Anti-glomerular basement membrane disease

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CASE PRESENTATION

A 47-year-old white man who worked as a professional musician presented to his general practitioner with a 3-week history of malaise, loss of appetite, slight weight loss, and dark urine. There was no history of respiratory symptoms and, in particular, no hemoptysis. He had had mild asthma for 10 years, which had been treated with inhaled bronchodilators. He was a lifelong nonsmoker and drank alcohol only occasionally. He had no family history of renal disease.

The general practitioner detected proteinuria and hematuria, and blood tests showed an elevated serum creatinine. The patient was referred to our renal unit the same day. On arrival, he looked well and was afebrile with no rash or edema. Pulse was 80 beats/minute and regular, and blood pressure was 145/70 mm Hg. Clinical examination of the heart, lungs, abdomen, and nervous system was normal. Urinalysis showed 2+ blood, 3+ protein, dysmorphic red cells, and granular and red cell casts. Serum creatinine was 550 μmol/L (6.3 mg/dL) and urea 21 mmol/L. Electrolytes were in the normal range, but albumin was reduced at 2.3 g/dL. Blood picture showed hemoglobin 10.7 g/dL; white blood cell count 9.6×10^9/L; and platelets 654×10^9/L. Oxygen saturation with the patient breathing room air was 94%, and a chest radiograph was normal.

An enzyme-linked immunosorbent assay (ELISA) for anti-glomerular basement membrane (anti-GBM) antibodies was positive at 80% (normal range, 0% to 15%), confirmed by positive binding on a Western blot using collagenase solubilized human GBM. Anti-neutrophil cytoplasm antibodies (ANCA) and anti-nuclear antibodies were negative, and complement levels were normal. Renal biopsy disclosed a focal segmental necrotizing glomerulonephritis in 17 of 27 glomeruli, with cellular crescents in most of them (Fig. 1A and B). In some glomeruli, there was rupture of Bowman's capsule, with a giant cell response. The interstitium contained a dense focal mononuclear cell infiltrate, and tubules had focal dedifferentiation with many red cell casts. Immunoperoxidase studies showed strong deposition of IgG in a linear pattern along the GBM, with weaker staining for IgM and C3 (Fig. 1C). The conclusion was that the biopsy revealed crescentic glomerulonephritis due to anti-GBM disease.

Treatment was started with oral prednisolone, 60 mg daily, and cyclophosphamide, 200 mg daily. Plasma exchange was performed daily for 14 days using 4 L exchanges for human albumin, with 500 mL fresh frozen plasma for 5 days following renal biopsy. The patient showed a good response to treatment. Anti-GBM antibodies fell to within the normal range by 2 weeks. His urine output increased rapidly to more than 2 L per day, and the serum creatinine slowly improved. He developed transient neutropenia at 3 weeks, which improved on discontinuation of cyclophosphamide for 7 days. No other adverse effects of treatment occurred. Cyclophosphamide was stopped after 3 months, and prednisolone was rapidly tapered by 3 months and discontinued by 6 months. Anti-GBM antibodies remained negative, and the serum creatinine fell to 180 μmol/L (2.0 mg/dL).

At last follow-up, after 3 years, the patient was well, and his serum creatinine had improved to 152 μmol/L (1.7 mg/dL). Urinalysis still showed small amounts of blood and protein. He was taking antihypertensive medication only.

DISCUSSION

Prof. CHARLES D. PUSEY (*Renal Section, Division of Medicine, Imperial College London; and Hammersmith Hospital, London, United Kingdom*): The patient presented illustrates the difficulty in making an early diagnosis of anti-GBM disease when only the kidney is involved. More important, this case demonstrates that patients with advanced renal failure can recover independent renal function if treated aggressively. The term "anti-GBM disease" is now widely used to describe patients in whom anti-GBM antibodies are associated with crescentic
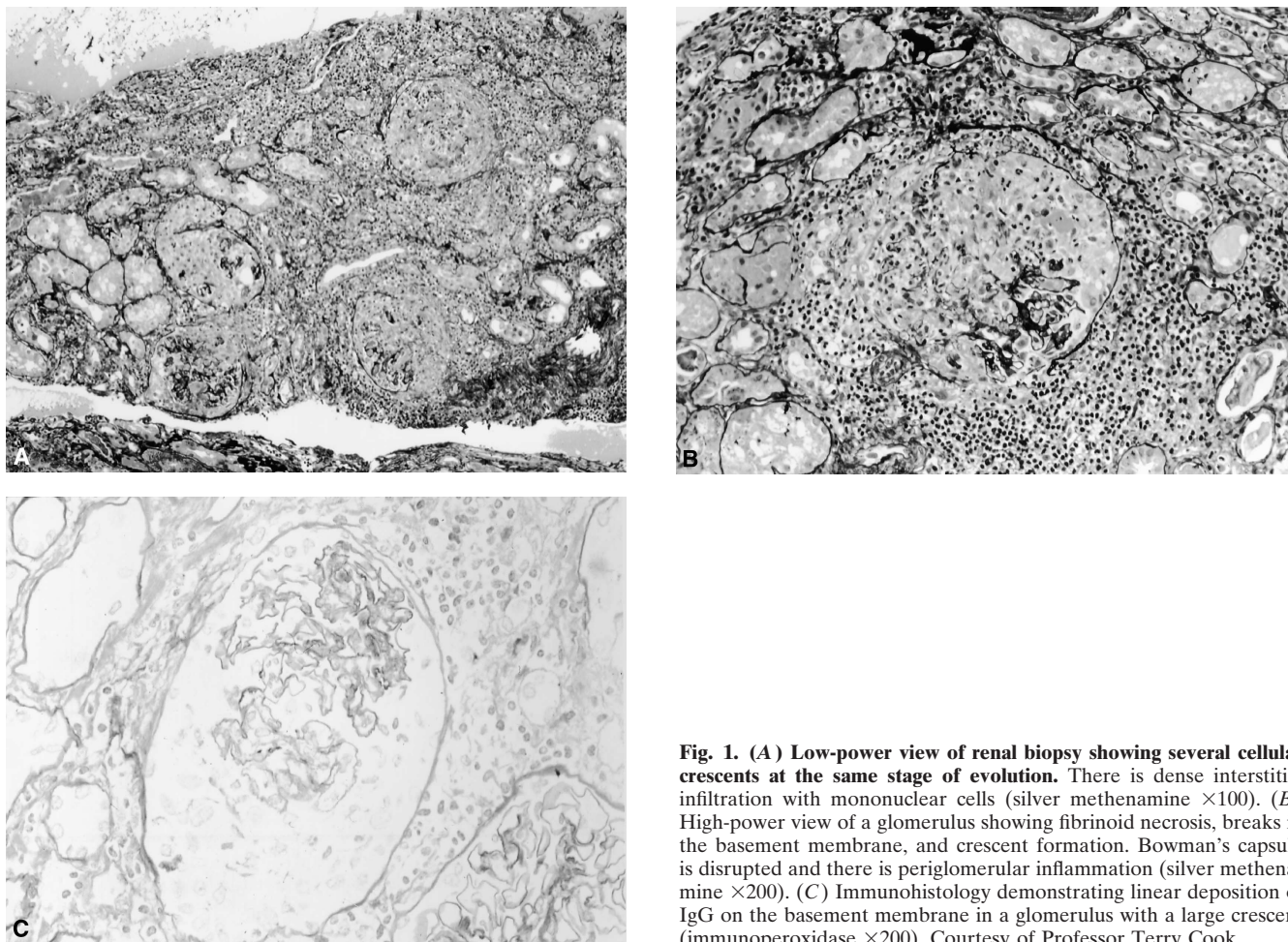
Fig. 1. (*A*) Low-power view of renal biopsy showing several cellular crescents at the same stage of evolution. There is dense interstitial infiltration with mononuclear cells (silver methenamine ×100). (*B*) High-power view of a glomerulus showing fibrinoid necrosis, breaks in the basement membrane, and crescent formation. Bowman's capsule is disrupted and there is periglomerular inflammation (silver methenamine ×200). (*C*) Immunohistology demonstrating linear deposition of IgG on the basement membrane in a glomerulus with a large crescent (immunoperoxidase ×200). Courtesy of Professor Terry Cook.

glomerulonephritis [1, 2], often in association with alveolar hemorrhage, and rarely with other clinical features such as retinopathy. Occasionally, patients present with pulmonary hemorrhage alone, without overt renal disease, although urinary abnormalities may be present and glomerular deposits of anti-GBM antibodies are often found if renal biopsy is performed. Anti-GBM disease is regarded by some as a specific and restricted form of systemic vasculitis, which affects only the capillary beds of selected organs containing the target auto-antigen. This auto-antigen has been identified as the α3 chain of type IV collagen.

Anti-GBM disease is also known as "Goodpasture's disease" or "Goodpasture's syndrome" [3, 4]. Briefly, the term Goodpasture's syndrome was first used in the 1950s by Stanton and Tange [5], who described a series of patients with pulmonary-renal syndrome, similar to the patient first reported by Goodpasture in 1919 [6]. It is not known whether these cases, or indeed Goodpasture's original case, had anti-GBM antibodies, because techniques for detecting them were not available at the time. The presence of antibodies to the GBM was first demon-

strated in the 1960s, following the development of immunofluorescence techniques for use in renal biopsies [7, 8]. Some authors now use the term Goodpasture's syndrome to describe the combination of severe glomerulonephritis and lung hemorrhage, as it was originally used, regardless of etiology. Others reserve this diagnosis for cases in which anti-GBM antibodies also have been detected. For simplicity, I think that the term anti-GBM disease has some merit, and I will use this synonymously with Goodpasture's disease to describe patients with anti-GBM antibodies accompanied by kidney and/or lung involvement.

Clinical features

The clinical features of anti-GBM disease have been reviewed recently [2, 3, 9] so I shall only summarize them briefly. There is a bimodal age distribution, with peak incidence in the third and sixth decades, and a slight overall excess of males. Most patients present with the combination of rapidly progressive glomerulonephritis and lung hemorrhage, although 30% to 40% present with isolated renal involvement. Renal disease can present with hematuria, accompanied by mild to moderate

proteinuria, with "acute nephritis," or with features of acute renal failure. Hypertension is usually a late feature, accompanying advanced renal failure and fluid retention. Lung disease, which is commoner in young men, presents with breathlessness and cough, sometimes accompanied by overt hemoptysis. Chest radiographs can reveal alveolar shadowing, but this appearance is nonspecific, and a more sensitive test is the corrected carbon monoxide transfer factor (KCO) [10]. Many patients also develop systemic features such as malaise, fatigue, and weight loss, and these might relate to anemia from pulmonary hemorrhage or to the effects of uremia. Additional clinical features, such as arthralgia and myalgia, have been reported, but it is difficult to know whether these in fact represent manifestations of an accompanying ANCA-positive systemic vasculitis.

Anti-GBM disease is a rare disorder with an incidence estimated at around one patient per million population. However, it might be responsible for up to 20% of cases of rapidly progressive glomerulonephritis. The disease is commoner in white populations and appears to be very rare in those of African origin. Although some have suggested that the incidence of anti-GBM disease is higher in spring and early summer, this is not the case in all series. There are some reports of localized "outbreaks" of the disease, possibly suggesting a relationship with infection. The commonest disease association is with ANCA-positive systemic vasculitis: ANCA are found in approximately 30% of patients with anti-GBM disease, and 5% to 10% of patients presenting with ANCA-positive systemic vasculitis have anti-GBM antibodies [11]. Occasionally, patients with membranous nephropathy develop crescentic glomerulonephritis in association with anti-GBM antibodies. Associations with urinary tract obstruction and with lithotripsy for ureteric calculi also have been reported. Patients with Alport's syndrome can develop anti-GBM disease in the allograft following renal transplantation.

Laboratory features

Almost all patients with Goodpasture's disease have circulating anti-GBM antibodies detectable by ELISA. Most of the available assays use purified bovine or sheep GBM enriched for the NC1 domain of the α3 chain of type IV collagen [α3(IV)NC1]. Although such assays are highly specific and sensitive, we confirm all positive results by Western blotting on collagenase-solubilized human GBM. Rarely, these standard assays are negative despite the presence of deposited anti-GBM antibodies in the kidney. We have found that low levels of circulating anti-GBM antibody can be detected in the serum of such patients using a sensitive biosensor assay [12]. Biosensors incorporating chips coated with recombinant α3(IV)NC1 are likely to be more widely used in the future.

In the correct clinical context, positivity in well-established assays can be used as an indication to start treatment when renal biopsy cannot be performed immediately. However, we prefer to perform renal biopsy in all patients who have evidence of renal involvement. We then can perform immunofluorescence studies and can assess the extent and activity of glomerulonephritis by light microscopy. Almost all specimens have linear deposits of IgG along the GBM, often accompanied by complement C3, and occasionally by other immunoglobulin isotypes, such as IgA and IgM. Light microscopy generally reveals widespread crescent formation. The disease is typically monophasic, with all glomeruli showing lesions of a similar age. This is in contrast to findings in ANCA-positive systemic vasculitis, in which lesions of different ages are often seen together. Lung histology is rarely obtained, but examination of pulmonary tissue can disclose alveoli containing red cells together with hemosiderin-laden macrophages. Immunofluorescence of lung tissue is technically difficult but can reveal intermittent linear deposits of IgG along the alveolar basement membrane.

Predisposing factors

Anti-GBM disease has been described in siblings and two sets of identical twins, although discordant twins also have been reported. As in other autoimmune diseases, anti-GBM disease has been strongly associated with the major histocompatibility complex (MHC). Several serologic studies over the last 20 years have documented a strong association with the MHC class II gene, HLA DR2 [13]. This association is consistent between different Caucasoid populations and, overall, HLA DR2 is present in approximately 80% of cases. More recently, genotyping studies have confirmed the association with HLA DRB1*1501 and 1502 (corresponding to the serologically defined HLA DR2 or DR15 genes) [14]. A meta-analysis of more than 130 patients revealed a hierarchy of associations, as observed in other autoimmune diseases, with some genes conferring susceptibility and others conferring resistance [15]. The strongest association was with HLA DRB1*1501 but, when the effect of this gene was excluded, subsequent analysis revealed an increased frequency of DRB1*04 and DRB1*03 and a decreased frequency of DRB1*07 and DRB1*01. Associations with DQ alleles reflected the linkage disequilibrium with DR alleles; in particular, an increased frequency of DQB1*06 (linked to DRB1*1501) was noted. The meta-analysis also revealed that both inherited DRB1 alleles had an effect on susceptibility, but that if one of the alleles was DRB1*1501, the effect of the second allele was either neutral (DRB1*04 and DRB1*03) or protective (DRB1*07 and DRB1*01). Subsequent functional studies showed that the protective alleles bound the majority of peptides derived from the α3(IV)NC1 sequence with greater affinity than the major susceptibility

allele, DRB1*1501 [16]. The authors suggest that protective alleles therefore might compete for peptide epitopes with the susceptibility alleles. However, the precise molecular basis of these MHC associations remains to be determined.

In X-linked Alport's syndrome, which is due to mutations in the α5 chain of type IV collagen, the supramolecular organization of the α3, α4, and α5 chains in the basement membrane is defective [17]. This alteration leads to progressive abnormalities of the GBM, and of basement membranes in the cochlea and eye, leading to the clinical features of Alport's syndrome. The absence of expression of α5 (and the associated lack of α3 and α4) means that these molecules within a renal allograft are regarded as foreign antigens [18]. The presence of anti-GBM antibodies in the allografts of such patients is relatively common, although clinical anti-GBM disease is infrequent. The immune response in such cases is often directed to the α5 chain, but also can be directed toward the α3 or α4 chains. Although much rarer, mutations in the α3 or α4 chain can lead to autosomally inherited Alport's syndrome [19], in which anti-GBM disease also can occur in renal transplants.

Although a strong association exists between anti-GBM disease and HLA DRB1*1501, this allele is present in as many as one-third of individuals in white populations. It is therefore clear that additional factors, either genetic or environmental, are required for disease expression. Environmental factors preceding diagnosis do not necessarily drive the autoimmune response; instead they might exacerbate pre-existing but unrecognized disease. Infectious agents have long been suspected as causative, for example, influenza in Goodpasture's original patient. Infection also might account for reports of clustering of cases. However, no specific infectious agent has yet been consistently linked to the disease. Exposure to hydrocarbons has been associated with the onset of symptoms, and case control studies have shown higher levels of anti-GBM antibodies (at borderline levels) in individuals exposed to inhaled hydrocarbons [20]. Cigarette smoking has frequently been reported in association with the development of disease [10], and relapse has been linked to the onset of smoking [21]. One hypothesis suggests that damage to the lung from infection, cigarette smoke, or hydrocarbons exposes a cryptic epitope within the alveolar basement membrane that triggers an autoimmune response to α3(IV)NC1. However, no convincing evidence for this theory has surfaced, and it seems more likely, at least for cigarette smoking, that the additional pulmonary insult allows or increases alveolar damage in patients who already have circulating anti-GBM antibodies. It has been suggested that, within the kidney, urinary infection, lithotripsy, or pre-existing glomerulonephritis likewise might reveal sequestered epitopes within the GBM and lead to development of anti-GBM disease in susceptible individuals. However, these conditions are relatively common, while subsequent anti-GBM disease is extremely rare.

Pathogenesis: Experimental models

I shall consider the role of animal models in illustrating disease mechanisms and suggesting new approaches to treatment. Two models of experimental anti-GBM disease are widely used. In experimental autoimmune glomerulonephritis (EAG), animals are immunized with GBM or α3(IV)NC1 and subsequently develop an autoimmune response that targets their own kidneys. In nephrotoxic nephritis (NTN), animals are injected with a heterologous antibody to GBM, which deposits in the kidney and causes transient injury (the heterologous phase). The animal then mounts its own immune response to the foreign immunoglobulin, which acts as a planted antigen on the GBM (the autologous phase). This model can be made more severe by pre-immunizing the animal with immunoglobulin from the species in which the anti-GBM antibodies are raised, such that immediate binding of the autologous antibody to the planted foreign immunoglobulin occurs (the accelerated or telescoped model of NTN). Although these models have been developed in various species, most recent studies have used either mice or rats.

Experimental autoimmune glomerulonephritis. Experimental autoimmune glomerulonephritis has been difficult to induce reliably in mice. However, in one study, the disease was strain-dependent and was linked to the MHC and to a Th1 dominant pattern of cytokine expression [22]. Nephritis could be transferred by autoantibodies (if T cell immunity in the recipient was intact), and by mononuclear cells. It has also proved possible to induce EAG in mice deficient in the inhibitory FcγRIIB, in a strain in which wild-type controls were resistant [23]. This suggests that the interaction of autoantibody with Fc receptors on leukocytes is an important step in the development of glomerular injury. In the FcγRIIB knockout animals, activation of leukocytes via FcγRI and FcγRIII was likely unopposed, although this has not been proved in this model. More recently, mice genetically engineered to produce only human antibodies developed nephritis following immunization with recombinant α3(IV)NC1. This disease was transferable by the "human" anti-GBM antibodies produced by immunized mice [24].

Experimental autoimmune glomerulonephritis in the rat is also strain-dependent, and the most consistent model is that induced in the Wistar-Kyoto (WKY) strain [25, 26]. These animals develop severe crescentic glomerulonephritis after immunization with crude GBM, and the disease can be induced with purified or recombinant α3(IV)NC1 [27, 28]. Also, EAG can be transferred by anti-GBM antibodies purified from the urine [29] or eluted from the kidneys of nephritic animals (abstract;

Reynolds J et al, *J Am Soc Nephrol* 12:639A, 2001). Wu et al induced EAG in WKY rats using a denatured form of recombinant α3(IV)NC1; in this system, glomerulonephritis was associated with sensitized T cells but not with antibody deposition [30]. The same group also demonstrated that EAG could be transferred by CD4+ T cells from nephritic rats, expanded in vitro by stimulation with α3(IV)NC1 [31]. No anti-GBM antibodies were detected in the recipients. This experiment provides the best direct evidence to date that T cells can cause autoimmune glomerulonephritis in mammals.

Taken together, these studies in EAG demonstrate that anti-GBM antibodies as well as antigen-specific T cells can be pathogenic under the right circumstances. Furthermore, autoimmune responses to α3(IV)NC1, the major antigen in human anti-GBM disease, are sufficient to induce crescentic glomerulonephritis. It thus seems likely that both humoral and cellular immunity can contribute to glomerular damage in Goodpasture's disease, although direct evidence of cell-mediated injury in patients is lacking.

Before the T cell transfer experiments, the role of T cells was implicated in EAG by the results of anti-T cell therapy. For example, in the Brown Norway (BN) rat, EAG can be inhibited by cyclosporine or by anti-CD4 antibodies [32]. In the WKY rat, blocking the major T cell co-stimulatory pathways is also effective in preventing disease. Separate studies have illustrated the role of blocking the B7/CD28 pathway with soluble CLTA4-Ig [33, 34] and blocking the CD40/CD40 ligand pathway with anti-CD40L monoclonal antibodies (abstract; Reynolds J et al, *J Am Soc Nephrol* 11:480A, 2000). Each of these approaches reduced both anti-GBM antibody production and severity of disease. A more direct role for effector T cells in EAG is suggested by a study in which anti-CD8 therapy did not affect circulating antibodies but significantly reduced glomerulonephritis [35]. An alternative strategy is to induce antigen-specific tolerance, mediated by regulatory T cell subsets. The induction of mucosal tolerance, by administering oral GBM prior to active immunization, was effective in reducing T cell responses to GBM as well as the development of glomerulonephritis [36]. Hopefully, one or more of these approaches to T cell immunotherapy will prove effective in human anti-GBM disease. Their clinical potential in glomerulonephritis is supported by reports of the effectiveness of anti-CD4 antibodies [37] and anti-thymocyte globulin [38] in patients with systemic vasculitis.

Nephrotoxic nephritis. Nephrotoxic nephritis in mice also can be difficult to induce. It seems to depend on the quality of the "nephrotoxic serum," typically raised in sheep. The disease is strain-dependent, with Th1-dominant strains developing crescentic glomerulonephritis in the accelerated model of NTN [39]. Considerable use has been made of knockout animals to demonstrate that the disease depends on Th1-related cytokines such as interleukin-12 (IL-12) and interferon γ [40, 41], and can be prevented by Th2-related cytokines such as IL-4 and IL-10 [42]. The role of chemokines in murine NTN [43] has been shown using mice deficient in monocyte chemoattractant protein-1 (MCP-1) or in chemokine receptors [44], and by therapeutic studies blocking MCP-1 [45]. In some mouse models of NTN, disease is critically dependent upon CD4+ T cells and can be induced in the absence of functioning B cells [46]. The importance of intrinsic renal cells in the pathogenesis of this model, presumably due to antigen presentation to T cells, is illustrated by the requirement for expression of MHC class II on renal cells [47]. However, other experiments in murine NTN support a major role for antibodies, as the disease can be prevented by lack of the stimulatory Fc receptors (FcγRI and FcγRIII) [48] and exacerbated by lack of the inhibitory receptor FcγRIIB [49]. Complement also appears to have an important role in the early stages of NTN. Mice deficient in C3 and C4 are protected from disease [50], whereas deficiency of complement-regulating proteins worsens disease [51], as does deficiency of C1q, probably by reducing clearance of glomerular neutrophils [52]. Recently, the role of proliferation of renal cells in murine NTN was demonstrated by a study in which Gas6-deficient mice were protected from disease [53].

Nephrotoxic nephritis in rats is similarly strain-dependent. In several strains, crescentic nephritis can be achieved using an accelerated model. However, in the WKY rat, a single injection of nephrotoxic serum leads rapidly and consistently to severe crescentic glomerulonephritis [54]. Nephrotoxic nephritis in the rat has been used to demonstrate the effectiveness of different forms of anti-inflammatory therapy [55], for example, blockade of inflammatory cytokines, such as tumor necrosis factor (TNF), IL-1, and migration inhibitory factor (MIF) [56, 57], or administration of anti-inflammatory cytokines, such as IL-4 and IL-11 [58, 59]. The use of macrophages transfected with adenovirus to express IL-4 has been reported as a form of "gene therapy" [60]. The inhibition of chemokines, for example, fractalkine or MCP-1, also has been effective in preventing disease [61, 62]. An alternative approach has been blockade of the adhesion molecules important in leukocyte migration across the endothelium. Blockade of both leukocyte function associated molecule-1/intercellular adhesion molecule-1 (LFA-1/ICAM-1) [63] and very late antigen-4/vascular cell adhesion molecule-1 (VLA-4/VCAM-1) [64] interactions has proved effective. The expression of several inflammatory cytokines and adhesion molecules depends on transcription factors such as nuclear factor-kappaB (NF-κB). Decoy oligodeoxynucleotides (ODN) to NF-κB reduce glomerular inflammation in NTN [65]. The progression of inflammation to scarring and renal failure can be inhibited by approaches such as antibodies to very late antigen-1 (VLA-1) [66].
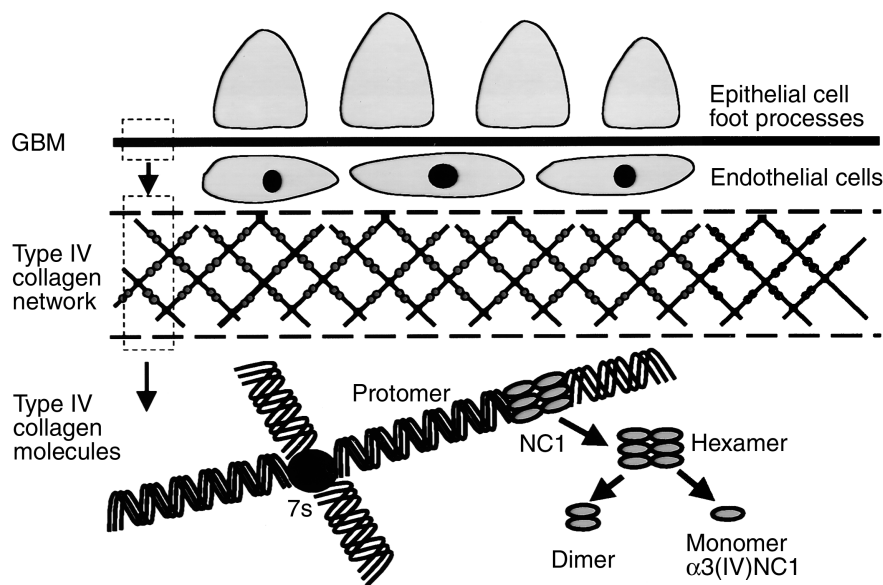
Fig. 2. Type IV collagen exists as a complex supramolecular network. Three individual α3(IV) chains are interwoven to form a triple helix, known as the protomer. The NC1 domains are arranged in a head-to-head fashion to form hexamers, and binding through the 7S domains completes the network structure. Hexamers can be dissociated to form dimers and monomers of NC1 domains, allowing identification of α3(IV)NC1 as the autoantigen (redrawn with permission from [72]).

As in EAG, it seems that there is a role for both anti-GBM antibodies and T cells in different animal models of NTN. Therapeutic studies, particularly in the rat, have identified a number of targets for novel anti-inflammatory therapy. Some of these are already being explored in human disease. The use of anti-TNF antibodies is currently being investigated in crescentic nephritis related to systemic vasculitis, and initial results seem promising (personal observations). Antibodies that block the adhesion molecule CD18 have been successful in similar patients [67]. It remains to be seen which of these novel approaches, or which combination, will be most useful in clinical practice.

Pathogenesis: Human anti-GBM disease

Anti-GBM disease is widely regarded as a good example of an autoantibody-mediated disease. Much of the research into pathogenesis therefore has involved studies of the specificity and pathogenicity of anti-GBM antibodies. More recently, the role of T cells has attracted increasing interest, particularly in coordinating the autoimmune response, but also in their potential as effector cells (as shown in animal models). I shall briefly consider what is known both about autoantibodies and T cells in this disease.

Strong evidence indicates that anti-GBM antibodies are directly pathogenic. The presence of these antibodies is consistently linked to development of disease, and antibody levels and severity of disease are broadly correlated in some series [68]. Even more convincing, anti-GBM disease recurs immediately in renal allografts if the recipient still has circulating antibodies [1]. The classic transfer experiments by Lerner et al, more than 30 years ago, showed that antibodies eluted from the kidneys of patients with anti-GBM disease could cause glomerulonephritis when injected into squirrel monkeys [69].

The main target of these autoantibodies is the noncollagenous domain (NC1) of the α3 chain of type IV collagen [α3(IV)NC1] [70, 71]. Figure 2 shows the localization of α3(IV)NC1 in the GBM [72]. This molecule is found together with α4 and α5 chains in certain specialized basement membranes, such as those of the kidney, lung, choroid plexus, retina, and cochlea [73]. The GBM also contains a separate network of α1 and α2 chains, which are present in all vascular basement membranes. Although sera from all patients recognize α3(IV)NC1, sera from some patients also can bind less strongly to other type IV collagen chains. Competition studies using monoclonal antibodies to α3(IV)NC1 suggest that antibodies from most patients bind to a common epitope [74]. The precise localization of this epitope was subsequently determined using chimeric recombinant molecules containing regions of α3(IV)NC1, together with regions from the non-antigenic α1(IV)NC1 [75–77]. The advantage of this approach is that the recombinant molecule should maintain its three dimensional structure, allowing recognition of conformational epitopes by anti-GBM antibodies. Several groups have identified a major epitope in the amino terminal region of α3(IV)NC1 and, in one study, clinical outcome correlated with the level of antibodies reactive with the construct containing α3 at the amino terminus [76]. Sera from some patients also react less strongly with a site in the carboxyl region of the molecule, and it is possible that certain autoantibodies cross-react with these two homologous sites, whereas others might recognize a conformational epitope formed by a combination of both sites [78]. The major amino terminal epitope has been mapped in detail by substituting

Table 1. Treatment of anti-glomerular basement membrane (anti-GBM) disease

Initial	
Plasma exchange	Daily, 4 L exchange for 5% human albumin solution; use 300 to 600 mL fresh frozen plasma within 3 days of any invasive procedure (e.g., biopsy) or in patients with pulmonary hemorrhage; continue for 14 days or until antibody levels are fully suppressed; withhold if platelet count $<70 \times 10^9$/mL, or hemoglobin <9 g/dL; watch for coagulopathy, hypocalcemia, and hypokalemia
Cyclophosphamide	Oral dosing at 2 to 3 mg/kg/day (round down to nearest 50 mg; reduce to 2 mg/kg/day in patients over 55 years); stop if white cell count $<4 \times 10^9$/mL and restart at lower dose when counts $>4 \times 10^9$/mL
Prednisolone	Oral dosing at 1 mg/kg/day (maximum 60 mg); reduce dose weekly to 20 mg by week 6 and then more slowly; no evidence for benefit of intravenous methylprednisolone and can increase infection risk (possibly use if plasma exchange not available)
Prophylactic treatments	Oral nystatin and amphotericin (or fluconazole) for oropharyngeal fungus infection; ranitidine or proton-pump inhibitor for steroid-promoted gastric ulceration; low-dose cotrimoxazole for *Pneumocystis carinii* pneumonia prevention; consider acyclovir as cytomegalovirus prophylaxis; consider calcium/vitamin D for prevention of osteoporosis (but relatively short course of steroids)
Maintenance	
Prednisolone	Reduce dose slowly from 20 mg at 6 weeks; stop completely by 6 months
Cyclophosphamide	Stop after 2 to 3 months; no further cytotoxic agents necessary

individual amino acids from the α1 to the α3 chain, and 9 residues critical for binding have been identified [79]. Hudson's group recently described the quarternary organization of the Goodpasture antigen, in particular where the major epitope is located within the NC1 hexamers formed from cross-linked α3/α5 heterodimers and α4/α4 homodimers [80].

Although anti-GBM disease is regarded as a prototypic autoantibody mediated disease, it is clear that T cells also play a vital role. We know that T cell help is required for the class-switched and highly specific antibody response, and T cell involvement also can be implied from the strong MHC associations. In addition to providing T cell help for antigen-specific B cells, effector T cells might contribute directly to tissue injury. Both CD4+ and CD8+ cells are found in affected glomeruli [81], but their specificity has not yet been determined. However, proliferation assays can detect T cells reactive with α3(IV)NC1 in the circulation [82]. Interestingly, T cells from normal individuals also show some proliferative capacity to the autoantigen in the absence of anti-GBM antibodies. We have found that α3(IV)NC1 is expressed in the thymus, so central tolerance should be present, but some T cells presumably escape this mechanism [83]. The finding of autoreactive T cells in healthy individuals is not unique to Goodpasture's disease and has been reported in other autoimmune conditions.

The frequency of T cells specific for α3(IV)NC1 is higher in patients than in controls [83]. This high frequency in patients diminishes with time from disease onset and reaches levels comparable to controls after a number of years [83]. Our recent data suggest that this reduction in frequency is accompanied by the emergence of a CD4+ CD25+ subset of regulatory T cells (abstract; Salama AD et al, *J Am Soc Nephrol* 13:664A, 2002). The epitopes of α3(IV)NC1 recognized by autoreactive

T cells have proved hard to define. The use of linear peptides spanning the α3(IV)NC1 sequence has yielded varying results. We have found it difficult to demonstrate T-cell responses to such peptides, but others have reported more promising findings using freshly isolated cells from acute patients (abstract; Cairns LS et al, *J Am Soc Nephrol* 11:473A, 2000). However, no dominant T-cell epitope has been clearly defined.

Treatment and outcome

Untreated anti-GBM disease has an almost universally poor outcome, with death from renal failure or lung hemorrhage [1]. The use of steroids alone conferred little benefit, but the introduction of combined treatment with plasma exchange, cyclophosphamide, and corticosteroids in the 1970s revolutionized the outcome in this disease [84]. The rationale of this approach is that plasma exchange rapidly removes the pathogenic autoantibodies, cyclophosphamide prevents further antibody synthesis, and steroids act as a powerful anti-inflammatory agent. A rapid reduction in anti-GBM antibody levels appears to be necessary in view of the speed of glomerular damage, and this cannot be achieved by drug therapy alone. Only one small trial has compared plasma exchange with drug treatment alone; this study demonstrated a more rapid fall in anti-GBM antibodies and suggested a trend toward a better outcome in the plasma exchange group [85]. However, trials are difficult in such a rare disease, and the improved outcome with plasma exchange reported in many series makes it unlikely that further trials will be attempted.

The treatment protocol used at the Hammersmith Hospital is shown in Table 1. It is important to note the intensive use of plasma exchange in this regimen, which is generally successful in reducing anti-GBM antibody levels to near normal within 2 weeks. Plasma exchange

Table 2. Outcome of patients with Goodpasture's disease

	Number of patients	1-year patient survival %	1-year renal survival %	Renal recovery if initial creatinine >600 μmol/L (6.6 mg/dL) % *treated patients*	Notes
Johnson et al [85]	17	94	45	0	Randomized prospective study of plasma exchange
Walker et al [86]	22	59	45	18	Australian single center; all plasma exchanged
Savage et al [68]	59	75	8.5	NA[a]	Data from multiple British centers;
	49	84	35	11	Hammersmith Hospital single center
Bouget et al [87]	14	79	29	0	French single center
Herody et al [88]	29	93	41	0	French single center; most plasma exchanged
Merkel et al [89]	35	89	40	6	Survival at time of analysis; all plasma exchanged
Daly et al [90]	40	—	20	0	All plasma exchanged

[a] NA, not available.

may be continued for longer if anti-GBM antibodies are still detectable, in the presence of clinical evidence of disease activity. In general, the disease does not relapse, and immunosuppressive drugs can safely be discontinued within a few months. Patients with both anti-GBM antibodies and ANCA receive the same initial treatment as those with pure anti-GBM disease, but the former then receive maintenance immunosuppression, as used in ANCA-positive systemic vasculitis. In our experience, these "double-positive" patients behave the same as those with isolated anti-GBM disease in their early response to treatment (abstract; Levy JB et al, *J Am Soc Nephrol* 12:113A, 2001), although their subsequent course can be more similar to that of ANCA-associated vasculitis.

In most recent series, 1-year patient survival in anti-GBM disease is between 75% and 90%. However, renal recovery is less common and depends on renal function at the start of treatment. Several series show that most patients starting treatment with a creatinine of less than 600 μmol/L (6.6 mg/dL) will recover renal function, but that recovery is rare in patients with an initial creatinine of more than 600 μmol/L (Table 2) [68, 86–90]. We recently analyzed the Hammersmith Hospital series of 71 patients who received the regimen I described [9]. This was a severely affected cohort: 39 patients required dialysis at presentation, 13 had an initial creatinine greater than 500 μmol/L (5.7 mg/dL), and 19 had a creatinine of less than 500 μmol/L. Pulmonary hemorrhage was present in 62% and appeared commoner in younger men. The patients who presented with creatinine less than 500 μmol/L had an excellent outcome at one year, with 100% surviving and 95% retaining independent renal function. Of those with a creatinine greater than 500 μmol/L, but not requiring dialysis immediately, 83% survived 1 year, and independent renal function was maintained in 82% of survivors. However, the outcome was not as good in dialysis-dependent patients, with 65% surviving 1 year, and only 8% of those survivors retaining

Table 3. One-year outcome in treated anti-glomerular basement membrane (anti-GBM) disease at Hammersmith Hospital

	Number	Patient survival %	Renal survival %
Creatinine <500	19	100	95
Creatinine >500	13	83	82
Dialysis	39	65	8
Total	71	77	53

independent renal function (Table 3). Analysis of renal outcome at 1 year, related to the percentage of crescents on renal biopsy and to the initial serum creatinine, showed that neither a high percentage of crescents nor a high serum creatinine precluded recovery. However, none of the patients with 100% crescents and undergoing dialysis recovered independent renal function.

Long-term follow-up of these patients (Fig. 3) showed that renal function was maintained over several years, with 5-year renal survival of 94% for those presenting with creatinine less than 500 μmol/L, and 50% for those with creatinine more than 500 μmol/L. These results are of particular interest, as they demonstrate that with intensive treatment many patients with a serum creatinine greater than 500 μmol/L can recover renal function. Today's case illustrates this outcome. We would argue that all patients not established on dialysis should be offered treatment with plasma exchange and immunosuppression. For those already on dialysis, the question of treatment is more difficult. It could be argued, in view of the low probability of recovery, that the risks of immunosuppression outweigh the potential benefit. However, for younger patients with evidence of recent crescents on biopsy, and who are not yet anuric, aggressive therapy still could be considered. The presence of lung hemorrhage, which resolved in 90% of our patients, provides a separate indication for intensive treatment, regardless of the severity of renal disease.

Summary

Anti-GBM disease is due to an autoimmune response to α3(IV)NC1, a major component of glomerular, alveo-
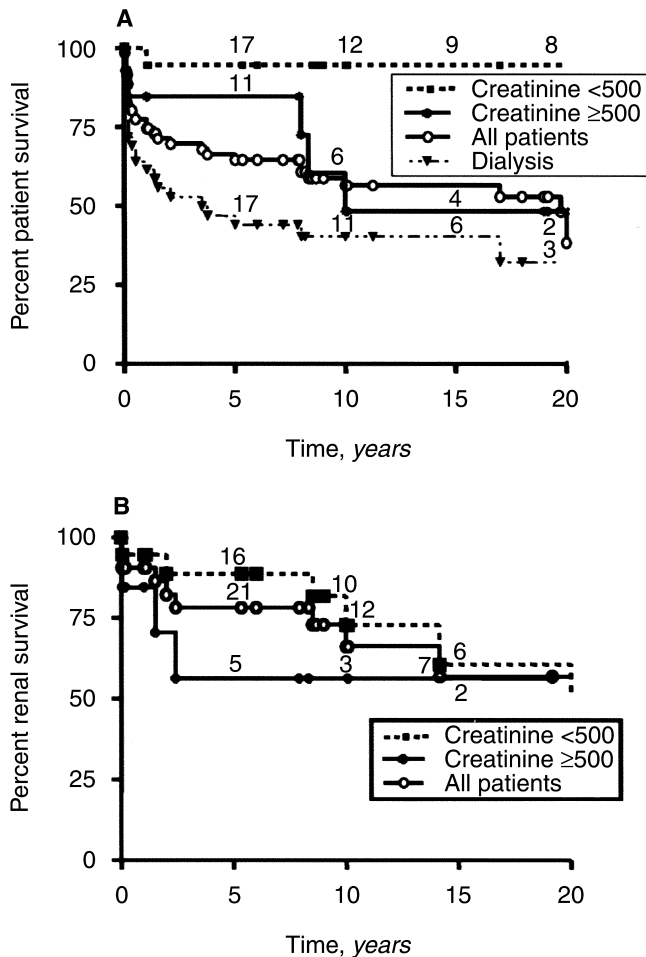
Fig. 3. Long-term patient and renal survival in treated patients with anti-GBM disease. (*A*) Patient survival. The difference between patients with an initial creatinine concentration less than 500 μmol/L and the other groups was significant (*P* = 0.0055). (*B*) Renal survival. Only patients who did not require dialysis at presentation are included. The difference between the two groups was not significant (redrawn with permission from [9]).

lar, and other specialized basement membranes. The epitopes of α3(IV)NC1 recognized by autoantibodies have been characterized at a molecular level, but T-cell epitopes are proving more elusive. Treatment with a combined regimen of prednisolone, cyclophosphamide, and plasma exchange has revolutionized the outcome of this condition. Most patients presenting with a creatinine of less than 500 μmol/L (5.7 mg/dL) will recover long-term independent renal function, as will about one-half of patients presenting with a creatinine greater than 500 μmol/L but not on dialysis. Renal recovery is rare in patients requiring dialysis before treatment can be started. Studies of experimental models of anti-GBM disease suggest that anti-GBM antibodies as well as antigen-specific T cells play a role in pathogenesis. The potential of new approaches to treatment, including regulation of the autoimmune response and modulation of

inflammatory mechanisms, has been demonstrated in these models. The future challenge is to determine whether these approaches can provide more effective and safer treatment for patients with anti-GBM disease or other types of crescentic glomerulonephritis.

QUESTIONS AND ANSWERS

DR. JOHN T. HARRINGTON (*Division of Nephrology, Tufts-New England Medical Center, Boston, Massachusetts*): Has the incidence of anti-GBM disease decreased since influenza vaccination has become standardized across western Europe and the United States? Also, why do you think your patients did so well, particularly those who had a serum creatinine above 500 μmol/L?

PROF. PUSEY: Your first question relates to the role of infection in causing or triggering this disease. It seems likely that Goodpasture's original patient had influenza, and there are several reports of clusters of cases, suggesting infection as a cause. There have also been a few studies relating infections, such as influenza, to development of disease. I do not know of any rigorous case controlled studies, and I am unconvinced that any specific infectious disease has been linked to anti-GBM disease. I think it is a little too early to say whether the incidence has changed following introduction of the influenza vaccine. The various series of rapidly progressive glomerulonephritis that I have reviewed in the literature appear to show a pretty constant incidence of anti-GBM disease. In most studies, it is probably the second most important cause to ANCA-positive rapidly progressive glomerulonephritis (RPGN). The incidence does not seem to be decreasing; on the other hand, it does not seem to be rising either. I do not think the flu vaccine has altered the incidence very much.

In response to your second question about outcome, in the series I presented, the majority of patients with a creatinine of over 500 μmol/L but not on dialysis recovered [9]. I think recovery might relate to the intensity of the plasma exchange regimen we use and the speed with which we use it, because, in many other published series, plasma exchange is used at a lower volume per exchange and at a lower frequency. For example, some centers might use 2.5 or 3.0 L two or three times a week. Even if they are attempting to use it daily, they probably do not because it is time-consuming for the nursing staff, and it involves a lot of equipment and extra effort. So there are drawbacks to plasma exchange inasmuch as it is difficult to deliver. By keeping our regimen at 14 daily 4 L exchanges, our practice differs from that in other series. I cannot tell you whether that is the only difference, but it is one difference that is apparent.

DR. INDRANIL DASGUPTA (*Birmingham Heartlands Hospital, Birmingham, U.K.*): Is there any justification for using plasma exchange in a patient with anti-GBM disease who requires dialysis at presentation but who

has no evidence, clinical or otherwise, of pulmonary hemorrhage?

PROF. PUSEY: If a patient has any evidence of lung hemorrhage, then that is a separate indication for plasma exchange. Lung hemorrhage rapidly kills you if untreated, but it responded in 90% of patients in our study [9]. Hemorrhage is not always obvious on a chest radiograph, and the patient does not always cough up blood; a falling hemoglobin disproportionate to the degree of renal failure might alert you. If so, then you can perform the KCO, a sensitive way of detecting lung hemorrhage [10]. If no lung hemorrhage is present and the patient already is on dialysis, then you have to think very hard. If the patient has severe renal failure but is not on dialysis, I would argue that you should treat, and our results support that argument, even though previous thoughts were that you should not treat patients with a creatinine of over 600 μmol/L. After dialysis is initiated, you need to tailor your decision to each individual. For a young patient who has recently started dialysis, particularly if the biopsy shows acute changes and some normal glomeruli, you would discuss the risks and benefits of treatment. In an elderly patient, who is less able to tolerate treatment, and in whom there is extensive scarring on biopsy, you should not treat. Thus, there would be exceptions in which you might treat dialysis-dependent patients, and that depends on their clinical state and renal biopsy.

PROF. JOHN FEEHALLY (*Department of Nephrology, Leicester General Hospital, Leicester, U.K.*): Charles, as you rightly pointed out, we are past the 25th anniversary of the treatment regimen, and we are all still doing exactly the same thing. My question is about how you would test new treatment. You have a treatment that works and saves lives, and the things that you might want to add as new treatments will be quite potent, such as anti-T-cell therapy or anti-TNF therapy. How are you going to add them without increasing toxicity? How are you going to decide what you leave out, and how are you going to judge success?

PROF. PUSEY: I will answer by referring to our practice in systemic vasculitis first. In systemic vasculitis, we use the same drugs, cyclophosphamide and prednisolone. In advanced cases, as you know, we also use plasma exchange, which might be superior to methylprednisolone in this situation. We have investigated anti-TNF antibodies as additional therapy in patients who present with systemic disease and renal involvement, with clinical features like those in the CYCAZAREM trial [91]. We then compared the rate of response with anti-TNF therapy, using the Birmingham Vasculitis Activity Score (BVAS), renal function, and markers of disease, with a well-studied cohort in CYCAZAREM. So we are using a historic comparator before designing a randomized controlled trial. We also have used anti-TNF antibodies in patients who are initially in remission, and then start

to relapse, or who show evidence of "grumbling" disease, and we add anti-TNF as the only change in therapy.

In Goodpasture's disease, we cannot study relapse because it very rarely relapses, but we could add anti-TNF antibodies to the existing therapy at presentation and look at the outcome compared with historic series from our own and other units. I think a historic comparison is probably the only way to go initially. If this approach appeared not to cause any harm, or even to show a possible benefit, then my aim would be to try and remove the more harmful parts of the current protocol successively—to reduce, for example, the high dose of steroids initially, or to limit the dose of cyclophosphamide. So the answer to your question is that it would be very difficult to do a trial when you have patients with a severe disease and a treatment that works. However, I am keen that we try and if our experience in vasculitis is very positive, that might convince us use a similar approach in anti-GBM disease.

PROF. ANDREW REES (*Department of Medicine and Therapeutics, Institute of Medical Sciences, Aberdeen, U.K.*): Charles, you alluded in passing to a very interesting group of patients who had linear staining of the GBM with essentially negative anti-GBM ELISA, but positive anti-GBM antibodies by a much more sensitive assay, the biosensor assay. Can you tell us more about the phenotype of those patients? Did they have severe nephritis and, in particular, is there any hint of a predominant role of the T cells in that particular group?

PROF. PUSEY: These patients, in fact, were fairly typical: they all had severe nephritis. They had linear deposits of immunoglobulin as detected by our renal pathologist, but the standard ELISA assays were negative. At the time, we treated them according to the immunofluorescence, because we were convinced by the clinical features and by the pathologic findings. Only when we subsequently went on to develop the biosensor assay did we look at these patients in more detail and find that they had detectable anti-GBM antibodies. They were toward the lower end of the limit that we detected on biosensor in known positive patients, but they were clearly different from all of the controls. I did not look specifically to see whether there were many more T cells in their glomeruli. The immune response in those patients might have been a little more tipped toward T cells and a little less toward antibodies.

PROF. REES: There of course are ancient examples from the 1970s of negative immunofluorescence on the first biopsy of a patient with crescentic nephritis, followed by positive immunofluorescence on the second biopsy shortly afterwards.

PROF. PUSEY: Yes, you are right, and this supports your suggestion for a predominant role for T cells at an early stage in some cases.

DR. JEREMY HUGHES (*Centre for Inflammation Re-*

search, University of Edinburgh Medical School, Edinburgh, U.K.): You mentioned clustering. It is certainly striking when you see it, because recently in Edinburgh we have had two unrelated patients who presented with this disease and who live on the same street just by a railway track and a gas station. Do you think that environmental agents, apart from infection, might play a role either in initiation or pathogenesis of this disease? My second question is whether there is a human equivalent of the Lewis rat, that is, someone who makes antibodies but is resistant to getting disease?

PROF. PUSEY: The environmental question has been widely addressed. As you know, a number of cases have developed after prolonged and excessive hydrocarbon exposure. A case control study in the north of England looked at workers in a factory who were either exposed to petroleum-based oils or not [20]. Those with considerable exposure appeared to have higher levels of anti-GBM antibodies, although usually not outside the normal range. So I think hydrocarbons indeed might be important. Smoking also might play a role and, by damaging the alveolar basement membrane, might release sequestered antigens and lead to an increased incidence of disease. However, making etiologic links is quite difficult, because it is also clear that smoking might just increase the degree of lung damage in those who already have anti-GBM antibodies. I would suggest that this is the mechanism in most of these cases; if you have anti-GBM antibodies circulating, then smoking will make the alveolar capillaries more permeable or activate macrophages, and the disease becomes manifest. In short, there are associations between anti-GBM disease and infection, hydrocarbons, and smoking. I am not absolutely convinced that these are proven etiologic factors. Clearly, something in the environment must be involved, because around 25% of us are walking around with DR2, yet only a very small percentage of us will get Goodpasture's disease. So the MHC associations alone do not go anywhere near to explaining why you get the disease, and other genetic and environmental factors presumably contribute to its development.

With regard to the rats, obviously the WKY strain is exquisitely sensitive; some other rats make a minor response, Brown Norway for example, and others appear to be completely resistant, like the Lewis. As you may know, we currently have a breeding program to study genetic linkages with disease in the WKY strain, and we found that F1 animals, that is, cross of Lewis and WKY, are totally resistant, suggesting that resistance is dominant. You can cross WKY rats with the F1 rats and make a backcross (BC1) generation. In these backcross animals, a proportion of them are susceptible, and a proportion of them are resistant. We have bred a large number of these, and we are doing a genome-wide linkage analysis to try and identify genetic factors over and

above the MHC. With regard to humans, a few patients have presented with hematuria and lung hemorrhage and do not appear to have developed crescentic nephritis, at least within the time they have been followed before treatment. These people might possess other genes that are protective. I would not be at all surprised if humans show a range of inflammatory responses to the deposition of a certain amount of antibody, but I do not have results to support this suggestion. Maybe if we can track down the susceptibility genes in the rats, we can use these as candidate genes in the patients.

DR. AJAY SINGH (*Renal Division, Brigham and Women's Hospital, Boston*): My question has to do with the issue of patients with Alport's syndrome developing what appears to be anti-GBM disease, particularly in the context of a second transplant. How do you treat this disease in the context of transplant immunosuppression, and what strategies do you use to prevent it?

PROF. PUSEY: The first thing is to be aware of the possibility and to get the diagnosis of Alport's right. Then you can monitor anti-GBM antibody production carefully. As you know, the production of anti-GBM antibodies and their deposition in the kidneys of patients with Alport's after a first allograft is not particularly common, maybe 10% to 20%. Severe nephritis is very uncommon. If we were giving a patient with Alport's a first allograft, we would monitor circulating anti-GBM antibodies regularly, make sure we did fluorescence or peroxidase studies on any renal biopsies, and consider the possibility of Goodpasture's disease carefully. If it looked as if this were developing, we would treat the patient using the strategy I previously outlined. In patients developing disease after a second graft, the disease seems to be much more aggressive. We have seen two such patients, tried to treat them, and failed in both. It looks like the stimulus of a second large dose of what is essentially an allo-antigen is just too much. For the benefit of those who do not look after this sort of patient, most cases of Alport's are X-linked and missing $\alpha5$ chains of type IV collagen, but they can get an immune response not only to $\alpha5$ but also to $\alpha3$ and $\alpha4$, which are not incorporated correctly in the collagen network. There are rare autosomally inherited cases of Alport's in which the patient is defective in $\alpha3$ or $\alpha4$ chains; anti-GBM disease in the grafts also has been reported [19]. So anti-GBM disease in Alport's patients who have undergone renal transplantation is not typical, because it involves a response to $\alpha5$ in many cases, which is not the case in the native kidney of patients with Goodpasture's disease. If it occurs in a second graft, which is what you are asking, it is very difficult to treat, and I would be interested to hear whether anyone has successfully treated these patients.

DR. SINGH: Have you treated patients with plasmapheresis before they get the second graft?

PROF. PUSEY: We have not. As it happens, they have

been referred to us with evidence of disease following a second graft, and they have been treated rapidly and aggressively but have not responded.

PROF. REES: We have attempted to prevent recurrence in one patient using the sort of regimens that people have used for preventing graft rejection in patients who have pre-formed antibodies to the graft, and we have failed. We used a combination of plasma exchange plus cytotoxic drugs.

PROF. CAROLINE SAVAGE (*MRC Centre for Immune Regulation, The Medical School, University of Birmingham, Birmingham, U.K.*): Charles, you put up a very good case for T cells as effector cells in anti-GBM disease. In fact, the current effective therapy that you outlined is geared to antibody removal and against the B cell. If you are really contemplating the introduction of anti-T-cell therapy in this disease, are you not in danger of removing the good regulatory T cells?

PROF. PUSEY: I think you are. If CD8+ T cells were involved in humans, which I think we have yet to demonstrate, then perhaps an anti-CD8 antibody in addition to conventional therapy would be a good approach. Standard immunosuppressive therapy, including cyclophosphamide, affects both B and T cells and clearly induces a response. But targeting pathogenic T cells specifically would be a good idea. I think one aspect of your question is that if you wanted to target activated T cells, they would be CD4+ and CD25+, as they express CD25+ as part of their activation state. These might be the bad guys. The same phenotype, CD4+ and CD25+, is present on some regulatory subsets, so you would perhaps want to target the bad ones but end up damaging the good ones. Ideally, you would spare the good ones, or perhaps even stimulate their activity. It is not a straightforward situation. On balance, in the acute phase of the disease, you would want to make sure you targeted autoaggressive T cells as hard as possible but did not inhibit development of regulatory T cells, which could be generated later. At present, we do not know enough about the phenotypes of the different regulatory subsets to be able to do this.

DR. ALAN SALAMA (*Renal Division, Brigham and Women's Hospital, Boston*): We looked at CD25+ regulatory cells in alloresponses in patients who had received anti-IL-3 therapy, and such therapy does not seem to preclude development of regulatory cells. So, partly, I believe there is a clone size effect. If you get rid of enough autoreactive T cells, the regulatory cells can then emerge. My second point relates to Andy Rees' question about T cells in patients who did not have detectable antibodies. We only studied one such patient in terms of T-cell responses. Actually, he had a very high T-cell response, so it might be useful to look at patients who do not have circulating antibodies to see whether they

are a subset of Goodpasture's patients who have a more T-cell–mediated disease.

DR. CHRISTOPHER WINEARLS (*Renal Unit, Oxford Radcliffe Hospital, Oxford, U.K.*): Charles, your case presentation includes a rather intriguing comment that, on renal biopsy, the interstitium contained a dense mononuclear cell infiltrate. Did the biopsy reveal antibody binding to the tubular basement membrane? Does tubular injury, perhaps related to deposition of antibody on the tubular basement membrane, affect renal function? Also could the treatment, which was remarkably effective in this case considering the glomerular injury, in fact be a result of dealing with tubular injury rather than glomerular injury?

PROF. PUSEY: That is a valid point. Indeed, in many studies of anti-GBM disease, one also finds deposition of IgG on the tubules. In fact, immunoperoxidase staining in the case I showed did demonstrate both glomerular and tubular deposition of immunoglobulin. It appears to be the distal tubules that express the Goodpasture antigen, rather than the proximal tubules; nonetheless, antibody is deposited in the tubules. This deposition might, for example, recruit macrophages or other inflammatory cells from the circulation and be responsible for the tubulointerstitial infiltrate. However, I think the tubulointerstitium can become inflamed via other mechanisms, one of which is possibly ischemic, because you can imagine that the crescentic glomeruli are not letting a lot of blood through to the interstitium. Another mechanism might be the flow of inflammatory mediators, such as cytokines, along the tubules, which could stimulate or injure the tubular cells from the luminal side. Third, as you saw in the high-power view of the biopsy specimen (Fig. 1B), when this disease is very severe within the glomerulus, you see breaks in Bowman's capsule, followed by direct spillover of cells outside the capsule. Often the interstitial inflammation is strongly periglomerular, as it was in this patient. I can therefore give you a number of potential mechanisms whereby tubulointerstitial nephritis might develop, although I cannot tell you exactly by which mechanism it occurs. I do think it is very important in determining prognosis of the disease, although we have not specifically examined it in our series.

DR. CHARLES TOMSON (*Renal Unit, Southmead Hospital, Bristol, U.K.*): You mentioned that anti-GBM disease is monophasic, and that sets it apart from many of the other autoimmune diseases that we see. Is there anything in the human or animal studies that tells us why it is monophasic?

PROF. PUSEY: Not directly, but I suspect one explanation is that the antigen responsible is "relatively" cryptic. The antigen is held within the NC1 hexamer within the basement membrane of the kidney and the lung. It is not immediately accessible to antigen-presenting cells or

T cells, and it is not usually exposed in an inflammatory milieu. It might be exposed, however, under certain circumstances. For example, lung infection can reveal alveolar antigen, and renal infection or obstruction can reveal kidney antigen. Indeed, several instances of anti-GBM disease have developed after either lithotripsy or obstruction of the kidney. It could just be that a human with a severe chest infection is like a rat immunized with GBM in Freund's adjuvant. If you allow antigen presentation in an inflammatory situation, you might stimulate an immune response. That state of stimulation then resolves, because either the infection gets better or we stop doing lithotripsy, and there is no longer an additional push of antigen into the immune system. Regulatory mechanisms can then come into play. I think that might be the case in Goodpasture's. The antigenic stimulus overcomes natural tolerance mechanisms for a while, and then the body tips back into equilibrium. It is the mechanisms by which that happens, and how we can push the human from activation of these T cells to tolerance, that we need to learn more about. Perhaps in auto-immune responses involving other antigens, such as DNA or histones in lupus, antigens might be more widespread, accessible, or prone to being presented.

PROF. PETER MATHIESON (*Renal Unit, Southmead Hospital*): Could you speculate on how much of the effectiveness of plasma exchange is due to the removal of circulating mediators rather than to the removal of the pathogenic antibody itself? Second, I have a practical question. In an acute renal failure practice, anti-GBM disease is probably the only disease in which a delay of a day or two makes a major difference to outcome, and yet it is a very rare disease. How can we balance these two things? Should we be sending serum samples from our referring hospitals to you or someone else who can do an anti-GBM assay so that we can deal with the delay that inevitably occurs in this country, and I guess in other health care systems, in transferring patients to a center where they can be managed?

PROF. PUSEY: In reply to your first question, we and others have also used immunoabsorption using protein A [92]. We have used it in one or two patients who, for various reasons, could not receive human blood products; their disease was quite advanced, and they did not recover despite rapid removal of antibody. Swedish groups have used protein A immunoabsorption more extensively in Goodpasture's disease, and I've seen isolated reports of patients who did not initially respond to plasma exchange but then did better with immunoabsorption [92]. The rationale is that you can remove immunoglobulin more quickly using protein A immunoabsorption. Despite these promising reports, we have not started using immunoabsorption as our major therapeutic strategy, because plasma exchange also removes inflammatory mediators, for example, complement components

and coagulation factors. By not removing them and focusing on the antibody, you might lose some of the therapeutic effect. We have become slightly trapped by the success of the current regimen. Much as I would like to be doing selective or even specific immunoabsorption, I think we are not yet in a position to do that.

With regard to your second question, I think that as part of your acute renal failure screen, in the presence of suspicious circumstances like proteinuria or hematuria, an urgent anti-GBM assay should be performed. Because the disease is treatable if diagnosed early, I think this approach is justified.

PROF. DAVID OLIVEIRA (*Division of Renal Medicine, St George's Hospital Medical School, London*): With respect to the mechanism of the protective effect of certain MHC alleles, there are, as you hinted, other mechanisms apart from determinant capture. For example, do you have any information on the restriction of the regulatory population? Or do you have any information on mechanisms of the dominant nonresponsiveness in your F1 hybrid rats?

PROF. PUSEY: Those are good questions, but I do not have a good answer yet. Antigen-specific regulatory T cells can be detected in patients following stimulation with $\alpha 3(IV)NC1$, but we do not know how the protective or susceptibility MHC alleles influence their development. In our rat models, it is clear that the MHC is one factor, because in WKY rats from different sources, with either a RT1-l or RT1-k haplotype, those with RT1-l get severe disease, whereas those with RT1-k get much less severe disease. In our breeding program, we have matched the MHC such that both the WKY and the Lewis rats are RT1-l, so no difference in MHC exists across the parental strains. The difference in responsiveness in F1 animals is therefore not directly related to MHC genes in that particular cross; it must depend on other genes influencing immune or inflammatory responses [93]. I agree that the MHC will have effects other than that on peripheral antigen presentation and could be involved in molding the T-cell repertoire. Our studies of infiltrating glomerular T cells in EAG show restricted T-cell receptor gene usage, and further work on this might be informative (abstract; Habib A-MN et al, *J Am Soc Nephrol* 13:172A, 2002). For example, we could look at different crosses and see whether the same CDR3 sequences are used.

DR. WILLIAM NELSON (*Renal Unit, Belfast City Hospital, Belfast, Northern Ireland*): Charles, can you characterize the small number of patients who relapse?

PROF. PUSEY: One of our patients who relapsed was a young man who was incapable of stopping smoking for any significant period. He did in fact give up smoking for a time after he came off intensive care during the initial illness, but then he started again. He had at least two episodes of relapse with lung hemorrhage and ne-

phritis, the first of which was successfully treated using the same regimen as before and which resulted in his stopping smoking. The final episode, when he started smoking again, led to severe lung hemorrhage, which he survived, then severe nephritis, which did not respond, and he ended up on dialysis.

The second patient who relapsed worked as a hairdresser and regularly used volatile aerosol hairsprays. We considered suggesting that she change her profession, but this was not practical because she was dependent on her job. She had a minor relapse, changed the sprays she used, and did not have another relapse. She is still well and under regular follow-up several years later. I do not know whether these anecdotes have any bearing on the incidence of relapse, but in both cases there was a potential environmental factor.

DR. NELSON: A patient of ours relapsed 18 months after an initial acute episode. He had negative anti-GBM titers on our conventional ELISA assay, linear IgG on the biopsy, and a positive ANCA. Has this been your experience?

PROF. PUSEY: Briefly, double-positive patients are initially similar to those with anti-GBM disease, that is, they are unlikely to come off dialysis. However, the continuing management of those patients should be along the lines of that for systemic vasculitis. Although the anti-GBM antibody goes away, and nearly always stays away, the ANCA tends to recur. Many of our patients, having recovered from their initial anti-GBM and ANCA-positive disease, are then treated with maintenance therapy as for other ANCA-positive patients. This is entirely consistent with the literature saying that patients with ANCA are liable to relapse [94]. If ANCA is the primary factor, it also begs the question whether ANCA-induced damage to the GBM releases GBM antigens in an immunogenic fashion and leads to development of anti-GBM disease. It would be interesting to look at the serum of your patient using a biosensor to see whether he has low levels of anti-GBM antibodies as well as ANCA.

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