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Increased Sialylation of Polymeric λ-IgA$_1$ in Patients With IgA Nephropathy

Joseph C.K. Leung, Sydney C.W. Tang, Daniel T.M. Chan, Sing Leung Lui, and Kar Neng Lai*

Division of Nephrology, Department of Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong

The mechanism of mesangial IgA deposition is poorly understood in IgA nephropathy (IgAN). Abnormal glycosylation of carbohydrate moieties in the hinge region of the IgA molecule has recently attracted much attention. In this report, we studied galactosylation and sialylation profiles in κ- and λ-IgA$_1$ from patients with IgAN. Total serum IgA$_1$ was isolated from patients with IgAN or healthy controls by jacalin-affinity chromatography. Six fractions of molecular weight (MW) 50–1,000 kDa were separated by fast protein liquid chromatography (FPLC). Four lectin-binding assays were used to study the sialylation and the presence of terminal galactose or N-acetylgalactosamine (GalNAc) in the O-linked carbohydrate moieties of κ- or λ-IgA$_1$. *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA) lectin recognize α(2,3)- and α(2,6)-linked sialic acid, respectively. Peanut agglutinin (PNA) and *Helix aspersa* (HA) lectin recognize terminal galactose and GalNAc, respectively. Reduced HA was demonstrated in macromolecular κ or λ-IgA$_1$ (300–825 kDa) isolated from patients with IgAN ($P < 0.05$ compared with healthy controls). Lambda- but not κ-IgA$_1$ from patients with IgAN bound less to PNA ($P < 0.05$). The α(2,3)-linked sialic acid content in λ- but not κ-IgA$_1$ of MW 150–610 kDa from patients was higher than that of controls ($P < 0.005$). The α(2,6)-linked sialic acid content in λ-IgA$_1$ (300–825 kDa) and κ-IgA$_1$ (150–610 kDa) from patients was also higher than that of controls. This unusual glycosylation and sialylation pattern of the λ-IgA$_1$ may have important implications for the pathogenesis of IgAN, as both the masking effect of sialic acid on galactose and the reduced galactosylation will hinder the clearance of macromolecular λ-IgA$_1$ by asialoglycoprotein receptor of hepatocytes. The negative charge from sialic acid may also favor mesangial deposition of macromolecular λ-IgA$_1$ in IgAN. J. Clin. Lab. Anal. 16:11–19, 2002.

Key words: IgA nephropathy; glycosylation; sialylation; IgA$_1$; polymeric IgA; λ-IgA

INTRODUCTION

IgA nephropathy (IgAN) is the most common type of glomerulonephritis worldwide. The hallmark of the disease is a mesangial deposition of polymeric IgA$_1$. Accumulating observations suggest that IgA nephropathy is associated with a regulatory abnormality in IgA synthesis. These observations include raised serum IgA levels (1,2), overproduction of IgA$_1$ by B lymphocytes in vitro (3,4), raised serum level of IgA-containing immune complexes (5,6) and the anionic nature of IgA (7,8). However, the pathogenesis of IgAN remains to be explored, since none of these abnormalities can adequately explain how IgA$_1$ is deposited in the glomerular mesangium.

IgA$_1$ is heavily glycosylated and consists of 8% carbohydrate. The five O-linked glycosylation sites in the hinge region of the CH1 and CH2 domains of the heavy chain on IgA$_1$ is a distinctive and unusual feature that is not found in IgA$_2$ (9). Analysis of serum IgA$_1$ from IgAN patients revealed significant alterations in the glycan of these side chains. Studies using carbohydrate-specific lectin-binding assays and mass spectrometry analysis suggested that the abnormality is a reduction in the terminal galactosylation of the O-linked carbohydrate moieties of the IgA molecule (10–12). It was suggested that reduced terminal galactosylation of O-linked moieties at the hinge region may lead to defective clearance of IgA and mesangial deposition (13). At the same time, few studies have examined the sialylation of the O-linked carbohydrate moieties of IgA in IgAN. The sialic acid content of IgA$_1$ could exert a profound influence on the binding of IgA molecules to mesangial cells. We had previously reported increased binding of polymeric λIgA from patients with IgAN to cultured human

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*Correspondence to: Professor K.N. Lai, Department of Medicine, Queen Mary Hospital, University of Hong Kong, Pokfulam, Hong Kong.
E-mail: knlai@hkucc.hku.hk

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mesangial cells (HMCs) (14). We also demonstrated that there was increased sialylation in polymeric IgA from IgAN patients (15). However, no information is available on the nature of sugar and glycan at the side chains of the κ-IgA or λIgA. In the present study, we used enzyme-linked lectin assays to investigate sialylation and glycosylation profiles in monomeric or polymeric κ- or λ-IgA isolated from patients with IgAN.

MATERIALS AND METHODS

Patients and Controls

Thirty-six Chinese patients (18 males and 18 females) with a clinical and renal immunopathologic diagnosis of primary IgAN were studied. They had been symptomatic for 12 months or more. The histologic diagnosis was confirmed by the presence of predominant granular IgA deposits, mainly in the glomerular mesangium and occasionally along the peripheral capillary basement membrane, by immunofluorescence studies, as well as by mesangial electron dense deposits in ultrastructural examination. Systemic lupus erythematosus, Henoch-Schonlein purpura, and hepatic disease were excluded by detailed clinical history, examination, and negative laboratory test for hypocomplementemia, anti-DNA antibody, or hepatitis B virus surface antigen. No significant renal impairment was documented in these patients, and their endogenous creatinine clearances were greater than 70 ml/min/1.73 m^2. Twenty milliliters of blood were collected from each patient at clinical quiescence. The serum was isolated and frozen at –20°C until isolation of IgA_1 by a jacalin-agarose affinity column. Serum IgA levels were determined by nephelometry.

Thirty-four healthy subjects (19 males and 15 females), comparable in age and race, and with no microscopic hematuria or proteinuria, were used as controls. Serum was collected as described above.

Isolation of Jacalin-Bound Proteins (JBPs) by Fast Protein Liquid Chromatography (FPLC)

JBPs were purified using a jacalin-agarose affinity column (Pierce Chemical Co., Rockford, IL) and were fractionated at room temperature by the FPLC system (Pharmacia, Uppsala, Sweden) as described previously (16). Briefly, monomeric IgA_1, polymeric IgA_1, and IgA_1-immune complexes were separated by FPLC following affinity chromatography with jacalin. The identity of IgA after FPLC was confirmed by anti-IgA affinity chromatography and an IgA sandwich enzyme-linked immunosorbent assay (ELISA) (16). Six pooled fractions were prepared for further analysis. Pooled fractions F1–F3 were high molecular mass IgA of MW 300–1,000 kDa. Pooled fractions F4–F6 were low molecular mass IgA of MW 50–300 kDa. The content of IgG and IgM in pooled fractions was measured by anti-IgG and anti-IgM ELISA, respectively. The pooled fractions were dialyzed and concentrated to 1 ml with Centriprep (Amicon, Beverly, MA) and stored at –70°C until use. The purity of IgA_1 fractions was confirmed by SDS-polyacrylamide gel electrophoresis and ELISA as described previously (16).

Enzyme-Linked Lectin Assays

The sugar-binding specificity of lectins has made them useful tools in glycoprotein research since sugars present in glycoconjugates can be analyzed selectively. Enzyme-linked lectin binding assays (ELLAs) have been employed to investigate the galactosylation of N- and O-linked carbohydrate moieties of IgA_1 in IgAN (15). Briefly, 96-well microtiter plates (Immunlon 2, Dynatech, Marnes la Coquette, France) were coated with 100 μl of rabbit anti-human IgA or anti-human κ light chain or anti-human λ light chain $F(ab')_2$ (Dakopatts, Kyoto, Japan) diluted 1:1,000 in carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C. The plates were then blocked by phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA) and 0.05% Tween 20 for 1 hr at room temperature and washed three times with PBS containing 0.05% Tween 20 (PBS-Tween). To avoid any increased binding of IgA due to elevated IgA concentrations in JBP samples from patients, JBP samples from patients or control were appropriately diluted to achieve comparable levels of total IgA. Then 100 μl of JBP samples, diluted in PBS-Tween containing 1% BSA, were incubated in the wells overnight at room temperature. The wells were washed three times with PBS-Tween. Next, 100 μl of 1:1,000 digoxigenin-labeled peanut agglutinin (PNA) (Boehringer Mannheim, Mannheim, Germany), digoxigenin-labeled *Maackia amurensis* agglutinin (MAA) (Boehringer Mannheim), digoxigenin-labeled *Sambuscus nigra* agglutinin (SNA) (Boehringer Mannheim), or horseradish peroxidase-labeled *Helix aspersa* (HA) (Boehringer Mannheim) in PBS-Tween were added to the wells and incubated for 4 hr at room temperature. After washing with PBS-Tween, the wells incubated with digoxingenin-labeled lectins were further incubated for 1 hr with 1:1,000 alkaline phosphatase conjugated anti-digoxigenin in PBS-Tween. After washing, 100 μl/well p-nitrophenyl phosphate (pNPP) (Sigma, St. Louis, MO) or O-phenyldiamine (OPD) (Sigma) were added to the plates and were incubated for a minimum of 30 min in the dark at room temperature. Finally, the reaction was terminated by addition of either 50 μl/well 2 M NaOH or 2M sulfuric acid, and the absorbances were measured at 450 nm or 490 nm using an ELISA reader. All samples were done in the same batch to avoid interbatch variation. Asialofetuin and transferrin were used as the positive and negative control, respectively, for PNA ELLA; α(2-3) sialyllactose and α(2-6) sialyllactose were used as positive controls for MAA and SNA, respectively. Results were expressed as the optical density (OD) unit.

Culture of HMC Culture

Isolation and characterization of HMCs were performed as previously described (16). Glomeruli were prepared from

the cortex of human cadaveric kidney judged to be not suitable for transplantation or from the intact pole of kidneys removed for circumscribed tumor. Histological examination of these kidney samples revealed no renal pathology. Glomerular cells were grown in RPMI 1640 medium supplemented with glutamine (2 mmol/L), N-[2-hydroxyethyl]-piperazine-N′-[2-ethanesulfonic acid] (HEPES) (10 mmol/L), penicillin (50 U/ml), streptomycin (50 μg/ml), and 12% fetal calf serum in an atmosphere of 5% CO_2/95% air. Mesangial cells have a stellate appearance and grow in clumps. They show a network of intracellular fibrils of myosin and they contract in the presence of 1 nmol/L of angiotensin II. Mesangial cells at the fourth to seventh passages were used.

Binding of IgA_1 Subfractions to HMCs

HMCs were grown to log phase and harvested by using 0.05% trypsin/0.02% EDTA for 5 min at room temperature. The cells were adjusted to 5 × 106/ml and 200 μl of cell suspension was used in binding assays. All staining was done at 4°C with staining buffer (PBS with 1% fetal bovine serum and 0.1% sodium azide). The cells were incubated with 100 μl of various subfractions of IgA_1 for 30 min. After incubation, the cells were washed with staining buffer and then further incubated with 100 μl of fluorescein isothiocyanate (FITC) conjugated $F(ab')_2$ fragment of rabbit anti-human IgA. Background control staining was achieved by reaction with preimmune F(ab′)2 fragment of fluorescein-labeled isotypic antibody. The stained cells were analyzed using a Coulter EPICS XL analyzer (Coulter Electronic, Miami, FL). A minimum of 5,000 fixed cells for each sample were analyzed. Fluorescence intensity was evaluated by comparing the mean fluorescence channels. The result was expressed as mean fluorescence intensity (MFI).

Statistics

The results are shown as median with range unless specified otherwise. For comparison between patient and control groups, the Mann-Whitney U test and the Spearman correlation analysis were used. Statistical significance was considered to be $P < 0.05$.

RESULTS

Purification of Monomeric and Polymeric JBPs

A typical chromatogram of JBP after FPLC is shown in Fig. 1. Six pooled fractions (F1–F6) were prepared according to their molecular weight. Pooled fractions F1–F3 were macromolecular IgA with MW 300–1,000 kDa.. Pooled fraction F4 was monomeric IgA with MW 150–300. Pooled fraction F5 was jacalin-purified α2-HS glycoprotein. Pooled fraction F6 contained little protein. Both F5 and F6 served as controls. No IgG or IgM was detected in fractions F4–F6. IgG and IgM represented 0.1% and < 0.01% of total protein, respectively, in fractions F1–F3 as measured by ELISA. IgA in fractions F1 and F2

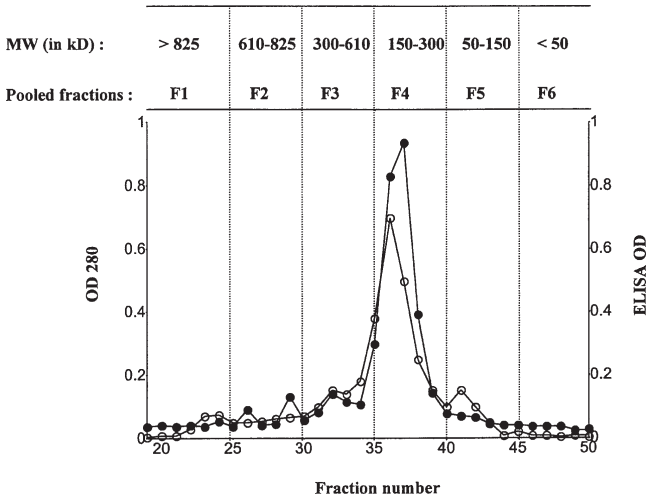

Fig. 1. Representative chromatogram illustrating fractionation of JBPs by the FPLC system. JBP was separated into six pooled fractions (F1–F6) with different molecular sizes. Protein absorbances at 280 nm are shown as open circles. IgA ELISA results are indicated as closed circles.

(MW > 610 kDa) was interpreted as polymeric IgA, and IgA in fraction F3 (MW 300–610) was interpreted as dimeric IgA.

The serum IgA levels in patients with IgAN (2.94 + 0.6 g/L, mean + standard deviation (SD)) were significantly higher than those of healthy controls (1.85 + 0.4 g/L, $P < 0.001$). To avoid any increased binding of IgA due to elevated IgA concentration in JBP samples from patients, JBP samples from patients or controls were appropriately diluted to achieve comparable levels of total IgA. Studies of IgA in FPLC fractions showed that monomeric IgA (F4) amounted to 92 + 4% (mean + SD) of total IgA for both healthy controls and patients. Similarly, macromolecular IgA (F1–F3) represented 6 + 2% of total IgA from either group of subjects. The ratio of monomeric to polymeric IgA did not differ between JBPs from patients and healthy controls.

Galactosylation Profiles of O-Linked Sugar in Serum IgA, Polymeric, and Monomeric Jacalin-Bound κ- or λ-IgA_1

The binding property of the lectins (PNA, MAA, SNA, and HA) is shown in Fig. 2. Positive reaction with PNA indicates the presence of galactose-β(1,3)-N-acetylgalactosamine [Gal β(1,3)-GalNAc]. However, a terminal sialic acid will block the core disaccharide accessibility by PNA. Positive reaction with MAA indicates the presence of sialic acid terminally α(2,3) linked to galactose. Positive reaction with SNA indicates the presence of sialic acid terminally (2,6) linked to galactose or N-acetylgalactosamine. HA binds only to ungalactosylated N-acetylgalactosamine (GalNAc) and does not bind to those with galactosylation.

Polymeric IgA (F2; MW 610–825 kDa) from patients with IgAN showed significantly lower binding to lectin HA than

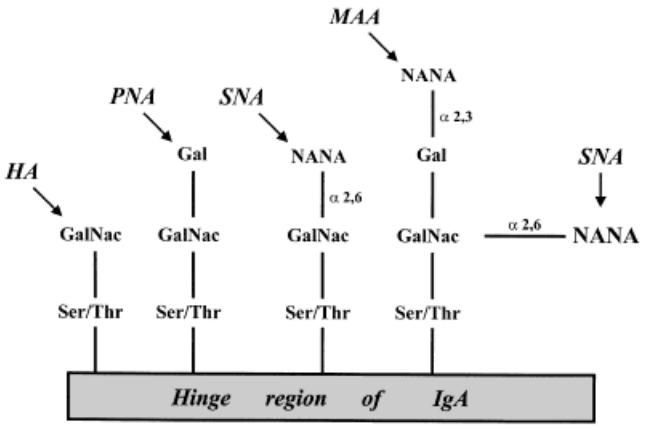

Fig. 2. Diagrammatic representation of the binding sites of lectins MAA, SNA, PNA, and HA on oligosaccharide side-chains in the hinge region of human IgA_1. Lectin MAA binds sialic acid that is α(2,3) linked to Gal. Lectin SNA binds sialic acid that is α(2,6) linked to GalNAc. Lectin PNA binds only those Gal without sialic acid, and lectin HA binds only ungalactosylated GalNAc.

IgA prepared from controls ($P < 0.05$) (Fig. 3A). A lower binding to HA was also evident for dimeric and polymeric κ-IgA (F2 and F3; MW 300–825 kDa; $P < 0.05$) and polymeric λ-IgA (F2; MW 610–825 kDa; $P < 0.05$) (Fig. 3B and C). Although the binding of monomeric total IgA, κ-IgA or λ-IgA (F4) from patients with IgAN was apparently higher than that of controls, the difference was not statistically significant ($P = 0.105$ for monomeric total IgA, $P = 0.164$ for monomeric κ-IgA, and $P = 0.324$ for monomeric λ-IgA).

Total dimeric IgA (F3; MW 300–610 kDa) from patients with IgAN showed significantly lower binding to lectin PNA than IgA prepared from controls ($P < 0.005$) (Fig. 4A). A lower binding to PNA was also evident for dimeric and polymeric λ-IgA from patients with IgAN (F2 and F3; MW 300–825) when compared with that of controls ($P < 0.05$ and $P < 0.005$, respectively) (Fig. 4C). Although the binding of monomeric total IgA or λ-IgA (F4) was apparently lower from patients with IgAN than those of controls, the difference was not statistically significant ($P = 0.1135$ for monomeric total IgA, and $P = 0.1094$ for monomeric λ-IgA).

The binding of dimeric total IgA (F3; MW 300–610 kDa) and dimeric λ-IgA (F3; MW 300–610 kDa) to MAA was significantly higher in patients with IgAN compared with controls ($P < 0.05$) (Fig. 5A and C). A higher binding of monomeric λ-IgA (F4) from patients with IgAN to MAA was also observed ($P < 0.05$) (Fig. 5C). However, there was no significant difference in binding to lectin MAA for other fractions.

Total dimeric and polymeric IgA (F2 and F3; MW 300–1,000 kDa) from patients with IgAN showed significantly higher binding to lectin SNA than to IgA prepared from controls ($P < 0.05$ and < 0.005, respectively) (Fig. 6A). A higher binding to SNA in patients with IgAN compared with controls was evident for dimeric κ-IgA (F3; MW 300–610 kDa ; $P < 0.05$) (Fig. 6B) and

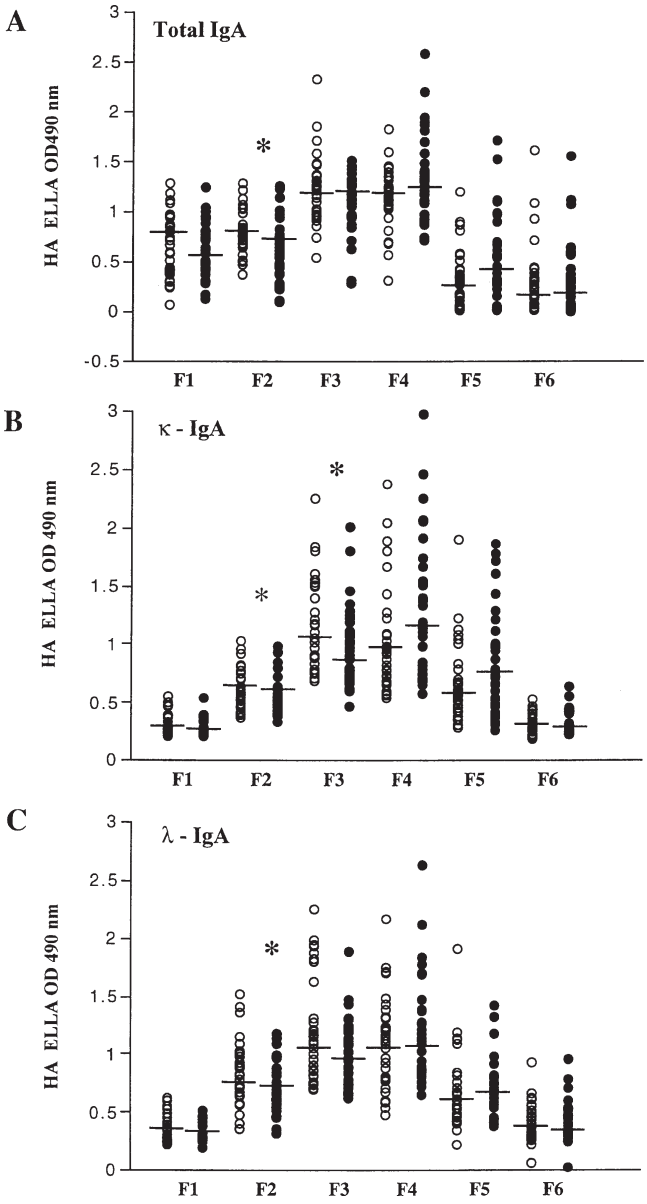

Fig. 3. Binding of lectin HA to (**A**) serum IgA, (**B**) κ-IgA, and (**C**) λ-IgA from patients with IgAN (closed circles) or healthy controls (open circles). Horizontal bars represent the median values. Statistical significance is expressed as * with $P < 0.05$, and as ** with $P < 0.005$.

for dimeric/polymer λ-IgA (F2 and F3; MW 300–1,000 kDa; $P < 0.05$ and $P < 0.005$, respectively) (Fig. 6C). Monomeric κ-IgA from patients with IgAN also showed a higher binding to SNA ($P < 0.05$) (Fig. 6B). There was no significant difference in binding to lectin SNA for other fractions.

Binding of the FPLC-Fractionated IgA to Cultured HMCs

The binding of IgA with different molecular sizes to cultured HMCs was examined by flow cytometry. The binding of dimeric

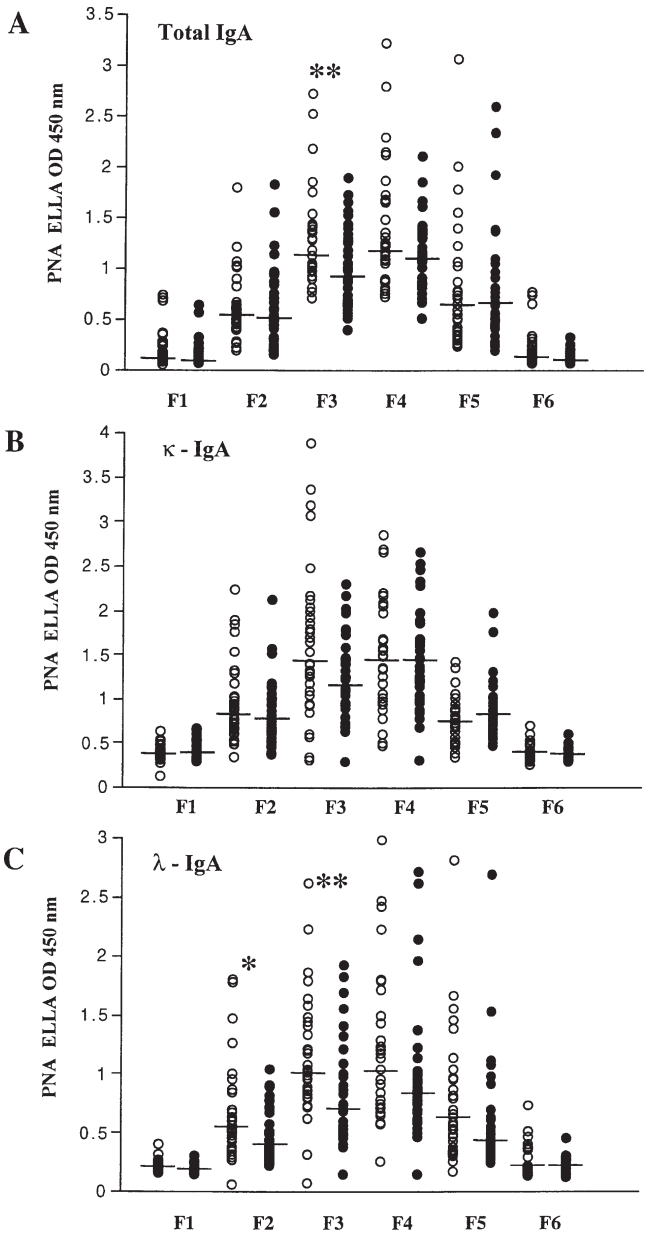

Fig. 4. Binding of lectin PNA to (**A**) serum IgA, (**B**) κ-IgA, and (**C**) λ-IgA from patients with IgAN (closed circles) or healthy controls (open circles). Horizontal bars represent the median values. Statistical significance is expressed as * with $P < 0.05$, and as ** with $P < 0.005$.

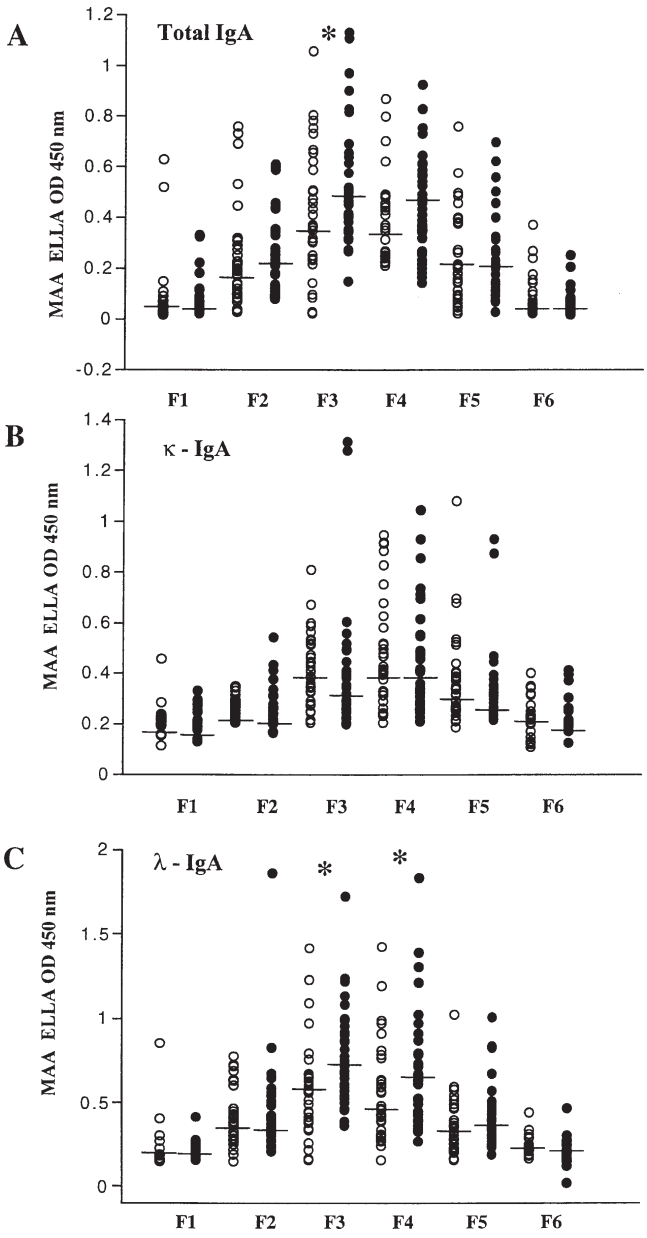

Fig. 5. Binding of lectin MAA to (**A**) serum IgA, (**B**) κ-IgA, and (**C**) λ-IgA from patients with IgAN (closed circles) or healthy controls (open circles). Horizontal bars represent the median values. Statistical significance is expressed as * with $P < 0.05$, and as ** with $P < 0.005$.

IgA fraction (F3; MW 300–610 kDa) from patients with IgAN was significantly higher compared to controls ($P < 0.001$) (Fig. 7). There was no difference in the binding to mesangial cells between patients and controls for other IgA fractions.

Correlation Analysis Between Binding Properties of IgA to Lectins and Mesangial Cells

The binding of different IgA fractions to various lectins (HA-, MAA-, PNA-, and SNA-ELLA) was compared with the binding to cultured HMCs to determine any correlation between the two binding properties. When all data (from both IgAN patients and controls) were included for analysis, the binding of dimeric and polymeric IgA to HMC significantly correlated with the MAA-ELLA (r = 0.4542, $P = 0.0004$) (Fig. 8A) or the PNA-ELLA (r = –0.4627, $P = 0.0005$) (Fig. 8B). A negative correlation also existed between PNA-ELLA and MAA-ELLA in dimeric/polymeric IgA (r = –0.2479, $P = 0.0109$) (Fig. 8C). The data were further analyzed sepa-

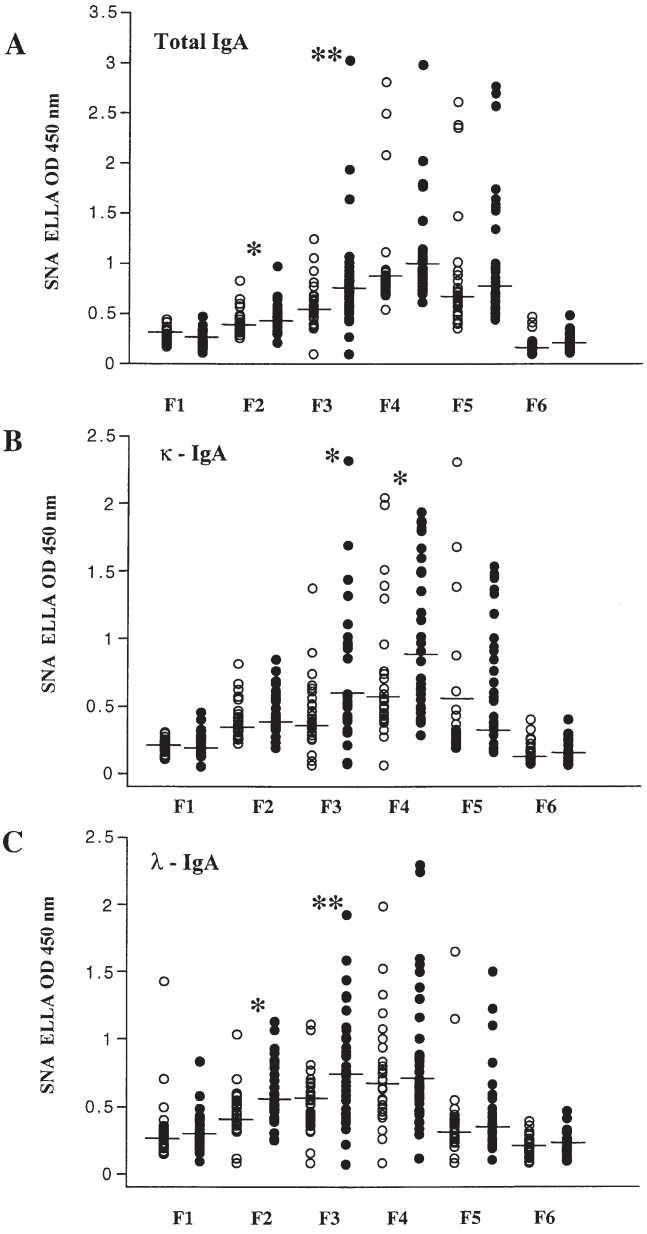

Fig. 6. Binding of lectin SNA to (**A**) serum IgA, (**B**) κ-IgA, or (**C**) λ-IgA from patients with IgAN (closed circles) or healthy controls (open circles). Horizontal bars represent the median values. Statistical significance is expressed as * with $P < 0.05$ and as ** with $P < 0.005$.

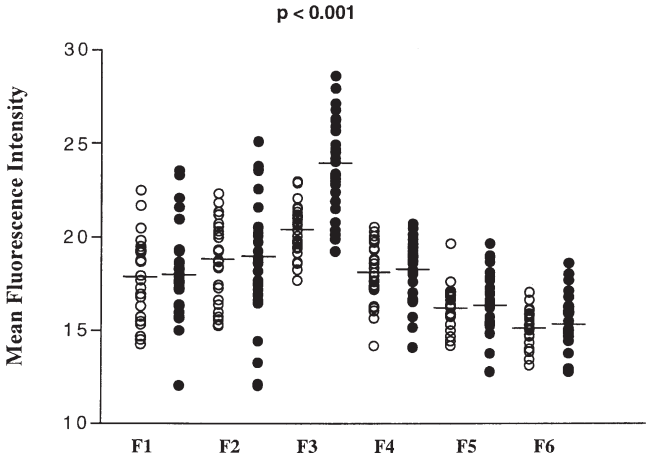

Fig. 7. Binding of FPLC-separated IgA from patients with IgAN (closed circles) or from healthy controls (open circles) to cultured HMCs.

rately within the group of patients or controls. In patients with IgAN, the binding of dimeric and polymeric IgA to HMC significantly correlated with the MAA-ELLA (Fig. 8A; closed circles; r = 0.3642, $P = 0.0458$) or the PNA-ELLA (Fig. 8B; closed circles; r = –0.3624, $P = 0.0312$). An apparent negative correlation existed between PNA-ELLA and MAA-ELLA in dimeric/polymeric IgA from these patients but the correlation failed to reach significance (Fig. 8C; closed circles; r = –0.2479, $P = 0.1915$). In the control subjects, a significant correlation existed between the binding of dimeric and polymeric IgA to HMC and the MAA-ELLA (Fig. 8A; open circles; r = 0.5080, $P = 0.0095$). However, such correlation was not observed between the binding of dimeric and polymeric IgA to HMC and the PNA-ELLA (Fig. 8B; open circles; r = –0.2922, $P = 0.1659$) or between PNA-ELLA and MAA-ELLA in dimeric/polymeric IgA from controls (Fig. 8C; open circles; r = 0.0131, $P = 0.9517$). No significant correlation was found between other lectin binding assays. Our findings suggested that oversialylation of polymeric IgA (determined by MAA-ELLA) was positively associated with increased binding of polymeric IgA to mesangial cells. An inverse correlation was observed between the binding of polymeric IgA to mesangial cells and the exposure of galactose residue at the hinge region of the IgA molecule (determined by PNA-ELLA). This is more likely to arise from increased terminal α(2,3) sialylation than from the reduction of terminal galactose of the carbohydrate side chain of polymeric IgA.

DISCUSSION

The present study examines sialylation and glycosylation profiles in monomeric and polymeric κ- and λ-IgA isolated from patients with IgAN. We had previously demonstrated that mesangial IgA is predominantly λ-IgA, and that there is an increased level of circulating λ-IgA_1 in patients with IgAN (16). These findings suggested that the immunochemical nature of λ-IgA plays an important pathogenetic role in primary IgAN. Studies of renal eluates revealed that 70% of the glomerular IgA had characteristics of genuine polymeric IgA (17). However, it remains obscure whether the predominant glomerular pIgA_1 deposits represent a selective mesangial uptake/deposition or nonselective trapping due to elevated circulating IgA_1 levels in response to unidentified antigen(s). We recently reported that reduced terminal galactosylation of IgA_1 in IgAN is confined to monomeric IgA_1, not to polymeric

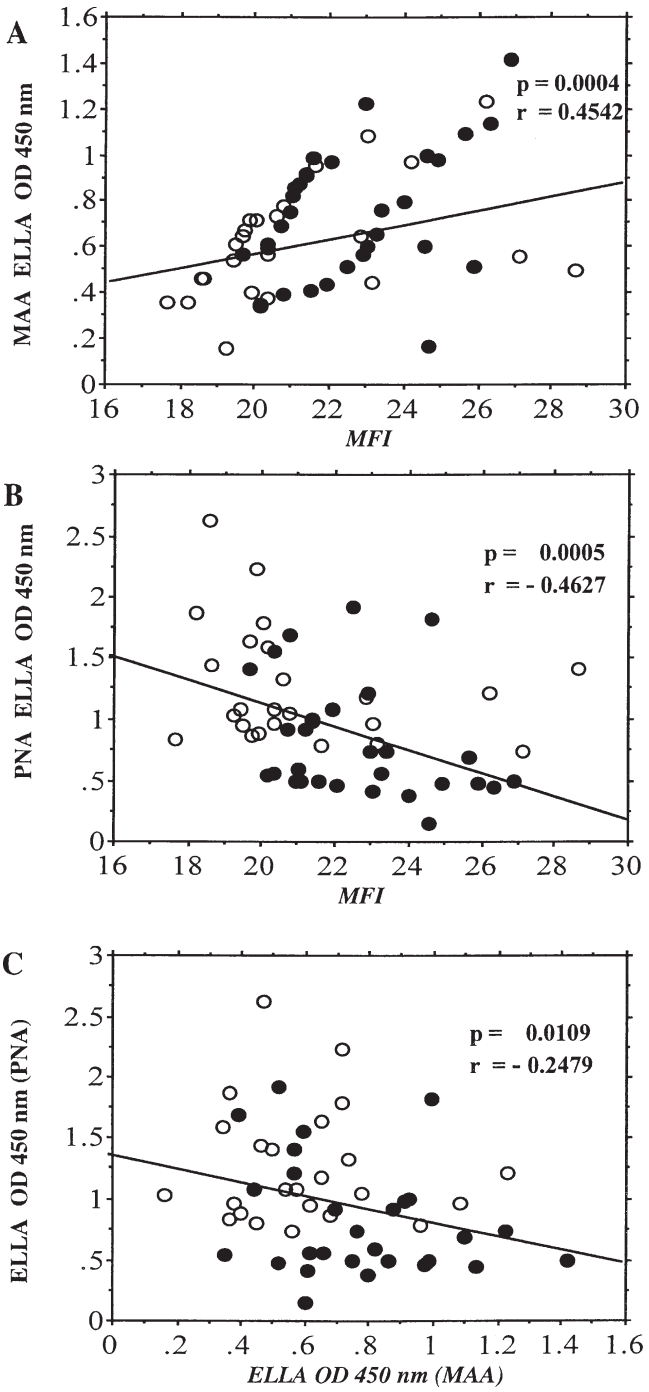

Fig. 8. Correlation analysis between the binding of IgA to HMCs, and results of (**A**) lectin-binding assay with MAA and (**B**) lectin-binding assay with PNA. The binding of polymeric IgA to HMC correlates positively with MAA ELLA ($P = 0.0004$) and negatively with PNA ELLA ($P = 0.0005$). **C:** There is a negative correlation between MAA ELLA and PNA ELLA ($r = -0.2479$, $P = 0.0109$). Data from patients with IgAN, and from healthy controls are expressed as closed circles and open circles, respectively.

IgA_1 (15). In contrast, oversialylation was demonstrated in polymeric IgA_1 (18). In the present study, we report the novel observation that O-glycan of polymeric λ-IgA_1 from IgAN patients is oversialylated. This observation was supported by the data from lectin-binding assays. The increase in MAA binding of polymeric λ-IgA_1 from patients with IgAN indicated an increase in α(2,3)-linked sialic acid content of polymeric λ-IgA_1. Oversialylation of polymeric λ-IgA_1 from IgAN patients was further confirmed by the reduction in lectin HA and PNA binding, as increased terminal α(2,3) sialic acid content will hinder the binding of IgA to HA or PNA that is specific for Gal β(1,3)-GalNAc and GalNAc, respectively. Furthermore, the positive correlation between mesangial binding of polymeric IgA and MAA-ELLA in IgAN patients suggested an oversialylation of polymeric IgA from these patients.

It has been agreed that IgA_1 molecules from patients with IgAN display altered glycan moieties (19–21). Structural alteration of the IgA molecule, manifested by decreased binding to plant lectins, has been speculated to bear pathological implication in IgAN. Oversialylation of IgA_1 had also been found in Sjogren syndrome (pSS) and it was speculated that oversialylation of IgA_1 from patient with pSS may prevent the recognition of IgA by receptors that were responsible for their clearance, thus resulting in an excess of IgA_1 and their complexes in circulation (22). We have demonstrated the oversialylation of polymeric λ-IgA_1 in the present study. The negatively-charged α(2,3)-linked sialic acid is mostly located in the oligosaccharides of the Fab and/or the hinge region of polymeric λ-IgA_1. These sugar moieties are large and bulky compared with the protein backbone. Any change in the carbohydrate moieties affects the tertiary structure as well as the electrostatic charges that are pivotal in the interaction and recognition with other molecules, such as asialoglycoprotein receptor (ASGP-R) and Fcα receptor (FcαR) (23–25). Recent consensus is that the hinge region of serum IgA_1 from patients with IgAN has reduced galactose content. Isolated hinge region protein of human IgA_1 displays the highest affinity among all chemical and proteolytic fragments of IgA_1 for the ASGP-R (26). Deficiency of galactose (Gal) residues in the hinge region may have a profound effect on the recognition of IgA_1 by ASGP-R and, hence, its catabolism. Studies using lectin-binding assays and carbohydrate analysis by mass spectrometry revealed a reduced terminal galactose content in the carbohydrate side chain at the hinge region of IgA_1 in IgAN (11,13). Using gas-liquid chromatography, Mestecky and coworkers (13) observed a marked deficiency of Gal residues in IgA_1 from patients with IgAN. Furthermore, a decreased reactivity of IgA_1 with the lectin jacalin was demonstrated, supporting the speculation that there was a reduction of galactoside content in the sugar moieties. Decrease in binding of IgA to galactose-specific lectins can result from the absence of Gal or the masking effect of sialic acid that is linked to galactose. The sialylated content in IgA_1 has also been reported to be higher in patients with IgAN than in healthy controls (12). Nevertheless, all of these findings were confined to serum IgA that is comprised of at least 80% monomeric IgA. There are few studies that focus on the glycosylation

and sialylation of polymeric IgA molecules that should be the main player for IgA deposition in IgAN. The α(2-3)-linked sialic acid content was increased in polymeric λ-IgA_1 but not in polymeric κ-IgA_1. Lectin PNA binds only to terminal galactose molecules not linked to sialic acid. Oversialylation in polymeric λ-IgA_1 was supported by decreased binding of polymeric λ-IgA_1 to PNA in patients with IgAN. Recently, Odani and coworkers (26) showed a decrease of GalNAc, Gal, and sialic acid in O-glycosylated hinge peptides from Fc fragments of total human serum IgA_1. Their results do not necessarily contradict our present findings, since polymeric IgA represents less than 15% of total serum IgA (27), and it is not surprising that oversialylation was not observed in reports when total serum IgA was studied. Similarly, reduction of terminal galactosylation in serum IgA from patients with IgAN was attributed to the predominant composition of monomeric IgA. To complicate the picture further, sialic acids can be located in both the Fab and Fc fragments of IgA molecules (28). The sialic acid content of the IgA Fab fragment (exclude the hinge region) has not been examined, and a clear picture of sialylation in IgA_1 cannot be drawn.

The abnormal glycosylation or sialylation of IgA can be the result of altered amino-acid sequences in IgA or altered glycosidase, galactosyltransferase, and/or sialytransferase activity. Alteration in the amino-acid sequence of IgA_1 could modify the template available for O-glycosylation and sialylation. This is less likely as the amino-acid sequence of the hinge region in IgA molecule is rather conserved inherently. Moreover, there is no difference in the genomic DNA and mRNA sequences for the IgA_1 hinge region in peripheral blood mononuclear cells between IgAN patients and controls (29). Nevertheless, the possibility that a minority clone of IgA plasma cells with altered amino-acid sequence cannot be excluded. For the latter case, reduced galactosylation of the O-linked glycans of IgA_1 resulting from B cell-restricted reduction of β1,3 galactosyltransferase activity has recently been reported (30). The present finding of oversialylation of O-glycans in polymeric IgA_1 from patients with IgAN may also be the result of abnormal O-glycan specific glycosyltransferase activity. Further experiments are needed to support this speculation.

Increased polymeric IgA-containing immune complexes are commonly found in patients with IgAN, and the glomerular IgA deposits are mainly polymeric (5,6). Eluted IgA from glomerular sections revealed predominantly polymeric IgA of anionic nature with a narrow pI of 4.5–5.6 (31). Serum IgA from these patients preferentially bound to cationic bovine serum albumin and mesangial cell membrane rendered cationic by preincubation with poly-L-lysine (32). These findings suggest that the anionic nature of polymeric IgA may be pivotal in its binding to mesangial cells. Our present results indicate that the anionic nature of polymeric λ-IgA_1 from patients with IgAN is, at least, partly due to oversialylation in the carbohydrate moieties of the O-glycans. Oversialylation of polymeric λ-IgA_1 could have two potential implications for the pathogenesis of IgAN. First, the clearance of polymeric λ-IgA_1 from the circulation by the ASGP-R in the reticulo-endothelial system may be affected by the masking effect of sialic acid on galactose. This favors its deposition in the glomerulus. Second, oversialylation of polymeric λ-IgA_1 molecules will increase the overall negative charge of the IgA molecule. Experimental studies have supported the notion that the charge of the IgA molecules can play a role in glomerular deposition. The electrostatic charge of immune complexes is important in mesangial binding, and animal experiments have revealed a preferential mesangial deposition of immune complexes of anionic nature (7,33). As the charge of carbohydrate moieties at the hinge region is mainly contributed by the highly negatively charged sialic acids, oversialylation would render the polymeric IgA_1 from patients with IgAN more anionic, thus favoring their binding to mesangial cells. This hypothesis is supported by our recent in vitro study showing that the binding of IgA to HMCs is charge-dependent. The binding of polymeric IgA can be increased by altering the cell surface charges with polycation, and decreased with polyanion (34). The good correlation of mesangial cell binding and MAA ELLA suggests that oversialylation of polymeric λ-IgA_1 could play an important role in the pathogenesis of IgAN. Further investigation of the cause of this immunochemical abnormality is warranted.

In conclusion, our study demonstrates a pathological mechanism for our previous observation of increased binding of polymeric λ-IgA_1 from patients with IgAN to cultured HMCs. The oversialylation of polymeric λ-IgA_1 may play an important role in the pathogenesis of IgAN.

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