Human mesangial cells in culture and in kidney sections fail to express Fc alpha receptor (CD89) #

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SUMMARY

The mechanism of deposition of IgA in the renal mesangium in primary IgA-nephropathy is poorly understood. It has been suggested that membrane receptors for IgA on mesangial cells (MC) of the kidney may be involved. To obtain more insight in the occurrence of the myeloid receptor for IgA (CD89) on MC, both in situ and in culture, rabbit- and goat polyclonal antibodies and mouse monoclonal antibody against recombinant CD89 were raised. Kidney sections from five control subjects and five patients with primary IgA-nephropathy failed to be positive for CD89 in the mesangium, using our polyclonal and monoclonal antibodies. Also, five primary human MC cultures assessed for CD89 expression showed no protein expression of CD89. Furthermore reverse transcription-PCR failed to detect mRNA expression of CD89 in the cultured MC. It was demonstrated that all five human primary MC bound human IgA in a dose-dependent manner, which was not inhibitable by blocking monoclonal anti-CD89 antibody (My43). In contrast, binding of IgA to U937 cells was blocked efficiently by My43. Finally, incubation of human MC with either human or rat IgA, led to increased interleukin-6 production, whereas only human IgA, but not rat IgA, was able to bind to human CD89. Therefore, it is concluded that human MC do not express CD89 (to a significant extent). These results strongly suggest that binding of IgA to human MC occurs via an IgA receptor distinct from CD89.

# Drs Westerhuis and van Zandbergen contributed equally to this study. J Am Soc Nephrol 10:770, 1999
INTRODUCTION

Primary IgA-nephropathy (IgAN) is the most common form of glomerulonephritis in humans, leading to progressive renal failure in nearly half of the patients. IgAN is characterized by deposition of mainly IgA1 in the mesangial area often associated with higher serum IgA1 levels. IgA deposition in the mesangium is thought to play a crucial role in the inflammatory process in this disease (1,2) but the mechanism responsible for IgA deposition remains unknown.

Different investigators have showed that IgA binds to rat mesangial cells (MC) (3,4). It was also shown that binding of IgA to MC leads to uptake and degradation of IgA and that IgA induces release of interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) (3). Previous results have shown that IgA binds to human MC (5-7), however, no specific receptor could be identified.

The DNA sequence of a receptor for the Fc tail of IgA expressed on myeloid cells was published earlier (8). Further investigations found expression of this IgA receptor, designated Fcα receptor or CD89, on the surface of neutrophils (9,10), eosinophils (11) and cells of the monocyte/macrophage lineage (12).

Very recently, mRNA expression of the Fc receptor CD89 has been suggested on human MC (13). Activation of human MC with IL-6 or TNFα led to increased binding of IgA accompanied by an increase of mRNA expression of CD89. However, no evidence was provided for the direct involvement in the binding of IgA to these cells via CD89 (13). Two additional studies showed an increase of mRNA expression of monocyte chemoattractant protein-1, IL-8, nuclear factor-κB, and also an increase of intracellular calcium release following interaction of MC with aggregated IgA (14,15). Further mRNA expression of CD89 was demonstrated in whole human glomeruli in 40% of patients with IgAN. In healthy individuals and patients with mesangial proliferative glomerulonephritis distinct from IgAN, no CD89 expression was found, suggesting an absence of CD89 expression on human MC in situ (16).

Because of these contradictory results, the aim of the present study was to determine whether CD89 is expressed on human MC in vivo and in vitro.

MATERIALS AND METHODS

Cell culture

The CD89-expressing human monocytic cell line U937 was cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin (all from Life Technologies, Breda, The Netherlands).
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The murine B-cell line IIA1.6 (17) was cultured in RPMI 1640 supplemented with 10 % FCS. IIA1.6 was transfected with CD89 cDNA, and stable cell surface expression was maintained by cotransfection of human γ-chain cDNA, as described previously (17). 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).

In the present study, primary human MC were obtained from five different normal donor kidneys. MC were cultured and characterized as reported in detail elsewhere (18,19). After outgrowth of the MC, the hillocks formed were lifted off the culture flasks and explanted into 24-wells culture plates (Greiner, Alphen aan de Rijn, The Netherlands) and subcultured in T25 or T75 flasks (Greiner) in 10 % FCS, 100 U/L penicillin and 100 µg/ml streptomycin (Life Technologies). Primary MC-cultures were used between subculture 3 and 10.

Monoclonal anti CD89 antibodies

The generation and production of recombinant soluble CD89 (sCD89) by transfected Chinese Hamster Ovary (CHO) cells using the pEE14 expression system will be described in detail elsewhere (G. v. Zandbergen, Manuscript in preparation). Soluble recombinant CD89 was isolated from the culture supernatant by affinity chromatography, using Sepharose-bound human IgA.

To generate new monoclonal antibodies, female BALB/C mice were immunized with purified sCD89 (8). Splenocytes isolated from immunized mice were fused with myeloma cells (SP20), using 50% polyethylene glycol. The cell suspension was diluted in RPMI 1640 supplemented with 10% FCS, hypoxantine (100 µmol), aminopterin (0.4 µM), thymidine (16 µM), 100 U/L penicillin and 100 µg/ml streptomycin. Cells producing anti-CD89 antibodies were subcloned by limiting dilution. Five clones producing anti-CD89 antibodies were expanded and the specificity determined by Western blotting. For this purpose, recombinant sCD89 was electrophoresed on 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels under reducing conditions and blotted onto nitrocellulose (20) and subsequently interacted with culture supernatant from A77, a well defined mouse monoclonal antibody (mAb) anti-CD89 (21), and the five new clones (7D7, 7G4, 2D11, 2E6, 2H8, 1/100). Finally, bound antibodies were detected using goat anti-mouse IgG-horseradish peroxidase, and detected by chemiluminescence.
**Polyclonal antibodies**

A rabbit and a goat were immunized with 100 µg of purified sCD89 at 4 weekly intervals. After 3 months, serum was tested for anti-CD89 reactivity by enzyme-linked immunosorbent assay (ELISA). The IgG fractions of the polyclonal antibodies were isolated by (NH₄)SO₄ precipitation followed by diethylaminoethyl Sephacel (Pharmacia, Uppsala, Sweden) anion exchange chromatography and tested on Western blots. For control purposes, rabbit- and goat IgG were also isolated from normal sera.

**Immunohistochemistry**

Pretransplant kidney tissue and biopsies from patients with proven IgAN (Department of Nephrology, University Hospital Leiden, The Netherlands) were snap-frozen in liquid nitrogen. Tissue specimens were processed for immunofluorescence according to standard procedures. As a control for tissue with CD89 antigen, normal donor spleen was used.

For analysis of CD89 on MC, primary MC were grown on glass coverslips for 24 h, washed in phosphate-buffered saline (PBS), and air-dried. As a positive control, U937 cells cultured on glass coverslips were used. Cryostat sections and coverslips were fixed in acetone for 10 min at room temperature.

The tissue sections and the glass coverslips were washed 3 times for 5 min with PBS and incubated with either polyclonal or monoclonal anti-CD89 antibodies for 1 h. As a positive control, we used W6-32, a mouse mAb anti-MHC-class I. After washing the tissue sections and coverslips the preparations were incubated with goat anti-mouse-FITC, rabbit anti-goat-FITC, and with goat anti-rabbit-FITC, respectively (Dako, Glostrup, Denmark).

**RNA Isolation and Reverse Transcription-PCR**

Total RNA from U937, IIA1.6 CD89-transfected cells, and human MC in the same passage as used for fluorescence-activated cell sorter (FACS) analysis. Also MC cultured in 20% FCS or stimulated with phorbol 12-myristate 13-acetate (10ng/ml)/Ionomycin (1 µg/ml) for 24 h were used. Total cellular RNA was isolated from 1 x 10⁶ cells, using RNAzol B (Cinna/Biotecx, Houston, TX), according to manufacturer’s instructions (22). Fixed amounts of total cellular RNA (1 µg) were reverse-transcribed into cDNA by oligo-(dT) priming, using Moloney murine leukemia virus reverse transcriptase (Life Technologies).

The amplification of cDNA by PCR was performed using the primers as shown in Figure 1. We tested seven different combinations of the above-described forward and reverse primers as shown. Furthermore we performed a nested PCR in which
we first used the forward primer (bp 90 to 109) combined with the reverse primer (bp 614 to 633). The product of this PCR was checked on agarose gel and then 1, 0.1, and 0.01 µg of the PCR product were again amplified by PCR using the forward primer (bp 119 to 138) and the reverse primer (bp 441 to 460). PCR amplification was performed under standard conditions (50 mM KCl, 10 mM Tris-HCl, pH 8.4, 20 mM MgCl₂, 0.06 mg/ml bovine serum albumin, 0.25 mM dNTP, 25 pmol of each primer, and 1 U of Taq polymerase: Perkin Elmer, Norwalk, CT) by 35 cycles of the following scheme: 1.5 min at 95°C, 2.5 min at 60 °C, 1.5 min 72°C, followed by 10 min of primer extension at 72 °C. MgCl₂ concentrations of 20 mmol/L was found to be superior compared with 15 or 25 mmol/L for the amplification of CD89. PCR products were analyzed on a 1% agarose gel containing ethidium bromide. Results were registered using a digital camera (Eagle eye: Stratagene, San Diego, CA), and for reason of clarity, the images were black/white inverted.

**Figure 1. Gene of CD89 and the used primers.** At the top, the gene of CD89 is shown containing five exons: S1, S2, extracellular-1 (EC1), EC2, and TM/C (transmembrane/cytoplasmic). The six different primers shown at the bottom are indicated by an arrow below the CD89 gene to illustrate their location. Three forward and three reverse primers were used in different combinations. Primers for GAPDH were used as positive control.

<table>
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<tr>
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**Isolation of human and rat IGA**

Human IGA was isolated from serum of an IGA myelomatosis patient as described previously (23). Rat hybridoma anti-dinitrophenol (DNP) IGA was isolated as described (24). Briefly, IGA containing ascites was precipitated using (NH₄)₂SO₄, resuspended, and dialyzed against PBS-2mM ethylenediaminetetra-acetic acid (EDTA). Specific IGA-anti-DNP was obtained by immunoabsorption using a DNP-lysine-coupled Sepharose affinity column. After washing, anti-DNP-specific IGA was eluted from the column with 0.1 M DNP. After removal of free DNP by chromatography on Dowex (1×2 to 400), the IGA-containing fractions
were pooled, concentrated, and subjected to gel filtration chromatography on a Sephacryl S-300 column to yield monomeric, dimeric and polymeric IgA. The purified IgA preparations were dialyzed against PBS and were shown to be devoid of detectable IgG and IgM by sandwich ELISA.

**FACS analysis**

To evaluate a possible trypsin sensitivity of CD89, U937 cells were treated with 0.05% trypsin/0.02% EDTA (all from Sigma) for 3 min at room temperature and assessed for CD89 expression by FACS (see below). No differences between trypsinized and nontrypsinized U937 cells were observed. Human MC were tested for binding of IgA after using the different detaching procedures described above and again no differences were detected. This suggests that the IgA binding molecules present at the surface of MC are not affected by the different detaching protocols. Therefore, 0.05% trypsin/0.02% EDTA was used for the detachment of MC.

FACS analyses for CD89 expression on U937 and MC were performed as follows: MC were detached with trypsin/EDTA, and portions of 0.25 x 10^6 cells per sample were washed twice in FACS buffer (PBS/1% bovine serum albumin/0.02% NaN₃) and incubated 1 h with the five different mouse mAb anti-CD89 (culture supernatant diluted 1:10 in FACS buffer) and the rabbit and goat polyclonal anti-CD89 IgG (both 5 µg ml). As a positive control, W6-32 was used (culture supernatant 1:10, IgG2a mouse mAb). After incubation, cells were washed twice with FACS buffer and incubated for 1 hour with goat anti-mouse IgG1-phycoerythrin (PE) polyclonal antiserum, goat anti-rabbit IgG-PE polyclonal antiserum, rabbit anti-goat–FITC and goat anti-mouse IgG-PE polyclonal antiserum, respectively (all from Dako). All staining procedures were performed at 4°C.

The binding of human IgA1 to U937 and MC was analyzed as follows: cells were washed twice with FACS buffer and incubated for 1 h with 400, 200 and 100 µg/ml purified IgA1. After incubation, the cells were washed and bound IgA was detected by incubation with mouse monoclonal antihuman IgA antibody (4E8) and subsequently with PE-labeled goat anti-mouse IgG1 polyclonal antiserum.

To demonstrate the specificity of binding of IgA to cellular CD89, studies were performed in which U937 and MC were incubated with IgA in the presence and absence of the CD89 blocking mouse mAb My43 (25). An isotype-matched irrelevant mouse mAb served as a control for My43.

To determine the specificity of rat and human IgA for human CD89, IIA1.6 CD89- transfected cells were tested for binding of human and rat IgA. A total of 100 µg/ml human IgA1 or rat dimeric IgA was added to the cells for 1 h. Then
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cells were washed and bound IgA was detected by incubation with mouse monoclonal antihuman IgA antibody (4E8)-biotin or rabbit polyclonal anti-rat IgA-biotin, respectively. After washing, cells were incubated for 1 h with streptavidin PE (Dako). The percentage positive cells were used in all FACS-experiments as a measure for CD89 expression and IgA binding.

Figure 2. Fluorescence-activated cell sorter (FACS) analyses of CD89 expression on U937 cells by six mouse monoclonal antibodies (mAb). A77, a defined Ms mAb anti-CD89, and five new mouse mAb anti-CD89 (2E6, 2D11, 7G4, 2H8, and 7D7) were tested on FACS using CD89-expressing U937 cells. Broken lines show the staining of the cells by an isotype-matched control antibody: the solid lines show the staining with the anti-CD89 mAb. The dotted lines in all panels indicate the mean staining obtained with A77.

**IL-6 production by human MC in vitro**

Subconfluent 48-well plates with human MC were washed 3 times with PBS and cultured for another 48 h in Dulbecco's modified Eagle's medium/0.5% FCS to bring the cells to a quiescent state. After washing the cells three times with medium, 100 µg of dimeric human and rat IgA was added to triplicate wells. After 72 h of incubation, supernatants were harvested and assessed for IL-6 production, using the IL-6 dependent murine hybridoma cell line B9 (26,27). Serial dilutions of human recombinant IL-6 were used as a standard.

Figure 3. FACS analysis of CD89 expression on U937 cells. Two polyclonal anti-CD89 antibodies (rabbit and goat) were tested on U937 cells. The broken lines show the staining of the cells using a normal control antibody, the solid lines show the staining with the polyclonal antibodies.
Chapter 4

Statistical analyses
All values are expressed as mean ±SD unless stated otherwise. Statistical analysis was performed using an unpaired t test. A P value <0.05 was considered significant.

RESULTS

Generation of anti-CD89 antibodies
Five mouse hybridoma cell lines producing anti-CD89 antibodies designated 7D7, 2D11, 2E6, 7G4, and 2H8 were selected for the present studies. In addition, two polyclonal Ab (goat and rabbit) were raised. The whole set of antibodies was tested for CD89 specificity by FACS, using CD89 expressing U937 (Figures 2 and 3) and IIA1.6 CD89-transfected cells. Besides the well-known mAb anti-CD89 A77, all of the new monoclonals were able to recognize CD89 to the same extent or better.

Figure 4. Western blot analysis for the characterization of the monoclonal and polyclonal anti-CD89 antibodies. Soluble recombinant CD89 was blotted and stained using the five new mouse mAb anti-CD89, A77, and two polyclonal anti-CD89 antibodies. As an example, results using A77, 7D7 and 7G4 are shown. As the negative control, we used an isotype-matched irrelevant mouse mAb. The other monoclonal and polyclonal antibodies were also positive using this method.

Furthermore, the new antibodies were tested by Western blot. All of the monoclonal and polyclonal antibodies showed a strong reaction with soluble CD89. The results for two of the monoclonal antibodies and A77 are shown in Figure 4.

Immunofluorescence of kidney and spleen cryostat sections
Kidney sections of control pretransplant biopsies and those of patients with IgAN failed to show any CD89-expressing cells in the mesangium: however, a strong reaction was observed in normal spleen cryostat sections using the above-described set of anti-CD89 antibodies (Figure 5). The positive cells morphologically resembled macrophages and monocytes. Staining of the control antigen MHC class I was positive for nearly all cells in glomeruli of controls, IgAN patients, and the spleen sections (Figure 5).
**Immunofluorescence of cells grown on glass coverslips**

MC from five of five primary MC cultures grown for 24 h on glass coverslips revealed no positive staining for CD89, using all our antibodies and A77 (Figure 5). On the other hand, CD89 expression on the monocytic cell line U937 was clearly present. As expected, expression of MHC class I was positive for both U937 cells and MC (Figure 6).
FACS analysis for CD89 expression

U937 and MC were also analyzed for CD89 protein expression by FACS analysis. Although U937 cells were strongly positive for CD89, all of the five primary MC were completely negative with all monoclonal and polyclonal antibodies (Figure 7). In contrast, MHC class I expression was clearly positive for both U937 and MC.

Figure 7. FACS analysis of CD89 expression on U937 and MC. CD89 expression was analyzed with five mouse mAb anti-CD89 and two polyclonal antibodies directed against CD89. Histograms of one monoclonal and one polyclonal antibody are shown as representative examples for two of the five examined primary MC cultures. As a positive control MHC class 1 detection by W6-32 is shown.

Reverse Transcription PCR for CD89

The results above indicate the absence of protein expression of CD89 on MC. Therefore, we analyzed U937, IIA1.6 CD89-transfected cells, and MC for mRNA expression of CD89, using reverse transcription (RT)-PCR. Reverse transcriptase and PCR of the mRNA showed for U937 cells and IIA1.6 CD89-transfected cells the expected positive band in each primer combination except primer combination 3+6 (Figure 8). In contrast, CD89-PCR analysis of cDNA from five primary MC cultures revealed no PCR product with the expected amplicon sizes of all primer combinations. Expression of GAPDH was detectable in all cases (Figure 8). Also, when cDNA was derived from MC cultured in 20% FCS or stimulated with phorbol 12-myristate 13-acetate (10ng/ml)/ionomycin (1 µg/ml), no mRNA from CD89 was found. Finally, we performed a nested PCR using three different concentrations (1, 0.1, and 0.01 µl) of the product of a first RT-PCR in a second PCR with internal primers. The CD89-expressing control cells were positive in all cases, whereas mRNA isolated from human MC failed to be positive for CD89, even after this second round of amplification (Figure 8).
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**Binding of human IgA to MC**

Incubation of MC with IgA and subsequent analysis by FACS revealed that all five MC bound human IgA1 with a mean of $19.9 \pm 3.2\%$ positive cells (ranging from 17.4 to 25.1%). To determine whether the binding of IgA to the MC was mediated by CD89, MC were exposed to IgA in the presence and absence of the mAb anti-CD89 My43 and subsequently assessed for binding of IgA. My43 did not reduce the binding of IgA1 to human MC ($18.1 \pm 4.4\%$), but it inhibited the binding of IgA to U937 cells by 94.8 % (Figure 9). The U937 cells without preincubation with IgA showed a basal MFI of 3.05. Cells incubated with IgA increased their mean fluorescence intensity (MFI) to 28.2 and those cells preincubated with the blocking anti-CD89 antibody (My43) showed minimal binding of IgA indicated by an MFI of 5.81.

![Figure 8. Reverse Transcription (RT)-PCR analysis to detect CD89 mRNA expression.](image)

(A) Results of RT-PCR obtained with eight different primer sets. None of the primer combinations for CD89 was able to detect mRNA of this receptor in five of five primary MC cultures, whereas only primer combination 3+6 was negative for U937- and IIA1.6-CD89-transfected cells. All other combinations showed positive bands. Control primers detecting mRNA expression of GAPDH was positive for all the examined cell lines. As a negative control, water was used. (B) Three examples of a primer combination as well as the result of the nested RT-PCR for CD89. GAPDH control is shown at the top.

![Table](image)

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**Production of IL-6 after incubation of MC rat and human IgA**

To determine the effect of dimeric rat and human IgA, MC were incubated with 100 µg of rat or human IgA and assessed for IL-6 production. The basal production was $1280 \pm 400$ U IL-6 per $10^5$ cells. Incubation of MC with 100 µg of
human IgA resulted in enhancement IL-6 production to $3170 \pm 1400$ U IL-6 per $10^5$ cells ($P<0.05$). Interestingly, rat IgA also induced an enhancement of IL-6, and resulted in production of $3586 \pm 1471$ U IL-6 per $10^5$ cells ($P<0.01$). Both results are significantly higher compared with MC cultured in medium alone (Figure 10).

**Figure 9. Effect of My43 on the binding of IgA1 to U937, and MC.** (A) Contour profiles of MC incubated without (left panel) and with (middle panel) human IgA. In this case, 17.7% positive cells were observed. The panel on the right shows the binding of IgA to MC in the presence of My43. Similar results were found using four other primary MC. (B) Contour profiles of U937 cells showing 43.8% positive cells with a mean fluorescence intensity (MFI) of 28.2 after incubation of U937 cells with IgA (middle panel). In the presence of My43, a reduction of IgA-binding to 3.8% respective a mean fluorescence intensity (MFI) of 5.81 was observed. U937 incubated without IgA are shown in the left panel (MFI 3.05). Comparable results were obtained in three different experiments.

**Figure 10** IgA-induced enhancement of interleukin-6 (IL-6) production by human MC. The effect of human (100 $\mu$g/ml) and rat IgA (100 $\mu$g/ml) on production of IL-6 by human MC compared to MC incubated in medium alone is shown. The results are means ± SD of three experiments.

**FACS analysis of binding of human IgA1 and rat dimeric IgA to CD89-transfected cells**

To test the specificity of human IgA for CD89, FACS-analysis using IIa1.6 CD89-transfected cells expressing recombinant human CD89 on their surface was performed. After incubation IIa1.6 CD89-transfected cells with human and rat IgA, a difference in binding was found.
Human IgA1 bound to approximately 100% of the transfected cells, whereas dimeric rat IgA did not show any detectable binding (Figure 11). No binding to nontransfected cells was found (data not shown).

**DISCUSSION**

Although deposition of IgA in the mesangium of IgAN patients is well documented, the involvement of the Fc receptor for IgA (CD89) on MC in the pathogenesis of the disease is controversial. In this study, we investigated whether CD89 is expressed on the surface of human MC in vivo and in vitro. Therefore, we developed five mouse mAb and two polyclonal antibodies directed against CD89. These antibodies were shown to be specific for CD89 and able to stain CD89 on cells in cryostat sections of spleen. However, we did not find CD89 reactivity in kidney sections of control subjects and patients with IgAN by using our new set of monoclonal and polyclonal antibodies completed with a well-known mouse mAb anti-CD89 A77. MC grown on glass coverslips were also negative for CD89. Furthermore, we performed FACS analysis and again CD89 protein expression was not detectable on human MC. However, various tissue and cell controls were positive for CD89.

To exclude a possible processing defect of CD89 by MC, we determined mRNA expression of CD89. Seven combinations of three forward and three reverse primers (Figure 7A) were used recognizing different parts of the extracellular domain 1 and extracellular domain 2 of CD89. This should also allow the detection of possible expression of splice variants of CD89 in human MC. All of these primer sets failed to reveal expression of CD89 in human MC, while with primer combination 1-6 U937 and IIa1.6 CD89-transfected cells were positive. Also, the very sensitive nested RT-PCR failed to detect CD89 mRNA expression in five of five human MC, whereas the control cells were clearly positive. Only primer
combination 7, which is identical to the primers used in an earlier publication (13), did not show an amplification band either in the controls or the MC. Taken together, these data strongly indicate that CD89 is neither expressed as a protein on the surface nor as mRNA in human MC. To exclude that MC derived from patients with IgAN are in contrast to “normal MC” able to express CD89, we stained five kidney sections from five different IgAN patients with our set of two polyclonal and five monoclonal anti-CD89 antibodies. They also did not show any positivity for CD89; furthermore, the “normal MC” showed binding of IgA independent of CD89.

These results are in contrast with earlier findings demonstrating CD89 mRNA expression in human and rat MC by Northern blot and RT-PCR analysis, respectively (6,13). Also, experiments performed with the primers used in the above mentioned studies (13), failed to detect CD89 mRNA in either quiescent or activated MC, or in our CD89-expressing controls (U937 and IIA1.6 CD89-transfected cells) as stated above. Based on the finding that 40% of patients with IgAN have CD89 mRNA expression in whole glomeruli, it has been suggested that MC in IgAN might express CD89 mRNA (16). However, it is known that influx of monocytes may occur in IgAN (28-30), which may explain these positive results. Furthermore, no CD89 expression was found in normal glomeruli and those isolated from patients with non-IgA mesangial proliferative glomerulonephritis, supporting our findings of the absence of CD89 mRNA in isolated human MC. However, in the present study, we could not find any CD89-positive monocyte in the mesangial area of patients with IgAN. We suggest that the sensitivity of the immunohistochemical staining is too low to detect CD89 expression on this infiltrating cell.

Recently, it was shown that stimulation of human MC with IL-6, TNFα, and interferon-γ enhances binding of IgA in vitro to MC (13). The investigators interpreted the enhanced binding of IgA and a simultaneous upregulation of mRNA of CD89 as evidence for enhanced CD89 protein expression. However, no direct evidence of CD89 receptor involvement in the binding of IgA was presented, because they did not perform blocking experiments with anti-CD89 antibodies.

A number of earlier investigations showed binding of IgA to human and rat MC (4-6,13,31); however, the direct involvement of CD89 on MC in the binding of IgA to the cells has not been addressed until now. Therefore, we examined the binding of IgA to human MC in the presence and absence of a blocking monoclonal antibody (My43) against CD89. My43 anti-CD89 inhibited the binding of IgA to U937, whereas no reduction of binding of IgA to MC was observed, indicating that the binding of IgA to MC is CD89-independent.
To confirm specificity of CD89 for human IgA, it was shown that only human but not rat IgA reacts with CD89-transfected IIA1.6 cells. Similar results were obtained using an ELISA-system, using recombinant soluble CD89 (data not shown). On the other hand, it was found that not only human but also rat IgA is able to enhance IL-6 production by human mesangial cells \textit{in vitro}, again indicating the presence of another IgA binding moiety on the surface of human MC.

A number of possible receptors, such as the asialoglycoprotein receptor (ASGP-R) and mannose receptor, could be involved. Since reliable poly- and monoclonal antibodies against the mannose receptor have been generated (32,33), and it has been suggested that activated mouse MC express this receptor (34), we investigated its expression on human MC. However, we did not find mannose receptor expression using FACS analysis either on resting or on activated human MC (data not shown).

ASGP-R is known to be expressed on human and rat hepatocytes and is involved in the clearance of galactose-terminal glycoproteins for example human IgA1 (35-38). Recently, it was reported that ASGP-R is expressed on human and rat MC using RT-PCR (31). In the above-mentioned study (31) binding and catabolism of IgA by human and rat MC was saturable and partly inhibitable by galactose but not by other carbohydrates.

Taken together, we have shown for the first time that CD89 is not expressed on human MC either \textit{in situ} or \textit{in vitro}. Furthermore, we found that binding of IgA to MC is not mediated via CD89 and that activation of MC after binding of IgA is independent of CD89. Therefore, we postulate that another receptor, different from CD89, is responsible for binding of IgA to MC.
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