PART XII
GLOMERULONEPHRITIS II
Chairman: Professor C Kleeman
Complement Deposition in Glomerular Diseases

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Summary

Biopsies from 400 patients affected by glomerular diseases, both ‘primary’ and secondary to systemic diseases, have been studied by immunofluorescence. Staining was performed for immunoglobulins, fibrogen and C_1q, C_4, C_3 and C_3A.

C_1q, C_4 and C_3 were positive in a high percentage of cases in focal glomerulosclerosis, membranoproliferative glomerulonephritis, lupus nephritis and essential cryoglobulinaemia glomerulonephritis.

C_1q and C_4 were very rarely present in focal proliferative glomerulonephritis and rheumatoid purpura glomerulonephritis. C_3A was found frequently only in acute glomerulonephritis.

Results are discussed with reference to their diagnostic value and to information about mechanisms of complement activation.

Introduction

Two different mechanisms of complement activation are possibly involved in human glomerulonephritis (GN), the classical and the alternate pathway (Götz and Müller-Eberhard, 1971).

Authors have studied the possible pathway involved in single nephropathies, both measuring serum levels of various components or by detecting the presence of these substances in glomeruli by immunofluorescence. The latter studies, initially limited to hypocomplementaemic GN only (Westberg et al, 1971, Rothfield et al, 1972), mainly correlated the presence of complement components in glomeruli with the pattern of deposits (Verroust et al, 1974), or with the immunoglobulins mainly present within glomerular structures (Berger et al, 1974).

In this study we investigate the presence of complement components in glomerular deposits in a wide group of GN, classified on the basis of histological diagnosis. Some further information about the immunohistological characteristics of single nephropathies may derive from this study, and the possible complement pathway involved may be postulated.
MATERIAL AND METHODS

We studied renal biopsies from 400 patients affected by different glomerular diseases, both ‘primary’ and secondary to systemic diseases. Details on the immuno-fluorescence techniques have been previously described (Tarantino et al, 1973). Besides sera anti-immunoglobulins, fibrinogen and albumin, biopsies were tested with rabbit anti-sera (Behringwerke) anti-C₃ and C₄ in all cases, anti-C₁q in 319 cases and C₃ Activator (C₃A) in 275 cases. Only for C₃A has the indirect method been used. ‘Primary’ GN have been grouped according to the classification of Churg, White and Habib (Churg et al, 1970).

RESULTS

Our results are shown in Table I. Besides the number and percentage of positive cases for the tested complement components, we reported the site of deposits and the predominant immunoglobulin classes observed in single nephropathies. Examination of the table allows a few observations:

(1) the exception of minimal change nephropathy and mesangial proliferative GN, C₃ was positive in a high percentage of cases.
(2) C₁q and C₄ have been found always with a lower frequency than C₃.
(3) In focal glomerulosclerosis (Figure 1), membranoproliferative GN (Figure 2), lupus nephritis (Figure 3) and essential cryoglobulinaemia GN, C₁q and C₄ were present in glomerular deposits in a high percentage of cases, together with C₃.
(4) In other nephropathies such as focal GN with mesangial IgA–IgG deposits and rheumatoid purpura GN, C₁q and C₄ were almost always absent.
(5) In membranous GN, acute exudative GN, and proliferative GN with crescents we observed fixation of C₁q and C₄ in 20–40% of cases.
(6) C₃A was positive in a good percentage of cases only in acute GN (Figure 4). In cryoglobulinaemia GN, C₃A was present in 40% of cases, but localised to hyaline thrombi.

On the basis of immunohistological patterns, we divided membranoproliferative GN into two groups, according to the presence of deposits of C₃ and immunoglobulins or of isolated C₃. We observed that in the former there was a high percentage of fixation for C₁q and C₄ (100% and 88% respectively), while in the latter this percentage was low (0% and 28% respectively).

DISCUSSION

Our findings are in good agreement with previous reports. Also other authors
Figure 1. Focal glomerulosclerosis. Focal and segmental fixation of serum anti-C₃ in subendothelial areas (x 300).

Figure 2. Membranoproliferative glomerulonephritis. Diffuse granular staining for C₁q along the basement membrane (x 300).
Figure 3. Lupus focal proliferative glomerulonephritis. Deposits of $C_{1q}$ in mesangial areas ($\times 300$).

Figure 4. Early acute poststreptococcal glomerulonephritis. Staining for $C_4$ activator as granules and nodules irregularly scattered throughout the glomerulus ($\times 300$).
<table>
<thead>
<tr>
<th>Nephropathy</th>
<th>Number of cases</th>
<th>Prominent site of deposits</th>
<th>Predominant Ig</th>
<th>C₃</th>
<th>nⁿ</th>
<th>%</th>
<th>C₄</th>
<th>nⁿ</th>
<th>%</th>
<th>C₃A</th>
<th>nⁿ</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal changes</td>
<td>34</td>
<td>s.end.</td>
<td>–</td>
<td>13/34</td>
<td>39</td>
<td>0/27</td>
<td>0</td>
<td>0/34</td>
<td>0</td>
<td>0/24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Focal glomerulosclerosis</td>
<td>35</td>
<td>s.end.</td>
<td>M</td>
<td>33/35</td>
<td>91</td>
<td>24/31</td>
<td>77</td>
<td>26/35</td>
<td>74</td>
<td>0/27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Membrane GN</td>
<td>47</td>
<td>s.ep.</td>
<td>G</td>
<td>40/47</td>
<td>85</td>
<td>7/39</td>
<td>17</td>
<td>19/47</td>
<td>40</td>
<td>0/29</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Exudative proliferative GN</td>
<td>16</td>
<td>s.ep.</td>
<td>(G)</td>
<td>16/16</td>
<td>100</td>
<td>3/13</td>
<td>23</td>
<td>5/16</td>
<td>31</td>
<td>9/12</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Mesangial proliferative GN</td>
<td>33</td>
<td>s.ep.mes.</td>
<td>–</td>
<td>20/33</td>
<td>60</td>
<td>1/22</td>
<td>4</td>
<td>2/33</td>
<td>6</td>
<td>0/17</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Proliferative GN with crescents</td>
<td>11</td>
<td>s.end.</td>
<td>G – (M)</td>
<td>10/11</td>
<td>91</td>
<td>4/9</td>
<td>44</td>
<td>5/11</td>
<td>45</td>
<td>1/6</td>
<td>16</td>
<td></td>
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<tr>
<td>Focal and segmental GN</td>
<td>70</td>
<td>mes.</td>
<td>A – G</td>
<td>64/70</td>
<td>91</td>
<td>3/51</td>
<td>6</td>
<td>7/69</td>
<td>10</td>
<td>0/48</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Membranoproliferative GN</td>
<td>42</td>
<td>s.end.</td>
<td>G – M</td>
<td>42/42</td>
<td>100</td>
<td>30/36</td>
<td>83</td>
<td>32/41</td>
<td>78</td>
<td>7/32</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Advanced and/or unclassified GN</td>
<td>16</td>
<td>s.end.</td>
<td>G – M</td>
<td>12/16</td>
<td>75</td>
<td>4/15</td>
<td>26</td>
<td>4/16</td>
<td>25</td>
<td>0/13</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Lupus nephritis                           | 42              | s.end.                     | G – M          | 40/42 | 95 | 23/32 | 72  | 30/42 | 71  | 4/32 | 12  |
| Rheumatoid purpura GN                     | 7               | mes.s.end.                 | A – G          | 5/7   | 71 | 0/7  | 0   | 0/7  | 0   | 1/5  | 20  |
| Goodpasture's syndrome GN                  | 3               | linear                     | G              | 3/3   | 100| 1/3  | 33  | 2/3  | 66  | 0/3  | 0   |
| Other (amyloidosis, Alport syndr., scleroderma, etc) | 23              | –                           |                | 13/23 | 56 | 2/17 | 12  | 3/23 | 13  | 0/12 | 0   |

* s.end. = sub-endothelial, s.ep. = sub-epithelial, mes. = mesangial
* number of positive cases/number of tested cases
have found early complement components quite frequently in focal glomerulonephritis, membranoproliferative GN, lupus nephritis (Berger et al., 1974; Michael and McLean, 1974) and very rarely in focal proliferative GN and rheumatoid purpura GN (Berger et al., 1974; Roy et al., 1973; Evans et al., 1973).

Nevertheless other authors have found no deposition of C₃A in acute GN or higher fixation for C₃ in membranous GN (Michael and McLean, 1974).

More complete information on immunohistological pattern in single nephropathies may be obtained from the use of sera against early complement components.

On the basis of complement fractions positivity, site and composition of deposits, we could distinguish four groups.

In a first group of nephropathies, with deposition of C₁q, C₄ and C₃, IgG and IgM predominate and deposits are localised in sub-endothelial areas: for this group the classical pathway of complement activation may be postulated.

In a second group with deposition of C₃ alone, mesangial IgA predominates in focal GN and rheumatoid purpura GN, and C₃ is present without immunoglobulins in the second group of membranoproliferative GN: the alternate pathway is possibly involved in these nephropathies.

A third group, with deposition of C₃ accompanied by C₁q and C₄ only in 20–40% of cases, includes membranous GN and proliferative GN with crescents: here IgG is often present in sub-epithelial areas and a long basement membrane respectively.

In a fourth group, with deposition of C₃ and C₃A, including only exudative-proliferative GN, IgG is sometimes present in sub-epithelial humps: for this condition both pathways of complement activation are possibly involved.

In all our cases we found a good relationship between the presence of complement components on the one hand and the composition and site of deposits on the other. These results, besides being a useful diagnostic help, provide some information on the possible mechanisms of complement activation in single nephropathies.

References

Götze, O and Müller-Eberhard, H J (1971) Journal of Experimental Medicine, 134, 90s
Open Discussion

STOCK (Israel) I think great care must be taken in concluding that the relative amounts of various immunoglobulins and complement components allow the conclusion that these are different disease entities, or represent different pathogenetic mechanisms. At least one important factor is the stage of the disease, which depends on the different clearing rates of immunoglobulins and of complement components. Again the rate of clearing is different in different situations within the glomerulus, for example whether the deposits are extracapillary or extramembranous or within basement membrane. The absence of immunoglobulins and early C1 components does not automatically mean that this is the alternate pathway, it could mean that the early components have already been cleared and only C3 is left.

BARBIANO di BELGIOJOSO I agree with you when you say that the finding of C3 without immunoglobulins or early complement components does not prove that the alternate pathway of complement activation is operating; in these cases this mechanism is speculative. But some authors have found a deposition of early components together with immunoglobulins in earlier biopsies and deposition of isolated C3 in a later biopsy in the same patient.