Predominant synthesis of IgA with lambda light chain in IgA nephropathy

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Predominant synthesis of IgA with lambda light chain in IgA nephropathy. The nature of the light chains in mesangial IgA deposits and serum IgA was studied in patients with IgA nephropathy. Immunofluorescence (IF) studies using murine monoclonal antibodies, rabbit and goat anti-human monospecific antisera were performed in kidney sections from 15 IgA nephritic patients with only IgA isotype detected in the renal biopsy. Lambda light chain IF was demonstrated in all biopsy specimens and kappa light chain IF in 11 renal biopsy specimens. The majority of renal biopsies showed a predominance of lambda light chain IF staining in the mesangial deposits. The concentration of individual immunoglobulins and their light chain fractions, and the kappa/lambda ratio were determined in the serum and the supernate from peripheral blood mononuclear cells culture of 30 IgA nephritic patients and 30 age-matched healthy controls. The IgA nephritic patients had a higher serum concentration of total IgA (P < 0.001) and a significantly lower IgA kappa/lambda ratio (P < 0.001) compared with the controls. The kappa/lambda ratio of supernatant IgA from IgA nephritic patients (N = 20) was also significantly lower than that of the normal subjects (N = 14), both in the unstimulated (P < 0.01) and pokeweed mitogen stimulated, peripheral blood mononuclear-cell culture (P < 0.05). Our results showed that patients with primary IgA nephropathy displayed a unique immunologic response characterized by a predominance of IgA with lambda light chain in circulation.

Primary IgA nephropathy characterized by mesangial deposition of IgA and C3 commonly occurs in young subjects with synpharyngitic hematuria [1]. This entity of glomerulonephritis has now been recognized to be the most common glomerulopathy in many countries [1–3].

IgA is present in the circulation as monomeric units, mainly of the A1 subclass, but at mucosal sites it is secreted actively as A1 or A2 dimers polymerized by J-chain and transported by binding to the secretory component [4]. Normal serum IgA contains approximately 90% IgA1 and 10% IgA2 [5]. Recent reports demonstrate that the glomerular IgA deposits consist predominantly of monomeric IgA1 with a minor proportion of IgA2 [6–8]. These findings suggest that the circulating IgA immune complex is deposited in the glomeruli of patients with IgA nephropathy irrespective of antibody subclass. Contrary to the IgA1 and IgA2 subclasses, our preliminary data suggest that predominant lambda light chain immunofluorescence (IF) occurs in the mesangial immunoglobulin deposits in IgA nephropathy [9], despite the fact that the normal ratio of kappa to lambda light chain immunoglobulin in human sera is approximately two to one [10]. Since the light chain IF may arise from the concomitant mesangial IgM and IgG deposits, one could argue that a definitive immunochemical characterization of the light chains in the mesangial IgA deposits in IgA nephropathy could be difficult. The present study was undertaken to confirm our previous finding in renal tissues and to determine the kappa/lambda light chain ratio of different immunoglobulins in serum and lymphocyte culture supernate from normal subjects and patients with IgA nephropathy.

Methods

Patients

A total of 15 percutaneous renal biopsy specimens from 15 patients with primary IgA nephropathy were studied. IgA nephropathy was diagnosed on the basis of the following pathologic criterion: focal or diffuse segmental mesangial proliferative glomerulonephritis on light microscopy; presence of predominant IgA deposits, mainly in the mesangium in immunofluorescence studies; and presence of electron dense deposits in the mesangium and paramesangial areas on ultrastructural examination [3]. These 15 kidney biopsies showed IgA deposits alone and formed the basis of our histopathologic studies. Systemic lupus erythematosus, Henoch-Schönlein purpura, and hepatic diseases were excluded by clinical and laboratory examinations. Renal tissue from 15 patients who died suddenly from trauma and other causes where postmortem examinations failed to reveal neoplastic, hepatic, autoimmune, or renal diseases were used as control.

An additional 15 patients with biopsy-proven IgA nephropathy were included in the studies of light chain ratio in serum immunoglobulins and 30 age-matched medical and nursing personnels acted as controls. The studies of immunoglobulins in serum and lymphocyte culture supernate were performed during an infection-free and clinical quiescent period.

Immunofluorescence studies

Renal biopsy specimens which demonstrated mesangial IgA deposits alone were examined by (a) direct immunofluorescence costained with 1:10 rhodamine-conjugated goat anti-human alpha heavy chain (F(ab')2 fragments) (Tago, Burlingame, California, USA) and 1:15 FITC-labeled rabbit anti-human...
lambda or kappa light chain (F(ab')₂ fragments) (Dakopatts, Copenhagen, Denmark); (b) direct immunofluorescence with 1:15 FITC-labeled goat anti-human lambda and kappa light chains (F(ab')₂ fragments) (Tago); and (c) indirect immunofluorescence with 1:15 murine monoclonal antibody against human lambda light chain and 1:75 murine monoclonal antibody against human kappa light chain (Becton-Dickinson, Mountain View, California, USA) followed by 1:10 FITC conjugated goat anti-mouse immunoglobulins (Coulter, Hialeah, Florida, USA). The slides were examined with a coded basis by two observers (F.M.L. and K.N.L.) under ultraviolet illumination, with an Orthoplan microscope equipped with a Ploem illuminator (Leitz, Heidelberg, FRG). The fluorescence intensity of the slide preparations was visually scored semiquantitatively as 0, 1+, 2+, 3+ and 4+ by two observers without the knowledge of previous immunofluorescence studies and the nature of anti-human light chain antisera.

The costained sections were examined in a fluorescence microscope with epilumination equipped with filters for narrow-band excitation and barrier filters LP515 for fluorescein and LP580 for rhodamine visualization. Alternate immunofluorescence examination was permitted by switching one filter consecutively to the other. Photographs were taken on standard Kodacolor film developed at 100 ASA.

**Antisera**

The properties and specificities of fluorescein-conjugated (FITC) rabbit anti-human lambda and kappa chain antisera (Dakopatts) had been tested as described in our previous communication [9]. Briefly, the antisera were purified by gel filtration and ion exchange chromatography. Their monospecificity was ascertained by Ouchterlony double immunodiffusion in agarose gel against Bence-Jones lambda and kappa chain, purified by gel filtration and immunoadsorption chromatography (Behringwerke AG, Marburg, FRG and our laboratory). The monospecificity of the goat anti-human lambda and kappa light chains, F(ab')₂ fragments, antisera (Tago) and murine monoclonal antibodies against human lambda and kappa light chains (Becton-Dickinson) were similarly ascertained. These antibodies had been further tested not to show any cross reactions using fresh, human lymphoid tissue. The molar fluorescein/protein ratios of the rabbit anti-human lambda and kappa light chain antisera were identical: 2.30. The molar fluorescein/protein ratio of goat anti-human lambda chain antiserum was 2.50, and this ratio was lower than the value of 3.08 for the goat anti-human lambda chain antiserum. The antibody concentrations of the antibodies were determined by radial immunodiffusion by the supplier. The antibody concentrations of the rabbit anti-human antisera were identical (100 µg/ml), but the antibody concentration of the goat anti-human kappa antiserum (0.7 mg/ml) was slightly higher than that of lambda antiserum (0.6 mg/ml). The kappa and lambda monoclonal antibody concentration was 125 µg/ml and 25 µg/ml, respectively.

**Lymphocyte culture and in vitro immunoglobulin production**

Peripheral blood mononuclear cells (PBMC) were separated from heparinized whole blood by density gradient centrifugation using Ficoll Hypaque (Nyegaard, Oslo, Norway). After three washes in TC-199 (Gibco, Chargrin Falls, Ohio, USA), the cells were resuspended at a concentration of 1 x 10⁶ cells/ml in complete culture medium (CCM) consisting of RPMI 1640 (Gibco) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml fungizone. One hundred µl aliquots of this cell suspension were dispensed into 96-well microculture flat-bottom plastic plates (Costar, Cambridge, Massachusetts, USA) and cultured in quadruplicates without mitogen and with pokeweed mitogen (PWM) (Sigma Chemicals, St. Louis, Missouri, USA) at a concentration of 1 µg/ml. The PBMC were incubated at 37°C in humid atmosphere of 5% CO₂ in air. The cell free supernates prepared from the lymphocyte cultures on the fifth day were stored at −40°C until assayed.

**Assay of immunoglobulins and determination of kappa/lambda ratios**

IgA, IgG and IgM in lymphocyte culture supernates were quantitated by enzyme-linked immunosorbent assay (ELISA). Purified immunoglobulin fractions of specific rabbit antisera to human α, γ and µ chains (Dakopatts) were used at 1:2000 dilution. Microtiter plates (Labsystems OY, Helsinki, Finland) were coated by adding 100 µl/well of the appropriate antiserum diluted in 0.015 M sodium carbonate-bicarbonate buffer, pH 9.6. The plates were incubated overnight at 4°C and washed 20 times with phosphate buffer saline, pH 7.4, containing 0.05% (vol/vol) Tween 20 (PBS-tween). Samples and immunoglobulin standards (Dakopatts) appropriately diluted in CCM were then introduced, 100 µl/well in duplicate. After incubation at 37°C for three hours, the plates were again washed 20 times with PBS-tween. The same antisera as used for coating, but conjugated with horseradish peroxidase and diluted 1:2000 in PBS-tween, were added, 100 µl/well as appropriate. Incubation at 37°C was continued for another two hours and the washing repeated. A freshly prepared substrate solution containing 10 mg urea hydrogen peroxide and 35 mg o-phenylene-diamine dihydrochloride (Sigma Chemicals) in 50 ml 0.20 M phosphate-citrate buffer, pH 5.0, was then added, 100 µl/well, and the plates incubated at 37°C for a further 30 minutes. Finally, the reaction was terminated by addition of 50 µl/well of 0.10 M citric acid, pH 3.0, and the absorbances were measured at 450 nm wavelength using a Dynatech MR 600 Microplate Reader (Dynatech Laboratories Inc., Alexandria, Virginia, USA). Immunoglobulin concentrations were determined by reference to their respective standard curves.

Kappa/lambda light chain ratios of immunoglobulins in sera and lymphocyte culture supernates were determined also by ELISA. Working standards were prepared from a commercial human serum standard (Behringwerke AG) with known overall kappa and lambda chain concentrations, using CCM as diluent. Employing the same procedure as for the immunoglobulin assay, antibodies to α, γ, and µ chains immobilized on microtiter plates were used to capture the respective immunoglobulins in these working standards, and 1:500 diluted peroxidase-conjugated anti-kappa and anti-lambda sera (Dakopatts) were used for quantitation of light chains on the captured immunoglobulins. By calculation of absorbance ratios, the kappa and lambda chain concentrations of individual immunoglobulins in the standard were determined, and the kappa/lambda ratios computed. The reliability of such further calibration was coun-
Table 1. Frequency of presence of each light chain immunofluorescence and its intensity in the mesangial deposits of 15 patients with IgA nephropathy

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Kappa positive</th>
<th>Lambda positive</th>
<th>Intensity of fluorescence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>lambda/kappa ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit anti-human (direct IF)</td>
<td>12</td>
<td>15</td>
<td>IgA (alpha) 43 lambda 38 kappa 15</td>
<td>2.53</td>
</tr>
<tr>
<td>Goat anti-human (direct IF)</td>
<td>11</td>
<td>15</td>
<td>IgA (alpha) 44 lambda 38 kappa 14</td>
<td>2.71</td>
</tr>
<tr>
<td>Murine monoclonal (indirect IF)</td>
<td>11</td>
<td>15</td>
<td>not done lambda 29 kappa 10</td>
<td>2.90</td>
</tr>
</tbody>
</table>

<sup>a</sup> The fluorescence scores were obtained by addition of the points from renal biopsy of individual patient, on a scale of zero to four.

Fig. 1. Costained IF of heavy and light chains in a patient with IgA nephropathy. (A) FITC rabbit anti-human lambda IF (3+), (B) Rhodamine-conjugated goat anti-human alpha IF (2+) in the same section as (a), and (C) FITC rabbit anti-human kappa IF (2+) in a serial deeper section. (magnification × 430).

Immunofluorescence studies

All 15 kidney biopsies were positive for mesangial IgA deposits as revealed by polyclonal antibodies, but neither IgG nor IgM was detected in the mesangium or the glomerular capillary basement membrane. The frequency with which deposits of lambda and kappa light chain were detected in the renal biopsies with different antisera is shown in Table 1. All 15 renal biopsy specimens (100%) showed mesangial lambda light chain IF, and 11 (73%) showed mesangial kappa light chain IF. The mesangial IF pattern of lambda and kappa light chains was similar to those of the alpha heavy chain when the renal biopsies were costained with the double immunofluorescence technique (Fig. 1). These IF findings confirmed that the light chain IF came directly from the mesangial IgA deposits, as there was no concomitant deposition of IgG or IgM in these glomeruli. Neither IgA, IgG, IgM, or two subclasses of light chain was found in any of the 15 control biopsy specimens.

The intensity of mesangial immunofluorescence is depicted in Table 1. With the use of three different sources of kappa and lambda light chain antisera at comparable antibody concentration, the fluorescence intensity score for lambda light chain was at least one to two points higher than that of kappa light chain in 13 patients, while the remaining two showed similar IF score. The IF scores for IgA, lambda and kappa light chains from
direct immunofluorescence using rabbit or goat antisera were similar, but lower IF scores were generally obtained in indirect IF studies using monoclonal antibodies.

Serum studies

The serum concentrations of individual immunoglobulins and their light chain fractions are present in Figure 2. Patients with IgA nephropathy had a significantly higher serum concentration of total IgA, kappa light chain IgA, and lambda light chain IgA when compared with healthy controls. The IgA kappa/lambda ratio was 0.91 ± 0.03 in IgA nephrotic patients, and this was significantly lower than the value of 1.20 ± 0.05 measured in normal subjects (P < 0.001). These findings indicated a predominance of lambda light chain containing IgA in the serum of patients with IgA nephropathy. No correlation was found between the total serum IgA and the kappa/lambda ratio in individual patients (r = 0.1768, not significant).

The serum IgG and IgM concentrations of the IgA nephritic patients were comparable to those of the normal subjects. Despite a different kappa/lambda ratio detected between the patients and the normal controls, the serum concentrations of kappa chain IgM and kappa chain IgG were nevertheless higher than those of lambda chain IgM and IgG in both patients and controls.

Lymphocyte culture supernates studies

The concentrations of individual immunoglobulins and their light chain fractions are shown in Figure 3. There was no difference observed in the numbers of B cells in the peripheral blood mononuclear cell cultures from normal individuals and patients with IgA nephropathy. The individual immunoglobulin concentrations in supernate from the unstimulated (spontaneous) and the PWM-stimulated lymphocyte cultures were similar in the patients and the normal subjects. The concentrations of IgG and IgM in the supernate increased significantly when the
peripheral blood mononuclear cells from IgA nephritic patients or controls were cultured with pokeweed mitogen (P < 0.01 for IgG, and P < 0.05 for IgM). Similarly, in vitro production of IgA increased when the peripheral blood mononuclear cells from the patients or the controls were stimulated with mitogen, but these changes failed to reach statistical significance. The mean concentration of lambda light chain IgA was higher than that of the kappa light chain IgA in supernate obtained from both the unstimulated and stimulated lymphocyte culture in patients with IgA nephropathy. The kappa/lambda ratio of supernatant IgA in patients was significantly lower than that of normal subjects in both unstimulated (P < 0.01) and stimulated lymphocyte cultures (P < 0.05). A significant difference of kappa/lambda ratio in supernatant IgM and IgG was not demonstrated between the patients and the normal subjects.

Discussion

Our recent study demonstrated that predominant lambda light chain IF staining occurred in glomerular immunoglobulin deposits in primary IgA nephropathy and these findings inferred that mesangial IgA deposits comprised predominantly lambda chain IgA [9]. Fifty-eight percent of our previous renal biopsies had concomitant mesangial IgM and/or IgG deposits, and the interpretation of the mesangial light chain IF could be difficult as the light chain IF could arise from the IgG and IgM deposits. In the present study, we have repeated these immunofluorescence studies with three different antisera in renal tissues from another 15 patients with mesangial IgA deposits alone to clarify the immunoochemical nature of the light chains of the mesangial IgA deposits. Lambda light chain IgA was present in all 15 biopsy specimens and kappa light chain IgA was demonstrated in 73% of the biopsy specimens. Although our measurement of fluorescence was only semiquantitative, it was apparent that the lambda IF was either equal to or stronger than the kappa IF in most renal biopsies studied with rabbit anti-human antisera. More meaningful was the fact that similar results were obtained in studies with goat anti-human light chain antisera in which the antibody concentration and fluorescein/protein ratio were higher for the kappa chain antisera. The IF patterns observed with murine monoclonal antibodies were similar to those of polyclonal antisera despite a less sensitive detection with monoclonal antibodies than polyclonal antibodies [11]. The higher incidence of positivity and the greater intensity of lambda light chain IF over kappa light chain confirm our original observation that the glomerular IgA deposits in IgA nephropathy are composed predominantly of lambda light chain IgA.

This observation in the nature of the light chains in IgA deposits in IgA nephropathy has an interesting bearing on the immunopathogenesis of primary IgA nephropathy. As inferred from the findings of glomerular and circulating IgA1 and IgA2 subclasses [6-8], predominant kappa chain IgA deposits should be expected as kappa chain immunoglobulin is the predominant immunoglobulin fraction in serum of normal subjects [10]. Our findings of predominant lambda light chain IgA deposits in glomerular mesangium could indicate that the deposition of circulating immune complexes in IgA nephropathy is highly selective due to the differences in the multimeric nature, size of the immune complexes and anionic charge of different subclasses of IgA [12]. The other possible immunopathologic mechanism is the altered ratio of light chains with a given specificity to an unidentified antigen leading to excessive production of lambda light chain IgA.

The kappa/lambda ratio of individual immunoglobulins have not been studied in detail in normal subjects or in disease. In healthy subjects, the normal ratio of serum kappa chain to lambda chain immunoglobulin is approximately two to one [10, 13, 14] and this ratio is obtained from either total serum (usually predominately IgG) or cord serum (containing only IgG). The kappa/lambda ratio of IgG is confirmed in our normal subjects and patients with IgA nephropathy. However, the kappa/lambda ratio of serum IgA has not been studied previously. Our present study demonstrates that the kappa/lambda ratios of IgA and IgM in normal subjects are significantly different from that of IgG. Patients with IgA nephropathy have a higher serum concentration of lambda chain IgA (P < 0.001) compared with healthy controls. Furthermore, their kappa/lambda ratio of serum IgA is significantly lower (P < 0.001). Nevertheless, a predominance of serum kappa chain IgG and IgM is demonstrated in patients with IgA nephropathy as observed in controls. These findings indicate that lambda chain IgA is the predominant circulating IgA fraction in these patients. The predominance of mesangial lambda IF is, therefore, mainly due to a non-selective deposition of polyclonal IgA immune complexes formed by a majority of lambda chain IgA.

In this study, we have further investigated the in vitro production of immunoglobulin by cultured lymphocytes to determine whether an altered antibody response is present in patients with IgA nephropathy. Although other workers had demonstrated a significant increase in vitro production of IgA by patients with IgA nephropathy [15-17], our results had shown that the IgA production by peripheral blood mononuclear cells was the same as that of the controls. Three other studies had also failed to demonstrate an increase of in vitro production of IgA in patients with IgA nephropathy [18-20]. Inconsistencies in the IgA immune system abnormalities found in IgA nephropathy may have some basis in methodological variations, although a similar ELISA method has been used in these studies. However, variability in patients selection and study design may also contribute. It is important to note that in the present study and the other two studies that failed to demonstrate an increase of in vitro production of IgA [18, 19], patients were examined in the quiescent phase of the disease whereas the clinical status of patients reported by other studies was not known [15-17]. As observed by the other two groups of investigators [18, 19], we have shown that the increase of in vitro production of IgA with mitogen stimulation was insignificant as compared with that of IgG or IgM. These findings suggest that the immunoregulatory function of lymphocytes varies with the disease activity and these variations probably contribute to the controversial findings in immune system abnormalities in patients with IgA nephropathy. Although our study of in vitro IgA production fails to demonstrate an IgA-specific B cell hyperactivity as suggested by Hale and coworkers [15], nevertheless, the immunoglobulin production by cultured B cells from our patients during clinical quiescence is quite specific as a significant quantity of lambda chain IgA in response to an unidentified antigen was produced. The kappa/lambda ratios of supernatant IgA obtained from both unstimulated and stimulated cultures are significantly different from...
those of healthy controls. Similar changes in supernatant IgG and IgM are not observed.

In conclusion, we have shown that mesangial IgA deposits in primary IgA nephropathy consist mainly of IgA with lambda light chain. These patients display a unique predominance of circulating lambda chain IgA. The distinct reduction of kappa/lambda ratio of IgA in serum and supernate from lymphocyte culture strongly supports that IgA nephropathy is an immune disorder with a specific antibody response.

Acknowledgments

Portions of this study were presented at the Second International Symposium of IgA nephropathy, Bari, Italy, April 10—11, 1987. This study was supported by a grant from the Croucher Foundation.

Reprint requests to Dr. Kar-Neng Lai, Department of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong.

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