Underglycosylation of IgA1 Hinge Plays a Certain Role for Its Glomerular Deposition in IgA Nephropathy

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Abstract. This study was performed to isolate and investigate the IgA1 that could accumulate in glomeruli (glomerulophilic IgA1). IgA1 was fractionated by the electric charge and the reactivity to Jacalin. Serum IgA1 of IgA nephropathy patients was separated and fractionated using a Jacalin column and subsequent ion-exchange chromatography. The fractions were divided into three groups of relatively cationic (C), neutral (N), and anionic (A). IgA1 was also divided into Jacalin low (L), intermediate (I), and high (H) affinity fractions by serial elution using 25, 100, and 800 mM galactose. The left kidneys of Wistar rats were perfused with 2, 5, or 10 mg of each group of IgA1. The rats were sacrificed 15, 30 min, 3 h, or 24 h after the perfusion. The accumulation of each IgA1 in the glomeruli was then observed by immunofluorescence. The IgA1 of the fractions N and H separated by the two methods was definitely accumulated in the rat glomeruli with a similar pattern. The electrophoresis revealed that the macromolecular IgA1 was increased in fraction H compared with other fractions. Therefore, Jacalin high-affinity IgA1(fraction H) was applied on a diethylaminoethyl column and divided into electrically cationic (HC), neutral (HN), and anionic (HA). Only the asialo-Galβ1,3GalNAc chain was identified in the fraction HA IgA1 by gas-phase hydrazinolysis. Furthermore, the IgA1 fraction was strongly recognized by peanut agglutinin, Vicia Villosa lectins, and antisynthetic hinge peptide antibody. These results indicated that the IgA1 molecules having the underglycosylated hinge glycopeptide played a certain role in the glomerular and extraglomerular uptake of IgA1 in IgA nephropathy.

The human IgA1 molecule, which is the predominant subtype to deposit in the glomeruli in IgA nephropathy (IgAN) (1), is known to have a unique glycosylated hinge, possessing O-glycan side chains (2). Previously, we succeeded in the specific release of galactosyl b1–3N-acetylgalactosamine (Galβ1,3GalNAc) side chains from the IgA1 hinge by gas-phase hydrazinolysis (3) and found an increase in the asialo-Galβ1,3GalNAc residue in patients with IgAN (4). On the other hand, Allen et al. found a decrease of galactose content in the IgA1 hinge using Vicia Villosa (VV), which is a lectin specifically recognizing the terminal N-acetylgalactosamine (GalNAc) residue (5). Tomana et al. also found the galactose (Gal) deficiency in the O-glycans in the IgA1 hinge using several GalNAc-specific lectins (6). Most recently, we suggested the decrease of the Gal and/or GalNAc residues in the IgA1 hinge glycopeptides in IgAN using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (7,8).

IgAN discovered by Berger and Hinglais (9) is recognized as a common form of glomerular disease throughout the world (10). The actual mechanism of the IgA deposition in the mesangial area which is characteristic in this disease has not yet been fully understood. The glomerular IgA deposition has been ascribed to the circulating immune complexes containing IgA antibodies (11). On the other hand, Sancho et al. found an increase in kidney uptake of soluble heat aggregates of polymeric IgA (12). We have reported the role of the asialo-Galβ1,3GalNAc residue for the formation of macromolecular IgA1 due to the conformational instability of the IgA1 molecule (4). Recently, we also observed that the IgA1-IgA1 interaction that had been found in IgAN (13) was inhibited by the synthesized IgA1 hinge peptide core as well as Galβ1,3GalNAc (14). These observations provided the possibility that the glomerular IgA deposition could occur not only due to the IgA immune complexes, but also due to the nonimmunologic formation of macromolecular IgA1 induced by the abnormal O-glycosylation in the IgA1 hinge.

Therefore, we hypothesized that patients with IgAN might have serum IgA1 possessing a characteristic structure in the hinge region, resulting in its glomerular deposition. The aim of the study is to separate the “glomerulophilic” IgA1 from patients with IgAN and to investigate the structure of the IgA1 hinge glycopeptides for establishing the hypothesis.

Materials and Methods

Patients

Among the patients with biopsy-proven IgA-N, 10 patients having severe glomerular IgA deposits were selected for this study. After
obtaining informed consent, the serum samples were collected at the
time of the diagnosis made by renal biopsy and used for this study
immediately afterward. Sera from four healthy individuals were also
used as controls.

Materials

The following materials were purchased from the sources indi-
cated: Sephacryl S-300 from Pharmacia Biotech (Uppsala, Sweden);
DEAE-Toyopearl from Tosoh Co. (Tokyo, Japan); Jacalin-agarose
from Vector Laboratories (Burlingame, CA); goat antihuman IgA
antibody from BIOSYS S. A. (Compiegne, France).

A rabbit polyclonal antibody against the synthetic IgA1 hinge
peptide (PVPSTPPTPSPSTPPTPSPS) (50% ammonium sulfate pre-
cipitates) reported in our previous study (15) and peroxidase-conju-
gated anti-rabbit IgG antibody were obtained from BIO-Synthesis,
Inc. (Lewisville, TX) and Organon Teknika Corp. (West Chester, PA),
respectively.

Fractionation of IgA1 by Ion-Exchange
Chromatography

If not stated, the affinity chromatography was carried out at room
temperature (RT). Ten to fifteen milliliters of sera (IgAN patients, $n = 3$;
healthy controls, $n = 2$) from each subject was treated with 50% amno-
nium sulfate precipitation and applied to the Jacalin column ($1 \times 20$ cm).
After the column was thoroughly washed with 0.01 M phosphate buffer,
0.15 M NaCl, pH 7.5, containing 0.01% sodium azide (phosphate-
buffered saline [PBS]) and 0.8 M glucose, IgA1 bound in the column was
eIuted with 0.1 M melibiose in PBS solution as described previously (4).
The eluate was dialyzed against 20 mM PB, pH 8.0, overnight
(O/N) at 4°C and applied to a DEAE-Toyopearl column ($1 \times 40$ cm)
equilibrated with the same buffer. IgA1 was eluted with a linear
gradient elution from 20 to 200 mM PB, pH 8.0. The obtained
relatively cationic, neutral, and anionic subfractions were separately
collected, dialyzed against distilled water (D/W), and lyophilized. A
sample of the chromatography is shown in Figure 1a.

![Figure 1a](image1)

**Figure 1.** (A) A sample of the ion-exchange chromatography of IgA1. (B) A sample of the serial elution pattern of IgA1 of Jacalin affinity
chromatography.
Fractionation of IgA1 by Reactivity to Jacalin

Ten to fifteen milliliters of sera of seven IgAN patients and two healthy control subjects was treated with 50% ammonium sulfate precipitation and applied to the Jacalin column. IgA1 molecules were then divided into Jacalin low-affinity IgA, which was eluted with 25 mM galactose (Gal), the Intermediate (eluted with 100 mM Gal), and Jacalin high-affinity IgA1 (800 mM Gal). Each fraction was dialyzed against D/W and lyophilized. A sample of the serial elution profile is shown in Figure 1b. Jacalin nonbound IgA was isolated from the Jacalin pass fraction using a Sepharose 4B column (1 × 15 cm) coupled with antihuman IgA antibody.

Animals

Experiments were performed in female Wistar rats weighing approximately 200 to 250 g, purchased from Shizuoka Jikken Doubutu, (Shizuoka, Japan).

Renal Perfusion of Human IgA1 in Rats

The procedure for the kidney perfusion of rats was basically similar to that of Davin et al. (16) The left kidney of a rat was exposed by midline skin incision under ether anesthesia and subsequently inhalation anesthesia (N2O 0.8 L/min, O2 0.8 L/min, Fentanyl 2%). The aorta was clamped above the left renal artery. A branch of the left renal artery was canulated from the aorta with a polyethylene tube (24 G, Terumo, Tokyo, Japan). The left kidney was injected with 0.3 ml of saline to remove the blood and to certify the perfused part of the kidney. Thereafter, 1 ml of PBS solution containing 2, 5, or 10 mg of each IgA1 fraction was injected at an approximate flow rate of 0.2 ml/min. After the injection, the clamping of the aorta was removed to reestablish normal blood flow. The rats were sacrificed at various time intervals (15 min, 30 min, 3 h, 24 h), and the perfused part of the kidneys was removed for immunofluorescence, light, and electron microscopy. The dose of the injected IgA and the time intervals are summarized in Table 1.

Histologic Examination

Renal tissue was fixed with buffered formalin and embedded in paraffin, and 4-µm sections were stained with periodic acid-Schiff (PAS). A second portion was embedded in OCT medium (Miles Laboratories, Elkhart, IN) immediately after sampling, frozen in liquid nitrogen, and stored at −80°C until use. Frozen sections were cut to 2 µm, fixed in absolute acetone for 10 min, and then rinsed in 0.01 M phosphate buffer containing 0.15 M saline, pH 7.4 (PBS). The sections were incubated with 1:20 diluted FITC-labeled goat antihuman IgA antibody (IgG fraction, Organon Teknika Corp.) with incubation for 45 min at RT. The stained sections were rinsed with PBS and observed under a fluorescence photomicroscope (Nikon Corp., Tokyo, Japan). They were graded as 0 to 3+ by the authors (Drs. Hiki

Table 1. Dose and time interval of the injected IgA, and the accumulation in rat glomeruli

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of IgA1</th>
<th>Removal Time after Injection</th>
<th>Intensity of Immunofluorescence</th>
<th>Ion-Exchange Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cationic</td>
<td>Neutral</td>
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<tr>
<td>IgA-N</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>2 mg</td>
<td>15 min</td>
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<tr>
<td>1</td>
<td>2</td>
<td>15</td>
<td>–</td>
<td>++</td>
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<td>2</td>
<td>5</td>
<td>30</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>1 and 3</td>
<td>5</td>
<td>3 h</td>
<td>NT</td>
<td>+++</td>
</tr>
<tr>
<td>Healthy controls A and B</td>
<td>5</td>
<td>30 min</td>
<td>–</td>
<td>–</td>
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<tr>
<td>IgA-N</td>
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<td>4 and 5</td>
<td>5 mg</td>
<td>3 h</td>
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<td>6 and 7</td>
<td>10</td>
<td>3</td>
<td>NT</td>
<td>+</td>
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<td>8, 9, and 10</td>
<td>10</td>
<td>24</td>
<td>NT</td>
<td>–</td>
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<tr>
<td>Healthy controls C and D</td>
<td>5</td>
<td>3</td>
<td>–</td>
<td>NT</td>
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Reactivity against Jacalin

<table>
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<th>Intermediate</th>
<th>High</th>
</tr>
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<tbody>
<tr>
<td>IgA-N</td>
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<tr>
<td>4 and 5</td>
<td>5 mg</td>
<td>3 h</td>
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<td>8, 9, and 10</td>
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<tr>
<td>Healthy controls C and D</td>
<td>5</td>
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Figure 2. Ion-exchange chromatogram of Jacalin high-affinity IgA1. A definite peak of the neutral fraction is observed.
Kobayashi). A third sample of renal tissue was fixed in 2% glutaraldehyde and embedded in Epon for electron microscopic examination.

**Size Analyses of the IgA1 Molecules by Native Polyacrylamide Gel Electrophoresis**

Polyacrylamide gel electrophoresis (PAGE) was performed under the nonreduced condition by the Phast system (Pharmacia), using PhastGel gradient 4-15 according to the instructions.

**Analysis of O-Glycans of Jacalin High IgA Fractions by Gas-Phase Hydrazinolysis**

Because of the positive results of fractions H and N on the histology, the rest of the injected Jacalin high-affinity IgA1 (fraction H) of each IgAN patient was pooled and divided by ion-exchange chromatography into cationic (HC), neutral (HN), and anionic (HA) fractions in the same manner. In this fractionation, a definite peak of the neutral fraction appeared (Figure 2).

The release of O-glycans from the IgA1 was performed according to our previous method of gas-phase hydrazinolysis (3). The solutions containing the IgA1 were dried under reduced pressure at 50°C. Approximately 2 mg of dried sample was treated with anhydrous hydrazine (Honen Co., Yokohama, Japan) at 65°C for 6 h using Hydraclub S-204 (Honen Co.). After the reaction, hydrazine was absorbed in concentrated sulfuric acid under reduced pressure. The dried sample was suspended in 250 ml of saturated sodium bicarbonate, and then 25 ml of acetic anhydride was added for acetylation. After 30 min of incubation at RT, this treatment was repeated. To desalt the solution, the sample was applied to a Dowex 50W column (X8, H form, 3 ml) (Muromachi Kagaku Kougyou, Tokyo, Japan) and washed with 15 ml of distilled water. The eluate containing the released oligosaccharides was then dried.

After the dried sample was pyridylaminated as previously reported (17), the pyridylaminated oligosaccharide mixture (PA-sugar) was analyzed using a PALPAK type N column (4.6 × 250 mm, Takara Shuzo, Tokyo, Japan) at a flow rate of 1.0 ml/min at 40°C. Two solvents, A and B, were prepared for the fractionation of PA-oligosaccharides. Solvent A consisted of 50 mM acetic acid adjusted to pH 7.3 with triethylamine and acetonitrile (15:85, vol/vol). Solvent B was 1 M acetic acid adjusted to pH 7.3 with triethylamine and acetonitrile (50:50, vol/vol). The column was equilibrated with solvent A. After the sample injection, the proportion of solvent B was increased linearly to 55% over 110 min. The peaks of each PA-oligosaccharide were then observed.

**Enzyme-Linked Immunosorbent Assay using Anti-Synthetic IgA1 Hinge Peptide Antibody, Peanut Agglutinin, and Vicia Villosa Lectins**

The procedures were carried out according to our previous reports (18). Each well of the microtitration plates (Limbro/Titertek, A Flow General Company, McLean, IL) was coated with 2 µg of the IgA1 fractions in 100 µl of 0.015 M carbonate buffer, pH 9.6, and the unreacted sites were blocked with PBS containing 1% bovine serum albumin (BSA) (fraction V, Sigma Chemical Co., St. Louis, MO).

One hundred microliters of anti-synthetic IgA1 hinge peptide (anti-sHP) antibody (1:100 dilution in PBS) or peroxidase-labeled peanut agglutinin (PNA) (Seikagaku Co., Tokyo, Japan) or VV lectins (5 µg/ml, Sigma Chemical Co.) in PBS was placed in each of the IgA1-coated and noncoated wells. After incubation for 3 h at RT, the plates were washed with PBS containing 0.1% BSA and 0.05% Tween 80 (PBS/BSA/Tween). For the analysis of anti-sHP antibody, 100 µl of peroxidase-conjugated anti-rabbit IgG (1:200 in PBS) was added to the wells, incubated for 1 h at RT, and then washed. All plates were exposed to an enzyme substrate consisting of 0.4 mg/ml O-phenylenediamine dihydrochloride (Sigma Chemical Co.) and 0.03% vol/vol of hydrogen peroxide in a solution containing 0.1 M disodium hydrogen phosphate and 0.05 M citric acid monohydrate. The developed color was read at 490 nm with a microplate reader (model 450; Bio-Rad Laboratories, Richmond, CA). All analyses

**Figure 3.** Grading samples of IgA1 deposits in rat glomeruli by immunofluorescence. A, −; B, +; C, ++; D, ++++. Magnification, ×460.
were performed in triplicate, and each absorbance level of 490 nm was reduced by that of the IgA1 noncoated well.

**Results**

**IgA1 Fractions Accumulating in Rat Glomeruli and Histology**

Definite positive staining was observed in the neutral and the Jacalin high-affinity IgA1 fractionated by the ion-exchange and Jacalin affinity chromatography, respectively (Table 1). No significant deposits were found in the IgA1 among other fractions in either method.

In the positive samples, IgA was accumulated in the glomerular capillary lumen and within the mesangial area. Typical grading samples of the immunofluorescence (IF) are shown in Figure 3a (−), b (+), c (++), and d (+++).

Histologic findings on electron microscopy (EM) and light microscopy (LM) showed the intraluminal precipitates compatible with the IgA precipitates on IF at 15 and 30 min (Figure 4a). At 3 h, in addition to these precipitates, the definite mesangial deposits, degranulated platelets, and prominent infiltration of polymorphonuclear cells (EM, Figure 4b; LM, Figure 4c) were observed. At 24 h, the precipitates and the histologic reaction had declined.

**Size Analyses of the IgA1 Molecules by Electrophoresis**

The cationic IgA1 fraction was mainly composed of monomer and dimer. Significant amounts of macromolecular IgA1 (approximate MW 1000 kD) were present in the neutral and anionic IgA1 fractions. However, there was no obvious difference in the distribution of the molecular weights between the neutral and anionic IgA1.

On the other hand, it was revealed that the relative contents of the macromolecule IgA1 was definitely increased in Jacalin high-affinity IgA1 compared with other fractions of IgA1 (Figure 5).

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**Figure 4.** Typical samples of the histologic findings. (a) Intraluminal precipitates compatible with the IgA precipitates on immunofluorescence are seen (15-min perfusion; ×3000). (b) The definite mesangial deposits and degranulated platelets in the lumen are seen (3 h; ×6000). (c) Typical samples of the histologic findings on light microscopy. The prominent accumulation of polymorphonuclear cells is observed (3 h; periodic acid-Schiff stain; ×480).
Analyses of Released O-Glycans from “Glomerulophilic” IgA1 Fraction by Gas-Phase Hydrazinolysis

As shown in Figure 6, two major peaks (peak A and B) and two minor peaks (peak C and D) were identified. In our previous study (19), it had been clarified that peaks A, B, C, and D were asialo-Gal\(\beta\)1,3GalNAc, NeuAc\(\alpha\)2,3Gal\(\beta\)1,3GalNAc, Gal\(\beta\)1,3(NeuAc\(\alpha\)2,6)GalNAc and NeuAc\(\alpha\)2,3Gal\(\beta\)1,3(NeuAc\(\alpha\)2,6)GalNAc, respectively. It was noted that the “glomerulophilic” IgA1 fraction (HN) had only an asialo-Gal\(\beta\)1–3GalNAc peak (Figure 6b).

Reactivities of PNA, VV Lectins, and Anti-sHP Antibody to “Glomerulophilic” IgA1

Enzyme-linked immunosorbent assay revealed that all reagents of anti-sHP antibody, PNA, and VV lectins strongly bound to the “glomerulophilic” IgA1 fraction among the Jacalin high-IgA1 fractions (Figure 7). The actual levels of 490-nm absorbance in each reagent were as follows [(HC) versus (HN, glomerulophilic) versus (HA)]: Anti-sHP antibody: 0.26 ± 0.05 versus 0.94 ± 0.10 versus 0.01 ± 0.07; PNA: 0.11 ± 0.01 versus 0.31 ± 0.03 versus 0.06 ± 0.00; VV: 0.01 ± 0.01 versus 0.12 ± 0.01 versus 0.01 ± 0.01.

Discussion

In this study, we could isolate and characterize the IgA1 molecules that could accumulate in rat glomeruli. We called the IgA “glomerulophilic” IgA1. The “glomerulophilic” IgA1 was located in the electrically neutral (N) and Jacalin high-affinity (H) fractions. For investigating the structure of O-glycans in the IgA1 hinge, two different approaches, the chemical analysis (gas-phase hydrazinolysis) and enzyme-linked immunosorbent assay (reactivity of PNA, VV, and anti-sHP antibody), were performed. These analyses provided the results reasonably explaining that the hinge of the “glomerulophilic” IgA1 was underglycosylated.

Only an asialo-Gal\(\beta\)1,3GalNAc peak was identified in the “glomerulophilic” IgA1 by the analyses of gas-phase hydrazinolysis. This was compatible with the finding of the increased binding of the “glomerulophilic” IgA1 to PNA lectin, which recognizes asialo-Gal\(\beta\)1,3GalNAc. These observations would reasonably explain our previous comparative studies of the increased reactivity of Jacalin to IgA1 in the patients with IgAN (20) and the increase in the relative content of asialo-Gal\(\beta\)1,3GalNAc in the IgA1 molecule (4).

It was clarified that not only sialic acid but other O-glycan component(s) in the hinge were also lacking in the “glomerulophilic” IgA1. The increased binding of the “glomerulophilic” IgA1 to VV lectin suggested the decrease of the galactose content resulting in the increase of the unsubstituted GalNAc residue in the IgA1 hinge because VV lectin binds to GalNAc but not to Gal residue. The galactose deficiency had been pointed out in the IgA1 molecules of patients with IgAN by Allen et al. (5) and Tomana et al. (6). The increased binding of VV lectin to “glomerulophilic” IgA1 seems to be inconsistent with the results of PNA lectin and the gas-phase hydrazinolysis. However, the phenomenon could occur because PNA lectin does not recognize sialylated-Gal\(\beta\)1,3GalNAc but does recog-
nize the asialo-Galβ1,3GalNAc residue. If the Galβ1,3GalNAc side chains are highly desialylated, PNA lectin could strongly bind to the IgA1 hinge even if the total number of Galβ1,3GalNAc residues is decreased due to the relative increase of the unsubstituted GalNAc residue that is recognized by VV lectin. Similarly, the increased binding of VV lectin to the “glomerulophilic” IgA1 is not necessarily inconsistent with the result of the gas-phase hydrazinolysis, because the gas-phase hydrazinolysis provided no information concerning the unsubstituted GalNAc residues, because it could not identify the unsubstituted GalNAc residues (3,21).

The “glomerulophilic” IgA1 was also strongly recognized by polyclonal anti-sHP antibody that reacted with the peptide core of the IgA1 hinge but not with O-glycan side chains. The results, therefore, suggested that the hinge region in the “glomerulophilic” IgA1 was highly deglycosylated because the results reflected the increased area of the exposed peptide core. The increased reactivity of the antibody was also observed in the IgA1 molecules of the IgAN patients in our recent study (15).

The actual reason(s) why the “glomerulophilic” IgA1 is located in the Jacalin high-affinity fraction is uncertain. The phenomenon seemed to be inconsistent with the observation that the hinge glycopeptide of the IgA1 was under-O-glycosylated, because Jacalin recognizes the O-glycan residues. There could be two possibilities for reasonably explaining the discrepancy. The first is the effect of the desialylation of O-glycans in the hinge. Even if the number of O-glycan side chains decreased, the total affinity of O-glycan to Jacalin could increase due to desialylation similar to the binding of PNA. It was observed by Hortin and Trimpe (22) that the binding of O-glycans to Jacalin, although not completely prevented, did appear to be reduced by sialic acid located on the outer side of the O-glycan chains. The second possibility is the self-aggregation of the “glomerulophilic” IgA1. The result of the native PAGE indicated that the Jacalin high-affinity IgA1 contains higher amounts of macromolecular IgA1 compared with other IgA1 fractionated by the Jacalin column. It had been observed that the reactivity of IgA1 to Jacalin was increased along with the increase in its molecular weight (20).

The cause(s) of the location of the “glomerulophilic” IgA1 in the electrically neutral fraction is also beyond current knowledge. The analyses of the molecular weights indicated that the cationic fraction was mainly composed of monomer and dimer, containing few macromolecules of IgA1. On the other hand, a large amount of macromolecular IgA1 was observed in the neutral and anionic IgA1 fractions. These results suggested that the “glomerulophilic” IgA1 was shifted into the relatively cationic range among the macromolecular IgA1. This could be partially explained by the result of this study that the “glomerulophilic” IgA1 had no sialic acid in the Galβ1,3GalNAc residues because sialic acid is negatively charged. Considering the difference in molecular weights between the cationic IgA1 fraction and the “glomerulophilic” IgA1 (neutral) fraction, it was also suspected that the aggregation itself also influenced the electric charge of the “glomerulophilic” IgA1 probably due to the change in its steric structure.
As far as the mechanism of the glomerular IgA deposition in IgAN is concerned, our previous studies clarified the presence of the IgA-IgA interaction resulting in the formation of macromolecular IgA1 (13) and the association of asialo-Galβ1,3GalNAc with the conformational instability of IgA1 in IgAN (4). Recently, it was found that desialylation of the IgA1 molecule by treatment with sialidase induced the self-aggregation of IgA1 (18). The present study had results consistent with those reported previously. It was clarified that the Galβ1,3GalNAc side chains identified by gas-phase hydrazinolysis were only of the asialo-type in the “glomerulophilic” IgA1, and the IgA1 was mainly composed of macromolecular IgA.

In general, it had been established that the mesangial localization of the immune complexes was associated with their molecular weights ranging up to 1000 kD or greater (23,24). However, the analyses of the molecular weights of IgA1 fractionated by ion-exchange chromatography suggested that the glomerular deposition of IgA1 cannot be fully explained only by the size of the IgA1 molecules because the anionic IgA1 also contained a considerable amount of macromolecular IgA1 as did the “glomerulophilic” IgA1 (neutral) fraction. However, the “glomerulophilic” IgA1 was relatively cationic in the macromolecular IgA1 fractions. This would be reasonably explained by the fact that the cationic macromolecule tended to be trapped in the glomeruli rather than the anionic one (25,26).

The increase in the IgA1 having affinity for type IV collagen (27), laminin (28), and fibronectin (29), which composed the glomerular basement membrane and mesangial matrix, had been found in patients with IgAN. Furthermore, Coppo et al. suggested an important role of O-glycans in the hinge for the binding of IgA1 to type IV collagen, laminin, and fibronectin (30). Our recent study also showed that the IgA1 molecules acquired the reactivity with these extracellular matrix proteins when they were treated with sialidase. After further treatment with β-galactosidase, the IgA1 (agalacto-IgA1) showed the highest affinity for these proteins compared with those of the asialo- and naked (treated with sialidase, β-galactosidase and N-acetylgalactosaminidase) IgA1 (18). Considering this previous in vitro study, it is reasonable to explain the phenomena observed in this study as follows: The desialylation of the IgA1 hinge was a prerequisite for the self-aggregation, and furthermore underglycosylation, especially undergalactosylation, played a key role(s) in the affinity toward the extracellular matrix proteins, resulting in the mesangial IgA deposition.

In this study, a mild but definite histologic response due to the glomerular deposition of IgA1 was observed. At 3 h after the perfusion, prominent accumulation of polymorphonuclear cells and degranulated platelets were observed in the glomerular capillary lumen. This phenomenon seems to be similar to that of the study reported by Davin et al. (16), who also observed the histologic response induced by the perfusion of polymeric IgA-ConA complexes into the rat kidney. In our study, the histologic reaction did not occur at 15 min and 30 min after the perfusion and disappeared after 24 h. These time course reactions and histologic characteristics were consistent with those of the experimental arthus-type nephritis reported by Shigematsu et al. (31).

This study provided the first information concerning the isolation and the structural characterization of the IgA1 molecules that could accumulate in rat glomeruli and induce the
histologic reaction. This information may provide further understanding of the actual mechanism of glomerular IgA deposition and the histologic reaction in IgAN.

Acknowledgments

This study was supported in part by grants from New Energy and Industrial Technology Development Organization (NEDO) and Asahi Chemical Industry. The authors thank Ms. Y. Tanaka and Ms. M. Saitoh for excellent technical assistance. The authors are also grateful to Professor H. Shigematsu of Shinshu University and the members of the Electron Microscope Laboratory Center of Kitasato University for their valuable suggestions and assistance with electron microscopy and photography.

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