EVIDENCE OF HIGH POLYMERIC IgA LEVELS IN SERUM OF PATIENTS WITH BERGER’S DISEASE AND ITS MODIFICATION WITH PHENYTOIN TREATMENT

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Summary

High levels of polymeric IgA were found in the serum of patients with IgA glomerulonephritis. In four of the patients the IgA percentage distribution was established by ultracentrifugation in sucrose density gradients before and after six months of phenytoin treatment. A decrease in polymeric IgA, adopting a pattern similar to the controls, was observed. These findings may have both pathogenic and therapeutic implications.

Introduction

The presence of IgA in the mesangium in a granular pattern constitutes IgA glomerulonephritis (IgA GN) as described by Berger and Hinglais [1] and Berger [2]. Very little is known about the pathogenesis of mesangial IgA deposits. The high serum IgA levels frequently found in these patients [3–6] and the frequency of disease recurrence after kidney transplantation [7] suggests a host abnormality, perhaps in the IgA itself. Circulating immune complexes containing IgA have not yet been demonstrated [8] and no experimental model for this type of lesion is available.

We have recently reported the presence of a large amount of serum IgA of high molecular weight in patients with Berger’s disease [9], and defined some biochemical characteristics of this IgA including the presence of J-chain and its affinity for free secretory component [10].

IgA GN was initially considered a benign entity but recent reports [4,6] have shown that deterioration of renal function is not uncommon, occurring in about 20% of patients after several years. No treatment has as yet been considered useful. Since a reduction of serum IgA is a common finding in epileptics receiving phenytoin therapy [11,12] we have studied the effects of this drug in a group of patients with Berger’s disease.
Material and Methods

The diagnosis of IgA nephropathy (Berger’s disease) was based, in 15 patients, upon clinical and laboratory data and on histological and immunofluorescent studies of percutaneous renal biopsy specimens [6]. Patients with clinical or biochemical evidence of liver disease, systemic lupus erythematosus, Henoch Schonlein syndrome and other systemic diseases were excluded. Sera from medical students were used as controls. Serum IgA preparations were stored in small aliquots at −20°C and processed within a short time.

Informed consent of the patients was obtained. The dose of phenytoin was 300mg day and it was the only drug taken by the majority of the patients during the study. Three of them also received a thiazide diuretic. Clinical, laboratory and immunological assessments were made at each hospital visit. The preliminary studies of the effects of phenytoin on serum IgA in 6 patients are reported here.

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

IgA purification The purification of serum IgA was performed by starch electrophoresis. The presence of IgA was looked for by double immunodiffusion, in the different fractions. The approximate IgA molecular weight of fractions from the anode and cathode side was examined by gel filtration on Sephadex G−200 superfine.

J−chain examination The presence of a J−chain was examined by alkaline urea acrylamide electrophoresis [13] in samples containing large amounts of IgA, obtained either from the anode fractions of the starch electrophoresis or from Ultragel Ac A34, that did not contain IgM, after total reduction with mercaptoethanol 10mM in urea 10M.

Affinity of the secretory component for polymeric IgA Human secretory component was isolated from human whey by affinity chromatography on IgM sepharose absorbent according to Underdown’s method [14]. The affinity of the secretory component for the polymeric IgA was evaluated by Brandzaeg’s method [15].

Phenytoin assay The serum concentration of phenytoin was measured by a commercially available radioimmunoassay (Amersham, England).
Results

In the patients with Berger’s disease studied before the beginning of phenytoin treatment there was a significant increase in the percentage of IgA in the fractions with sedimentation constants between 9–13 S, 13–17 S and 17–21 S and a significant decrease between 5–9 S in relation to the controls (Table I). In four patients the percentage distribution of IgA was similar to the controls and did not change with time.

**TABLE I. Effect of Phenytoin Treatment on Serum IgA Percentage Distribution after Ultracentrifugation in Sucrose Density Gradient (pH 7.4) in Patients with IgA Mesangial Glomerulonephritis**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>5–9 S</th>
<th>9–13 S</th>
<th>13–17 S</th>
<th>17–21 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>4</td>
<td>58.94 ± 3.15 //</td>
<td>33.38 ± 1.46</td>
<td>7.27 ± 1.59</td>
<td>0.60 ± 0.24</td>
</tr>
<tr>
<td>After 6 months treatment</td>
<td>4</td>
<td>79.12 ± 4.55</td>
<td>18.23 ± 4.03</td>
<td>2.11 ± 0.98</td>
<td>0.53 ± 0.19</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>75.08 ± 3.17</td>
<td>21.12 ± 3.45</td>
<td>3.41 ± 0.95</td>
<td>0.38 ± 0.16</td>
</tr>
</tbody>
</table>

// Mean ± SD. * paired t-test

To determine whether the increases in 9–21 S fractions were caused by IgA as immune complexes the serum of some of the patients was studied at pH 7.4 and 2.8, the IgA percentage being determined in the same way. There was no significant change in the 9–17 S fraction which represents more than 98% of serum IgA. These results are consistent with a covalent structure for these forms of high molecular weight IgA, suggesting that the circulating IgA is not playing a role as a circulating immune complex.

IgA samples with high molecular weight obtained from the anode side of starch electrophoresis [16] did not contain IgM or IgG. In 5 of these patients the presence of J-chain by urea alkaline acrylamide electrophoresis was found in the IgA samples with high molecular weight (Figure 1). Affinity of the secretory component for polymeric IgA was also shown in the 5 patients studied.

These data and the results of reduction-alkylation studies [9,10], yielding fragments of lower molecular weight, are consistent with a marked increase of polymeric IgA in the sera of these patients in relation to normal human sera.

In a group of four patients with high serum polymeric IgA levels, the percentage distribution was established before and after six months of phenytoin treatment. There was a decrease in polymeric IgA and an increase in monomeric IgA adopting a pattern similar to the controls (Table I). In two other patients with a normal IgA percentage distribution there was also a decrease in polymeric forms — 20.66 ± 3.69 vs 14.65 ± 1.66 (9–13 S), 5.04 ± 1.07 vs 2.03 ± 1.97 (13–17 S) and 0.76 ± 0.09 vs 0.48 ± 0.03 (17–21 S) —, and an
increase in monomeric forms (73.51 ± 1.60 vs 82.84 ± 3.65) suggesting that the action of phenytoin was more marked on the IgA of high molecular weight. In these six patients the serum phenytoin concentrations were in the therapeutic range.

**Discussion**

This study confirms our previous findings that a large amount of serum IgA with high molecular weight is present in patients with IgA glomerulonephritis. The results of analytical ultracentrifugation at pH 2.8, which dissociates the non-covalent forces binding antigen to antibody, are against the serum IgA playing any role as an immune complex.

The existence of true polymers requires certain requisites, such as the presence
of J-chain and affinity for the secretory component [17]. Both requisites were observed in the patients studied.

The absence of secretory IgA in serum or in the glomerular mesangium of these patients [18,19] is against the possibility that polymeric IgA diffuses back into the vascular compartment from the gastrointestinal tract. The true origin of this IgA remains to be elucidated. The marked increase of IgA bearing peripheral blood lymphocytes and a significantly decreased IgA specific suppressor T cell activity [20] suggest a probable primitive lymphocyte abnormality.

It has been shown that aggregated serum proteins injected into experimental animals are localised preferentially in the mesangium [21]. The large amount of high polymeric IgA levels found in the serum of patients with Berger's disease might follow the same route.

The diminution of the high polymeric IgA levels observed after 6 months of phenytoin treatment is of particular interest. It is possible that the normalisation of polymeric IgA levels could decrease the load of abnormal IgA presented to the mesangium, thereby allowing it to eliminate the polymeric IgA. We have observed that serum from patients with Berger's disease suppresses polymorphonuclear leucocyte chemotaxis [22], an aspect previously described in patients with IgA myeloma in fractions corresponding to polymeric forms [23]. It is likely that the polymeric IgA decreases the IgG-mediated phagocytosis by human polymorphonuclear leucocytes [24]. We are presently evaluating whether or not both (chemotaxis and phagocytosis capacity) become normal after the decrease of polymeric IgA levels following phenytoin treatment.

How phenytoin diminishes the serum IgA levels is not yet known. Fontana et al [25] have suggested that a predisposition to phenytoin-induced low serum IgA levels occurs only in epileptic patients in whom some constitutional factors are present. This may account for the differences observed with time in the serum IgA levels measured by radial immunodiffusion in patients with Berger's disease. Phenytoin affects many enzymes and it is difficult to speculate on its mechanism of action. Depression of one or more parameters of cellular and/or humoral immune responses was found in 60% of the patients treated with phenytoin [26]. Phenytoin reduces DNA synthesis in vitro and in vivo. This may be a major mechanism by which the drug causes immunosuppression. To support and there are data suggesting that the fall in IgA levels is due to failure of synthesis rather than an increase in catabolic rate [27].

Even though a complete and careful clinical study, including repeated renal biopsies, is needed to prove whether this therapy is effective, the IgA changes described and their normalisation by phenytoin treatment (or eventually by other drugs able to reduce serum IgA) offers a pathogenic and therapeutic approach to this nephropathy.

Acknowledgments

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References

1 Berger, J and Hinglais, N (1968) *J. Urol. Nephrol.*, 74, 694
10 López-Trascasa, M, Egido, J, Sancho, J, Hernando, L (1979) (Submitted for publication)
13 Reisfeld, RA and Small, PAJ (1966) *Science.*, 152, 1253
16 Gavrilova, EM, Egorov, AM and Shakhmina, KC (1976) *Biokhimiya*, 41, 684
21 Michael, AF, Fish, AJ and Good, RA (1967) *Lab. Invest.*, 17, 14
23 Van Epps, DE and Williams, RC (1976) *J. Exp. Med.*, 144, 1227

Open Discussion

KOENE (Nijmegen) I want to ask you if you think that the IgA polymers might be responsible for the cryoglobulinaemia that is sometimes found in patients with Berger’s disease?

EGIDO We have not searched for cryoglobulins in the serum of these patients. But in France, Cordonnier has found them sometimes, but the IgA was never contained in the cryoglobulin itself.

KATER (Utrecht) Did you ever find indications of the presence of polymeric IgA in skin biopsies or kidney biopsies of patients with Morbus Berger?

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EGIDO  Probably the failure to find polymeric IgA at the mesangium level in the literature was due to the attempt to find the secretory IgA. We think that the IgA deposited in the mesangium is in the polymeric form, but not containing secretory component, because in serum fractions with high molecular weight IgA the secretory component was never found. Returning to your question, we are now looking for true polymeric IgA in the renal mesangium based upon the studies performed in the serum, that is the affinity of secretory component for polymeric IgA, but at the moment I have no results to give you.

VRIESMAN (Maastricht) Since you often find in Berger’s disease both IgA and IgG deposited in the mesangium, one wonders if you also looked for the presence of IgG aggregates in the blood?

EGIDO  This study is concerned only with the biochemical aspects of IgA, because, as you know IgA seems to be the immunoglobulin commonly involved in this nephropathy, among other reasons because some cases contain only IgA in the mesangium and the high serum IgA levels frequently found in these patients, etc.

BERGSTROM (Stockholm) I would like to ask you, did you see any clinical effects or benefits of phenytoin treatment in these patients; I mean decrease in haematuria for instance?

EGIDO  Here, I have presented only the biochemical study of serum IgA and the effects of the phenytoin treatment on this IgA. Logically we are doing a complete study of the clinical and immunological aspects and also we want to perform repeated renal biopsies. At the moment I have not complete data to give you, but roughly the frequency of macroscopic haematuria seems to diminish. However I think that the true effects of treatment in this nephropathy, with so prolonged a course, will be based upon the biochemical study of IgA, and the effects on the IgA deposited in the renal mesangium, because as you know the haematuria sometimes disappears spontaneously or occasionally runs an intermittent course in patients without treatment. I think it will be very difficult to assess on clinical grounds the beneficial effects of a drug in the nephropathy we are talking about.