Immunofluorescent Localization of Immunoglobulins, Complement, and Fibrinogen in Human Diseases. II.
Acute, Subacute, and Chronic Glomerulonephritis *

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Gamma globulin and complement have been demonstrated in renal glomeruli of patients with glomerulonephritis (1–10). Certain problems, which are the subject of the present investigation, remain to be elucidated: 1) the type of immunoglobulin (Y₂, Y₁M, or Y₁A) present in the renal lesion and its relation to complement localization; and 2) the significance of fibrinogen localization. Kidneys from patients with acute, subacute, and chronic glomerulonephritis were examined by the fluorescent antibody technique to assess the nature of protein deposition in the renal parenchyma.

Methods
Specimens from 17 patients with acute, subacute, and chronic glomerulonephritis were quick-frozen in dry ice and isopentane at −70°C. The methods of tissue fixation, preparation of fluoresceinated antisera, and fluorescence microscopy have been described in a previous paper (11). Histological examination was performed on paraffin sections stained with hematoxylin and eosin and subjected to the periodic acid Schiff reagent after diastase treatment. Phosphotungstic acid hematoxylin (PTAH) and Lendrum stains for detection of fibrin were also employed.

Major clinical and pathological findings pertinent to the present study are summarized in Table I. Any patient with clinical symptoms suggestive of systemic lupus erythematosus (SLE) was excluded. All patients with subacute and chronic glomerulonephritis were uremic and hypertensive on their final admissions to the hospital, except for two patients (No. 5 and 16). Autopsy tissues were obtained in all cases except for renal biopsies obtained in three patients with acute glomerulonephritis.

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### TABLE 1
Clinical and pathological findings in patients with glomerulonephritis

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Kidney weights combined</th>
<th>Gross appearance of kidneys</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Clinical history</th>
<th>Proteinuria</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/100 ml</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Acute glomerulonephritis</td>
<td></td>
<td></td>
<td>8</td>
<td>M</td>
<td>W</td>
<td>URI and scarlet fever 4 weeks before admission. Hematuria and hypertension on admission. Died in acute pulmonary edema.</td>
<td>15</td>
<td>1+</td>
</tr>
<tr>
<td>1</td>
<td>180</td>
<td>Swollen, pale gray surface with petechiae</td>
<td>175</td>
<td>130</td>
<td>11</td>
<td>Postnecrotic cirrhosis, URI before admission. Oliguria and hematuria on admission.</td>
<td>23</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>†</td>
<td>†</td>
<td>55</td>
<td>F</td>
<td>W</td>
<td>Sore throat 6 weeks before admission. Mild persistent hematuria on admission.</td>
<td>10</td>
<td>1+</td>
</tr>
<tr>
<td>3</td>
<td>†</td>
<td>†</td>
<td>28</td>
<td>F</td>
<td>W</td>
<td>URI and gross hematuria 3 months before admission. Mild persistent hematuria.</td>
<td>12</td>
<td>2+</td>
</tr>
<tr>
<td>Subacute glomerulonephritis</td>
<td></td>
<td></td>
<td>49</td>
<td>F</td>
<td>W</td>
<td>Photophobia and leg edema for 1 month. Expired after peritoneal dialysis for severe oliguria.</td>
<td>19</td>
<td>1+</td>
</tr>
<tr>
<td>5</td>
<td>235</td>
<td>Smooth, pale, many petechiae</td>
<td>200</td>
<td>100</td>
<td>2-3+</td>
<td>Acute glomerulonephritis (clinical) 3 months before admission, followed by facial and leg edema. Died in comatose state.</td>
<td>100</td>
<td>2-3+</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>Smooth, pale, many petechiae</td>
<td>530</td>
<td>Smooth, pale, many petechiae</td>
<td>46</td>
<td>M</td>
<td>W</td>
<td>Renal disease in childhood. Albuminuria and hypertension for many years. Marked oliguria terminally.</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>Slightly granular, surface pale gray</td>
<td>225</td>
<td>Smooth, pale, many petechiae</td>
<td>75</td>
<td>F</td>
<td>W</td>
<td>Flank pain for 1 week and hematuria (duration unknown). Expired with severe vomiting, dehydration, and oliguria.</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td></td>
<td></td>
<td>25</td>
<td>M</td>
<td>N</td>
<td>Five months of hypertension. Terminal intermittent convulsions and congestive heart failure.</td>
<td>125</td>
<td>3-4+</td>
</tr>
<tr>
<td>9</td>
<td>310</td>
<td>Finely granular, pale, slight contraction</td>
<td>175</td>
<td>Finely granular, pale, with few petechiae, marked contraction</td>
<td>51</td>
<td>M</td>
<td>N</td>
<td>Sore throat 8 months before admission followed by fatigue, dyspnea, and ankle edema. Died after a convulsive seizure.</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>130</td>
<td>Finely granular, pale, marked contraction, medullary cysts</td>
<td>27</td>
<td>F</td>
<td>W</td>
<td>One month of pruritis, weakness, and anorexia. Died in comatose state.</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>185</td>
<td>Granular, pale, marked contraction</td>
<td>25</td>
<td>M</td>
<td>W</td>
<td>Sore throat with fever and hematuria 7 years before admission. Died in acute pulmonary edema.</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>150</td>
<td>Granular, pale, marked contraction</td>
<td>32</td>
<td>M</td>
<td>W</td>
<td>Acute glomerulonephritis 21 years ago; hypertension noted 11 years ago, controlled by medication. Died in acute pulmonary edema.</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>125</td>
<td>Finely granular, pale, with few petechiae, marked contraction.</td>
<td>22</td>
<td>F</td>
<td>N</td>
<td>Nephrotic syndrome 11 years ago followed by persistent hypertension. Died in congestive heart failure.</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>160</td>
<td>Finely granular, pale, few petechiae, marked contraction</td>
<td>28</td>
<td>F</td>
<td>N</td>
<td>Painless hematuria and subsequent fatigue and dyspnea. Peritoneal dialysis for progressive oliguria.</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>360</td>
<td>Slight granularity, pale, with multiple petechiae</td>
<td>76</td>
<td>M</td>
<td>N</td>
<td>Weakness for 3 weeks. Anuria and coma terminally.</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>160</td>
<td>Granular, pale, contraction, with few flexibites</td>
<td>28</td>
<td>M</td>
<td>W</td>
<td>Recurrent edema and albuminuria for many years. Died in coma.</td>
</tr>
</tbody>
</table>

* BUN = blood urea nitrogen.
† Renal biopsy.
‡ URI = upper respiratory infection.
distribution in glomeruli at any stage of glomerulonephritis, but occasionally focal interstitial fluorescence was observed in the subacute and chronic stages. Tubular epithelium was stained by anti-γ1A-serum (Figure 2), in all stages of glomerulonephritis, but occasionally weak fluorescence with anti-γ2- and γ1M-sera was also present. Gamma1A localization in tubular epithelium was most marked in cases of chronic glomerulonephritis with severe proteinuria. Deposits of immunoglobulins were rarely observed in the walls of the small arterioles, some of which showed fibrinoid degeneration (Patient 6).

BetαC-globulin localization. Complement was deposited in the renal glomerulus in a pattern similar to γ2- and γ1M-globulins. The glomerular fluorescence was least intense in cases of chronic glomerulonephritis. When sections from these kidneys were incubated with fresh human serum before treatment with fluoresceinated anti-complement serum, a marked increase in fluorescence was noted (Figure 3). Acute and subacute glomerulonephritis showed more significant in vivo deposition of complement in glomeruli. Complement was not present in tubular epithelium, although an occasional cast was positively stained. Blood vesi-
in a few glomeruli when sections were incubated with antifibrinogen serum.

Membranous and interstitial deposition of fibrinogen in glomeruli (Figure 4A) and diffuse localization in some crescents were observed in subacute and chronic glomerulonephritis. Many glomeruli in which fibrinogen could be localized also con-

**Fibrinogen localization.** In acute glomerulonephritis, patchy interstitial staining was observed...
tained γ₂- and γ₁M-globulins. Partially or almost completely hyalinized glomeruli in advanced glomerulonephritis contained undulating membranous structures brightly stained with antifibrinogen serum and weakly stained by anti-γ₂-sera (Figure 4B). Blood vessels with immunoglobulins and complement deposition showed fibrinogen deposits as well. PTAH and Lendrum stains revealed rare focal fibrin deposits in crescents and glomeruli of subacute and chronic glomerulonephritis. A membranous fibrin distribution was not demonstrable by these stains.

Alpha₂-macroglobulin and albumin localization. Occasional glomeruli exhibited small interstitial foci of α₂-macroglobulin and albumin deposition. Tubular epithelium was weakly stained, and some tubular casts fluoresced after treatment of sections with the antisera to these proteins (Figure 5).

Discussion

Previous investigations have demonstrated the presence of γ-globulin and complement in the renal lesions of acute, subacute, and chronic glomerulonephritis (1-10). In the present study immunoglobulin deposition noted in renal glomeruli and small blood vessels was qualitatively similar to that seen in SLE. Gamma₂- and γ₁M-globulins were localized in glomeruli, whereas γ₁A-globulin was demonstrable in tubular epithelium. The number of glomeruli stained and the intensity of fluorescence were less than that observed in SLE kidneys. Vascular involvement, although less severe than in SLE, is also a feature of glomerulonephritis (12).

Deposits of immunoglobulins were usually limited to nonhyalinized glomeruli showing thickening of the mesangium and basement membrane. A recurrent deposition of immunoglobulin and complement affecting fewer glomeruli from patients with subacute and chronic glomerulonephritis may account for the lack of uniform glomerular involvement in contrast to SLE. The development of membranous glomerulonephritis in normal isologous renal transplants from some identical twins (13) supports the hypothesis that host activity persists in the late stages of the disease.

Similar to SLE, it appears that γ₂- and γ₁M-globulins localized in the kidney are antibody components of immune complexes. Although faint localization of complement was demonstrated in chronic glomerulonephritis, in vitro fixation with human complement induced bright fluorescent staining of glomeruli, indicating that complexes in glomeruli may be partially unsaturated in vivo or that complement disappears from the lesions more rapidly than the γ-globulin components. Gamma₁A-globulin, however, was found only in tubules unassociated with complement.

In acute glomerulonephritis small amounts of fibrinogen are present in a patchy distribution in renal glomeruli, in contrast to subacute and chronic glomerulonephritis, in which primarily a membranous distribution is present. The propensity of fibrinogen localization for the later stages of glomerulonephritis suggests that it is localized in previously damaged renal glomeruli, although in experimental Masugi nephritis, fibrinogen deposition has been demonstrated in the early stages of the disease (14). A concomitant abnormality of the coagulation system may contribute to fibrinogen trapping in the glomerulus. Fibrinogen persists in older lesions, as indicated by its presence.
in partially hyalinized glomerulus, and it may contribute to the glomerulosclerotic process (15). Demonstration of this protein in SLE (11), toxemia of pregnancy (16), renal cortical necrosis (17), periarteritis nodosa (18), and malignant nephrosclerosis (19, 20) indicates that it is not a unique feature of glomerulonephritis. The failure of histochemical stains to detect the membranous distribution of fibrinogen may be related to the occurrence of urea soluble fibrinogen polymers in the basement membrane (21).

The striking similarity of the immunoglobulin, complement, and fibrinogen localization in patients with glomerulonephritis and SLE may imply a common immunologic pathogenesis. The selective deposition of two immunoglobulins and complement in renal glomeruli, and the similarity of the glomerular distribution of γ-globulin and complement to experimental immunologic renal disease (22, 23) support the hypothesis that antibody is an etiologic factor. Differences in the antigenic moieties of the immune complexes and in the intensity of the antibody response to these antigens may be reflected in the more prolonged course of chronic glomerulonephritis.

Summary

Gamma_{\text{a}} and γ_{\text{M}}-globulins in association with complement and fibrinogen have been localized in the glomeruli of kidneys showing acute, subacute, and chronic glomerulonephritis. Gamma_{\text{a}}-globulin was present in tubular epithelium in the absence of complement. Complement was also fixed to glomeruli in vitro. The similarity of immunological findings in systemic lupus erythematosus and glomerulonephritis implies a common injury induced by antigen-antibody complexes and possibly by fibrinogen.

Acknowledgments

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References