Antineutrophil-Cytoplasmic Antibodies and Antiglomerular Basement Membrane Antibodies in Goodpasture's Syndrome and in Wegener's Granulomatosis

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ABSTRACT
Antiglomerular basement membrane (anti-GBM) diseases-including Goodpasture's (GP) syndrome and Wegener's granulomatosis (WG) are systemic diseases, which may be diagnosed by means of circulating autoantibodies. Possible overlap syndromes may exist; however, they remain imperfectly defined.
We analyzed sera from 31 patients with WG and from 23 patients with anti-GBM disease. All underwent biopsy. Anticytoplasmic antibodies (ANCA) were demonstrated by indirect immunofluorescence (IIF); a perinuclear (P-ANCA) or diffuse-cytoplasmic (C-ANCA) staining was discerned. In addition, myeloperoxidase (MPO) antibodies (P-ANCA) and protein 3 (SP3) antibodies (C-ANCA) were analyzed by specific ELISA systems. Anti-GBM antibodies (anti-NC1 antibodies) were detected by ELISA and immunoblotting; the globular domain NC1 of collagen IV was employed as antigen. All 31 WG patients, as defined by clinical and histological criteria, showed ANCA by IIF. Twenty-nine of 31 showed a C-ANCA pattern; all were also positive for SP3 antibodies by ELISA. Three of 31 WG patients were P-ANCA positive by IIF and also had anti-MPO antibodies by ELISA. In one of these patients, SP3 antibodies were additionally found by IIF and by ELISA (double positive). No patient with WG had anti-NC1 antibodies. All 23 serum samples from patients with GP syndrome (N = 19) or anti-GBM glomerulonephritis (N = 4) had anti-NC1 antibodies. In seven of these patients, low titers of anti-MPO antibodies were detected by ELISA; however, the IIF for ANCA was negative. None of these seven patients had extraglomerular vasculitis. In addition, the clinical prognosis of these patients was similar to that of those patients who lacked these antibodies. One anti-NC1 positive-GP patient was also positive for SP3 antibodies (ELISA and IIF). On renal biopsy, an extraglomerular vasculitis was found in addition to the anti-GBM disease. We conclude that: (1) anti-NC1 antibodies are specific for GP syndrome or anti-GBM glomerulonephritis, (2) C-ANCA, i.e., SP3 antibodies may indicate WG, (3) low titers of MPO-antibodies occur in about one third of GP patients, and (4) anti-NC1 antibodies and SP3 antibodies (C-ANCA) may rarely occur concomitantly in GP patients with accompanying extraglomerular vasculitis.

Key Words: Goodpasture, Wegener, myeloperoxidase, protein 3, NC1, anti-GBM antibodies

Goodpasture described a patient with lung hemorrhage and proliferative glomerulonephritis during the influenza epidemic of 1917 to 1918 (1). The syndrome that today bears his name (Goodpasture's [GP] syndrome) implies the presence of antiglomerular basement membrane (anti-GBM) antibodies (2) and often, but not always, includes hemoptysis that may be scant to massive. Wegener described patients with upper and lower airway disease who had concomitant glomerulonephritis (3). The pathologic hallmark was the presence of necrotizing granulomas (Wegener's glomerulonephritis [WG]). Both conditions are systemic diseases, which today may be diagnosed more easily by the demonstration of circulating autoantibodies. Facilitated diagnosis also allows identification of patients with both diseases before the advent of the full-blown clinical picture, which would have been recognized by their original describers. The anti-GBM antibodies in GP syndrome react with the globular NC1 domain of basement membrane collagen IV as the target antigen (4–8).
WG, antibodies directed against cytoplasmic antigens of human granulocytes may be detected (anticytoplasmic antibodies [ANCA]) [9–11]. Upon indirect immunofluorescence microscopy (IIF), either a diffuse cytoplasmic staining (C-ANCA) or a perinuclear staining (P-ANCA) may occur. The antigen responsible for the diffuse cytoplasmic staining was recently discovered to be protein 3 (SP3)/myeloblastin (12–14). Possibly, a subgroup of C-ANCA may react with other granule constituents as the target antigen (CAP 57) [11]. The corresponding autoantigen for P-ANCA is primarily myeloperoxidase (MPO), although a similar pattern may rarely be produced by antibodies to elastase and to lactoferrin [12,15,16]. SP3 autoantibodies do occur primarily in WG [9,10,16,17]. However, they have also been recently found in patients with cystic fibrosis, with and without associated vasculitis, in clinically defined microscopic polyarteritis, and in human immunodeficiency virus-infected patients at low titers (17–20). The autoantibodies to MPO were originally detected in WG [9,10,16,17]. However, they have also been found rarely in other immune-mediated diseases, such as systemic lupus erythematosus, Henoch-Schoenlein purpura, Churg-Strauss disease, and rheumatoid arthritis, and may also occur in WG [11,17,21,22]. Both types of ANCA occur frequently in idiopathic, pauci-immune crescentic glomerulonephritis [18,23]. Because the clinical courses of vasculitis patients positive for C-ANCA and P-ANCA may be quite similar, a controversy surrounding the nomenclature of systemic vasculitis has developed and was recently discussed comprehensively by Falk et al. [11,16].

O’Donoghue et al. first reported that ANCA may also be demonstrable in anti-GBM disease [24]. Subsequently, Jayne et al. observed that 30% of anti-GBM–positive sera were also positive for ANCA. Conversely, anti-GBM antibodies were found in 8% of patients who were ANCA positive [25]. However, anti-GBM antibodies were demonstrated with an unpurified collagenase digest of GBM as the antigen. Furthermore, a discrimination between SP3 antibodies and MPO antibodies by ELISA was not possible in that study. To shed more light on this matter, we determined the frequency of ANCA in sera of patients with GP and also measured anti-GBM antibodies (anti-NC1 antibodies) with a highly specific assay in patients with WG.

MATERIALS AND METHODS

Demonstration of Anti-GBM (Anti-NC1) Antibodies

Anti-GBM antibodies were demonstrated by ELISA and immunoblot by using the globular NC1 domain of collagen IV as the antigen [7]. Briefly, the NC1 domain was isolated from collagenase digests of human GBM, which were preextracted with 6 M guanidine-HCl (GuHCl)–0.05 M Tris-HCl (pH 7.5) for 12 h. The supernatant after centrifugation was passed through an anion exchange column (DE 52, Pharmacia, Freiburg, Germany). Unbound material was pooled, concentrated, and then applied to a molecular sieve column (Bio-Gel A-1.5m; BioRad, München, Germany) equilibrated with 0.05 M Tris-HCl–0.1 M CaCl₂ (pH 7.5). The fractions containing NC1 were identified after dissociation in 6 M GuHCl–0.05 M Tris-HCl (pH 7.5) by an inhibition ELISA, pooled, and concentrated. Purity was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Detection of Anti-GBM Antibodies by ELISA

Microtiter plates were coated with 50 μL of GBM-NC1 (10 μg/mL) in 6 M GuHCl. After incubation with probes and reference sera, bound immunoglobulin G (IgG) was detected with alkaline phosphatase-labeled rabbit anti-human IgG (Behring Werke, Marburg, Germany). p-Nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) was used as substrate, and absorbances were read at 405 nm on an ELISA reader (Dynatec, Denkendorf, Germany).

SDS-PAGE and Immunoblotting

SDS-PAGE was performed in a horizontal slab gel electrophoresis unit (LKB Instrument GmbH, Gräfelfing, Germany) with a 5 to 22.5% gradient polyacrylamide gel as previously described [7,8]. Proteins were then electrophoretically transferred to a 0.45-μm nitrocellulose paper (Schleicher & Schüll, Dassel, Germany) overnight with a constant current of 100 mamp. Human sera diluted 1:10 to 1:20 in phosphate-buffered saline were allowed to react with the blotted proteins. Horseradish peroxidase-conjugated F(ab)₂-fragments of rabbit anti-human IgG-Fc (Jackson Immunoresearch Lab., Avondale, PA) were used as secondary antibody at 1:2,000 dilution to detect bound IgG antibodies. After 2 h of incubation at room temperature and appropriate washing, 4-chloro-1-naphthol was used to visualize the bound antibodies.

Demonstration of ANCA

ANCA were demonstrated both by IIF on human granulocytes and by specific ELISA systems.

Demonstration of ANCA by IIF

For IIF, preparations of cytopspun human leukocytes obtained from healthy volunteers were fixed in ethanol (100%) by methods described at the First International Workshop on ANCA in Copenhagen.
The leukocytes were then incubated with human sera and washed, and bound antibodies were visualized by fluorescein isothiocyanate-conjugated anti-human IgG antibodies. Two staining patterns were discerned—a cytoplasmic pattern (C-ANCA) and a peninuclear pattern (P-ANCA).

ELISA for C-ANCA/(SP3) Antibodies

C-ANCA were also demonstrated by an ELISA, using a partially purified antigen that was extracted from the alpha fraction of human neutrophils. The specificity and sensitivity for C-ANCA have been recently described (26). Briefly, human granulocytes were prepared by Percoll-gradient centrifugation (Biochrom, Berlin, Germany) followed by destruction of the remaining erythrocytes by lysis buffer. The granulocytes were then subjected to high pressure in a nitrogen bomb (350 psi for 20 min). After equilibration with nitrogen and rupture of the cell membranes, the intracellular alpha granules were prepared by density centrifugation on a Percoll gradient. Thereafter, the alpha granules were disrupted by adding Triton X-100. This preparation was then used as C-ANCA antigen.

For the ELISA, microtiter plates were coated with 100 μL of the C-ANCA preparation (1 μg/mL; overnight; 20°C) in phosphate buffer (pH 11.6). After incubation with probes and reference sera (diluted 1:80 in phosphate-buffered saline/2% BSA) for 1 h at 20°C, bound IgG antibodies were detected with peroxidase-labeled rabbit anti-human IgG (Behring Werke, Marburg, Germany). After being washed, 4-chloro-1-naphthol was added and absorbances (optical densities [OD]) were read at 492 nm on an ELISA reader (Dynatec). The mean of 50 serum samples (mean±0.1 ± 0.02) from healthy volunteers, plus fivefold SD, was initially defined as cut-off (0.2). The standard sera were then pooled. Because the cutoff/mean ratio was two, positive/negative (P/N) ratios were calculated routinely (ODtest serum/ODpooled serum). Ratios above two were regarded as positive. The assay was shown to be specific and sensitive for C-ANCA and was therefore considered to detect SP3 antibodies. MPO antibodies did not react in the ELISA. Serial dilution studies disclosed that IIF and ELISA were of comparable sensitivity.

ELISA for MPO Antibodies

In this ELISA, purified MPO was used as the antigen, which was isolated by previously described methods (27). Briefly, a granule extract of human leukocytes was prepared by homogenization of the cells in 0.2 M sodium acetate buffer—1.0 M NaCl (pH 4.0). After centrifugation at 30,000 x g for 10 min, the pellet was rehomogenized and washed five times. The pellet was then dialyzed at 4°C overnight against 0.05 M Tris-HCl, 0.1 M NaCl, and 0.001 M dithiothreitol (pH 8.0). The precipitate was removed by centrifugation at 1,100 x g for 10 min. The supernatant was concentrated and applied to a Sephadex G-75 superfine column (Pharmacia) equilibrated with the same buffer. The first peak was eluted, concentrated, dialyzed against 0.02 M Na-acetate—0.1 M NaCl (pH 5.0) and applied to a CM-cellulose column. MPO was then isolated by a linear salt gradient (0.1 to 0.4 M NaCl). Purity of the preparation was analyzed by SDS-PAGE.

For the ELISA, microtiter plates were coated with 50 μL of MPO (200 μg/mL) solubilized in Triton X-100 (0.01%) for 48 h at 4°C. After incubation with probes and reference sera, bound IgG was detected with alkaline phosphatase-labeled rabbit anti-human IgG (Behring Werke). p-Nitrophenyl-phosphate was used as substrate, and absorbances were read at 405 nm on an ELISA reader. The mean of 50 serum samples (mean±0.05 ± 0.007) from healthy volunteers, plus sevenfold SD, was initially defined as cut-off (0.1). Thereafter, the standard sera were pooled. Similar to the C-ANCA ELISA, P/N ratios were calculated routinely (ODtest serum/ODpooled serum) and ratios above two were regarded as positive. The assay reliably detected MPO antibodies in various control sera, whereas sera from healthy volunteers and from patients with various other autoantibody-mediated diseases, including sera containing rheumatoid factor, were always negative. Serial dilutions of MPO antibodies' positive sera showed that the ELISA was more sensitive in detecting the antibodies than was IIF.

Patients

The original descriptions of GP and WG have been mentioned earlier. For our purposes, the term GP implies anti-GBM nephritis with concomitant lung hemorrhage (or signs of pulmonary involvement on chest radiography) (2). If the anti-GBM disease was confined to the kidneys, the term anti-GBM disease was used as a more general one. WG was defined as a necrotizing granulomatous vasculitis of the upper and/or lower respiratory tract in the presence of glomerulonephritis with scant or no immunofluorescence staining (28). Patients who suffered from pauci-immune necrotizing and crescentic glomerulonephritis and who had C-ANCA were considered to have limited Wegener's disease.

We investigated serum samples from 24 patients with biopsy-proven active anti-GBM antibody-mediated disease (Table 1). In three patients, the biopsy material was not adequate. However, in these patients, anti-NC1 antibodies were positive by ELISA and immunoblot. Moreover, all three patients suffered from hemoptysis and renal insufficiency and were therefore considered to have anti-GBM-mediated disease. Thirteen patients suffered from GP.
TABLE 1. Features of patients with anti-NC1 antibody-associated diseases

<table>
<thead>
<tr>
<th>Age (yr.)</th>
<th>Clinical Symptoms</th>
<th>Serum Creatinine (μmol/L)</th>
<th>Crescents (%)</th>
<th>Treatment</th>
<th>IF</th>
<th>Antibodies</th>
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<td>H</td>
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<td>IgG, C3</td>
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*Abbreviations: H, hemoptysis; R, renal involvement; L, lung involvement (x-ray); HD, chronic intermittent hemodialysis; TX, kidney transplant; ND, not done; neg, negative; C, corticosteroids; Cy, cyclophosphamide; PS, plasma separation; P/N, positive/negative ratio.

Eight patients had no clinical evidence for hemoptysis but suffered from glomerulonephritis with linear IgG deposits alone. However, four of these patients showed roentgenographic evidence for pulmonary involvement. Lung and renal biopsies in these patients showed a linear staining for IgG along the GBM and the lung alveolar basement membranes. Two patients presented with hemoptysis and no renal insufficiency. However, in these patients, microscopic hematuria was demonstrable and renal biopsies showed linear staining for IgG along the GBM in both patients. In all of these patients, special attention was paid to additional signs of other organ involvement, such as cutaneous vasculitis, iridocyclicitis, arthralgias, seizures, and peripheral neurologic defects, on the initial examination as well as on the follow-up evaluations.

In addition, 31 serum samples from patients with active WG (N = 27) or limited Wegener's disease (N = 4) were investigated (Table 2). The diagnosis in 27 patients was confirmed by the demonstration of granulomatous vasculitis, either on nasal or on lung biopsy. Renal biopsy was performed in 24 patients and showed various degrees of pauci-immune necrotizing and crescentic glomerulonephritis. In one patient with disease limited to the kidney, a renal granuloma was demonstrable. Four patients suffered from hemoptysis. In 13 other patients, lung involvement was documented by roentgenographic examination. Twenty-one patients had ear, nose, or throat symptoms. Nearly all of these patients also suffered from arthralgias, including those with disease limited to the kidney. A retro-orbital granuloma was found in four patients.

The serum creatinine values on admission and the final serum creatinine concentrations are given in
Tables 1 and 2. In addition, the treatment applied is outlined briefly.

RESULTS

The clinical data, including the patient outcomes, are summarized in Tables 1 and 2. In addition, the results of the serum examinations for autoantibodies are given in Figures 1 and 2.

ANCA were demonstrable by IIF microscopy in all 31 serum samples from patients with WG. Twenty-nine out of 31 serum samples showed diffuse cytoplasmic staining (C-ANCA) and were also positive in the SP3 ELISA. The remaining two serum samples showed a perinuclear staining. In these two serum samples, anti-MPO antibodies could be demonstrated by ELISA and on IIF. In one serum sample, both SP3 antibodies and MPO antibodies were found. In both patients with MPO antibodies alone, the diagnosis of WG was made by lung biopsy, which demonstrated a necrotizing, granulomatous vasculitis. No serum from patients with WG was positive for anti-NC1 antibodies either on ELISA or on immunoblotting (Figure 1). One patient died because of septicemia.

In all 23 serum samples from patients with biopsy-proven anti-GBM-mediated disease and in the 2 serum samples from patients with inadequate biopsy, anti-NC1 antibodies were detected both by anti-NC1 ELISA and by immunoblotting. On immunoblot, all serum samples stained positive for monomers and dimers of the NC1 hexamer. In 7 out of 23 serum samples, low titers of anti-MPO antibodies were de-
DISCUSSION

The diagnosis of pulmonary-renal syndromes caused by WG and microscopic artheritis has been greatly facilitated by the detection of ANCA (9-11,16). These diseases may now be identified as readily as are lupus erythematosus and GP syndrome. Moreover, the demonstration of ANCA in about 90% of immunohistochemically negative crescentic glomerulonephritis supports the notion that this form of glomerulonephritis may be an oligosymptomatic form of vasculitis (18,23). A role for ANCA in the pathogenesis of these disorders is supported by the fact that ANCA titers usually parallel the clinical activity of the disease (29). Such a possible role has been substantiated further by in vitro experiments. For instance, in vitro incubation of MPO-ANCA- and SP3-ANCA-positive sera with cytokine-primed neutrophils induces a degranulation and an oxidative burst (30,31). A similar mechanism may also be relevant in vivo. In addition, the target antigen of SP3-ANCA is deposited not only in the azurophilic granules but also on the cell surface. This observation has recently been demonstrated by immunoelectron microscopy. Thus, the cell surface expression may allow a reactivity with the circulating antibodies (32).

It would be of great clinical importance if the diagnosis of the underlying immune process in pulmonary-renal syndromes and rapidly progressive glomerulonephritis could be established reliably by simple serologic tests. However, in a recent report, 30% of anti-GBM-positive sera were also positive for ANCA. Similarly, anti-GBM antibodies were also found in about 8% of ANCA-positive sera (25). On the basis of these findings, as well as on additional clinical data, the authors of that report suggested that overlap syndromes may commonly exist. Our results do not support such a view. We were unable to find any anti-NC1 antibodies in patients with biopsy-proven WG. In addition, no signs of linear deposition of immunoglobulins along basement membranes could be detected histologically in our WG patients. These findings indicate that SP3 antibodies (C-ANCA) sensitively point to WG as the underlying disease process. The high incidence of anti-GBM antibodies in ANCA-positive sera in the study of Jayne et al. (25) may possibly be because of the use of an unpurified collagenase digest of human GBM as antigen. The GBM is built from several molecules including collagen IV, laminin, nidogen/entactin, thrombospondine, heparan-sulfate proteoglycane, and possibly other hitherto undetected substances (33). In addition, the preparation usually also con-
tains adherent membrane fragments of the glomerular endothelial and epithelial cells. A crude GBM digest contains many of these molecules and their degradation products, all of which are potential candidates for a reaction with ANCA. We used the purified globular NC1 domain of type IV collagen isolated from GBM (7,8) as antigen in our study. This antigen contains the specific epitopes recognized by anti-GBM antibodies in GP syndrome. In support of our findings, Pusey et al. recently showed that most anti-GBM antibodies found in patients with ANCA were not directed against the Goodpasture antigen (34).

Conversely, in anti-NC1-mediated diseases, about one-third of the serum samples showed low titers of MPO antibodies by ELISA. However, IIF, which in our hands is the less-sensitive technique, was always negative. Moreover, upon renal biopsy, no signs of extraglomerular vasculitis could be detected. In addition, no evidence for small-vessel vasculitis could be clinically identified. Furthermore, the occurrence of MPO antibodies in addition to anti-NC1 antibodies also did not influence the clinical outcome of these patients. A beneficial effect on the clinical outcome was theoretically possible, because glomerulonephritis in Wegener’s vasculitis or microscopic polyarteritis may be more successfully treated by immunosuppression than is GP.

In one older patient, SP3 antibodies were found in addition to high titers of anti-NC1 antibodies. In this patient, the diagnosis of an anti-GBM-mediated rapidly progressive glomerulonephritis was made by the demonstration of linear immunofluorescence for IgG along glomerular capillaries. In addition, C-ANCA could be demonstrated by IIF and by ELISA. Furthermore, extraglomerular vasculitis was also found upon renal biopsy. This observation indicates that the presence of anti-NC1 antibodies and C-ANCA may rarely occur when GP syndrome is associated with extraglomerular vasculitis. The occurrence of ANCA in about one third of anti-GBM-positive patients in the study of Jayne et al. may primarily be due to the occurrence of MPO antibodies. The authors were not able to further analyze this issue because their ELISA recognized both MPO and SP3 antibodies.

We conclude that anti-NC1 antibodies are specific for GP syndrome or anti-GBM glomerulonephritis. In addition, SP3 antibodies (C-ANCA) may indicate WG. Low titers of anti-MPO antibodies may occur in about one third of patients with GP syndrome. Our GP patients with such antibodies had neither an increased incidence of vasculitis nor a better prognosis compared with patients who lacked these antibodies. The simultaneous presence of anti-NC1 antibodies and SP3 antibodies may rarely occur when GP is associated with extraglomerular vasculitis. Whether these "double"-positive patients may have a more favorable outcome remains to be established.

ACKNOWLEDGMENTS
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REFERENCES
15. Falk RJ, Jennette JC: Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vascul-
ANCA and GBM Antibodies in GP and WG