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Expression of Fc α/μ Receptor by Human Mesangial Cells: A Candidate Receptor for Immune Complex Deposition in IgA Nephropathy

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IgA nephropathy is characterized by the deposition of IgA immune complexes in the glomerular mesangium, but the mechanisms responsible for this are not well understood. Human mesangial cells (HMCs) can bind IgA but do not express known IgA receptors. We show here that primary HMCs express mRNA for a novel receptor, the Fc alpha/mu receptor (Fcα/μR), and that receptor expression is upregulated by IL-1. We also detected mRNA for a novel receptor variant in HMCs that may encode a soluble form of the receptor. Fcα/μR was expressed in a heterologous system which showed that the receptor was approximately 58 kDa in weight and was only minimally N-glycosylated. As predicted from the characteristics of the murine homologue, the expressed human Fcα/μR was able to bind IgA and IgM, but not IgG. These results suggest that Fcα/μR may be the receptor responsible for mesangial IgA deposition in IgA nephropathy.

Key Words: **Fc alpha/mu receptor; IgA nephropathy; mesangial cells; glomerular mesangium; IgA; IgM.**

IgA nephropathy (IgAN) is the most common form of glomerulonephritis and is the underlying cause of renal failure in up to 10% of patients in dialysis and renal transplantation programs. The condition has a variable natural history, leading over a period of several years to end stage renal failure in approximately one-third of those affected (1). The pathogenesis of IgAN, and the factors determining susceptibility and disease progression are incompletely understood, but abnormalities have been described in both the systemic and mucosal compartments of the immune system (2, 3). Although circulating levels of IgA may be increased in patients (4, 5), and the IgA is underglycosylated (6, 7), the characteristic diagnostic feature is the presence of IgA-containing immune complexes in the glomerular mesangium. However, the mechanism by which these complexes are deposited in the mesangium, and the nature of their pathophysiological role, is unknown. Human mesangial cells (HMCs) cultured *in vitro* can bind monomeric and polymeric IgA in a dose-dependent, inhibitable manner but do not express known cell surface IgA receptors such as the Fc alpha receptor (CD89), the polymeric immunoglobulin receptor or the asialoglycoprotein receptor (5, 8–11). This evidence has led several authors to suggest that HMCs possess a novel IgA receptor, but its identity has remained elusive. Mesangial cell activation following IgA binding and receptor signaling could underlie the mesangial cellular proliferation and extracellular matrix accumulation seen in IgAN (12). Alternatively, impaired clearance of nephritogenic IgA immune complexes might occur if the deposited IgA failed to interact normally with the mesangial cell receptors in IgAN. A better understanding of these potentially receptor-mediated events is an important step toward defining the pathophysiology of IgAN more clearly.

A novel murine receptor capable of binding the Fc portions of IgA and IgM was recently identified (13). The receptor was expressed by mouse B lymphocytes and macrophages, and receptor messenger RNA was detected in some other tissues including kidney. The cDNA sequence of this Fc alpha/mu receptor (Fcα/μR) predicts a transmembrane glycoprotein that, unlike CD89, has a single immunoglobulin-like domain. A human homologue of Fcα/μR has also been identified on the basis of sequence similarity but no functional data have been described (13). We hypothesized that this human homologue may be the unidentified receptor responsible for IgA binding on HMCs.

The nucleotide sequences of the wild-type and variant forms of the Fc alpha/mu receptor have been submitted to GenBank under Accession Nos. AY063125 and AY063126.

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MATERIALS AND METHODS

Isolation of HMCs and cell culture. Primary HMC cultures were established from glomeruli obtained from the normal pole of tumour nephrectomy specimens using a standard sequential sieving technique (14). Cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (10 mg/ml), insulin (10 μg/ml), transferrin (5.5 μg/ml), and selenium (6.7 ng/ml) at 37°C, 6% CO_2 in a water saturated atmosphere. COS-7 cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (10 mg/ml). All tissue culture reagents were obtained from Life Technologies, Paisley, UK. In experiments to examine receptor regulation, confluent HMCs were stimulated for 24 h with either IL-1α or TNFα (AMS Biotechnology, Abingdon, UK) at a concentration of 50 ng/ml.

RNA extraction and RT-PCR. Total RNA was extracted from resting or stimulated HMCs between passages 2–4 using TRIzol reagent (Life Technologies), and 3 μg was reversed transcribed to cDNA using oligo dT priming and Superscript II enzyme (Life Technologies). PCR to detect the human Fcα/μR was performed with specific primers (sense, 5′-GAC AAC TAC CAA GGC TGA TAG G-3′; antisense, 5′-TCT GTC CCT CAG GGT CCT GGA T-3′) and a "modified hot start" using *Taq* DNA polymerase (Promega, Madison, WI). Cycling conditions were denaturation at 94°C for 90 s followed by 40 cycles of annealing at 60°C for 60 s, extension at 72°C for 60 s and denaturation at 94°C for 30 s. A final extension at 72°C for 10 min was performed. The cDNA samples were also subjected to PCR for β-actin as an internal control (sense primer, 5′-GGG GTA TGC CCT CCC CCA TGC CAT CCT GCG-3′; antisense primer, 5′-TTG GCG TAC AGG TCT TTG CGG ATG TCC ACG-3′). PCR products were resolved on 1.2% agarose gels and bands visualized by ethidium bromide staining.

Cloning of human Fcα/μR sequence. PCR was used to amplify the full length Fcα/μR sequence using HMC cDNA (sense primer, 5′-AGG AGG GCA GGA TGG AAA ATG-3′; antisense primer, 5′-GGG TCC TGG ATT TCT CTC TG-3′) and *Pfu* DNA polymerase (Stratagene, La Jolla, CA). The product was purified from an agarose gel and 3′ adenine overhangs were added by incubation with *Taq* DNA polymerase at 72°C for 15 min. The fragment was then subcloned into the pcDNA3.1/V5/His/TOPO expression plasmid (Invitrogen, Groningen, The Netherlands) in frame with a V5 epitope tag. Identity and orientation were confirmed by sequencing and restriction digest.

Expression of the human Fcα/μR sequence. COS-7 cells were transfected with plasmid containing human Fcα/μR cDNA using FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN). Control cells were transfected with the same plasmid containing irrelevant DNA. After 48 h cells were lysed by the addition of ice-cold lysis buffer [1% (v/v) Triton X-100, 50 mM Tris–HCl, pH 7.5, 0.25% (w/v) sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM vanadate, and 1 mM NaF] containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 1 μg/ml each of chymostatin, leupeptin, antipain, and pepstatin). Insoluble debris was removed by centrifugation at 15,000*g* for 15 min. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transferred to nitrocellulose, and receptor expression was examined by immunoblotting using a monoclonal antibody with specificity for the V5 epitope tag (Invitrogen). An HRP-conjugated anti-mouse immunoglobulin secondary antibody (Amersham–Pharmacia Biotech, Little Chalfont, UK) was used and bands were visualized by enhanced chemiluminescence (Amersham–Pharmacia Biotech) and autoradiography. Lysates of some transfected cells were treated overnight at 37°C with 5 munits of recombinant N-glycanase (Glyko, Upper Heyford, UK) prior to immunoblotting with the anti-V5 antibody.

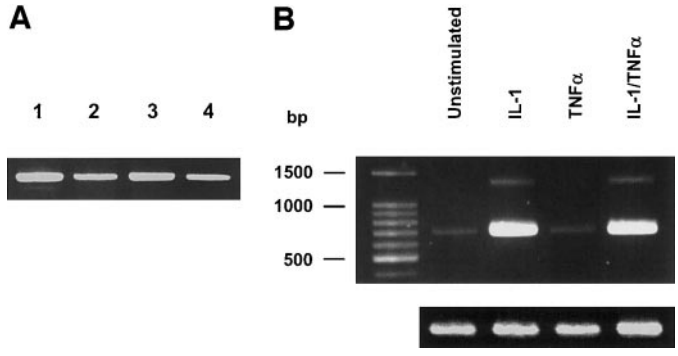

FIG. 1. Expression of Fcα/μR by human mesangial cells. (A) RT-PCR demonstrating endogenous Fcα/μR expression (702-bp amplicon) in HMC lines 1–4. (B) Cultured HMCs were stimulated for 24 h with IL-1 and TNFα either alone or in combination, each at a concentration of 50 ng/ml. The lower panel shows β-actin expression for each sample.

Ligand binding studies. For ligand binding studies, COS-7 cells transfected with either the full length human Fcα/μR sequence or with irrelevant DNA in the same plasmid were grown on glass coverslips for 48 h. The cells were washed with PBS and incubated with 10 μg/ml of FITC-labeled human IgA, IgM, or IgG (Jackson Laboratories, West Grove, PA) in PBS for 45 min at 4°C. The cells were then washed twice with PBS and mounted using DAPI-containing mounting medium (Vector Labs, Burlinghame, CA) to counterstain cell nuclei. Antibody binding to cells was analyzed using a fluorescence microscopy imaging system (Improvision, Coventry, UK).

RESULTS AND DISCUSSION

Expression and Regulation of Fcα/μR mRNA by Human Mesangial Cells

Expression of Fcα/μR mRNA by primary HMCs in culture was examined using RT-PCR. Fcα/μR message was detected in all of four HMC lines tested (Fig. 1A). One of the 702-bp amplicons was sequenced to confirm identity. To determine whether receptor message could be regulated by inflammatory cytokines, HMCs were stimulated for 24 h with either IL-1 or TNFα at a concentration of 50 ng/ml. Semiquantitative RT-PCR was performed by reducing the number of cycles to 35. Treatment with IL-1, but not with TNFα, resulted in upregulation of Fcα/μR expression when compared to unstimulated cells (Fig. 1B). There was no synergistic effect of both cytokines in combination and upregulation of receptor mRNA by IL-1 was confirmed in a second HMC line.

Cloning and Genomic Organization of Human Fcα/μR

To further characterize the human homologue of Fcα/μR, and to establish whether it could bind IgA and IgM as had been reported for the mouse receptor, the full length open reading frame was cloned and subsequently expressed in a heterologous system. RT-PCR was used to amplify a 1614-bp fragment containing the

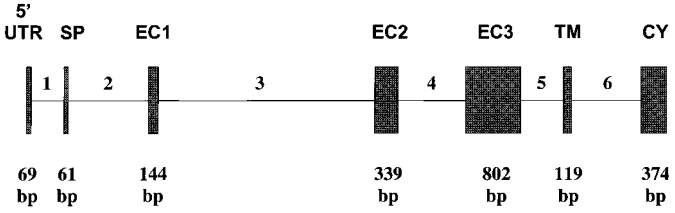

FIG. 2. Genomic organization of Fcα/μR. The full-length receptor was cloned from a mesangial cell line and the sequence compared with the NCBI human genome database. The seven exons (shaded boxes) and six introns (numbered) are shown to scale, and the size of each exon is indicated.

predicted full length coding sequence from HMC cDNA and this was ligated into an expression plasmid. The identity of the insert was confirmed by sequencing, and the exon structure of the gene was deduced by comparison with data from the National Center for Biotechnology Information human genome database (Fig. 2). The Fcα/μR gene is sited on chromosome 1q32 and consists of 7 exons and 6 introns spanning approximately 10 kb. The mature protein is composed of 522 amino acids. The first exon, 5′ UT, encodes the start of the 5′ untranslated region (5′ UTR) while the second exon, SP, contains the rest of the 5′ UTR as well as the ATG translation initiation codon and the 10-amino-acid signal peptide sequence. The extracellular region is encoded by 3 exons, EC1–EC3 with EC2 coding for an immunoglobulin-like domain. A single sixth exon, TM, encodes the membrane proximal extracellular portion, the 20-amino-acid transmembrane region and the proximal portion of the cytoplasmic domain. The remainder of the 62-amino-acid cytoplasmic tail is encoded by exon CY, which also encodes the start of the 3′ untranslated region.

HMCs Express a Variant Fcα/μR Transcript

An additional product of about 1300 bp in size can be seen in Fig. 1B following stimulation with IL-1. This band could also be seen faintly in unstimulated HMCs (data not shown). The band was excised from agarose, gel purified, subcloned and sequenced. Sequencing revealed that this represented a novel form of Fcα/μR with an in frame insertion of 624 bp of sequence which comprises the complete intron 5 of the genomic sequence. This intron separates exons EC3 and TM and is spliced out in the wild-type transcript. The sequence of this variant predicts that the first codon following divergence from the wild type sequence is TAA, a stop codon. As the exon coding for the transmembrane domain is downstream from this stop codon, translation of this larger transcript would yield a soluble, circulating form of the receptor. This would be consistent with the existence of soluble forms of other Fc receptors which have been described, including the Fc alpha receptor (CD89) (15, 16).

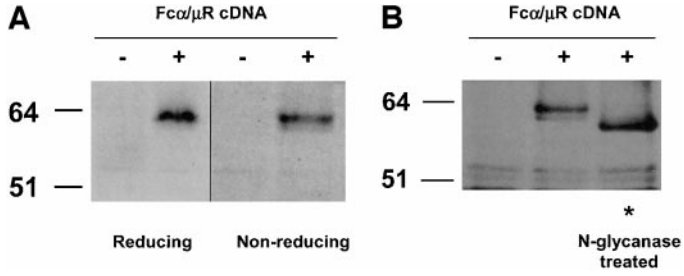

FIG. 3. Fcα/μR expression in COS-7 cells. (A) Immunoblot of transfected COS-7 cell lysates for tagged Fcα/μR protein using an antibody specific for the V5 epitope. Cells were transfected with either Fcα/μR DNA (+) or with irrelevant DNA (−) and lysates separated under reducing and non-reducing conditions. (B) Lysate treated for 24 h with N-glycanase (*). Numbers represent molecular weight markers in kilodaltons.

Expression and Antibody Binding Characteristics of Fcα/μR

Fcα/μR cDNA was used to transfect COS-7 cells and lysates were prepared for immunoblotting. As there is not yet an antibody available with specificity for human Fcα/μR, we used an antibody directed against the V5 epitope tag encoded by the expression plasmid. Expression of V5 epitope-tagged Fcα/μR fusion protein by transfectants was demonstrated by immunoblotting (Fig. 3A). The product of the tagged Fcα/μR gene was approximately 63 kDa under reducing conditions and there was no mobility shift observed under non-reducing conditions indicating that the receptor was expressed as a monomer. The V5 tag accounts for about 5 kDa of weight. The protein was only minimally N-glycosylated, as treatment with N-glycanase to remove N-linked carbohydrate groups revealed a core of approximately 60 kDa (Fig. 3B). This is in contrast to the Fc alpha receptor which is relatively heavily N-glycosylated (17).

To define the antibody binding specificity of the human Fcα/μR, transfected COS-7 cells were incubated with either fluorescein-labeled IgA, IgM, or IgG antibodies and examined using fluorescence microscopy. Cells transfected with Fcα/μR cDNA were able to bind IgA and IgM but did not bind IgG (Fig. 4), whereas cells transfected with irrelevant DNA did not bind immuno-

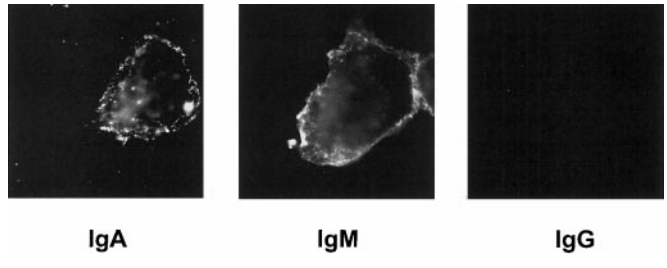

FIG. 4. Fluorescence micrographs showing antibody binding to COS-7 cells transfected with Fcα/μR DNA.

globulin of any isotype (data not shown). The binding of IgM by transfected cells was stronger than that of IgA.

The finding of a novel human IgA-binding receptor expressed by HMCs is of importance as this Fcα/μR must be a candidate for mediating immune complex deposition in IgAN. This is also the first report to show that the human Fcα/μR is able to bind IgA and IgM. A recent report demonstrated that the transferrin receptor (TfR) was also capable of IgA binding and that HMCs expressed this receptor (18). As TfR is ubiquitously expressed by dividing cells, it is not yet clear what factors regulate restriction of IgA binding to this receptor. TfR has significantly higher affinity for monomeric compared with polymeric IgA and how this relates to the polymeric aggregates of IgA seen in the mesangium in IgAN is also unclear. The relative contributions of TfR and Fcα/μR to IgA binding by HMCs will require further study. It is possible that receptor variants, or polymorphisms affecting receptor expression levels, underlie the variable course of IgAN and explain why only certain patients progress to end stage renal failure. Variation in receptor function or expression, in conjunction with abnormally glycosylated circulating IgA, may be permissive for abnormal glomerular IgA deposition or clearance and susceptibility to disease. Studies to examine to relative affinity of Fcα/μR for normally and abnormally glycosylated forms of IgA will be important. That Fcα/μR is upregulated by IL-1 is of significance, as increased intrarenal expression of this inflammatory cytokine has been described in IgAN (19, 20). IL-1 produced by infiltrating monocytes, or by local intraglomerular cells, could result in upregulation of mesangial Fcα/μR expression and promote IgA deposition in the mesangium.

In summary, these experiments show that HMCs express mRNA for Fcα/μR and a potential soluble variant, and that an inflammatory cytokine implicated in the pathogenesis of IgAN, IL-1, is able to upregulate receptor expression levels. We have also demonstrated that the human version of Fcα/μR is able to bind IgA and IgM. Insight into the role of this receptor should further the understanding of the pathophysiology of IgAN and may identify novel therapeutic strategies.

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