THE PRESENCE OF J CHAIN IN MESANGIAL IMMUNE DEPOSITS OF IgA NEPHROPATHY

U Donini, S Casanova, N Zini, P Zucchelli

Malpighi Hospital, Bologna, Italy

Summary

In order to identify polymeric IgA in the mesangial IgA deposits of 33 patients, the presence of J chains by a PAP method was investigated. In 9/27 cases of primary IgA nephropathy (PlgANP) and in 4/4 cases of Henoch-Schönlein glomerulonephritis (HSGN) J chains were absent. The remaining cases, two of them with IgA glomerulonephritis associated with alcoholic cirrhosis (ACLgAGN), were J-positive. The two groups, PlgANP J-positive and PlgANP J-negative, were compared according to clinical features. The only difference detected was the time length between the disease onset and the renal biopsy, being shorter in the negative group.

Introduction

Polymeric IgA has been considered important in the pathogenesis of primary IgA nephropathy (PlgANP), Henoch-Schönlein nephritis (HSGN) and glomerulonephritis associated with alcoholic cirrhosis (ACLgAGN). Its presence was essential in causing experimental PlgANP [1]. Nevertheless, different investigators studying serum polymeric IgA in patients with PlgANP have obtained differing results [2–4].

The aim of this work was to assess if the mesangial IgA, deposited in the glomeruli of patients with PlgANP, HSGN, ACLgAGN, was monomeric or polymeric. We examined the glomerular immune deposits for the presence of J chain, a small glycopeptide which is linked to α and µ chain in polymeric immunoglobulins. We found two groups of patients, one J-positive and the other J-negative. We then compared the clinical features of these two groups.

Material and methods

Patients

We studied 33 patients, 27 with PlgANP, without any laboratory or clinical evidence of systemic and liver diseases. All these patients had diffuse mesangial
deposits of IgA, some also had C3 deposits (96.29%), moderate IgM (33.3%) and IgG (40.7%). Four patients had the clinical features of Henoch-Schönlein purpura. All four had predominant IgA deposits, which were associated with C3 (100%) and IgG (25%). No IgM was present. Two patients with AC IgAGN had heavy mesangial IgA deposits, which were accompanied with C3 (100%) and IgM (50%). In one of these cases renal tissue was obtained at autopsy. As controls, another ten patients suffering from different nephropathies were studied. Two had prominent IgG glomerular deposits, five had IgM deposits and three were without any deposits (Table I).

**TABLE I. Number and percentages of J-positive cases in the different groups of patients studied and frequency of positivity for IgA, IgM, IgG**

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>J</th>
<th>IgA</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IgA nephropathy</td>
<td>27</td>
<td>18 (66.6%)</td>
<td>27</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Henoch-Schönlein glomerulonephritis</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IgA glomerulonephritis associated with alcoholic cirrhosis</td>
<td>2</td>
<td>2 (100%)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Membranous glomerulonephritis</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Focal and segmental glomerulosclerosis</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse granulomatosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Minimal change glomerulonephritis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Antisera**

FITC-conjugated rabbit antisera to human IgG, IgA, IgM, C3, FITC-conjugated goat antiserum to rabbit immunoglobulins (Igs) and FITC-conjugated rabbit antiserum to goat Igs were obtained from Behring. FITC-labelled goat antiserum and unlabelled rabbit and goat antisera to human J chain were obtained from Nordic Laboratories, and unlabelled swine antiserum to rabbit Igs and rabbit PAP complex from DAKO.

**Absorption of antisera and specific staining controls**

FITC-conjugated goat antiserum to rabbit Igs and FITC rabbit antiserum to goat Igs were absorbed exhaustively with human Igs until they produced no fluorescence on mouse liver sections containing heat aggregated human Igs as previously described [7].

By Ouchterlony double diffusion anti-J antiserum had a weak but evident precipitating line against reference J-chain (Nordic). Immuno-electrophoresis and Ouchterlony double diffusion, showed no precipitating reaction of unabsorbed
anti-J chain sera against human IgG, IgA, IgM and colostrum IgA (Behring). On the other hand, by immunofluorescence, antisera to J chain was found to react with IgG deposited in glomeruli in the two cases of membranous glomerulonephritis used as controls. Therefore these antisera were absorbed with purified human IgG until the staining of IgG immune deposits disappeared (Figure 1). Three mg of IgG per 1ml of anti-J antiserum were needed.

Figure 1. Control staining with anti-J antiserum absorbed with human IgG. A) Membranous glomerulonephritis: lack of the reaction. B) Bone marrow smear of patient with IgA myeloma: two plasma cells are J-positive (PAP method, 250x – 300x)

The cross-reaction of absorbed anti-J antisera with α chain was excluded by the following steps. When the bone marrow smear of a patient with IgA myeloma was stained with anti α chain antiserum, many plasma cells presented a positive reaction. However when new smears of the same bone marrow were stained with anti-J antiserum absorbed with IgG, only a few plasma cells showed a positive reaction (Figure 1). After absorption with purified colostrum IgA coupled to CNBr-activated Sepharose 4B (Pharmacia) anti-J antiserum did not react with the positive control (IgA myeloma bone marrow smears).

**Immunofluorescence technique**

Renal tissue was processed as described elsewhere [5]. Cryostat renal sections were air dried, fixed in acetone, treated for 20 min at 4°C with 0.1M glycine-HCl buffer, pH 3.2, containing 6M urea and washed overnight with PBS at 4°C. The sections were stained by direct and indirect immunofluorescence using specific antisera. The same treatment was performed on bone marrow smears of patients with IgA myeloma. Observation was made by Zeiss photomicroscope II equipped with epifluorescence condenser III RS and XBO 75W/2 xenon light source. Specific fluorescence was graded from 0–4+.

**Immunoperoxidase technique**

Cryostat sections were stained by the peroxidase anti-peroxidase (PAP) immunohistochemical technique [6]. The peroxidase activity was demonstrated by
incubating the sections for 10 min at 20°C in Hankser-Yates medium (Polysciences), followed by treatment with diaminobenzidine-4HCl medium and osmium tetroxide in order to amplify the staining reaction. Intensity of reaction was scored from 0 to 4+.

Choosing of the immunohistochemical technique

At first we compared the different immunohistochemical techniques in order to establish which would be the best.

By direct and indirect immunofluorescence, we found ten cases which were J-positive (three cases with 3+ and seven cases with 2+/1+ of positivity). These cases were stained also by PAP method. The positivity for J chain was found to be in six cases 4+ and in four cases 3+. Therefore we decided that immunofluorescence was unsatisfactory for our investigation, since several results were doubtful.

The 33 cases studied and the ten control cases, were examined using PAP method on cryostat sections treated with urea-glycine buffer. Each case was stained with a) anti-alfa chain antiserum, b) anti-J antiserum absorbed with human IgG. As J-positive we considered only the cases with a minimum of 25 per cent intensity grade for J-chain vis a vis a 100 per cent IgA (Figures 2 and 3).

Figure 2. Primary IgA nephropathy. A) Heavy mesangial IgA deposits. B) The same case with positivity for J chain reaction (PAP method, 250x)

Figure 3. Henoch-Schönlein glomerulonephritis. A) Heavy mesangial IgA deposits. B) The same case was negative for J chain reaction (PAP method, 250x)
**Statistical analysis**

The value of the significance for the differences between J-positive and J-negative groups was evaluated by means of the student t test and \( \chi^2 \) test for the ordinal and the nominal variables respectively.

**Results**

The results obtained are shown in Table I.

Nine of the J-positive and three of the J-negative cases had neither IgG or IgM deposits. In the other cases the positivity for IgG and IgM was minor compared with IgA positivity.

The frequency of cases with IgM deposits was 6/20 for the J-positive group and 4/13 for J-negative group. In our study we excluded the possibility that J-positivity was due to IgM by the following reasoning. The frequency of IgM mesangial deposits is the same in both groups. However in two control cases with mild deposits of IgM the reaction for J chain was negative. Furthermore in the six positive cases for both IgM and J chain, the J chain positivity was stronger than that of IgM. Therefore we considered this J-positivity resulted from the presence of polymeric IgA.

**TABLE II.** Primary IgA nephropathy. Comparison between J-positive and J-negative cases, frequency, percentage and mean values of the different parameters

<table>
<thead>
<tr>
<th></th>
<th>J + ve (18 cases)</th>
<th>J - ve (9 cases)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria &gt; 1g/day</td>
<td>8 (44.4%)</td>
<td>4 (44.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrohaematuria</td>
<td>10 (55.5%)</td>
<td>6 (66.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Presence of hypertension</td>
<td>5 (27.7%)</td>
<td>2 (22.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>P creat (mg/dl): mean value range</td>
<td>1.16</td>
<td>1.18</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.6 – 2</td>
<td>0.7 – 1.3</td>
<td></td>
</tr>
<tr>
<td>Serum IgA &gt; 400mg/dl</td>
<td>8 (44.4%)</td>
<td>3 (33.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years): mean value</td>
<td>34.29</td>
<td>28.4</td>
<td>NS</td>
</tr>
<tr>
<td>Males</td>
<td>16 (88.8%)</td>
<td>7 (77.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Time between the onset of the</td>
<td>2.66</td>
<td>1.28</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>disease and renal biopsy (years):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular sclerosis &gt; grade 3</td>
<td>2 (11.1%)</td>
<td>1 (11.1%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

We examined the PlgANP patients in two groups: one J-positive and the other J-negative. In Table II we compare the clinical features, glomerular sclerosis grade and time length between onset of the disease and renal biopsy of these two groups.

The different parameters were not significantly different in the two groups.
except for the time length between disease onset and renal biopsy. The J-positive group had the illness for a longer period.

Discussion

Our data of J-positivity in 100 per cent of cases with AC1AgAGN agrees with results of others who have found high polymeric IgA values in the serum and kidneys of such patients [3,8]. In rats, hepatocytes can synthesise secretory component and transport polymeric IgA from blood to bile [9,10]. If this hepatocyte function exists also in man, its failure could explain the high amount of polymeric IgA which is frequently found in AC1AgAGN.

All our cases of HSGN and 33.3% of cases with PIgANP were J-negative. Other reports found a lower frequency of J-negative cases [11,12]. We believe that this discrepancy comes from the different methods and subjective evaluations used. Precipitating tests are incapable of uncovering undesired cross-reaction of antisera used in immunohistochemical techniques. Immunohistochemical control tests are required. Using the PAP method we could perform exhaustive absorption of the antisera without losing the specific sensitivity of the reaction. In any case the presence of few amounts (1+) of J chain does not mean that immune deposits are predominantly constituted of polymeric IgA. In our study we were looking for the cases where the presence of polymeric IgA could be considered a major component of the IgA deposit.

We compared the clinical features of the two groups: J-positive and J-negative. From the different parameters studied, we found that the only significant difference between the two groups was in the time length of the disease. At the time of biopsy, the J-negative patients had a more recent onset of clinical signs.

The first stage of the illness could be due to pathological monomeric IgA. The recurrence of PIgANP after renal transplantation has been found to correlate with abnormal amounts of conglutinin reactive IgA, predominantly monomeric IgA [4]. It is possible that this alteration of monomeric IgA facilitates its mesangial deposition. Moreover the deficiency of specific IgA suppressor T-cells activates the IgA system [13].

Peripheral blood lymphocytes of patients with PIgANP can synthesise in vitro polymeric IgA [14]. Furthermore serum IgA rises during mucosal inflammatory diseases [15]. In this way, a larger amount of PIgA could intermittently circulate in the plasma and at such times it could be possible to find high polymeric IgA values in the serum and in the immune deposits.

References

Open Discussion

BERTHOUX (St Etienne) I will make a comment; in 1969 Berger reported this disease as IgA plus IgG. We all know that now we find most frequently IgA and IgM, for instance in our experience we found IgM in 70 per cent of the cases. You found J-positivity in 65 per cent so how can you rule out the presence of small amounts of IgM in your biopsy. I agree that IgA must be of mucosal origin and may be polymeric but I don’t think you can solve it by your methods.

DONINI In 33 per cent of the cases studied in this work IgM deposits were present but these deposits were minor. We excluded that the positivity for J chain was due to IgM deposits, because the frequency of IgM positivity was the same in the two groups, J-positive and J-negative. Furthermore the J-positivity was stronger than IgM positivity. For these reasons we concluded that J-positivity was not IgM dependent.

RITZ (Heidelberg) Your findings are in agreement with Dr Waldherr of our group who also demonstrated J chain but I share the concern of Dr Berthoux, with respect to compounding problems resulting from the concomitant presence of IgM and I am much more hesitant to conclude from the presence of J chain that there is polymeric IgA. I think the final answer will have to come from elution studies.

DONINI We did not examine eluates from these cases. We have excluded that the J-positivity was due to IgM but I agree that absolute certainty on this point can only come from elution studies.

VALENTYN (Leiden) I would like to congratulate you on this study because I think it is fascinating data. I am not very sure whether your conclusion that there are two types of IgA nephropathy based on the presence or absence of J chain isn’t a little bit too premature. There are a lot of technical problems in investigating the J chain in those patients, one of which is IgM. We have observed several patients with exclusively IgA that were also J chain positive and in patients with IgA nephropathy, the IgA in a double staining technique is capable of binding secretory component itself, which is indirect evidence of course for the presence of J chain. You treated all your biopsies with glycine.
urea. Did you have any problems with untreated biopsies? Did you really have to pre-treat your biopsy material as there are several studies that claim that they have demonstrated the J chain without pre-treatment. What is your experience with that?

DONINI We found that without treating the sections by urea glycine buffer the J reactivity sometimes was positive and sometimes it was not very positive. We decided that it was better to treat the sections with urea glycine buffer because the results were more consistent.