Treponemal Antigen in Immunopathogenesis of Syphilitic Glomerulonephritis

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A patient with syphilitic glomerulonephritis had a renal biopsy and was treated for secondary syphilis. Light, electron, and immunofluorescence microscopic studies revealed an acute proliferative glomerulonephritis with subepithelial, intramembranous, and subendothelial immune complex deposits containing IgG, IgA, IgM, C4, and C3. Similar local deposits containing predominantly IgM were noted in areas of mesangial proliferation. Indirect fluorescent antibody studies employing rabbit treponemal antibody and sheep antirabbit globulin conjugate revealed the presence of treponemal antigen in the glomerular deposits. This finding provides strong evidence for the immunopathogenesis of the glomerular lesion as well as a causal link with Treponema pallidum. (Am J Pathol 82:479-492, 1976)

It is well documented that glomerulonephritis is a destructive inflammatory process of the glomerulus often accompanied by tubular, interstitial, and vascular abnormalities. In over 85% of the cases, the initial glomerular insult appears to involve two possible immunologic mechanisms. In the first, antibodies specific for antigens comprising the glomerular basement membrane (GBM) itself initiate the insult. In the second, preformed circulating antigen–antibody complexes trapped in the glomerular filter initiate the immunologic insult. Although syphilitic renal disease has been recognized for more than a century, the basis of this association and the mechanism of renal injury has not been clearly linked to treponemal antigen. Early reports theorized that the renal involvement was directly associated with the presence of Treponema pallidum localized in the kidney parenchyma since most cases responded to antisyphilitic treatment. Subsequent studies showed the presence of immune complex deposits along the glomerular basement membrane both by immunofluorescence and electron microscopy. These studies suggested for the first time that the immune

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complex mechanism of immunologic insult was a tenable explanation for syphilitic glomerulonephritis. However, attempts to demonstrate treponemal antigen or antibody failed until very recently, when Gamble and Reardan \(^{18}\) reported eluting treponemal antibody from glomerular immune-complex deposits. These antibodies were subsequently shown to be reactive in the standard fluorescent treponemal antibody absorption confirmatory test for syphilis. In the report described below, use of the indirect fluorescent antibody (IFA) technique with nonhuman syphilis reagents demonstrated the presence of treponemal antigen in the glomerular immune-complex deposits in a patient with syphilitic glomerulonephritis.

**Case Report**

A 34-year-old black male was admitted to the Martland Hospital, Newark, N.J., on December 16, 1974, because of a 4-day history of swelling of the face, scrotum, and legs associated with a 17-kg weight gain. His previous health had been excellent, and he denied renal disease or hypertension in the past. Physical examination revealed a blood pressure of 170/110 mm Hg and 4+ pitting edema of scrotum and legs. Multiple nonpruritic, dark brown maculopapular lesions were symmetrically distributed on the palms of the hands and soles of the feet. Generalized lymphadenopathy was observed. Routine urinalysis revealed 4+ proteinuria, an abnormal urine sediment with ten to fifteen red blood cells, two to four white blood cells, occasional fine granular casts and a 24-hour urinary protein of 13.3 g. The total serum protein was 5.1 g/dl; albumin, 1.2 g/dl; blood urea nitrogen, 80 mg/dl; and serum creatinine, 2.1 mg/dl. Serum antinuclear antibody tests and lupus erythematosus blood smear preparations were negative. The serum C3 concentration was 65 mg/dl (normal 80 to 140) and immunoelectrophoresis studies revealed elevated levels of IgG. The automated reagin test was reactive at 32 dils, and the fluorescent treponemal antibody absorption (FTA/Abs) test was reactive. Percutaneous renal biopsy was performed on the fourth hospital day. The patient was given three weekly 2.4 million unit doses of benzathine penicillin G intramuscularly commencing on the seventh hospital day. He remained hypertensive (150/80 to 180/120) for 14 days. During the 5½ week hospital course, the patient became edema free; his body weight decreased by 16 kg and his 24-hour urinary protein decreased to 500 mg. His serum albumin level rose to 2.8 mg/dl and the blood urea nitrogen and creatinine levels were 14 mg/dl and 0.9 mg/dl, respectively. Repeated urine and serum protein electrophoretic studies performed in the course of the patient’s hospital stay revealed nonselective proteinuria initially, with subsequent improvement as the patient’s clinical condition improved. The patient refused a follow-up biopsy and was discharged from the hospital against our advice on January 24, 1975. He has failed to return for follow-up examination.

**MATERIALS AND METHODS**

**Light Microscopy**

Half of the biopsy specimen was fixed in formalin and processed in the usual manner at Martland Hospital. The other half was studied by immunofluorescence and electron microscopy at the Saint Barnabas Medical Center.

**Electron Microscopy**

A portion of the renal biopsy specimen was divided into small blocks, about 1 cu mm in size, fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in
Epon 812 after a dehydration process. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Carl Zeiss EM9S-2 electron microscope.

**Fluoresceinated Antiseras**

Fluorescein isothiocyanate (FITC)-labeled goat antiseras directed against human IgG, IgA, IgM, C3, C4, and fibrinogen (Hyland Laboratories) were made monospecific through appropriate absorptions with tissue powders and antigens according to methods previously described. An antiserum directed against rabbit γ-globulin and made in a sheep was FITC-labeled for use in IFA studies employing rabbit antiserum directed against *Treponema pallidum*. Both of these reagents were kindly supplied by Clinical Sciences, Inc., Whippany, N.J. The FITC-labeled sheep antirabbit globulin was further absorbed with normal human serum (NHS) prior to use. The rabbit antitreponemal antibody was reactive with the Venereal Disease Research Laboratory screen test at a 1:64 dilution and produced a strong 4+ reactive FTA-Abs test.

**Immunohistochemical Studies**

Direct fluorescent antibody studies were performed on consecutive section of a needle biopsy specimen of kidney that was snap-frozen in liquid nitrogen. The sections were cut at 4 to 6 μ and dried for 30 minutes. The sections were washed in phosphate-buffered saline (PBS), pH 7.2, blotted, and placed in a moist chamber. Each antiserum was applied to the appropriate sections and incubated at room temperature for 30 minutes. The slides were washed for 30 minutes with three changes of PBS. Excess PBS was blotted and two drops of a 9:1 glycerol-buffer solution, pH 8.0, were applied to the sections and then covered with a glass cover slip. The slides were examined immediately with a Gillet and Sibert fluorescence microscope equipped with a halogen light source, an interference filter, a BG 38 red suppression filter, and a type H secondary filter. Photomicrographs were taken with a Gillet and Sibert Autolynx Camera on high speed Ektachrome (daylight) film.

IFA studies were carried out employing the rabbit antitreponemal serum and the FITC-labeled sheep antirabbit γ-globulin on normal human kidney sections, clinical biopsy tissue obtained from 2 patients with systemic lupus erythematosus, and the biopsy tissue obtained from the patient according to standard procedures described by Coons and Kaplan. Blocking experiments included prior absorption of the rabbit antitreponemal serum with a suspension of Nichols stain of *T. pallidum*. Approximately 10⁶ organisms/ml of antiserum were mixed and incubated for 1 hour at 37°C and 4°C, respectively. The antiserum was then centrifuged at 25,000g in a Sorvall RC-3 refrigerated centrifuge for 30 minutes. This antiserum was then used to perform IFA studies on frozen sections obtained from the patient’s biopsy tissue.

Fluorescent treponemal antibody absorption and antinuclear antibody studies were carried out using Nichols stain on *T. pallidum* and rat liver sections, respectively (Clinical Sciences, Inc., Whippany, N.J.), according to the manufacturer’s Fluoro-Kit instructions.

**Results**

**Light Microscopic Findings**

Light microscopic studies (Figures 1 and 2) based on the examination of Epon-embedded 1-μ-thick sections stained with toluidine blue revealed that all observed glomeruli were markedly hypercellular due to endothelial and mesangial cell proliferation and the numerous leukocytes. The capillary loops were distended and filled with the proliferated swollen endothelial cells, leukocytes, and a few red blood cells. The glomerular
capillary wall was slightly thickened, and the juxtaglomerular apparatus was prominent. The cytoplasm of the proximal convoluted tubular cells was swollen and focally degenerated. Some tubules contained red blood cells and cellular debris. The blood vessels were unremarkable.

**Electron Microscopic Findings**

Electron microscopic studies (Figures 3–6) revealed irregularly thickened glomerular capillary basement membranes with mainly subepithelial electron-dense deposits characterized as “humps” of varying sizes. Subendothelial (Figure 5) and intramembranous electron-dense deposits were also noted. The foot processes of the swollen epithelial cells were diffusely fused. Deposits were also noted outside the epithelial cells (Figure 6). The mesangial matrix was markedly increased with mesangial cell proliferation, and it contained electron-dense deposits (Figure 4). Nearly all capillary loops were distended and filled with proliferated swollen endothelial cells, leukocytes, and occasional red blood cells (Figure 3). A portion of the proximal tubular epithelium was refluxed into the Bowman’s space. Red blood cells were occasionally found in the Bowman’s space, tubular lumen, and interstitium. There were occasional degenerating epithelial cells in both the proximal and distal tubules. The intertubular interstitium was focally infiltrated by leukocytes.

A summary of electron microscopic findings in this case and previously studied cases is shown in Table 1.

**Direct Immunohistochemical Studies**

The results of routine fluorescent antibody studies (Figures 7–10) revealed 3+ to 4+ large granular IgG, C3, C4, and IgA deposits in a lobular pattern along the glomerular basement membrane in all observed glomeruli. These same constituents were also observed as diffuse fine granular deposits along the glomerular capillary walls. The larger deposits were unique in that they showed a peripheral accentuation of staining intensity. Mesangial cell deposits were also noted. IgM staining was found predominantly in areas of mesangial proliferation. Fibrinogen staining was restricted to local areas adjacent to Bowman’s space and within areas of mesangial proliferation.

**Indirect Immunohistochemical Studies**

Indirect fluorescent antibody studies using FITC-labeled sheep antirabbit globulin (Figure 9) revealed 2 to 3+ granular glomerular basement membrane deposits similar to those observed in the direct fluorescent antibody-stained sections obtained from the patient’s biopsy. IFA studies
Table 1—Summary of Electron Microscopic Findings of the Glomerulus in Acquired Syphilitic Glomerulonephritis Prior to Therapy

<table>
<thead>
<tr>
<th>Cellular and Subcellular Changes</th>
<th>Tourville</th>
<th>Falls(a)</th>
<th>Braunstein(^b)</th>
<th>Bhorade(^c) No. 1</th>
<th>Bhorade(^c) No. 2</th>
<th>Hellier(^d)</th>
<th>Gamble(^e)</th>
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<tr>
<td>Basement membrane thickening</td>
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<td>Diff</td>
<td>+</td>
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<td>Subendothelial deposits</td>
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<td>+</td>
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<td>ID</td>
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<td>Intramembranous deposits</td>
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<td>Tubuloreticular structures</td>
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\( + = \) Changes present, ID = ill-defined deposits, not confined to any particular aspect of the membrane; Diff = diffuse; Norm = normal; Segm = segmental.
performed on frozen sections of kidney obtained from 2 patients with lupus nephritis (Figure 10) revealed minimal nonspecific glomerular staining. Consecutive sections stained with antisera directed against human IgG and C3 revealed 4+ diffuse granular glomerular basement membrane deposits. Absorption of the rabbit antitreponemal serum with Nichols stain of T. pallidum completely blocked the 4+ granular staining observed prior to absorption. The residual staining was similar to that shown in Figure 10. Indirect fluorescent antibody studies carried out with normal autopsy kidney tissue showed minimal nonspecific glomerular staining.

**Discussion**

The clinical events and renal biopsy findings in this case closely resemble the observations reported in a number of studies describing syphilitic nephritis. Subepithelial immunoglobulin and complement deposits have been observed by electron microscopy and immunofluorescence in several previous case reports.\textsuperscript{12-14} When such deposits are found in glomeruli, it is assumed that the glomerular disease may be caused by immune complexes. The mere presence of these deposits, however, does not prove they are there in the form of immune complexes.\textsuperscript{2} Previous attempts to demonstrate direct evidence for the presence of treponemal antigen have been unsuccessful.\textsuperscript{12-14} The identification of treponemal antigen in the subepithelial immune complex deposits in this case provides direct evidence for the immunopathogenesis of the glomerular lesion. These findings are in accord with the reported demonstration of treponemal antibody in a similar case of syphilitic glomerulonephritis.\textsuperscript{15} The finding of both treponemal antigen and antibody in these 2 cases strongly suggests an immunopathogenesis of the disease and a causal link with T. pallidum in syphilitic glomerulonephritis. The syphilitic form of glomerulonephritis should now be included with the other proven immune complex–induced renal injuries such as poststreptococcal glomerulonephritis,\textsuperscript{20,21} systemic lupus erythematosus,\textsuperscript{22} malaria,\textsuperscript{23} carcinoembryonic antigen \textsuperscript{24,25} and hepatitis B.\textsuperscript{26,27} In most cases, however, the simultaneous detection of both antigen and antibody has not been demonstrated except in systemic lupus erythematosus.\textsuperscript{22} The glomerular insult in this case appears to be more severe than in most previously reported cases. The prolonged clinical course and marked proliferative changes as shown by light and fluorescent microscopy and particularly by electron microscopy (see Table 1) support this view. The initial C3 determination in this case was decreased, while in other case reports C3 levels were reported normal, and in some cases \textsuperscript{12} was undetectable in the glomerular deposits by direct immunohistochemical studies. The local-
ization of the immune complex deposits from areas of mesangial proliferation to areas outside the epithelial cells suggests a state of dynamic immune-complex association and dissociation. The possible dissociation of at least some of the glomerular complexes could have conceivably freed-up treponemal antigenic sites, making them available to react with the rabbit treponemal antibody. It is interesting to speculate that the timing of the percutaneous renal biopsy procedure, the severity of the immunologic insult, and the possible dynamic dissociation of the immune complexes (particularly in the larger humps) are factors that may play a role in the success or failure in demonstrating treponemal antigen in the immune complex deposits associated with syphilitic glomerulonephritis. This could help explain the earlier unsuccessful attempts to demonstrate treponemal antigen in syphilitic glomerulonephritis.

The presence of 4+ staining IgA deposits has not been described in earlier case reports of syphilitic glomerulonephritis. It is generally accepted that IgA does not bind complement via the classic pathway of complement activation. IgA is capable of activating complement via the alternate pathway. This, coupled with the observation that the alternate pathway of activating complement may be involved in membranoproliferative glomerulonephritis, suggested that a similar mechanism may be active in this case. Although insufficient tissue was available to study this possibility completely, the presence of C4 in the glomerular deposits indicates that the classic pathway of complement activation was active. Further studies are necessary to assess whether the alternate pathway of complement fixation is associated with syphilitic glomerulonephritis.

The negative antinuclear antibody studies and absence of staining in the lupus nephritis biopsy specimens using the IFA technique with treponemal antibody provides evidence for the specificity of the IFA studies in this patient. In addition, prior absorption of the rabbit treponemal antiserum with Nichols stain of T. pallidum completely blocked the 4+ FTA-Abs test produced prior to absorption. Acid elution studies were not possible due to insufficient tissue and the presence of only three glomeruli in the frozen sections.

The results of this study complement the report by Gamble and Rearden, and the two studies together firmly established the immunopathogenesis of syphilitic glomerulonephritis.

References


[Illustrations follow]
Legends for Figures

Figure 1—Glomerulus showing hypercellularity due to endothelial and mesangial cell proliferation with numerous leukocytes blocking and distending the capillary loops (Epon-embedded 1-μm-thick section with toluidine blue stain, × 300).

Figure 2—Higher magnification of Figure 1 showing the capillary basement membrane with subepithelial (arrowheads) and subendothelial (arrow) dense nodules. (Epon-embedded 1-μm-thick section with toluidine blue stain, × 1100).

Figure 3—Electron micrograph of a glomerular capillary loop showing subepithelial electron-dense deposits (arrows) associated with basement membrane thickening and epithelial cell foot-process fusion. The capillary loop is distended and filled with endothelial cells and leukocytes attached to the basement membrane. Note the branches of the markedly increased mesangial matrix surrounding proliferated mesangial cells and reaching the capillary basement membrane. (Uranyl acetate and lead citrate, × 4800)
Figure 4—Electron micrograph showing electron-dense deposits in the mesangial matrix (arrows) (Uranyl acetate and lead citrate, × 17,900).

Figure 5—Electron micrograph of a segment of the thickened glomerular capillary basement membrane showing numerous subendothelial (straight arrows) and one subepithelial (curved arrow) electron-dense deposit (Uranyl acetate and lead citrate, × 9800).

Figure 6—Electron micrograph showing electron-dense deposits (straight arrows) localized outside the epithelial cells. Note detached cytoplasm of the epithelial cell (curved arrow). (Uranyl acetate and lead citrate, × 7400)
Figure 7—FITC-labeled antihuman IgG-stained section showing fine and large granular glomerular basement membrane deposits. Similar IgA staining deposits were observed. (× 330)

Figure 8—FITC-labeled anti-human C3-stained consecutive section showing fine and large granular glomerular basement membrane deposits (× 330).

Figure 9—FITC-labeled sheep anti-rabbit globulin stained section using rabbit antitreponemal antibody and the IFA procedure. The granular staining pattern is due to antibodies directed against Treponema pallidum antigens. (× 330)

Figure 10—FITC-labeled sheep antirabbit globulin-stained section from a patient with lupus nephritis similar to Figure 5, showing nonspecific minimal staining. Consecutive sections stained with antihuman IgG and C3 revealed 4+ diffuse granular glomerular basement membrane deposits. (× 330)