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Mesangial expression of angiotensin II receptor in IgA nephropathy and its regulation by polymeric IgA1

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Background. Enhanced gene expression for the renin-angiotensin system (RAS) is detected in glomerular mesangial cells in IgA nephropathy (IgAN). Preliminary studies showed a reduced glomerular gene expression of angiotensin II subtype 1 receptor (AT1R), suggesting a regulatory response to high intrarenal angiotensin II (Ang II) concentration in IgAN.

Methods. We examined the effect of polymeric IgA1 (pIgA1) from patients with IgAN on the expression of Ang II receptors in cultured human mesangial cells (HMC).

Results. Polymeric IgA1 from patients with IgAN down-regulated the expression of AT1R in HMC in a dose-dependent manner. When similar experiments were conducted with addition of an angiotensin-converting enzyme inhibitor (captopril) or an AT1R antagonist (losartan), there was a significant increase in the expression of AT1R. Blockade of Ang II with captopril or losartan alone resulted in a stepwise increase of AT1R in cultured HMC. Down-regulation of Ang II subtype 2 receptor (AT2R) was not observed in HMC cultured with pIgA1 from patients with IgAN. The acute suppressive effect of pIgA1 from IgAN on the expression of AT1R was confirmed in HMC incubated with IgA isolated from 15 IgAN patients, 15 healthy subjects, and other glomerulonephritides control subjects. Reduced glomerular expression of AT1R (but not AT2R) was also demonstrated in renal biopsies from patients with IgAN.

Conclusion. Our findings demonstrate an altered AT1R expression in HMC in response to raised intrarenal Ang II in IgAN. Our in vitro studies also support that an imbalance of AT1R and AT2R activity in HMC following exposure to pIgA plays a significant pathogenetic role in the inflammatory injury in IgAN.

The renin-angiotensin system (RAS) has been recognized as a key factor in the progression of chronic renal failure. Angiotensin II (Ang II) plays a central role as a mediator of glomerular hemodynamic adaptation and injury. It has been suggested that Ang II-induced mesangial cell contraction with efferent arteriolar vasoconstriction initiates intraglomerular hypertension, and may eventually lead to enhanced matrix formation and renal fibrosis following increased synthesis of transforming growth factor-β (TGF-β) [1]. Pharmacologic blockade of this system, by either angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II subtype-1 receptor (AT1R) antagonist, retards progression of glomerulosclerosis [2]. However, renal expression and regulation of the components of the RAS have not been well studied in human subjects. Changes in the plasma RAS do not reflect local expression of the RAS in kidney [3, 4]. Study of intrarenal expression and regulation of the RAS is necessary for evaluating the role of the renin system on the kidney, and hence, the therapeutic target in chronic renal diseases.

IgA nephropathy (IgAN), now recognized to be the most common glomerulonephritis worldwide, runs an indolent but slowly progressive course leading to end-stage renal failure (ESRF) in 30% to 40% of patients over 30 years [5]. The hallmark of the disease is characterized by mesangial deposition of polymeric IgA1 (pIgA1), proliferation of mesangial cells, increased synthesis of extracellular matrix, and infiltration by macrophages, monocytes, and T cells [6]. Recent data suggest serum IgA from patients with IgAN are different from those of healthy subjects and can exert pathophysiologic effect on target cells [7, 8].

Genes encoding for renin, angiotensinogen, and angiotensin-converting enzyme (ACE) are detected in cultured mesangial cells and in mesangial cells in kidney tissues from patients with IgAN [9]. However, the information of the RAS in IgAN remains scarce and is mainly limited to ACE-related polymorphism. So far, the results on the pathogenetic role of different ACE genotypes in IgAN remain divergent and inconclusive [10]. Both AT1R and Ang II subtype 2 receptor (AT2R) are expressed in the normal human kidney, as well as in

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patients with glomerular disease [11]. The histologic distribution of these receptors supports the notion that both receptors may have a physiologic role in normal and diseased kidneys in humans. In the present study, we studied the Ang II receptor expression in kidney of IgAN patients. Polymeric IgA1 isolated from patients with IgAN was examined to determine any regulatory effect on the expression of these receptors in human mesangial cells (HMC).

METHODS

Experimental design

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the institutional ethics committee for studies in human. All subjects (patients and healthy control patients) gave their written informed consent for serum and tissue collections.

We first examined the glomerular expression of AT1R and AT2R in patients with IgAN. Renal tissues were obtained from nine normotensive patients with mild IgAN (grade 1) admitted consecutively for diagnostic renal biopsy with the presentation of microscopic hematuria. The severity of renal pathology was classified into grade 1, 2, or 3, as previously described [12], and grade 1 pathology indicated mesangial proliferation with no crescent, sclerosis, or tubulointerstitial changes. These nine patients had normal creatinine clearance (>80 mL/min/1.73m^2) with proteinuria ranging from 0.5 to 1.3 g/day. They had not previously received ACEI or AT1R antagonists. Next, we studied the dose- and time-response profile of the acute effect of pIgA1 from patients with IgAN on the expression of Ang II receptors in cultured HMC. Polymeric IgA1 were isolated from sera of five of these patients selected randomly. The same IgA preparations were used in the subsequent study of the Ang II production and expression of AT1R in HMC following prolonged exposure to pIgA1.

For comparison of the immediate regulatory effect of pIgA1 isolated from different groups of subject on the expression of Ang II receptors in cultured HMC, pIgA1 was prepared from sera of another 15 patients with IgAN randomly recruited from the clinic. Their histologic diagnosis was made at least 18 months prior to the study, and their serum creatinine remained stable over the previous 12 months. Their proteinuria ranged from 0.1 to 2.9 g/day. Their mean endogenous creatinine clearance was 74.6 ± 23.0 mL/min./1.73 m^2. These patients varied in the histologic severity with grade 1 in 5, grade 2 in 8, and grade 3 in 2. Control subjects included healthy volunteers and patients with other glomerulonephritides. None of these 15 patients with IgAN or patients with other glomerulonephritides received ACEI or AT1R antagonists for blood pressure control during the study period.

Materials

RPMI 1640 and fetal bovine serum (FBS) were obtained from Life Technologies (Rockville, MD, USA). Jacalin agarose was obtained from Pierce (Rockford, IL, USA). Rabbit polyclonal antibodies against antihuman AT1 or AT2 receptor were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Superose FPLC column was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Consumables for electrophoresis were obtained from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

Morphologic and immunohistologic studies

All patients who underwent renal biopsy or control subjects for blood collection were administered a sodium-controlled diet (50 mmol/L per day) the week before biopsy or blood collection. Diet compliance was checked by evaluating the urinary excretion of sodium the day before biopsy or blood collection.

As previously mentioned, renal tissues were obtained from nine normotensive patients with mild IgAN (grade 1) consecutively admitted for diagnostic renal biopsy with the presentation of microscopic hematuria. Control renal tissues were obtained from the intact pole of kidneys removed for single circumscribed tumor in five normotensive subjects (comparable in age, sex, and race). Renal biopsy specimens were processed for light, immunofluorescent, and electron microscopy by standard methods [13]. The glomerular expression of AT1R or AT2R was detected by immunohistochemical staining using specific polyclonal antibodies for individual Ang II receptor. Briefly, dewaxed 5-μm paraffin sections were incubated with 0.5% hydrogen peroxide for removal of endogenous peroxidase activity. Nonspecific binding was blocked by incubation of the slides for 30 minutes with blocking buffer [5% normal goat serum and 3% bovine serum albumin in phosphate-buffered saline (PBS)]. The sections were then incubated with anti-AT1R or anti-AT2R antibody (5 μg/mL) overnight. The bound antibodies were visualized as brown color using the Dako Envision Plus System. To ensure the specificity of the staining, we performed the following labeling controls: (*1*) the primary antibodies were substituted with preimmune rabbit immunoglobulins; (*2*) staining was carried out without either the primary antibodies or the peroxidase-labeled polymer; and (*3*) the primary antibodies were preincubated with 5 mg/mL carboxyl terminal peptides of AT1R or AT2R. Some sections were counterstained with hematoxylin before mounting. The intensity of the slide preparations was semiquantitatively scored at 0, 1+, 2+, 3+, and 4+ by two of the authors independently without the knowledge of the nature of antibodies using a grading as previously described [13]. Ten

consecutive glomerular cross sections (gcs) were counted at high-power field (× 400), and staining was graded on a scale of (0) for no staining, (1+) for <5% of labeled cells per gcs stained, (2+) for 5% to 10% of labeled cells per gcs stained, (3+) for 10% to 15% of labeled cells per gcs stained, and (4+) for >15% of labeled cells per gcs stained.

Purification and characterization of polymeric and monomeric IgA1 by jacalin affinity chromatography and fast protein liquid chromatography (FPLC)

Following the histologic examination for Ang II receptors, we then proceeded to determine whether IgA from patients with IgAN exerted any regulatory effect on the expression of these receptors in HMCs in vitro. IgA was isolated from sera obtained from five of these patients selected randomly for in vitro time and dose response studies. IgA1 was fractionated at room temperature by the fast protein liquid chromatography (FPLC) system (Pharmacia) as described previously [14]. Briefly, monomeric IgA1 (mIgA1) and pIgA1 were separated by FPLC following jacalin affinity chromatography. The identity of IgA after FPLC was confirmed by immunoblotting and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Every IgA preparation from each individual patient was separated into mIgA1 or pIgA1 according to molecular weight. Chromatography fractions with MW 300 to 1000 kD were high-molecular-mass IgA or pIgA1, whereas fractions with MW 50 to 300 kD were low-molecular-mass IgA or mIgA1. Distinct mIgA1 and pIgA1 fractions were separately prepared from each individual patient for subsequent incubation studies. The content of IgG in the fraction was measured by an anti-IgG enzyme-linked immunosorbent assay (ELISA). The fractions were dialyzed and concentrated to 2 mL with Centriprep (Amicon, Beverly, MA, USA) and stored at −70°C until use. The purity of IgA1 fractions was confirmed by SDS-PAGE and ELISA [14]

Culture of human mesangial cell culture

Isolation and characterization of HMCs were performed as previously described [14]. 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 FBS 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 Ang II. Mesangial cells from a single nephrectomy sample at fourth to seventh passage were used in our experiments.

Treatment of human mesangial cells with different IgA preparations or RAS blockade

Human mesangial cells were grown to log phase and harvested using 0.05% trypsin. The trypsinized-recovered cells were subcultured onto 6-well culture plates (1×10^6 per wells). The cells were arrested by growing 24 hours in medium containing 0.5% FBS before exposure to IgA preparations or RAS blockade at increasing concentrations (0, 0.125, 0.25, 0.5, 1, or 2 mg/mL for IgA, and 0, 1, 10, 100, 1000, and 10,000 nmol/L for captopril or losartan) for 6 or 24 hours at 37°C in replicates. Preliminary experiments were conducted showing these incubation periods were convenient and were associated with optimal synthesis of mRNA (6 hours) and protein (24 hours) for Ang II receptors. Cells were harvested for RNA extraction and total cell extract preparation. The AT1R or AT2R content of the mesangial cells was studied by Western blotting. Culture supernatants were also collected for determination of Ang II.

Gene expression of AT1 and AT2 receptors in cultured mesangial cells

Specific primers for AT1R, AT2R, and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were designed from known GeneBank sequences (AT1R NM–004835; AT2R U16957; GAPDH X01677). The sequences of each primers were as follows: (*1*) AT1R, sense primer 5′-GAT GAT TGT CCC AAA GCT GG-3′ and antisense primer 5′-TAG GTA ATT GCC AAA GGG CC-3′; (*2*) AT2R, sense primer 5′-AAG AAG AAA TCC CTG GCA AGC-3′ and antisense primer 5′-CTT GGT CAC GGG TTA TCC TGT-3′; and (*3*) GAPDH, sense primer 5′-ACCACAGTCCATGCCATCAC-3′ and antisense primers 5′-TCCACCACCCTGTTGCTGTA-3′. We performed reverse transcription and polymerase chain reaction (PCR) as described [15] using the following profile: first cycle, 94°C for three minutes, 55°C for one minute, 72°C for one minute; second to 30th cycles, 95°C for 45 seconds, 55°C for 40 second, 72°C for 45 seconds. The final cycle was 94°C for one minute and 72°C for 10 minutes. The PCR products from AT1R or AT2R and control (GAPDH) amplicons were mixed and separated by 1.5% wt/vol agarose gels, stained with ethidium bromide, and the gel image was captured and analyzed using the Gel Doc 1000 Gel Documentation System and Quantity One software (Bio-Rad Laboratories, Ltd.). We semiquantitated the result of Ang II receptor mRNA yield as a ratio of AT1R or AT2R amplicon to GAPDH amplicon. We took all necessary steps cautiously to ensure the validity of the results as previously described [16].

Determination of Ang II receptor protein in cultured human mesangial cells

Homogenized human mesangial cells treated with different IgA preparation or following RAS blockade were harvested and dissolved in protein extraction buffer containing protease inhibitor cocktails, and the protein concentrations were measured by a modified Lowry method using bovine serum albumin as standard (DC protein assay kit; Bio-Rad). The extracts were spun at 10,000*g* for 5 minutes at 4°C to remove cell debris, and 50 μg of protein electrophoresed through a 15% SDS-PAGE gel before transferring to a polyvinylidene fluoride (PVDF) membrane. After blocking for 1 hour at room temperature in blocking buffer (1% gelatin in PBS with 0.05% Tween 20), the membrane was incubated for 16 hours with anti-AT1R (1:1000) or anti-AT2R in PBS-Tween. The membrane was washed and incubated for 2 hours at room temperature with a peroxidase-labeled goat antirabbit immunoglobulin (Dako) and detected with enhanced chemiluminescence (ECL) plus chemiluminescent detection reagent (Amersham Pharmacia Biotech, Arlington, IL, USA). The Western blotting images were scanned on a flatbed scanner and the density of the bands was quantitated using ImageQuant software (Molecular Dynamic, Sunnyvale, CA, USA). Densitometry results were reported as average arbitrary integrated values (units). The results of Ang II receptor proteins obtained by immunoblotting were further validated using flow cytometry as previously described [14].

Determination of Ang II in supernatant from cultured human mesangial cells

Angiotensin II was measured by an enzyme immunoassay using an anti-Ang II Fab' monoclonal antibody labeled with acetylcholinesterase [17] (SPI bio, Massy Cedex, France). The minimum detectable concentration was 1 pg/mL, and the intra-assay coefficient of variation was 7%.

Comparison of the regulatory effect of pIgA1 from IgAN patients and control subjects on the expression of Ang II receptors in mesangial cells

The effect of pIgA1 from patients with IgAN on the expression of Ang II receptors in mesangial cells was further studied in another 15 Chinese patients with primary IgAN, as previously mentioned. Twenty milliliters of blood were collected from each patient at clinical quiescence (a period free of macroscopic hematuria or mucosal infection and urinary erythrocyte count <10,000/mL in uncentrifuged urine). The serum was isolated and frozen at −20°C until for isolation of IgA1 by a jacalin-agarose affinity column. Serum IgA level was determined by nephelometry.

Fifteen healthy subjects (8 males and 7 females), matched in age and race with no microscopic hematuria or proteinuria, were used as healthy control subjects. Patients with Henoch-Schonlein purpura (HSP) ($N = 10$), lupus nephritis ($N = 10$), minimal change nephropathy (MCN) ($N = 10$), and membranous nephropathy (MGN) ($N = 10$) were recruited as disease control subjects. Serum was similarly collected from these individuals for purification of IgA.

Detection of Ang II-induced early apoptosis in human mesangial cells by Western blotting

Cell extracts were prepared from homogenized human mesangial cells treated with different IgA preparations, or following RAS blockade for 24 hour as described above. Early apoptosis was detected by Western blotting using monoclonal anti-poly[ADP-ribose] polymerase (PARP) antibody (Cell Signaling Technology) that recognized the 89 kD cleaved PARP fragment. The membrane was washed and incubated for 2 hours at room temperature with a peroxidase-labeled goat antimouse immunoglobulin (Dako) and the reaction was detected with ECL plus chemiluminescent detection reagent (Amersham).

Ang II production and expression of AT1R in HMC following prolonged exposure to pIgA

In order to find out whether short and prolonged exposure to pIgA exerts different regulatory effect on HMC, HMC were subcultured onto 6-well culture plates (0.5×10^6 per wells). The cells were exposed to pIgA preparation from four patients with IgAN (0.5 mg/mL) at different time intervals (from 12 hours to 16 days). Culture medium was changed every four days with fresh pIgA preparation (0.5 mg/mL) added. At each time point, culture supernatants were saved for assay of Ang II. Total cell lysate was prepared for analysis of AT1R and AT2R expression by immunobloting. In parallel experiments, cells were harvested at each time point, and cell proliferation was determined by expression of proliferating cell nuclear antigen (PCNA) using flow cytometry.

Cell proliferation assay

Mesangial cells were harvested and fixed with absolute methanol for 10 minutes at −20°C followed by permeabilization with 0.5% NP-40 for 5 minutes. The cells were washed once with staining buffer (PBS containing 1% FBS, 10% normal human serum, and 0.01% sodium azide). The cells were incubated with a fluoroscein isothiocyanate (FITC)-conjugated monoclonal anti-PCNA antibody (PCNA; clone PC10; Dako) for 30 minutes at room temperature. The cells were then washed twice with staining buffer and resuspended in PBS containing 1% FBS for flow cytometry analysis.

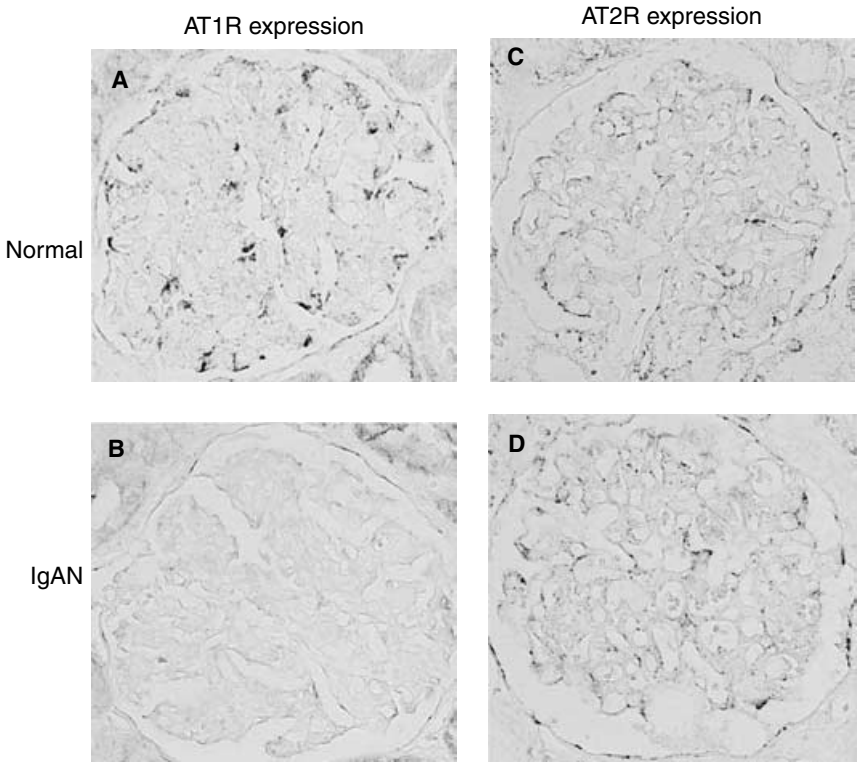

Fig. 1. Representative glomerular staining of angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) in normal kidney and in IgAN. Decreased glomerular expression of AT1R was observed in renal biopsies from patients with IgAN. In contrast, no difference in glomerular expression of AT2R was observed in glomeruli from controls or from patients with IgAN (hematoxylin and eosin, magnification ×200).

Isotype-matched antibody was used as a control, and at least 5000 cells were counted. The results were expressed as mean fluorescence channel (MFI).

Statistics

All data (from patients or cell culture experiments) were expressed as mean ± standard deviation (SD). Intergroup differences for continuous variables were assessed by the unpaired *t* test except in concentrations exceeding the in vivo relevance (for pIgA1) or the therapeutic level (for captopril or losartan). The mRNA expression or protein synthesis of Ang II receptors in cultured cells following exposure to different concentrations of IgA preparations or RAS blockade were analyzed with multivariate analysis of variance (ANOVA) for repeated measures. All *P* values quoted are two-tailed, and the significance is defined as $P < 0.05$.

RESULTS

Glomerular expression of Ang II receptors

Glomerular immunoreactive AT1R or AT2R protein was found within mesangial cells in normal glomerulus (Fig. 1A and C). Both arteriolar endothelium and glomerular epithelial cells contained immunoreactive AT1R and AT2R protein. These receptors were also detected in tubular epithelium (picture not shown). In kidney biopsies from patients with IgAN, immunostaining revealed a pattern of reduced AT1R protein but not in AT2R protein (Fig. 1B and D).

The intensity of the slide preparations was visually scored independently from 0 to 4+ by two pathologists without the knowledge of the nature of antibodies. In general, there was good concordance of the score, and no sample had a discordance of score greater than one grade. Glomerular immunostaining for AT1R was significantly lower in patients with IgAN (mean score 1.0 vs. 1.8 in control subjects, $P = 0.012$) (Fig. 2A). Similar findings were not detected in glomerular immunostaining for AT2R (mean score of 2.33 in IgAN vs. 3.0 in control subjects, $P = 0.255$) (Fig. 2B).

Expression of Ang II receptors in cultured human mesangial cells

Inhibition of the ACE following incubating the HMC with captopril led to an increased gene expression of AT1R in a dose-dependent manner that peaked at a concentration of 1000 nmol/L (Fig. 3A). Similarly, blockade of the AT1R by specific antagonist also resulted in a stepwise increase in gene expression of AT1R in HMC, and the effect peaked at a concentration of 1000 nmol/L. The findings of AT1R protein expression in cultured mesangial cells incubated with either ACEI or AT1R antagonist were parallel to those of mRNA signals (Fig. 3B). More interestingly, pIgA1 from patients with IgAN significantly down-regulated the gene and protein

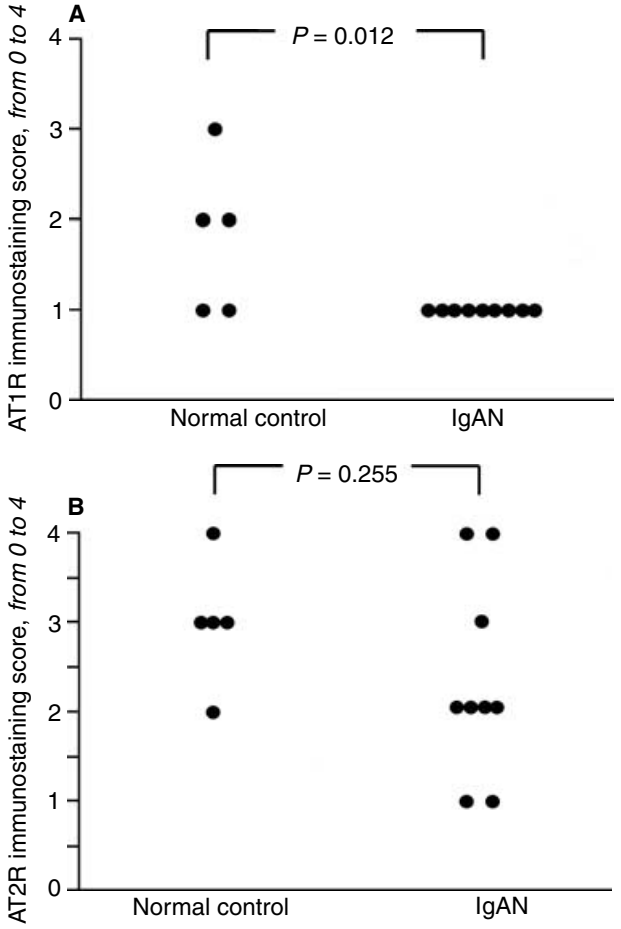

Fig. 2. (*A*) Glomerular expression of angiotensin II type 1 receptor (AT1R) was reduced in renal biopsies from patients with IgAN when compared with normal kidney. (*B*) Glomerular expression of angiotensin II type 2 receptor (AT2R) was not reduced in renal biopsies from patients with IgAN when compared with normal kidney.

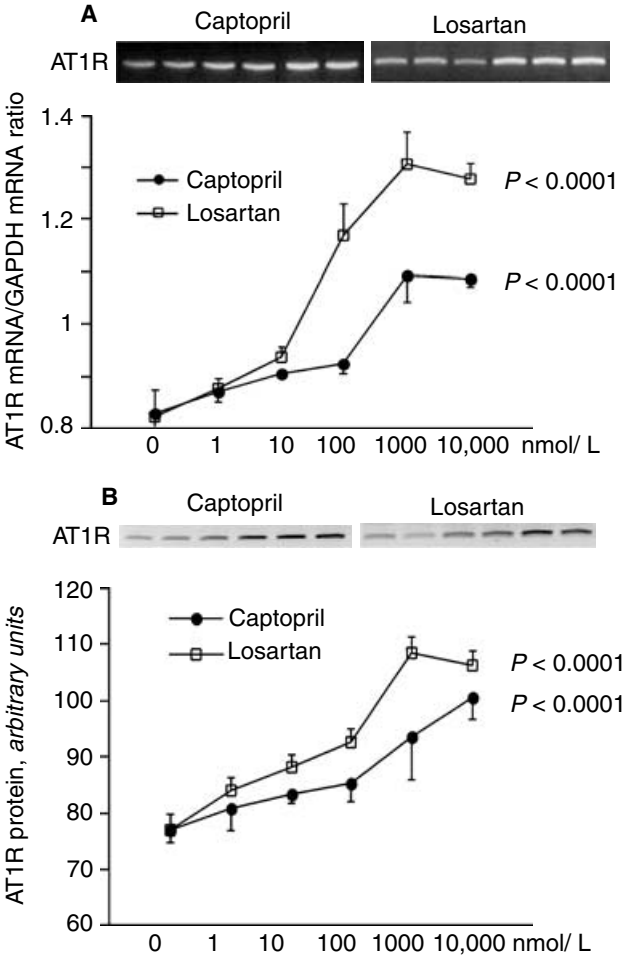

Fig. 3. (*A*) Up-regulation of angiotensin II type 1 receptor (AT1R) mRNA in cultured human mesangial cells incubated with captopril or losartan [a group and "within" dose interaction, $P < 0.0001$ by multivariate analysis of variance (MANOVA)]. Measurement of AT1R mRNA in human mesangial cells (HMC) incubated with captopril at different concentrations differed significantly with each other ($P < 0.05$) except for measurements between 0 and 1 nmol/L, between 1 and 10 nmol/L, and between 10 and 100 nmol/L. Measurement of AT1R mRNA in HMC incubated with losartan at different concentrations differed significantly with each other ($P < 0.05$) except for measurements between 0 and 1 nmol/L, and between 1 and 10 nmol/L. The results represent the mean ± SD of five separate experiments. (*B*) Increased AT1R protein synthesis in cultured HMC incubated with captopril or losartan (a group and "within" dose interaction, $P < 0.0001$ by MANOVA). Measurement of AT1R protein in HMC incubated with captopril at different concentrations differed significantly with each other ($P < 0.05$) except for measurements between 0 and 1 nmol/L, between 0 and 10 nmol/L, and between 1 and 10 nmol/L. Measurement of AT1R protein in HMC incubated with losartan at different concentrations differed significantly with each other ($P < 0.05$). The results represent the mean ± SD of five separate experiments.

expression of AT1R in a dose-dependent manner reaching a 8% and 17% protein decrease at concentrations of 0.25 and 0.5 mg/mL, respectively (Fig. 4). Despite mIgA1 from patients with IgAN also down-regulated the protein expression of AT1R in a similar dose-dependent manner, yet the magnitude was small. Similar experiments were also performed with pIgA1 from five matched healthy volunteers. Polymeric IgA1 from patients with IgAN exerted more down-regulation of the gene and protein expression of AT1R in HMC than that from healthy volunteers, especially at concentration higher than 0.25 mg/mL (Fig. 5).

In contrast, up-regulatory effect on AT2R protein expression was not observed in cultured HMC incubated with either ACEI or AT1R antagonist (Fig. 6A). Similarly, pIgA1 isolated from patients with IgAN exerted no effect on the AT2R protein expression in cultured mesangial cells (Fig. 6B). The findings of gene expression of AT2R in cultured mesangial cells incubated with captopril, losartan, or IgA preparations were parallel to those of protein synthesis (data not shown).

The results of Ang II receptor proteins obtained by immunoblotting were validated by flow cytometry. The correlation coefficients between immunoblotting and flow cytometry measurements for AT1R and AT2R were 0.907 and 0.926, respectively.

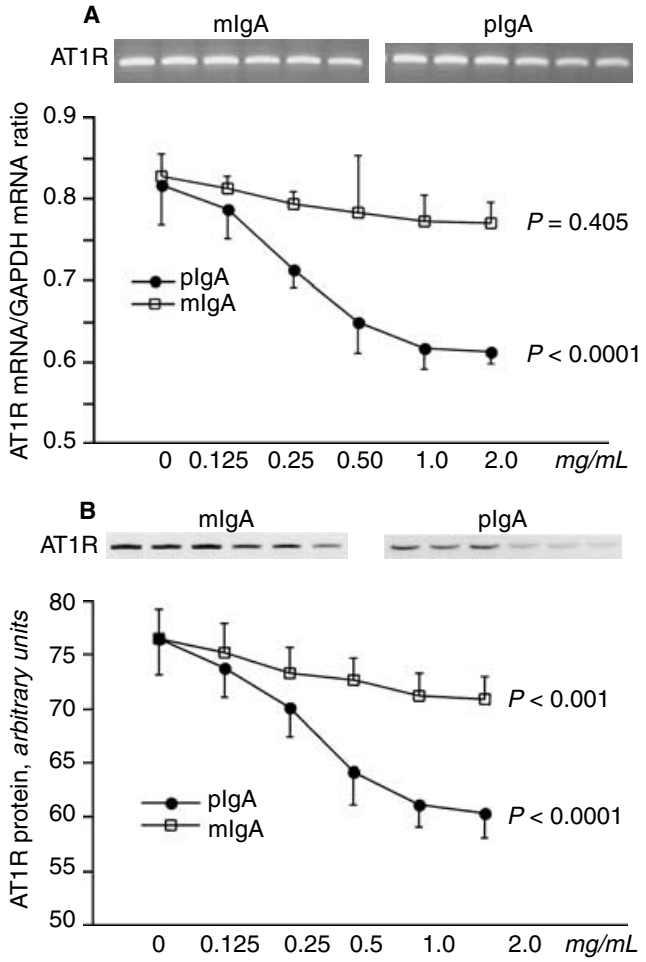

Fig. 4. (*A*) Down-regulation of angiotensin II type 1 receptor (AT1R) mRNA in cultured human mesangial cells incubated with pIgA1 preparations from IgAN patients [a group and "within" dose interaction, $P < 0.0001$ by multivariate analysis of variance (MANOVA)]. Measurement of AT1R mRNA in HMC incubated with pIgA at different concentrations differed significantly with each other ($P < 0.05$) except for measurements between 0 and 0.125 mg/mL, and between 0.5 and 1 mg/mL. No down-regulation of AT1R mRNA in cultured human mesangial cells incubated with mIgA1 preparations from IgAN patients was observed. The results represent the mean ± SD of five separate experiments. (*B*) Decreased AT1R protein synthesis in cultured human mesangial cells incubated with pIgA1 preparations from IgAN patients (a group and "within" dose interaction, $P < 0.0001$ by MANOVA). Measurement of AT1R protein in HMC incubated with pIgA1 at different concentrations differed significantly with each other ($P < 0.05$). Incubation of human mesangial cells with mIgA preparation from IgAN patients resulted in a reduction in AT1R protein synthesis, but of a small magnitude. The results represent the mean ± SD of five separate experiments.

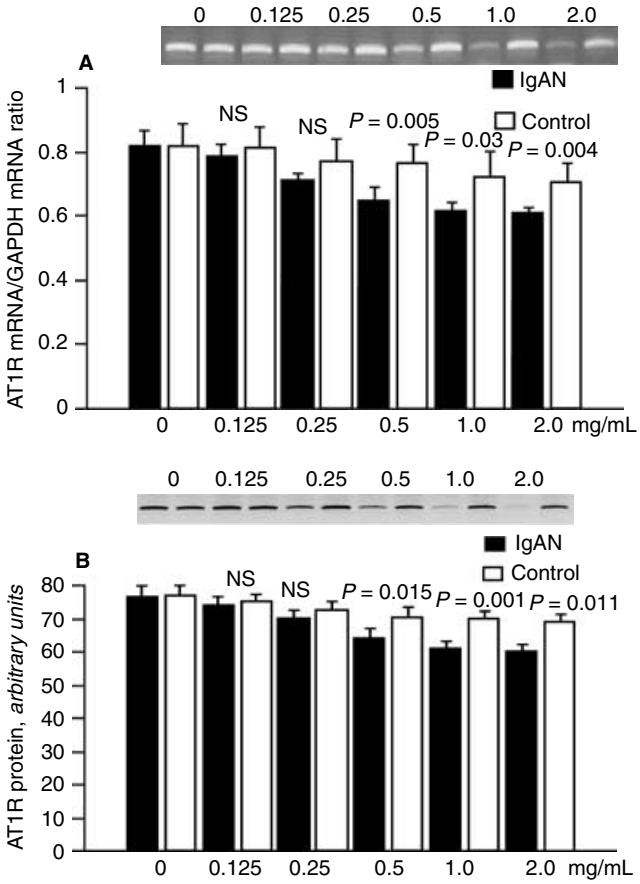

Fig. 5. Expression of angiotensin II type 1 receptor (AT1R) in human mesangial cells (HMC) incubated with pIgA1 from patients with IgAN ($N = 5$) or from matched healthy volunteers ($N = 5$). Polymeric IgA1 from patients with IgAN exerted more down-regulation of the gene and protein expression of AT1R in HMC than that from healthy volunteers, especially at concentration higher than 0.25 mg/mL. The results represent the mean ± SD.

Effect of blocking the RAS in cultured HMC preincubated with IgA

Next, we preincubated HMC with optimal dose of IgA for 4 hours to down-regulate the AT1R expression. Polymeric IgA1 (0.5 mg/mL) suppressed the AT1R expression more than mIgA1 at equivalent concentration or control medium (Fig. 7). The mesangial expression of AT1R increased when HMC preincubated with pIgA1 was further treated with captopril (100 nmol/L) or losartan (100 nmol/L). Identical experiments done with mIgA1 demonstrated a similar pattern for AT1R expression, but the magnitude of the down-regulation was smaller than pIgA1. AT2R expression in HMC was not altered by preincubation with pIgA1 or mIgA (Fig. 8A). Subsequent treatment with captopril or losartan exerted no change in AT2R expression.

Comparison of the regulatory effect of pIgA1 from IgAN patients and control subjects on the expression of Ang II receptors in mesangial cells

The suppressive effect of pIgA1 in patients with IgAN on the AT1R expression in mesangial cells was compared between 15 IgAN patients with 15 healthy control subjects comparable in age and sex. The serum IgA level in patients with IgAN (2.96 ± 1.13 g/L) was significantly higher than that of healthy control subjects (1.81 ±

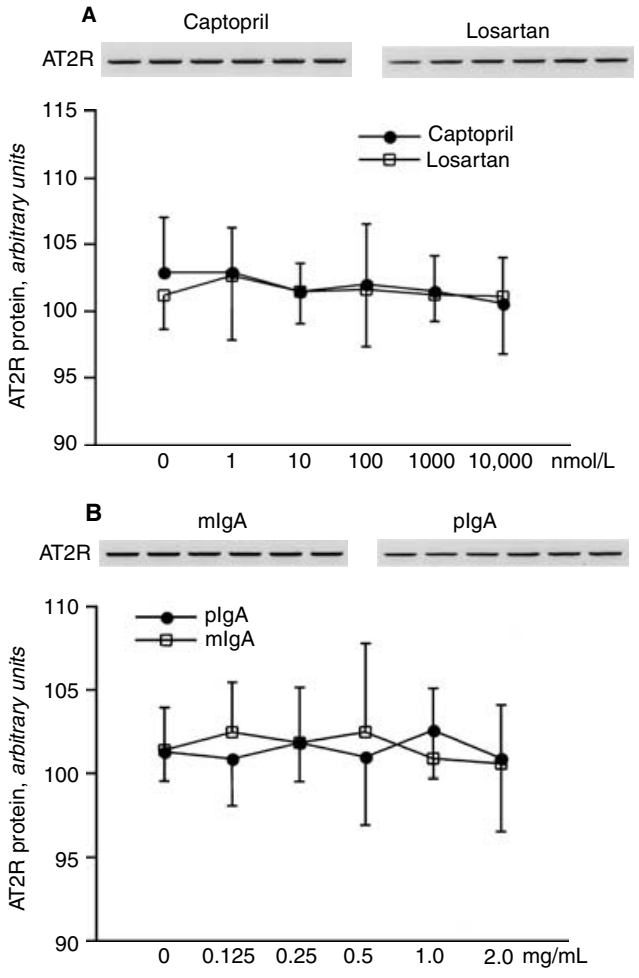

Fig. 6. (*A*) Neither angiotensin-converting enzyme inhibitor (ACEI) nor angiotensin II type 1 receptor (AT1R) antagonist exerted any regulatory effect on angiotensin II type 2 receptor (AT2R) protein expression in cultured HMC. (*B*) pIgA1 or mIgA1 isolated from patients with IgAN exerted no effect on the AT2R protein expression in cultured mesangial cells. The results represent the mean ± SD of five separate experiments.

0.82 g/L, $P < 0.001$). No IgM was detected in the IgA fractions. IgG represented 0.1% of total protein in IgA fractions as measured by ELISA. Studies of IgA in FPLC fractions by IgA ELISA showed that mIgA1 amounted to 90% of total IgA1 from either control subjects or patients (data not shown). Despite the IgA was isolated during clinical quiescence, pIgA1 from patients induced a significantly less AT1R than cells incubated with pIgA1 from healthy control subjects ($P < 0.025$ and < 0.005 for mRNA and protein, respectively) (Fig. 9).

Human mesangial cells were also incubated with pIgA1 from different disease groups to explore whether IgA preparations from other glomerulonephritides also suppressed the AT1R expression in HMC. Polymeric IgA1 prepared from patients with other glomerulonephritides did not exert similar suppressive effect as observed in pIgA1 isolated from patients with IgAN (Fig. 9). In contrast, the effect of pIgA1 isolated from different glomerulonephritides (including IgAN) on the mesangial expression of AT2R did not differ from that of healthy control subjects (Fig. 8B).

A good negative correlation was demonstrated between the Ang II levels in the cell supernatant and the expression of AT1R of human mesangial cells incubated with pIgA1 from patients with IgAN ($r = -0.61, P < 0.02$) (Fig. 10). Similar correlation did not exist between the supernatant level of Ang II and the expression of AT2R of HMC following incubation with pIgA1 from patients with IgAN.

Ang II-induced early apoptosis in human mesangial cells incubated with IgA

As Ang II-induced apoptosis in renal proximal tubular cells and cardiomyocytes is mainly mediated through activation of AT2 receptors [18, 19, 20], we tested for early apoptosis in HMC incubated with pIgA from patients with IgAN using a monoclonal anti-PARP antibody (Fig. 11A). The supernatant concentration of Ang II (4 to 6 pg/mL or 10^{-11} mol/L) in HMC released by incubating with pIgA was too low to induce apoptosis via the binding to AT2R. Similarly, the supernatant concentration of Ang II in HMC following incubation with AT1R antagonist failed to induce apoptosis. In contrast to human renal proximal tubular cells that became apoptotic at an Ang II concentration of 10^{-9} mol/L [21], we showed that apoptosis was inducible in HMC only at an Ang II concentration $\geq 10^{-7}$ mol/L (Fig. 11B).

Ang II production and expression of AT1R in HMC following prolonged exposure to pIgA

The supernatant concentration of Ang II peaked between day 1 and day 2 following initial culture with RMPI 1640 medium supplemented with pIgA preparation from patients with IgAN (Fig. 12). Following the change of fresh culture medium (with added pIgA) every four days, the cultured HMC continued to synthesize Ang II, and maintained a steady concentration toward the end of prolonged culture (day 16).

Following initial culture with RMPI 1640 medium supplemented with pIgA preparation from patients with IgAN, there was a stepwise reduction of AT1R expression in cultured HMC in response to raised Ang II concentration in the supernatant (Fig. 13). The maximum suppression occurred on day 4 before the change of culture medium. Subsequent to the initial suppression of AT1R in the first 4 days, the down-regulation was gradually reversed despite the change of fresh culture medium (with added pIgA) every four days. At day 16, the expression of AT1R increased to values comparable to that on day 2.

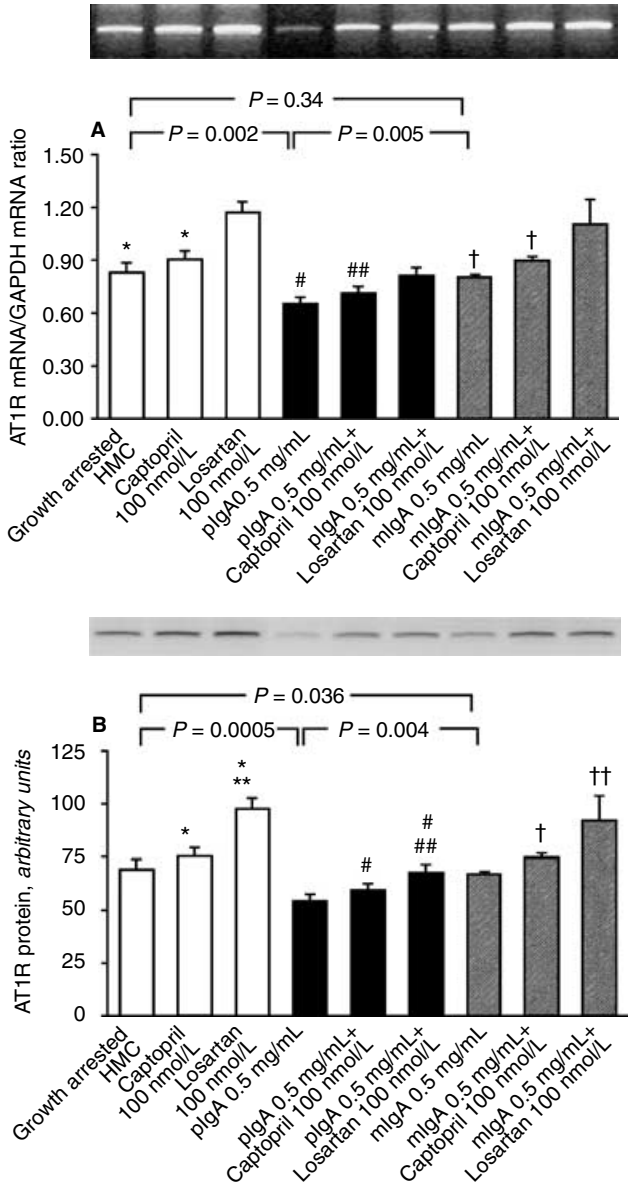

Fig. 7. (*A*) Gene expression of angiotensin II type 1 receptor (AT1R) following blocking the renin-angiotensin system (RAS) in cultured human mesangial cells (HMC) preincubated with IgA1 preparations. Incubation with losartan (100 nmol/L) up-regulated the gene expression of AT1R in resting HMC. *Signifies $P < 0.002$ as compared with HMC incubated with losartan. Incubation with losartan (100 nmol/L) partly reversed the down-regulatory effect of pIgA1 (0.5 mg/mL) on the gene expression of AT1R in HMC. Similar effect was not seen with captopril (100 nmol/L). # and ## signify $P < 0.0025$ and $P < 0.02$, respectively, as compared with pIgA1-treated HMC that were subsequently incubated with losartan. Incubation with losartan (100 nmol/L) partly reversed the down-regulatory effect of mIgA1 (0.5 mg/mL) on the gene expression of AT1R in HMC. †Signifies $P < 0.03$ as compared with mIgA1-treated HMC that were subsequently incubated with losartan. The results represent the mean ± SD of five separate experiments. (*B*) AT1R synthesis following blocking the RAS in cultured human mesangial cells (HMC) preincubated with IgA1 preparations. Incubation with captopril (100 nmol/L) or losartan (100 nmol/L) up-regulated the protein synthesis of AT1R in resting HMC. *Signifies $P < 0.01$ as compared with growth-arrested HMC and **signifies $P < 0.05$ compared with HMC incubated with captopril. Incubation with captopril (100 nmol/L) or losartan (100 nmol/L) partly reversed the down-regulatory effect of pIgA1 (0.5 mg/mL) on the protein synthesis of AT1R in HMC. #Signifies $P < 0.01$ as compared with HMC preincubated with pIgA alone, and ## signify $P < 0.01$ compared with pIgA1-treated HMC that were subsequently incubated with captopril (100 nmol/L). Incubation with captopril (100 nmol/L) or losartan (100 nmol/L) partly reversed the down-regulatory effect of mIgA1 (0.5 mg/mL) on the protein synthesis of AT1R in HMC. †Signifies $P < 0.001$ compared with HMC preincubated with mIgA alone, and †† signify $P < 0.05$ compared with mIgA1-treated HMC that were subsequently incubated with captopril (100 nmol/L). The results represent the mean ± SD of five separate experiments.

No alteration was observed with AT2R during prolonged culture.

There was progressive increase in cell proliferation of the cultured mesangial cells (MC) following initial culture with RMPI 1640 medium supplemented with pIgA preparation from patients with IgAN (Fig. 14). Following the change of fresh culture medium (with added pIgA) every four days, the cultured HMC continued to proliferate with maximal activity on day 8. Cell proliferation was maintained toward the end of prolonged culture (day 16) at activity comparable to that on day 2. In contrast, the proliferative activities of MC cultured with RMPI 1640 medium with or without supplement of IgG from the same patient were lower than that with pIgA. Furthermore, the proliferative activities were not maintained with prolonged culture.

DISCUSSION

Increased Ang II activity is implicated in various renal pathologic settings. There are two main Ang II receptors (AT1R and AT2R) in human, and these receptors are detected in the vasculature, in glomeruli, and in tubular segments [11]. However, the molecular mechanism and the Ang II receptor subtypes involved in different renal diseases have not been fully identified. More recently, the effects on renal tubules or glomerular mesangial cells following the blockade of the RAS provide initial understanding of the role of these Ang II receptors in renal pathophysiology. ACE inhibition or AT1R blockade consistently produce natriuresis, whereas AT2R blockers have no effect [22]. Luminal AT(1A) (basolateral type 1A) receptor mediates a biphasic regulation of bicarbonate absorption in proximal tubules by luminal Ang II in rodent, while no evidence is obtained for a role of AT2R [23]. In contrast, Ang II induces apoptosis in proximal tubules (human and rodent) via AT2R but not via AT1R [18, 21]. Angiotensin II exerts a hypertrophic effect in primary cultured mesangial cells [24], and induces cellular proliferation upon interaction with platelet-derived growth factor [25]. While RAS blockade by AT1R antagonist prevents proliferation of mesangial cell [25], AT2R stimulation exerts a potent antiproliferative effect in cultured rat mesangial cells [26]. The observation that AT2R

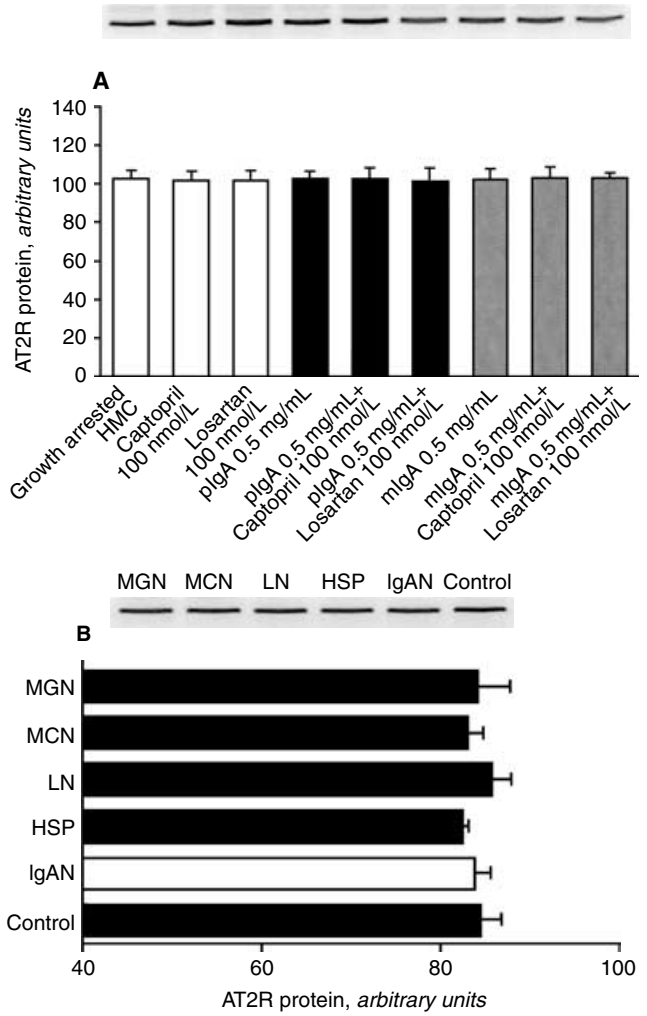

Fig. 8. (*A*) Angiotensin II type 2 receptor (AT2R) synthesis in human mesangial cells (HMC) was not altered by preincubation with pIgA1 or mIgA. Subsequent treatment with captopril or losartan exerted no change in AT2R expression. The results represent the mean ± SD of five separate experiments. Similar findings were detected in gene expression of AT2R (data not shown). (*B*) No difference in AT2R protein synthesis is demonstrated in cultured human mesangial cells incubated with pIgA1 (0.5 mg/mL) from patients with IgAN when compared with pIgA1 from healthy or disease controls. The results represent the mean ± SD. Similar findings were detected in gene expression of AT2R (data not shown). HSP, Henoch-Schonlein purpura; MCN, minimal change nephropathy; MGN, membranous nephropathy.

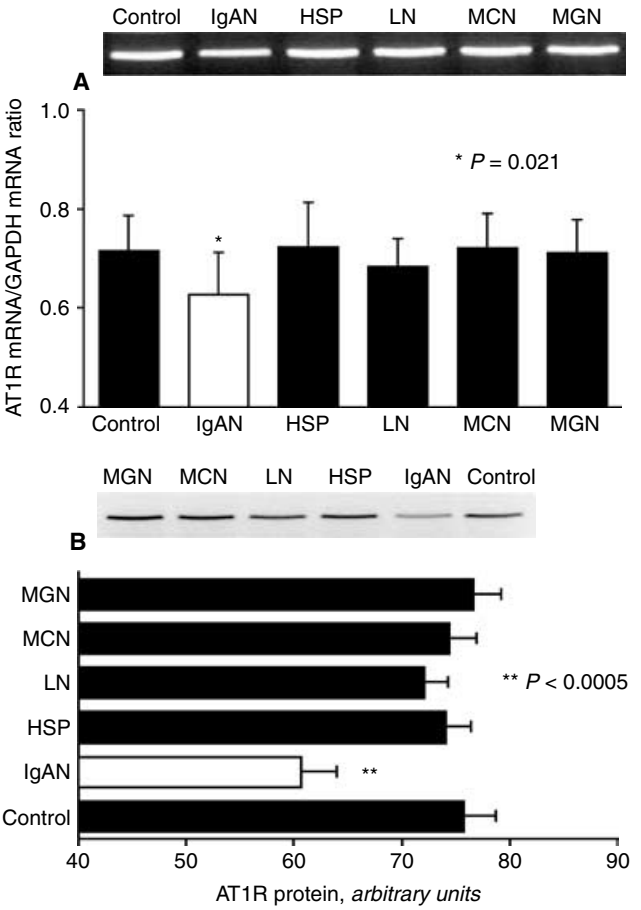

Fig. 9. (*A*) Angiotensin II type 1 receptor (AT1R) gene expression and (*B*) AT1R protein synthesis in cultured human mesangial cells incubated with pIgA1 (0.5 mg/mL) from normal controls, IgAN, HSP, lupus nephritis, MCN, and MGN. All IgAN patients and disease controls were in clinical quiescence. Polymeric IgA1 from patients with IgAN induced a greater suppression of AT1R mRNA or protein by HMC than pIgA1 from healthy or disease controls. * and ** signify $P < 0.025$ and $P < 0.005$ in AT1R mRNA and AT1R protein, respectively, compared with HMC incubated with pIgA1 from healthy or disease controls. HSP, Henoch-Schonlein purpura; MCN, minimal change nephropathy; MGN, membranous nephropathy. The results represent the mean ± SD.

expression is impaired in stroke-prone spontaneously hypertensive rats (SHR) kidney in vivo and in mesangial cells in vitro suggests the unbalanced expression of Ang II receptors with exaggerated AT1R signaling during early life in SHR may play a role in the programming for hypertension and related renal injury [26].

The RAS has been implicated in the development of progressive renal fibrosis in IgAN [27, 28]. Local Ang II hyperactivity is indirectly demonstrated by functional tests in IgAN patients [29]. Angiotensin II plays a pivotal role in the glomerulosclerosis through induction of TGF-β expression in mesangial cells [30]. Despite the presence of gene encoding for renin, angio-tensinogen, and ACE in mesangial cells in human kidney [9], little information is available about the RAS regulation in the human kidney, and particularly, in kidney diseases. The information of mesangial expression of Ang II receptors in glomerular disease is even scarcer. Using ^{125}I-labeled Ang II autoradiographic technique, Hale et al [31] first suggested a local down-regulation of Ang II receptors despite proliferation of glomerular mesangium in IgAN. In isolated glomeruli from 14 patients with IgAN, Wagner et al [32] reported a reduced gene expression of the AT1R. Although not significantly, an interesting trend toward higher AT1R mRNA levels was observed in patients on ACE inhibitors in that study. Most recently,

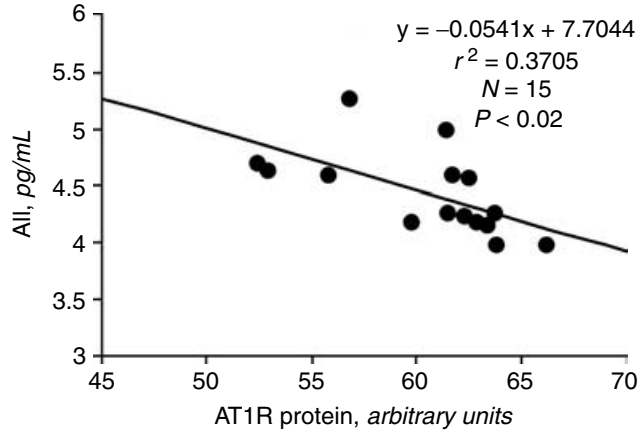

Fig. 10. Correlation between the Angiotensin II levels in the cell supernatant and angiotensin II type 1 receptor (AT1R) concentration of human mesangial cells incubated with pIgA1 from patients with IgAN.

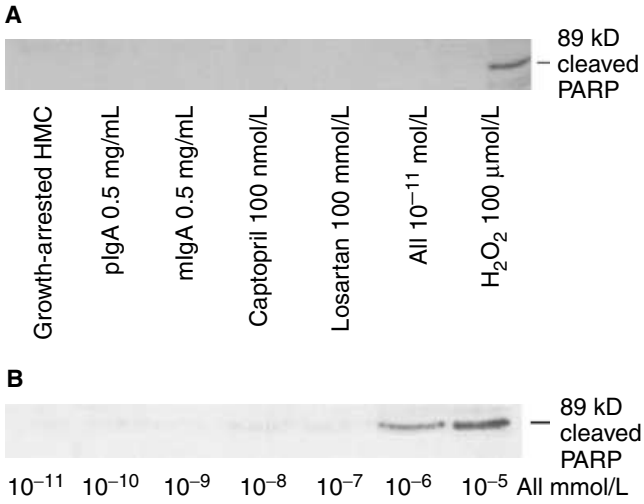

Fig. 11. (A) Western blot analysis of human mesangial cells incubated with culture medium with or without addition of Angiotensin II (Ang II) (10^{-11}mol/L), captopril (100 nmol/L), losartan (100 nmol/L), pIgA from IgAN (0.5 mg/mL), or mIgA from IgAN (0.5 mg/mL). Blots were probed with a monoclonal anti-PARP antibodies. Cleaved PARP fragments (89 kD) were not detected in these blots. Human mesangial cells incubated with H_2O_2 (100 µmol/L) presented a positive control. (*B*) Western blot analysis of human mesangial cells incubated with culture medium containing increasing concentration of Ang II. Cleaved PARP fragments (89 kD) were only just detected in blots with Ang II concentration of 10^{-7}mol/L.

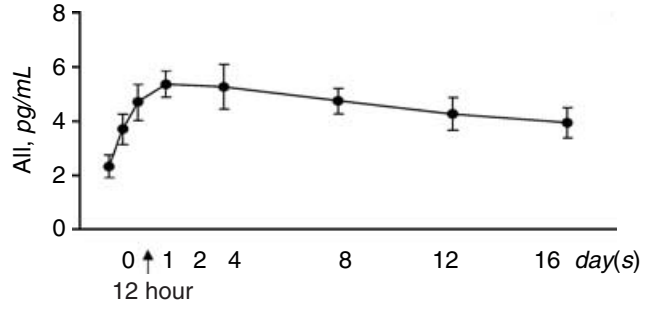

Fig. 12. Supernatant concentration of Angiotensin II (Ang II) in human mesangial cells undergoing prolonged culture. The cells were exposed to pIgA preparation from four patients with IgAN (0.5 mg/mL) at different time interval (from 12 hours to 16 days). Culture medium was changed every four days with fresh pIgA preparation (0.5 mg/mL) added. The concentration of Ang II in fresh culture medium (with added pIgA) before use for cell culture was 0 pg/mL.

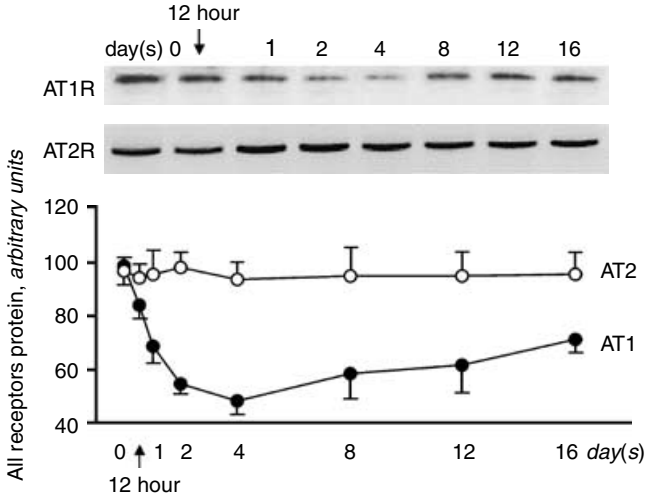

Fig. 13. Angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) protein expression in human mesangial cells undergoing prolonged culture. The cells were exposed to pIgA preparation from four patients with IgAN (0.5 mg/mL) at different time interval (from 12 hours to 16 days). The results represent the mean ± SD of four separate experiments. The upper panels depict representative Western blot analysis for AT1R and AT2R protein.

Del Prete et al [28] reported an overexpression of RAS genes (renin, angiotensinogen, and ACE) in kidney biopsy of IgAN. In isolated glomeruli, an inverse relationship between these RAS genes and mRNA encoding for AT1R or AT2R was observed. However, a paradoxic finding of lower glomerular Ang II receptor mRNA levels in controls than IgAN was observed since normal control subjects had lower intrarenal RAS activities.

In the present study, we examined the Ang II receptor expression in glomerular mesangial cells in IgAN. The understanding of the local expression of RAS and Ang II receptors in mesangial cells is important in IgAN, as the nephritis is developed following mesangial deposition of IgA mainly of the pIgA subclass. Although tubulointerstitial damage is a better prognostic predictor than glomerular damage in IgAN, IgA deposits are rarely detected in renal tubules [33]. These pathologic findings suggest the tubulointerstitial damage in IgAN is not a direct event between pIgA1 and renal tubules, but rather an indirect sequel possibly mediated through soluble factor(s) released following interaction between IgA deposits and glomerular mesangial cells. Recently, we have demonstrated that pIgA1 from patients with IgAN is capable of inducing macrophage migration inhibitory factor and tumor necrosis factor-α production, and up-regulating

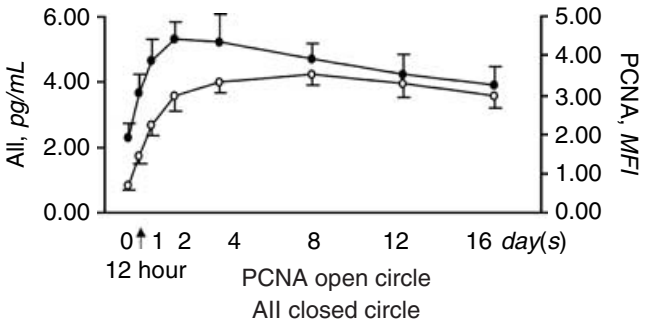

Fig. 14. Proliferative activity (measured by expression of PCNA) in human mesangial cells undergoing prolonged culture. The cells were exposed to culture medium alone, culture medium with added pIgA preparation from patients with IgAN (0.5 mg/mL), or culture medium with added IgG preparation from patients with IgAN (0.5 mg/mL). Culture medium was changed every four days. There was progressive increase in cell proliferation of the cultured mesangial cells (MC) exposed to pIgA preparation from patients with IgAN, but not for MC exposed to culture medium alone or culture medium supplemented with IgG from patients with IgAN.

renin, Ang II, and TGF-β synthesis in HMC [34, 35]. In this study, we detected immunoreactive AT1R or AT2R protein in mesangial cells in glomerulus from normal or IgAN kidney. These receptors were also detected in tubular epithelium and arteriolar endothelium. Semiquantitative analysis showed a reduction of AT1R protein in HMC from IgAN, but not for the AT2R protein. Our finding is compatible with those of Hale et al [31] and Wagner et al [32], supporting a local down-regulation of AT1R in IgAN resulting from the negative feedback due to enhanced intraglomerular RAS, and hence, Ang II activity [36, 37]. Our finding contrasts that of Del Prete et al [28], in which gene expression rather than protein translation for Ang II receptors and whole glomerulus rather than mesangial cells were studied. Furthermore, our studied subjects had a lower sodium-controlled diet (40% less) than those studied by Del Prete et al [28].

Although an extension of our histologic findings to an animal model of IgAN or an Ang II receptor knockout animal model could be potentially useful in validating our postulation, existing experimental [38] or transgenic [39] mouse models of IgAN are far from satisfaction because none of them truly demonstrates all the essential clinical and immunopathologic features of human IgAN. Information inferred from transgenic mice does not even correlate with human data [40]. The structure of human IgA1 has no parallel, and IgA clearance mechanisms, systemic and mucosal compartmentalization, and handling differ across the species [41]. Instead, we further evaluated our histologic findings by in vitro experiments studying the regulatory effect of IgA on the expression of these receptors in cultured HMC. Polymeric IgA1 and mIgA1 were selectively isolated from serum of our patients with mild disease to determine the time- and dose-related effect of different IgA fractions on the RAS in HMC. For subsequent inhibitory studies and comparisons between patients and controls, a concentration of 0.5 mg/mL was used for all IgA preparation in cell culture. This is comparable to the serum concentration of pIgA concentration (i.e., 0.35 mg/mL) in IgAN patients with an average serum total IgA of 3 g/L, of which 11% to 13% are IgA1 of polymeric nature. In resting HMC, incubation with either an ACEI (captopril) or an AT1R antagonist (losartan) resulted in increased expression of AT1R in a dose-dependent manner. With decreased binding of Ang II to its receptor on HMC following blockade of the RAS, the increased expression of AT1R is likely to mediate via the negative feedback loop. More interestingly, pIgA1 from patients with IgAN also significantly down-regulated the gene and protein expression of AT1R in a dose-dependent manner, reaching a 17% protein reduction at a concentration of 0.5 mg/mL. The AT1R expression was restored to that of resting cells when HMC preincubated with pIgA from patients with IgAN were treated with either captopril or losartan. AT2R expression was not affected by incubation with pIgA1 at these concentrations. The supernatant concentration of Ang II (10^{-11} mol/L) in HMC released by incubating with pIgA was too low to induce apoptosis via the binding to AT2R [21]. One may argue that our finding in resting cells may differ when similar experiments are conducted in proliferating cells [42]. We accepted our present experimental condition as the in vitro findings parallel those in the renal biopsy from IgAN patients. Our initial in vitro studies were performed using IgA isolated from randomly selected patients with mild histologic grading during clinical quiescence. The suppressive effect of pIgA1 from patients with IgAN on the RAS in mesangial cells was confirmed in another 15 patients with various histologic grading. Polymeric IgA1 from IgAN patients down-regulated the AT1R expression in HMC when compared with cells incubated with pIgA1 from healthy or disease control subjects comprising different glomerulonephritides. Of note is the different finding between IgAN and HSP, despite the latter having similar renal histopathology. This could be due to the difference in the size of circulating IgA immune complexes, the subclass of IgA (λ− versus κ−light chain) deposited in the mesangium, and the regulation of B cells by T lymphocytes between the two glomerulonephritides (reviewed in [43]). The regulatory effect of pIgA1 from patients with IgAN on the AT1R and Ang II synthesis in HMC shown in this and our recent studies [34, 35], and the recent finding of increased nitric oxide generation under blockade of RAS only in IgAN patients but not in healthy subjects [44] reflects the uniqueness and importance of the RAS, as well as the unique pathogenetic role of pIgA1 in IgAN.

The most interesting issue is the pathophysiologic significance of the down-regulation of mesangial AT1R in

IgAN. Regulation of Ang II receptors in renal tissues is complex. In general, AT1R stimulates cell proliferation while AT2R regulates cell growth inhibition and apoptosis [45, 46]. Previous studies showed a down-regulation of glomerular AT1R mRNA in chronic renal failure, including diabetic nephropathy and IgAN [32, 47]. Ang II infusion studies in animal showed a reduction of AT1R mRNA level in mesangial cells following exposure to Ang II [36, 37]. The down-regulation is presumed to mediate through the negative feedback due to enhanced intraglomerular Ang II activity. This hypothesis is supported by the dose-response findings between RAS blockade and the expression of AT1R, and the negative correlation between supernatant Ang II levels and AT1R expression in HMC incubated with pIgA in our in vitro experiments. It has been speculated that a decrease in AT1R mRNA levels mirrors an adaptive response to high intrarenal Ang II levels in human glomerulonephritis and diabetes mellitus [32]. The obvious question is why such adaptive mechanism operates in glomerulonephritides such as IgAN. In IgAN, we hypothesize that this could arise from the differential expression of Ang II receptors in mesangial cells following the release of Ang II by polymeric IgA. In renal proximal tubular cells exposed to Ang II, the growth-stimulatory effects through AT1R may be counter-balanced by AT2R-mediated apoptosis and growth inhibition [18, 48]. Despite the fact that pIgA from patients with IgAN induces Ang II release from HMC, our novel findings suggest that the Ang II level (10^{-11} mol/L) is unlikely to be sufficient to stimulate the apoptotic (or even the antiproliferative) effect via enhanced expression of AT2R. Our data reveal the Ang II concentration required to induce apoptosis in HMC ($>10^{-7}$ mol/L) is 100-fold higher than that of human renal proximal tubules (10^{-9} mol/L) [21]. Unbalanced Ang II receptor expression with exaggerated AT1R has shown to contribute to hypertension and related renal injury in different experimental models [26, 49]. Hence, we postulate that the defective counterbalance of AT1R by AT2R in HMC exposed to pIgA from IgAN patients leads to an adaptive down-regulation of the AT1R. An immediate down-regulation of mesangial AT1R expression will ameliorate the proliferative and inflammatory changes induced by Ang II released by pIgA. However, we envisage this adaptive mechanism in HMC may gradually be altered with chronic exposure to Ang II released by pIgA in IgAN. Failure to suppress the AT1R expression continuously in the presence of defective AT2R activation is likely to permit the development of proliferative and inflammatory processes in the glomerulus that may finally lead to progression of renal deterioration in IgAN. This hypothesis has lent support from our study of HMC following prolonged exposure to pIgA from IgAN. The adaptive down-regulation of AT1R gradually disappeared despite the maintenance of significant Ang II concentration in the supernatant. Furthermore, there was progressive increase in cell proliferation following prolonged culture of HMC with exposure to pIgA, but not with plain culture medium or IgG from the same disease subject.

CONCLUSION

Our findings support an altered AT1R expression in HMC in response to raised intrarenal Ang II concentration in IgAN. An imbalance of AT1R and AT2R activity in HMC following exposure to pIgA may play a significant pathogenetic role in the glomerular inflammation in IgAN.

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REFERENCES

1. Ketteler M, Noble NA, Border WA: Increased expression of transforming growth factor-β in renal disease. *Cur Opin Nephrol Hyperten* 3:446–452, 1994
2. Hilgers KF, Mann JFE: ACE inhibitors versus AT1 receptor antagonists in patients with chronic renal failure. *J Am Soc Nephrol* 13:1100–1108, 2002
3. Nakamura N, Soubrier F, Menard J, *et al*: Nonproportional changes in plasma renin concentration, renal renin content, and rat renin messenger RNA. *Hypertension* 7:855–859, 1989
4. Wagner J, Volk S, Haufe CC, *et al*: Renin gene expression in human kidney biopsies from patients with glomerulonephritis or graft rejection. *J Am Soc Nephrol* 5:1469–1475, 1995
5. Schena FP: A retrospective analysis of the natural history of primary IgA nephropathy worldwide. *Am J Med* 89:209–215, 1990
6. Lai KN, Ho RT, Leung JCK, *et al*: Increased mRNA encoding for transforming growth factor-β in CD4+ cells from patients with IgA nephropathy. *Kidney Int* 46:862–868, 1994
7. Lai KN, Leung JCK: Heat-aggregated IgA prepared from patients with IgA nephropathy increases calcium mobilization and superoxide production of human neutrophils in vitro. *Nephron* 64:129–135, 1993
8. Lai KN, Leung JCK, Li PKT: Heat-aggregated IgA prepared from patients with IgA nephropathy increases priming of human neutrophils to produce inositol triphosphate following FMLP stimulation in vitro. *Nephron* 69:1–8, 1995
9. Lai KN, Leung JCK, Lai KB, *et al*: Gene expression of the renin-angiotensin system in human kidney. *J Hypertension* 16:91–102, 1998
10. Galla JH: Molecular genetics in IgA nephropathy. *Nephron* 88:107–112, 2001
11. Mifune M, Sasamura H, Nakazato Y, *et al*: Examination of angiotensin II type 1 and type 2 receptor expression in human kidneys by immunochemistry. *Clin Exp Hypertens* 23:257–266, 2001
12. Lai KN, Ho CP, Chan KW, *et al*: The nephrotic range proteinuria—A good predictive index of progressive disease in IgA nephropathy? *Quart J Med* 57:677–688, 1985

13. Yang N, Nikolic-Paterson DJ, Ng YY, *et al*: Reversal of established rat crescentic glom-erulonephritis by blockade of macrophage migration inhibitory factor (MIF): Potential role of MIF in regulating glucocorticoid production. *Mol Med*. 4:413–424, 1998
14. Leung JCK, Tang SCW, Lam MF, *et al*: Charge dependent binding of polymeric IgA1 to human mesangial cell in IgA nephropathy. *Kidney Int* 59:277–285, 2001
15. Lai KN, Li FK, Lan HY, *et al*: Expression of aquaporin 1 in human mesothelial peritoneal cells and its upregulation by glucose in vitro. *J Am Soc Nephrol* 12:1036–1045, 2001
16. Leung JCK, Tsang AW, Chan TM, Lai KN: Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. *J Am Soc Nephrol* 11:241–249, 2000
17. Volland H, Pradelles P, Ronco P, *et al*: A solid-phase immobilized epitope immunoassay (SPIE-IA) permitting very sensitive and specific measurement of angiotensine II in plasma. *J Immunol Methods* 228:37–47, 1999
18. Bhaskaran M, Reddy K, Radhakrishanan N, *et al*: Angiotensin II induces apoptosis in renal proximal tubular cells. *Am J Physiol Renal Physiol* 284:F955–F965, 2003
19. Goldenberg I, Grossman E, Jacobson KA, *et al*: Angiotensin II-induced apoptosis in rat cardiomyocyte culture: A possible role of AT1 and AT2 receptors. *J Hypertens* 19:1681–1689, 2001
20. Busche S, Gallinat S, Bohle R, *et al*: Expression of angiotensin AT1 and AT2 receptors in adult rat cardiomyocytes after myocardial infarction. *Am J Pathol* 157:605–611, 2000
21. Weidekamm C, Hauser P, Hansmann C, *et al*: Effect of AT1 and AT2 receptor blockade on angiotensin II induced apoptosis of human renal proximal tubular epithelial cells. *Wien Klin Wochenschr* 114:725–729, 2002
22. Xie MH, Liu FY, Wong PC, *et al*: Proximal nephron and renal effects of Dup753, a non-peptide angiotensin II receptor antagonist. *Kidney Int* 38:473–479, 1990
23. Zheng Y, Horita S, Hara C, *et al*: Biphasic regulation of renal proximal bicarbonate absorption by luminal AT(1A) receptor. *J Am Soc Nephrol* 14:1116–1122, 2003
24. Ray PE, Aguilera G, Kopp JB, *et al*: Angiotensin II receptor mediated proliferation of human fetal mesangial cells. *Kidney Int* 40:764–771, 1991
25. Higueruelo S, Romero R: Angiotensin II requires PDGF-BB to induce DNA synthesis in rat mesangial cells cultured in an exogenous insulin free medium. *Nephrol Dial Transplant* 12:694–700, 1997
26. Goto M, Mukoyama M, Sugawara A, *et al*: Expression and role of angiotensin II type 2 receptor in the kidney and mesangial cells of spontaneously hypertensive rats. *Hypertens Res* 25:125–133, 2002
27. Remuzzi A, Perico N, Sangalli F, *et al*: ACE inhibition and ANG II receptor blockade improve glomerular size-selectivity in IgA nephropathy. *Am J Physiol* 276:F457–F466, 1999
28. Del Prete D, Gambaro G, Lupo A, *et al*: Precocious activation of genes of the renin-angiotensin system and the fibrogenic cascade in IgA glomerulonephritis. *Kidney Int* 64:149–159, 2003
29. Coppo R, Amore A, Gianoglio R, *et al*: Angiotensin II local hyperactivity in progression of IgA nephropathy. *Am J Kidney Dis* 21:593–602, 1993
30. Kagami S, Border WA, Miller DE, Noble NA: Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. *J Clin Invest* 93:2431–2437, 1994
31. Hale GM, Howarth GS, Aarons I, *et al*: Quantitation of glomerular angiotensin II receptors in IgA nephropathy. *Clin Nephrol* 32:5–9, 1989
32. Wagner J, Gehlen F, Ciechanowicz A, Ritz E: Angiotensin II receptor type 1 gene expression in human glomerulonephritis and diabetes mellitus. *J Am Soc Nephrol* 10:545–551, 1999
33. Habib R, Niaudet P, Levy M: Schonlein-Henoch purpura nephritis and IgA nephropathy, in *Renal Pathology, with Clinical and Functional Correlations*, 2nd ed., edited byTisher CC, Brenner BM, Philadelphia, JB Lippincott, 1994, pp 472–523
34. Leung JC, Tang SC, Chan LY, *et al*: Polymeric IgA increases the synthesis of macrophage migration inhibitory factor by human mesangial cells in IgA nephropathy. *Nephrol Dial Transplant* 18:36–45, 2003
35. Lai KN, Tang SC, Guh JY, *et al*: Polymeric IgA1 from IgA nephropathy up-regulates synthesis and signal transduction of transforming growth factor-β in human mesangial cell via the renin-angiotensin system. *J Am Soc Nephrol* 14:3127–3137, 2003
36. Makita N, Iwai N, Inagami T, Badr K: Two distinct pathways in the down-regulation of type-1 angiotensin II receptor gene in rat glomerular mesangial cells. *Biochem Biophys Res Commun* 185:142–146, 1992
37. Chansel D, Bizet T, Vandermeersch S, *et al*: Differential regulation of angiotensin II and losartan binding sites in glomeruli and mesangial cells. *Am J Physiol* 266:F384–393, 1994
38. Montinaro V, Gesualdo L, Schena FP: The relevance of experimental models in the pathogenetic investigation of primary IgA nephropathy. *Ann Med Interne (Paris)* 150:99–107, 1999
39. Zheng F, Kundu GC, Zhang Z, *et al*: Uteroglobin is essential in preventing immunoglobulin A nephropathy in mice. *Nat Med* 5:1018–1025, 1999
40. Coppo R, Chiesa M, Cirina P, *et al*: In human IgA nephropathy uteroglobin does not play the role inferred from transgenic mice. *Am J Kidney Dis* 40:495–503, 2002
41. Monteiro RC, Van De Winkel JG: IgA Fc receptors. *Annu Rev Immunol* 21:177–204, 2003
42. Blagosklonny MV: Apoptosis, proliferation, differentiation: In search of the order. *Semin Cancer Biol* 13:97–105, 2003
43. Davin J, ten Berge IJ, Weening JJ: What is the difference between IgA nephropathy and Henoch-Schonlein purpura nephritis? *Kidney Int* 59:823–834, 2001
44. Roccatello D, Mengozzi G, Gigliola G, *et al*: Effect of angiotensin II blockade on nitric oxide blood levels in IgA nephropathy. *Nephrol Dial Transplant* 15:988–993, 2000
45. Matsubara H: Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ Res* 83:1182–1191, 1998
46. Franco M, Paniagua R, Herrera-Acosta J: Renal effects of renin-angiotensin system blockade. *Curr Opin Nephrol Hypertens* 7:153–158, 1998
47. Cao Z, Bonnet F, Candido R, *et al*: Angiotensin type 2 receptor antagonism confers renal protection in a rat model of progressive renal injury. *J Am Soc Nephrol* 13:1773–1787, 2002
48. Wolf G: Angiotensin II and tubular development. *Nephrol Dial Transplant* 17(Suppl 9):48–51, 2002
49. Sahajpal V, Ashton N: Renal function and angiotensin AT1 receptor expression in young rats following intrauterine exposure to a maternal low-protein diet. *Clin Sci* 106:607–614, 2003