Pathogenic role of (S)IgA in IgA nephropathy

Oortwijn, B.D.

CHAPTER 3

Comparable binding of monomeric and polymeric IgA to the novel IgA Fc receptor, Fcα/μR.

Beatrijs D. Oortwijn[1], Reinier N. van der Geest[1], Anja Roos[1], Kenneth J. McDonald[2], Mohamed R. Daha[1], Cees van Kooten[1]

[1]Dept. Nephrology, C3-P, Albinusdreef 2, 2333 ZA, Leiden University Medical Center, Leiden, The Netherlands [2]Renal Unit and University Department of Medicine & Therapeutics, Western Infirmary, Glasgow, United Kingdom

Summary

Aberrant interaction between IgA and IgA-receptors has been implicated as an important pathogenic factor in IgA nephropathy (IgAN). Several IgA receptors have been characterized but most of these receptors are not present on mesangial cells. Recently a novel Fc receptor for IgA (Fcα/μR) has been identified in B cells and macrophages. It has been shown that Fcα/μR is abundantly expressed in the kidney. In this study we produced recombinant human Fcα/μR proteins and investigated their interaction with human IgA and IgM.

Using RT-PCR we showed that human renal mesangial cells express Fcα/μR and that the expression is upregulated following IL-1 stimulation of the mesangial cells. Human Fcα/μR chimeric proteins (Fcα/μRIg), consisting of the extracellular domain of Fcα/μR and the human IgG1 Fc-tail, were generated and produced in CHO cells. The interaction of Fcα/μRIg with IgA was tested with two different assays. First the binding of different forms of IgA and IgM to Fcα/μRIg was measured directly by ELISA. In a second assay the competition with binding of labelled mIgA to Fcα/μRIg by different forms of IgA and IgM were investigated. All forms of IgA and IgM showed a dose dependent binding to Fcα/μRIg. The binding of monomeric and polymeric IgA to Fcα/μRIg were comparable. Enzymatically deglycosylated IgA obtained by PNGase F and neuraminidase treatment showed a reduced interaction with the Fcα/μRIg. In conclusion, using recombinant chimeric proteins, we showed that IgA binds to Fcα/μR and that the interaction of IgA with Fcα/μR is partially dependent on glycosylation of IgA. This study provides additional insight in the interaction of IgA with Fcα/μR.

INTRODUCTION

Receptors for the Fc portion of immunoglobulins are present on different cell types of the immune system and play important roles in a wide array of immune responses, like antibody dependent cellular cytotoxity and phagocytosis. IgA is the predominant isotype produced and it plays a critical role in the protection of the host against environmental pathogens at mucosal surfaces (1).

IgA nephropathy (IgAN) is the most common type of primary glomerulonephritis worldwide. The disease presents a spectrum of clinical symptoms, leading to progressive renal failure in a substantial proportion of patients. The hallmark of the disease is deposition of IgA in the glomerular mesangium (2-4). In glomeruli of patients with IgAN mostly high molecular weight IgA1 is detected, sometimes together with IgM and/or C3 (5-7). The deposition of IgA in the mesangium is associated with renal inflammation, however extensive studies still have not revealed a specific mechanism of deposition.

Binding of IgA to mesangial cells has been studied before (8,9) and has revealed that the binding of polymeric IgA to mesangial cells is stronger than that of monomeric IgA. Moreover, recently our group showed that secretory IgA, present in low concentrations in the circulation, has the best binding to mesangial cells in vitro as compared to serum IgA (10). Several studies have shown that aberrant O-glycosylation of circulating IgA from IgAN patients, may result in increased Tn antigen (GalNAcβ1-Ser/Thr) expression (11-13) and that binding of IgA from patients with IgAN to mesangial cells is better as compared to that of IgA from healthy individuals (9). However, this finding is still controversial (14).

Several IgA receptors have been described in literature. The best-known receptors, the polymeric Ig receptor (pIgR), the asialoglycoprotein receptor and CD89 have already been described to be absent on mesangial cells (15-17). Recently a novel IgA receptor, Fcα/μR, located on chromosome 1, has been identified. Expression of transcripts of this receptor has been demonstrated in several tissues including kidney, small intestine, lymph node and appendix (18,19). Furthermore, transcription of the Fcα/μR was demonstrated in mesangial cells, and shown to be upregulated by IL-1, a cytokine implicated in the pathogenesis of IgAN (20). Studies in mice have shown surface expression of Fcα/μR in mature but not in immature B lymphocytes. Furthermore, stimulated B lymphocytes were able to bind IgA and IgM (19). Of interest is the finding that rat IgA and both human and rat IgM binds to mouse Fcα/μR (18). However, the interaction of human Fcα/μR with human IgA and IgM has not been reported until now.

Therefore, the aim of the present study was to analyse the interaction of human IgA and IgM to recombinant human Fcα/μR. We show that two different recombinant Fcα/μRs react with IgM and with different molecular forms of IgA in ELISA and that monomeric IgA has similar binding as polymeric IgA to Fcα/μR. Furthermore, the binding of monomeric IgA to Fcα/μR is affected by its glycosylation.

MATERIAL AND METHODS

Production of Fcα/μRIg

Two forms of Fcα/μRIg fusion protein were produced following cloning of the complete extracellular part of the receptor into the pME-Neo Fc vector (long Fcα/μRIg) (21) or of the N-terminal Ig domain without the long stalk (short Fcα/μRIg). The XHO-1 restriction site in the linker between the fusion product and the human IgG1 Fc tail and Eco-RI at the beginning of the extracellular part of the receptor were used. PCR was used to amplify the extracellular part of the Fcα/μR using a pcDNA3.1/ His/ TOPO vector containing the whole receptor (20) (Table 1) and PWO polymerase (5U/μl, Roche). The purified product obtained after agarose gel electrophoresis was subcloned in the pME-Neo Fc vector previously used for the production of Fc-$(CD89)_2$ (21) and the identity and orientation were confirmed by sequencing and restriction digestion. The vector was subsequently introduced into Chinese hamster ovary (CHO) cells using electroporation and selected for neomycin resistance with G418 (400 μg/ml, Gibco). Single clones with stable expression were obtained, and clones with the highest production as determined by ELISA were selected (long Fcα/μRIg: 4 μg/ml, short Fcα/μRIg 1.7 μg/ml). Supernatants of CHO cells producing long or short Fcα/μRIg were applied to a prot G or HiTrap Mabselect SuRe columns and after washing the column with PBS, bound proteins were eluted with 0.1M citric acid pH 3.0. Fractions were neutralized immediately with 1M Tris-HCl pH 10, and tested for human IgG content by ELISA.

Table 1: Primer sequences

		Sequence (5'-3')
Long Fcα/μRIg	Forward	CTT CGA ATT CAT GCC CCT CTT CCT CAT ACT GTG CC
	Reverse	TCA ACT CGA GCC GAG AGC TGC TTT CAT CTT CTG G
Short Fcα/μRIg	Forward	CTT CGA ATT CAT GCC CCT CTT CCT CAT ACT GTG CC
	Reverse	ATT ACC TCG AGC CCA GCA GCT GGA GTG GCT GT
Fcα/μR	Forward	GAC AAC TAC CAA GGC TGA TAG G
	Reverse	TCT GTC CCT CAG GGT CCT GGA T

Cytospin preparations

Cells were harvested by trypsin digestion and subsequently incubated with 1 % paraformaldehyde for 30 min at 4 ºC. After washing with PBS, the cells were centrifuged onto glass slides and air dried. After blocking with PBS/ 1 % BSA for 1 hour RT, the cytospin preparations were incubated with 10 μg/ml monoclonal anti human IgG (HB43, ATCC) for 1 hour RT. Binding of HB43 was visualized with Oregon Green conjugated goat anti mouse IgG (Molecular probes, Leiden, The Netherlands).

IgG ELISA

In order to determine the chimeric protein concentrations we used an IgG ELISA as described before (22). Plates were coated with rabbit anti human IgG (Jackson, West Grove, PA, USA) in carbonate buffer (pH 9.6) (100 μl/ well) overnight at RT. Subsequently, the plates were washed with PBS/ 0.05 % Tween. Plates were incubated with chimeric protein or IgG in PBS/ 1 % BSA/ 0.05 % Tween for one hour at 37 ºC. After washing, bound IgG was detected with goat anti human IgG biotin, followed by streptavidin conjugated to HRP (Zymed).

Enzyme activity of HRP was developed using ABTS (Sigma). The O.D. at 415 nm was measured.

RNA extraction and RT-PCR

Total RNA was extracted from mesangial cells using RNeasy mini kit (Qiagen, Valencia, CA). OD260/280 ratio was measured to determine the quantity and purity of RNA preparations. Fixed amounts of total cellular RNA (1 µg) were reverse transcribed into cDNA by oligo(dT) priming, using M-MLV reverse transcriptase (Gibco/Life Technologies, Breda, The Netherlands). PCR to detect the human Fcα/µR was performed with specific primers (Table 1) using AmpliTaq DNA polymerase (Applied Biosystems, Roche, Mannheim, Germany). The PCR reaction was performed as described before (20). The cDNA samples were also subjected to PCR for GAPDH as an internal control (23). PCR products were resolved on 1 % agarose gels and bands were visualized by ethidium bromide staining.

Purification of IgA, IgG and IgM

Serum was used as a starting source for IgA purification, using methods described before (24). In brief, serum was applied to an anti-IgA (clone HisA 43, kindly provided by dr J. van den Born, Free University Medical Center, Amsterdam) affinity column. The column was washed sequential with 0.5 x PBS and 1 M NaCl, and subsequently bound IgA was eluted with 0.1 M glycine/ 0.3 M NaCl (pH 2.8). Directly after elution the fractions were neutralized with 1 M Tris pH 8.0. The eluted protein fractions that contained IgA, as assessed by ELISA (25), were pooled and dialysed against PBS containing 2 mM EDTA.

Purified IgA was size-separated into monomeric IgA (mIgA) and polymeric IgA (pIgA) using a HiLoadTM 16/60 HR200 Superdex prep grade gelfiltration column (120 ml, Amersham Pharmacia, Roosendaal, The Netherlands). Pools of monomeric and polymeric IgA were made and analysed for total IgA content using ELISA (25).

Secretory IgA (SIgA) was obtained from Sigma. Human IgG and IgM, free of IgA, were isolated as described before (26).

Assessment of immunoglobulin binding to Fcα/µRIg

Binding of IgA and IgM to Fcα/µRIg constructs was analysed by ELISA. Ninety-six well Nunc Maxisorb microtitre plates (Gibco/Invitrogen, Carlsbad, CA) were coated overnight with long Fcα/µRIg (5 µg/ml) in carbonate buffer (pH 9.6) (100 µl/well) at room temperature. Subsequently, the plates were washed with PBS/ 0.05 % Tween and incubated with different concentrations of IgA or IgM in PBS/ 1 % BSA/ 0.05 % Tween for one hour at 37 ºC. After washing, bound IgA was detected with biotin labelled monoclonal anti human IgA (4E8), bound IgM with biotin labelled monoclonal anti human IgM (HB57). After incubation of one hour the plates were washed and bound conjugates were detected with streptavidin conjugated to HRP. Enzyme activity of HRP was developed using ABTS (2, 2'-azino-bis (3-ethyl benzathioline-6-sulphonic acid)) (Sigma, St. Louis, MO). The O.D. at 415 nm was measured using a microplate biokinetics reader (EL312e, Biotek Instruments, Winooski, Vermont, USA).

In order to compare the interaction of different immunoglobulins to the Fcα/µRIg constructs we set up a competition ELISA. For this purpose, the dose dependent binding of dig labelled mIgA was tested to immobilized long and short Fcα/µRIg. Subsequently, plates were coated with long Fcα/µRIg (5 µg/ml) in carbonate buffer (pH 9.6) (100 µl/well) overnight at room temperature and after extensive washing with PBS/ 0.05 % Tween, incubated with IgA,

IgM or IgG in PBS/ 1 % BSA/ 0.05 % Tween for one hour at 37 ºC. After one hour, dig-conjugated mIgA (final concentration 1 μg/ml) was added to the plate. The amount of mIgA-dig bound was detected subsequently using $F(ab)_2$ anti-Dig Abs conjugated to HRP.

Glycosidase treatment of IgA

In order to investigate whether the IgA glycosylation is important for the interaction with Fcα/μR, mIgA was enzymatically deglycosylated. Therefore, monomeric IgA (800 μg) was first digested with N-glycosidase F (PNGase F), cloned from *flavobacterium meningosepticum* and expressed in *E. coli* (6 U/ 200 μg IgA, Roche) in 0.02 M sodium phosphate buffer pH 7.2 with 2 mM EDTA. The first sample, to be used as control, was IgA with incubation buffer (mock IgA (800 μg)); the second sample was IgA with enzymes (treated IgA (800 μg)). After 72 hours of incubation at 37 ºC this IgA was further digested with neuraminidase from *Arthrobacter ureafaciens* (6 mU/200 μg IgA, Roche, Mannheim, Germany), β (1-3,4,6) galactosidase isolated from a cloned gene expressed in *E. coli* (7 mU/ 200 μg IgA, Prozyme), or combinations of the enzymes. This deglycosylation step was performed in 50 mM sodium acetate pH 5.6 for 72 hours at 37 ºC. After treatment, samples (10 μg) were analysed by loading on a 10 % reduced SDS-PAGE gel and Coomassie stained.

Flow cytometry

NHMC (normal human mesangial cells, Cambrex, USA) were expanded according to the protocol provided by the manufacturer. Experiments with NHMC were performed in RPMI with 10 % FCS, 1 % non-essential amino acids, 0.5 % transferrin/insulin/selenium, 1 % sodium pyruvate, 1 % L-glutamine (all purchased at Gibco/ life Technologies, Paisley, Scotland). Cells were harvested by trypsinization (0.02 % (w/v) EDTA/ 0.05 % (w/v) trypsin in PBS from Sigma (St. Louis, MO)), and washed with FACS buffer (0.5 x PBS containing 1 % BSA/ 2.8 % glucose/ 0.01% NaN3) and incubated with monomeric and polymeric serum IgA, and SIgA (Sigma). Following incubation for 1 hour at 4 ºC, cells were washed and incubated for one hour at 4 ºC with monoclonal anti-IgA Ab 4E8 (IgG1) (25). IgA binding was visualized using PE-conjugated goat anti-mouse IgG1 (Southern Biotechnology, Birmingham, AL) and assessed for fluorescence intensity by flow cytometry (FACSCalibur, Cell quest software; BD Biosciences). Dead cells, identified by propidium iodide uptake, were excluded from analysis.

Binding of IgA to mesangial cells was also determined following activation of mesangial cells for 48 hours with IL-1 (10 ng/ml).

RESULTS

Construction of chimeric forms of Fcα/μR

In order to investigate the interaction of different molecular forms of IgA with Fcα/μR, two different Fcα/μRIg fusion proteins were generated. Long Fcα/μRIg was generated by fusion of the complete extracellular part of the receptor with the human IgG1 Fc tail, whereas short Fcα/μRIg was generated by fusion of the extracellular part containing the Immunoglobulin-like domain with the human IgG1 Fc tail (Figure 1A). Recombinant soluble long and short Fcα/μRIg were produced in CHO cells. Because no specific reagents were available for detection of human Fcα/μR, we tested the cells (Figure 1B) and supernatants for the presence of the IgG Fc-tail. Untransfected cells were negative for the human IgG1 Fc tail, whereas both fusion

proteins were produced by a number of cell lines.

Supernatants of the two transfected cell lines were applied to protein G columns and after acid elution one peak each was collected and tested in the human IgG ELISA (Figure 1C). The human IgG ELISA was positive for both human IgG and the chimeric proteins.

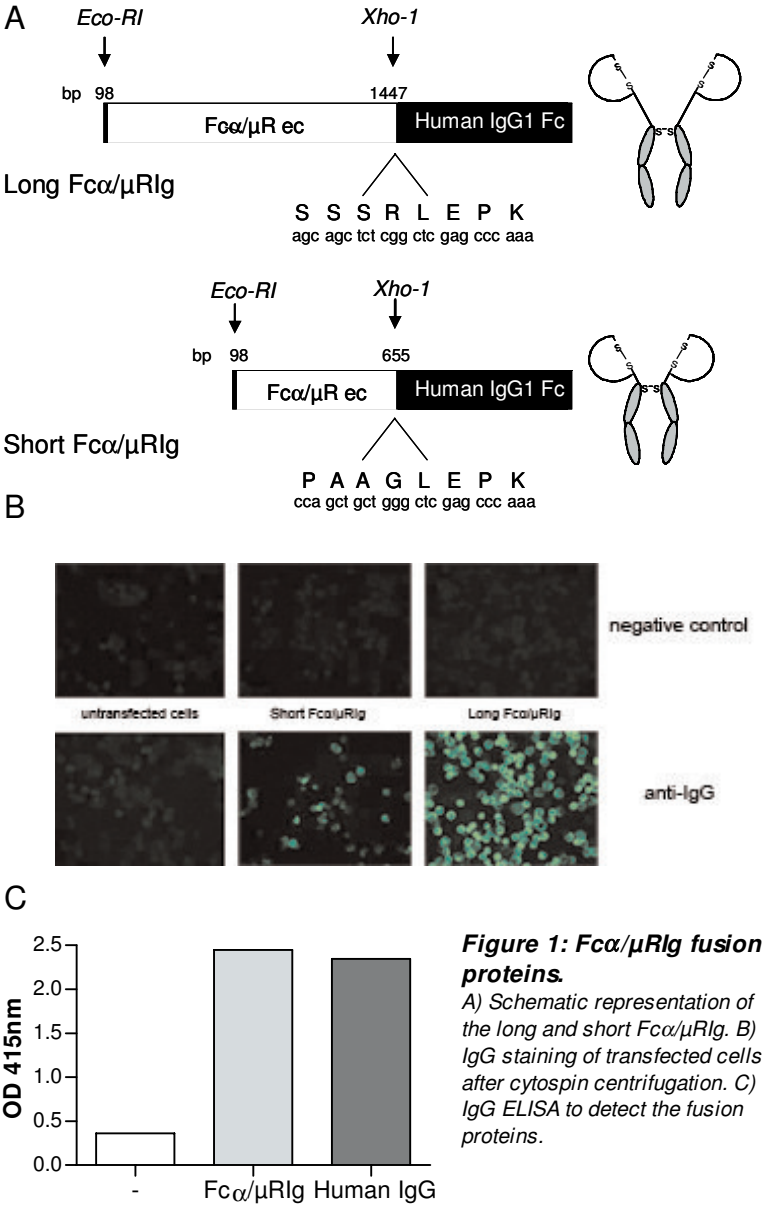

Figure 1: Fcα/μRIg fusion proteins.
A) Schematic representation of the long and short Fcα/μRIg. B) IgG staining of transfected cells after cytospin centrifugation. C) IgG ELISA to detect the fusion proteins.

Dose dependent binding of IgA and IgM to Fcα/μR in ELISA

To investigate the interaction of different molecular forms of IgA with Fcα/μR, long Fcα/μRIg was coated in ELISA plates and binding of IgA and IgM was assessed. The binding of IgA was dose-dependent over a wide range of concentrations (Figure 2A). Binding of monomeric and polymeric IgA to Fcα/μR were similar. We have reported earlier that in serum low concentrations of SIgA are present (10). Therefore we also tested the binding of SIgA to Fcα/μR. Binding of purified serum IgA to Fcα/μR was stronger than that of SIgA. Also IgM exhibited a dose dependent binding to Fcα/μR (Figure 2B).

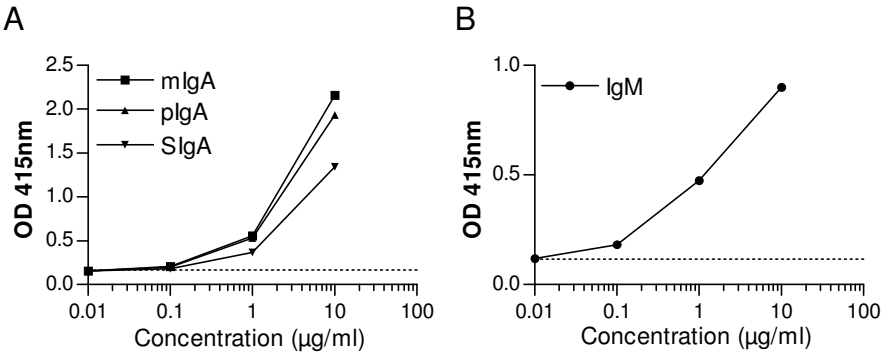

Figure 2: Binding of IgA and IgM to Fcα/μR in ELISA.
A) Dose dependent binding of IgA to immobilized long Fcα/μRIg (5 μg/ml). IgA binding was detected with a monoclonal anti-human IgA antibody B) Dose dependent binding of IgM to immobilized long Fcα/μRIg (5 μg/ml). IgM binding was detected with a monoclonal anti-human IgM antibody. Dotted line represents the negative control.

Inhibition of binding of mIgA-Dig to Fcα/μR by monomeric and polymeric IgA

Results presented above demonstrated that several forms of IgA and IgM bind to Fcα/μR. However, the detection antibody for binding of IgA and IgM were different and therefore it was not possible to compare the relative amounts of binding of IgA and IgM to Fcα/μR. To obtain more quantitative comparison of the binding of IgA and IgM to Fcα/μR, a competition ELISA was set up. First, the dose dependent binding of mIgA-Dig to Fcα/μR was tested (Figure 3A). Binding of mIgA-Dig to Fcα/μR was dose dependent and the binding of mIgA-Dig to Fcα/μR was similar for long Fcα/μRIg and short Fcα/μRIg. Short Fcα/μRIg contains the immunoglobulin domain of the Fcα/μR without the long extracellular stalk, whereas long Fcα/μRIg consists of the whole extracellular part of the Fcα/μR. The similar binding of mIgA-dig to long Fcα/μRIg and short Fcα/μRIg, suggests the involvement of the immunoglobulin like domain of Fcα/μR for binding to mIgA.

Based on the results, we then selected a dose of 1 μg/ml mIgA-Dig for subsequent competition experiments with different forms of IgA and IgM (Figure 3B). The binding of mIgA-Dig to Fcα/μR was inhibited with mIgA and pIgA in a dose dependent manner, while IgG was unable to influence the binding of mIgA-Dig to Fcα/μR. Furthermore, SIgA showed a reduced capacity to inhibit mIgA-Dig binding to

Fcα/μR. Using IgM as a competitor; we did find some inhibition even at relatively low concentrations. However, IgM inhibited the mIgA-Dig binding with a maximum of 30 %, indicating that SIgA and IgM have a much lower affinity for Fcα/μR than mIgA or pIgA.

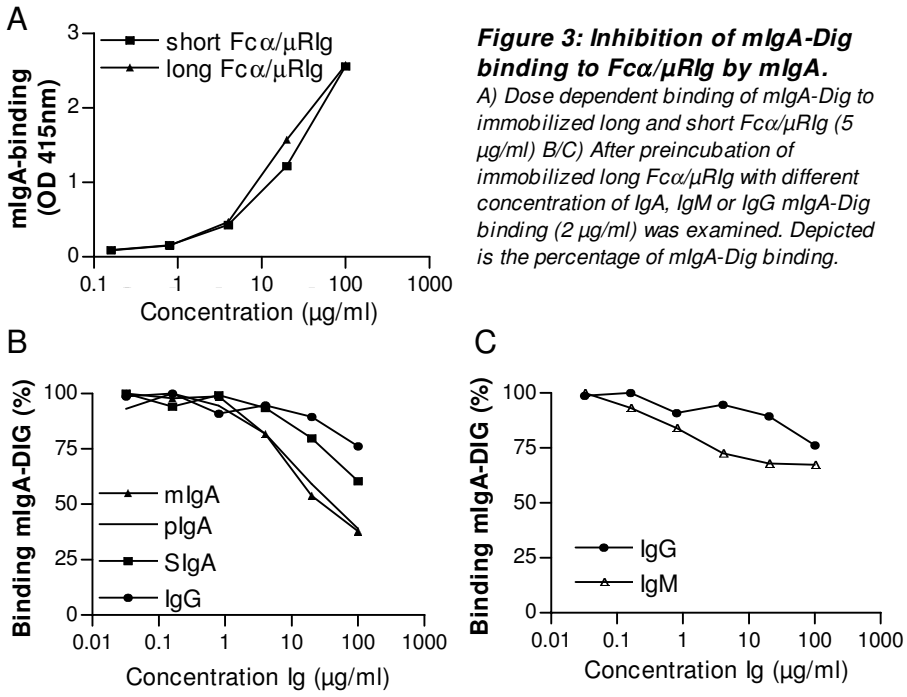

Figure 3: Inhibition of mIgA-Dig binding to Fcα/μRIg by mIgA.
A) Dose dependent binding of mIgA-Dig to immobilized long and short Fcα/μRIg (5 μg/ml) B/C) After preincubation of immobilized long Fcα/μRIg with different concentration of IgA, IgM or IgG mIgA-Dig binding (2 μg/ml) was examined. Depicted is the percentage of mIgA-Dig binding.

Deglycosylation of mIgA is associated with reduced binding to Fcα/μR

Since previously published results suggest that glycosylation of IgA can be important for the interaction with its receptors (27,28), we investigated whether glycosylation differences of IgA affect its interaction with Fcα/μR. Therefore, IgA was treated with neuraminidase, galactosidase, PNGase F or with combinations of these enzymes (28), and tested this treated IgA for binding to Fcα/μR (Figure 4). Treatment of mIgA with a combination of galactosidase and neuraminidase did not alter the binding of IgA to Fcα/μR (Figure 4). Removal of the N-glycans of IgA by treatment of IgA with PNGase F resulted in reduced inhibition of the binding of mIgA-Dig to Fcα/μR. This reduction in binding of IgA to Fcα/μR was even more pronounced after treatment of IgA with a combination of PNGase F and neuraminidase. These results suggest that N-glycans and sialic acids positively affect the interaction of IgA with Fcα/μR.

No difference in interaction of IgA with stimulated mesangial cells

We hypothesized that after IL-1 stimulation the IgA binding to mesangial cells was increased. Therefore, we first analyzed the IgA binding to mesangial cells with-

out stimulation of the mesangial cells (Figure 5A). After incubation of different molecular forms of IgA with mesangial cells the binding was examined by FACS. Binding of mIgA to mesangial cells was very low. In contrast, pIgA and SIgA showed a clear binding to mesangial cells.

The results presented above indicate IgA binding to mesangial cells. It has been suggested that Fcα/μR is present on mesangial cells, and thereby could play a role in IgAN (20). Therefore, we investigated whether Fcα/μR was detectable in mesangial cells (Figure 5B). The Fcα/μR transcript was present in mesangial cells and was up-regulated after stimulation of mesangial cells with IL-1, as described before (20).

The binding of IgA to mesangial cells was examined after mesangial cell stimulation with IL-1. Although the transcript of Fcα/μR is up-regulated in mesangial cells after IL-1 stimulation, there was no increase in IgA binding after stimulation of mesangial cells (Figure 5C). This was observed with all different molecular forms of IgA.

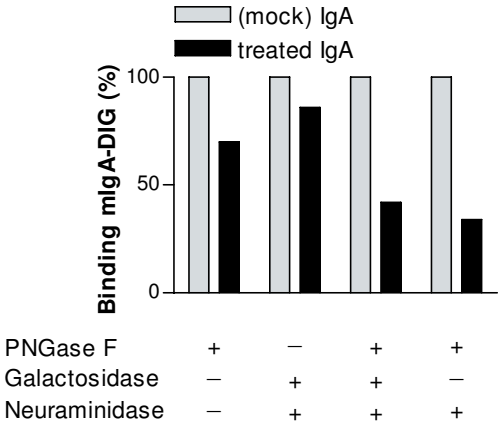

Figure 4: Interaction of deglycosylated IgA with Fcα/μR.
A) Inhibition of mIgA-Dig binding (2 μg/ml) with mock treated IgA (10 μg/ml) or deglycosylated IgA (10 μg/ml) to immobilized long Fcα/μRIg, depicted is the percentage of mIgA-Dig inhibition B) mIgA-Dig binding after preincubation of immobilized long Fcα/μRIg with different concentration mock treated or PNGase F and neuraminidase treated IgA. Depicted is the percentage of mIgA-Dig binding.

DISCUSSION

To our knowledge this is the first time that the interaction of human IgA and human IgM to human Fcα/μR was studied directly. In literature it has been suggested that the binding of mouse IgM to mouse Fcα/μR was stronger as compared to the binding of mouse IgA (18). In the present study we cannot confirm the mouse study in the human situation, in that the interaction of human IgA and human IgM with human Fcα/μR was strongest for IgA. Furthermore, this interaction of IgA with Fcα/μR was affected by the glycosylation of IgA. The binding of IgA and IgM was similar for both fusion proteins investigated, suggesting an important role for the immunoglobulin-like domain of the receptor in the interaction with IgA and IgM.

The mouse Fcα/μR is homologous to the human Fcα/μR (18). Strong binding of rodent IgM and IgA to mouse Fcα/μR was observed, but in contrast to rodents in humans 90 % of circulating IgA is monomeric (29). In this study we found that the interaction of human Fcα/μR was similar for monomeric and polymeric IgA. This

interaction of IgA with Fcα/μR is different as compared to the interaction of IgA with another IgA receptor of the Ig superfamily, the FcαRI/CD89 (28,30-32).

Fcα/μR has been described to be present on mesangial cells and was suggested to play a role in IgAN. With size fractionation of eluted proteins from kidney sections, it was shown that deposited IgA was mostly of high molecular weight nature (5). Furthermore, elution of deposits from glomeruli from a patient with IgA nephropathy, revealed a high accumulation of SIgA in the IgA deposits (10). Moreover, in vitro binding studies showed that high molecular weight IgA bound better to mesangial cells than monomeric IgA. In the present study we show that the Fcα/μR interacts similar with monomeric and polymeric IgA. Furthermore we showed an up-regulation of Fcα/μR transcript in mesangial cells after IL-1 stimulation. However, high molecular weight IgA binds better to mesangial cells as compared to monomeric IgA and we could not detect differences in IgA binding to IL-1 stimulated mesangial cells. Altogether this suggests an additional receptor on mesangial cells.

In IgAN an aberrantly O-glycosylated IgA1 molecule has been described. With enzymatic treatment of IgA we investigated whether the interaction of IgA with

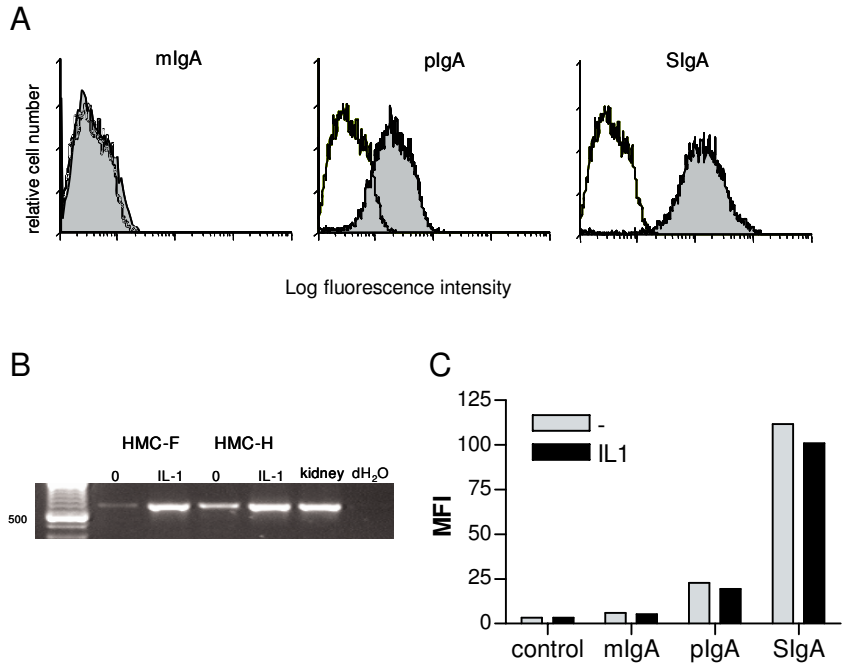

Figure 5: Interaction of IgA with stimulated mesangial cells.
A) mesangial cells were incubated with different molecular forms of IgA (200μg/ml) and assessed for IgA binding by flow cytometry. Filled histograms represent the binding of IgA, the open histograms represent the control staining with secondary antibodies B) mRNA expression of Fcα/μR in mesangial cells with and without IL-1 stimulation. Kidney was used as positive control and H_2O as negative control. C) FACS analysis with IL-1 stimulated mesangial cells and different molecular forms of IgA. Depicted is the mean fluorescence intensity (MFI).

Fcα/μR was glycosylation dependent. After treatment of IgA with neuraminidase and galactosidase, to generate a glycan structure as described to be present on IgA from IgAN patients, no difference in the interaction of IgA with Fcα/μR was observed. However, after removal of the sialic acid and N-glycan residues a clear reduction in binding of IgA to Fcα/μR was observed, suggesting that the charge of the sialic acid residues together with the N-glycan structure is important for the IgA- Fcα/μR interaction. However, it is not clear whether the carbohydrates itself or the possible change in confirmation as a result of the treatment play a role in this process.

Mouse Fcα/μR can mediate endocytosis of immune-complexes composed of IgM and S. aureus (18). The underlying mechanism of this internalization is not known yet, but with receptor mutants it is suggested that the di-leucine motif is important in this process (18). For human Fcα/μR it is not known whether the receptor is able to internalize immune-complexes with IgA or IgM and if so then which mechanism could be of importance for this process. Therefore it would be very interesting to investigate, whether human Fcα/μR is also able to mediate endocytosis of IgA immune-complexes.

The Fcα/μR immunoglobulin like domain has the same motif as the first extracellular immunoglobulin like domain of the polymeric Ig receptor (pIgR) (18). IgA and IgM are also able to bind to pIgR, and this binding is described to be on the first extracellular domain of the pIgR (33). The binding of IgA and IgM to pIgR is restricted to IgA (dimeric IgA (dIgA)) and IgM containing the J chain (34). The binding of IgA and IgM was similar for the long Fcα/μRIg and the short Fcα/μRIg, suggesting an important role for the immunoglobulin like domain of the Fcα/μR for the interaction with IgA and IgM. As mentioned before the binding of IgA to the pIgR is restricted to dIgA (34), whereas monomeric IgA has the best interaction with Fcα/μR, suggesting another role for the Fcα/μR as compared to the role of pIgR.

In conclusion, we showed that IgA and IgM are able to interact with Fcα/μR. A similar binding of monomeric and polymeric IgA to Fcα/μR was observed. Furthermore, we showed that this interaction is affected by the glycosylation of the IgA molecule. The mechanism of interaction of IgA with Fcα/μR is still not clear however our data suggest an important role for the immunoglobulin like domain of the extracellular part of the Fcα/μR. This study provides more insights in the interaction of IgA with Fcα/μR.

Acknowledgements

This work was financially supported by the Dutch Kidney Foundation (BDO: C99.1822; AR: PC95)

REFERENCES

1. Mestecky J, Moro B, Underdown BJ: Mucosal Immunoglobulins, chap. 9, in Mucosal Immunology, 2 ed., edited by Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR, San Diego, Academic Press, 1999, pp 133-152
2. Berger J, Hinglais N: Les depots intercapillaires d'IgA-IgG. J Urol Nephrol (Paris) 74:694-695, 1968
3. Donadio JV, Grande JP: IgA nephropathy. N Engl J Med 347:738-748, 2002
4. Feehally J: IgA nephropathy--a disorder of IgA production? QJM 90:387-390, 1997

5. Monteiro RC, Halbwachs-Mecarelli L, Roque-Barreira MC et al.: Charge and size of mesangial IgA in IgA nephropathy. Kidney Int 28:666-671, 1985
6. Floege J, Feehally J: IgA nephropathy: recent developments. J Am Soc Nephrol 11:2395-2403, 2000
7. Van Es LA, de Fijter JW, Daha MR: Pathogenesis of IgA nephropathy. Nephrology 3:3-12, 1997
8. Novak J, Vu HL, Novak L et al.: Interactions of human mesangial cells with IgA andIgA-containing immune complexes. Kidney Int 62:465-475, 2002
9. Leung JC, Tang SC, Lam MF et al. : Charge-dependent binding of polymeric IgA1 to human mesangial cells in IgA nephropathy. Kidney Int 59:277-285, 2001
10. Oortwijn BD, van der Boog PJ, Roos A et al.: A pathogenic role for secretory IgA in IgA nephropathy. Kidney Int 69:1131-1138, 2006
11. Allen AC, Bailey EM, Barratt J et al.: Analysis of IgA1 O-glycans in IgA nephropathy by fluorophore-assisted carbohydrate electrophoresis. J Am Soc Nephrol 10:1763-1771, 1999
12. Coppo R, Amore A: Aberrant glycosylation in IgA nephropathy (IgAN). Kidney Int 65:1544-1547, 2004
13. Hiki Y, Tanaka A, Kokubo T et al.: Analyses of IgA1 hinge glycopeptides in IgA nephropathy by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Am Soc Nephrol 9:577-582, 1998
14. Wang Y, Zhao MH, Zhang YK et al.: Binding capacity and pathophysiological effects of IgA1 from patients with IgA nephropathy on human glomerular mesangial cells. Clin Exp Immunol 136:168-175, 2004
15. Barratt J, Greer MR, Pawluczyk IZ et al.: Identification of a novel Fcalpha receptor expressed by human mesangial cells. Kidney Int 57:1936-1948, 2000
16. Leung JC, Tsang AW, Chan DT et al.: Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. J Am Soc Nephrol 11:241-249, 2000
17. Westerhuis R, van Zandbergen G, Verhagen NA et al.: Human mesangial cells in culture and in kidney sections fail to express Fc alpha receptor (CD89). J Am Soc Nephrol 10:770-778, 1999
18. Shibuya A, Sakamoto N, Shimizu Y et al.: Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes. Nat Immunol 1:441-446, 2000
19. Sakamoto N, Shibuya K, Shimizu Y et al.: A novel Fc receptor for IgA and IgM is expressed on both hematopoietic and non-hematopoietic tissues. Eur J Immunol 31:1310-1316, 2001
20. McDonald KJ, Cameron AJ, Allen JM et al.: Expression of Fc alpha/mu receptor by human mesangial cells: a candidate receptor for immune complex deposition in IgA nephropathy. Biochem Biophys Res Commun 290:438-442, 2002
21. van der Boog PJ, van Kooten C, van Zandbergen G et al.: Injection of recombinant Fc{alpha}RI/CD89 in mice does not induce mesangial IgA deposition. Nephrol Dial Transplant 19:2729-2736, 2004
22. de Fijter JW, van Nisselrooij NI, Schroeijers WE et al.: Decreased cytokine-induced IgA subclass production by CD40-ligated circulating B cells in primary IgA nephropathy. Nephrol Dial Transplant 13:285-292, 1998
23. Nauta AJ, De Haij S, Bottazzi B et al.: Human renal epithelial cells produce the long pentraxin PTX3. Kidney Int 67:543-553, 2005
24. van der Boog PJ, van Zandbergen G, de Fijter JW et al.: Fc alpha RI/CD89 circulates in human serum covalently linked to IgA in a polymeric state. J Immunol 168:1252-1258, 2002
25. de Fijter JW, van den Wall Bake AW, Braam CA et al.: Immunoglobulin A subclass measure

ment in serum and saliva: sensitivity of detection of dimeric IgA2 in ELISA depends on the anti body used. J Immunol Methods 187:221-232, 1995
26. Roos A, Essers M, Gijlswijk-Janssen D et al.: Both IgG and IgM anti-pig antibodies induce com plement activation and cytotoxicity. Xenotransplantation 8:3-14, 2001
27. Carayannopoulos L, Max EE, Capra JD: Recombinant human IgA expressed in insect cells. Proc Natl Acad Sci U S A 91:8348-8352, 1994
28. Oortwijn BD, Roos A, van der Boog PJ et al.: Monomeric and polymeric IgA show a similar association with the myeloid Fc?RI/CD89. Mol Immunol 44:966-973, 2007
29. Endo T, Radl J, Mestecky J: Structural differences among serum IgA proteins of chim panzee, rhesus monkey and rat origin. Mol Immunol 34:557-565, 1997
30. Reterink TJ, van Zandbergen G, van Egmond M et al.: Size-dependent effect of IgA on the IgA Fc receptor (CD89). Eur J Immunol 27:2219-2224, 1997
31. van Zandbergen G, van Kooten C, Mohamad NK et al.: Reduced binding of immunoglobulin A (IgA) from patients with primary IgA nephropathy to the myeloid IgA Fc-receptor, CD89. Nephrol Dial Transplant 13:3058-3064, 1998
32. Fanger MW, Shen L, Pugh J et al.: Subpopulations of human peripheral granulocyes and monocytes express receptors for IgA. Proc Natl Acad Sci U S A 77:3640-3644, 1980
33. Beale D: Cyanogen bromide cleavage of bovine secretory component and its tryptic fragments. Int J Biochem 20:873-879, 1988
34. Hendrickson BA, Rindisbacher L, Corthesy B et al.: Lack of association of secretory compo nent with IgA in J chain-deficient mice. J Immunol 157:750-754, 1996