Properties of circulating IgA molecules in Henoch–Schönlein purpura nephritis with focus on neutrophil cytoplasmic antigen IgA binding (IgA-ANCA): new insight into a debated issue*

R. Coppo¹, P. Cirina¹, A. Amore¹, R. A. Sinico², A. Radice² and C. Rollino³, for the Italian Group of Renal Immunopathology Collaborative Study on Henoch–Schönlein purpura in adults and in children

¹Nephrology and Dialysis Department, Regina Margherita Children’s Hospital, Torino, ²Nephrology and Dialysis Department, S Carlo Hospital, Milano, ³Nephrology and Dialysis Department, Giovanni Bosco Hospital, Torino, Italy

Abstract

Background. The presence and the pathogenetic role of circulating IgA reacting with neutrophil cytoplasmic antigens (IgA-ANCA) in patients with Henoch–Schönlein purpura (HSP) is still debated. This study was aimed to investigate some characteristics of serum IgA and macromolecular IgA in HSP patients, focusing on IgA-ANCA.

Methods. Eighty-seven HSP patients with biopsy proved renal involvement (51 adults and 36 children) enrolled in a multicentre study of the Italian Group of Immunopathology were investigated.

Results. Significantly high levels of IgA immune complexes were found in both adults (P < 0.05) and children (P < 0.01), while the binding of IgA to jacalin, was significantly low in children with HSP (P < 0.01) only. Two series of ELISA were done for IgA-ANCA, in two different laboratories. Increased binding to PMN crude extracts (P < 0.01) without any modification in IgA binding to proteinase 3 was found by either specific ELISA. Conversely, the binding of IgA to myeloperoxidase (MPO) was found to be significantly (P < 0.05) increased with positive values in 25% of patients by one assay only. Three of four sera with positive IgA-MPO ANCA exhibited binding in Western-blot studies with the MPO preparation used in ELISA to a 28-kDa species. D-galactose and N-acetyl-glucosamine decreased the binding of serum IgA to MPO more in HSP than in controls (P < 0.05).

Conclusions. The conflicting reports on IgA-ANCA may reflect some atypical characteristics of the reaction which can be detected only by some ELISAs. We suggest that not an antigen-antibody reaction but a lectinic interaction due to abnormal composition of IgA carbohydrate side chains may account for the IgA-ANCA reaction in patients with HSP nephritis.

Key words: ANCA; IgA-ANCA; Henoch–Schönlein purpura; IgA glycosylation; lectins

Introduction

A multicentre collaborative study of the Italian Group of Renal Immunopathology has recently evaluated histological and clinical features of 219 patients (136 adults and 83 children) presenting IgA nephropathy secondary to Henoch–Schönlein purpura (HSP) [1]. This large cohort of patients was thought to be suitable to analyse some immunological factors possibly related to the pathogenesis of HSP nephritis and a study was aimed to investigate abnormalities in circulating IgA of the patients enrolled in the main study and still in follow-up.

We measured circulating IgA immune complexes (IgAIC), mixed IgA-IgGIC, IgA-fibronectin complexes and IgA with altered galactosylation. We especially focused on the reaction between serum IgA and neutrophil cytoplasmic components (IgA-ANCA), whose presence in sera of HSP patients is still debated. After the first report of increased reactivity of serum IgA with acidic neutrophil extracts [2], the following published investigations have sent contrasting messages [3–9]. There is a general agreement that IgA molecules from these patients react more than controls with neutrophil extracts; however, doubts remain on the kind of reactivity with the lysosomal enzymes, namely myeloperoxidase and serine proteinase which are the targets of IgG-ANCA in several other vasculitides [10,11]. IgA ANCA have been detected in variable percentages of patients with HSP [2–9]; however, the immunofluorescence pattern is atypical [8,9], the target of the reactivity is still unknown and fibronectin [9] and yet not identified membrane-associated proteins [8] have been proposed.

Our collaborative study allowed the investigation of a cohort of patients which, for the homogeneous
criteria, including renal biopsy material review, number of patients enrolled of wide age range, is unique in the literature. Moreover, sera were examined by different laboratories, that in the past had either positive or negative results in IgA-ANCA detection in HSP [4,9]. The results obtained suggest that patients with IgA nephropathy secondary to HSP have in circulation IgA molecules bearing various abnormal reactivities which can be due to altered glycosylation. This abnormal glycosylation supports an increased reactivity with the ANCA antigens. Of interest, the detection of the IgA-ANCA binding can be evidenced only with particular assays, thus suggesting a role of different methodologies in the conflicting results from the recent literature.

Subjects and methods

Patients

Sera were collected from 87 patients enrolled in the main study (51 adults and 36 children) still in follow-up. All patients satisfied the selection criteria of IgA-dominant glomerular immune deposits in biopsy material available for review, palpable purpura (slightly raised haemorrhagic skin lesions without thrombocytopenia) and/or bowel angina (abdominal pain worsening after meals or bowel ischaemia with bloody diarrhoea).

Total serum IgA

Serum IgA levels were detected by nephelometry (Beckman Array Protein System, Brea, CA, USA)

Macromolecular IgA

IgAIC detected by the conglutinin solid-phase assay (K-IgAIC), mixed IgA-IgGIC and IgA-fibronecin (IgA/F) aggregates were analysed as described in detail elsewhere [12].

Serum IgA specific properties

IgA molecules binding to the lectin jacalin, supposed to have abnormal galactosylation [13], were measured as described before [12].

Rheumatoid factor

IgA rheumatoid factor was detected as described elsewhere [14].

Detection of IgA-ANCA

Neutrophil antigens employed. Proteinase 3 (PR3) was purified in Laboratory 2 as described [9]. Myeloperoxidase (MPO) was purchased from Calbiochem (La Jolla, CA). Acid neutrophils extracts were prepared from normal volunteer myeloid cells isolated on a Hypaque gradients, lysed in wells uncoated with antigen and processed with the addition (MPO) was purchased from Calbiochem (La Jolla, CA). This assay was performed in 10 sera positive in ELISA 1, identified in Laboratory 2 as described [9]. Myeloperoxidase ELISA 3 (Laboratory 1)

Neutrophil antigens employed. Proteinase 3 (PR3) was purified in Laboratory 2 as described [9]. Myeloperoxidase (MPO) was purchased from Calbiochem (La Jolla, CA). Acid neutrophils extracts were prepared from normal volunteer myeloid cells isolated on a Hypaque gradients, lysed in wells uncoated with antigen and processed with the addition of serum, as done in ELISA 2. Results were calculated as in ELISA 2.
**Analysis of the interactions between cytoplasmic lysosomal enzymes and IgA-ANCA**

To analyse the lectin sugar-dependent interactions, the following sugars (Sigma) were used for the assay: 0.1 M mannos (Man); 0.1 M N-acetyl-glucosamine (GlcNac); 0.1 M galactose (Gal); 0.1 M N-acetyl-galactosamine (GalNac). Each serum, diluted in PBS to get the final concentration of IgA 20 mg/dl, was diluted again 1:2 in PBS or in each sugar and incubated for 1 h at room temperature. Samples without and with the sugars were tested, in duplicated, in consecutive wells. The ELISA was then performed as described above (ELISA 1) and results calculated as percentage of inhibition of each sugar: OD value without sugar—OD value with sugar divided by OD value without sugar $\times 100$.

In five sera positive for IgA-ANCA, the effect of co-incubation with poly-L-lysine (10 $\mu$g/ml), jacalin (50 $\mu$g/ml), fibronectin (10 $\mu$g/ml), neutrophil extracts (100 $\mu$g/ml), purified MPO (1.6 $\mu$g/ml), sialic acid (0.05 M) was similarly evaluated.

**Western blot for IgA ANCA**

Purified MPO (Calbiochem, Lot no. B10818) was diluted in acetate buffer 50 mM, pH 6, NaCl 100 mM, and further diluted in Laemmli sample buffer without mercaptoethanol. Five micrograms of boiled MPO were loaded in a 10% polyacrylamide separating gel (Pharmacia, LKB, Uppsala, Sweden) with 4% acrylamide stacking gel according to Laemmli [15], using a Bio Rad apparatus (Mini-Proteinn II, Bio Rad Laboratories, Richmond, CA) at constant amperage, 100 volts. After transfer for 60 min at 100 volts, 250 mA in 25 mM Tris, 192 mM glycine, $v^20$% methanol, pH 8.3, the nitrocellulose strips were blocked with 10% BSA (Sigma) with HSP overnight. Four HSP sera positive in ELISA for binding to MPO, 5 HSP sera negative and five healthy controls were blotted for 3 h at room temperature. The strips were washed with PBS-Tween and incubated overnight with $^{125}$I-labelled anti-human IgA (Amersham, NL). The binding was detected by autoradiography. The presence of MPO antigenic fragments in the blot was assessed by a polyclonal rabbit anti-MPO (Dako, Glostrup, Denmark) diluted 1/3000 and alkaline phosphatase labelled anti-rabbit antibodies.

**Statistics**

Statistical calculations were performed using a standard statistical package (Statview 512 Brainpower, Calabasas, CA). For comparison of mean levels of each test data among the various groups the ANOVA analysis of variance was used. A $P$ value $<0.05$ was considered to be statistically significant. Linear regression analysis was used for testing the relationship between two variables. Stepwise multiple regression analysis was performed to evaluate the influence of several data (dependent variables) on one result series (independent variable).

**Results**

**Serum IgA**

IgA levels $>400$ mg/dl were found in 31% of adults and values higher than the individual ranges for age in 33% of children with HSP.

**Macromolecular IgA**

Mean levels of conglutinin-binding circulating IgAIC (Table 1) were significantly higher than controls in the whole group ($P<0.01$) as well as in the two subgroups of adults ($P<0.05$) and children ($P<0.01$).

Mean levels of IgA/IgGIC were significantly increased in adults only ($P<0.05$).

In contrast, mean levels of mixed IgA/IgGIC were similar to controls.

**IgA rheumatoid factor** mean levels were significantly increased in adults only ($P<0.05$). However, normal range for children was not available.

**Binding of IgA to jacalin**

Mean values were significantly reduced in children with HSP only ($P<0.01$) and similar to controls in adults (Table 1).

**Correlations of macromolecular IgA and the clinical data**

Multivariate analysis failed to show any statistical correlation among different forms of macromolecular IgA or IgA with abnormal binding to different probes and systemic or urinary signs of activity of HSP.

**Binding of IgA to sonicated neutrophil extracts**

By ELISA 1 assay, mean OD values of the binding of IgA to neutrophil extracts in HSP patients were significantly higher than controls ($0.75\pm0.59$ OD vs

---

**Table 1. Macromolecular IgA and IgA with abnormal binding to different probes in HSP patients**

<table>
<thead>
<tr>
<th></th>
<th>Adult HSP</th>
<th>Adult controls</th>
<th>Children HSP</th>
<th>Children controls</th>
<th>Positive values adults (%)</th>
<th>Positive values children (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_IgAIC$</td>
<td>$0.30\pm0.26^*$</td>
<td>$0.18\pm0.14$</td>
<td>$0.50\pm0.28^{**}$</td>
<td>$0.21\pm0.20$</td>
<td>16</td>
<td>51</td>
</tr>
<tr>
<td>$IgA-IgGIC$</td>
<td>$0.28\pm0.13$</td>
<td>$0.24\pm0.10$</td>
<td>$0.16\pm0.11$</td>
<td>$0.15\pm0.10$</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>$IgA/F$</td>
<td>$0.34\pm0.20^*$</td>
<td>$0.22\pm0.14$</td>
<td>$0.32\pm0.26$</td>
<td>$0.25\pm0.18$</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>$IgA-jac$</td>
<td>$0.23\pm0.14$</td>
<td>$0.24\pm0.17$</td>
<td>$0.16\pm0.11^*$</td>
<td>$0.21\pm0.16$</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>$IgA-RF$</td>
<td>$0.22\pm0.34^*$</td>
<td>$0.12\pm0.07$</td>
<td>$0.15\pm0.26$</td>
<td>n.d.</td>
<td>20</td>
<td>8</td>
</tr>
</tbody>
</table>

$K_IgAIC$, conglutinin binding IgAIC; $IgA-IgGIC$, mixed IC; $IgA/F$, IgA-fibronectin complexes; $IgA-jac$, IgA binding to jacalin; $IgA-RF$, IgA rheumatoid factor; $^*P<0.05$ vs controls; $^{**}P<0.01$ vs controls.
0.49 ± 0.23 OD, \( P < 0.01 \)). Of the 59 sera tested 12 (20.3%) had positive values, i.e. > mean + SD, (32.3% of adults and 4% of children) and 6 (10%) had values exceeding the 3rd SD, which included all the data in healthy controls (Figure 1).

By performing ELISA 1, mean OD values of the binding of IgA to MPO were significantly higher than controls (0.45 ± 0.37 OD vs 0.30 ± 0.12 OD, \( P < 0.05 \)). Positive data were found in 15/59 (25.4%) of patients (32.3% of adults and 16% of children), and very high values exceeding mean + 3SD were found in 10 (16.9%), (Figure 1). No increased binding was found for PR3 (0.46 ± 0.36 OD vs 0.44 ± 0.49 OD, \( P = \text{n.s.} \)) and only two patients (2.9% of adults and 4% of children) had positive values.

By using ELISA 2, the mean OD values of the binding of IgA to MPO (0.26 ± 0.13 OD vs 0.22 ± 0.07 OD, \( P = \text{n.s.} \)) and PR3 (0.13 ± 0.06 OD vs 0.10 ± 0.04 OD, \( P = \text{n.s.} \)) found in HSP patients were not significantly different from those of controls. Each serum was negative in the final evaluation, since positive data, found in 16/90 (17.7%) for MPO (22.6% of adults and 10.8% of children) and 8/88 (9%) for PR3 (9.4% of adults and 8.1% of children) turned out to be negative when the binding of IgA to non coated wells was subtracted. Figure 2 reports the comparison of the two series of data obtained in the two laboratories by ELISAs 1 and 2. To facilitate the comparison with the respective values in healthy controls, results are expressed as ratio between values in patients and in controls.

Since different result series (ELISAs 1 and 2) were confirmed in additional checks by either laboratories, ELISA 3 was performed in 10 sera positive by ELISA 1 but negative by ELISA 2. ELISA 3 gave data similar to ELISA 1, as 9/10 cases still showed values higher than mean + 2 SD in healthy controls similarly tested and calculated.

**Western blot for IgA ANCA**

In a reference gel in which markers of different MW had been run, four clear bands were evidenced, the greatest of 28 kDa and three others between 38 and 50 kDa. The specificity for MPO was confirmed by the reaction with anti MPO antibodies (Figure 3).

Four sera with positive binding to MPO by ELISA 1 were tested: of them three showed a strong reactivity with the band of 28 kDa. This reactivity was not found with the other positive HSP patient nor with the healthy or diseased controls (Figure 4).

**Correlations of IgA-ANCA with other laboratory, clinical, and histological variables**

Since only ELISA 1 provided a consistent number of positive data, correlations were searched using this series of data only.

Serum IgA were prediluted, in healthy controls and in HSP patients, to get the final levels of 20 mg/dl, hence any interference with the results due to age related or individual variation in IgA levels was avoided.

No correlation was found with class of renal biopsy and clinical presentation at onset. However, both clinical onset and renal biopsy were been done 1–5 years...
proteinuria or the presence of significant microscopic haematuria. Conversely, patients with positive IgA binding to MPO had a significant association ($P < 0.05$) with extracapillary proliferation, outcome in dialysis or impaired renal function. Similar associations were not found for IgA binding to neutrophil extracts. The multivariate analysis showed that the binding of IgA to neutrophil extracts is significantly related to the binding to MPO and jacalin ($P < 0.01$).

**Inhibition of binding of IgA to neutrophil cytoplasmic antigens by sugars**

The binding of IgA to neutrophil extracts was reduced by 20–30% of basal values by the addition of Man, GlcNAc and Gal and the effect of Man was significant higher than in control sera ($P < 0.05$) (Figure 5). The binding of IgA to purified MPO was inhibited by 20–30% of basal values. GlcNAc and Gal exerted a significantly different effect in comparison to controls ($P < 0.05$ for both sugars) (Figure 6).

In five sera with positive binding to neutrophil extracts the coincubation with different molecules variously modified the basal binding (Table 2). At the

Fig. 3. SDS 10% acrylamide gel electrophoresis. Lane 1, MW markers. Lane 2, Calbiochem MPO blot (5-μg/slot) in denaturating (boiling) non-reducing conditions. Lane 3, Western blot. A rabbit polyclonal anti-MPO reagent (Dako) blotted MPO in 4 bands: the greatest of 28 kDa and the others between 38 and 50 kDa.

before; therefore this lack of correlation was somehow expected. Hence we looked at correlations with the present general and renal status, i.e. active purpura and systemic signs, blood creatinine, proteinuria and microscopic haematuria. No correlation was found with the result of either test and the amount of proteinuria or the presence of significant microscopic haematuria. Conversely, patients with positive IgA binding to MPO had a significant association ($P < 0.05$) with extracapillary proliferation, outcome in dialysis or impaired renal function. Similar associations were not found for IgA binding to neutrophil extracts. The multivariate analysis showed that the binding of IgA to neutrophil extracts is significantly related to the binding to MPO and jacalin ($P < 0.01$).

**Inhibition of binding of IgA to neutrophil cytoplasmic antigens by sugars**

The binding of IgA to neutrophil extracts was reduced by 20–30% of basal values by the addition of Man, GlcNAc and Gal and the effect of Man was significant higher than in control sera ($P < 0.05$) (Figure 5). The binding of IgA to purified MPO was inhibited by 20–30% of basal values. GlcNAc and Gal exerted a significantly different effect in comparison to controls ($P < 0.05$ for both sugars) (Figure 6).

In five sera with positive binding to neutrophil extracts the coincubation with different molecules variously modified the basal binding (Table 2). At the

Fig. 4. Western blot on Calbiochem MPO in denaturating, non-reducing conditions. Lane 1, IgA-ANCA negative HSP serum. Lanes 2 and 3, IgA-ANCA positive HSP sera by ELISA 1 for binding to purified MPO. A band of 28 kDa was clearly evidenced.

Fig. 5. Reduction of the binding of IgA to neutrophil extracts in HSP patients and controls by the addition of various sugars.

Fig. 6. Inhibition of the binding of IgA to purified MPO in HSP patients and controls by the addition of various sugars.
which was obtained as well by Laboratory 2 which

Discussion

Table 2. Inhibitory effects on the binding of IgA to neutrophil cytoplasmic antigens

| Binding of IgA to Binding of IgA to |
|----------------------|----------------------|
| Neutrophil extracts (%) | purified MPO (%) |
| Neutrophil extracts | HSP<sup>1</sup> | Controls | HSP<sup>2</sup> | Controls |
| Neutrophil extracts | 1 | 6 | 0.7 | 2.8 |
| MPO | 4 | 0.1 | 3 | 4 |
| Fibronectin | 2 | 4 | +6<sup>*</sup> | 5 |
| Jacalin | +3 | +12 | +11<sup>*</sup> | +2 |
| Poly-L-lysine | +65 | +50 | +46 | +17 |
| Sialic acid | 10<sup>*</sup> | 0.5 | 0.8 | 0.4 |

<sup>1</sup>P<0.05 vs inhibitory effect in controls.
<sup>2</sup>Five sera from HSP patients positive for binding of IgA to neutrophil extracts.

concentration employed, neutrophil extracts as well as MPO failed to exert a significant inhibition on the binding of IgA to neutrophil extracts. The binding was enhanced by the cationic molecule poly-L-lysine in both HSP and controls and inhibited by negatively charged sialic acid, particularly in HSP (P<0.05 vs controls).

The binding of IgA to MPO was enhanced as well by coincubation with poly-L-lysine, while sialic acid failed to influence the binding. Also the coincubation with fibronectin enhanced the binding of IgA to MPO and the difference with controls was statistically significant (P<0.05). The binding of IgA to MPO was insignificantly inhibited by either purified MPO and neutrophil extracts.

Discussion

Table 2. Inhibitory effects on the binding of IgA to neutrophil cytoplasmic antigens

| Binding of IgA to Binding of IgA to |
|----------------------|----------------------|
| Neutrophil extracts (%) | purified MPO (%) |
| Neutrophil extracts | HSP<sup>1</sup> | Controls | HSP<sup>2</sup> | Controls |
| Neutrophil extracts | 1 | 6 | 0.7 | 2.8 |
| MPO | 4 | 0.1 | 3 | 4 |
| Fibronectin | 2 | 4 | +6<sup>*</sup> | 5 |
| Jacalin | +3 | +12 | +11<sup>*</sup> | +2 |
| Poly-L-lysine | +65 | +50 | +46 | +17 |
| Sialic acid | 10<sup>*</sup> | 0.5 | 0.8 | 0.4 |

<sup>1</sup>P<0.05 vs inhibitory effect in controls.
<sup>2</sup>Five sera from HSP patients positive for binding of IgA to neutrophil extracts.

The IgA-dominant renal deposits in a clinical setting of systemic vasculitis put HSP nephritis in between two completely different disease entities, primary IgA nephropathy (IgAN) and idiopathic vasculitis. The relationships between idiopathic IgAN and the IgAN secondary to HSP are not yet clearly defined, even though several pathogenetic mechanisms seem to be common, like hyperactive immune system with enhanced IgA response and increased levels of IgAIC in patients with similar renal histological features (reviewed in [16]). In agreement with these reports, we detected abnormal levels of circulating macromolecular IgA or IgA rheumatoid factor in 20–50% of patients with HSP nephritis. These data were not correlated with urinary signs of activity, proteinuria or renal function. This lack of correlation with the phases of clinical activity differs from previously published reports [17], possibly because the cross-sectional design of our study did not allow the analysis of patients in very active phases of HSP.

Primary IgAN and IgAN related to HSP differ by the features of systemic vasculitis. However, HSP is a peculiar kind of vasculitis, without pulmonary or respiratory tract involvement, fever and cachexia, which characterizes most vasculitides. Moreover, HSP has abundant IgA deposits while the immunoglobulin deposits are scanty in other systemic vasculitides. Several data suggest a pathogenetical role for IgG-ANCA in most vasculitides and crescentic glomerulonephritis [10,11,18,19]. While IgG ANCA were generally found to be negative in patients with HSP [8,9], conflicting data have been reported on the presence of IgA-ANCA [2–9] and this issue remains controversial. IgA-ANCA are described in 28–79% of patients with HSP, in some cases related with the phases of clinical activity [6]. IgA directed to PMN acid extracts obtained by cell sonication have been more frequently observed; however, these antibodies failed to show clear character of specificity for IgAN idiopathic or related to HSP in comparison to other nephropathies [3].

We compared the results of two series of ELISA done in different laboratories which in the past reported either positive [4] or negative [9] data for IgA-ANCA, with the hope of getting further details on this discrepancy. The binding of IgA to sonicated neutrophil extracts was significantly increased in comparison to controls and some 33% of patients displayed positive values. No increased binding was found for PR3 by either test. Differences in results were limited to the binding to MPO. Indeed, prevalence of positive data reached 25% with ELISA 1, while ELISA 2 gave negative data for each serum. This difference was not due to different control populations, nor to calculations taking into account the aspecific binding of IgA to plastic surface. The only major difference between the two tests was the use of human or bovine serum albumin as protein saturating uncoated plastic sites and serum dilutions. Human serum albumin, used by Laboratory 1 provided with satisfying low background, which was obtained as well by Laboratory 2 which used BSA. To avoid the binding of IgA to coating BSA, in ELISA 2 sera were preincubated in BSA. This step does not influence the specific binding of IgA to MPO in case of a true antigen-antibody reaction, but might decrease this binding in case of carbohydrate or electrical charge interactions. Indeed the binding of IgA to MPO failed to be reduced by preincubation with either MPO or acid extracts, again indicating a poor role for antigen-inhibition effects, while it was affected by electrical charge (poly-L-lysine and sialic acid) and by carbohydrates. Jacalin and fibronectin, which can bind and bridge IgA to other glycoproteins [13,20], enhanced the binding of IgA to MPO.

These data are consistent with a lectinic binding between IgA and neutrophil cytoplasmic antigens. The multiple carbohydrate interactions, which mediate this binding are only partially affected by the addition of MPO or neutrophil extracts, since these molecules do not represent unique specific targets as in the case of antigen–antibody reactions. It is conceivable that circulating IgA in HSP may have disturbances in glycosylated residues similar to those found in primary IgA nephropathy [21–24]. IgA is a highly glycosylated molecule, bearing O-linked glycans rich in GalNAc and Gal in the hinge region, and N-linked bi-branched...
hydrate abnormalities and neutrophil cytoplasmic anti-

On the basis of these observations we aimed our study to investigate the hypothesis of a role played by carbohydrate interactions in the reactivity between IgA and neutrophil cytoplasmic antigens.

The binding of IgA to neutrophil extracts and to MPO was inhibited by the addition of Man, GlcNAc and Gal, and the effects were significantly higher than in controls.

Our data suggest that in HSP nephritis the increased binding of IgA to neutrophil extracts is mediated by an increased exposure of Man, suggesting truncation of N-terminal biantennary chains of IgA.

The binding to MPO was inhibited by GlcNAc and Gal. These data are consistent with the hypothesis that the binding is mediated by a truncation of O-linked glycans.

It is not clear whether these abnormalities are related to the pathogenesis of the HSP nephritis or are an epiphenomenon.

HSP nephritis can be experimentally induced by lypopolisaccharide injection [26]. The glycosylation pattern of serum IgA can be regulated by genetic factors as well as by cytokines produced during mucosal infections. The resulting abnormal glycosylated residues of IgA might facilitate the binding to neutrophil cationic enzymes released by polymorphonuclears during infections.

Circulating complexes formed by IgA bearing carbohydrate abnormalities and neutrophil cytoplasmic antigens may lead to lower polymorphonuclear and T-lymphocyte enhancement and granuloma formation, than IgG-ANCA complexes and may conditionate their mesangial delivery.

However, the inconstancy of the finding of IgA with increased binding to neutrophil cytoplasmic antigens in HSP may indicate as well that this reactivity is devoided of a clear pathogenetical role.

Besides these considerations, our study gives new insight into the debated issue of IgA-ANCA in HSP, suggesting that the reactivity is not due to an antigen-antibody recognition, but reflects some atypical characteristics of circulating IgA in these subjects, which bear modified carbohydrate side chains leading to abnormal lectin interactions with polymorphonuclear cytoplasmic antigens.

Appendix

Italian Group of Renal Immunopathology Collaborative study on Henoch–Schönlein purpura in adults and in children.

Coordinating Group:
Chairman
R. Coppo
Scientific coordinators
G. Barbiano di Belgioioso, L. Cagnoli, G. Colasanti, A. Lupo, F. P. Schena.

Pathology committee

Clinical committee

Laboratory committee


References

Received for publication: 18.2.97
Accepted in revised form: 17.6.97