascular injury.

In vitro studies have shown that priming of neutrophils with cytokines such as TNFα, TGFβ, or IL-8 leads to the translocation of both MPO and PR3 to the neutrophil surface, where they are available for ANCA binding (Fig. 5). Neutrophils from patients with active vasculitis exist in a primed or pre-activated state, with increased expression of CD66b, CD64, and CD63, together with PR3, on the cell surface [39, 40]. ANCA IgG bind the surface-expressed antigens via their $F(ab')_2$ portions and trigger neutrophil activation by ligation of constitutively expressed Fcγ receptors, FcγRIIa and FcγIIIb [41-43]; some signaling also can occur following antigen ligation by ANCA $F(ab')_2$ [44]. This antigen binding results in intracellular signaling cascades that include tyrosine phosphorylation, PKC translocation, and PI 3-kinase activation, but not phospholipase D activation (abstract; Ben-Smith et al, *J Am Soc Nephrol*, 10:570A, 2000) [43]. Signal transduction activates a respiratory burst with the release of reactive oxygen species and leukotrienes, degranulation of azurophilic granules, and secretion of proinflammatory cytokines [45-48]. Adhesion via β2 integrins is also required for neutrophil activation by ANCA [49], and might be involved in ANCA's ability to convert neutrophil rolling adhesion to firm adhesion [50].

ANCA also induce accelerated neutrophil apoptosis. This process depends both on prior priming with TNFα and on neutrophil activation with a resultant respiratory burst, as accelerated apoptosis is not seen in the presence of catalase or with neutrophils from individuals with chronic granulomatous disease that lacks NADPH oxidase [51]. However, the accelerated apoptosis has some unusual features. The ANCA-induced apoptosis is dysregulated: the morphologic changes of apoptosis develop without accelerated surface expression of phosphatidylserine, necessary for successful clearance of such apoptotic cells by scavenging phagocytes. Failure to clear these neutrophils in vivo can result in progression to secondary necrosis (Fig. 5) and lead to the intravascular leukocytoclasis that typifies vasculitic lesions.

How might these effects relate to endothelial injury? In vitro, neutrophil activation by ANCA can cause endothelial cell injury and can convert neutrophil rolling adhesion to firm adhesion [50] and can cause endothelial cell injury [52]. Stimulation of neutrophils by IL-8, secreted by monocytes and neutrophils in response to ANCA [53, 54], produces rapid actin cytoskeletal reorganization and can inhibit neutrophil transendothelial migration [55]. TNFα down-regulates the expression of the IL-8 receptor CXCR2, but not CXCR1, and thus reduces the ability of neutrophils to respond to an IL-8 chemotactic gradient while increasing superoxide release, which is mediated by CXCR1 [56]. Collectively, these effects could result in intravascular retention of neutrophils. In vivo, this intravascular retention might lead to delayed migration or even trapping of neutrophils within capillary circulatory systems such as the glomerulus. Increased neutrophil-endothelial adhesion can increase the localization of activated neutrophils to endothelial cells [50]. Reactive oxygen species, cytokines, proteolytic enzymes,

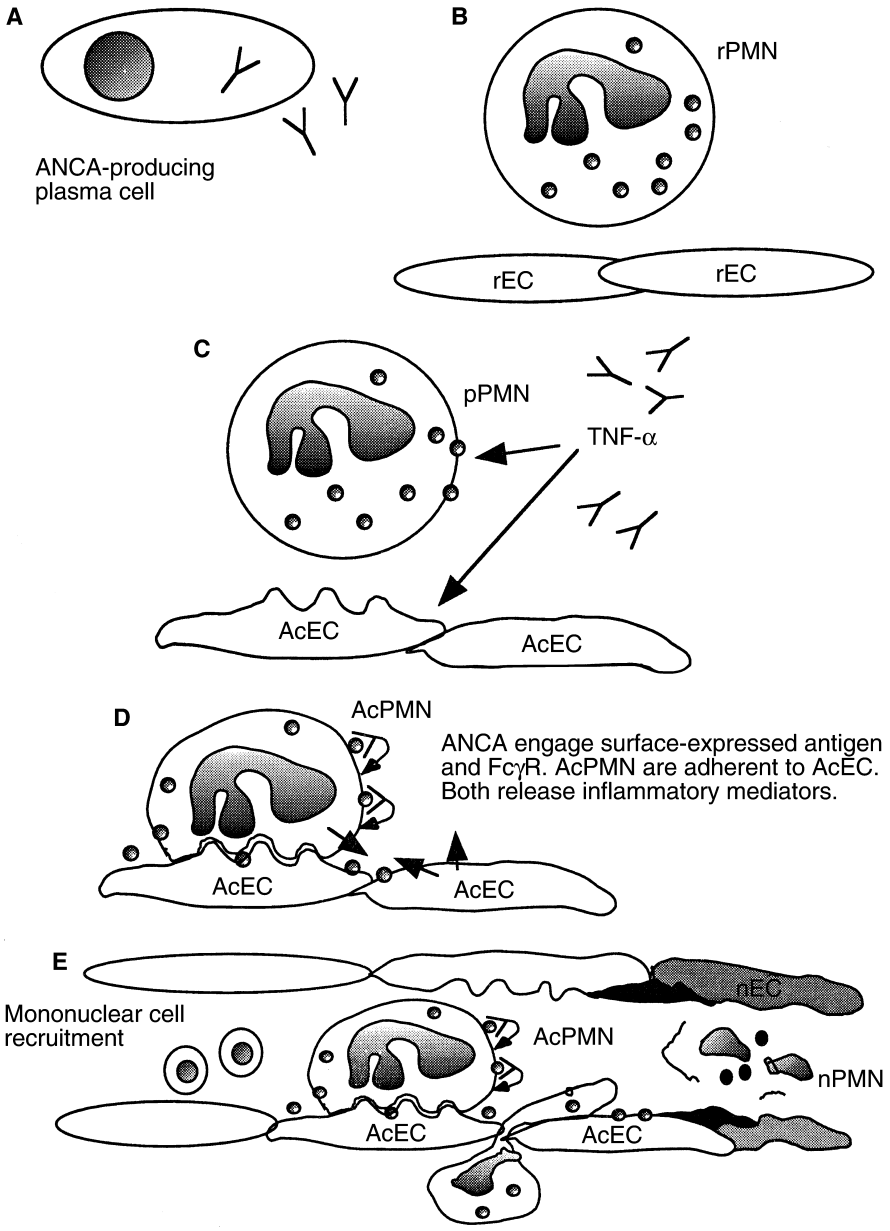

Fig. 5. Stages in the initiation and development of vasculitic lesions. (*A*) Production of high-affinity ANCA IgG from plasma cells. The events leading to this are unclear but are likely to require T-cell help within lymphoid tissues. (*B*) Circulating neutrophils (PMN) and vascular endothelial cells (EC) are in a resting (r) state until: (*C*) infection leads to local generation of cytokines such as TNFα, which prime neutrophils (pPMN) to surface-express PR3 or MPO [●] and induce endothelial cell activation (AcEC); (*D*) ANCA bind and activate neutrophils (AcPMN) following engagement of antigen [by $F(ab)_2$] and FcγR (by Fc). PMN become adherent with EC through adhesion molecule interactions. Release of reactive oxygen radical and inflammatory mediators (cytokines, proteases →) from PMN and EC initiates endothelial damage. Anti-endothelial cell antibodies may contribute to EC activation. (*E*) Released IL-8 causes delay of PMN migration and/or retention of AcPMN within the microcirculation. Primed PMN undergo accelerated apoptosis in response to ANCA and progress to secondary necrosis (nPMN) within the microcirculation. Intravascular thrombosis and EC necrosis develop (nEC). PR3 binding to EC may contribute. Amplification of inflammation follows recruitment of mononuclear cells (monocytes, T-cells) into lesions.

myeloperoxidase, nitric oxide, and secondary neutrophil necrosis all might contribute to directly damaging the endothelial cell [reviewed in 31]. That the earliest vasculitic lesions are thrombotic, necrotic, contain small numbers of neutrophils, and are associated with the presence of lysed leukocytes within the vessels [57] is consistent with the activity of such mechanisms. Further, the number of activated neutrophils in vasculitic renal biopsies correlates with the degree of renal impairment, as assessed by serum creatinine [58], while activated neutrophils are present within the circulation, and their extent of activation, as measured by surface expression of PR3, correlates with disease activity [59].

Monocytes, known to express MPO and PR3, can interact with ANCA and increase injury [reviewed in 31]. Further, the endothelium can contribute to injury both by increasing its adhesive and prothrombotic properties in response to cytokines and by acting as a target for binding of ANCA antigens [reviewed in 31]. Both PR3 and MPO can bind to endothelial cells; indeed, specific receptors for PR3 can be present on endothelial cells [60]. ANCA binding to these surface-expressed antigens can induce endothelial cell cytotoxicity [61], MPO-induced detachment [61], and PR3-induced apoptosis [62]. Contrary to initial reports [63], endothelial cells do not express PR3 and hence are not direct targets for PR3-ANCA [60, 64, 65]. However, the presence of anti-endothelial cell antibodies in some patients might contribute to en-

dothelial activation [66]. Such endothelial activation or injury could precede and promote the vasculitic lesions.

Proteinase 3 and MPO also are expressed on neutrophils undergoing apoptosis [67]. Pro-inflammatory responses can follow from the presence of ANCA antigens on the surface of apoptotic neutrophils, as opsonization of these cells by ANCA increases their uptake by macrophages [40]. Macrophage phagocytosis of opsonized apoptotic cells stimulates further inflammatory cytokine release by macrophages, including TNFα, and leads to persistence of the inflammatory reaction and further tissue damage.

As association between a deficiency of α1-antitrypsin, the main inhibitor of proteinase 3, and PR3-ANCA-positive vasculitis suggests that the neutrophil-ANCA-proteinase 3 loop is an important determinant of tissue injury in vasculitis. The *PiZ* allele, which codes for a nonsecreted form of the inhibitor, is associated with severe disease and poor prognosis [68]. Polymorphisms in Fcγ receptors bound by ANCA on the surface of neutrophils and monocytes also have been studied in view of their potential effects on neutrophil activation by ANCA. We have not found associations between the development of ANCA-associated vasculitis and alleles of *FcγRIIa* (*H131, R131*) or *FcγRIIIb* (*NA1, NA2*) [69, 70]. However, patients with vasculitis are more likely to develop renal failure if they have the *FcγRIIa-R131* allele [69].

While substantial evidence exists that ANCA contribute to initiation and early pathogenesis of vascular damage, the questions of how they develop in the first place and why tolerance is lost to PR3 and MPO remain. Since ANCA are high-affinity, IgG subclass-switched antibodies, it is reasonable to presume that memory B-cells have developed in germinal centers with T-cell help as part of a secondary immune response. During the late stages of vasculitic injury, ANCA contribute little, being overshadowed by monocyte/macrophages and T-cells that are recruited to lesional sites [12].

Therapy

Untreated, the prognosis of ANCA-associated vasculitis with renal involvement is poor, with few patients surviving past one year. Current immunosuppressive regimens allow survival rates of greater than 80% at one year. The greatest threat comes from acute pulmonary hemorrhage, which carries a high mortality.

Treatment needs to be tailored to the different stages of disease and can be divided into therapy to induce remission, maintain remission, or treat relapse. One also should tailor therapy to the severity and extent of disease; non-renal Wegener's granulomatosis, presence of renal disease, or disease with life-threatening pulmonary hemorrhage need different approaches. I will focus here on patients with renal involvement.

Induction-of-remission therapies require high-dose corticosteroids and cyclophosphamide. Combined therapy will induce remission in more than 90% of patients [27]. The details of the dose, route, and duration of therapy vary. A commonly used approach is prednisolone commencing at 1 mg/kg/day (to a maximum of 80 mg), reducing to 10 mg/day by 3 months [71]. Cyclophosphamide has advocates for both daily oral and intermittent pulse regimens. Many nephrologists use 2 mg/kg/day, adjusted for age and renal function, for three months, provided the white blood cell count remains above 4×10^9/L. This approach is supported by the preliminary results from CYCAZAREM, a European collaborative, prospective, randomized controlled trial examining the benefit of using azathioprine instead of cyclophosphamide for maintaining remission [72]. It has been suggested that intravenous pulse cyclophosphamide therapy is associated with a lower cumulative dosage and fewer adverse effects, but clinical trials are required to confirm this and to determine the relative effectiveness of intermittent pulse versus daily oral therapy on control of disease activity. Unfortunately, existing randomized prospective controlled trials do not provide a definitive answer. When the plasma creatinine at presentation is greater than 500 μmol/L (5.7 mg/dL), I believe that one should escalate therapy by adding intravenous pulses of methylprednisolone, 15 mg/kg (maximum 1 g) for three days, or by commencing a course of seven 3-liter plasma exchanges. Intravenous immunoglobulin also has been used during induction of remission, but its efficacy has not been tested in prospective controlled trials.

The choice of maintenance therapy has to be balanced between the risks of disease recurrence (which vary between 25% and 50% over the first 3 to 5 years) and toxicity of therapy. One approach is to substitute azathioprine for cyclophosphamide after the first 3 to 6 months, while reducing the prednisolone to very low levels or discontinuing it altogether [71]. Alternatives to azathioprine include methotrexate [73], although this drug is contraindicated if the serum creatinine is >2 mg/dL, or mycophenolate mofetil [74]. At present it is unclear whether continuation of azathioprine beyond 18 to 24 months helps reduce the long-term likelihood of relapse. Trimethoprim-sulfamethoxazole also can be useful in reducing the risk of relapse during maintenance therapy [36], perhaps in association with immunosuppressive therapy.

Patients with vasculitis require 3-month review over the long term to detect early signs of disease recurrence. Some evidence indicates that patients are more likely to relapse if ANCA reappear in the circulation or if there is a sustained rise in ANCA titers [22, 24]. Therapy for relapse depends on its severity and the time at which the relapse occurs relative to therapy for the vasculitis. Thus, choice of therapy will vary between an increase in corticosteroid dosage to reinstitution of a full course of corticosteroid and cyclophosphamide therapy.

It is always important to distinguish tissue damage caused by active vasculitis from that caused by nonhealing scars inflicted by the disease itself or by toxic treatment. Only acute disease is amenable to immunosuppressive therapy. Tools have been designed to help the clinician with the assessment of acute disease and tissue damage. As we saw in the case presentation, the Birmingham Vasculitis Activity Score (BVAS) is a validated scoring system for assessing disease activity solely due to vasculitis [75]. It can be used to monitor patients, evaluate their responses to therapy, and screen for relapse. Other scoring systems include the Vasculitis Activity Index, the Disease Extent Index, and the Groningen Vasculitis Score [reviewed in 76]. The Vasculitis Activity Index suffers from the disadvantage that it does not differentiate chronic from acute damage. The Disease Extent Index (ELK, standing for ear, nose, throat, lung, and kidneys) was limited in its scope but has now been extended to include additional organs. The Groningen Vasculitis Score was designed for use only in Wegener's granulomatosis and has the disadvantage that the assessment requires a biopsy, thus limiting its use. The Vasculitis Damage Index (VDI), which aims to provide information on chronic damage, scores the accumulation of nonhealing scars due to effects of disease (for example, end-stage renal failure) or therapy (for example, infertility due to cyclophosphamide) [75]. Over time, the VDI score will stay the same (if there is no further damaging event) or increase if the patient continues to suffer further tissue-damaging events.

What of patients with end-stage renal failure? Providing the disease is in remission, there is no contraindication to transplantation, even if ANCA are still detectable in the serum. Evidence suggests a low relapse rate in patients with functioning transplants, and relapses often can be effectively treated by utilizing cyclophosphamide [77].

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston, Massachusetts, USA*): Cytokines are ubiquitous and tumor necrosis factor is alleged to be involved both in rheumatoid arthritis and Wegener's granulomatosis. What "tells" TNF to attack the joints in rheumatoid arthritis and the kidneys in Wegener's?

Prof. Savage: I think that the tumor necrosis factor in these diseases is initiating or amplifying injury in the presence of co-factors that might be site- or disease-specific. Local TNFα production is increased in renal biopsies of ANCA-associated glomerulonephritis, and TNFα concentrations correlate with disease activity. In some inflammatory disorders, including rheumatoid arthritis, the *TNF-303* (A) allele of the promoter has been associated with increased TNFα production. Two studies have excluded an association of the promoter polymorphism *TNF-303* with ANCA-associated vasculitis, however [78, 79]. This exclusion suggests that factors controlling TNFα production differ between vasculitis and rheumatoid arthritis.

Prof. John Saville (*Professor of Medicine, Centre for Inflammation Research, Royal Infirmary, Edinburgh, Scotland*): You have emphasized the role of oxidant stress in promoting tissue injury in ANCA-associated disease. Given that antioxidants such as vitamin E confer benefit in atheromatous disease—in which oxidative stress, endothelial injury, and dysregulated apoptosis are prominent—is there any evidence that antioxidants might be of benefit, particularly in the problematic area of maintenance therapy after remission of vasculitis has been induced?

Prof. Savage: Dr. Lorraine Harper in our group performed a small pilot study. She looked at oxygen radical production from neutrophils from patients in remission who had no evidence of disease activity, although they were still receiving low doses of prednisolone and azathioprine. She found that their baseline superoxide production was still greater than that of normal controls. She then gave the patients 10 days of vitamin C and vitamin E treatment and re-measured the superoxide production from neutrophils; it had fallen to normal levels. This is interesting for two reasons. First, the decrease suggests that even when patients are in clinical remission, they are not totally normal. That notion is also supported by, for example, elevated von Willebrand factor levels that persist during clinical remission. Second, the vitamin C and vitamin E study suggests that it is possible to modify neutrophil oxidant production.

Prof. Andrew Rees (*Department of Medicine and Therapeutics, University of Aberdeen, Institute of Medical Sciences, Aberdeen, Scotland*): One of the really striking things about the ANCA-associated diseases is the bias in the clinical phenotype that is associated with the particular ANCA specificities, PR3 with granulomatous disease and MPO with vasculitis without granulomas. Why should that be the case?

Prof. Savage: Microscopic polyangiitis splits more or less equally between PR3-ANCA and MPO-ANCA without any obvious difference in the phenotype. So to consider that PR3-ANCA associate with granuloma formation might be misleading. However, disease induction might take different routes. In Wegener's, a nasal mucosal factor might initiate disease associated with specific cytokine profiles and associated with the early granuloma. As the Wegener's evolves, it becomes more vasculitic and systemic with development of PR3-ANCA; in other words, it becomes more like microscopic polyangiitis. Interestingly, we have found that IgG from MPO-ANCA-positive patients are more activating than are IgG from PR3-ANCA-positive patients [80]. Our results

differ from a study from Franssen, who found that PR3-ANCA were more activating than MPO-ANCA [81]; however, this study was difficult to interpret, as the MPO-ANCA used were no more activating than normal control IgG. If these effects are relevant to the presence or absence of granulomata, one would have to speculate that PR3-ANCA with the very lowest neutrophil-activating potential are associated with granuloma formation. It is not clear why MPO-ANCA are more activating than PR3-ANCA; the difference cannot be explained by increased expression of MPO on the cell surface, as PR3 is expressed on more cells than MPO. Neither can IgG subclasses account for the difference; IgG1 and IgG4 are the predominant isotypes in both MPO-ANCA and PR3-ANCA. Although IgG3 ANCA has been suggested as more activating, PR3-ANCA contains more IgG3 than does MPO-ANCA.

Dr. Jeremy Duffield (*Department of Renal Medicine, Royal Infirmary, University of Edinburgh*): You described the well-recognized association between clinical infection and disease induction or relapse. One hypothesis is that the infection leads to release of pro-inflammatory cytokines that might up-regulate the immune response. If your model of disease initiation is correct, one might expect sterile inflammation, such as operative wounds or trauma, to lead to disease induction. Is there any evidence for this?

Prof. Savage: That's a very interesting point. It has been recognized for 30 years that relapses of vasculitis can follow an intercurrent infection. A systematic search to determine whether sterile inflammation has a similar effect would be valuable. I don't know of such a study.

Prof. Michael Dillon (*Great Ormond Street Hospital for Children, London, England*): This patient's initial presentation and subsequent relapse were preceded by upper respiratory tract symptoms. Kawasaki disease manifests evidence of an infective precipitant, and superantigens have been invoked. In addition, support exists for a superantigen role in Wegener's granulomatosis [35]. Brogan et al, working in our department, similarly have demonstrated superantigen involvement in childhood vasculitides [abstract; Brogan et al, *Clin Exp Immunol* 120(Suppl 1): 46, 2000]. What is your view about the potential superantigen etiology of vasculitic episodes?

Prof. Savage: If a link between *Staphylococcus aureus* carriage in the nose and relapse of Wegener's is proven, one hypothesis is that this is due to a superantigen. In addition, three studies that have found expansions of T-cells bearing particular Vβ receptors in patients with Wegener's granulomatosis and microscopic polyangiitis [35, 82, 83] might support a role for superantigens in disease initiation.

Dr. Alan Watson (*City Hospital, Nottingham, England*): The patient was a poultry farmer and you alluded to an increasing incidence of vasculitis in a rural area of the country. Does cluster analysis suggest that further detailed epidemiologic analysis might lead to identification of a facilitating infection?

Prof. Savage: Other than the association with nasal carriage of *Staphylococcus aureus* that I described, other searches for facilitating infections have not been productive. However, a systematic epidemiologic study might provide further insights.

Prof. Stephen Powis (*Centre for Nephrology, Royal Free and University College Medical School, London, England*): Have any family-based studies attempted to quantify the overall genetic component of ANCA-associated vasculitis?

Prof. Savage: The literature contains a handful of reports of both Wegener's granulomatosis and microscopic polyangiitis occurring in sibling pairs or in a parent and child. As yet, these kindreds have not been subjected to detailed genetic analysis.

Prof. Charles Pusey (*Renal Section, Imperial College, London, England*): I was struck by the marked rise in the MPO-ANCA titer that occurred several weeks before the clinical relapse, and by the extent of interstitial damage in the second renal biopsy. Do you think that some subclinical renal damage might have occurred before the relapse was diagnosed? Do you think that any change in treatment is justified on the basis of a rising ANCA titer?

Prof. Savage: It is possible that continuation of low-dose azathioprine and prednisolone caused partial disease suppression, so although clinically inactive by our criteria, there was continuing tissue injury. In retrospect, the rising ANCA titer might have indicated occult disease activity. As discussed, the predictive value of a rise in ANCA titer for relapse was 57% in one study. Treating on the basis of changing serology alone, however, might expose some patients to unnecessary risks from increased immunosuppression.

Prof. Alex Davison (*St. James's University Hospital, Leeds, England*): I was very impressed that the second biopsy showed a large amount of interstitial damage. It suggested to me that something had been going on in spite of your apparently very effective therapy. In view of the fact that serology is not particularly helpful, what is the place of a protocol biopsy to determine subsequent therapeutic management once remission is achieved?

Prof. Savage: We have used protocol biopsies at six months in patients with Henoch-Schönlein vasculitis whom we have treated with immunosuppressive drugs. Therapy is then continued or discontinued depending on the results of the biopsy [84]. I think a protocol biopsy would be worth considering in ANCA-associated vasculitis, provided it was undertaken within the context of a clinical trial. It also would help to address whether particular subgroups of patients are more prone to continuing

occult disease activity, for example, those who remain ANCA-positive or who have a rising ANCA titer.

Dr. Neil Iggo (*Royal Sussex County Hospital, Brighton, England*): Of course we are concerned about the toxicity of treatment, but we should not forget that until cyclophosphamide was introduced, Wegener's was an almost universally fatal illness. Given that more than 50% of people who have a recurrence of ANCA develop a relapse, and given that the CYCAZAREM trial has shown that 3 months and 12 months of cyclophosphamide are as safe or as toxic, depending on your point of view, do you think that the time has come to organize a trial of 3 months of initial treatment with cyclophosphamide and then treat any serologic recurrence of ANCA positivity with a further 3 months of cyclophosphamide to see whether that approach could prevent relapse, and to determine whether that regimen is safe?

Prof. Savage: I think that comes back to an earlier question of whether we are missing ongoing tissue injury and whether a protocol renal biopsy would be justified. Unless we can be sure that a rising ANCA titer correlates with continuing occult disease activity, I would not re-introduce cyclophosphamide.

Dr. David Reaich (*South Cleveland Hospital, Middlesborough, England*): Please comment on the value of renal biopsy. I was intrigued that you opted to biopsy the patient on the second occasion when relapse was already apparent. You alluded to the difficulty in deciding whether to re-introduce immunosuppression with the rising ANCA. Would it perhaps not be more appropriate to perform a biopsy at that point to decide whether to amplify the immunosuppression before the relapse?

Prof. Savage: The reason we did the second biopsy was because the clinical symptoms were relatively mild: the patient had a positive ANCA but had had a positive ANCA for some time; the CRP had increased, but only to 37 mg/L, and she had recently had upper respiratory tract infections; and the creatinine had risen modestly with recrudescence of hematuria. The renal biopsy was very helpful in confirming the clinical suspicion of relapse. I think it would be difficult to justify a renal biopsy in the presence of recurrence of ANCA or a rising ANCA titer unless a change was found in the urine sediment or renal function.

Dr. Ian Roberts (*Renal Pathologist, John Radcliffe Hospital, Oxford, England*): As a pathologist, I am interested in knowing what impact disease activity in the renal biopsy specimen has on the way you manage the patient, and what drugs you give.

Prof. Savage: Let me give you two examples. Presence of disease activity in a patient who is dialysis-dependent at presentation, providing there are some viable glomeruli left, is an indicator that full therapy will be useful, that is, cyclophosphamide, prednisolone, and either plasma exchange or methylprednisolone. Presence of disease activity in a patient who might have a relapse will guide the decision to re-introduce therapy, and the type of therapy can be guided by the severity of the relapse.

Prof. Peter Mathieson (*Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, England*): Why is cyclophosphamide more effective at remission induction than azathioprine, yet, as shown by the CYCAZAREM trial, it is no better than azathioprine for remission maintenance?

Prof. Savage: Cyclophosphamide induces remission in more than 90% of patients and in many patients induces the ANCA to fall. When cyclophosphamide is discontinued, the plasma cells can slowly become active again, and azathioprine might be less effective at controlling these. It is clear that cyclophosphamide is not good at establishing tolerance, because relapse is common and often associated with ANCA.

Dr. Harrington: Could you expand on the issue of oral versus intravenous cyclophosphamide for the treatment of Wegener's?

Prof. Savage: Pulses of intravenous cyclophosphamide have been used very effectively in the control of lupus nephritis, and it was because of its effectiveness in that setting that it was introduced for treatment of vasculitis. The potential benefit of using intermittent pulses of cyclophosphamide, whether given orally or intravenously, is that the patient receives approximately one-half the total dose of cyclophosphamide that would have been administered orally. Given the toxicity of cyclophosphamide, that might be very beneficial. The question is whether intermittent pulses of cyclophosphamide are as effective for induction of remission as daily oral cyclophosphamide. A recent meta-analysis (unpublished data) suggested that they are, but at the expense of an increased number of relapses. The CYCLOPS study, organized by the European Vasculitis Study Group, should confirm or refute this.

Dr. John Bone (*Royal Liverpool Hospital, Liverpool, England*): Cyclophosphamide-based regimes carry a big risk of side effects. Would it not be reasonable to reserve cyclophosphamide for severe disease involving lung hemorrhage and perhaps use mild regimes involving azathioprine from the outset for patients with less-systemic disease?

Prof. Savage: I think there is a move to do just that in patients with limited Wegener's, for example, to use methotrexate in preference to cyclosphamide in patients without renal involvement. This has been tested by the EUVAS trial "NORAM," which will report in 2001. Once there is clinical evidence of systemic disease, even if this is mild without major tissue injury, it is important that the disease be controlled quickly to limit tissue damage and prevent progression. Cyclophosphamide is currently the best drug we have to do this.

Dr. Lynda Stuart (*MRC Centre for Inflammation*

Research, Edinburgh University, Edinburgh): I was intrigued by the fact that antigen-specific T-cells are present in peripheral blood. Are any trials looking at T-cell-specific treatment, such as antibodies against CD40 ligand or CTLA4Ig?

Prof. Savage: Martin Lockwood undertook studies using anti-T-cell therapies, anti-CD4 and -CD56, for treatment of vasculitis. In many patients those therapies were very valuable for inducing and maintaining remission. Anti-CD40 ligand antibodies have been associated with a thrombotic risk (unpublished data), so they might be hazardous in vasculitis, which carries an increased incidence of thrombotic events.

Dr. Gill Gaskin (*Imperial College School of Medicine, London*): Would you treat all generalized ANCA-associated vasculitis in the same way, irrespective of the phenotype or the ANCA specificity?

Prof. Savage: I like to tailor immunosuppression to severity of the disease. Our practice is guided by this principle. For example, we did not prescribe plasma exchange or methylprednisolone for the patient we have discussed. However, if there had been pulmonary hemorrhage, we would have had no hesitation in doing so. I would like a greater choice of therapies to allow us to adjust treatment to suit the age of the patient, the presence of other co-morbidity, as well as the severity and the type of organ involvement. As to whether I would treat patients with PR3-ANCA or MPO-ANCA differently, I have already alluded to a greater neutrophil activating capacity with IgG from MPO-ANCA-positive patients [80]. Despite this, several studies, including those of Franssen and Geffriaud-Ricouard [85, 86], have not shown any difference in outcomes between either antibody group. Some, but not all the studies, have suggested that PR3-ANCA is associated with a higher relapse rate than is MPO-ANCA. However, several studies, including CYCAZAREM, indicate an increased likelihood of relapse in Wegener's compared with microscopic polyangiitis (abstract; Jayne et al, *J Am Soc Nephrol* 10:105A, 1999). If anything, we should direct therapy toward trying to reduce the incidence of relapse in patients with Wegener's granulomatosis.

Dr. Caroline Whitworth (*Royal Infirmary of Edinburgh, NHS Trust, Edinburgh, Scotland*): What duration of maintenance treatment would you recommend for patients who do not have a clinical relapse? Do you differentiate between PR3-ANCA–positive and MPO-ANCA–positive microscopic polyangiitis? Would you continue for a year or beyond that for both categories?

Prof. Savage: At present practices differ. Some clinicians discontinue therapy after 18 to 24 months; others, including myself, continue indefinitely with low-dose azathioprine and/or prednisolone. We do not know whether the latter approach reduces the incidence of relapse. Earlier we discussed the greater propensity of patients with Wegener's to relapse compared with patients who have microscopic polyangiitis. Perhaps we should consider maintenance therapy for patients who are most likely to relapse. At present, I do not differentiate between PR3-ANCA– and MPO-ANCA–positive microscopic polyangiitis patients for the reasons discussed earlier.

Prof. Eberhardt Ritz (*Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany*): Stegmar et al reported that trimethoprim-sulfamethoxazole given prophylactically reduces the number of recurrences [36], and I wonder why this is not prescribed routinely. Do you have experience to the contrary, or is there a consensus in the vasculitis community that the data are not sufficiently solid?

Prof. Savage: There was a 20% incidence of side effects in the study you cite, and this might be the reason why trimethoprim-sulfamethoxazole is not used routinely. We do not know whether the effect was due to eradication of nasal carriage of *Staphylococcus aureus* or another antibacterial or immunosuppressive effect of the drug. If an association between *S. aureus* nasal carriage and increased likelihood of relapse is confirmed, it might be possible to eradicate *S. aureus* using less-toxic agents such as mupirocin.

NOTE ADDED IN PROOF

The following articles, quoted in text, are now in press:

Ben-Smith A, Dove SK, Martin A, Wakelam MJO, Savage COS: Anti-neutrophil cytoplasm autoantibodies from patients with systemic vasculitis activate neutrophils via distinct signalling cascades compared to conventional Fcγ receptor ligation. *Blood*, in press

De Groot K, Adu D, Savage COS: The value of pulse cyclophosphamide in ANCA-associated vasculitis. Meta-analysis and critical review. *Nephrol Dial Transplant*, in press

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