GLOMERULAR LESIONS PRODUCED BY AUTOLOGOUS SERUM AND AUTOLOGOUS IgG MODIFIED BY TREATMENT WITH A CULTURE OF A β-HAEMOLYTIC STREPTOCOCCUS

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PLATE I

ALTHOUGH the epidemiological relation between certain types of group-A β-haemolytic streptococcus and human glomerulonephritis is well established (Rammelkamp, 1957), the pathogenesis of the glomerular lesions and the factors concerned in the progression of acute nephritis to chronic disease are poorly understood (Vernier, 1969).

Immunohistological studies in this disease reveal nodular deposits of IgG and β2C on the glomerular basement-membranes (Michael et al., 1966) suggestive of a pathogenic series of events similar to those observed in acute serum sickness in experimental animals (Dixon, Feldman and Vazquez, 1961). It seems unlikely that constant exposure to streptococcal antigens is involved in the production of the chronic disease, although chronic serum sickness can be produced experimentally in this way (Michael et al., 1964).

Results of a number of studies in man and laboratory animals suggest that streptococcal antigen-antibody complexes (Lindberg, Vosti and Raffel, 1967; Treser et al., 1970), tissue antibodies cross-reacting with streptococcal antigens (Markowitz, Armstrong and Kushner, 1960; Rapaport et al., 1969) and streptococcus-altered basement-membrane (Lawrence, 1959) play a role in the development and progression of glomerular lesions.

We have suggested an alternative hypothesis. Streptococcal products may alter the chemical composition of IgG, rendering the immunoglobulin auto-immunogenic and this may lead to the production of an autologous immune complex disease (McIntosh et al., 1970). We have demonstrated alterations in the carbohydrate composition of IgG exposed to type-12 group-A streptococcus in vitro and suggested that such alteration may be due to enzymic

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change or to interaction of a product of the organism with immunoglobulin such as occurs when gamma-globulin is treated with protein A from *Staphylococcus aureus* (McIntosh, Kulvinskas and Kaufman, 1971).

The purpose of the present investigation was to test our hypothesis in the experimental animal.

**MATERIALS AND METHODS**

*The test streptococcus.* A group-A type-12 β-haemolytic streptococcus was isolated from the pharynx of a patient with acute post-streptococcal glomerulonephritis. The strain was subcultured in Todd-Hewitt broth at 37°C. After centrifugation, the organisms were inoculated into several tubes of Todd-Hewitt broth and grown overnight. The pooled cultures were refrigerated and used as stock culture suspension. On the day of the scheduled experiment, 8-ml volumes of stock suspension were transferred to 40-ml volumes of Todd-Hewitt broth and incubated at 37°C for 24 hr and centrifuged. Thereafter the supernatant broth was decanted from each culture and the bacterial sediment was washed repeatedly by centrifugation with 40 ml of Dulbecco's solution at 37°C and finally suspended in 10 ml of Dulbecco's solution. In order to determine the numbers of viable organisms in these final test suspensions, blood agar plates were seeded with 0-1 ml of serial dilutions and, after incubation, haemolytic colonies were counted.

*The test animals.* Ninety New Zealand White rabbits weighing c. 2.5 kg were used in six groups of 15 (see below). Prior to study, 24-hr specimens of urine were collected and qualitative estimation of blood protein determined with Hemacombustix (Ames Labs). No animals used in the study showed any evidence of haematuria or proteinuria.

*Group I.* 50 ml of blood was withdrawn from the ear-vein of each rabbit; the serum was removed and incubated with $3 \times 10^4$ organisms at 37°C for 24 hr. The serum was then centrifuged twice at 15,000g; the supernatant of each treated serum was separately passed through a Millipore filter and injected intravenously into the donor animal.

*Group II.* 50 ml of blood was withdrawn from the ear-vein of each animal. IgG was isolated by half-saturation with (NH₄)₂SO₄, and purified by gel filtration on Sephadex G-200 (Flodin and Killander, 1962) followed by chromatography on diethylaminoethyl (DEAE) cellulose (Tomasi and Kunkel, 1964). The IgG was tested for purity by immuno-electrophoresis (Scheiddegger, 1955) and immunodiffusion in agar gel (Ouchterlony, 1958). The IgG was dialysed against distilled water, lyophilised and dissolved in sterile Dulbecco’s solution. After centrifugation at 15,000g, the supernatant solution was incubated at 37°C with $3 \times 10^4$ micro-organisms for 24 hr, centrifuged at 15,000g, and passed through a Millipore filter. The IgG was then re-isolated by ammonium sulphate precipitation, and by Sephadex G-200 gel and DEAE ion-exchange chromatography. After Millipore filtration and centrifugation at 15,000g the IgG was reinjected into the donor animal.

*Group III.* These animals received autologous serum treated in the same manner as for Group I, but micro-organisms were not introduced into the incubated serum.

*Group IV.* These animals received injections of their IgG treated in the same manner as for group II, but micro-organisms were not introduced into the incubated solution.

*Group V.* These animals received untreated autologous serum.

*Group VI.* These animals received autologous IgG in Dulbecco’s solution.

For all of the animals, antistreptolysin O (ASO) determinations were performed weekly. Detection of other streptococcal antibodies was attempted by double-layer immunofluorescence as we have previously described (McIntosh et al., 1971). Urine was studied quantitatively for blood and protein twice weekly, and urine was also promptly aspirated from the bladder of killed animals for these studies.
Histological and immunohistological studies

Animals were killed at the onset of significant proteinuria (>300 mg per 100 ml) or haematuria or at the end of 6 wk. Portions of cortex were fixed in formalin for light microscopy. Other portions were placed in vials of isopentane, quick-frozen in liquid nitrogen and saved for immunohistological studies. Immunofluorescence procedures have been described by us previously (McIntosh et al., 1970). Localisation of rabbit IgG and β1C was performed by a direct fluorescence technique (Coons, 1956). Fluorescein-conjugated antisera to rabbit IgG were obtained from Hyland Labs (California), antisera to rabbit β1C were kindly supplied by Dr T. S. Edgington (Scripps Clinic, La Jolla, California). Localisation of streptococcal antibodies was performed by double-layer immunofluorescence with serum from a patient with pharyngitis; the serum had a high titre of activity against type-12 M protein as measured by haemagglutination-inhibition (Fox, 1964) by Dr Eugene Fox (La Rabida, University of Chicago Institute) and marked antistreptokinase and antistreptococcal hyaluronidase activities.

Biological tests on materials used. Samples of all materials introduced into the animals were cultured bacteriologically to check that they were not contaminated. The biological properties of all solutions were tested for the ability to fix complement and to induce inflammatory changes in unsensitised guinea-pigs by intradermal injection. These methods have been previously described (Barnett et al., 1970).

RESULTS

In none of the animals was antibody to streptococcal antigens demonstrable by the methods that we used. Streptococcal antigens were not localised in the glomeruli of any of the animals. Cultures of the preparations injected yielded no streptococci. None of the test solutions fixed complement or was associated with inflammatory changes in guinea-pig skin when injected intradermally. Blood cultures performed were negative for all animals.

None of the control animals in groups III, IV, V and VI showed proteinuria or haematuria; no renal abnormalities were detected by light microscopy and no deposits of host IgG or β1C were observed in the glomeruli.

Animals in group I were divided into two subgroups. In group IA, animals died or were killed less than 6 wk after the injection. Of these ten animals, three died during the first 3 days of the experiment, apparently owing to injury sustained in handling of the animals or from the injection. The other seven developed haematuria and proteinuria within 10–14 days of the experiment. Glomerular changes noted in these animals varied from focal segmental mild cellular proliferation with some increases in PAS-positive material in the mesangium (fig. 1) to diffuse hypercellularity, capsular adhesions and the presence of polymorphonuclear leucocytes in the glomeruli (fig. 2). Nodular deposits of IgG and β1C globulin were localised on the glomerular capillary loops and in the mesangium (fig. 3). In the five animals in subgroup IB, which were killed at 6 wk, abnormal findings were comparatively mild or absent. Animals in group II were divided into subgroups A and B on the same basis as for group I. Findings were similar to those recorded for groups IA and IB respectively. The results of our investigations are summarised in the table.
### Table

**A summary of the observations made on the test animals**

<table>
<thead>
<tr>
<th>Test animals*</th>
<th>Day†</th>
<th>Degree of</th>
<th>Observations on sections of renal tissue</th>
<th>Degree of immunofluorescence§ specific for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>proteinuria‡</td>
<td>haematuria‡</td>
<td>Histology by light microscopy</td>
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<td>Group IA (given treated serum)</td>
<td>2 (D)</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
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<tr>
<td></td>
<td>2 (D)</td>
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<td>3 (D)</td>
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<td>0</td>
<td>Normal</td>
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<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>Focal endothelial and mesangial proliferation</td>
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<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>Focal segmental cellular proliferation with increase in PAS-positive material in the mesangium</td>
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<tr>
<td></td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>Moderately severe proliferative glomerulonephritis</td>
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<tr>
<td></td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>Focal glomerulitis</td>
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<td>2</td>
<td>Diffuse proliferative glomerulonephritis</td>
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<td></td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>Focal cellular proliferation</td>
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<td>Focal glomerulitis</td>
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<td>Normal</td>
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<td></td>
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<td>Group IIA (given treated IgG)</td>
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<td>Focal glomerulonephritis</td>
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<td>0</td>
<td>0</td>
<td>Normal</td>
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</table>

* Animals in groups IA and IIA developed early haematuria and proteinuria; those in groups IB and IIB did not and were killed at 6 wk.
† Day of killing or death (D).
‡ 0 = Nil; tr = trace; 1, 2, 3 = increasing degrees (see text).
§ − = Nil; 1, 2, 3 = increasing degrees of immunofluorescence; SA = streptococcal antigen.
Glomerular lesions produced by modified IgG

Fig. 1.—Representative glomerulus from an animal with focal lesions. Haematoxylin and eosin. ×250.

Fig. 2.—Representative glomerulus from an animal with moderately severe renal disease. Periodic acid-Schiff. ×250.

Fig. 3.—Immunofluorescent staining of glomerulus from animal in group I with FITC-conjugated goat anti-rabbit-IgG showing nodular deposits on the capillary loops and in the mesangium. ×250.
GLOMERULAR LESIONS PRODUCED BY MODIFIED IgG

DISCUSSION

Several studies have been performed in experimental animals in which renal lesions were induced by administering streptococcal organisms (MacNider, 1924; Horn, 1937; Kobernick, 1952; Tan, 1964; Lindberg et al., 1967; Becker and Murphy, 1968; Rapaport et al., 1969). These excellent studies either produced alterations not analogous to post-streptococcal glomerulonephritis in man or used methods unlike those that excite it; moreover, they lacked detailed immunological studies and there was incomplete clarification of the mechanisms involved. Studies in animals and in man have so far not satisfactorily explained the progression of acute to chronic glomerulonephritis nor the recurrence of nephritis apparently following infection not only by streptococci but by viruses and other micro-organisms.

It is well recognised that acute post-streptococcal glomerulonephritis is an immune-deposit disease. We do not dispute that the acute disease may be brought about by soluble complexes of specific streptococcal antigen and antibody. However, we have suggested that alteration of immunoglobulins either by combination with antigen or as a result of treatment with micro-organisms may lead to the production of immunoglobulins that have the biological properties of immune complexes; alternatively, modified IgG may be immunogenic and this may result in the formation of complexes of an immunoglobulin and its specific antibody of the same or different subclass (Barnett et al., 1970; McIntosh et al., 1970). The latter mechanism would lead to an autologous immune-complex disease similar to that described by Edgington, Glassock and Dixon (1967), and could bring about the development of chronic disease in a susceptible host. The results of the studies of Zinneman, Levi and Seal (1968) support this possibility.

There is no evidence that streptococcal organisms were introduced into the animals in our investigation, and the results obtained with the control animals eliminate the possibility that aggregated IgG was responsible for the disease. The results of the present study are consistent with those of our previous studies (McIntosh et al., 1970), and suggest that an autologous IgG altered by culture products of a streptococcus is capable of producing immune-complex disease.

SUMMARY

The theory that culture products of type-12 group A β-haemolytic streptococci may enzymically render IgG auto-immunogenic was investigated with reference to the production of an autologous immune-complex disease in rabbits.

Six groups of 15 animals were used. Autologous serum that had been incubated with a culture of the test streptococcus was centrifuged and freed from bacteria by filtration and then reintroduced into the donor animals in group I. Purified autologous IgG was similarly treated with streptococcal culture and the bacteria-free material was reintroduced into the donor animals in group II. Control animals in groups III and IV were treated similarly to those in groups I and II, except that streptococci were not included in the incubation
step. Animals in groups V and VI received untreated autologous serum and untreated autologous IgG respectively.

Fifty per cent. of the animals in groups I and II developed haematuria and proteinuria, and showed glomerular lesions with deposition of host IgG and $\beta_1$C on the basement-membranes; no streptococcal antigens could be detected in the kidney. Cultures of the injected preparations yielded no streptococci and results of blood culture and antistreptolysin O determinations were negative for all of the animals. The control animals in groups III–VI showed no abnormalities.

The results of this study support the hypothesis that streptococcal modification of IgG may be related to the development of immune-deposit disease.

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REFERENCES


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