Absence of CD89, Polymeric Immunoglobulin Receptor, and Asialoglycoprotein Receptor on Human Mesangial Cells

JOSEPH C. K. LEUNG, ANITA W. L. TSANG, DANIEL T. M. CHAN, and KAR NENG LAI

Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong.

Abstract. IgA nephropathy (IgAN) is characterized by raised serum IgA and predominant mesangial IgA deposits of polymeric nature. The expression of IgA receptor molecules in white blood cells and glomerular mesangial cells has recently attracted much attention in relation to the uptake of IgA by these cells. This study investigates the expression of IgA Fc receptor (FcαR1 or CD89), asialoglycoprotein receptor (ASGPR), and polymeric Ig receptor (pIgR) in cultured glomerular mesangial cells. Using a sensitive nested reverse transcription-PCR, mRNA encoding for FcαR1, pIgR, or the H2 chain of ASGPR was not demonstrated on human mesangial cells. U937, HepG2, and HT29 cell lines, used as positive controls, strongly expressed the FcαR1, ASGPR, and pIgR mRNA, respectively, under similar experimental conditions. Flow cytometry also demonstrated the presence of surface proteins for FcαR1, ASGPR, and pIgR on the respective control cell lines but not on human mesangial cells. Expression of FcαR1 mRNA on cultured U937 cells was upregulated by tumor necrosis factor-α. However, tumor necrosis factor-α, interleukin-1β, or transforming growth factor-β failed to induce the expression of FcαR1 on human mesangial cells. Human serum IgA or secretory IgA bound to human mesangial cells, HepG2, or the U937 cell line in a dose-dependent manner. The binding of purified IgA to human mesangial cells was not blocked by preincubation with human IgG, IgM, orosomucoid, asialoorosomucoid, anti-CD89 antibody (My43), or anti-secretory component antibody. The present study concluded that there was an absence of FcαR1, ASGPR, or pIgR on human mesangial cells. These findings suggest that the predominant binding of human IgA to human mesangial cells is mediated by other mechanisms.

IgA nephropathy (IgAN), recognized as the most common glomerulonephritis worldwide, is characterized by the mesangial deposition of IgA_1 subclass. Immunoregulatory abnormalities in IgA synthesis have been documented in IgAN, and these include raised serum IgA levels (1,2), overproduction of IgA_1 by B lymphocytes *in vitro* (3,4), raised serum levels of IgA-containing immune complexes (5,6), and IgA of anionic nature (7,8). Nevertheless, how IgA molecules interact with mesangial cells leading to glomerular and interstitial injury in IgAN remains unclear.

There are three known IgA receptors: FcαR1 (CD89), asialoglycoprotein receptor (ASGPR), and polymeric Ig receptors (pIgR). The human FcαR is a membrane glycoprotein that contains two extracellular Ig-like domains, a membrane-spanning region, and a cytoplasmic tail of 31 amino acids. FcαR1 binds both the monomeric and dimeric forms of IgA_1 and IgA_2 (9,10). Transfection studies in leukocyte showed that the FcαR1 does not bind IgG (11). FcαR1 was originally found to be expressed by neutrophils, monocytes, macrophages, and eosinophils (12). It was proposed that FcαR1 plays a role in the removal of IgA-antigen complexes from the circulation (13).

The asialoglycoprotein receptor is a C-type lectin that recognizes galactose and *N*-acetylgalactosamine residues of desialylated glycoproteins and mediates endocytosis of serum glycoproteins (14,15). It has been suggested that ASGPR provides a degradative pathway in glycoprotein homeostasis. The human ASGPR is an integral transmembrane glycoprotein composed of two units, H1 and H2. In rat, expression of cDNA of both H1 (RHL-1) and H2 (RHL-2/3) is required to generate high-affinity asialoglycoprotein binding sites in transfected hepatoma and fibroblast cells, even though each subunit has a functional carbohydrate domain (16). Although ASGPR is thought to be exclusively present in liver cells, several investigators have shown that mRNA for rat RHL-1 and RHL2/3 are widely expressed in different tissues and cell lines (17,18).

pIgR is an integral membrane secretory component localized on the basolateral surface of secretory epithelial cells. It mediates the transepithelial transport of polymeric Ig, particularly, polymeric IgA (19,20). pIgR is detected in most human secretory epithelia, including intestine, bronchus, salivary glands, renal tubule, and uterus (21). The pIgR neutralizes extracellular and intracellular pathogens in mucous membranes by epithelial transport of polymeric IgA-pathogen complexes and then excretes them via epithelial transcytosis (22).

Recent works reported the detection of mRNA for FcαR1 in cultured mesangial cells (23,24). The expression was upregulated by interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α),

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Correspondence to Dr. Kar Neng. Lai, Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong. Phone: 852 285 54251; Fax: 852 281 62863; E-mail: knlai@hkucc.hku.hk

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or interferon-γ (IFN-γ) (25). These results suggested that receptors for the Fc portion of IgA (FcαR1) may be responsible for the mesangial binding of IgA-immune complexes. Lately, *in vitro* studies have indirectly suggested that rat and human mesangial cells (HMC) possess ASGPR with specificity for the terminal galactose residues of several glycoproteins, including IgA_1 (26). This raises the notion that ASGPR participates in the mesangial deposition of IgA_1. In the present study, we studied the protein and gene expression of these IgA binding receptors in HMC and other cell types. The effect of various cytokines on the regulation of receptor expression was examined.

Materials and Methods

Materials

Monoclonal anti-human FcαR1: clone A59 was purchased from Pharmingen (San Diego, CA); clone A3 was obtained from Coulter-Immunotech (Miami, FL); and clone My43 was a gift from Dr. R. C. Monterio (Necker Hospital, Paris, France). Rabbit monospecific anti-human ASGPR H1 and H2 antisera were kindly provided by Dr. M. A. Shia (Boston University School of Medicine, Boston, MA). Monoclonal and polyclonal antibodies were used for the analysis of pIgR: clone GA-1 was obtained from Sigma (St. Louis, MO), and polyclonal antibody was purchased from Dako (Kyoto, Japan). Fluorescein-conjugated rabbit anti-mouse Ig and the FITC isotypic control antibodies used for flow cytometry were obtained from Dako. TNF-α, IL-1β, and transforming growth factor-β (TGF-β) were purchased from Boehringer Mannheim (East Sussex, United Kindgom). Serum IgA and secretory IgA were obtained from ICN Pharmaceuticals (Aurora, OH). All other chemicals were obtained from Sigma. Asialo-orosomucoid was prepared from desialylation of human orosomucoid with neuraminidase (0.03 U/mg protein) by incubation for 8 h at 37°C in 0.1 M sodium acetate buffer, pH 5.0. The FITC-labeled IgA was obtained from Pierce (Rockford, IL). The fluorescein-conjugated IgA was prepared from normal human serum IgA with a fluorescein: protein ratio of 3.1 moles FITC per mole IgA. The FITC-labeled IgA contained 9.5% of high molecular mass IgA as determined by size exclusion chromatography.

Cell Lines, Tissue, and Cell Culture

Cell lines were obtained from American Type Culture Collection (Rockville, MD), and these included the monocytic line U937 and THP-1, the myeloid line K562, the hepatocellular carcinoma line HepG2, the T cell leukemia line Jurkat, the promyelocytic line HL60, and the colonic adenocarcinoma line HT29. Human colonic samples obtained during laparotomy from patients undergoing bowel surgery were stored at −70°C until extraction for total RNA. Isolation and characterization of HMC were performed as described previously (27). Glomerular cells from 10 different donors were studied. Glomeruli were prepared from the cortex of human cadaveric kidney judged 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 supplemented with glutamine (2 mmol/L), Hepes (10 mmol/L), penicillin (50 U/ml), streptomycin (50 μg/ml), and 20% fetal calf serum in an atmosphere of 5% CO_2/95% air. Mesangial cells have a stellate appearance and grow in clumps. They show a network of intracellular fibrils of myosin, and they contract in the presence of 1 nmol/L angiotensin II. In studies of gene expression or protein synthesis, cultured cells grown to confluence in T75 tissue culture medium were first equilibrated with serum-free medium for 48 h before incubating with serum-free culture medium, TNF-α, TGF-β, or IL-1β overnight for 16 h at 37°C. Total RNA was extracted and stored at −70°C until analysis of gene expression. In duplicated sets of experiments, cells were harvested for detection of cell surface receptors by flow cytometry.

Total RNA Extraction and Reverse Transcription-PCR

Total RNA was extracted from 2×10^6 cells using Qiagen RNeasy kit (Hilden, Germany). All RNA samples prepared from 2×10^6 cells were dissolved in 20 ml of diethyl pyrocarbonate-H_2O and were stored at −70°C until assay. The quality of RNA was checked by formaldehyde agarose gel electrophoresis, and 2 μl of total RNA contained approximately 0.1 μg of RNA. The mRNA was reverse-transcribed with Superscript II using random hexamers (Life Technologies BRL, Gaithersburg, MD). The receptor expression was examined by nested PCR using specific pairs of primer under specific conditions (Table 1). For the second round of PCR, 1 μl of first-round PCR product was used. Twenty microliters of the PCR products was analyzed by agarose gel electrophoresis and staining with ethidium bromide. High level of sensitivity with nested PCR may easily lead to false positive results due to minute contamination. The following precautions were taken to ensure the validity of the results. (*1*) Precautions to avoid carryover of PCR product included physical separation of pre-PCR and post-PCR mixtures and aliquots of reagents. (*2*) The results were considered valid only if these were consistent in repeated (four times) independent experiments. For PCR-involved human glomerular mesangial cells, total RNA were isolated from 10 different donors and the PCR results were considered valid only if all findings from mRNA of different donors were consistent. (*3*) Positive controls included literature-cited cell lines that are known to express specific receptors. (*4*) Negative controls included cells that are not known to produce the receptors, and a reaction mixture without RNA or DNA was run in each experiment. (*5*) Negative controls for PCR consisted of reagent control in which RNA was replaced by diethyl pyrocarbonate-distilled H_2O. (*6*) Confirmation of PCR signal for RNA was performed by repeating the PCR without reverse transcription (RT). (*7*) Further validation of PCR signal for RNA was achieved by treating the extracted RNA with DNase-free RNase before RT-PCR. (*8*) Integrity of the RT products from RNA isolated from various cell types was ensured by examining the gene expression of a housekeeping gene, glyceraldehyde 3-phosphate-dehydrogenase (GAPDH).

Verification of the PCR Products

The PCR product of the mRNA encoding for the IgA receptor was verified by direct sequencing with standard technique (27). Briefly, amplified PCR products were electrophoresed in 1.5% low melting point agarose gel (Life Technologies). Gel slices corresponding to the correct products were cut out. DNA from the excised fragments was purified by the QIAEX gel extraction kit (Qiagen). The sequencing primers were labeled at the 5′ end with 3 U/ml T4 polynucleotide kinase (New England Biolabs, Beverly, MA) in 70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 10 mM dithiothreitol with 5 ml [γ^{32}P]-adenoside triphosphate (ATP > 5000 Ci/mmol) (Amersham, Buckinghamshire, United Kingdom) at 37°C for 30 min, followed by 5 min of incubation at 90°C. Labeled primers were stored at −20°C until use. Cycle sequencing reaction was performed using a double-stranded DNA cycle sequencing system (Life Technologies). The sequence was analyzed by electrophoresis in 6% polyacrylamide gel (Life Technologies) with 7 M urea in 1× TBE (90 mM Tris-HCl, 90 mM borate, 2

Table 1. Primer sequence and size of PCR products for nested PCR[a]

PCR Product	5′ to 3′ Sequence of PCR Primers	Size (bp)
FcαR1		
outer primer	CCT GTG TCT TGT GCT CTG TC GAG GAA GTG AAA GCG GAA AG	799
inner primer	TCG GGG TCT GGT GTT GAT GC GGA AAG TGT GGT GGG GAA GC	415
ASGPR H1		
outer primer	CTC GCC TCC TCC TGC TCT CC CAC CCT ATC CTT CCC CTT CC	885
inner primer	AGC AAC TTC ACA GCG AGC AC TCC CGT CCA CCC ACT TCC AG	448
ASGPR H2		
outer primer	GCA GGC TGA ATC CCA GGA GA CCA TTG AAG AGG CTG ACG AT	1072
inner primer	TGG GCT GAG GCG GAG AAG TA AGG TGT GGG GTA TGG GTT AG	382
pIgR		
outer primer	GCC CGA GCT GGT TTA TGA AG AGC CGT GAC ATT CCC TGG TA	694
inner primer	GCA GTG GGG AAA ACT GTG AC GCT GCG GGG AAT CGT GGA CT	245
GAPDH	TGA AGG TCG GAG TCA ACG GAT TTG GT CAT GTG GGC CAT GAG GTC CAC CAC	984

[a] FcαR1, IgA Fc receptor; ASGPR, asialoglycoprotein receptor; pIgR, polymeric immunoglobulin receptor; GAPDH, glyceraldehyde 3-phosphate-dehydrogenase.

mM ethylenediaminetetra-acetic acid) at 1500 V for 3 h, vacuum-dried, and exposed to Kodak X-OMAT AR x-ray film (Eastman Kodak, Rochester, NY) in an intensifying screen at −70°C overnight.

Flow Cytometry

Various cell lines and HMC were grown to log phase and harvested using 0.05% trypsin/0.02% ethylenediaminetetra-acetic acid for 5 min at room temperature. The cells were adjusted to 5×10^6 per ml, and 200 μl of cell suspension was used in staining for various IgA receptors. All staining was done at 4°C with staining buffer (phosphate-buffered saline with 1% fetal bovine serum and 0.1% sodium azide). Background control staining was achieved by reaction with either preimmune FITC- $F(ab')_2$ or isotype-matched mouse IgG. The stained cells were analyzed using a Coulter EPICS XL analyzer (Coulter Electronic, Miami, FL). A minimum of 5000 fixed cells for each sample was analyzed. Fluorescence intensity was evaluated by comparing the mean fluorescence channels. The result was expressed as mean fluorescence intensity.

The possibility that IgA receptors are degraded by trypsinization was examined by comparing values from U937 cells with or without trypsinization. Moreover, after trypsinization, mesangial cells were cultured overnight in suspension with shaking to allow regeneration of surface proteins. The expression of surface proteins for CD89, ASGPR H1, H2, and pIgR was tested after overnight culture.

Inhibition Study

The effect of various potential blocking proteins on IgA binding was examined by flow cytometry. A total of 1×10^6 cells in staining buffer was incubated with different blocking proteins for 30 min at 4°C. After incubation, the cells were washed once with staining buffer before incubation with FITC-IgA. After incubation, the stained cells were washed and analyzed by flow cytometry as described.

Statistical Analyses

The results are expressed as mean ± SD. For comparison between patient and control groups, the unpaired *t* test was used.

Results

Expression of mRNA for FcαR1, pIgR, and ASGPR

Figure 1 depicts results of post-RT nested PCR for FcαR1. After the first round of PCR amplification, no visible PCR product was detected for FcαR1 except a band of PCR product with a size of 499 bp, which was the correct size for chloramphenicol acetyltransferase RNA that served as RT-PCR positive control. After the second round of PCR amplification, amplicons of FcαR1 were detected in U937, TNF-α-activated U937, THP-1, TNF-α-activated THP-1, and HL60. Visible amplicon for FcαR1 was not detected in K562, TNF-α-activated K562, HepG2, Jurkat, colonic tissue, HT29, or HMC.

Figure 2 illustrates results of post-RT nested PCR for pIgR. After the first round of PCR amplification, amplicons for pIgR were only found in colonic tissue. After the second round of PCR amplification, amplicons for pIgR were detected in TNF-

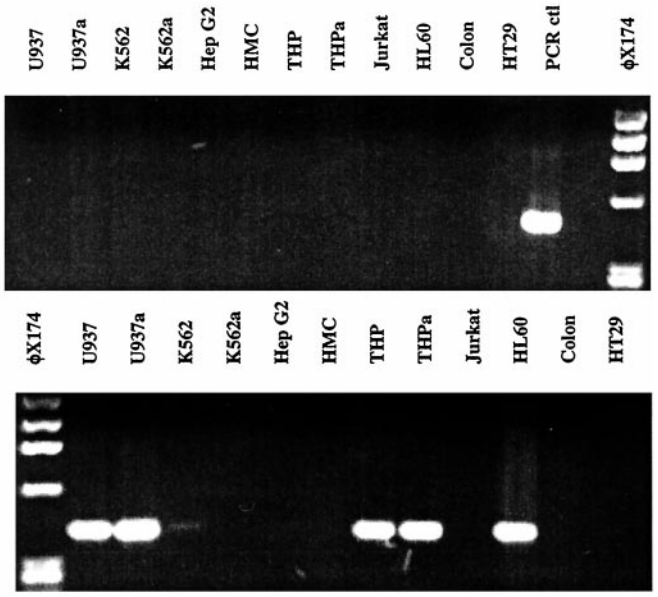

Figure 1. Demonstration of nested PCR products of FcαR1. (Top Panel) Results after first-round PCR. (Bottom Panel) Results after second-round PCR. U937a, K562a, and THPa represent cells activated with 1000 U/ml tumor necrosis factor-α (TNF-α). PCR ctl is the amplification of chloramphenicol acetyltransferase (CAT) gene, which serves as positive control for reverse transcription (RT)-PCR reaction.

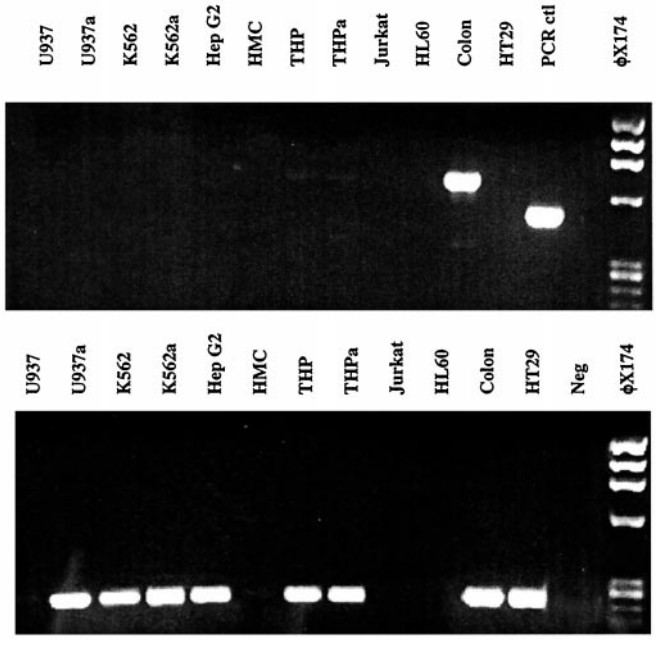

Figure 2. Demonstration of nested PCR products of polymeric Ig receptor. (Top Panel) Results after first-round PCR. (Bottom Panel) Results after second-round PCR. U937a, K562a, and THPa represent cells activated with 1000 U/ml TNF-α.

α-activated U937, K562, TNF-α-activated K562, HepG2, THP-1, TNF-α-activated THP-1, colonic tissue, and HT29. Visible amplicon for pIgR was not detected in unstimulated U937, Jurkat, HL60, or HMC.

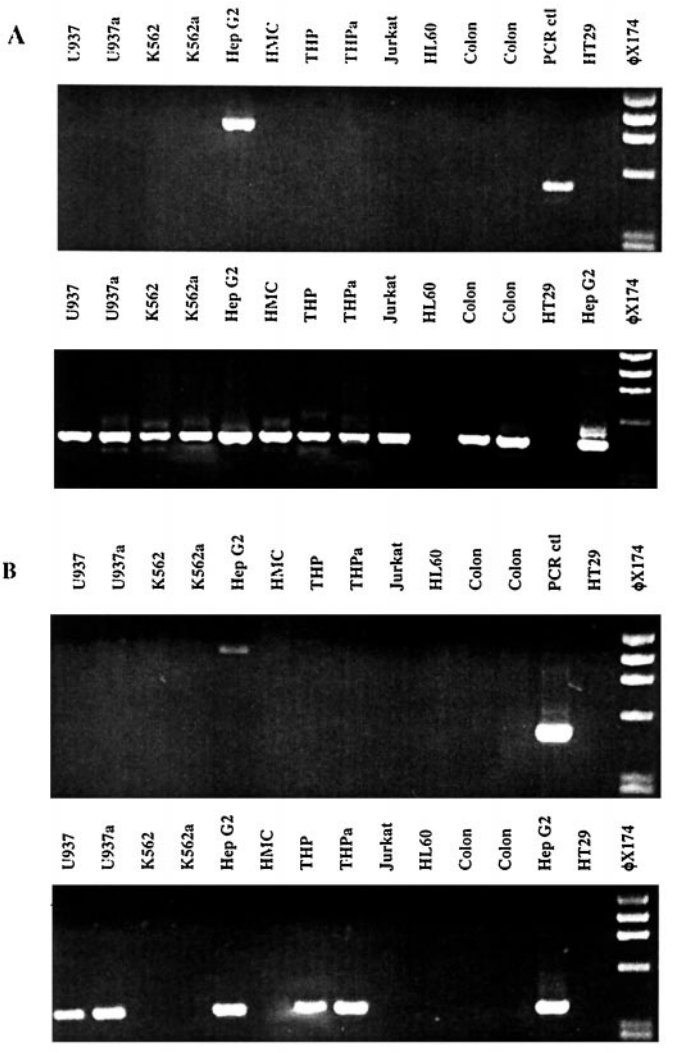

Figure 3. Demonstration of nested PCR products of asialoglycoprotein receptor H1 chain (A) and H2 chain (B). (Top Panels) Results after first-round PCR. (Bottom Panels) Results after second-round PCR. U937a, K562a, and THPa represent cells activated with 1000 U/ml TNF-α. PCR ctl is the amplification of CAT gene, which serves as positive control for RT-PCR reaction.

Figure 3 shows results of post-RT nested PCR for H1 and H2 chains of ASGPR. Amplicons for both H1 and H2 chains of ASGPR were only found in HepG2 after the first round of PCR amplification. After the second round of PCR amplification, amplicons for both H1 and H2 chains of ASGPR were detected in U937, TNF-α-activated U937, THP-1, TNF-α-activated THP-1, and HepG2. While only amplicons for H1 were detected in K562, TNF-α-activated K562, colonic tissue, and HMC, amplicons for H2 chain of ASGPR remained undetectable in these cells. No signal was found in HL60 and HT 29 after nested PCR.

To confirm the identity of amplified nucleotides, representative amplicons for PCR were sequenced. The nucleotide sequences were identical to those of sequences documented in GenBank (data not shown).

Table 2. Dose-dependent binding of IgA and expression of IgA receptors on cell surface of various types of cells[a]

Cell Type	Binding of sIgA and IgA									
	Secretory IgA (mg/ml)					Serum IgA (mg/ml)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
U937	3.25	11.20	19.58	29.41	32.15	2.97	8.56	15.56	26.58	28.65
HMC	3.16	7.45	21.72	30.56	35.54	2.89	6.58	22.54	35.68	41.46
HepG2	2.69	5.68	12.56	17.89	21.47	2.87	4.56	11.07	15.26	18.59
HT29	3.12	4.56	5.23	6.78	6.90	2.91	4.05	5.07	5.98	6.23

	Expression of IgA Receptor			
	CD89	ASGPR		pIgR
		H1	H2	
U937	+++	neg	neg	neg
HMC	neg	neg	neg	neg
HepG2	neg	++	++	neg
HT29	neg	neg	neg	+

[a] For IgA binding, results are expressed as the average MFI of two individual experiments. MFI, mean fluorescence intensity; sIgA, secretory IgA; HMC, human mesangial cells. Other abbreviations as in Table 1.

neg, MFI is no different from that of the isotypic control; +, MFI is 1.2- to twofold greater than that of the isotypic control; ++, MFI is 2- to 2.5-fold greater than that of the isotypic control; +++, MFI is at least 2.5-fold greater than that of the isotypic control.

Expression of Surface IgA Receptors

Table 2 summarizes the findings of a flow cytometry study for the expression of FcαR1, pIgR, or ASGPR on cell surface of U937, HepG2, HT29, and HMC. HMC did not express any of these receptors on the cell surface, whereas U937 and HT29 expressed FcαR1 and pIgR, respectively. HepG2 expressed both H1 and H2 chains of ASGPR. All four cell types bound to human serum IgA or secretory IgA in a dose-dependent manner.

There was no difference in the expression of CD89 on U937 cells with or without trypsinization (data not shown). After trypsinization, HMC expressed no CD89, ASGPR H1 or H2, or pIgR after overnight culture in suspension (data not shown).

Expression of FcαR1 after Stimulation by Cytokines

The receptor expression for FcαR1 on surface of U937 was studied with flow cytometry using three different monoclonal antibodies. Expression of FcαR1 was upregulated after overnight incubation with 1000 U/ml TNF-α (Table 3). However, expression of FcαR1 was not demonstrated on HMC under similar experimental conditions with or without addition of TNF-α.

Table 4 shows the quantification for the expression of FcαR1 on U937 or HMC using clone A59. With U937, the FcαR1 protein was readily detectable on the cell surface without stimulation. The mean fluorescence intensity increased after incubation with 1000 U/ml TNF-α (21.05 ± 2.79 *versus* 10.75 ± 1.13, $P < 0.01$), but not with IL-1β or TGF-β. Expression of FcαR1 on HMC was not demonstrated under similar experimental conditions with or without TNF-α, IL-1β, or TGF-β.

Table 3. Semiquantitative analysis of the expression of FcαR1 expression on U937 and HMC with and without stimulation by TNF-α (1000 U/ml) determined by flow cytometry using anti-FcαR1 clone, A59, A3, or My43[a]

Cell Type	Anti-CD89 mAb		
	A59	A3	My43
U937	++	+	+
U937; TNF-α, 1000 U/ml	+++	++	++
HMC	neg	neg	neg
HMC; TNF-α, 1000 U/ml	neg	neg	neg

[a] TNF-α, tumor necrosis factor-α; mAb, monoclonal antibody. Other abbreviations as in Tables 1 and 2.

Inhibition Study

Binding of FITC-labeled human IgA (FITC-IgA) to HMC, U937, HepG2, or HT29 was significantly inhibited by preincubation with 5 mg/ml human serum IgA or human secretory IgA isolated from colostrum ($P < 0.01$ or $P < 0.05$ for HT29) (Table 5). The FcαR1 blocking antibody (clone My43) significantly blocked the binding of FITC-IgA to U937 ($P < 0.01$) but not to HMC or HepG2. The binding of FITC-IgA to U937 was partially inhibited when preincubated with IgG. Binding of FITC-IgA to HepG2 was marginally inhibited by asialo-orosomucoid ($P < 0.05$). Binding of FITC-IgA to HT29 was significantly inhibited by IgM or anti-secretory component ($P < 0.01$). There was no inhibition for binding of FITC-IgA to HMC with other proteins tested (IgG, IgM, orosomucoid,

Table 4. Quantitative analysis of the expression of FcαR1 expression on U937 and HMC with and without stimulations by IL-1β (4 ng/ml), TGF-β (2 ng/ml), or TNF-α (1000 U/ml) determined by flow cytometry using anti-FcαR1 clone A59[a]

Cell Type	Preincubated with	MFI
HMC	Isotypic control	6.70 ± 0.17
HMC	Plain culture medium[b]	6.94 ± 0.35
HMC	IL-1β, 4 ng/ml	6.88 ± 0.38
HMC	TGF-β, 2 ng/ml	7.00 ± 0.25
HMC	TNF-α, 1000 U/ml	6.86 ± 0.15
U937	Isotypic control	3.28 ± 0.30
U937	Plain culture medium[b]	10.75 ± 1.13[c]
U937	IL-1β, 4 ng/ml	11.61 ± 0.85[c]
U937	TGF-β, 2 ng/ml	9.97 ± 1.15[c]
U937	TNF-α, 1000 U/ml	21.05 ± 2.79[c,d]

[a] Results are expressed as mean MFI ± SD of five individual experiments. IL-1β, interleukin-1β; TGF-β, transforming growth factor-β. Other abbreviations as in Tables 1 through 3.
[b] Serum-free plain culture medium.
[c] $P < 0.01$ compared with isotype control.
[d] $P < 0.01$ compared with medium control.

asialo-orosomucoid, My43, or anti-human secretory component).

Discussion

Receptors for IgA including FcαR1, pIgR, and ASGPR are expressed on various types of cells. The FcαR1 is found on human neutrophils (polymorphonuclear), monocytes, macrophages, eosinophils, and probably certain populations of lymphocytes. The pIgR is expressed on the basolateral surfaces of glandular epithelia and sinusoidal surfaces of hepatocytes. The ASGPR is expressed on the hepatocyte involving the clearance of glycoproteins with terminal galactose. In HMC, the existence of IgA receptors has not been fully resolved. Previous works have suggested that mesangial cells possess Fc receptors for IgA (23–25). However, recent works from several groups of investigators failed to demonstrate the expression of FcαR1 (CD89) by HMC (28–31), despite the fact that mesangial cells showed Fc-dependent IgA binding that was saturable and dose-dependent. It has been suggested that a novel IgA Fc receptor may be present instead of the prototype FcαR1 (31). In the present study, we confirmed that the FcαR1 is not expressed on the surface of cultured HMC, using highly sensitive nested RT-PCR and flow cytometry. On the contrary, the presence of FcαR1 on the monocytic cell line U937, used as a positive control, was well documented. We found that expression of FcαR1 on U937 was upregulated by TNF-α, but not by IL-1β or TGF-β. Bagheri *et al.* (25) reported that the proinflammatory cytokines IL-6, IFN-γ, and TNF-α upregulated mRNA expression for FcαR on HMC. Using the similar dose of cytokines, we failed to demonstrate the expression or induction of FcαR1 by TNF-α, IL-1β, or TGF-β. It remains unclear why FcαR1 is detected by some laboratories and not by others. Table 6 summarizes all published findings in the expression of FcαR1 on HMC. Most of them are *in vitro* studies using RT-PCR or Northern blot hybridization for detection of FcαR1 expression on cultured HMC. One may argue that the expression of FcαR1 will be lost as the number of cell passages increases. However, we had examined the FcαR1 expression in five individual lines of HMC using nested RT-PCR, and FcαR1 expression was not demonstrated from the first to the tenth passage (data not shown). Kashem *et al.* (32) had studied microdissected glomeruli from fresh renal biopsy of patients with IgAN. The expression of FcαR1 was only confined to 40% of biopsy samples, and the expression of FcαR1 correlated with the serum IgA level and the severity of the hematuria. Furthermore, neither Kashem *et al.* (32) nor we (unpublished data) were able to detect FcαR1 in the mesangium by immunofluorescence examination. It is possible that FcαR1 is only expressed on the mesangial cells in some of the patients with IgAN. With such a highly sensitive technique like PCR, the signals obtained from homogenized glomeruli were more likely to derive from infiltrating macrophages or neutrophils. Human and murine studies revealed that polymeric IgA (pIgA) had a stronger affinity and higher binding sites/cell to mesangial cells than monomeric IgA (33,34). If the mesangial binding of circulating IgA (>90% are monomeric) was only via the FcαR, such a manifold difference should not be expected. Although we and other investigators failed to demonstrate the expression of FcαR1 on HMC, our *in vitro* finding that human IgA binds to cultured mesangial cells in a dose-dependent manner leads us to postulate that there must be other receptors or mechanisms governing the mesangial binding of IgA_1.

Asialoglycoprotein receptor and pIgR are potential candidates for mediation of IgA binding to HMC. Using *in vitro* binding studies, Gómez-Guerrero *et al.* (26) first demonstrated that human and rat mesangial cells were able to specifically bind, internalize, and degrade I^{125}-labeled asialo-orosomucoid (ASOR) that was rich in terminal galactose. They also detected RHL-1 and RHL-2 transcripts in RNA extracted from rat mesangial cells. With IgA_1 partially inhibiting the binding of ASOR to HMC, they concluded that HMC expressed ASGPR. In this study, we found that RNA isolated from a number of cell types expressed the H1 transcript of the ASGPR, but the H2 transcript of human ASGPR remained undetected by nested RT-PCR. Furthermore, surface protein was not detected by flow cytometry. This suggests that expression of both H1 and H2 transcripts is essential for the translation or membrane transportation of ASGPR, and there may be posttranscriptional regulation. In contrast, using the same sets of primers and antibodies, we were able to detect ASGPR mRNA and protein on the control cell lines HepG2. Moreover, ASOR only barely inhibited the binding of FITC-IgA. All of this evidence suggests that ASGPR is unlikely to be expressed on cultured HMC. Data from our study point to some differences for the expression of ASGPR on rat and HMC. Next, we examined the expression of pIgR on HMC. Neither transcript nor surface protein for pIgR was detected in HMC. On the basis of these findings, the bulk of IgA bound to HMC is likely to be

Table 5. Inhibition study of competitor proteins on binding of FITC-IgA to HMC, HepG2, U937, or HT29[a]

Category	MFI			
	HMC	U937	HepG2	HT29
Isotype control	5.20 ± 0.14	3.37 ± 0.29	4.94 ± 0.79	5.04 ± 0.22
FITC-IgA, 0.5 mg/ml	14.98 ± 0.71	12.85 ± 1.00	14.44 ± 1.43	6.34 ± 0.46
Preincubation with the following proteins before FITC-IgA incubation				
IgA, 5 mg/ml	6.34 ± 0.10[b]	4.03 ± 0.74[b]	5.95 ± 1.04[b]	5.68 ± 0.41[c]
secretory IgA, 5 mg/ml	7.45 ± 0.60[b]	4.04 ± 0.51[b]	5.42 ± 0.45[b]	6.06 ± 0.25
IgG, 5 mg/ml	14.96 ± 0.19	11.98 ± 1.14	13.31 ± 1.25	6.22 ± 0.36
orosomucoid, 5 mg/ml	15.22 ± 0.52	12.99 ± 0.99	14.25 ± 1.77	6.21 ± 0.20
ASOR, 5 mg/ml	15.13 ± 1.43	12.55 ± 1.43	12.30 ± 1.42[c]	6.35 ± 0.32
IgM, 5 mg/ml	15.45 ± 1.11	11.71 ± 1.55	14.23 ± 2.19	5.12 ± 0.20[b]
My43, 50 mg/ml	15.61 ± 1.12	3.70 ± 0.29[b]	13.44 ± 1.55	6.20 ± 0.25
antisecretory component, 50 mg/ml	15.23 ± 1.64	11.77 ± 1.13	13.34 ± 1.55	5.45 ± 0.26[b]

[a] Results are expressed as MFI ± SD of five individual experiments. ASOR, asialo-orosomucoid. Other abbreviations as in Tables 1 through 4.
[b] $P < 0.01$ compared with FITC-IgA.
[c] $P < 0.05$ compared with FITC-IgA.

Table 6. Summary of published data in the expression of FcαR1 on HMC[a]

Category	Method	Remarks	Reference
Detection of FcαR1			
cultured HMC	PCR, NB	Upregulated by IL-6, TNF-α	25
microdissected glomeruli	PCR	Positive in 40% of IgAN, negative in healthy donor	32
cultured HMC	NB		23
No detection of FcαR1			
cultured HMC	PCR	Amplified products with partial homology to CD89	28
cultured HMC	PCR		29
cultured HMC and section	PCR, Imm		30
cultured HMC	PCR, NB, FACS		31

[a] NB, Northern blotting; IgAN, IgA nephropathy; Imm, immunostaining; FACS, flow cytometry. Other abbreviations as in Tables 1 through 4.

mediated by mechanism(s) other than the known IgA receptors.

Structural changes of the hinge region of the IgA_1 molecule have been speculated to bear pathologic implication in IgAN (35,36). Deficiency of terminal galactose in the hinge region may have a profound effect on the recognition of IgA_1 by ASGPR and, hence, its catabolism. Oligosaccharides in the hinge region carry negatively charged sialic acid that is large and bulky compared with the protein backbone. Any change in the carbohydrate moieties affects the tertiary structure as well as the electrostatic charge, factors that are pivotal in the interaction with and recognition of other molecules such as ASGPR and FcαR1 (36,37). Earlier studies revealed a reduced terminal galactosylation of serum IgA_1 in IgAN (37,38). However, these findings failed to provide a pathogenetic mechanism in IgAN because more than 90% of serum IgA_1 are monomers, whereas mesangial IgA deposits are mainly polymeric and anionic in nature. Moreover, pIgA from patients with IgAN exhibited an increased binding to HMC (33). Recently, we found that the anionic nature of pIgA from patients with IgAN is at least partly due to oversialylation of the *O*-linked carbohydrate moieties at the hinge region (39). Oversialylation of pIgA has two potential implications on the pathogenesis of IgAN. First, the masking effect of sialic acid may hinder the binding of pIgA to ASGPR, which is specific for terminal galactose residues. Hence, these large macromolecular IgA not effectively removed from the circulation by the ASGPR in the reticulo-endothelial system will be deposited in kidney, skin, and choroid. Moreover, increased FcαR1 occupation by IgA_1 and decreased FcαR1 expression had been observed in the blood phagocytic cells from patients with IgAN (13). Thus, a defect in FcαR1-mediated clearance of IgA immune com-

plexes may contribute to the pathogenesis of IgAN. Second, oversialylation renders pIgA from patients with IgAN more anionic, and this enhances the mesangial deposition of IgA immune complexes (7,8). In this study, we have demonstrated the presence of ASGPR in myelomonocytic cell lines in addition to HepG2 cells. It is intriguing to speculate that the reduced terminal galactosylation of serum or monomeric IgA_1 (37–39) observed in IgAN may be the sequela of increased binding of monomeric IgA_1 with *O*-linked carbohydrate rich in terminal galactose to hepatic and/or white cells rather than mesangial cells. Additional studies are warranted in this area.

In conclusion, the present study demonstrated the absence of FcαR1, ASGPR, and pIgR on HMC. We suggest that the binding of human IgA to HMC is mediated by other novel receptors or through other mechanisms.

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