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IgA nephropathy: characterization of the polymeric nature of mesangial deposits by *in vitro* binding of free secretory component

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SUMMARY

IgA nephropathy, as Berger defined it, is characterized by mesangial deposits of IgA, which are easily visualized by immunofluorescence on kidney biopsies. The structure (mono- or dimeric) of these IgA has not been clearly defined so far. Fifteen renal biopsies were studied to find out whether these IgAs are serum monomers, or are polymers from a different origin. This was done by tissue fixation *in vitro* of free secretory component, which was then visualized by immunofluorescence (IF). In all 15 cases, the IgA deposits were shown to lack bound secretory component, but were able to bind, specifically, with the free secretory component. The presence of J chain in these deposits was also evidenced by indirect IF. These findings favour the hypothesis that these immunoglobulins are polymeric.

INTRODUCTION

IgA nephropathy is a chronic disease characterized by episodic, macroscopic or microscopic haematuria and/or proteinuria, usually occurring some time after respiratory or gastroenteric infections.

Berger was the first to demonstrate that it differs from other glomerulopathies in its long-term mode of evolution and because of the presence of IgA and C3 mesangial deposits, which can be visualized in the glomeruli by immunofluorescence (IF) on kidney biopsies (Berger, 1969). IF is now routinely used for the diagnosis of this type of nephropathy (Berger's disease), but its aetiology remains unclear.

Nevertheless, the frequency of mucosal infections occurring previous to haematuric episodes in patients with Berger's disease is strong evidence favouring the hypothesis that mesangial IgA are secretory in nature.

Two types of IgA, of different origins (sytemic or mucosal) are present in the body. Serum IgA are mainly monomeric 7S immunoglobulins (Heremans, 1974), while secretory IgAs are 11S dimers, consisting of two identical molecules linked by a peptide called joining piece or J chain (Brandtzaeg, 1973a). In the secretions, secretory IgAs also differ from serum IgAs because of the presence of a specific glycopeptide called the secretory component (SC) (Brandtzaeg, 1973b). This molecule prevents the IgA from being digested by the proteases of the secretions. It is produced by mucosal and glandular epithelial cells and binds to dimeric IgAs from the mucosa when they move through the epithelium to reach the lumen. SC is also secreted in small quantities as free secretory component (FSC) (i.e. in colostrum and bile). Initially, some authors (Dobrin, Knudson & Michael,

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1975; McCoy, Abramowsky & Tischer, 1974) failed to visualize the presence of SC in kidney biopsies and doubted that these IgAs had such a secretory structure. This might be attributed to the different secretory sites for mucosal IgA and SC (mucosal plasma cells and epithelium respectively) and to the two-step mode of formation of secretory IgA.

In this work, we used two types of experiment to try to assess the dimeric nature of mesangial IgA in Berger's disease: (i) A study of the ability of purified FSC to be bound *in vitro* (assuming that the deposits have been demonstrated to lack bound secretory component, BSC). This was thought possible because the specificity of this binding phenomenon has been evidenced *in vitro* (Mach, 1970). Indirect IF was used with highly specific antibodies, after incubation with FSC, on frozen-cut kidney sections, and (ii) Demonstration by IF of the presence of J chain in these deposits.

MATERIAL AND METHODS

Patients. Of 130 kidney biopsies sent to the laboratory for routine IF testing, 20 were found to have glomerular deposits of IgA. Five of these were associated with a well defined Henoch–Schönlein syndrome and were therefore excluded from this study, leaving 15 biopsies to be tested further.

The most frequent indication given by the clinicians was that the patients had had one or more instances of microscopic or macroscopic haematuria. In some cases the circumstances of its appearance (i.e. after episodes of rhinopharyngeal infection) and a concomitant proteinuria had suggested the diagnosis of an IgA nephropathy; in other cases, the diagnosis was made only after immunohistochemical study.

Antisera. Fluorescein-conjugated sera prepared against human IgA, IgG, IgM, IgE (H-chain-specific), C1q, C3, fibrin, and SC were purchased from Hoechst-Behring (Paris) and Sebia (Paris) Laboratories, as well as the non conjugated anti-C9 and anti-C3 proactivator antisera. The specificity of all these antisera had previously been confirmed by immunochemical techniques (immunodiffusion, immunoelectrophoresis) as well as immunohistological testing. The latter included labelling of deposits in specimens of known pathology (i.e. plasmocytomas for anti-Ig sera) and checking the absence of non-specific tagging.

Fluorescein-conjugated anti-rabbit-IgG serum came from the Institut Pasteur (Paris). Double-labelling experiments were performed with rhodamine-conjugated anti-human-IgA immune serum raised in rabbits (Cappel, USA).

Non-conjugated rabbit anti-human-J chain serum was obtained from Nordic Laboratories, and Dr J. Mestecky kindly gave us some rabbit anti-human-J chain serum. The specificity of Nordic's antiserum was assessed by electrophoresis of dithioerythritol-reduced secretory IgA in polyacrylamide gel with sodium dodecyl sulfate, followed by two-dimensional immunoelectrophoresis in an agarose gel containing the antiserum. After this treatment, only one peak was observable, indicating the absence of cross-reactivities of the serum with components of sIgA other than the J chain.

Some cross-reactivities appeared nevertheless with the anti-SC serum. Its specificity was enhanced by adsorption with polymerized monomeric IgA. This sorbent was prepared from isolated serum IgA according to the method described by Avrameas & Ternynck (1967).

IF was performed by 30 min of incubation with the antisera, followed by three washes in PBS. For indirect IF, this procedure was followed by a second incubation, with the labelled serum, and by another series of washes.

A Leitz Ortholux UV microscope equipped with a Ploem-type vertical epi-illuminator was used for observation, after the samples had been mounted in glycerol/saline mixture under a coverslip.

Secretory component. FSC was prepared from human colostrum, following, with minor modifications the technique described by Kobayashi (1971).

Five millilitres of colostrum was delipidated by centrifugation, and the pH was lowered to 4·2 with acetic acid to precipitate the casein. After centrifugation, the pH of the supernatant was readjusted to 7·2 with 1N NaOH. This fat- and casein-free sample was applied to a column (100 × 2·5 cm) packed with AcA 34 gel (LKB) equilibrated with 0·01 M phosphate buffer. The elution was performed with the same buffer and was monitored by photometric measurement of the protein

concentration of the eluate at 280 nm. Four peaks could be seen on the optical density recording chart, and the fractions under each peak were gathered into four pools. Each pool was challenged in a double immunodiffusion test (Ouchterlony technique) against anti-SC and anti-IgA-alpha-chain specific sera. The first peak appeared to contain both IgA and FSC (or at least SC), and the second only FSC, while the two others were negative to both antisera.

The pool representing the second peak was concentrated by inverted dialysis against Carbowax, rechromatographed on DEAE cellulose, according to Mogi (1975), and eluted through a stepwise phosphate buffer gradient. The 0·01 M phosphate buffer eluted FSC, as shown by double immunodiffusion analysis. This test was performed with anti-SC, -IgG. -IgM, -IgA, and -lactoferrin antisera, and only a single line of precipitation was visualized, indicating that the preparation was pure FSC. The second buffer and the final saline wash eluted more proteins (as shown by photometric recording), but they were negative against the same antisera in the Ouchterlony test. The FSC solution was kept at +4°C with sodium azide.

Tissue sections. Immediately after being taken, the biopsies were snap-frozen at −196°C, and they were then stored at −70°C. Sections 4 μm thick were cut with a Slee (London) cryostat immediately before they were incubated with the various antisera.

Each kidney was tested with a panel of fluorescein-conjugated sera prepared against IgA, IgG, IgM, IgE, C1q, C3, SC, and fibrin. Indirect fluorescent staining was also performed, with rabbit anti-human-C9 and -human-C3 proactivator followed by fluorescein-conjugated anti-rabbit-IgG.

In vitro *FSC fixation.* Tissue sections were covered with a drop of FSC (working dilution: 0·24 mg/ml) and incubated in a moist chamber for 1 hr at 37°C (Mach, 1970). They were then washed three times for 5 min in PBS at room temperature, and incubated for another 30 min with fluorescein-conjugated anti-SC, at room temperature. After three more washes in PBS, the sections were covered with glycerol/saline mixture and a coverslip and observed under a Leitz Ortholux UV microscope.

An identical section incubated only with anti-SC labelled anti-serum was always used as a negative control.

Double labelling. In order to demonstrate the IgA specificity of FSC fixation, a double labelling procedure was performed for IgA and FSC. After the last washes of the technique described above, the slides were incubated again, with a drop of rhodamine-conjugated anti-IgA, for 30 min; then, washed three times in PBS, mounted in glycerol/saline under a coverslip, and observed with a Leitz Ortholux UV microscope equipped with a Ploem-type vertical illuminator and interference filters for narrow band excitation and selective filtration of green and red fluorescence.

RESULTS

In all 15 biopsy specimens, the glomerular mesangium showed a bright fluorescence with anti-IgA, anti-C3, and anti-C9 antisera. This is characteristic of IgA nephropathy as Berger (1969) described it. Table 1 shows the results of immunofluorescent staining of the biopsies with the other antisera. There were C3 and C9 deposits in the capillaries and/or arterioles for, respectively, 12 and 13 patients. C1q deposits were found in some cases, either with an endomembranous localization or in the glomerular mesangium. There were IgM deposits in seven cases: four times with an endomembranous localization, and three times in the mesangium. Fibrin was present in many of the biopsies (seven), but only twice in the glomeruli and not in the same localization as IgA and FSC. Finally, one kidney showed positive fluorescence with the anti-IgG serum, and C3-proactivator was visualized in four patients' biopsies.

For the most specific exploration, which was performed to determine the nature of the IgA, the results were the same in all 15 cases, and can be described as follows:

(a) Direct immunofluorescent staining with a properly adsorbed anti-SC antiserum showed no glomerular labelling, except, very faintly, in two cases. Only a slight fluorescence of the epithelium of the tubules in five cases could be detected, a finding which confirms previous observations (Dobrin *et al.*, 1975; McCoy *et al.*, 1974).

(b) In contrast, bright fluorescent deposits were seen in the mesangium after incubation of the slides

Table 1. Immunofluorescent staining of the 15 kidneys studied

Patient	C3 Vascular	C9 Vascular	C1q Endo-membranous	C1q Mesangial	IgM Endo-membranous	IgM Mesangial	Fibrin	C3 Proactivator
1	+	+	−	−	−	−	−	−
2	−	−	+	−	+	−	+	−
3	+	−	−	+	−	−	−	+
4	+	+	−	−	−	−	−	−
5	−	+	−	−	−	+	+	−
6	−	+	+	−	−	+	+	−
7	+	+	+	−	+	−	−	−
8	+	+	+	+	+	−	+	+
9	+	+	−	−	−	+	−	−
10	+	+	+	−	−	−	+	+
11	+	+	−	−	−	−	−	−
12	+	+	−	−	−	−	+	+
13	+	+	−	−	−	−	+	−
14	+	+	±	−	±	−	−	−
15	+	+	−	−	−	−	−	−

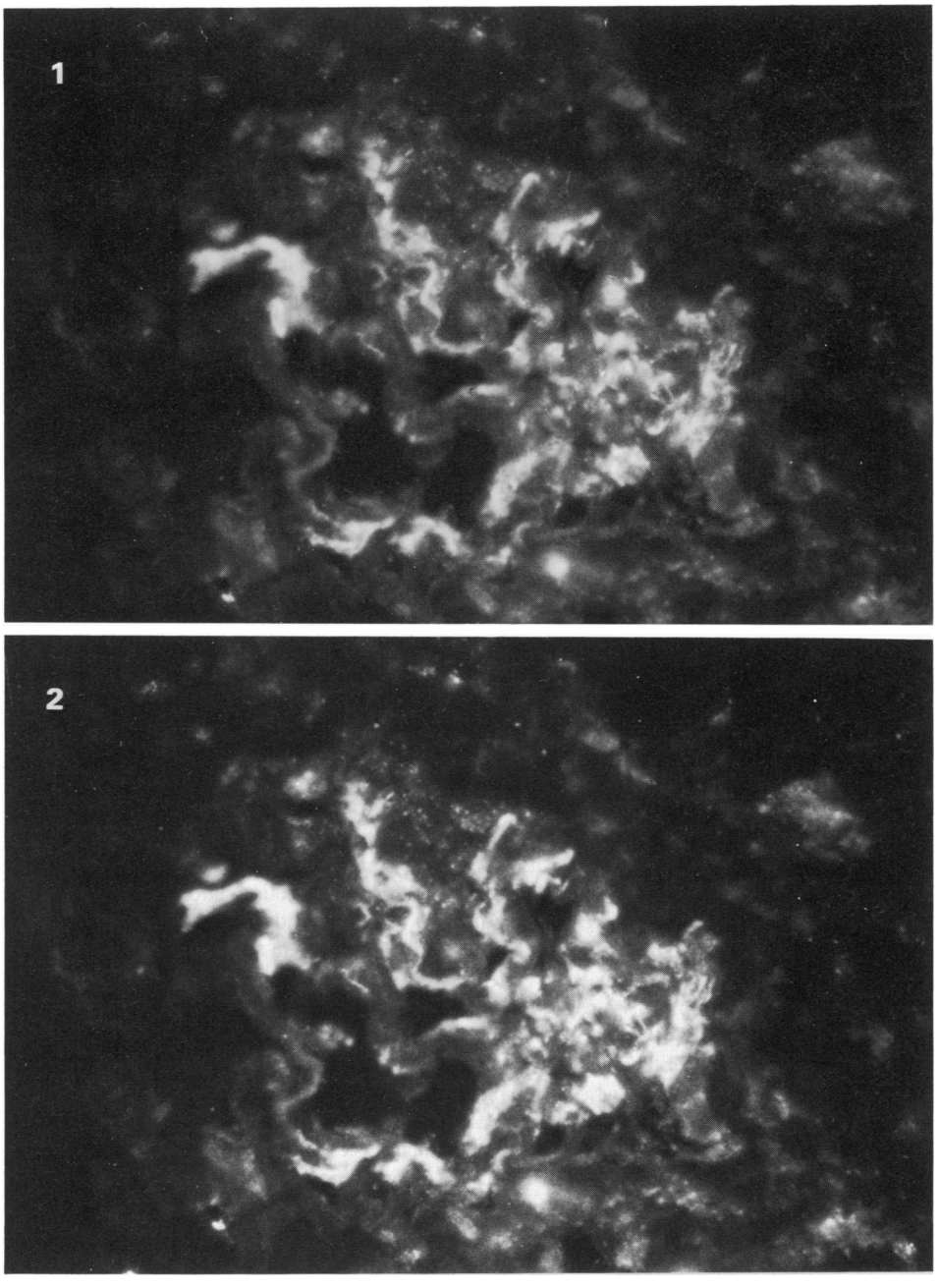

Fig. 1. Mesangial deposits visualized after incubation with free secretory component followed by labelling with purified anti-SC fluorescein-conjugated serum.

Fig. 2. Double labelling of the glomerulus in Fig. 1 incubated with rhodamine-conjugated anti-alpha-chain serum. The two images are nearly identical.

with FSC and fluorescent staining of the fixed SC by monospecific anti-SC antiserum. With rhodamine-conjugated anti-IgA antiserum, in the double labelling process, it appeared obvious that the two antisera labelled the same deposits (Fig. 1 & 2). It can therefore be assumed that the fixation of FSC was not incidental but was directly related to the specific affinity of di- or polymeric IgA for it.

The specificity of all the reagents had been verified previously with appropriate controls, including, for anti-SC serum, the incubation of ileal biopsies (where the mucosal IgA-secreting plasma cells were not labelled while the epithelial cells displayed a bright fluorescence), and of sections of an IgA-secreting plasmocytoma (which remained unlabelled). Immunoelectrophoresis gave more evidence of the purity of the FSC prepared.

Lastly, sections from kidneys with no glomerular lesion were treated the same way, with FSC for 1 hr, then anti-SC fluorescent serum. The glomeruli remained unlabelled, which assess that *in vitro* FSC fixation cannot be attributed to some mesangial tropism.

(c) J chain was visualized in all cases as fluorescent spots in mesangial areas of the glomeruli where IgA were present. This labelling was fainter than that of fixed FSC or IgA, and showed up only parts of the deposits. J chain could be visualized in kidneys lacking IgM deposits. In control experiments, in which the specific anti-J chain anti-serum was omitted, no mesangial fluorescence was observed. The image can therefore be attributed to the presence of J chain in mesangial IgA deposits.

DISCUSSION

Di- or polymeric IgA secreted by mucosal plasma cells can fix secretory component. Such fixation usually occurs when dimeric IgAs cross the epithelial cells and reach secretions in the lumen of the respiratory passages or the digestive tract, but mucosal IgA can also reach the peripheral blood as dimeric IgA lacking SC. Mach (1970) previously showed that FSC can be combined with native di- or polymeric IgA by simple incubation at 37°C for 1 hr. He also showed that this binding was specific, since it did not occur between FSC and IgG or serum albumin, or between FSC and artificially polymerized 7S IgA. Later, Brandtzaeg (1975) demonstrated that only dimeric 11S IgA possessing a J chain, and 19S IgM (that also have a J peptide), could bind non-covalently to FSC.

Those authors worked *in vitro* with purified preparations of all components, but this type of bonding has been shown to occur *in vivo* as well, together with other kind of links, i.e. disulfide bonds (Underdown, De Rose & Plaut, 1977; Newcomb, Normansell & Stanworth, 1968). Demonstration of the ability of deposited IgA to fix FSC in tissue sections shown to lack BSC would favour the presence of polymeric IgA, particularly if J chain could be evidenced in these IgAs as well. This is what was done in the present study, for IgA deposits in the glomerular mesangium of kidney biopsies from 15 patients with so called Berger's disease. There was no mesangial labelling with an anti-SC antiserum (only a slight fluorescence of the tubular cells, which confirms previous observations [McCoy, 1974]) but when the sections were incubated with FSC before being challenged by the anti-SC fluorescein-conjugated antiserum, the deposits were visualized, with the same localization as with an anti-IgA rhodamine-conjugated antiserum (double labelling).

J chain was visualized as spots in mesangial areas of IgA-containing glomeruli. This is consistent with the localization of J chain in the IgA dimer, which leaves few or no antigenic determinants of this small peptide accessible. Moreover, if the mesangial deposits in Berger's disease are, as they seem, immune complexes, the steric hindrance due to molecular structures surrounding the J chain must be even stronger. Nevertheless, there probably is some catabolism of these complexes *in situ*, which might act as an 'eraser' and make some J chain antigens more apparent. Similar images might be due to the presence of IgM, but, as stated before, J peptide labelling was observed in kidneys lacking IgM mesangial deposits.

It therefore seems that the polymeric character of IgA in the mesangial deposits of Berger's disease can be ascertained. Since these polymeric IgAs are essentially produced by mucosal plasma cells (Brandtzaeg, 1973a; Brandtzaeg, 1974; Mogi *et al.*, 1979), their presence in renal deposits suggest that they come from secreting mucosae and not, as previously thought, from systemic IgA-secreting structures (spleen, lymph nodes). McCoy *et al.* (1974) and Dobrin *et al.* (1975) had

already attempted to demonstrate the secretory nature of mesangial IgA, but they based their investigations on the identification of FSC in the deposits, whereas this molecule is bound to dimeric IgAs when they cross the epithelial cells. These complete sIgAs usually do not return to the blood flow and are therefore unlikely to pass through the kidneys.

Rifai *et al.* (1979) tried to induce renal lesions in mice with different preparations of IgA. They obtained nephropathies only with polymeric IgA.

Aetiologically speaking, it is important to emphasize the fact that the clinical manifestations of Berger's disease appear some time after infectious events such as rhinopharyngitis. Moreover, some authors report good clinical results in these patients after tonsilectomy or thorough dental treatment. The importance of IgA-monitored immunological defences in the respiratory and digestive tracts may suggest that an increase of IgA production in these areas leads to an increase of polymeric circulating IgA. This has been demonstrated in this disease by Lopez-Trascasa *et al.* (1980), and also in Henoch–Schönlein syndrome (Levinsky & Barratt, 1979). The formation of immune complexes is very likely as well, since C3 and C9 are visualized in the mesangial deposits.

These molecules and/or complexes would then deposit in the mesangium, inducing a lesion and the pathology consecutive to such an alteration. The rare cases when immunofluorescence of the glomeruli appeared with the anti-SC serum might account for the presence of mucosal lesions allowing small quantities of secretory IgA to pass from the lumen to the mucosa and the peripheral blood. This is consistent with an observation of Thompson, who reported the presence of low levels of sIgA in the serum of patients with gastroenteric inflammatory syndroms (Thompson, Asquith & Cooke, 1969).

More recently, controversy arose when André *et al.* (1980) demonstrated the presence of IgA2 in large amounts in the mesangium of patients with IgA nephropathies, while Conley, Cooper & Michael (1980) reported the predominance of IgA1 on the kidney of another series of such patients. These findings might not be as contradictory as they seem, since IgA2 represent only 40% of mucosal IgA.

The major remaining problem now, is the identification of the antigen responsible for the formation of these antibodies.

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