IgA1 and IgA2 IN CIRCULATING IMMUNE COMPLEXES AND IN RENAL DEPOSITS OF BERGER’S AND SCHÖNLEIN-HENOCH GLOMERULONEPHRITIS

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Summary

IgA subclasses in circulating immune complexes (IgA1IC), serum immunoglobulins and mesangial deposits in Berger’s and Schönlein-Henoch glomerulonephritis (GN) were studied. Both IgA1IC and IgA2IC were significantly higher in Berger’s and Schönlein-Henoch GN than in healthy people. In phases of clinical activity both IgAIC subclasses further increased. The IgA1/IgA2 ratio was found not to differ from controls in either groups of patients. An increase in polymeric IgA was observed in Berger’s and in Schönlein-Henoch GN. Both IgA subclasses were found in mesangial deposits.

Introduction

The analysis of IgA subclasses in plasma and renal tissue of patients affected by IgA nephropathies, Berger’s and Schönlein-Henoch glomerulonephritis (GN), gives some insight into the still controversial pathogenesis of these nephropathies [1,2]. Normal serum IgA, 85 per cent of which consists of monomers, contains 90 per cent of IgA1, and only 10 per cent of IgA2, whereas IgA1 and IgA2-producing cells are almost equally represented in normal mucosa and IgA from these cells is mainly polymeric.

We have studied 31 Berger’s GN and 14 Schönlein-Henoch GN cases, by investigating IgA subclasses in circulating immune complexes (IgAIC), in serum immunoglobulins (IgA) and in renal deposits. Serum polymeric IgA was also measured.

Materials and methods

Patients

Thirty-one Berger’s GN (48 sera) and 14 Schönlein-Henoch GN (27 sera) were studied. The phase of clinical activity was judged on the basis of microscopic
haematuria (more than 100 red blood cells/microscopic field) or of a significant increase in microscopic haematuria.

IgA1C

A previously modified conglutinin solid phase assay [3] utilising purified antIgA antibodies, labelled with alkaline phosphatase, was employed to detect the amount of IgA containing IC. Results were expressed in µg/ml of heat aggregated secretory IgA added to a pool of normal human sera (NHS).

IgA1IC and IgA2IC

An amplified conglutinin solid phase assay [4] was employed with unconjugated sheep anti-human alpha-1 and alpha-2 chains (Nordic Immunologic Laboratories) and purified rabbit anti-sheep immunoglobulins, labelled with alkaline phosphatase. Results were expressed in µg/ml of heat aggregated secretory IgA in NHS.

Dimeric IgA

Radial immunodiffusion technique, after reduction of the IgA by 1,4-dithioerythritol and alkylation with iodoacetamide [5].

Total IgA immunoglobulins

Immunodiffusion plates (Behring).

IgA1/IgA2 immunoglobulins

Solid phase inhibition test. Purified myeloma IgA1 and IgA2 proteins (kindly provided by Professor A O Carbonara and Dr De Marchi) were adsorbed on plastic surfaces. The inhibition of binding of both antisera induced by the sera to be tested was measured with anti-sheep IgG labelled with alkaline phosphatase. The ratio between the OD at 400nm with the anti IgA2 and that obtained with anti IgA1 was assumed to be the IgA1/IgA2 ratio.

Renal immunofluorescence (IF)

Direct IF technique with anti IgA (Behring). Indirect IF was applied on 16 kidney specimens, employing the above mentioned unconjugated anti IgA1 and IgA2, and fluorescein-coupled rabbit anti-sheep immunoglobulins. Two different antisera were employed: in a pilot study (on five renal biopsies) the anti IgA2 antisera displayed a cross reactivity with purified IgA1, whereas a second antisera (employed on 11 renal biopsies) did not show any significant cross reactivity.

Results and discussion

In both Berger's and Schönlein-Henoch GN we have found mean values of total
TABLE I. Detection of IgA class (IgAIC) and IgA subclass immune complexes (IgA1IC and IgA2IC)

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Number of sera</th>
<th>IgAIC µg IgA aggr/ml</th>
<th>IgA1IC µg IgA aggr/ml</th>
<th>IgA2IC µg IgA aggr/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy people</td>
<td>34</td>
<td>34</td>
<td>6.5±9.9</td>
<td>4 ±10.5</td>
<td>11.3±30.4</td>
</tr>
<tr>
<td>Berger's GN</td>
<td>31</td>
<td>48</td>
<td>61.6±121.2*</td>
<td>352.2±548.6*</td>
<td>244.1±424.2*</td>
</tr>
<tr>
<td>Schönlein-Henoch GN</td>
<td>14</td>
<td>27</td>
<td>197.7±328.1*</td>
<td>583.8±788.5*</td>
<td>409.7±589.2*</td>
</tr>
</tbody>
</table>

* p<0.01 in comparison with healthy people

IgA containing circulating immune complexes (IgAIC) to be significantly greater (p<0.01) than those detected in healthy people (Table I). The IgAIC values were significantly elevated (p<0.02) in the Schönlein-Henoch than in the Berger group and they were found to be significantly higher (p<0.02) in the clinically active phase than during inactive stages. The IgAIC data correlate significantly with the amount of microscopic haematuria (r=0.26, p<0.01).

The mean values of IgA1 and IgA2 subclasses in the IgAIC were found to be significantly greater (p<0.001) in both groups of patients than in healthy people (Table I).

On analysing the results obtained in various clinical phases (Table II) it was observed that in the clinically inactive phases of the diseases, both the IgA nephritides studied differ from healthy people only in the values of IgA1IC (p<0.01). The amounts of IgA2IC were similar to those observed in the normal population, even though a significant difference (p<0.001) was found between the mean values of IgA2IC obtained in the Berger and in the Schönlein-Henoch groups when studied in clinically inactive disease.

During the phases of clinical activity in Berger’s GN both kinds of IgAIC rose significantly (p<0.001). In Schönlein-Henoch GN both IgAIC subclasses rose in the active phase but the differences between various clinical stages was significant

TABLE II. IgA1IC and IgA2IC (µg IgA aggr/ml) in various clinical phases

<table>
<thead>
<tr>
<th></th>
<th>Active phase</th>
<th>Inactive phase</th>
<th>Student’s test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berger’s GN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA1IC</td>
<td>568 ±660.9</td>
<td>45.6± 32.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgA2IC</td>
<td>314.6±375.2</td>
<td>36.8± 86.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schönlein-Henoch GN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA1IC</td>
<td>881.5±919.2</td>
<td>135 ±278.8</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>IgA2IC</td>
<td>644.4±696.6</td>
<td>4.1± 10.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
only for the IgA2IC (p<0.001). The difference between IgA1IC mean values in the active and inactive phase of Schönlein-Henoch GN was not statistically significant because of persisting small amounts of IgA1IC even in inactive disease. Comparing the data obtained in the phase of clinical activity, higher mean amounts of IgA1IC were observed in the Schönlein-Henoch than in the Berger group (p<0.01).

On analysing the IgA subclasses in IgA immunoglobulins, no significant IgA1/IgA2 ratio changes were observed in either group in comparison with healthy people, even in Berger's GN where the mean values of total IgA were found to be significantly higher than controls (p<0.001).

No correlation was observed between the IgA subclass ratio in circulating immune complexes and in serum immunoglobulins (r=0.22, p>0.1).

In both groups of patients an increase of plasma IgA after use of reductive-alkylating agents, thought to be due to a dissociation of polymeric IgA, was observed (27.5% of sera from Berger's GN and 50% of sera from Schönlein-Henoch GN). In Berger's GN a significant correlation was observed between the total IgA and polymeric IgA values (r=0.44, p<0.01).

**TABLE III. IgA subclasses in mesangial deposits. First batch of antisera with some cross reactivity between anti-IgA2 and IgA1. Second batch of antisera without any significant cross reactivity.**

<table>
<thead>
<tr>
<th>First batch of antisera</th>
<th>Number of patients</th>
<th>Intensity of fluorescence*</th>
<th>Mean value of IgA1/IgA2 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgA</td>
<td>IgA1</td>
</tr>
<tr>
<td>Berger's GN</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Schönlein-Henoch GN</td>
<td>3</td>
<td>3.6</td>
<td>3</td>
</tr>
<tr>
<td>Second batch of antisera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berger's GN</td>
<td>8</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Schönlein-Henoch GN</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

* The fluorescence scores were obtained on the scale of zero to six.

In all the 16 kidney biopsies examined deposits of both IgA1 and IgA2 subclasses were found (Table III). Differences in results may be due to differing activity of reagent materials. In a pilot study of five renal biopsies, employing antisera having some cross reactivity between the anti IgA2 and alpha-1 chain, results similar to those of André [1] were obtained, with a predominance of IgA2 deposits in all cases. Different antisera without any significant cross reactivity gave completely different results, with a prevalence of the IgA1 over the
IgA2 deposits. Nevertheless, detectable IgA2 deposits were still evident in each case.

In conclusion our data confirm the hypothesis of the pathogenic role of circulating IgA containing immune complexes in IgA nephropathies. Moreover the significant presence of IgA2 reactivity in circulating IgAIC and mesangial deposits of these glomerulonephritides, together with high values of IgA which increase after reductive-alkylating agents, thought to be polymeric, suggest a mucosal origin of IgA in circulating IC and renal deposits, in agreement with experimental models [6,7]. Moreover some different immunological aspects between Berger's and Schönlein-Henoch GN, might account for different clinical behaviours possibly through a greater complement activation and consequent vasculitic lesions due to more IgA1IC in Schönlein-Henoch GN.

References

2 Conley ME, Cooper MD, Michael FJ. Clin Invest 1980; 66: 1432
5 André F, André C. Biol Gastroenterol 1976; 9: 147
7 Isaacs K, Miller F, Lane B. Clin Immunol Immunopathol 1981; 20: 419

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Open Discussion

RITZ (Heidelberg) There is currently controversy whether IgA1 or IgA2 is deposited in mesangium. I agree with your conclusion that the findings vary with the reagent used. Dr Waldherr in our group repeated the experiment of André, reported in the New England Journal of Medicine, where he found IgA2 using Nordic Co antisera. We have also found predominantly IgA2. However using the Beckton Dickinson monoclonal antibody used by Conley reported in J Clin Invest we confirmed his finding of exclusive IgA1 deposition in mesangium, although IgA2 was demonstrable in casts and tubular cells.

MERY (Chairman) I think that is a very important point.

COPPO I think that really the most important problem in evaluating the IgA sub-classes in circulating IgA containing immune complexes and in renal deposits of patients affected by IgA nephropathy is in having available reagents with good specific activity. In fact as I have pointed out our results changed according to whether we used reactive material with or without cross reactivity between the anti IgA2 antibodies and the Alpha 1 chain. But we had different results when we used antisera with different specific activity between the anti
IgA1 and anti IgA2 antibodies even when they displayed the same cross reactivity. In our first group of patients we performed the indirect immunofluorescence on the renal tissue employing antisera in which about 75 per cent of the activity was of the anti IgA1, using the antisera used by André we found a predominance of IgA2 deposits. But we had different results employing antisera with the same cross reactivity, that of the previous batch, but different in the ratio of the specific activity between the anti IgA1 and the anti IgA2 antibodies. In this lot the anti IgA2 was far less active than the anti IgA1 and the ratio of the specific activity between the two antibodies was about 34 per cent: we had a completely different result with a prevalence of the IgA1 over the IgA2 deposits. We checked the specific activity and the cross reactivity of the antibodies employed in a solid phase assay ELISA. The conclusion is that there is a very great variability in specific activity and in cross reactivity from one batch of antisera to another and we have to take into account that problem.

VALENTYN (Leiden) I have a comment and a question. We have examined several of the commercial monoclonal antibodies including the Nordic and the Beckton Dickinson antibodies and compared with the monoclonal antibodies produced in our own laboratory and extensively tested elsewhere. Using our own monoclonal antibody in a direct immunofluorescence assay in patients with IgA nephropathy we were able to demonstrate exclusively IgA1. We have never found IgA2 in the mesangium of those patients and using other antisera, including Nordic, we found very conflicting results finding IgA1 as well as IgA2 which also goes for the Beckton Dickinson antisera. I think that for immunofluorescence techniques it is very doubtful whether the Nordic antisera is satisfactory. They are probably adequate for precipitation techniques, but for immunofluorescence is doubtful. You were showing data about conglutinin binding immune complexes in 30 patients and these values were derived from something like 40 samples, so you had more samples than you had patients. How many patients with IgA nephropathy were actually positive with the conglutinin binding assay?

COPPO The percentage of positive results in all the 75 serum samples examined for IgA containing immune complexes was about 30 per cent, but personally I think that the percentage of positive results is not very important because the levels of circulating IgA containing immune complexes is fluctuating in IgA nephropathy and they are related to the clinical phases of activity; therefore in these cases the analysis of results only as a percentage of positive data, in my personal opinion, does not really mean anything.

VALENTYN (Leiden) I think the percentage might be important once you give a pathogenetic importance to the presence of those immune complexes. As you probably know we presented last year in Athens data that conglutinin binding complexes in patients with IgA nephropathy are present in about 30 to 35 per cent of patients. However, in patients with haematuria due to other causes we find the same incidence of conglutinin binding complexes, over 30 per cent,
and the same was observed for patients with Henoch-Schönlein purpura. I think the presence of conglutinin binding complexes in both these groups of patients is not very specific and if it was specific I was hoping that you could show us that there was a direct correlation between the presence and the sub-class in the circulation and a sub-class in the deposits. Were you able to demonstrate such a direct correlation between the presence in the circulation and the deposits?

COPPO Of course all cases displayed IgA deposits in mesangial areas, but the presence of IgA containing circulating immune complexes was observed only during the phases of clinical activity. It is possible to find IgA deposits in the mesangium but during a period of clinically inactive disease the IgA containing immune complexes will be normal. But in the same patient they can increase during periods of clinical activity.

VALENTYN How do you explain the other 70 per cent of patients?

COPPO They were mostly in a stage of non-active disease. Certainly not 100 per cent of the cases showed positive results for IgA1 during the phases of clinical activity, but the difference we observed between the values of IgA1 and IgA2 during different phases of clinical activity is I think of some meaning.

BERTHOUX (Saint Etienne) I think your contribution is very important. With André we were using rabbit polyclonal and IgA1 and A2 antibodies. We checked with Beckton Dickinson monoclonal antibodies and in fact we found most cases of A1 and only one out of 10 A2. If you are using monoclonal antibodies from another supplier you find A1 and A2 and even with monoclonal antibodies you don't find the same thing.

COPPO We checked for cross reactivity between the anti IgA2 antibodies and Alpha 1 chain and we have in fact seen, in two different batches of antisera from Nordic, a very high level of cross reactivity ranging between 75 and 85 per cent. Later on we obtained another batch of antisera which was good from the beginning and moreover we performed affinity chromatography on these antisera and we obtained only a slight amount of cross reactivity, as little as 10 per cent of cross reactivity between the anti IgA2 and the Alpha 1 chain, and so I think that the reactive material we used was purified enough.

MERY I would like to ask if you have been able to study the presence of the secretory component in mesangial IgA?

COPPO No, we have not performed this study.