PART XVII

NEPHROLOGY: GLOMERULAR DISEASE I  

Chairmen: P Verroust  
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PART XVIII

NEPHROLOGY: GLOMERULAR DISEASE II  

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PART XIX

NEPHROLOGY POSTERS II  

Chairmen: J Stewart Cameron  
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MACROPHAGE FUNCTION IN PRIMARY AND SECONDARY GLOMERULONEPHRITIS

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Summary

The function of the mononuclear phagocyte system was assessed in vivo in 85 patients with primary and secondary glomerulonephritis, by measuring the clearance of IgG–sensitised $^{51}$Cr-labelled autologous erythrocytes. Eleven per cent of patients in clinical remission were found to have a delayed clearance, whereas impaired macrophage function was present in 62.5 per cent of the patients with major urinary abnormalities.

Blockade of mononuclear phagocyte system, induced at least in part by unidentified factors, might have a role in development and perpetuation of glomerular injury.

Introduction

Experimental models and immuno-histochemical data suggest that immune complexes (IC) might play a role in the pathogenesis of many glomerular diseases [1]. This concept is supported by the detection of IC-like material in both the circulation and the affected tissues, where deposition might result in immunologically mediated damage.

The mononuclear phagocyte system (MPS) is thought to remove IC from the bloodstream in animals [2]. Blockade of the MPS might lead to decreased clearance of IC and their enhanced deposition in the kidney.

We studied the functional activity of the MPS in 85 patients affected by primary or secondary glomerulonephritis to investigate whether an impaired removal of immunologically active substances is present in these nephropathies. In addition the relationship between the presence of IC in serum and the Fc-receptor function of MPS was investigated.

Patients and Methods

Patients

Patients included 19 cases of membranous glomerulonephritis (MGN) (16 males, mean age 41.5, 20–65 years), 17 primary IgAGN (16 males, mean age 37.1,
11–55 years), eight membranoproliferative GN (5 males, mean age 35.6, 18–50 years), nine focal glomerulosclerosis (FGS) (6 males, mean age 37.2, 19–60 years), two GN with mesangial IgM deposits (IgMGN) (50 and 61 year old women), 13 lupus nephritis (LN) (all females, mean age 41.8, 27–55 years), three polyarteritis (P) (1 male, mean age 49, 27–65 years), four cryoglobulinaemias (C) (all females, mean age 56.2, 45–65 years), five Henoch-Schönlein purpura nephritis (HS) (3 males, mean age 31.6, 19–50 years). Five cirrhosis – associated GN (C GN) (all males, mean age 57, 50–65 years).

The diagnosis in the patients with systemic disease was based on both clinical and histological data (renal biopsy having been performed in every case). Twenty-nine patients (3MGN, 6 IgAGN, 2 MPGN, 5 FGS, 5LN, 2 P, 3 C, 1 HS, 2 C GN) were studied during a clinical stage of spontaneous or therapy-induced remission (as defined by the absence of urinary abnormalities or the presence of trace amounts of haematuria or proteinuria). Ten patients (4 MGN, 1 FGS, 3 IgAGN, 2 HS) presented with non-nephrotic proteinuria and/or persistent moderate haematuria. The others had nephrotic-range proteinuria and/or severe haematuria (>25 red blood cells (RBC)/high power microscopic field), with signs of systemic vasculitis (fever or arthralgia or cutaneous manifestation or gastrointestinal symptoms) in the cases of secondary GN.

Healthy subjects

Twenty normal volunteers acted as controls for the studies of macrophage function. Sera from 30 healthy subjects were analysed for the detection of IC.

Fc-receptor function of macrophages

The procedure described by Crome [3] recently developed by Frank [4], was employed with slight modifications [5]. Three times washed autologous RBC were labelled with $^{51}$Cr and sensitised with an amount of IgG anti-Rh (D) (CTS Lyon) selected to obtain a clearance of half-time (T 1/2) in normals of about 35 minutes.

IC detection

The detection of IgG containing IC (IgGIC) was performed by employing a modified Clq solid phase assay as previously described [6]. The conglutinin solid phase assay was used for measuring IgAIC levels [7]. Results were expressed here in optical density (OD) units.

Typing for HLA and D-locus-related antigens

Tissue typing was performed on peripheral blood leucocyte by the Medical Genetics Institute of the University of Turin.

Statistical analyses employed the Mann-Whitney U-Test and the r correlation.
**Results**

**Fc-receptor function of macrophages**

The mean values of T 1/2 in patients with MPGN, IgAGN and LN were significantly higher than those in healthy people (Table I). Half-lives exceeding the upper 95 per cent confidence limit (95th percentile) in healthy people were considered as delayed. Prolonged T 1/2 were observed only in three of 29 patients (1 MPGN and 2 LN) in clinical remission as defined above. Conversely, in the presence of urinary abnormalities, seven of 16 cases of MGN, nine of 11 IgAGN, four of 6 MPGN, one of four FGS, one of two IgMGN, seven of eight LN, one of one P, one of one C, two of four HS, two of three C GN had delayed RBC clearance.

**TABLE I. Fc-receptor function of macrophages in 85 patients with primary and secondary glomerulonephritis**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>T 1/2 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy people</td>
<td>20</td>
<td>32.9 (27–46)</td>
</tr>
<tr>
<td>Membranous GN</td>
<td>19</td>
<td>47.3 (15–136)</td>
</tr>
<tr>
<td>Primary IgAGN</td>
<td>17</td>
<td>59.5* (22–180)</td>
</tr>
<tr>
<td>Membranoproliferative GN</td>
<td>8</td>
<td>46.5* (26–66)</td>
</tr>
<tr>
<td>Focal glomerulosclerosis</td>
<td>9</td>
<td>33.2 (25–48)</td>
</tr>
<tr>
<td>GN with mesangial IgM deposits</td>
<td>2</td>
<td>33 (29–47)</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>13</td>
<td>59** (25–92)</td>
</tr>
<tr>
<td>Polyarteritis</td>
<td>3</td>
<td>45 (30–69)</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>4</td>
<td>37.3 (23–70.5)</td>
</tr>
<tr>
<td>Henoch-Schönlein purpura nephritis</td>
<td>5</td>
<td>49.8 (21–92)</td>
</tr>
<tr>
<td>Cirrhosis-associated GN</td>
<td>5</td>
<td>45 (11–81)</td>
</tr>
</tbody>
</table>

*p < 0.01, **p < 0.001, N = number of subjects

**IC levels**

IgGIC levels were significantly higher than normal control values only in LN (mean value 21μg Agg IgG Eq/ml, range 0–94 versus 2.2, range 0–16, p < 0.02). In this subset IgGIC levels were found to exceed the 90th percentile in normals of 8μg Agg IgG Eq/ml in each patient with urinary abnormalities. However the correlation with T 1/2 values did not reach significant levels (r = 0.4). Low amounts of IgGIC were detected in variable percentages in other nephropathies. Sixteen point six per cent in MGN, 25 per cent in IgAGN and MPGN, 40 per cent in HS.

The mean levels of IgAIC in patients with IgAGN (0.97 OD 400nm, range 0.3–3), HS (0.9 OD 400nm, range 0.23–1.83) and C GN (0.8 OD, 400nm, range 0.55–1.33) were significantly higher (p < 0.01) than those in healthy controls (0.25 OD 400nm, range 0.17–0.61). As reported in a previous study [5]
a good correlation was confirmed in primary and secondary IgA nephropathies, between IgAIC levels and T 1/2 values.

**Typing for HLA and D-locus-related antigens**

The frequency of DR3 positive subjects in MGN was about 63 per cent (36.6% B8 DR3). Fifty-seven per cent of DR3 positive MGN patients had normal RBC clearance. No difference in clinical status was found by comparing this group with the one of DR3 positive subjects with prolonged RBC half-life. The mean T 1/2 values of DR3 positive MGN patients were not different from the negative subjects with the same nephropathy. No HLA-A, B, DR types was prevalent in IgAGN patients who had Fe-receptor dysfunction. Five out of eight MPGN patients were typed for HLA-A, B DR antigens: four of five were DR3-positive and three of them had a prolonged RBC clearance.

**Discussion**

Defective macrophage function affected a conspicuous number of GN patients with urinary abnormalities, whereas this defect was rarely found in patients in remission. The mechanism responsible for the macrophage impairment is unclear. In primary and secondary IgA-nephropathies, a role – as blocking factors – might be played by circulating IgA-containing materials. Indeed the levels if IgAIC correlated significantly with the magnitude of the clearance defect. In LN high levels of IgGIC were present in all patients with nephrotic proteinuria and/or severe haematuria. These cases were also characterised by a delayed immune clearance. However the correlation between these two parameters did not attain statistical significance, suggesting that the dynamics of IC production and macrophage saturability could be non-consensual or even individual. Impaired macrophage function was present, to variable degrees in all patient groups. However only one of nine patients with FGS had a slightly prolonged clearance half-time. In the other cases of primary or secondary GN a clearance defect affected almost exclusively the patients showing urinary abnormalities, but IC were detected, at low levels, in a few. Recently it was suggested that Fe-receptor dysfunction might be linked to some HLA-A, A, B, DR types [8]. In the present study an unsuspected prevalence, although in a quite small group of patients, of DR3 positive subjects seemed to characterise MPGN patients with MPS dysfunction. However, in MGN patients — one of the most extensively studied group — in which a high frequency of DR3 positive subjects was observed, the attempt to relate macrophage dysfunction with the presence of this antigen failed. Therefore, although an intrinsic intracellular defect — perhaps related to an active phase of the disease — could not be excluded, these data might also suggest that still undetectable immunologically active substances might account for the macrophage dysfunction in some human GN. Whatever the mechanism involved, the frequent finding of macrophage dysfunction in patients with urinary abnormalities suggest a role of this defect in the development of glomerular injury.
Open Discussion

VALLENTINE (Leiden) I am impressed by the high number of patients that you have with decreased Fc clearance. The number of patients that you have with decreased function is remarkably higher than most other studies that have been described in glomerulonephritis. As you know one of the most important factors of studying Fc clearance is the amount of IgG molecules that you have coated on the erythrocytes. The amount of IgG molecules will determine how long the clearance studies will be. What kind of studies did you do to standardise the amount of IgG of your erythrocytes to show that your assay is standardised?

ROCCATELLO As I told you before, we incubated sensitised erythrocytes with $^{125}$I-labelled anti-IgG to evaluate the number of coated sites. To standardise our test, each time we changed our batch of anti-D immunoglobulins we verified, by using this method, that we obtained the same degree of sensitisation in normal subjects and in pathological ones.