A POSSIBLE COMMON PATHOGENESIS OF THE MESANGIAL IgA GLOMERULONEPHRITIS IN PATIENTS WITH BERGER’S DISEASE AND SCHÖNLEIN-HENOCH SYNDROME

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Summary

High serum levels of polymeric IgA, partially as immune complexes, have been found in patients with Berger’s disease and Schönlein-Henoch syndrome. Polymeric IgA was also found in the renal mesangium in both entities as judged by its affinity for the human secretory component. These data reinforce the clinical and morphological suspicion that both entities may represent variations of the spectrum of the same disease.

Introduction

The possible relationship between Berger’s disease and Schönlein-Henoch syndrome has been debated for the last few years. Several lines of evidence suggest that IgA plays a predominant role in both diseases: serum IgA levels have been elevated in a high percentage of cases. The IgA has been found constantly in the renal mesangium but sometimes also in the skin vessels [1,2]. Recurrence of Berger’s disease and Schönlein-Henoch nephritis in the transplanted kidney has been documented [3,4].

The precise role and origin of the IgA has not been elucidated. We have previously demonstrated the presence of high levels of polymeric IgA, partially as immune complexes, in the serum of patients with Berger’s disease [5,6]. Recently, in an animal model of IgA nephropathy, polymeric IgA was observed to be critical for renal deposition of complexes and induction of nephritic histological changes. In contrast, monomeric IgA immune complexes failed to produce glomerular deposits [7].

In the present paper we show that high serum levels of polymeric IgA, partially as immune complexes, are frequently found in patients with Berger’s disease and Schönlein-Henoch syndrome. Furthermore this polymeric IgA was also present in the renal mesangium of both entities.
Material and methods

The diagnosis of mesangial IgA glomerulonephritis (Berger’s disease) was based upon the presence of IgA in the glomerular mesangium whether accompanied or not by C3 and other immunoglobulins. Patients with clinical or biochemical evidence of liver disease or systemic diseases were excluded. The diagnosis of Schönlein-Henoch syndrome was based upon the presence of a mesangial glomerulonephritis as described above accompanied by the typical systemic symptoms.

Sera from nine medical students and staff members were used as controls. All sera from controls and patients were handled in the same manner. Venous blood samples were allowed to clot at 37°C for 1 hour and centrifuged at 2000g at room temperature for 15 minutes. The sera obtained plus 0.02% sodium azide were stored in small aliquots at −20°C and processed within a week.

Percentage distribution of serum IgA Serum samples of 50μl were diluted 1/10 and centrifuged in 5–40% sucrose density gradients in Tris-ClH 0.15 M, pH 7.4 and Gly-ClH 0.15 M, pH 2.8 buffers. The centrifugation was performed in a Spinco L-2 ultracentrifuge with a SW-50R1 rotor, during 16 hours at 170,000g. IgM (19 S), IgG (7 S) and bovine serum albumin (4.5 S) were used as markers. Fractions of 200μl were collected from the bottom; 1.5ml of buffer borate saline 0.9% were added to each tube and read by spectrophotometry U.V. at 280nm. Each fraction was assayed for IgA by a competitive double antibody RIA [6].

IgA purification The purification of serum IgA was performed by starch block electrophoresis. The presence of IgA was sought by double immunodiffusion in the different fractions. The approximate IgA molecular weight of fractions from the anodic and cathodic sides was examined by gel filtration on Sephadex G-200 superfine [6].

J-chain examination The presence of J-chain was examined by alkaline urea polyacrylamide gel electrophoresis [8] in samples of heavy IgA, obtained either from anodic fractions of the starch electrophoresis or from Ultrogel Ac A34, that did not contain IgM, after total reduction with mercaptoethanol 10mM in urea 10M.

Affinity of the secretory component for the polymeric serum IgA Human secretory component was isolated from human whey by affinity chromatography on an IgM absorbent according to Underdown's method [9]. The affinity of the secretory component for the polymeric IgA, based upon the Brandtzaeg’s method [10], was performed as previously described [6].

Immunofluorescence studies Kidney biopsies were mounted in Ames OCT compound snap-frozen in liquid nitrogen and stored at −70°C for further processing. Cryostat sections were cut at 3 microns and stained with commercially obtained antisera to human IgG, IgA, IgM, C4, C3, fibrinogen and albumin (Meloy Labora-
tories, Springfield, Virginia). The monospecificity of the antisera was confirmed by immunoelectrophoresis.

Rabbit antihuman secretory component (SC) (FITC conjugated) was obtained from Dakopatts (Dammark). The purity and specificity of this reagent was proven as follows: immunoelectrophoresis and Ouchterlony double diffusion analysis showed no precipitation reaction against normal human serum IgA, IgG and both light chains, but reacted with free human SC, isolated as described by Underdown [9], and with human colostrum. This antiserum was further purified by passing it subsequently through a Sepharose 4B column (Pharmacia Fine Chemical, Uppsala) conjugated to human IgA, IgG and both light chain types. The resulting antiserum gave a single precipitant line with SC.

In some cases J chain was sought by an indirect antibody technique (anti J chain, Nordic Laboratories, Holland) after treatment of cryostat sections with 6M urea in glycine–HCL buffer (pH 3.2) for 1h at 4°C. This method probably 'unfolds' Ig polymers exposing J chain determinants [10].

The specificity of staining was further ascertained by the absorption of labelled antisera with an immunogen (Ig, SC or J chain) and by blocking of staining with unlabelled antisera.

**Secretory component binding** To examine the presence of polymeric IgA at the mesangial level, kidney sections, as described above, were incubated with purified SC in a moist chamber at room temperature and washed intensively with PBS [10]. Several concentrations of SC (ranging from 10 to 500μg/ml) at different incubation periods were employed to determine the optimal conditions for SC binding. Afterwards all experiments were performed with 150μg/ml of SC and 30 min of incubation time. Slides were then incubated with the fluorescent anti SC antiserum (at 1/15 dilution) for another 30 min. Patients with other nephropathies, with or without IgA deposits, and sections from normal human kidneys were used as controls. A group of patients with diffuse IgM mesangial deposits constituted a positive control group because only J chain containing 19 S IgM and polymeric or dimeric IgA show specific non covalent affinity for free SC in vitro. Patients with IgA mesangial GN and IgM deposits were excluded.

**Results**

**Study of the polymeric IgA in the serum** To determine the percentages of IgA with different molecular size we performed sucrose density gradients ultracentrifugation. In patients with Berger's disease as we have previously found [6] there was a significant increase in the IgA percentages in the fractions with sedimentation constants between 9–21 S and a significant decrease between 5–9 S in relation to the controls. This was also found in patients with Schönlein-Henoch syndrome although it was less significant.

Taking into account that most dimeric and trimeric IgA is expected in 9–13 S fractions we divided the patients according to the existence of high IgA levels in these fractions in relation to controls (Table I). The 9–13 S (mean ± SD) in these groups with high IgA levels were 42.99 ± 16.66 for the patients with Berger's
TABLE I. IgA serum levels in 9–13 S fractions after ultracentrifugation in sucrose density gradient (pH 7.4) in patients with IgA mesangial glomerulonephritis

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Normal levels</th>
<th>High levels*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berger’s disease</td>
<td>21</td>
<td>6</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>Schönlein-Henoch syndrome</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td>Primary glomerulonephritis</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values higher than mean plus 2 standard deviations from normal controls (21.12 ± 3.45%)

disease and 39.34 ± 8.87 for the patients with Schönlein-Henoch syndrome.

In some patients with IgA mesangial GN (both groups) the IgA fractions with high molecular weights 13–21 S, decreased by 10–15% when submitted to pH 2.8 suggesting the presence of a small proportion of IgA as immune complex and the majority in a covalent form (data not shown).

The presence of J chain and the affinity for the secretory component were used as criteria to ensure that forms of high molecular weight IgA are true IgA polymers. Five patients with Berger’s disease and 2 patients with Schönlein-Henoch were studied. In all cases the presence of J chain and the affinity of the SC for the high molecular IgA was found.

These data and the results of reduction-alkylation studies [6], yielding fragments of lower molecular weight, are compatible with a marked increase of polymeric IgA in the sera of some patients with IgA mesangial GN in relation to normal human sera.

Immunofluorescence study of kidney IgA The frequency of a positive secretory component binding test in kidney biopsies is shown in Table II. No patients with

TABLE II. Secretory component binding by glomerular IgA in patients with different glomerulonephritides

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>IgA</th>
<th>Secretory component binding*</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berger’s disease</td>
<td>20</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Schönlein-Henoch syndrome</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>Lupus membranous nephropathy</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mesangiocapillary GN</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mesangiocapillary GN</td>
<td>5</td>
<td>(-)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgM mesangial GN</td>
<td>4</td>
<td>(-)</td>
<td>4</td>
<td>100†</td>
</tr>
<tr>
<td>Normal human kidneys</td>
<td>4</td>
<td>(-)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Secretory component (SC) binding: kidney sections were incubated with purified SC (150μg/ml) in a moist chamber at room temperature for 30 min and washed with PBS. Slides were then incubated with fluorochrome-labelled anti-Sc treated as described in Material and methods. †This group was used as a positive control because only J chain containing 19 S IgM and polymeric or dimeric IgA show specific non-covalent affinity for free SC in vitro

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Figure 1. Immunofluorescent micrographs of glomeruli from patients with IgA mesangial glomerulonephritis (a, b, d, e, f) and IgM glomerulonephritis (c). (a, d) glomeruli from a patient with Berger's disease: (a) stained for IgA (α chain); (d) secretory component (SC) binding by glomerular IgA (stained for SC). (b, e) Glomeruli from a patient with Schönlein-Henoch nephritis: (b) stained for IgA (α chain); (e) SC binding by glomerular IgA (stained for SC). (c) Glomerulus from a patient with IgM GN used as a positive control for the SC binding (stained for SC). (f) Glomerulus from a patient with Berger's disease treated with 6M urea in glycine – HCl buffer (pH 3.2) for 1h at 4°C (stained for J chain). More details in Material and methods.
IgA deposits (plus IgG and/or C3, but not IgM), distinct from patients with either Berger's disease or Schönlein-Henoch syndrome gave a SC fixation. As expected the four patients with IgM mesangial GN were positive.

The mesangial immunofluorescence pattern of the IgA fixing the SC was similar to that obtained, with the IgA intensity being generally less important (Figure 1). Some kidney sections from patients with IgA mesangial GN showed slight staining when incubated directly with the fluorescent anti-SC. This observation was also reported by McCoy et al [11] suggesting that SC was present coincidentally with α-chain in the glomeruli in some patients with glomerulonephritis.

In the few cases in which J chains were sought slight staining was observed (Figure 1).

Discussion

Both Berger’s disease and Schönlein-Henoch syndrome have many features in common [12,13]. Clinically only extrarenal manifestations can separate one picture from the other. The IgA seems to play a predominant role in both situations although its exact role is unclear. The granular appearance of IgA deposits in the mesangium suggests an immune complex disease. In this paper we confirm on a larger series our previous findings of high levels of polymeric IgA, partially as immune complexes, in the serum of patients with Berger’s disease [6]. Furthermore this feature has also been found in some patients with Schönlein-Henoch syndrome. The normal serum IgA pattern found in some patients could suggest that timing of the IgA study might be critical.

The nature of the IgA deposited in the glomerular mesangium of patients with IgA mesangial GN remains to be identified. Several studies have failed to reveal glomerular localisation of secretory IgA [14,15], though others have occasionally found it [11,16]. Our approach to this problem was somewhat different. Based upon the specific binding of secretory component to IgA [17] and on the existence of J (joining) chain in polymeric IgA and IgM, we tried to demonstrate the presence of polymeric IgA in the mesangium. Secretory component binding has been successfully applied by Brandtzaeg to study immune cells in the intestinal epithelium, and circulating human B cells [10]. Our results suggest thereby the existence of polymeric IgA in the mesangium of a large number of patients with IgA mesangial GN.

There are some other arguments in favour of the existence of polymeric IgA in these situations. We have recently observed that the serum of both patients with Berger’s disease and Schönlein-Henoch syndrome suppressed polymorphonuclear leucocyte chemotaxis (Egido et al, manuscript in preparation), an aspect previously described in patients with IgA myeloma in fractions corresponding to polymeric forms [18]. Interestingly the high levels of polymeric IgA become normal after treatment with phenytoin, a drug that selectively decreases the serum IgA levels [5,6].

These two entities may be pathogenetically related and mediated by polymeric IgA, partially as immune complexes. The basic defect could consist of an abnormal tendency to a high degree of IgA polymerisation. This IgA, in relation to
upper respiratory tract infections, or any other injury, could form a certain pro-
portion of immune complexes. Recently in an animal model of IgA nephropathy,
polymeric IgA was observed to be critical for renal deposition of complexes and
induction of nephritic histological changes. In contrast, monomeric IgA immune
complexes failed to produce glomerular deposits [7].

In summary, the presence here shown of high levels of polymeric IgA in serum
and kidneys of patients with Berger’s disease and Schönlein-Henoch syndrome
further support the hypothesis of a common pathogenesis for the two entities.

Acknowledgments

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References

2. Baart de la Faille-Kuyper EH, Kater L, Kuijten RH, Kooiker CJ, Wagenaar SS,
   Zouwen P van der, Dorhout Mees EJ. *Kidney Int* 1976; 9: 424
   1974; 18: 343
5. Lopez Trasca M, Egidio J, Sancho J, Hernando L. *Proc EDTA 1979*; 16: 513
   1977; 14: 111
    Takeuchi J. *Quart J Med, NS 1978*; 188: 495
15. Whitworth JA, Leibowitz S, Kennedy MC, Cameron JS, Chantler C. *Clin Nephrol*
    1976; 5: 33