Kidney International, Vol. 59 (2001), pp. 277–285

Charge-dependent binding of polymeric IgA_1 to human mesangial cells in IgA nephropathy

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Charge-dependent binding of polymeric IgA_1 to human mesangial cell in IgA nephropathy.

Background. IgA nephropathy (IgAN) is characterized by raised serum IgA_1 and predominant mesangial IgA_1 deposits of polymeric nature. The mechanism of polymeric IgA_1 ($pIgA_1$) deposition in the kidney mesangium is poorly understood in IgAN. It has been suggested that increased sialic acid content and anionic charge of the $pIgA_1$ molecules may be operational in the IgA_1 deposition in human mesangial cells (HMCs). The present study examined the binding of $pIgA_1$ with different surface charges to HMCs. The binding characteristics of IgA_1 to HMCs in the presence of polycation (poly-L-lysine) or polyanion (heparin) were also investigated.

Methods. IgA_1 was purified in sera from patients with IgAN and from healthy controls by jacalin affinity chromatography. IgA_1 was further separated into $pIgA_1$ and monomeric IgA_1 ($mIgA_1$) by fast protein liquid chromatography (FPLC). $pIgA_1$ or $mIgA_1$ with different net charges on their surface were resolved by ion exchange chromatography (IEC) with a Mono Q column. The binding characteristics of $pIgA_1$ and $mIgA_1$ to HMCs in the presence or absence of polycation or polyanion were examined by flow cytometry.

Results. In patients with IgAN, the absolute amount of $mIgA_1$ and $pIgA_1$ is significantly higher than that of healthy controls ($P < 0.001$). There was significant increase in binding of $pIgA_1$ from patients with IgAN to HMC and cell lysate. $pIgA_1$ that interacted strongly with the ion exchanger also bound more to HMCs when compared with IgA_1 interacted weakly with the ion exchanger ($P < 0.001$). The anionic charged $pIgA_1$ from patients was significantly higher than that of healthy controls ($P < 0.001$). Preincubation with poly-L-lysine increased the binding of $pIgA_1$ to HMCs. The binding of $pIgA_1$ to HMCs was decreased by preincubation with heparin.

Conclusions. The binding of IgA to HMCs is charge dependent. Polymeric IgA with the highest net negative charge binds more to HMCs. Preincubation with polyanion decreased the binding of polymeric IgA to HMCs. These results suggest an important role for anionic charge in IgA_1 deposition onto the kidney mesangial cells.

Key words: anionic charge, polycation, charge dependent IgA binding, deposits of IgA, kidney mesangial cells, glomerulonephritis, cell injury.

Received for publication April 26, 2000
and in revised form July 6, 2000
Accepted for publication July 24, 2000

Immunoglobulin A nephropathy (IgAN), characterized by mesangial deposition of IgA of IgA_1 subclass, is now recognized as the most common type of glomerulonephritis worldwide. Immunoregulatory abnormalities involving IgA_1 synthesis in IgAN have been documented, and these include overproduction of IgA_1 by B lymphocytes in vitro [1, 2], a raised serum level of IgA, IgA-containing immune complexes [3, 4], and IgA of an anionic nature [5, 6]. However, the pathogenesis of IgAN remains obscure, since none of these abnormalities can adequately explain how IgA_1 is deposited in the glomerular mesangium and how these IgA_1 deposits cause glomerular injury.

The majority of serum proteins, including IgA, carry carbohydrate components. IgA_1 is heavily glycosylated and consists of 8% carbohydrate. The five O-linked glycosylation sites at the hinge region between the CH_1 and CH_2 domains of the α chain on IgA_1 are distinctive and unusual features that are not found in most of the serum proteins, including IgA_2 [7]. There are at least five different O-glycan structures at the hinge region of the $\alpha 1$ chain in IgA_1, with 64% of them being monosialylated or disialylated [8]. Because of different site occupancy and occurrence of a mixture of the five O-glycan types, an array of IgA_1 consisting of a mixture of O-glycoforms is found. This phenomenon is called microheterogeneity of carbohydrates, which means that IgA_1 could have a variety of carbohydrate structures despite the same amino acid sequence [9]. Microheterogeneity exists in healthy individuals, and factors controlling the occupation of potential O-glycosylation sites, their galactosylation and sialylation, remain unknown. The O-glycosylation at the hinge region of IgA_1 has recently attracted much attention with regard to the hepatic clearance of IgA_1 by asialoglycoprotein receptor (ASGPR). Andre, Le Pogamp, and Chevet first reported reduced binding of IgA_1 to jacalin due to an unusual glycosylation of IgA_1 in IgAN [10]. Other studies using carbohydrate-specific lectin binding assays and matrix-assisted laser desorption/ionizing mass spectrometry analysis (MALDI-MS) suggested that the abnormality is a reduction in the ter-

minal galactosylation of O-linked glycans of the IgA_1 molecule [11, 12]. It was suggested that reduced terminal galactosylation of O-linked glycans at the hinge region may modify the conformational stability of the IgA_1 molecules and, consequently, the interaction with the receptors, extracellular matrix, and the surface proteins that may favor a reduced catabolism and clearance [13]. However, few studies have examined the sialylation of the O-linked glycans of IgA_1 in IgAN. Changes in the highly negatively-charged sialic acid could exert a profound effect on the binding of IgA_1 molecules to mesangial cells. Contradictory results have been reported in the sialic acid content of IgA_1. Dueymes et al showed that the sialic acid content was increased in IgAN [14]. Hiki et al reported that the glycans at the hinge region of IgA_1 from IgAN patients contained less N-acetylneuraminic acid (NeuNAc) residues attached to GalNAc [15]. Tomana et al also showed that there was an increased binding of GalNAc-specific lectins after the removal of NeuNAc [16]. Our previous study reported a reduction of terminal glycosylation in monomeric IgA_1 ($mIgA_1$), but not in polymeric IgA_1 ($pIgA_1$) isolated from patients with IgAN [17]. Instead, an oversialylation of IgA_1 was demonstrated in $pIgA_1$. We speculate that oversialylation will alter the overall charge of IgA_1 molecules, thus favoring their deposition onto the mesangial cell surface. To test this hypothesis, we purified $pIgA_1$ fractions with different charges and examined their binding characteristics to cultured human mesangial cells (HMCs). The effect of altering the cell surface charge on IgA_1 binding to HMCs was investigated.

METHODS

Materials

RPMI 1640 and fetal bovine serum were obtained from Life Technologies (Rockville, MD, USA). Fluorescein or peroxidase-conjugated rabbit anti-human IgA and FITC-isotypic control antibodies used for flow cytometry were obtained from Dako (Kyoto, Japan). Jacalin agarose was obtained from Pierce (Rockford, IL, USA). Superose Fast Protein Liquid Chromatography (FPLC) column and Mono Q column were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Consumable for electrophoresis was obtained from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

Patients and controls

Thirty Chinese patients (16 males and 14 females) with a clinical and renal immunopathologic diagnosis of primary IgAN were studied. They had been symptomatic for 12 months or more, with proteinuria ranging from 0.4 to 2.9 g/day, and were between 19 and 45 years of age (mean ± SD, 28.2 ± 6.3 years). IgAN was diagnosed 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 mesangial electron-dense deposits in ultrastructural examination. Systemic lupus erythematosus, Henoch-Schönlein purpura, and hepatic disease were excluded by detailed clinical history, examination, and negative laboratory testing 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 for isolation of IgA_1 by a jacalin-agarose affinity column. Serum IgA levels were determined by nephelometry.

Thirty healthy subjects (14 males and 16 females), comparable in age and race with no microscopic hematuria or proteinuria, were used as controls. Serum was similarly collected from these individuals.

Culture of human mesangial cell culture

Isolation and characterization of HMCs were performed as previously described [18]. Glomeruli were prepared from the cortex of human cadaveric kidney judged to be unsuitable for transplantation or from the intact pole of kidneys removed for circumscribed tumor. Histologic 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.

Purification and characterization of polymeric and monomeric IgA_1 by jacalin affinity chromatography and fast protein liquid chromatography

IgA_1 was purified using a jacalin-agarose affinity column, and IgA_1 was fractionated at room temperature by the fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) as described previously [19]. $mIgA_1$ and $pIgA_1$ 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). Two pooled fractions from fractions 20 to 33 ($pIgA_1$ fractions) and from fractions 34 to 50 ($mIgA_1$ fractions) were prepared for further analysis. $pIgA_1$ was high molecular mass IgA with

molecular weights between 250 and 1000 kD. $mIgA_1$ was low molecular mass IgA with molecular weights between 100 and 250 kD. The content of IgG in the fraction was measured by an anti-IgG ELISA. The pooled fractions were dialyzed and concentrated to 2 mL with Centriprep (Amicon, Beverly, MA, USA) and stored at −70°C until use. The purity of IgA_1 fractions was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ELISA [19]. The isoelectric point (pI) was determined by isoelectric focusing (IEF) as described previously [20].

Separation of polymeric and monomeric IgA_1 fractions with different charges of by anion exchange chromatography

One milliliter $pIgA_1$ or $mIgA_1$ pooled fraction was first dialyzed with binding buffer (20 mmol/L Tris-HCl, pH 8.0) before applying onto a Mono Q HR 5/5 column. The column was first equilibrated with 20 mL of binding buffer, and the bound IgA was eluted with 12.5 mL of elution buffer (a linear salt gradient from 0 to 1 mol/L NaCl in 20 mmol/L Tris-HCl, pH 8.0). Subfractions of 250 μL were collected throughout the elution. Fifty microliters of the eluted subfractions were stored for cell lysate binding ELISA. The rest of subfractions eluted with 0.2 to 0.35 mol/L NaCl in 20 mmol/L Tris-HCl was pooled as polymeric or $mIgA_1$ bearing less anionic charges (pIgA P1 or mIgA P1). The subfractions eluted with 0.35 mol/L to 0.55 mol/L NaCl in 20 mmol/L Tris-HCl were pooled as polymeric or $mIgA_1$ bearing more anionic charges (pIgA P2 or mIgA P2). The pooled subfractions were dialyzed and concentrated to 500 μL. IgA concentration in the pooled subfractions was assayed by a sandwich ELISA as described previously [19]. The pooled subfractions were then stored at −70°C until used.

The purified subfractions from patients or controls used for subsequent binding studies to HMCs were appropriately diluted to achieve comparable levels of IgA_1. This was performed in order to avoid any increased binding of IgA_1 due to elevated IgA_1 concentrations in purified IgA_1 subfractions.

Binding of IgA_1 subfractions to human mesangial cells

Human mesangial cells were grown to log phase and were harvested by using 0.05% trypsin/0.02% ethylenediaminetetraacetic acid (EDTA) for five minutes at room temperature. The cells were adjusted to 5×10^6 per mL, and 200 μL of cell suspension were used in binding assays. All staining was done at 4°C with staining buffer [phosphate-buffered saline (PBS) with 1% fetal bovine serum and 0.1% sodium azide]. The cells were incubated with 100 μL various subfractions of IgA_1 for 30 minutes. After incubation, the cells were washed with staining buffer and then further incubated with 100 μL of fluorescein-conjugated $F(ab')_2$ fragment of rabbit anti-human IgA. Background control staining was achieved by reaction with preimmune FITC-$F(ab')_2$. The stained cells were analyzed using a Coulter EPICS XL analyzer (Coulter Electronic, Miami, FL, USA). A minimum of 5000 fixed cells for each sample was analyzed. Fluorescence intensity was evaluated by comparing the mean fluorescence channels. The result was expressed as mean fluorescence intensity (MFI).

Binding assay of IgA_1 subfractions to human mesangial cell lysate

Human mesangial cells were grown to log phase. The cells were then lyzed with cold lysis buffer containing 1% Triton X-100, 0.1% SDS, 5 mg/mL sodium deoxycholate, 0.5 μg/mL leupeptin, 1 mmol/L EDTA, 1 μg/mL pepstatin, and 0.2 mmol/L phenylmethylsulfonyl fluoride (PMSF). Immulon 2 microtiter plates (Dynatech, Marnes la Coquette, France) were coated with 100 μL mesangial cell lysate in 0.1 mol/L carbonate-bicarbonate buffer at a final concentration of 10 μg/mL, pH 9.6. The plates were incubated overnight at 4°C and were then washed three times with PBS-Tween. After blocking the nonspecific binding sites with 3% bovine serum albumin in PBS, the plates were again washed three times with PBS-Tween. One hundred microliters of diluted fractions were introduced. After incubation at 37°C for two hours, the plates were washed three times with PBS-Tween before adding 100 μL of rabbit anti-human IgA with horseradish peroxidase (HRP) conjugate (1:10000). After incubation, the plates were washed three times with PBS-Tween, and 100 μL freshly prepared substrate solution containing 0.034% (wt/vol) O-phenylenediamine powder in 10 mL citrate/phosphate buffer with urea hydrogen peroxide were added. The plates were incubated at room temperature for a further 10 minutes before the reaction was stopped with 100 μL 2 mol/L sulfuric acid. Finally, the reaction was terminated by addition of 50 μL per well 1 mol/L sulfuric acid. The absorbances were measured at 490 nm using a Microplate Reader (Labsystem, Helsinki, Finland).

Treatment of human mesangial cells with poly-L-lysine or heparin

Human mesangial cells were grown to log phase and harvested by using 0.05% trypsin. The cells were allowed to recover overnight in suspension with gentle shaking. The trypsinized-recovered cells were adjusted to 5×10^6 per mL, and 100 μL of cell suspension were used for the experiments. The cells were then incubated with poly-L-lysine or heparin at different doses for five minutes at 4°C. After incubation, the cells were washed with PBS, and 10 μg of different IgA_1 subfractions were added before incubating for further 30 minutes. After incubation, the amount of IgA_1 subfractions bound by the mes-

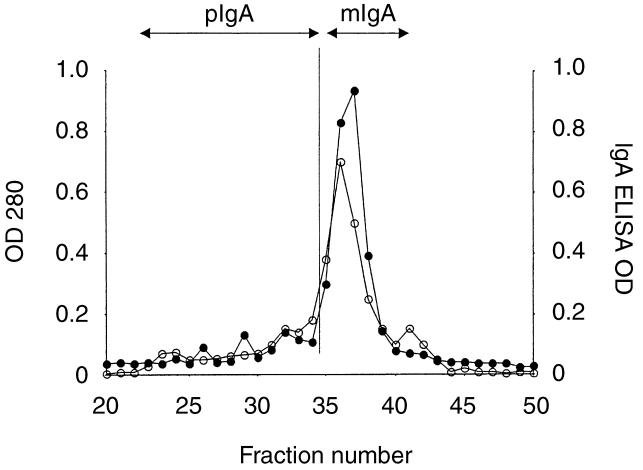

Fig. 1. Separation of jacalin-purified IgA_1 by size exclusion chromatography with fast-protein liquid chromatography (FPLC). The protein concentration eluted from the column was express as OD 280 unit (○). One-milliliter fractions were collected and analyzed by ELISA for the content of IgA (●).

angial cells was determined by flow cytometry as described previously in this article.

Statistics

The results are expressed as mean ± SD. The Student's unpaired *t* test was used to compare values from both patient and control groups.

RESULTS

Purification of monomeric and polymeric IgA_1

A typical chromatogram of IgA_1 separated by FPLC is shown in Figure 1. Pooled fractions 20 to 33 were $pIgA_1$ with molecular weights between 250 and 1000 kD. Pooled fractions 33 to 40 were $mIgA_1$ with molecular weights between 120 and 250 kD. No IgG or IgM was detected in the $mIgA_1$ fractions, and IgG represented 0.1% of total protein in $pIgA_1$ fractions, as measured by ELISA. The serum IgA level in patients with IgAN (3.02 ± 1.54 g/L) was significantly higher than that of healthy controls (1.84 ± 0.82 g/L, $P < 0.001$). Studies of IgA_1 in FPLC fractions by IgA ELISA showed that $mIgA_1$ amounted to 92% of total IgA_1 from either healthy controls or patients (data not shown). Similarly, $pIgA_1$ represented 8% of total IgA_1 from either group of subjects. The ratio of monomeric to $pIgA_1$ did not differ between patients and controls. Figure 2 depicts results of SDS-PAGE and IEF analysis of the pooled fractions of polymeric and $mIgA_1$. $mIgA_1$ fractions consisted of mainly $mIgA_1$ with a size of 160 kD, and there was no $pIgA_1$ copurified in these fractions. $pIgA_1$ fractions contained mainly dimeric IgA1 with small $mIgA_1$ contamination because of the incomplete separation of the dimeric IgA_1 from $mIgA_1$. The pI of pIgA ranged from 5.8 to 6.9, whereas that of mIgA was from 5.0 to 6.9. There was no significant difference in the range of pI distribution between $mIgA_1$ and $pIgA_1$ isolated from patients and control (data not shown).

Separation of polymeric and monomeric IgA with different charges by anion exchange chromatography

Figure 3 illustrates the results of separating $pIgA_1$ or $mIgA_1$ by ion exchange chromatography (IEC) using the Mono Q column. The bound IgA_1 was eluted using gradient of increasing ionic strength with NaCl. $pIgA_1$ was eluted between 0.32 mol/L NaCl and 0.48 mol/L NaCl. The major peak of the eluate was at 0.36 mol/L NaCl with a minor peak at 0.42 mol/L NaCl. $mIgA_1$ was eluted between 0.27 mol/L NaCl and 0.55 mol/L NaCl. The major peak of eluate was at 0.32 mol/L NaCl with several minor peaks at 0.48 mol/L, 0.51 mol/L, and 0.54 mol/L NaCl. There was no difference in the pattern of chromatograms between IgAN patients and controls for $pIgA_1$ or $mIgA_1$ separated by the Mono Q column (data not shown). Figure 4 shows the levels of IgA_1 in different subfractions eluted from the Mono Q column. The IgA_1 levels in subfractions pIgA P1, pIgA P2, and mIgA P1 were significantly higher in IgAN patients than controls ($P < 0.001$). The amount of pIgA P2 (that were more anionic in nature) represented 2.0 to 2.5% of total purified IgA_1 from IgAN patients or healthy controls.

Binding of IgA_1 subfractions to human mesangial cells and mesangial cell lysate

Figure 5 depicts the binding of IgA_1 subfractions separated by the Mono Q column to HMC. There was no significant difference between binding to HMC in $mIgA_1$ (including mIgA P1 and mIgA P2) from patients or controls. In contrast, $pIgA_1$ with more anionic surface charge (pIgA P2) exhibited a higher binding to HMCs among the IgA_1 subfractions (MFI = 2.32 ± 0.21 vs. MFI of pIgA P1 = 1.32 ± 0.13, $P < 0.001$). Moreover, the binding of pIgA P1 and pIgA P2 subfractions from IgAN patients to HMC was significantly higher than the corresponding values from controls ($P < 0.001$).

Figure 6 illustrates a typical binding pattern of IgA_1 subfractions from a patient with IgAN to HMC lysate. The binding of $pIgA_1$ subfractions to mesangial cell lysate was much higher than that of $mIgA_1$. The more anionic subfractions of $pIgA_1$ exhibited the highest binding to HMC lysate. A similar pattern of binding was also observed in subfractions isolated from normal controls (data not shown).

Binding of IgA_1 subfractions to human mesangial cells after preincubation with polyions

Figure 7 illustrates the effect of preincubating HMC with poly-L-lysine or heparin on the binding of IgA_1

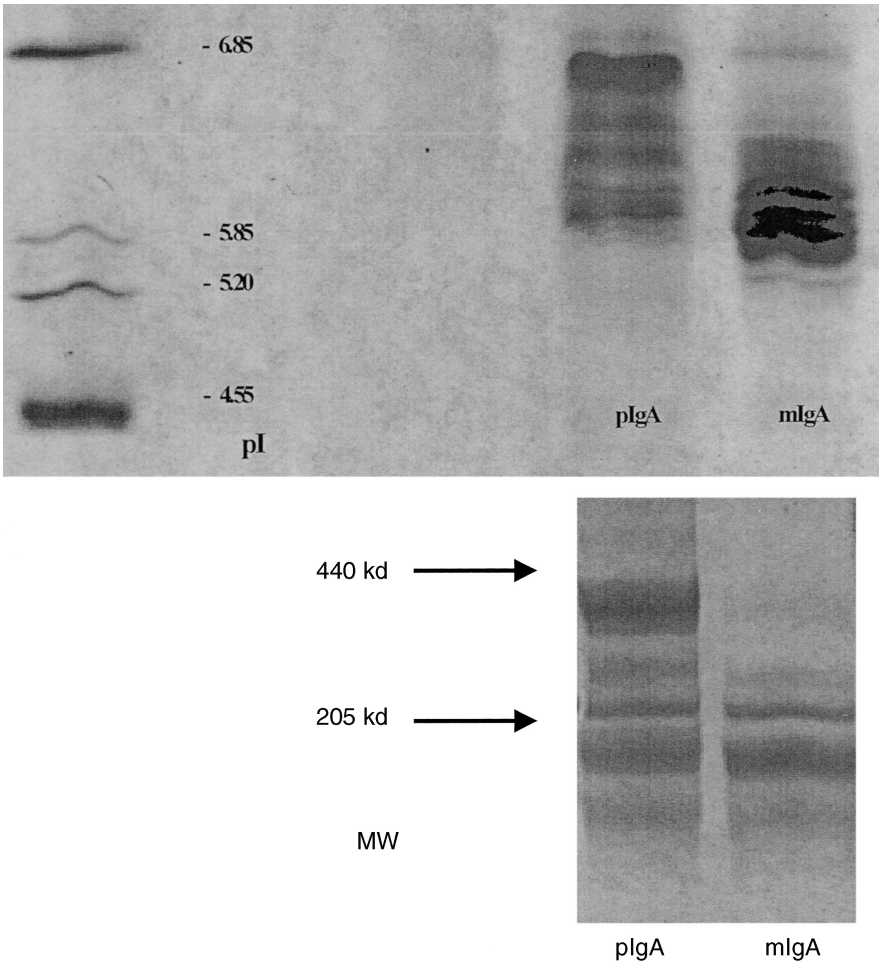

Fig. 2. Isoelectric focusing (IEF; upper) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; lower) analysis of purified pooled $pIgA_1$ and $mIgA_1$ fractions.

subfractions. Preincubation with poly-L-lysine (0.625 to 20 μg/mL) significantly increased the binding of pIgA, or $mIgA_1$ to HMCs in a dose-dependent manner. Similarly, preincubation with heparin (0.625 to 20 U/mL) significantly decreased the binding of IgA_1 to HMCs in a dose-dependent manner. Figure 8 summarizes the effect of preincubating the HMCs with polyanion or polycation on the binding of different IgA_1 subfractions from both groups of subjects. Preincubation with 5 μg/mL poly-L-lysine increased the binding of $pIgA_1$ or $mIgA_1$ to HMCs in both patients and controls ($P < 0.001$). Preincubated HMCs with heparin (5 U/mL) significantly reduced the binding of $pIgA_1$ or $mIgA_1$ to HMCs from patients and control ($P < 0.001$). In all experimental conditions, the binding of IgA_1 from patients with IgAN was significantly higher than that of controls ($P < 0.001$).

DISCUSSION

Serum IgA consists of an assortment of glycoforms with different O-glycosylation pattern. Structural changes at the hinge region of the IgA_1 molecule have been speculated to bear pathological implication in IgAN [21, 22]. Deficiency of terminal galactose in O-glycans at the hinge region may have a profound effect on the recognition of IgA_1 by ASGPR and, hence, its catabolism. Oligosaccharides at the hinge region carry negatively charged sialic acid that is 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 with and recognition by other receptors such as ASGPR and FcαR1 [13, 22]. Earlier studies revealed a reduced terminal galactosylation of serum IgA_1 in IgAN [11, 22]. However, these findings failed to provide a pathogenetic mechanism in IgAN, as over 85% of serum IgA_1 are monomers, whereas mesangial IgA_1 deposits are mainly polymeric and anionic in nature. We have shown that pIgA from patients with IgAN exhibited an increased binding to HMCs [18]. Recently, we found that the anionic nature of pIgA from patients with IgAN is due, at least partly, to oversialylation of the O-glycans at the hinge region [17]. Oversialylation of pIgA has two potential implications on the pathogenesis of IgAN. First, the

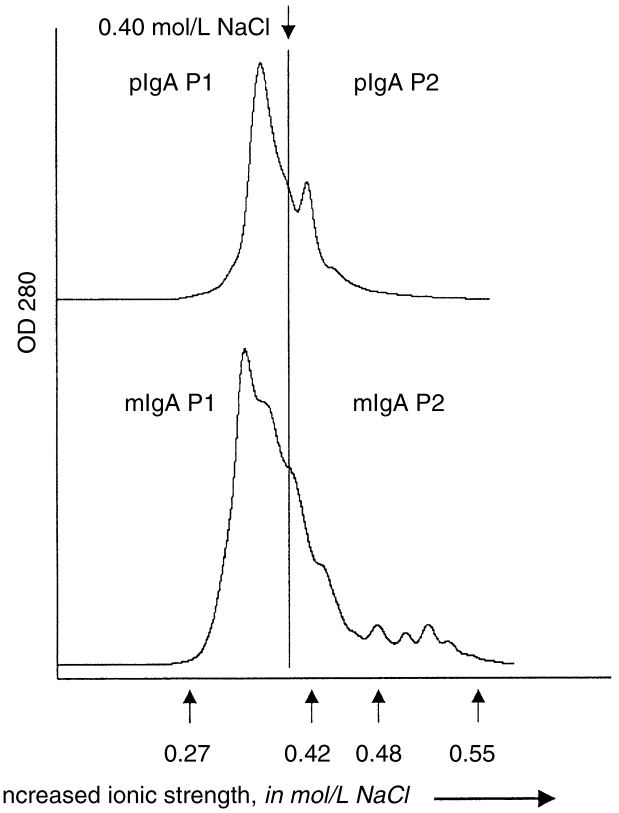

Fig. 3. Separation of polymeric $pIgA_1$ ($pIgA_1$; upper chromatogram) and monomeric IgA_1 ($mIgA_1$; lower chromatogram) by anion exchange chromatography with a Mono Q column. The fractions eluted with less than 0.4 mol/L NaCl were pooled as pIgA P1 or mIgA P1, and those fractions eluted with more than 0.4 mol/L NaCl were pooled as pIgA P2 and mIgA P2.

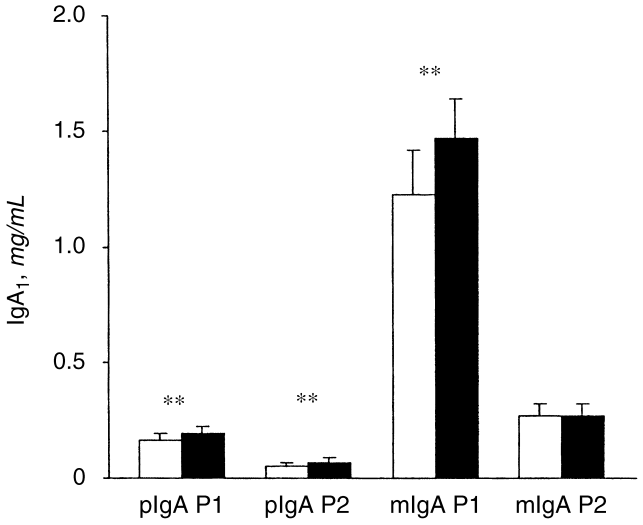

Fig. 4. IgA_1 content of purified pooled, Mono Q-separated IgA_1 subfractions. The IgA_1 level for subfractions pIgA P1, pIgA P2, and mIgA P1 were significantly higher in patients (■) than controls (□); $^{**}P < 0.001$.

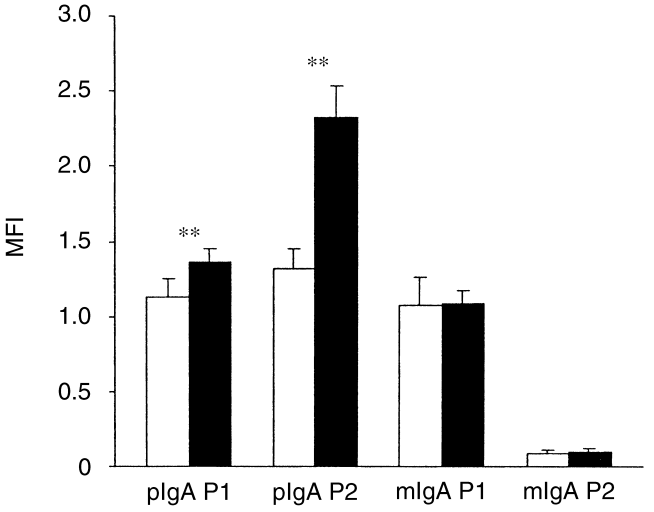

Fig. 5. Binding of pooled Mono Q-separated IgA_1 subfractions to cultured human mesangial cells (HMCs). Results were expressed as mean fluorescence intensity (MFI). pIgA1 subfractions (pIgA P1 and pIgA P2) bound more than $mIgA_1$ subfractions to HMC. The binding for subfractions pIgA P1 and pIgA P2 were significantly higher in patients (■) than controls (□); $^{**}P < 0.001$.

masking effect of sialic acid may hinder the binding of pIgA to ASGPR that is specific for terminal galactose residues. Hence, these large macromolecular IgA not effectively removed from the circulation by the ASGPR in the reticulo-endothelial system will be filtered and deposited in kidneys. In fact, it has been reported that increased sialylation causes elongation of glycans in IgM and reduces the clearance by the ASGPR [23]. Second, oversialylation renders pIgA from patients with IgAN more anionic, and this enhances the mesangial deposition of IgA immune complexes. These assumptions prompt us to examine whether the serum IgA_1s from patients with IgAN are bearing different levels of anionic charges and, if so, whether they bind differently to HMC. Herein, we have developed a novel scheme of purification to separate anionic-charged $pIgA_1$ from sera of human subjects. We found that the level of anionic-charged $pIgA_1$ (that is, $pIgA_1$ P2) was increased in patients with IgAN, yet the level of anionic-charged $mIgA_1$ (that is, $mIgA_1$ P2) remained unaltered. There was no change in the distribution of isoelectric point (pI) of polymeric IgA isolated by FPLC from patients or controls. This may be due to the fact that the increased amount of anionic-charged $pIgA_1$ (that is, $pIgA_1$ P2) was small as compared with the total polymeric IgA level, and the difference could not be reflected by the IEF analysis. Increased proportion of anionic IgA_1 and reduced cationic portion had been reported in serum of patients with IgAN [20, 24]. In this study, we found that the increased anionic IgA_1 was predominantly polymeric, and these anionic $pIgA_1$s had the highest affinity to mesangial cells despite

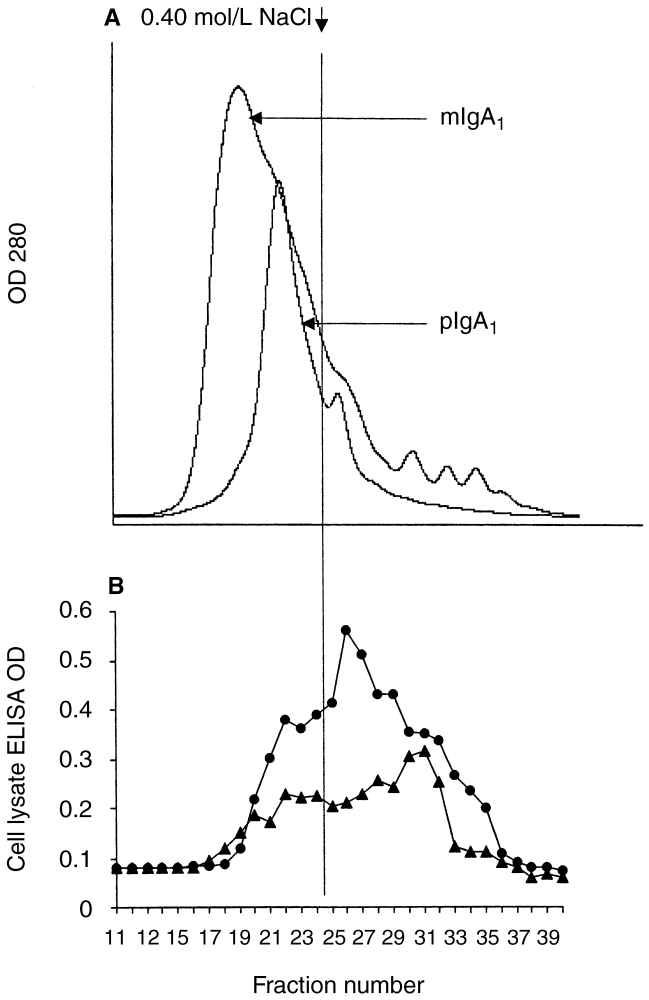

Fig. 6. Binding of $pIgA_1$ (●) and $mIgA_1$ (▲) from patients with IgAN to cell lysates prepared from cultured HMCs. Upper chromatogram (*A*) shows the elution pattern from Mono Q column. Lower figure (*B*) represents the result of ELISA assay.

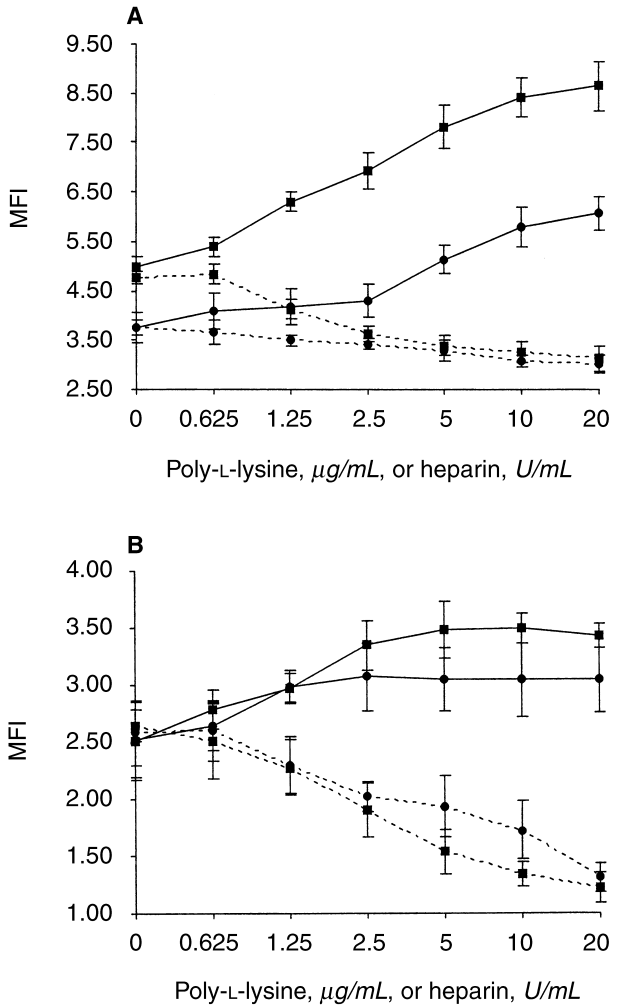

Fig. 7. Binding of $pIgA_1$ (*A*) and $mIgA_1$ (*B*) from patients with IgAN (■) or control (●) to cultured HMCs after preincubation with increasing dose of poly-L-lysine (μg/mL; solid line) or heparin (U/mL; dotted line).

that they only represented a very small percentage of total purified IgA_1. This is consistent with the previous finding of mesangial IgA_1 deposits in renal biopsies by Monterio et al [25]. They found that 64% of the eluted IgA from the biopsy in IgAN was polymeric and anionic in nature. Recently, O-glycans in IgA_1 from patients with IgAN had been analyzed by fluorophore-assisted carbohydrate electrophoresis. Allen et al demonstrated a significant increase in the percentage of O-glycans with single N-acetyl galactosamine (GalNAc) units, but there was no difference in the percentage of sialylated glycans [26]. Their observation does not necessarily contradict our finding for which $pIgA_1$ are more anionic. It is possible that both undergalactosylated and oversialylated IgA_1 molecules may be presented in the total bulk of IgA_1 purified from the same individual because of the variable occupancy of the five potential O-glycosylation sites at the hinge region. It should be pointed out that whether an individual IgA_1 molecule is sialylated cannot be simply predicted from the percentage of different glycan components. The exact site occupancy of O-glycan is an important factor in determining the physiochemical properties of the IgA_1 molecule. Besides electrostatic interaction, it has been proposed that the binding of human IgA_1 to HMCs may be mediated through specific IgA receptors such as FcαR (CD89), ASGPR, and polymeric immunoglobulin receptors [27–29]. However, the possibility of these receptors being actively involved in mediating IgA deposition is not supported by latest studies documenting their absence in mesangial cells [30–32]. Lately, novel receptor for IgA had been reported in mesangial cells, which is distinct from the myeloid FcαR CD89 [33]. This novel receptor binds pIgA with high affinity. It is possible that both receptor-mediated binding and charge-dependent binding operate together to

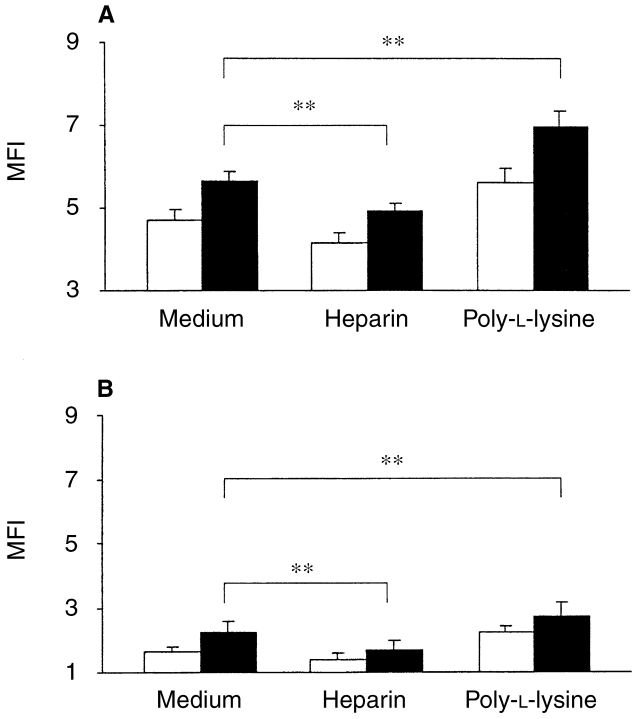

Fig. 8. Binding of pooled $pIgA_1$ (*A*) or $mIgA_1$ (*B*) in patients (■) or control (□) to cultured HMCs after preincubating the cells with 5 μg/mL poly-L-lysine or 5 U/mL heparin. Results were expressed as mean fluorescence intensity (MFI). Poly-L-lysine significantly increased the binding of $pIgA_1$ or $mIgA_1$ in patients with IgAN, whereas heparin significantly decreased the binding ($P < 0.001$ in both cases). In all experimental conditions, the binding of IgA_1 from patients with IgAN was significantly higher than that of controls ($P < 0.001$).

mediate pIgA binding to mesangial cells. Further investigation is warranted.

To elucidate further the role of electrostatic charge on mesangial deposition of IgA, we deliberately altered the surface charge by preincubating cultured HMCs with polyanion or polycation. Preincubation with polycations increased the binding of IgA_1 to HMCs, whereas preincubation with polyanion hindered the binding. HMCs secrete abundant amount of polyanionic matrix components that are thought to play a role in the molecular sieving by the mesangium. These polyanionic moieties include N-sulfated glycosaminoglycan, heparan sulfate, laminin, and fibronectin [34, 35]. A loss of the anionic sites on HMCs may result in increased glomerular permeability to proteins or other macromolecules. Polycation can neutralize the anionic charge, and polyanion can compete with anionic IgA for the same cationic binding site on mesangial cells. Polycations had been shown to interact with the polyanion and neutralize the zeta potential (electric potential at the plane of hydrodynamic shear) generated by the negative charge [36]. Neutralization of the negatively charged sites of cultured rat mesangial cells by poly-L-lysine stimulated prostaglandin E_2 synthesis and increased cytosolic-free calcium level [37]. It was suggested that neutralization of anionic sites on mesangial cells might affect the inflammatory response. With chronic inflammatory process, polyanionic sites on the mesangial cells could be altered by enzymatic attack, and degradation of anionic moieties occurred because of cationic enzymes released from activated platelets or leukocytes [38]. These enzymes include acid or neutral proteinase, endoglycosidase, and heparinase. Moreover, endogenous cationic proteins such as platelet factor 4 that released from activated platelets or leukocyte are capable of neutralizing glomerular polyanionic sites [39]. Neutralization or damage of glomerular polyanionic sites may compromise the electrostatic barrier to circulating IgA_1, thus leading to glomerular deposition. The therapeutic potential of polyanions for treating of IgAN awaits further exploration. Heparin is known to have anti-inflammatory properties, including complement inactivation and suppression of leukocyte functions [40]. Heparin inhibits migration and mitogenesis of cultured mesangial cells [41]. Recently, the anti-apoptotic potential of heparin was demonstrated in a variety of kidney cells, including HMCs [42]. Based on our present findings, further investigation should be taken to determine whether heparin could ameliorate the progression of IgAN by abrogating further mesangial IgA_1 deposition.

In conclusion, increased levels of anionic-charged $pIgA_1$ were found in IgAN. Although anionic-charged $pIgA_1$ only represents a small fraction of total serum IgA, in vitro studies show that its binding to mesangial cells is the highest among all fractions of serum IgA_1. Our findings suggest that the anionic property of IgA plays a crucial role in mesangial IgA_1 deposition in patients with IgAN.

ACKNOWLEDGMENTS

The study was supported by the Research Grant Committee (Hong Kong) grant number 7064/99 M and the Croucher Foundation.

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