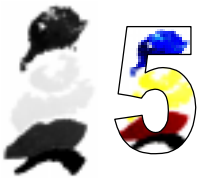

Reduced binding of immunoglobulin A (IgA) from patients with primary IgA nephropathy to the myeloid IgA Fc-receptor, CD89 [#]

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SUMMARY

Primary IgA nephropathy (IgAN) is associated with elevated levels of circulating IgA and is characterized by deposition of primarily IgA1 in the renal mesangium. It has not yet been clarified which mechanisms govern the deposition of IgA1 in the mesangium. One of the factors, which may play a role in trapping of IgA in the mesangial area, is the interaction of IgA with specific IgA receptors (FcαRI, CD89) on the mesangial cells. In the present study IgA derived from patients with IgAN and controls was investigated for its interaction with human CD89, expressed on the surface of the murine B cell line IIA1.6. IgA binding to CD89 expressing cells was specific, concentration dependent and binding of dIgA and pIgA occurred in a more efficient fashion than that of mIgA. IgA binding to CD89 directly from serum of patients compared to controls showed no significant difference. However these experiments are affected by differences in IgA concentration and combinations of different sizes of IgA. Using purified fractions of mIgA, dIgA, and pIgA isolated from serum, a significantly reduced binding of mIgA to CD89 from patients compared to controls was observed. Finally, the binding of aIgA2 to CD89 was less inhibited using mIgA from patients with IgAN compared to controls. The reduced binding of mIgA to CD89 seems to contradict a direct role for CD89 in deposition of IgA. However reduced binding of mIgA to CD89 may affect IgA clearance, leading to higher serum IgA. Furthermore, since it has been demonstrated that mIgA can interfere with binding of di- and pIgA, CD89 could still contribute to pIgA deposition in the mesangial area.

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INTRODUCTION

Primary IgA nephropathy (IgAN) is a common form of primary glomerulonephritis with a broad spectrum of clinical presentations, leading to progressive renal failure in a substantial proportion of patients *(1,2)*. The disease is characterized by increased production of plasma IgA1 by the bone marrow and by deposits of IgA1 in the glomerular mesangium *(2,3)*. The mesangial IgA has been found to consist at least in part of polymeric IgA (pIgA). The mechanisms responsible for the mesangial depositions remain unclear *(2)*.

IgA1-containing immune complexes are thought to be of pathogenic importance in IgAN. An important elimination mechanism of IgA-containing immune complexes *in vivo*, next to clearance via the asialoglycoprotein receptor, is thought to occur via IgA Fc receptor mediated phagocytosis *(4,5)*. It has been found that the binding of IgA-immune complexes can activate macrophages and thus lead to the elimination of IgA-containing immune complexes *(4)*. In patients with IgAN a defective clearance of IgA has been suggested *(6)*. Other IgA mediated effector functions like ADCC, superoxide generation, and release of cytokines and inflammatory mediators have been linked to tissue damage *(4)*. These functions are regulated via binding of IgA to its cognate Fc receptor termed FcαRI or CD89 *(4,7)*. In addition, pIgA and dIgA, but not mIgA, bind efficiently to mesangial cells and enhance IL-6 expression *(8)*.

The IgA Fc receptor, CD89 has been cloned from the monocytic cell line U937 *(7)*. CD89 is expressed on neutrophils, eosinophils, and cells of the monocyte and macrophage lineage *(4)*. The binding of IgA to CD89 is size dependent: polymeric IgA and dimeric IgA bind to a better extent to the receptor than monomeric IgA *(9)*. Controversial data concerning expression of CD89 on mesangial cells has been reported *(10,11)*. Nevertheless CD89 might be involved in binding and deposition of IgA in the kidney causing tissue damage via CD89 effector functions.

In the present study we investigated the binding characteristics of IgA from sera of patients with IgAN and controls to CD89. We found that monomeric IgA of patients with IgAN shows a diminished binding to CD89 compared to controls.

PATIENTS AND METHODS

Patients

Serum samples were collected from 23 patients with IgAN (19 males, 4 females) mean age 36 years (range 19-59 years). All patients had clinically quiescent disease, with biopsy-proven IgAN, defined by mesangial deposits of IgA as the

dominant isotype. Laboratory data of patients were: creatinine clearance > 80 ml/min; urinary protein < 2g/24h. As controls we used serum samples from 17 matched healthy volunteers. Neither patients, nor controls, had symptoms or signs of mucosal infection in the 2 weeks preceding the study.

Reagents

CD89 was detected by the mAb A77 (mouse IgG1), kindly provided by Dr. R. C. Monteiro *(12)*. For inhibition experiments culture supernatants from clone MY43 (mouse IgM) were used *(13)*, kindly provided by Dr. Li Shen. A panel of mAbs with specificity for human IgA, IgA1 and IgA2 resp. clone 4E8, clone 69-11.4 and clone 16-512-H5 (all mouse IgG1) *(14)*, were used for detection of human IgA. Mouse mAbs were either detected with PE-labeled goat-anti-mouse IgG1 polyclonal antiserum by flow cytometry (Southern Biotechnology, Birmingham, AL) or with HRP-labeled goat-anti-mouse IgG polyclonal antiserum by ELISA (Tago, Burlingame, CA).

Cell culture

The murine B-cell line IIA1.6 *(15)* was cultured in RPMI 1640 medium (Life Technologies, Breda, The Netherlands) supplemented with 10% heat-inactivated FCS (Life Technologies). IIA1.6 was transfected with CD89 cDNA and stable cell surface expression was maintained by cotransfection of human γ-chain cDNA, as described previously *(15)*. The CD89-transfectants were grown in the same medium supplemented with geneticin (G418, 0.8 mg/ml; Life Technologies) and methotrexate (MTX, 10 mmol/l; Pharmachemie, Haarlem, The Netherlands). The human monocytic like cell line, U937 was cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS.

Detection of CD89

CD89-transfected and control cells were tested for CD89 expression by FACS analysis using CD89 mAb A77. Cells ($5x10^5$) were washed twice with FACS buffer (PBS/0.5%BSA) and incubated for 1 h at 4°C with A77 culture supernatant diluted in FACS buffer. Following incubation, cells were washed twice (FACS buffer) and incubated for 1 h with a goat anti-mouse IgG1-PE polyclonal antiserum diluted in FACS buffer. After washing twice with FACS buffer, cells were fixed in 1% paraformaldehyde in PBS and analyzed on a FACScan (Becton Dickinson, San Jose, CA). Data acquisition and analysis were conducted with Lysis II (Becton Dickinson).

Purification of IgA

Human IgA from sera of multiple myeloma patients with IgA1 or IgA2 paraproteins was isolated by euglobulin, $ZnSO_4$ and $(NH_4)_2SO_4$ precipitation followed by DEAE Sephacryl (Pharmacia, Uppsala, Sweden) anion exchange chromatography, and Sephacryl S-300 (Pharmacia) gel filtration, as described previously *(16)*. The IgA preparations were shown to contain <0.1% of IgG or IgM by ELISA and were free of other protein contaminants as assessed by SDS-PAGE analysis under reducing conditions.

Binding and detection of purified IgA to CD89-transfected cells

CD89-transfected and control cells were tested for binding of purified IgA preparations, by FACS analysis. Cells were washed twice (FACS buffer) and incubated for 1 h with varying concentrations of purified IgA preparations, diluted in FACS buffer. Following incubation, cells were washed and bound IgA was detected by incubation with a panel of mAbs with specificity for human IgA, IgA1 or IgA2 and subsequently with PE-labeled goat anti-mouse IgG1 polyclonal antiserum. The geometric Mean Fluorescence Intensity (MFI) was used as a measure for binding of IgA.

Inhibition of IgA binding to CD89-transfected cells

To define the specificity of IgA binding to CD89, blocking studies were performed using CD89 mAb My43. Briefly, My43 culture supernatant diluted 1/5 in FACS buffer or an isotype-matched irrelevant control mAb was added to nontransfected or CD89-transfected IIA1.6 cells and incubated for 30 min at 4°C. Varying concentrations of purified IgA were added and cells were analyzed for IgA binding by FACS analysis as above.

Quantification of IgA in sera

Sera from patients with IgAN ($n=23$) and controls ($n=17$) were analyzed for total IgA, IgA1 and IgA2 concentrations by ELISA using 4E8, 69-11.4, and 16-512-H5 mAbs, respectively *(14)*.

Fractionation of serum IgA

Randomly selected sera from patients with IgAN ($n=6$) and controls ($n=6$) were separated by size on a 1.5 x 90 cm Sephacryl S300 column. Briefly, 0.5 ml aliquots of sera were diluted with an equal volume of PBS layered on the column and fractions of 1.25 ml/6 min were collected. The fractions were analyzed for

total IgA by ELISA and the binding of IgA to CD89-transfected IIA1.6 cells was tested as described above.

Binding of IgA (IgA1 and IgA2) directly from serum of patients and controls to CD89-transfected cells

Binding of IgA directly from sera of patients with IgAN and controls was determined as follows: sera were diluted 1/50 in FACS buffer and added to nontransfected or CD89-transfected IIA1.6 cells. After incubation for 1 h at 4°C, cells were analyzed for total IgA, IgA1 and IgA2 binding, with respectively mAb 4E8, 69-11.4 and 16-512-H5 as described above.

Statistical Analysis

All statistical calculations were performed using SPSS (for windows, release 6.0 software package). The concentrations of IgA and IgA subclasses in sera of patients and controls were transformed logarithmically prior to analysis. Comparisons between groups were performed by one-way analysis of variance (ANOVA), followed by Scheffe's procedure. The binding of IgA to CD89 expressed in MFI, was normally distributed, and consequently analyzed by (two-tailed) *t*-test for independent samples and expressed as arithmetic means $\pm$ SEM. Binding curves of patient and normal IgA to CD89 were analyzed using regression line statistics. For all tests *P* values <0.05 were considered significant.

RESULTS

FACS analysis of IgA binding to CD89

In order to assess binding of IgA to CD89 we used CD89-transfected IIA1.6 cells as a model *(15)*. These CD89-transfected IIA1.6 cells stained 100% positive for CD89 using mAb A77, whereas nontransfected cells were negative (Figure 1A). In agreement with this observation purified IgA bound strongly to CD89-transfected IIA1.6 cells, while hardly any binding was seen to nontransfected cells (Figure 1A).

To confirm specificity, binding of IgA to CD89-transfected IIA1.6 cells was assessed in the presence of the inhibitory mAb My43. Addition of My43 resulted in near complete inhibition of IgA binding to CD89-transfected IIA1.6 cells. No inhibition was found with an isotype-matched control antibody (Figure 1B). Detection of IgA binding with the mAb 4E8 was more sensitive when compared to detection with an anti-IgA polyclonal reagent as described previously *(9)* (Figure 2).

Using this system we could clearly detect binding to CD89 transfected cells of purified pIgA and dIgA, but also binding of mIgA was clearly visible (Figure 2).

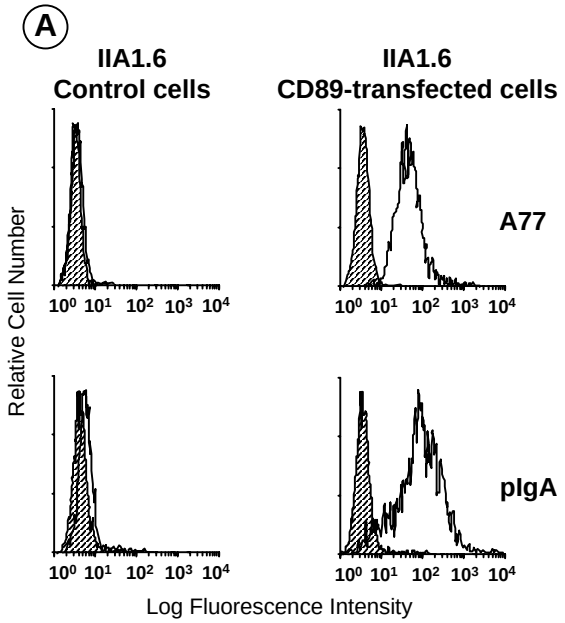
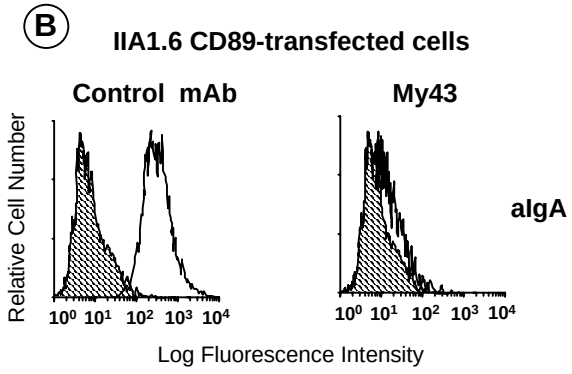


Figure 1. FACS analysis of IgA binding to CD89-transfected IIA1.6 cells A. *FACS histograms of IIA1.6 control cells (left) and CD89-transfected IIA1.6 cells (right) are shown. Surface expression of CD89 was detected with mAb A77(anti-CD89) (top) and binding of polymeric IgA (50µg/ml) to CD89 was detected with mAb 4E8 (anti-IgA) followed by Gt-anti-Ms IgG1-PE (bottom). Filled histograms represents the conjugate controls, open histograms represent either CD89 expression (top) or pIgA binding (bottom).* ***B.*** *FACS histograms of CD89-transfected IIA1.6 cells are shown. IgA binding was detected as described above, using aggregated IgA (50µg/ml) in the presence of an irrelevant IgM mAb (left) or in the presence of IgM mAb MY43 (anti-CD89) (right).*



Next, we assessed the binding to CD89-transfected IIA1.6 cells of IgA directly from whole serum. Incubation of CD89-transfected IIA1.6 cells with varying dilutions of serum resulted in a dose-dependent binding of IgA to the cells. No detectable binding to non-transfected IIA1.6 cells was observed. When we studied other isotypes, we could not demonstrate any binding of IgM or IgG to CD89 transfected cells (data not shown). The binding of IgA was be linear in a dilution range of serum from 1/20 to 1/800 (data not shown).

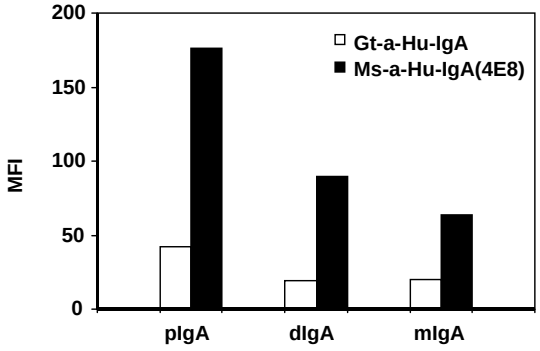


Figure 2. Detection of IgA binding to CD89-transfected cells with IgA specific antibodies. *PIgA, dIgA and mIgA were purified from sera of a myeloma patient and tested for binding to CD89 transfected cells. Binding of 25 µg/ml of pIgA, dIgA and mIgA was analyzed with either Gt-α-Hu-IgA polyclonal detected with PE conjugated Rb-α-Gt polyclonal (open bars), or Ms-α-Hu-IgA mAb (4E8) detected with PE conjugated Gt-α-Ms IgG1 polyclonal (filled bars). Indicated is the MFI, as a measure for binding, which is calculated as described in patients and methods.*

Binding of IgA from sera to CD89-transfected IIA1.6 cells

To assess the binding to CD89-transfected IIA1.6 cells of IgA directly from whole serum, sera from patients with IgAN and controls were tested for binding to CD89-transfected IIA1.6 cells at a fixed dilution of 1/50 (Table 1). As shown for purified IgA preparations, also serum IgA did not show binding to nontransfected cells (data not shown). However, in all cases binding to CD89-transfected IIA1.6 cells of IgA directly from whole serum was evident. Patients with IgAN had significantly higher concentrations of IgA (1.88 ± 0.26 mg/ml *vs.* 1.09 ± 0.08 mg/ml; $P < 0.001$) and IgA1 (1.65 ± 0.26 mg/ml *vs.* 1.05 ± 0.14 mg/ml ; $P<0.01$) in their sera, while no significant difference was found for IgA2 (0.25 ± 0.02 mg/ml *vs.* 0.20 ± 0.02 mg/ml ; $P=0.17$). Interestingly, the higher serum IgA concentrations in patients with IgAN did not result in enhanced binding to CD89-transfected IIA1.6 cells as compared to controls (Figure 3). The binding of the IgA subclasses, IgA1 and IgA2 showed no difference between patients and controls (Table 1).

Table 1. IgA binding to CD89-transfected cells

	IgA1	IgA2
Controls	69.59 ± 2.7	25.65 ± 2.3
Patients	70.70 ± 3.4	31.30 ± 2.3

Binding of serum IgA in MFI ± SEM. of patients with IgAN (n=23) and controls (n=17) to CD89-transfected cells. Binding of IgA1 or IgA2 was determined as described in the patients and methods section.

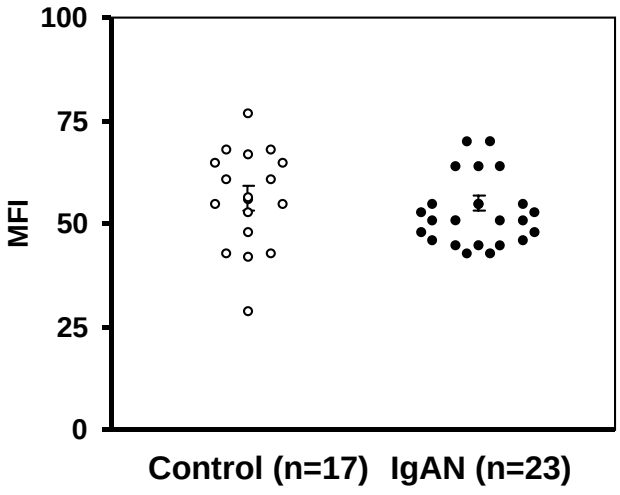


Figure 3. Binding to CD89-transfected IIA1.6 cells of IgA directly from serum of IgAN patients and controls. *Sera of patients (closed circles) with IgAN (n=23) and controls (open circles)(n=17) were tested for binding to CD89-transfected IIA1.6 cells. Binding of IgA was detected as described in figure 1. Indicated is the MFI, as a measure for binding, which is calculated as described in patients and methods.*

Size fractionation of serum IgA

We demonstrated previously that purified pIgA binds better to CD89 than mIgA *(9)*. Therefore the amount of IgA bound to CD89 is not only determined by con-

centrations of IgA as described above, but also by the content and ratio of polymeric, dimeric and monomeric IgA in the sera. In order to obtain insight in the effect of size on the binding to CD89 of IgA from patients with IgAN, sera of patients and controls were first fractionated by gel-filtration chromatography (Figure 4).

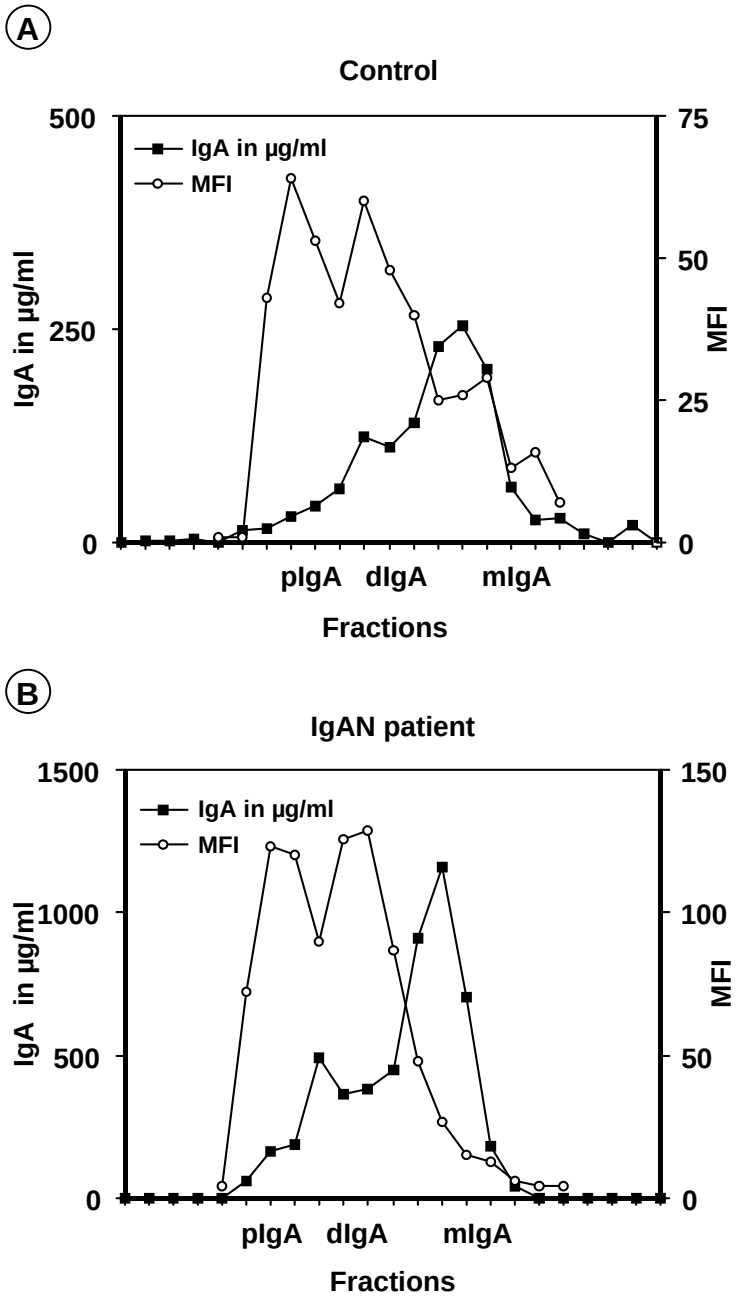


Figure 4. Fractionation of IgAN and control serum on S300 Sephacryl. *Sera of controls **(A)** with IgAN and patients **(B)** were fractionated by size on a S300 Sephacryl column. Fractions were test-ed for IgA concentrations (closed squares) and asses-sed for binding to CD89-transfected IIA1.6 cells (open circles). One representative example from each group, which contains 6 individuals, is shown.*

The percentages of polymeric, dimeric, and monomeric IgA, calculated from the IgA concentration in the sera samples after fractionation, were not different between patients and controls (Table 2). When the binding of the IgA containing fractions was assessed on CD89-transfected IIA1.6 cells, pIgA and dIgA containing fractions of patients with IgAN and controls showed significantly higher MFI binding values compared to mIgA containing fractions. Interestingly, mIgA from patients with IgAN showed reduced MFI values compared to monomeric IgA from controls, even though the mIgA concentrations in the tested fractions of the patients with IgAN was ~3-fold higher as compared to the controls (Figure 4).

Binding of pIgA, dIgA and mIgA to CD89-transfected cells

Next we assessed binding to CD89-transfected IIA1.6 cells of peak fractions containing pIgA, dIgA and mIgA. These fractions chosen showed no overlap in size as determined by gradient gel analysis (data not shown). First these fractions were tested at a concentration of 25 μg/ml. At these fixed concentrations, mIgA of patients with IgAN showed a strongly decreased binding to CD89 as compared to controls (Figure 5A).

Table 2. IgA size distribution from patients with IgAN and controls

	pIgA	dIgA	mIgA
Controls	13.36 ± 1.9	28.92 ± 1.4	57.72 ± 2.0
Patients	9.70 ± 1.0	27.92 ± 2.1	62.38 ± 2.9

Distribution of polymeric, dimeric and monomeric IgA from fractionated sera of patients with IgAN (n=6) and controls (n=6) given as percentages ± SEM.

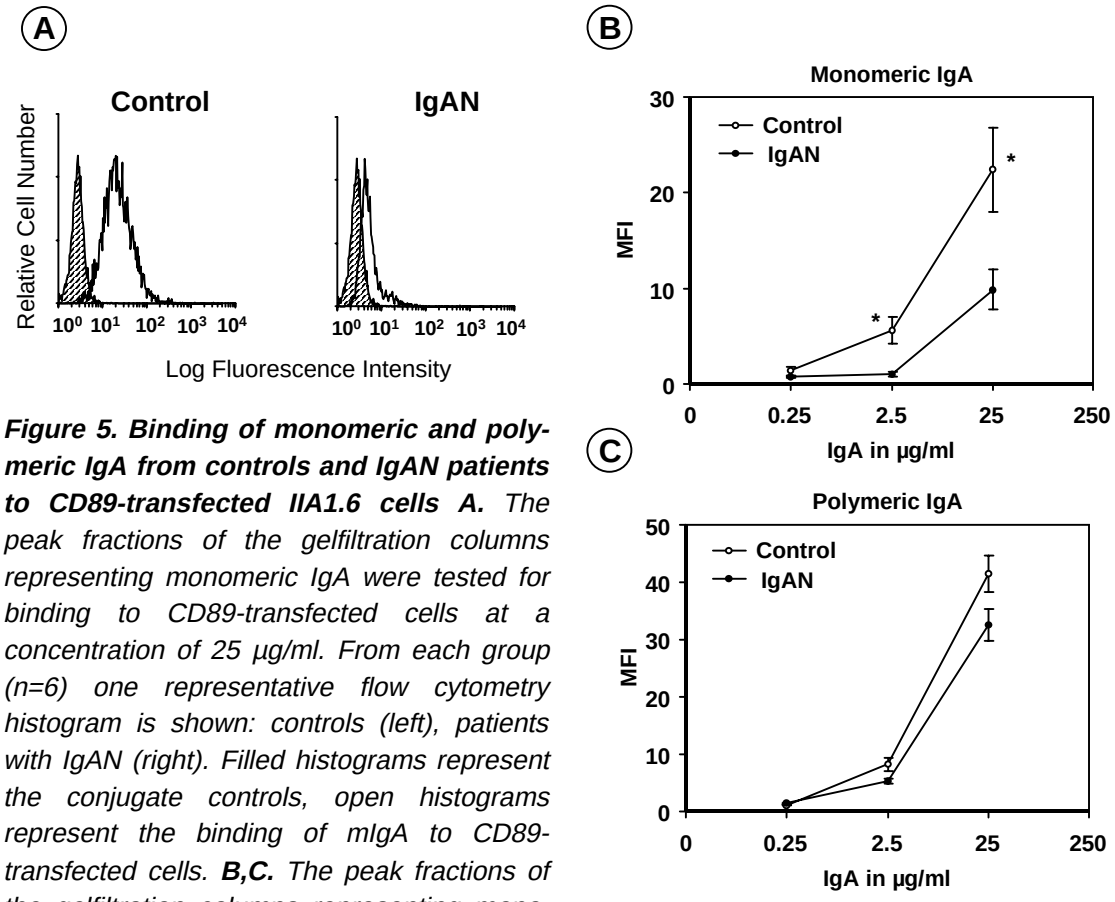


Figure 5. Binding of monomeric and polymeric IgA from controls and IgAN patients to CD89-transfected IIA1.6 cells A. *The peak fractions of the gelfiltration columns representing monomeric IgA were tested for binding to CD89-transfected cells at a concentration of 25 µg/ml. From each group (n=6) one representative flow cytometry histogram is shown: controls (left), patients with IgAN (right). Filled histograms represent the conjugate controls, open histograms represent the binding of mIgA to CD89-transfected cells.* ***B,C.*** *The peak fractions of the gelfiltration columns representing monomeric (B) and polymeric (C) IgA from sera of patients (closed circles) with IgAN (n=6) and controls (open circles) (n=6) were tested for binding to CD89-transfected IIA1.6 cells. IgA was tested in a dose response with the fixed concentration of resp. 0.25, 2.5, 25 µg/ml. Mean ± SEM are depicted, (*:P<0.05).*

When tested in a dose response, there was a significantly ($P < 0.05$) lower binding (MFI) of mIgA to CD89 from patients with IgAN as compared to controls (Figure 5B). Binding to CD89-transfected IIA1.6 cells of dIgA and pIgA of patients was not significantly reduced as compared to dIgA and pIgA of controls (Figure 5C).

Binding and inhibition of IgA to U937

To exclude that the observed differences were caused by the cell-transfectant, similar experiments were performed with cells, which naturally express CD89. For this purpose the monocytic like cell line U937 was used. Control experiments showed a significant expression of CD89 on U937 and binding of purified IgA which was completely inhibited by MY43 (data not shown). When purified fractions of monomeric IgA from patients and controls was tested for binding, we also found a significantly reduced binding of mIgA from IgAN patients (data not shown).

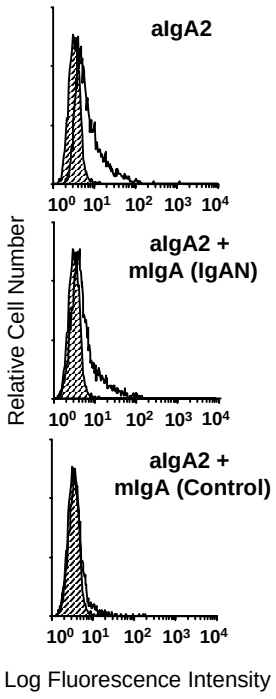
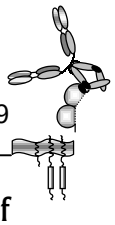


Figure 6. Inhibition of aIgA2 binding to U937 cells. *Inhibition of heat aggregated IgA2 (50 µg/ml) binding to U937 by preincubation with mIgA (50 µg/ml) of controls (n=3; 34.23±4.43%), patients with IgAN (n=3; 11.68±2.70*). From each group (n =3) one representative flow cytometry histogram is shown: aIgA2 (top), inhibition of aIgA2 binding by mIgA from patients with IgAN (middle) and as above with mIgA from controls (bottom). Filled histograms represent the conjugate controls, open histograms represent the binding of aIgA2 to U937 cells. Values in percentage aIgA2 binding inhibition ± SEM (*:P < 0.05).*

The reduced binding of mIgA to CD89 might lead to a reduced interference with pIgA binding and therefore favour pIgA deposition via CD89 interaction. To assess the capacity of mIgA from patients with IgAN and controls to interfere with binding of pIgA, U937 cells were pre-incubated with purified fractions of mIgA and specific binding of aIgA2 was tested. Isolated mIgA from controls ($n = 3$) inhibited aIgA2 binding with $34.23 \pm 4.43\%$, whereas mIgA isolated from patients with IgAN ($n = 3$) showed a significantly reduced inhibition of $11.68 \pm 2.70\%$ ($P < 0.05$) (Figure 6).

DISCUSSION

In this study we assessed the binding of IgA of different sizes to IIA1.6 cells transfected with human CD89. We used either purified IgA or IgA directly from patient and control sera to assess binding to CD89. Comparison of binding between



equal concentrations of serum or purified IgA to CD89 revealed a similar level of binding. This was found both for control sera and for sera of IgAN patients. Therefore we think it is unlikely that other serum proteins are affecting the binding of IgA to CD89. The advantage of this study compared to previous experiments *(9)* is the more sensitive detection of IgA binding to CD89-transfected cells (Figure 2). This difference is most pronounced in the detection of mIgA binding. Using this novel system, a significantly lower binding to CD89-transfected IIA1.6 cells of mIgA from patients with IgAN as compared to controls was found. This lower degree of binding to CD89-transfected IIA1.6 cells of mIgA was less evident for dIgA and pIgA. An explanation why dIgA and pIgA do not show a reduced reactivity with CD89 may be because they react with multiple CD89 molecules on a single cell, which results in an increased avidity. Specific binding of IgA to CD89 could also be detected using U937 cells. However, since this monocyte like cell line had a ten-fold lower expression of CD89, this resulted in a lower degree of IgA binding. Nevertheless the same decrease in binding of mIgA in patients as compared to controls was detected (Figure 6B).

Polymeric-IgA1 is considered to be a pivotal pathogenic factor In IgAN *(1,2)*. In contrast, in an experimental model it has been demonstrated that mIgA may protect the glomerulus against immune complexes containing pIgA *(17)*. In the line of our results this could imply that in patients with IgAN, a reduced binding of mIgA to CD89 might consequently result in a higher chance of binding of pIgA. Heat aggregated myeloma IgA2 (aIgA2) was used for binding to CD89 transfected cells in the absence or presence of mIgA isolated from patients with IgAN or controls. These serum IgA fractions consist for $\pm$ 85% of IgA1 and therefore will not interfere with our detection system. The result, which is depicted in Figure 6, shows that indeed mIgA isolated from controls results in a significantly stronger inhibition of the aIgA2 binding, as compared to mIgA from IgAN patients. The fact that we demonstrated a comparable binding to CD89 of IgA1 and IgA2, would also imply an increased deposition of IgA2. However, it is thought that deposits of IgA in IgAN mainly consist of IgA1 *(1,2)*.

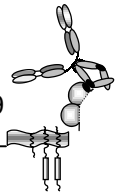
Binding of IgA-containing immune complexes to mesangial cells induces release of inflammatory factors such as superoxide anion, platelet-activating factor (PAF), IL-6 and TNFα *(4,8,18)*. The binding of IgA to mesangial cells is not solely dependent on the presence of CD89. For instance IgA or IgA-containing immune complexes can be bound via their mannose residues and targeted for endocytosis via the mannose receptor *(19)*. Expression of the mannose receptor on mesangial cells can be upregulated *in vitro* by TNFα and IL-1α *(19)*. Therefore it is most likely that multiple receptors might be responsible for binding of IgA in the kidney.

An important elimination mechanism of IgA-IC *in vivo* next to clearance via the asialoglycoprotein receptor is thought to occur via CD89-mediated phagocytosis *(4)*. In IgAN patients, an impaired endocytosis of surface-bound IgA by freshly isolated monocytes and neutrophils was found after cross-linking of the IgA Fc receptors *(20)*. This might contribute to the higher serum IgA concentrations found in IgAN patients leading to the deposition of IgA-IC in the kidney *(6,21)*. In addition, also CD89 on mesangial cells might be involved in clearance of deposited IgA containing immune complexes from the glomeruli, since IgA aggregates are taken up and catabolized by mesangial cells over time *(18)*. Therefore we think it is most likely that a failure of mesangial clearance could favor accumulation of IgA-IC on mesangial cells and thereby promote disease progression.

An aberrant interaction between IgA and its Fc receptor in IgAN patients leading to a defective clearance of IgA might be caused either by a defect in IgA or alterations of the Fc receptor. It has been demonstrated that IgA binds to CD89 via its Cα2 and Cα3 region *(22)*. Deglycosylation of the N-linked carbohydrate residues in this Cα2 and Cα3 region interrupts binding to CD89 *(22)*. In IgAN patients a lack of galactose residues in the O-linked sugars of the IgA1 hinge region has been reported *(6,21,23,24)*. Since these residues are normally recognized by the asialoglycoprotein receptor, but could also be of importance for IgA interactions with CD89, this could be a possible explanation for escape of IgA containing immune complexes from effective receptor mediated clearance, thus leading to higher serum IgA levels and deposition of IgA-IC's *(25,26)*.

Reports of recurrent IgA deposits in normal kidneys transplanted in IgAN recipients provide evidence that the basic abnormality in this condition lies within the IgA immune system rather than in the kidney *(1,2)*. The observation that the IgA deposits disappear when a graft containing mesangial IgA deposits is accidentally transplanted in a recipient not suffering from IgAN, further strengthens the idea that systemic factors are involved in the pathogenicity *(27)*. Nevertheless, alterations of CD89 expression have been described in patients with IgAN including dysfunction *in vivo* of CD89 on blood phagocytic cells *(20)*. Furthermore, the different splice variants of CD89 that have been described so far, which lack certain parts of the extracellular IgA binding domain, could possibly play a role in the pathogenesis of IgAN *(4)*.

In conclusion we have demonstrated a significantly reduced binding to CD89 of mIgA from patients with IgAN. This might have direct consequences for the clearance of the IgA molecule. In addition because of the reduced interference with pIgA binding, this could also contribute to pIgA deposition in the kidney.



REFERENCES

1. **Feehally, J.** 1997. IgA nephropathy--a disorder of IgA production? *QJM 90:387.*

2. **van Es, L. A., J. W. de Fijter, and M. R. Daha.** 1997. Pathogenesis of IgA nephropathy. *Nephrology 3:3.*

3. **van den Wall Bake, A. W., M. R. Daha, J. J. Haaijman, J. Radl, A. van der Ark, and L. A. van Es.** 1989. Elevated production of polymeric and monomeric IgA1 by the bone marrow in IgA nephropathy. *Kidney Int 35:1400.*

4. **Morton, H. C., M. van Egmond, and J. G. J. van de Winkel.** 1996. Structure and function of human IgA Fc receptors (Fc alpha R). *Crit Rev Immunol 16:423.*

5. **Maliszewski, C. R., L. Shen, and M. W. Fanger.** 1985. The expression of receptors for IgA on human monocytes and calcitriol-treated HL-60 cells. *J Immunol 135:3878.*

6. **Roccatello, D., G. Picciotto, M. Torchio, R. Ropolo, M. Ferro, R. Franceschini, G. Quattrocchio, G. Cacace, R. Coppo, and L. M. Sena.** 1993. Removal systems of immunoglobulin A and immunoglobulin A containing complexes in IgA nephropathy and cirrhosis patients. The role of asialoglycoprotein receptors. *Lab Invest 69:714.*

7. **Maliszewski, C. R., C. J. March, M. A. Schoenborn, S. Gimpel, and L. Shen.** 1990. Expression cloning of a human Fc receptor for IgA. *J Exp Med 172:1665.*

8. **Reterink, T. J. F., W. E. M. Schroeijers, L. A. van Es, and M. R. Daha.** 1996. Dimeric and polymeric IgA, but not monomeric IgA, enhance the production of IL-6 by human renal mesangial cells. *Mediat Inflamm 5:191.*

9. **Reterink, T. J., G. van Zandbergen, M. van Egmond, N. Klar-Mohamad, C. H. Morton, J. G. van de Winkel, and M. R. Daha.** 1997. Size-dependent effect of IgA on the IgA Fc receptor (CD89). *Eur J Immunol 27:2219.*

10. **Westerhuis, R., G. van Zandbergen, N. A. M. Verhagen, N. Klar-Mohamad, M. R. Daha, and C. van Kooten.** 1999. Human mesangial cells in culture and in kidney sections fail to express Fc alpha receptor (CD89). *J Am Soc Nephrol 10:770.*

11. **Gomez-Guerrero, C., E. Gonzalez, and J. Egido.** 1993. Evidence for a specific IgA receptor in rat and human mesangial cells. *J Immunol 151: 7172.*

12. **Monteiro, R. C., M. D. Cooper, and H. Kubagawa.** 1992. Molecular heterogeneity of Fc alpha receptors detected by receptor-specific monoclonal antibodies. *J Immunol 148:1764.*

13. **Shen, L., R. Lasser, and M. W. Fanger.** 1989. My 43, a monoclonal antibody that reacts with human myeloid cells inhibits monocyte IgA binding and triggers function. *J Immunol 143:4117.*

14. **de Fijter, J. W., A. W. van den Wall Bake, C. A. Braam, L. A. van Es, and M. R. Daha.** 1995. Immunoglobulin A subclass measurement in serum and saliva: sensitivity of detection of dimeric IgA2 in ELISA depends on the antibody used. *J Immunol Methods 187:221.*

15. **Morton, H. C., I. E. Van den Herik-Oudijk, P. Vossebeld, A. Snijders, A. J. Verhoeven, P. J. Capel, and J. G. J. van de Winkel.** 1995. Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. *J Biol Chem 270:29781.*

16. **Hiemstra, P. S., J. Biewenga, A. Gorter, M. E. Stuurman, A. Faber, L. A. van Es, and M. R. Daha.** 1988. Activation of complement by human serum IgA, secretory IgA and IgA1 fragments. *Mol Immunol 25:527.*

17. **Chen, A., L. F. Sheu, S. L. Ding, and W. H. Lee.** 1996. Monomeric immunoglobulin A protects glomerulus against polymeric immunoglobulin A immune complex in experimental immunoglobulin A nephropathy. *Lab Invest 74:737.*

18. **Gomez-Guerrero, C., M. J. Lopez-Armada, E. Gonzalez, and J. Egido.** 1994. Soluble IgA and IgG aggregates are catabolized by cultured

rat mesangial cells and induce production of TNF-alpha and IL-6, and proliferation. *J Immunol 153:5247*.

19. **Liu, Z. H., G. E. Striker, M. Stetler-Stevenson, P. Fukushima, A. Patel, and L. J. Striker**. 1996. TNF-alpha and IL-1 alpha induce mannose receptors and apoptosis in glomerular mesangial but not endothelial cells. *Am J Physiol 270:C1595*.

20. **Monteiro, R. C., B. Grossetete, A. T. Nguyen, P. Jungers, and A. Lehuen**. 1995. Dysfunctions of Fc alpha receptors by blood phagocytic cells in IgA nephropathy. *Contrib Nephrol 111:116*.

21. **Tomana, M., K. Matousovic, B. A. Julian, J. Radl, K. Konecny, and J. Mestecky**. 1997. Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. *Kidney Int 52:509*.

22. **Carayannopoulos, L., J. M. Hexham, and J. D. Capra**. 1996. Localization of the binding site for the monocyte immunoglobulin (Ig) A-Fc receptor (CD89) to the domain boundary between Calpha2 and Calpha3 in human IgA1. *J Exp Med 183:1579*.

23. **Lasseur, C., A. C. Allen, C. Deminiere, M. Aparicio, J. Feehally, and C. Combe**. 1997. Henoch-Schonlein purpura with immunoglobulin A nephropathy and abnormalities of immunoglobulin A in a Wiskott-Aldrich syndrome carrier. *Am J Kidney Dis 29:285*.

24. **Chuang, P. D. and S. L. Morrison**. 1997. Elimination of N-linked glycosylation sites from the human IgA1 constant region: effects on structure and function. *J Immunol 158:724*.

25. **Allen, A. C., S. J. Harper, and J. Feehally** . 1995. Galactosylation of N- and O-linked carbohydrate moieties of IgA1 and IgG in IgA nephropathy. *Clin Exp Immunol 100:470*.

26. **Mestecky, J., M. Tomana, P. A. Crowley-Nowick, Z. Moldoveanu, B. A. Julian, and S. Jackson**. 1993. Defective galactosylation and clearance of IgA1 molecules as a possible etiopathogenic factor in IgA nephropathy. *Contrib Nephrol 104:172-82:172*.

27. **Sanfilippo, F., B. P. Croker, and R. R. Bollinger**. 1982. Fate of four cadaveric donor renal allografts with mesangial IgA deposits. *Transplantation 33:370*.