

Integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ as a receptor for IgA1 in human glomerular mesangial cells in IgA nephropathy

Yoshikatsu Kaneko, Tadashi Otsuka, Yohei Tsuchida, Fumitake Gejyo and Ichiei Narita

Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata 9518510, Japan

Correspondence to: Y. Kaneko, Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Niigata 9518510, Japan; E-mail: kanekoy@med.niigata-u.ac.jp

Received 14 May 2011, accepted 26 December 2011

Abstract

IgA nephropathy (IgAN) is characterized by mesangial deposition of IgA1 and galactose-deficient IgA1 is expected to play a pathogenic role. However, the identity of the receptor for IgA1 is still controversial. Hence, the aim of this study was to explore the receptor for galactose-deficient IgA1. Human monoclonal IgA1 was treated with exoglycosidase and FITC-conjugated control, asialo- and agalactosyl-IgA1 was used as a probe to detect the receptor in cultured human mesangial cells. Tumor necrosis factor- α or transforming growth factor- $\beta 1$ treatment accelerated IgA1-binding on mesangial cells, and these effects were diminished by the addition of dexamethasone, whereas these changes were not dependent on galactose-deficiency of IgA1. According to comprehensive gene expression analysis, we focused on integrin $\beta 1$. Pre-treatment by Mn^{2+} , which activates integrin by changing its structure, enhanced the binding of IgA1 in cultured mesangial cells. Furthermore, pre-incubation with collagens specifically enhanced binding of IgA1 in the cultured human mesangial cells without activation by Mn^{2+} . Collagen type IV distributed in the mesangial region of the glomeruli as well as Bowman's capsule and tubular basal membrane in IgAN patients, and the IgA1 with collagen type IV induced proliferative signals on mesangial cells by phosphorylating extracellular signal-regulated kinase more effectively than the IgA1 alone. Immunoprecipitation assay revealed the binding of IgA1 and integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimer and down-regulation of integrin $\alpha 1$, $\alpha 2$ and $\beta 1$ expression in human mesangial cells induced by each specific small interfering RNA diminished the ability to bind IgA1 probe. Integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ would be a candidate receptor for IgA1.

Keywords: collagen, dexamethasone, O-glycan, transforming growth factor, tumor necrosis factor

Introduction

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis and is one of the main causes of end-stage renal disease throughout the world (1, 2). Typical features of IgAN consist of glomerular mesangial IgA deposition associated with focal or diffuse expansion of mesangial regions with cells and matrix and persistent hematuria and mild proteinuria over many years; furthermore, 20–30% of patients develop progressive renal insufficiency ≥ 20 years after initial discovery of the disease (1, 3). Several mechanisms of IgA deposition on mesangial cells have been proposed, including the formation of IgA-immunogen complexes derived from environmental or infectious antigens (4), or as the result of IgA–IgG complex formation (5, 6), IgA1-soluble Fc α receptor (CD89) complex (7), or IgA self-aggregation (8, 9). Corticosteroids and renin-angiotensin

system-blocking agents are used for the treatment of IgAN (10); however, no specific and fundamental therapy has yet been proposed since the precise mechanism of the development of IgAN is still to be elucidated (11).

Several recent studies reported the importance of aberrant O-linked glycosylation in the IgA1 hinge region for the pathogenesis of IgAN (12). Of the two subtypes of IgA, IgA1 and IgA2, the deposits in the glomerular mesangial region are considered to be composed of polymeric IgA1 (13, 14). IgA1 is characterized and distinguished from IgA2 by its proline-rich hinge region, which consists of 23 amino acids and 9 potential O-glycation sites possessing 4 or 6 O-glycans (15–17). These O-glycans starting from serine or threonine residues (17, 18) are considered to play a protective role against IgA1 self-aggregation and adhesion to

extracellular matrix proteins (19). Recent studies demonstrated that reduced galactosylation of *O*-glycans in the IgA1 hinge region was observed both in the serum and in the glomeruli of IgAN patients (20–22). Galactose-deficient IgA1 exhibits enhanced binding capacity to human mesangial cells (23) as the result of accelerated self-aggregation or increased binding capacity to extracellular matrices (19), and thus, galactose-deficient IgA1 is expected to play a pathogenic role in the development of IgAN (24).

On the other hand, the identity of the receptor for IgA1 in mesangial cells is still controversial. Three known IgA receptors, CD89, polymeric Ig receptor (pIgR) and asialoglycoprotein receptor (ASGP-R), have been proposed as candidates for IgA1 (25). However, pIgR is localized primarily in human secretory epithelia (26), and its expression has not been confirmed in human mesangial cells (27). ASGP-R is a C-type lectin and binds desialylated glycoproteins. Some groups have reported on the existence of ASGP-R in human mesangial cells (28, 29), but others could not identify it (4, 27). In human mesangial cells, the expression of CD89, which is mainly expressed in blood cells and binds to the Fc region of monomeric and polymeric IgA, is also still controversial. Several groups detected CD89 in human mesangial cells (30, 31), while others failed to detect its expression (4, 27, 32). Fc α/μ receptor was proposed as a candidate IgA receptor (33), but its pathogenic role is still to be determined. Transferrin receptor was originally identified from the lysate of monocytoïd cell line as a monomeric IgA1 receptor (34) and then polymeric IgA with galactose deficiency was found to interact with transferrin receptor more efficiently (35). Polymeric IgA1 was reported to induce up-regulation of transferrin receptor expression, resulting in activation of human mesangial cells (36). Moura *et al.* reported that IgA–transferrin receptor interaction in cultured human mesangial cells was inhibited by monoclonal anti-transferrin receptor antibody or transferrin itself by ~70% (34, 36) or with soluble transferrin receptor by ~50% (35, 36). These data suggest that human mesangial cells could still possess alternative galactose-deficient IgA1 receptors besides transferrin receptor. Hence, the aim of the present study is to identify the receptor for galactose-deficient IgA1 in mesangial cells using IgA1 probe with galactose-deficient *O*-glycans in its hinge region.

Methods

Study design

The protocol of the present study adhered to the Declaration of Helsinki and was approved by the ethics committee of our institute. Written informed consent for using kidney biopsy specimens was obtained from all the participants.

Cells and reagents

Normal human mesangial cells were purchased from DS Pharma Biomedical (Osaka, Japan). Mouse monoclonal anti-human integrin $\beta 1$ (clone HUTS-21 for flow cytometry and MAR4 for immunohistochemistry), anti-human integrin $\alpha 1$ (SR84), $\alpha 2$ (12F1), $\alpha 3$ (C3 II.1), $\alpha 5$ (IIA1), αv (13C2),

anti-human transferrin receptor (M-A712) and anti-human CD36 (CB38) antibodies as well as mouse IgG1 and IgG2a isotype controls were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA) and used for immunohistochemistry. For flow cytometry, each mAb with PE conjugation was used. Collagen types I, III, IV and V, laminin and fibronectin extracted from human placenta were purchased from Sigma–Aldrich (St Louis, MO, USA). Mouse monoclonal anti-collagen type I (COL-1), type III (FH-7A), type IV (COL-94), laminin (LAM-89) and fibronectin (IST-4) antibodies (Sigma–Aldrich) were used for immunohistochemistry.

FITC-conjugated galactose-deficient IgA

One milligram of monoclonal human IgA κ derived from myeloma cells (Acris Antibodies, Herford, Germany) was incubated with 0.25 U of neuraminidase with or without 0.1 U of β -galactosidase (Sigma–Aldrich) in 0.05 M acetic acid, pH 5.0, for 72 h at 37°C. *In vitro* desialylation and degalactosylation were confirmed by lectin ELISA using biotinylated *Jacalin* lectin, *Sambucus Nigra* lectin, *Peanut Agglutinin* lectin and *Vicia Villosa* lectin (Vector Laboratories, Burlingame, CA, USA) as described (23). FITC was conjugated using FITC conjugation kit according to the manufacturer's instructions (Sigma–Aldrich) with monoclonal IgA1 or polyclonal IgA1 purified from the sera of IgAN patients by agarose-bound *Jacalin* (Vector laboratories) and used as a probe for receptor in cultured mesangial cells.

Cell culture

Human mesangial cells characterized by α -smooth muscle actin (+), E-selectin (–), CD 14 (–), CD 16 (–), CD 62E (–) and podocalyxin (–) were cultured in CS-C complete medium (DS Pharma Biomedical) in 5% CO₂ incubator at 37°C. For *in vitro* cytokine stimulation, 100 ng ml^{–1} recombinant human tumor necrosis factor (TNF)- α or transforming growth factor (TGF)- $\beta 1$ (R & D Systems, Minneapolis, MN, USA) with or without 200 ng ml^{–1} dexamethasone (Sigma–Aldrich) was added in the culture medium and incubated for 72 h. Comprehensive gene expression analysis of cultured human mesangial cells treated with TNF- α , TGF- $\beta 1$, TNF- α with dexamethasone, TGF- $\beta 1$ with dexamethasone and control cells was conducted using GeneChip Human Genome U133 Plus 2.0 (Affimetrix, Santa Clara, CA, USA). Among the 9094 genes, 1455 genes up-regulated by TNF- α and TGF- $\beta 1$ and down-regulated by addition of dexamethasone and in control condition were selected by template matching method with Pearson correlation coefficient of ≥ 0.6 .

Flow cytometry analysis

To detect the binding of probe IgA1, cultured human mesangial cells were incubated with FITC-conjugated galactose-deficient IgA1 (100 μ g ml^{–1}) for 30 min on ice in PBS with 3% BSA and 0.02% NaN₃ and then analyzed by FACSCalibur (Becton, Dickinson and Company). Manganese chloride, calcium chloride or magnesium chloride was added to the cells at each concentration 30 min before

incubation with FITC-conjugated probe IgA. Collagen, laminin and fibronectin were incubated with FITC-conjugated probe IgA for 30 min on ice in advance and used for flow cytometric analysis.

Immunohistochemistry of kidney biopsy specimen

For the detection of each integrin or extracellular matrix, 4 μm of frozen section of kidney biopsy specimen was fixed in cold methanol. After incubation with 5% goat serum for blocking of non-specific reaction, mouse mAb specific for each integrin or extracellular matrix was incubated on the specimen for 90 min at room temperature, followed by incubation with tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG polyclonal antibody or FITC-conjugated goat anti-mouse IgM polyclonal antibody (Sigma–Aldrich). Deposited IgA was detected by FITC-conjugated goat anti-human IgA polyclonal antibody (Novus Biologicals, Littleton, CO, USA). Diagnosis of IgAN was based on kidney biopsy that revealed the presence of dominant glomerular mesangial deposits of IgA as assessed by immunofluorescence examination. Biopsy specimens of the patients with systemic disease that would cause IgA deposition in glomeruli, such as hepatic glomerulosclerosis, Henoch–Schönlein purpura, carcinoma or rheumatoid arthritis, were excluded.

Western blotting

Cultured human mesangial cells (3×10^5) were incubated with galactose-deficient IgA1 ($100 \mu\text{g ml}^{-1}$) or galactose-deficient IgA1 pre-incubated with type IV collagen ($25 \mu\text{g ml}^{-1}$) at 37°C for the indicated time and were lysed and electrophoresed in polyacrylamide gel in a reduced condition. The cell lysate was then transferred to polyvinylidene difluoride membrane, blocked with blocking reagent, reacted with rabbit polyclonal anti-extracellular signal-regulated kinase (ERK) 1/ERK2 and phosphorylated ERK1/ERK2 antibodies (R & D Systems), followed by incubation with HRP-conjugated sheep anti-rabbit IgG antibody (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). Immune complexes were visualized by Lumivision PRO (Aisin Seiki, Aichi, Japan) after reaction with electrochemiluminescence reagent (GE Healthcare Bio-Sciences). The density of each band was quantified by ImageJ software provided by National Institutes of Health.

Immunoprecipitation

Cultured human mesangial cell lysate (3×10^5) in 10 mM Tris–HCl, pH 7.5, buffer containing 0.15 M NaCl, 0.5 mM EDTA, 1% NP-40, 50 mM Mn^{2+} and 1% protease inhibitor cocktail (Sigma–Aldrich) was incubated with 10 μg of mouse monoclonal anti-integrin $\alpha 1$ (TS2/7; AbD Serotec, Kidlington, UK), $\alpha 2$ (HAS3; R & D systems), $\beta 1$ (4B7R; R & D systems) antibody or control mouse IgG bound with Protein G-beads together with biotinylated galactose-deficient IgA1 ($100 \mu\text{g ml}^{-1}$). Immune complex was precipitated, electrophoresed in polyacrylamide gel in a reduced condition, transferred to polyvinylidene difluoride membrane and reacted with peroxidase-conjugated goat anti-biotin antibody (Sigma–Aldrich) for detection of co-precipitated biotinylated IgA1.

Small interfering RNA

Small interfering RNAs (siRNAs) specific for integrin $\alpha 1$, $\alpha 2$, $\beta 1$ and transferrin receptor as well as control siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). These siRNAs were transfected into cultured human mesangial cells in RPMI 1640 medium containing 10% fetus bovine serum with cationic lipid reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Seventy-two hours after the transfection, the effect of siRNA was confirmed by flow cytometry as decreased cell surface expression of each specific molecule.

Statistical analysis

Statistical differences were calculated with one-way analysis of variance followed by Fisher's protected least significant difference. $P < 0.05$ was considered significant. SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Desialylation and degalactosylation of IgA1 enhanced binding affinity to cultured human mesangial cells

Human monoclonal IgA1 κ generated from myeloma cells was treated with neuraminidase with or without β -galactosidase in order to investigate the role of terminal *N*-acetylneuraminic acid or galactose of *O*-glycans in the hinge region of IgA1 (Fig. 1A). *In vitro* desialylation and degalactosylation were confirmed by lectin ELISA using biotinylated *Jacalin*, *Sambucus Nigra* lectin, *Peanut agglutinin* and *Vicia Villosa* lectin, which specifically recognize *N*-acetylgalactosamine, terminal sialic acid, terminal galactose and exposed *N*-acetylgalactosamine, respectively, and then control IgA, desialylated (deSial) IgA and desialylated and degalactosylated (deSial/deGal) IgA was conjugated with FITC to be used as a probe for the receptor in mesangial cells. *In vitro* desialylation alone produced significant amounts of *O*-glycans with exposed *N*-acetylgalactosamine (Fig. 1B) and also enhanced binding activity of IgA1 in mesangial cells (Fig. 1C and D). No substantial increase in IgA1-binding capacity was observed by additional degalactosylation of IgA1.

TNF- α and TGF- $\beta 1$ up-regulated IgA1 receptors, whereas additional dexamethasone quenched their effect

To reproduce the inflammatory circumstances in the patients with IgAN, the mesangial cells were cultured with or without TNF- α or TGF- $\beta 1$, and dexamethasone was added in the same culture medium to investigate the effect of corticosteroid, which was commonly employed as a therapy for IgAN. FITC-conjugated deSial/deGal IgA1 probe bound $\sim 4.5\%$ of the cultured human mesangial cells. TNF- α or TGF- $\beta 1$ treatment increased the expression of the receptor for deSial/deGal IgA1, and more interestingly, these effects were clearly diminished by addition of dexamethasone in the same culture medium with TNF- α or TGF- $\beta 1$ (Figs. 2A and B). To verify the universality of the regulatory effect of TNF- α

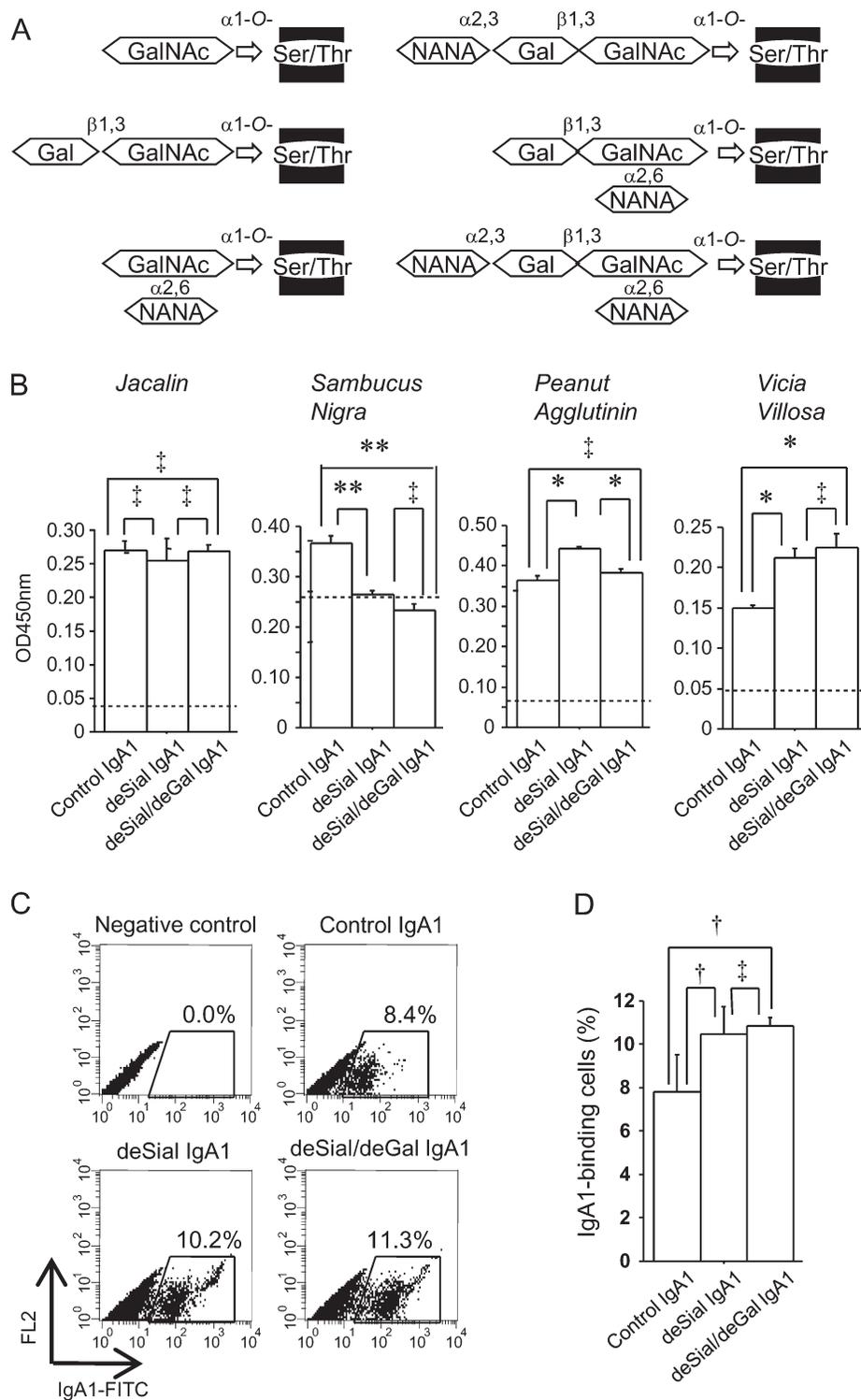


Fig. 1. Desialylated or desialylated and degalactosylated IgA1 showed enhanced binding activity in cultured human mesangial cells. (A) *O*-glycans in the hinge region of human IgA1 consist of six different kinds of structures. NANA, *N*-acetylneuraminic acid; Gal, galactose; GalNAc, *N*-acetylgalactosamine. (B) Control IgA1, desialylated (deSial) IgA1 or desialylated and degalactosylated (deSial/deGal) IgA1 were characterized for terminal sialic acid (*Sambucus Nigra*), galactose (*Peanut agglutinin*) or exposed *N*-acetylgalactosamine content (*Vicia Villosa*) by lectin ELISA. Jacalin, which has affinity toward *N*-acetylgalactosamine, was employed as a quantitative control. Cut-off value is shown as a dotted line determined as the value of absorbance without IgA1 samples. (C) FITC-conjugated deSial or deSial/deGal IgA1 probe bound cultured human mesangial cells more efficiently than control IgA1 probe. (D) Desialylation alone caused statistically significant increase of the number of IgA1-binding cultured mesangial cells; however, additional degalactosylation caused no increased binding capacity ($n = 3$). These are the representative of three independent experiments. † $P < 0.05$, * $P < 0.01$, ** $P < 0.001$, ‡not significant.

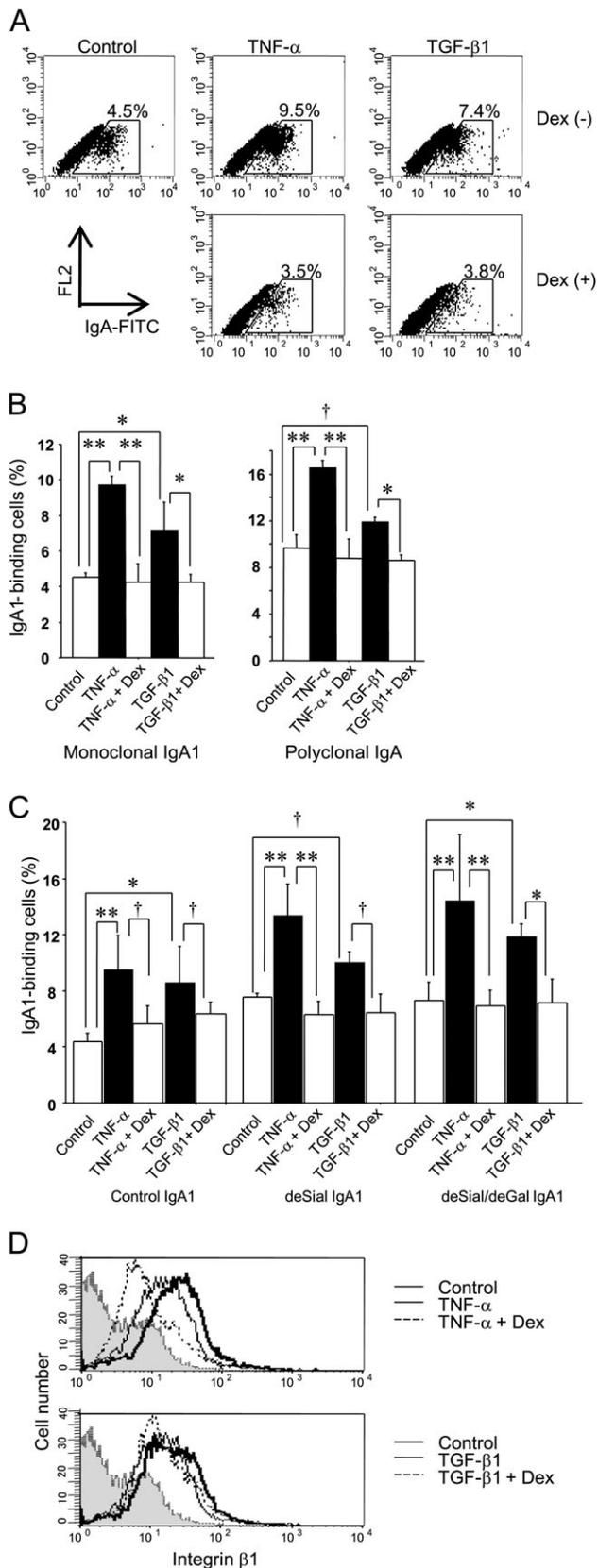


Fig. 2. TNF- α and TGF- $\beta 1$ up-regulated IgA1 receptors, whereas additional dexamethasone diminished their effect. (A) Cultured

or TGF- $\beta 1$ and dexamethasone on the binding of IgA1 in mesangial cells, polyclonal IgA1 was purified from the sera of IgAN patients and also used as the probe after FITC conjugation. This polyclonal IgA1 probe also detected the same regulatory effects of the receptor expression (Fig. 2B). Furthermore, control IgA1, deSial IgA1 and deSial/deGal IgA1 probes were also tested to the mesangial cells with each treatment, and again, although regulatory effect of TNF- α or TGF- $\beta 1$ and dexamethasone were observed in the same way, deSial IgA1 and deSial/deGal IgA1 demonstrated the same level of binding capacity in mesangial cells in each treatment (Fig. 2C).

Integrin $\beta 1$ as a candidate receptor for galactose-deficient IgA1

According to these data, we conjectured that these two different types of cytokines, TNF- α and TGF- $\beta 1$, would commonly induce galactose-deficient IgA1 receptor gene expression and additional dexamethasone would suppress it. Following this hypothesis, we extracted total RNA from the mesangial cells treated with TNF- α , TGF- $\beta 1$, TNF- α with dexamethasone, TGF- $\beta 1$ with dexamethasone or without any treatment as a control to conduct comprehensive gene expression analysis using DNA array (Table 1). Among the candidate genes of the IgA1 receptor, we focused on integrin $\beta 1$, whose mRNA expression was up-regulated by both TNF- α and TGF- $\beta 1$ and down-regulated by additional dexamethasone treatment. Integrin $\beta 1$ forms a heterodimer with an integrin α subunit (37). Among the variety of integrin α subunits capable of forming a heterodimer with $\beta 1$ subunit, $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 8$ and αv were reported to be expressed on human mesangial cells (38–40). We examined the cell surface expression of integrin $\beta 1$ by flow cytometry to confirm whether this molecule was actually regulated by TNF- α , TGF- $\beta 1$ and dexamethasone treatment. Figure 2(D) shows that, in accordance with the results of DNA array analysis, the cell surface expression of integrin $\beta 1$ was induced in the cultured mesangial cells stimulated with TNF- α or TGF- $\beta 1$ and additional dexamethasone down-regulated its expression.

Mn²⁺ activated IgA1 receptor

Integrin α and β subunits have Mg²⁺ and Ca²⁺-binding sites, and an integrin heterodimer changes its structure from 'bent

human mesangial cells were incubated with TNF- α or TGF- $\beta 1$, with or without additional dexamethasone (Dex), for 72 h and were subjected to incubation with FITC-conjugated galactose-deficient IgA1 probe for detection of IgA1 receptor. (B) Both monoclonal IgA1 probe and polyclonal IgA1 probe detected the same regulatory effect of TNF- α , TGF- β and dexamethasone in the IgA1 binding ($n = 3$). (C) Control, deSial and deSial/deGal IgA1 showed the same regulatory effect of TNF- α , TGF- β and dexamethasone in the IgA1 binding; however, additional degalactosylation caused no increased binding capacity ($n = 3$). (D) Expression of integrin $\beta 1$ was enhanced in the cultured human mesangial cells stimulated with TNF- α (upper) or TGF- $\beta 1$ (lower) (bold line) and that was down-regulated by additional dexamethasone (dotted line). Shaded histogram represents isotype antibody control. These are the representative of three independent experiments. † $P < 0.05$, * $P < 0.01$, ** $P < 0.001$, ‡not significant.

Table 1. Gene expression analysis in cultured human mesangial cells regulated by TGF- $\beta 1$, TNF- α and dexamethasone

Gene	Control	TGF- $\beta 1$	TGF- $\beta 1$ + dex	TNF- α	TNF- α + dex
C1q and TNF-related protein 2	122.6	248.8	44.6	444.4	19.3
Cadherin 11, type 2	322.2	962.2	544.9	600.4	291.1
CD274	2174.9	3679.8	1503.9	6263.2	2693.5
Filamin A interacting protein 1-like	2009.6	2923.0	1397.7	2264.0	1107.7
Glutamate receptor interacting protein 1	69.5	153.5	52.8	134.6	11.1
Integrin $\beta 1$	134.1	220.2	58.8	143.9	23.0
IL 1 receptor antagonist	226.5	400.1	116.1	799.2	174.6
LDL receptor-related protein 12	943.2	1062.6	891.6	1173.3	582.7
Myosin X	274.8	570.1	435.6	510.3	319.6
Protocadherin 10	90.5	238.1	137.1	164.4	93.9
Tensin 1	101.1	227.5	181.2	216.7	111.0

Signal strength of several typical genes up-regulated by TGF- $\beta 1$ and TNF- α and down-regulated by dexamethasone treatment were shown in alphabetical order. TNF; tumor necrosis factor, LDL; low-density lipoprotein.

form' to 'extended form' to acquire an activated state in the presence of Mn^{2+} (41). To confirm the contribution of integrins to IgA1 binding, cultured human mesangial cells were treated with Mn^{2+} for integrin activation and Ca^{2+} or Mg^{2+} for inactivation before incubation with FITC-conjugated deSial/deGal IgA1 probe. Figure 3 showed that Mn^{2+} dramatically activated the receptor for IgA1 in a dose-dependent manner, whereas Ca^{2+} or Mg^{2+} induced only slight changes.

Distribution of integrin α subunits in the glomeruli of IgAN patients

To identify the α subunit involved in IgA1 binding, $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$ and αv expressions on kidney biopsy specimens of IgAN patients were examined by immunohistochemical study and their localizations were compared with the distribution of IgA deposition. The integrin $\alpha 1$ and $\alpha 3$ expression was observed globally in mesangial cells and all tubule epithelial cells. The integrin $\alpha 2$ subunit also existed globally in mesangial cells and the part of the tubule epithelial cells, and the distribution of $\alpha 1$, $\alpha 2$ or $\alpha 3$ was closely overlapped that of IgA deposition. Expression of the integrin $\alpha 5$ was absent both in the glomeruli and in the tubule epithelial cells, and the integrin αv subunit was weakly and segmentally expressed in the mesangial region alone (Fig. 4).

deSial/deGal IgA1–collagen complex facilitated IgA1 deposition and proliferative response in cultured human mesangial cells

Considering the close interaction between the integrin heterodimers and the extracellular matrices, we speculated that IgA1 might be able to deposit as a complex with extracellular matrix. To examine the contribution of the extracellular matrices in IgA1–integrin interaction, several types of collagen, laminin and fibronectin were pre-incubated with FITC-conjugated deSial/deGal IgA1 probe and then applied on cultured mesangial cells. Surprisingly, pre-incubation of deSial/deGal IgA1 with collagen types I, III, IV and V enhanced the binding of IgA1 in cultured mesangial cells dose dependently without any activation by Mn^{2+} . On the other hand, the enhancing effect of fibronectin, a ligand of integrin $\alpha 5/\beta 1$, $\alpha 8/\beta 1$ or $\alpha v/\beta 1$ heterodimer, and laminin, a

ligand of integrin $\alpha 3/\beta 1$ heterodimer (42), was minimal (Fig. 5). Following these results, we examined the distributions of each extracellular matrix in the glomeruli of kidney biopsy specimens of IgAN patients. In accordance with previous reports (39, 43, 44), collagen type IV and fibronectin were detected in the mesangial region, as well as in capillary walls, Bowman's capsule and basal membrane of tubular cells, and the localization of these two extracellular matrices overlapped the distribution of IgA deposits in the glomerular region. On the other hand, collagen type I, type III and laminin were absent in the mesangial region and were observed in Bowman's capsule and basal membrane of tubular cells (Fig. 6). To clarify the biological effect of IgA1–collagen type IV complex in mesangial cells, deSial/deGal IgA1 pre-incubated with collagen type IV or deSial/deGal IgA1 alone was added in cultured mesangial cells, and ERK phosphorylation was examined by western blotting. Figure 7 indicates that deSial/deGal IgA1 pre-incubated with collagen type IV induced more prominent phosphorylation of ERK in cultured human mesangial cells than deSial/deGal IgA1 alone did.

Direct interaction between IgA1 and integrin $\alpha 1$, $\alpha 2/\beta 1$ heterodimers

Considering the results that IgA1 bound mesangial cells all by itself and IgA1 pre-incubated with collagen facilitated both binding affinity and proliferative signals, we speculated that receptor for IgA1 would be an integrin heterodimer which possesses binding affinity with type IV collagen, namely, integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$. To confirm the direct interaction between IgA1 and integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ heterodimer, cultured mesangial cell lysate was incubated with biotinylated deSial/deGal IgA1 and then integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ –IgA1 complex was immunoprecipitated with anti-integrin $\alpha 1$ -, $\alpha 2$ - and $\beta 1$ -specific antibodies, respectively. Figure 8A demonstrates that biotinylated IgA1 was co-precipitated as a complex with integrin heterodimer. In the cultured human mesangial cells, the integrin $\alpha 1$ expression was also enhanced by TNF- α or TGF- $\beta 1$ treatment, whereas the integrin $\alpha 2$ expression was down-regulated by dexamethasone (Figure 8B). Finally, to verify the interaction between IgA1 and integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ heterodimer in

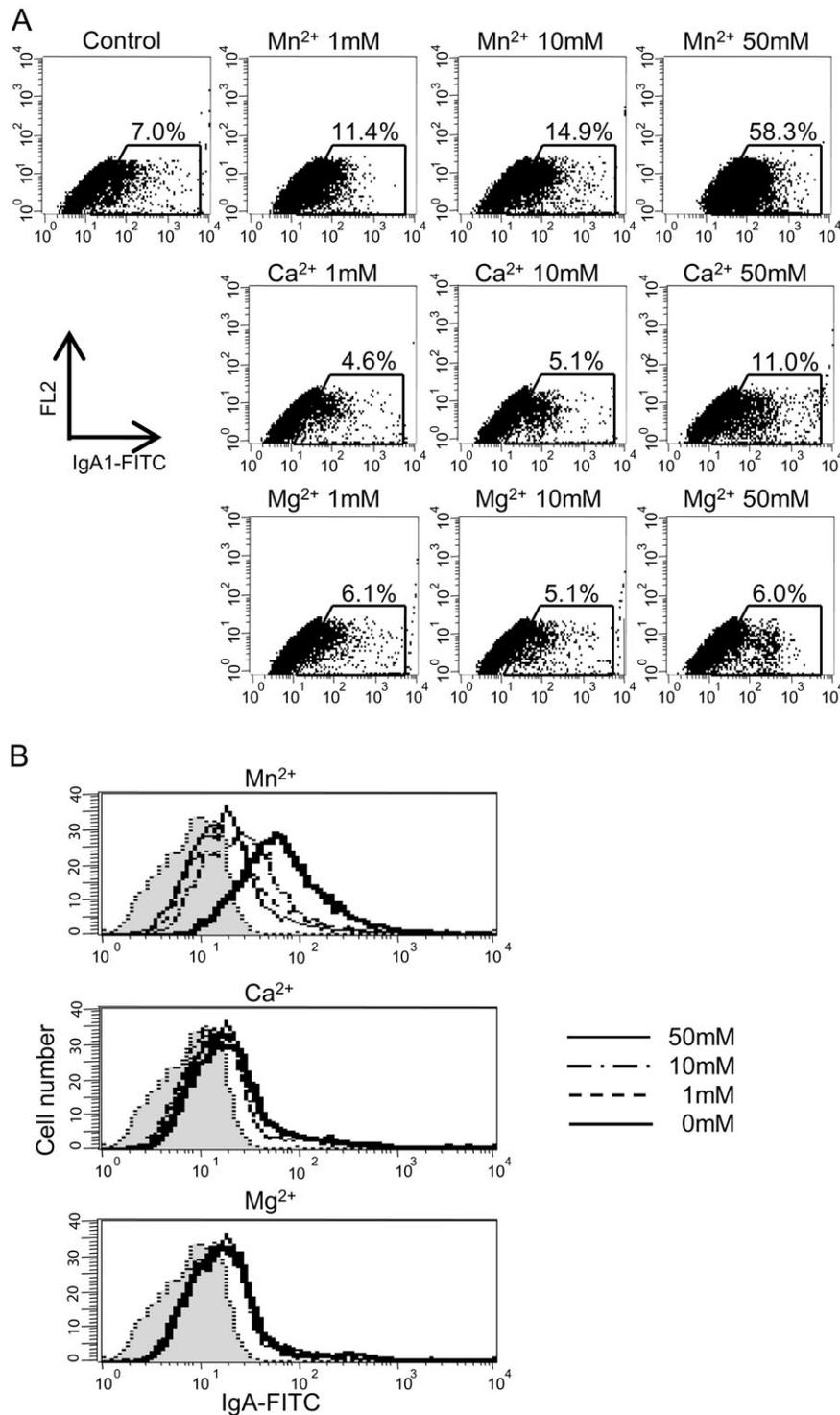


Fig. 3. Manganese ion enhanced IgA1 binding in mesangial cells. (A) Cultured mesangial cells were incubated with the indicated concentrations of Mn^{2+} , Ca^{2+} or Mg^{2+} for 30 min on ice prior to exposure to FITC-conjugated galactose-deficient IgA1 probe. (B) Histogram represents each fluorescence peak of FITC-conjugated IgA1 probe bound with cultured human mesangial cells after pre-incubation with indicated concentrations of Mn^{2+} , Ca^{2+} or Mg^{2+} . Fluorescence peaks of the histogram were positively shifted dose dependently by Mn^{2+} (upper) but not by Ca^{2+} (middle) or Mg^{2+} (lower). Shaded histogram represents negative control (FITC-IgA1 not added). These are the representative of three independent experiments.

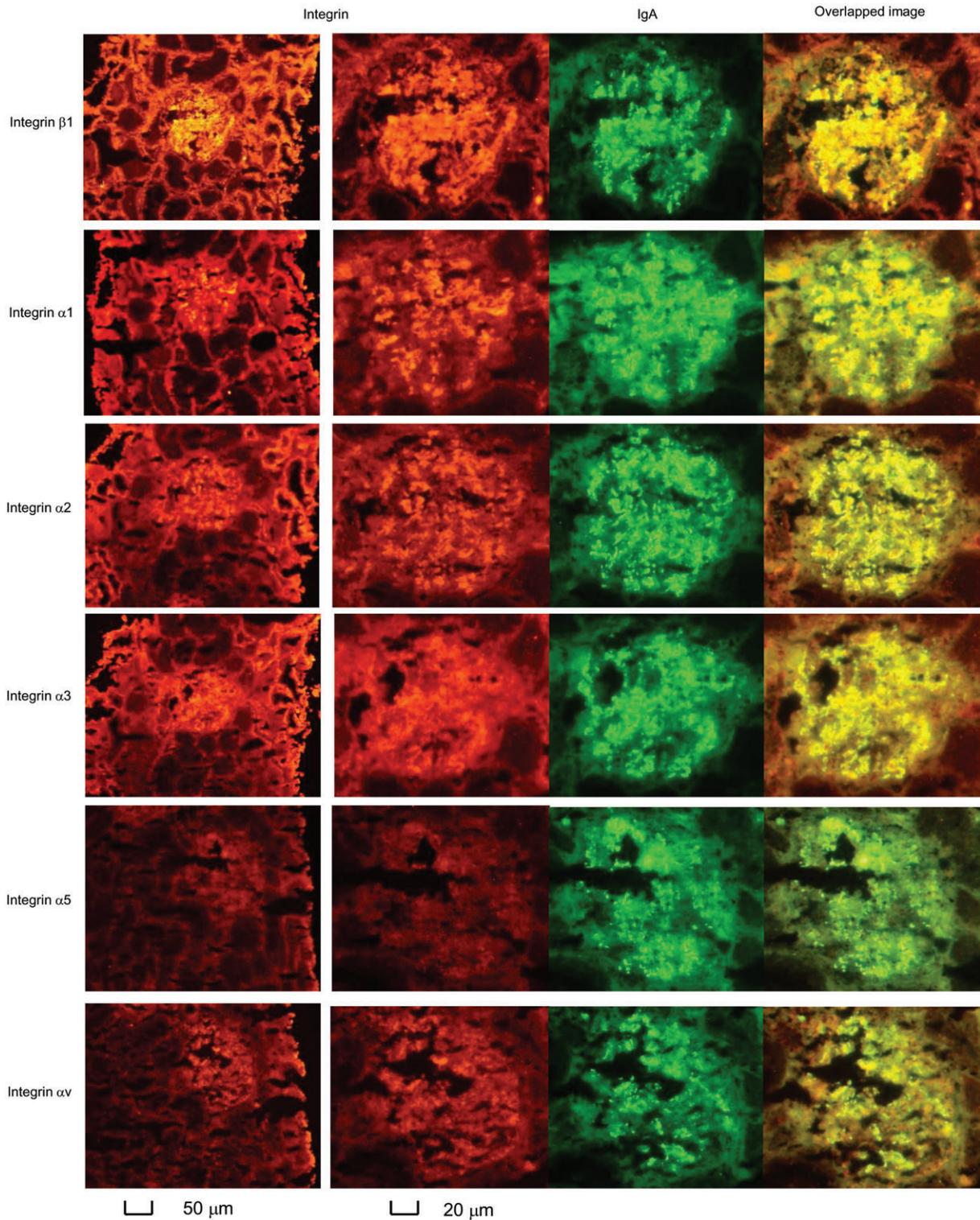


Fig. 4. Expression of integrin β and α subunits in mesangial cells. Frozen kidney biopsy specimens from IgAN patient were reacted with mAb specific for each integrin α or β subunit (red) to examine its localization in glomerulus and tubular cells and for human IgA (green) to compare the distribution of IgA deposit with that of integrin subunits in the mesangial region ($\times 100$ magnification for the left column and $\times 200$ magnification for the rest). These are the representative of five independent experiments.

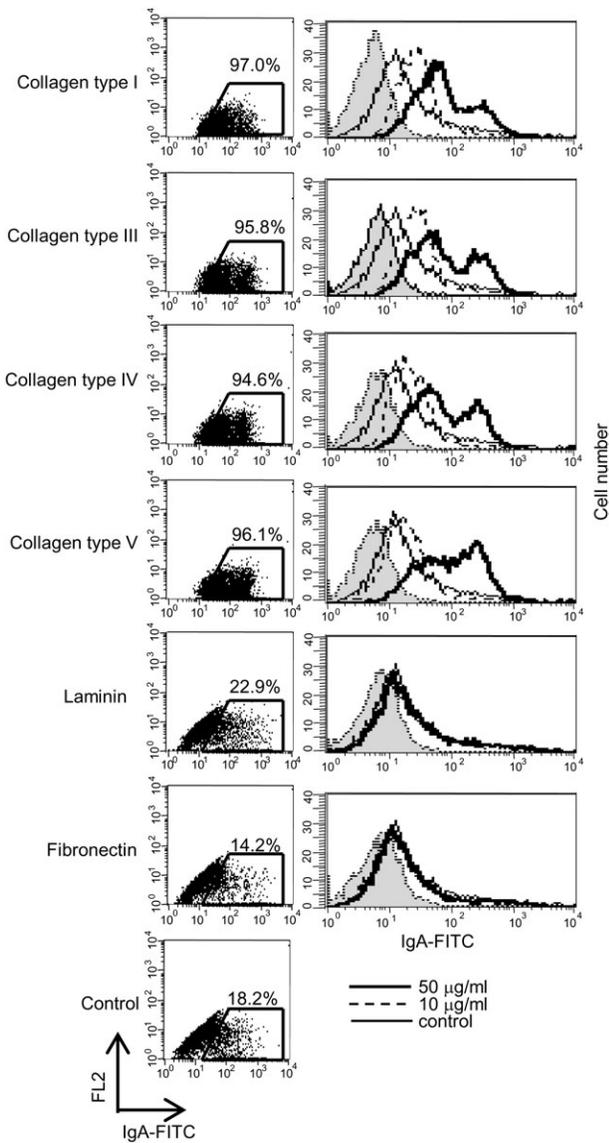


Fig. 5. Galactose-deficient IgA1 with collagen bound mesangial cells more efficiently. Galactose-deficient IgA pre-incubated with indicated concentrations of collagen type I, III, IV, V, laminin or fibronectin for 30 min in advance were reacted with cultured mesangial cells without Mn^{2+} activation, and FITC-IgA1 binding with cultured mesangial cells was examined by flow cytometry. Dot blots indicate that pre-incubation with $50 \mu\text{g ml}^{-1}$ collagen enhanced FITC-IgA1 binding. Histograms demonstrate the positive dose-dependent shift of the peaks of fluorescence by FITC-IgA1 probe after pre-incubation with the indicated concentrations of various types of collagens but not with laminin or fibronectin. Shaded histogram represents negative control (FITC-IgA1 not added). These are the representative of three independent experiments.

mesangial cells, siRNA specific for integrin $\alpha 1$, $\alpha 2$, $\beta 1$ or transferrin receptor gene had been administered to cultured human mesangial cells. Down-regulative effect of cell surface expression was in accordance with the specificity of administered siRNA, and siRNA specific for integrin $\beta 1$ induced down-regulation of integrin $\alpha 1$, $\alpha 2$ and $\alpha 3$ simultaneously. Cell surface expression of transferrin receptor

was not affected by siRNA specific for integrin $\alpha 1$ and $\alpha 2$, although integrin $\beta 1$ -specific siRNA rather up-regulated transferrin receptor expression in cell surface (Supplementary Figure 1, available at *International Immunology Online*). Binding ability with deSial/deGal IgA1 was significantly decreased by down-regulation of integrin $\alpha 1$, $\alpha 2$ and $\beta 1$ but not by that of transferrin receptor (Fig. 8C). These specific losses in the ability to bind deSial/deGal IgA1 were also demonstrated after pre-treatment with Mn^{2+} (Fig. 8D); however, pretreatment with collagen type IV diminished the losses of binding ability, presumably due to far higher affinity of IgA1–collagen complex compared with IgA1 by itself (Fig. 8E). According to these findings, we conclude that integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimers are involved in IgA1 deposition in mesangial cells.

Discussion

We have identified integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimer as a new candidate IgA1 receptor in human mesangial cells and have shown the possibility of its involvement in the development of IgAN through the induction of proliferative stimulation as a complex with collagen in mesangial cells.

Integrins are cell adhesion molecules that mediate cell–cell, cell–extracellular matrix and cell–pathogen interactions with non-covalently associated α and β subunits (37, 45). They function as signal transmitters through a variety of signaling pathways including phosphatidylinositol 3-kinase-Akt pathway, mitogen-activated protein kinase/ERK pathway or Rho family GTPase pathway to modulate many aspects of cell behavior such as proliferation, survival/apoptosis, cytoskeletal organization, gene expression and differentiation (42). In the glomerulus, among the 19 different integrin α subunits and 8 different integrin β subunits forming at least 25 kinds of $\alpha\beta$ heterodimers (41), integrin $\beta 1$ is the most prominent and widely distributed integrin subunit detected in mesangial, endothelial and epithelial cells, especially in areas where these cells attach to glomerular basement membrane or extracellular matrix in normal and diseased kidney (46–48). The $\alpha 1$ subunit is expressed widely in both mesangial cells and endothelial cells, whereas the $\alpha 2$ subunit is restricted in mesangial cells and distal tubular cells, and both $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimers serve as collagen receptors (42). The $\alpha 3$ subunit is abundantly distributed in mesangial, endothelial and epithelial cells and $\alpha 3/\beta 1$ heterodimer functions as a laminin receptor (42). The $\alpha 5$ subunit localizes in blood vessels, and some investigators report its distribution in mesangial cells, whereas αv subunit is detected in mesangial cells and tubular cells (38–40, 47), and the $\alpha 8$ subunit is exclusively expressed in mesangial cells (40). The $\alpha 5/\beta 1$, $\alpha v/\beta 1$ and $\alpha 8/\beta 1$ heterodimers are receptors for extracellular matrices that contain RGD sequences such as fibronectin (42). We examined the localization of these α subunits in human glomerulus with monoclonal antibodies, with the exception of $\alpha 8$, and our data were in accordance with previously reported findings except for $\alpha 5$ (Fig. 4).

Integrin heterodimers are activated in the presence of Mn^{2+} (41). Our data in Fig. 3 indicate the possibility that IgA1 receptors in cultured human mesangial cells are

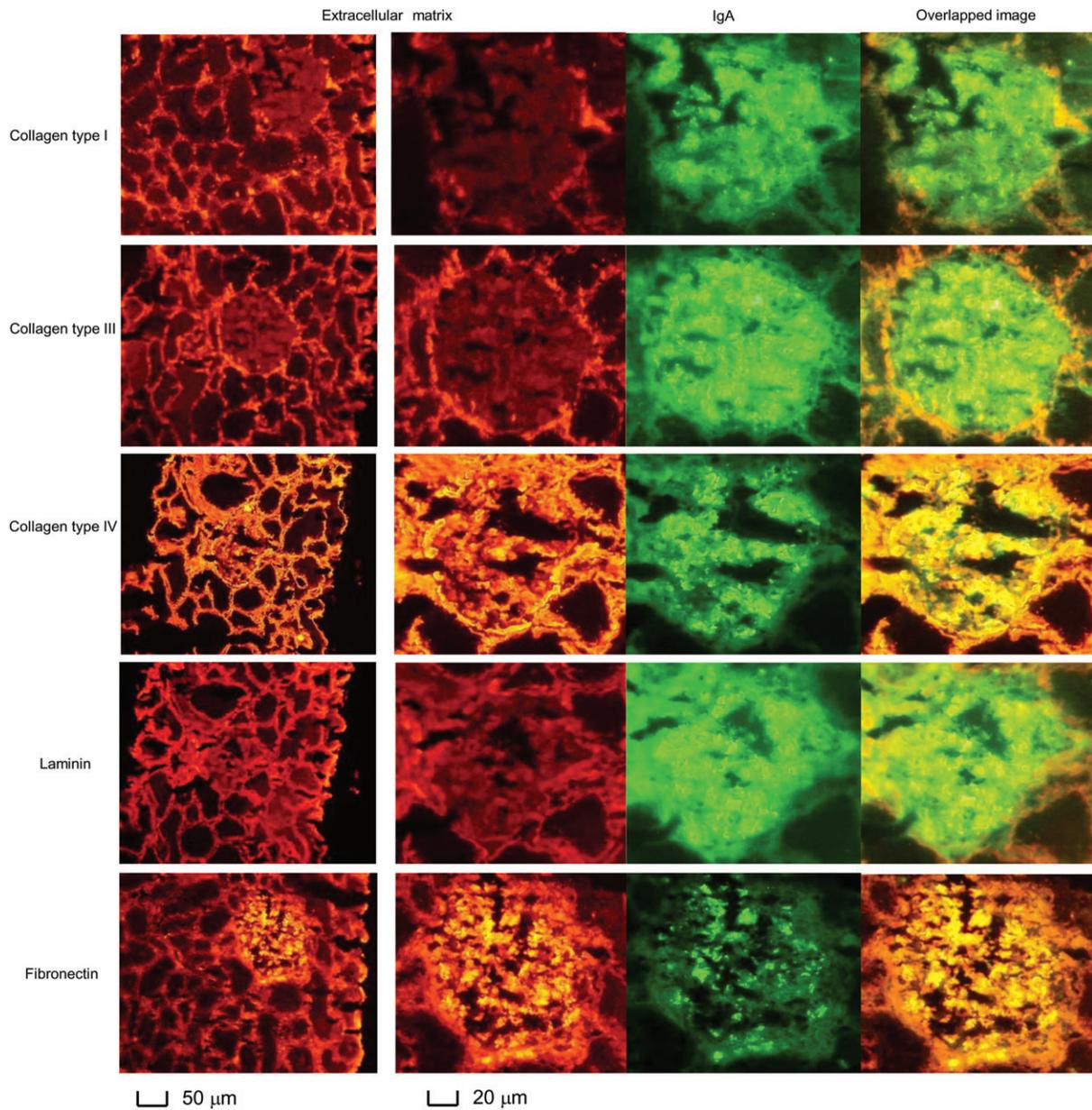


Fig. 6. Distribution of extracellular matrices in the kidney of IgAN patients. Frozen kidney biopsy specimens of IgAN patient were reacted with mAb specific for each extracellular matrix (red) to examine its distribution in glomerulus and tubulointerstitial regions and for human IgA (green) to compare the distribution of IgA deposit with that of extracellular matrices in the mesangial region ($\times 100$ magnification for the left column and $\times 200$ magnification for the rest). These are the representative of five independent experiments.

usually in an inactivated state and are activated in the presence of Mn^{2+} . While the activation by Mn^{2+} strongly supports the involvement of integrin heterodimers in IgA1 binding, these findings could also explain why only several percent of cultured mesangial cells could capture IgA1 probe even though each mesangial cell expressed integrin $\alpha 1$, $\alpha 2$ and $\beta 1$ subunits on its cell surface. We speculate that this is because integrin heterodimers in the cultured mesangial cells are normally in an inactivated state and the inactivated integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ heterodimer could possess very weak affinity toward IgA1.

More interestingly, IgA1 bound mesangial cells much efficiently by pre-incubation with various types of collagens. Even though co-localization of collagen type IV and IgA deposits in the mesangial region of kidney biopsy specimens from IgAN patients did not necessarily provide us with direct evidence to prove that IgA was deposited as a complex with collagen type IV, considering the increased tendency of galactose-deficient IgA1 to adhere the extracellular matrices (19, 49, 50), complex formation would have increased the affinity to the integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimer resulting in facilitation of the binding in

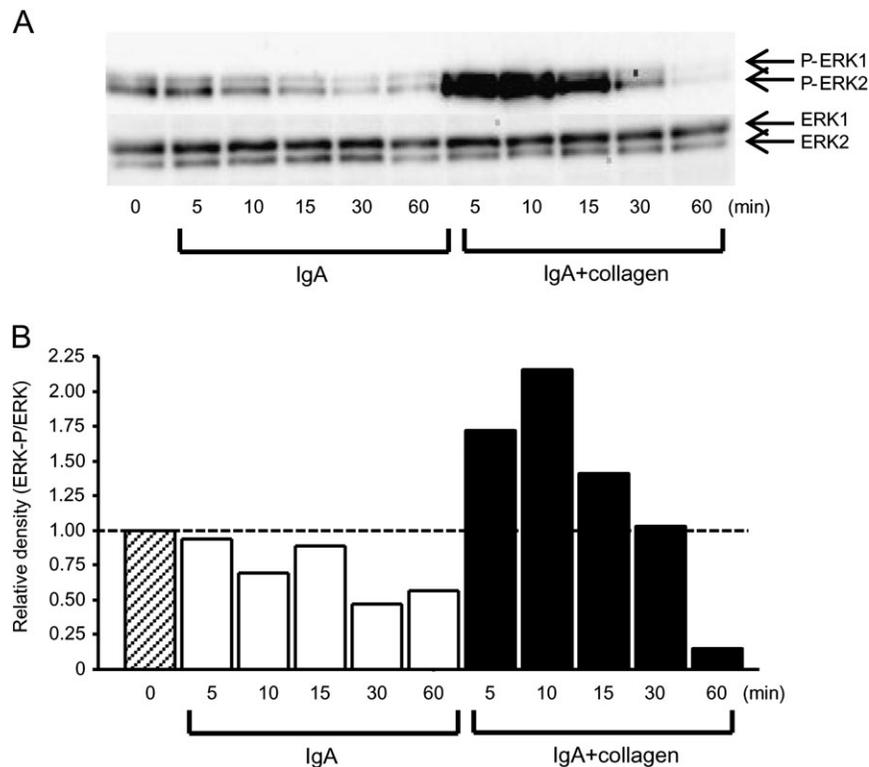


Fig. 7. deSial/deGal IgA1-collagen type IV complex-induced proliferative signals more prominently in mesangial cells. (A) deSial/deGal IgA1 or a mixture of deSial/deGal IgA1 and collagen type IV was co-incubated with cultured mesangial cells for the indicated times, and the phosphorylation of ERK (P-ERK) was determined by western blotting. (B) Relative density of P-ERK1 and P-ERK2 band against ERK1 and ERK2 band was quantified by densitometry in each condition (IgA, white and IgA with collagen type IV, black) and compared with the relative density at 0 min as 1.0 (striped). These are the representative of three independent experiments.

mesangial cells even in its inactivated state. We also speculated that this collagen-IgA1 complex would have possessed far higher affinity toward integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimers than IgA1 alone and would have overcome the down-regulation of these heterodimers induced by siRNA treatment (Fig. 8E). Similarly, proliferative stimulation would be induced more efficiently by IgA1-collagen type IV complex than by IgA1 alone, probably due to originally lower affinity of IgA1 to the integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ heterodimer compared with that of IgA1-collagen complexes, even though the existence of other different and specific receptor for IgA1-collagen complex has not been completely excluded. Alternative collagen receptor, CD36, was absent in both native mesangial cells in IgAN patients and cultured mesangial cells (Supplementary Figure 2, available at *International Immunology Online*).

Collagen type IV is a constituent of mesangial matrix and is reported to be increased in amount in various forms of glomerular injury (51–53), and its production is induced by TGF- β or platelet-derived growth factor (PDGF) in diseased kidney (54–57). We are tempted to conjecture that IgA1 generated in an IgAN patient might filter into the mesangial region to form complexes *in situ* with collagen type IV produced in the glomerulus under TGF- β or PDGF stimulation, otherwise circulating IgA1-collagen complexes, if pres-

ent, could bind to any cells that express integrin $\alpha 1/\beta 1$ or $\alpha 2/\beta 1$ heterodimers. And, thus, IgA1-collagen type IV complexes generated *in situ* or polymeric IgA1 might be deposited in mesangial cells via integrin $\alpha 1/\beta 1$ heterodimers possibly up-regulated by pro-inflammatory cytokines, such as TNF- α , or by growth factors such as TGF- β as well as via integrin $\alpha 2/\beta 1$ heterodimers. Enhanced binding affinity of IgA in the sera of IgAN patients toward collagen type IV (58) would strongly support this conjecture. We also speculate that steroid therapy might directly reduce the expression of integrin $\alpha 2/\beta 1$ heterodimers in mesangial cells as well as the inhibition of TGF- $\beta 1$ production (59), resulting in a curative effect in IgAN patients.

IgA-fibronectin complex has also been proposed by several investigators as playing a role in the pathogenesis of IgAN on the basis of the elevated serum level of circulating IgA-fibronectin complexes (60) or the findings for a mouse model of IgAN in which IgA-fibronectin complex formation was not prevented by genetically lacked uteroglobin through interaction with the heparin binding site of fibronectin (61) resulting in the glomerular mesangial deposition of IgA along with fibronectin and collagen type IV (57, 62). However, serum uteroglobin levels incorporated in IgA-fibronectin complexes were rather elevated in the patients with IgAN (58), and IgA-fibronectin complexes themselves were also increased in patients with Henoch-Schönlein

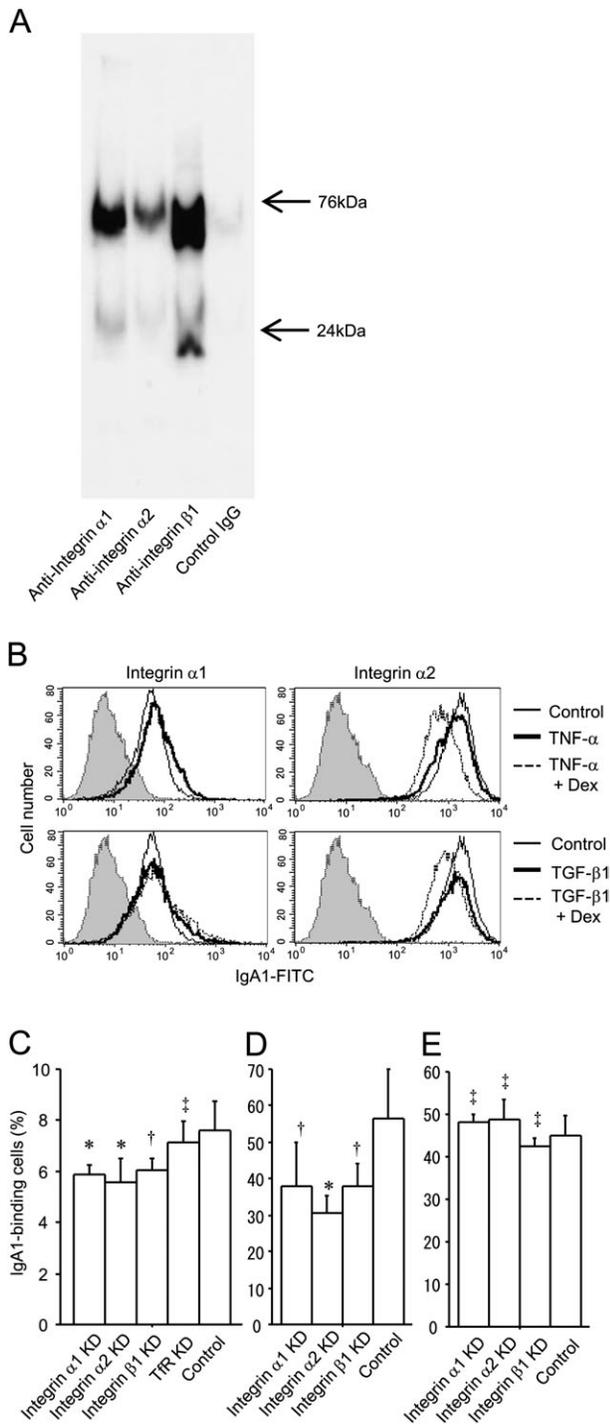


Fig. 8. Integrin $\alpha 1$ and $\alpha 2$ down-regulation induced loss in the ability to bind IgA1 probe. (A) Cultured human mesangial cell lysate co-cubated with biotinylated deSial/deGal IgA1 was reacted with each specific anti-integrin or control antibody for immunoprecipitation, and IgA1 in immune complexes were visualized by western blot using anti-biotin antibody. (B) Cultured human mesangial cells were co-cultured with TNF- α (upper) or TGF- $\beta 1$ (lower) with or without the addition of dexamethasone (Dex). Expression of integrin $\alpha 1$ (left) was enhanced with TNF- α or TGF- $\beta 1$ (bold line), whereas integrin $\alpha 2$ expression (right) was down-regulated by dexamethasone (dotted line). Shaded histogram represents isotype antibody control. (C) Cultured human mesangial cells treated with siRNA specific for

purpura without renal involvement and cirrhotic patients without urinary abnormalities (63). Thus, the role of IgA-fibronectin complex in the pathogenesis of IgAN is still controversial.

In the present study, we employed deSial/deGal IgA1 probe for the exploration of IgA1 receptor in mesangial cells except for Figs. 1 and 2. Absence of galactose in the hinge region of IgA1 was reported to be crucial for the development of IgA-IgG complex formation (5, 6) or IgA self-aggregation (8, 9, 19), but in this *in vitro* experimental conditions, desialylation alone could enhance binding affinity to mesangial cells and additional degalactosylation did not trigger extra binding affinity. We would rather conjecture that integrin $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimers are not specific receptors limited to deSial/deGal IgA1 but for IgA1 with variety of *O*-glycans in its hinge region, and binding affinity would be different according to the different structure of *O*-glycans. These subjects are still to be clarified in further investigations; however, this study provides us with a new candidate of IgA1 receptor in mesangial cells and proposes an innovative mechanism of IgA1 deposition in the pathogenesis of IgAN. We believe that these findings will lead us to a new fundamental therapeutic strategy against this disease.

Supplementary data

Supplementary data are available at *International Immunology Online*.

Funding

This work was supported by a grant-in-aid for Young Scientists (B) (No. 21790800) to Y. K. and a grant-in-aid for Scientific Research (B) (No. 20390234) to I.N. from the Japan Society for the Promotion of Science, Ministry of Education, Culture, Sports, Science and Technology, the Kanae Foundation for the Promotion of Medical Science to Y.K and the Study Group on IgAN to Y.K.

Acknowledgements

We thank Naofumi Imai, Katsura Minowa, Takae Watanabe and Hiroko Aita for expert technical assistance. Preliminary data from this study were presented in a poster session at the World Congress of Nephrology, April, 2011, Vancouver, Canada.

Disclosure

All the authors have no conflicting interests.

integrin $\alpha 1$, $\alpha 2$, $\beta 1$, transferrin receptor (TfR) or control siRNA were reacted with FITC-conjugated deSial/deGal IgA1 probe. Bar graph shows the percentage of probe IgA1-binding cells determined by flow cytometry. (D) Cultured mesangial cells treated with those siRNA were reacted with probe IgA1 with 10 mM Mn^{2+} . (E) Cultured mesangial cells treated with those siRNA were reacted with probe IgA1 pre-incubated with 25 $\mu g ml^{-1}$ collagen type IV. These are the representative of three independent experiments. † $P < 0.05$, * $P < 0.01$, ‡not significant compared with control siRNA treatment.

References

- 1 Donadio, J. V. and Grande, J. P. 2002. IgA nephropathy. *N. Engl. J. Med.* 347:738.
- 2 Levy, M. and Berger, J. 1988. Worldwide perspective of IgA nephropathy. *Am. J. Kidney Dis.* 12:340.
- 3 Nachman, P. H., Jennette, J. C. and Falk, R. J. 2008. Primary glomerular disease. In Brenner, B. M., ed., *The Kidney*, 8th edn p.1024. Saunders Elsevier, Philadelphia, PA.
- 4 Novak, J., Vu, H. L., Novak, L. *et al.* 2002. Interactions of human mesangial cells with IgA and IgA-containing immune complexes. *Kidney Int.* 62:465.
- 5 Tomana, M., Matousovic, K., Julian, B. A. *et al.* 1997. Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. *Kidney Int.* 52:509.
- 6 Suzuki, H., Fan, R., Zhang, Z. *et al.* 2009. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J. Clin. Invest.* 119:1668.
- 7 Launay, P., Grossetete, B., Acros-Fajardo, M. *et al.* 2000. Fc α receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease): evidence for pathogenic soluble receptor-IgA complexes in patients and CD89 transgenic mice. *J. Exp. Med.* 191:1999.
- 8 Kamata, T., Nogaki, F., Fagarasan, S. *et al.* 2000. Increased frequency of surface IgA-positive plasma cells in the intestinal lamina propria and decreased IgA excretion in hyper IgA (HIGA) mice, a murine model of IgA nephropathy with hyperserum IgA. *J. Immunol.* 165:1387.
- 9 Wang, Y., Zhao, M. H., Zhang, Y. K. *et al.* 2004. Binding capacity and pathophysiological effects of IgA1 from patients with IgA nephropathy on human glomerular mesangial cells. *Clin. Exp. Immunol.* 136:168.
- 10 Komatsu, H., Fujimoto, S., Hara, S. *et al.* 2008. Effect of tonsillectomy plus steroid pulse therapy on clinical remission of IgA nephropathy: a controlled study. *Clin. J. Am. Soc. Nephrol.* 3:1301.
- 11 Barratt, J. and Feehally, J. 2006. Treatment of IgA nephropathy. *Kidney Int.* 69:1934.
- 12 Narita, I. and Gejyo, F. 2008. Pathogenetic significance of aberrant glycosylation of IgA1 in IgA nephropathy. *Clin. Exp. Nephrol.* 12:332.
- 13 Tomino, Y., Sakai, H., Miura, M. *et al.* 1982. Detection of polymeric IgA in glomeruli from patients with IgA nephropathy. *Clin. Exp. Immunol.* 49:419.
- 14 Lomax-Smith, J. D., Zabrowarny, L. A., Howarth, G. S. *et al.* 1983. The immunochemical characterization of mesangial IgA deposits. *Am. J. Pathol.* 113:359.
- 15 Putnam, F. W., Liu, Y. S. V. and Low, T. L. K. 1979. Primary structure of a human IgA1 immunoglobulin. IV. Streptococcal IgA1 protease digestion, Fab and Fc fragments, and the complete amino acid sequence of the $\alpha 1$ heavy chain. *J. Biol. Chem.* 254:2865.
- 16 Iwase, H., Tanaka, A., Hiki, Y. *et al.* 1996. Estimation of the number of O-linked oligosaccharides per heavy chain of human serum IgA1 by matrix-associated laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) analysis of the hinge glycopeptide. *J. Biochem.* 120:393.
- 17 Arnold, J. N., Wormald, M. R., Sim, R. B. *et al.* 2007. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25:21.
- 18 Mattu, T. S., Pleass, R. J., Willis, A. C. *et al.* 1998. The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fc α receptor interactions. *J. Biol. Chem.* 273:2260.
- 19 Kokubo, T., Hiki, Y., Iwase, H. *et al.* 1998. Protective role of IgA1 glycans against IgA1 self-aggregation and adhesion to extracellular matrix proteins. *J. Am. Soc. Nephrol.* 9:2048.
- 20 Tomana, M., Novak, J., Julian, B. A. *et al.* 1999. Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. *J. Clin. Invest.* 104:73.
- 21 Hiki, Y., Odani, H., Takahashi, M. *et al.* 2001. Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. *Kidney Int.* 59:1077.
- 22 Moldoveanu, Z., Wyatt, R. J., Lee, J. Y. *et al.* 2007. Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. *Kidney Int.* 71:1148.
- 23 Zhang, J. J., Xu, L. X., Zhang, Y. *et al.* 2006. Binding capacity of *in vitro* deglycosylated IgA1 to human mesangial cells. *Clin. Immunol.* 119:103.
- 24 Xu, L. X. and Zhao, M. H. 2005. Aberrantly glycosylated serum IgA1 are closely associated with pathologic phenotypes of IgA nephropathy. *Kidney Int.* 68:167.
- 25 Gómez-Guerrero, C., Suzuki, Y. and Egido, J. 2002. The identification of IgA receptors in human mesangial cells: in the search for "Eldorado". *Kidney Int.* 62:715.
- 26 Krajci, P., Solberg, R., Sandberg, M. *et al.* 1989. Molecular cloning of the human transmembrane secretory component (poly-Ig receptor) and its mRNA expression in human tissues. *Biochem. Biophys. Res. Commun.* 158:783.
- 27 Leung, J. C. K., Tsang, A. W. L., Chan, D. T. M. *et al.* 2000. Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. *J. Am. Soc. Nephrol.* 11:241.
- 28 Pacifico, F., Laviola, L., Ulianichi, L. *et al.* 1995. Differential expression of the asialoglycoprotein receptor in discrete brain areas, in kidney and thyroid. *Biochem. Biophys. Res. Commun.* 210:138.
- 29 Gómez-Guerrero, C., Duque, N. and Egido, J. 1998. Mesangial cells possess an asialoglycoprotein receptor with affinity for human immunoglobulin A. *J. Am. Soc. Nephrol.* 9:568.
- 30 Gómez-Guerrero, C., González, E. and Egido, J. 1993. Evidence for a specific IgA receptor in rat and human mesangial cells. *J. Immunol.* 151:7172.
- 31 Bagheri, N., Chintalacharuvu, S. R. and Emancipator, S. N. 1997. Proinflammatory cytokines regulate Fc α R expression by human mesangial cells *in vitro*. *Clin. Exp. Immunol.* 107:404.
- 32 Diven, S. C., Caffisch, C. R., Hammond, D. K. *et al.* 1998. IgA induced activation of human mesangial cells: independent of Fc α R1 (CD89). *Kidney Int.* 54:837.
- 33 McDonald, K. J., Cameron, A. J. M., Allen, J. M. *et al.* 2002. Expression of Fc α/μ receptor by human mesangial cells: a candidate receptor for immune complex deposition in IgA nephropathy. *Biochem. Biophys. Res. Commun.* 290:438.
- 34 Moura, I. C., Centelles, M. N., Arcos-Fajardo, M. *et al.* 2001. Identification of the transferrin receptor as a novel immunoglobulin (Ig) A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. *J. Exp. Med.* 194:417.
- 35 Moura, I. C., Arcos-Fajardo, M., Sadaka, C. *et al.* 2004. Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. *J. Am. Soc. Nephrol.* 15:622.
- 36 Moura, I. C., Arcos-Fajardo, M., Gdoura, A. *et al.* 2005. Engagement of transferrin receptor by polymeric IgA1: evidence for a positive feedback loop involving increased receptor expression and mesangial cell proliferation in IgA nephropathy. *J. Am. Soc. Nephrol.* 16:2667.
- 37 Luo, B. H., Carmen, C. V. and Springer, T. A. 2007. Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* 25:619.
- 38 Adler, S. 1992. Integrin receptors in the glomerulus: potential role in glomerular injury. *Am. J. Physiol.* 262:F697.
- 39 Jin, D. K., Fish, A. J., Wayner, E. A. *et al.* 1996. Distribution of integrin subunits in human diabetic kidneys. *J. Am. Soc. Nephrol.* 7:2636.
- 40 Bieritz, B., Spessotto, P., Colombatti, A. *et al.* 2003. Role of $\alpha 8$ integrin in mesangial cell adhesion, migration, and proliferation. *Kidney Int.* 64:119.
- 41 Takagi, J. and Springer, T. A. 2002. Integrin activation and structural rearrangement. *Immunol. Rev.* 186:141.
- 42 Hynes, R. O. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell* 110:673.
- 43 Kanahara, K., Yorioka, N., Arita, M. *et al.* 1994. Immunohistochemical studies of extracellular matrix components and integrins in IgA nephropathy. *Nephron* 66:29.
- 44 Zhu, D., Kim, Y., Steffes, M. W. *et al.* 1994. Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. *Kidney Int.* 45:425.

- 45 Hynes, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69:11.
- 46 Kerjaschki, D., Ojha, P. P., Susani, M. *et al.* 1989. A β_1 -integrin receptor for fibronectin in human kidney glomeruli. *Am. J. Pathol.* 134:481.
- 47 Simon, E. E. and McDonald, J. A. 1990. Extracellular matrix receptors in the kidney cortex. *Am. J. Physiol.* 259:F783.
- 48 Canton, A. D. 1995. Adhesion molecules in renal disease. *Kidney Int.* 48:1687.
- 49 Cederholm, B., Wieslander, J., Bygren, P. *et al.* 1988. Circulating complexes containing IgA and fibronectin in patients with primary IgA nephropathy. *Proc. Natl Acad. Sci. USA* 85:4865.
- 50 Shinkai, Y., Karai, M., Osawa, G. *et al.* 1990. Antimouse laminin antibodies in IgA nephropathy and various glomerular diseases. *Nephron* 56:285.
- 51 Nakayama, H., Oite, T., Kawachi, H. *et al.* 1998. Comparative nephritogenicity of two monoclonal antibodies that recognize different epitopes of rat Thy-1.1 molecule. *Nephron* 78:453.
- 52 Yagi, M., Yamamoto, T., Kato, S. *et al.* 1998. Long-term observation of glomerulonephritis induced by multiple injections with anti-Thy-1 antibody in rats. *Pathol. Int.* 48:491.
- 53 Adler, S. G., Feld, S., Striker, L. *et al.* 2000. Glomerular type IV collagen in patients with diabetic nephropathy with and without additional glomerular disease. *Kidney Int.* 57:2084.
- 54 Ignatz, R. A. and Massagué, J. 1986. Transforming growth factor- β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem.* 261:4337.
- 55 Kamata, T., Muso, E., Yashiro, M. *et al.* 1999. Up-regulation of glomerular extracellular matrix and transforming growth factor- β expression in RF/J mice. *Kidney Int.* 55:864.
- 56 Naito, T., Nitta, K., Ozu, H. *et al.* 1997. Clinical assessment of the significance of platelet-derived growth factor in patients with immunoglobulin A nephropathy. *J. Lab. Clin. Med.* 130:63.
- 57 Zheng, F., Kundu, G. C., Zhang, Z. *et al.* 1999. Uteroglobin is essential in preventing immunoglobulin A nephropathy in mice. *Nat. Med.* 5:1018.
- 58 Coppo, R., Chiesa, M., Cirina, P. *et al.* 2002. In human IgA nephropathy uteroglobin does not play the role inferred from transgenic mice. *Am. J. Kidney Dis.* 40:495.
- 59 Haramaki, R., Tamaki, K., Fujisawa, M. *et al.* 2001. Steroid therapy and urinary transforming growth factor- $\beta 1$ in IgA nephropathy. *Am. J. Kidney Dis.* 38:1191.
- 60 Baldree, L. A., Wyatt, R. J., Julian, B. A. *et al.* 1993. Immunoglobulin A-fibronectin aggregate levels in children and adults with immunoglobulin A nephropathy. *Am. J. Kidney Dis.* 22:1.
- 61 Chowdhury, B., Zhang, Z. and Mukherjee, A. B. 2008. Uteroglobin interacts with the heparin-binding site of fibronectin and prevents fibronectin-IgA complex formation found in IgA-nephropathy. *FEBS Lett.* 582:611.
- 62 Zhang, Z., Kundu, G. C., Yuan, C. J. *et al.* 1997. Severe fibronectin-deposit renal glomerular disease in mice lacking uteroglobin. *Science* 276:1408.
- 63 Davin, J. C., Vecchi, M. L., Nagy, J. *et al.* 1991. Evidence that the interaction between circulating IgA and fibronectin is a normal process enhanced in primary IgA nephropathy. *J. Clin. Immunol.* 11:78.