Engagement of Transferrin Receptor by Polymeric IgA1: Evidence for a Positive Feedback Loop Involving Increased Receptor Expression and Mesangial Cell Proliferation in IgA Nephropathy

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IgA nephropathy (IgAN), the most common primary glomerulonephritis in the world, is characterized by IgA immune complex–mediated mesangial cell proliferation. The transferrin receptor (TfR) was identified previously as an IgA1 receptor, and it was found that, in biopsies of patients with IgAN, TfR is overexpressed and co-localizes with IgA1 mesangial deposits. Here, it is shown that purified polymeric IgA1 (pIgA1) is a major inducer of TfR expression (three- to four-fold increase) in quiescent human mesangial cells (HMC). IgA-induced but not cytokine-induced HMC proliferation is dependent on TfR engagement as it is inhibited by both TfR1 and TfR2 ectodomains as well as by the anti-TfR mAb A24. It is dependent on the continued presence of IgA1 rather than on soluble factors released during IgA1-mediated activation. In addition, pIgA1-induced IL-6 and TGF-β production from HMC was specifically inhibited by mAb A24, confirming that pIgA1 triggers a TfR-dependent HMC activation. Finally, upregulation of TfR expression induced by sera from patients with IgAN but not from healthy individuals was dependent on IgA. It is proposed that deposited pIgA1 or IgA1 immune complexes could initiate a process of auto-amplification involving hyperexpression of TfR, allowing increased IgA1 mesangial deposition. Altogether, these data unveil a functional cooperation between pIgA1 and TfR for IgA1 deposition and HMC proliferation and activation, features that are commonly implicated in the chronicity of mesangial injuries observed in IgAN and that could explain the recurrence of IgA1 deposits in the mesangium after renal transplantation.


Glomerular mesangium contains contractile specialized cells, the mesangial cells, located between the capillary endothelial cells and the basal membrane of the glomeruli. Mesangial cells are thought to regulate the blood flow through selected capillary loops and the uptake of macromolecules by phagocytosis (1–3). Because of their intracapillary location and their capacity to synthesize cytokines and other inflammatory molecules, mesangial cells are in a critical position to initiate and mediate glomerular damage. Thus, mesangial cell proliferation is an early pathologic alteration characteristic of many forms of immunologically mediated glomerulonephritis, such as IgA nephropathy (IgAN), mesangial proliferative glomerulonephritis, and diabetic nephropathy (4).

IgAN is a primary glomerulonephritis of common incidence worldwide. Clinical features include hematuria and proteinuria, and the disease is characterized histologically by the deposition of IgA1-containing complexes in glomerular mesangium, matrix expansion, and mesangial cell proliferation. IgA1 deposits are often associated with deposits of IgG, of the complement component C3, and less frequently of IgM. IgAN prognosis is unfavorable because 20 to 30% of patients develop progressive renal failure within 20 yr of disease activity (4). Recurrence of IgA1 deposits after transplantation indicates that circulating rather than local kidney abnormalities are crucial to the development of IgAN (5).

Alterations in IgA circulating levels and structure are associated with IgAN. Serum IgA levels are two- to three-fold enhanced in one half of the patients. In addition, polymeric:monomeric IgA ratio is increased in their serum (6). Furthermore, pathogenesis of IgAN involves IgA1 immune complex formation and alterations of IgA1 glycans composition (7). Indeed, patients with IgAN express a subpopulation of serum IgA1 that are hypogalactosylated (8–10). Altered O-glycans present in IgA1 hinge region can be recognized by naturally occurring antiglycans IgG and IgA, thus forming immune complexes (7) that are deposited in the mesangium (11).

Binding of IgA1 complexes on mesangial cells elicits cell activation involving an increase in intracellular Ca2+, phospholipase (PLC)-γ1 activation, production of inositol trisphosphate...
proliferation and IL-6 and TGF-

IgA1-mediated mesangial cell activation suggested the existence of a receptor for IgA1 on these cells. However, none of the known IgA receptors, such as FcαRI, polymeric Ig receptor (pIgR), and asialoglycoprotein receptor (ASGPR), is expressed on mesangial cells (4). Recently, we identified the transferrin (Tf) receptor (TfR) as an IgA1 receptor (15). TfR binds polymeric IgA1 (pIgA1) but neither monomeric IgA1 nor IgA2 and preferentially IgA1 complexes and hypoglycosylated IgA1 (16). TfR mesangial expression is upregulated in IgAN and Henoch-Schönlein purpura nephritis but also in other nephritides involving IgA deposits (17). TfR co-localized with IgA1 deposits in the mesangium from these patients (17).

Our study was designed to characterize the cellular consequences of IgA1/TfR interaction. We show that pIgA1 induces TfR expression in a time- and dose-dependent manner. In addition, engagement of TfR by pIgA1 induces mesangial cell proliferation and IL-6 and TGF-β secretion, indicating an original TfR signaling pathway activated by pIgA1. Finally, serum from patients with IgAN but not from normal individuals induces an IgA-dependent upregulation of TfR expression, suggesting a link among altered IgA glycosylation, TfR expression, and mesangial cell activation observed in IgAN.

Materials and Methods

Antibodies and Reagents

IL-1β, TNF-α, and IL-6 were from R&D Systems (Lille, France). LPS was from Sigma (St. Louis, MO). The anti-human TfR (CD71) mAb A24 (y2b2x) has been previously described (15), and the isotype-matched mAb 30.9 directed against the rat FcεRI chain was used as a control (18). The anti-IgA mAb CH-EB6-8 (y1κ) was from American Type Culture Collection (Manassas, VA). Human myeloma IgA1 (Dou) was purified as described (15). pIgA1 fractions were separated by gel filtration on Superdex 200 columns through HPLC (Amersham Biosciences, Uppsala, Sweden; >99% pure). Polyclonal anti-human transferrin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was coupled to activated Sepharose 4B beads (Amersham) according to the manufacturer’s recommendations. Tf-free IgA was purified by gel filtration and immunoadsorption through anti-Tf Sepharose 4B columns as described (15). Human serum IgG was purified by ammonium sulfate precipitation and DEAE ion exchange chromatography.

IgA1 depletion of sera from normal individuals and from patients with IgAN was performed using jacalin-coupled Sepharose beads. Briefly, jacalin was purified from jackfruits on DEAE column after extraction in PBS and coupled to CNBr-activated Sepharose 4B beads (Pharmacia, St. Quentin, France). Sera (250 μl) were incubated three times for 30 min with 200-μl beads on a rotating wheel at room temperature to remove IgA1.

Expression and Purification of TfR1 and TfR2 Ectodomains

Soluble (s) versions of human TfR1 and TfR2 (kindly provided by P. Bjorkman, CalTech, CA) were expressed in a lytic baculovirus/insect cell expression system as described previously (19). The construct for the control soluble form of the extracellular domain of CD89 (FcuRI) that contained a 6-His tag in the C-terminus was cloned in pFASTBAC1 baculovirus expression system (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Recombinant proteins were purified as described (19).

Cells

HMC were purchased from Clonetech (San Diego, CA). Cells were cultured in RPMI 1640, supplemented with L-glutamine (2 mM), 5 μg/ml insulin, 20% FCS (Life Technologies, Gaithersburg, MD), 7 mM glucose, 50 U/ml penicillin, and 50 μg/ml streptomycin in a 5% CO2 atmosphere. Studies were performed between passages 4 and 8.

Proliferation Assay

HMC were trypsinized, resuspended in RPMI 1640 with 0.5% FCS, added in triplicate at the concentration of 1 × 104 cells/well in 96-well tissue culture plates (Falcon, Oxnard, CA), and starved for 24 h before experiments. Proliferation of quiescent HMC was induced using IL-1β or TNF-α at 10 ng/ml, IL-6 at 100 ng/ml (R&D Systems), LPS at 10 μg/ml, or pIgA1 at 0.5 mg/ml for 48 h. For the inhibition experiments, mAb A24, mAb 30.9 (10 μg/ml), sTfR1, or sTfR2 (50 μg/ml) was added 15 min before addition of IgA1 or cytokines for 24 h. Proliferation was measured over 18 h, after pulses with 1 μCi/ml [3H]thymidine (American Type Culture Collection, Rockville, MD) and harvested on filters with a 96-well Harvester (Pharmacia), and the incorporation of [3H]thymidine was measured with a β-plate microscintillation counter (LKB; Pharmacia).

For depletion experiments, culture supernatants of HMC that were stimulated for 24 h with pIgA1 were adsorbed three times with an excess of either anti-IgA (CH-EB6-8) or irrelevant mAb (30.9) coupled to Sepharose beads. Depleted supernatants were used as a stimulating agent on quiescent HMC for 24 h, and cell proliferation was measured as described above.

Reverse Transcription–PCR

Total RNA was purified with Trizol (Invitrogen) and was reverse-transcribed using Advantage RT-for-PCR kit (Clontech, Palo Alto, CA) with oligo-dT primers according to the manufacturer’s instructions. TR cDNA were amplified using primers (sense 5′-GGCTTGTAGTTGAAAATCAATTTC and anti-sense 5′-GCTTTCTGACGCTCTGCAG) on the basis of the human TR sequences obtained from GenBank (accession no. M11957). β-Actin cDNA were amplified using sense primer 5′-GGCTATGCGCTCCCCCATGCATCCTGCG and anti-sense primer 5′-TGGGTCTA-CAGGCTTGTGGGATGTCGAG. Amplification was performed with initial denaturation at 94°C for 3 min, followed by 25 cycles at 94°C for 45 s, 60°C for 45 s, 72°C for 2 min, and a single final extension at 72°C for 5 min. The reaction mixture that lacked cDNA was used as a negative control. PCR products were analyzed in a 1.5% agarose gel.

Flow Cytometry Analysis

Cells (0.25 × 10⁶) were preincubated with 10 μl of human IgG (10 mg/ml) for 15 min on ice to mask FcyR. TR expression was examined using an indirect immunofluorescence assay in which cells that were preincubated with human IgG were incubated with 10 μl of biotinylated A24 (0.1 mg/ml) for 30 min on ice before washing and incubation with streptavidin-PE for 20 min at 4°C. After washes, APC-labeled streptavidin (Southern Biotechnology Associates, Birmingham, AL) was used as a developing reagent. Immunofluorescence was finally analyzed by flow cytometry (FACSCalibur; Becton Dickinson, Le Pont-de Claix, France).

ELISA

IgA concentration in the various sera was determined by a sandwich ELISA. Wells in 96-well plates were coated with 50 μl of anti-human IgA mouse mAb CH-EB6-8 at 5 μg/ml in borate buffer saline for 2 h at room temperature. Nonspecific sites were saturated for 2 h at room temperature in PBS that contained 1% BSA and 0.1% NaN₃. Sera (between 1:100 and
1:10,000 dilution) then were added for 16 h at 4°C in the wells, and wells were washed and incubated with a polyclonal anti-IgA antibody coupled to alkaline phosphatase (1:1000 dilution). Optical density was measured at 405 nm after addition of alkaline phosphatase substrate (Sigma), and the IgA concentration was determined by comparison with an IgA standard curve. IL-6 and TGF-β secretions were measured from culture cell supernatants using kits from R&D Systems following the manufacturer’s instructions.

**Statistical Analyses**

The results were analyzed by independent sample two-tailed t test, and evaluation for correlation was conducted by linear regression analysis. Results are presented as means ± SD.

**Results**

**plgA1 Induce TfR Expression on HMC**

Because TfR expression is enhanced in IgAN and co-localizes with mesangial deposits of IgA1 (17), we examined the ability of plgA1 to induce TfR expression on HMC. Incubation of HMC with plgA1 but not IgG resulted in an increased surface expression of TfR (Figure 1A). This correlated with a dramatic increase of TfR1 transcripts (Figure 1B). Time-course analysis showed that induction of TfR expression was observed as soon as after 2 h of incubation with plgA1 and increased steadily for the following 22 h (Figure 1, C and D). In dose-response experiments, the increase in TfR synthesis (Figure 2A) and cell surface expression (Figure 2, B and C) was detected with concentrations as low as 62.5 μg/ml plgA1 and did not reach a plateau up to the maximal concentration tested. Altogether, these data indicate that enhanced receptor expression was due to neosynthesis rather than to an increased exposure to the plasma membrane of the TfR that is stored in the recycling compartments. We conclude that plgA1 is a novel inducer of TfR gene transcription leading to TfR membrane expression.

**plgA1 Is a More Potent Inducer of TfR Expression than Proinflammatory Cytokines**

Proinflammatory cytokines such as IL-6, IL-1β, and TNF-α can upregulate TfR expression up to 1.35-fold in HepG2 hepatoblastoma cell line (20). Cytokine-induced TfR upregulation is dependent on ferritin H induction and occurs at both transcriptional and posttranslational levels after a 12- to 24-h lag time.
As mesangial cells are sensitive to these cytokines, we compared the potency of these factors with that of pIgA1 in the induction of TfR expression. Thus, quiescent HMC were incubated with optimal concentrations of pIgA1, IL-6, IL-1β, or TNF-α as well as LPS. Whereas pIgA1 induced a three-fold increase in TfR expression, the cytokines, as well as LPS, induced an increase of no more than 1.5-fold in TfR expression (Figure 3). Thus, pIgA1 is a major inducer of TfR expression on quiescent HMC.

**pIgA1-Induced Mesangial Cell Proliferation Involves TfR**

Mesangial cell proliferation is a characteristic of IgAN. It was previously shown that IgA can induce mesangial cell proliferation (14). Therefore, we asked whether the blocking of TfR by a specific mAb (A24) or by the ectodomains of the TfR1 or 2 could inhibit pIgA1-induced mesangial cell proliferation. Figure 4A shows that pIgA1 induced a 70% increase in HMC proliferation. MAb A24 specifically blocked this IgA-induced mesangial cell proliferation, whereas the isotype control (mAb 30.9) did not.

MAb A24 could block pIgA1-mediated cell proliferation by one of two ways: either through specific inhibition of pIgA1-mediated functions or by interfering with iron metabolism. Indeed, anti-TfR antibodies are known to inhibit cell proliferation of highly proliferative cells by depriving these cells of their iron uptake, because iron is essential in mitochondrial metabolism (22,23). To rule out such an effect of mAb A24 in our experimental conditions, we first examined the antiproliferative effect of mAb A24 on the spontaneous proliferation of HMC. MAb A24 had no effect on the low proliferative, quiescent mesangial cells (Figure 4B). To test whether mAb A24 inhibited HMC proliferation induced by other stimuli, we performed experiments with proinflammatory cytokines that are known to induce mesangial cell proliferation. MAb A24 had no significant effect on cytokine-induced HMC proliferation (Figure 4C). Therefore, the anti-TfR mAb A24 specifically blocks pIgA1-induced HMC proliferation.

Whereas mAb A24 inhibits TfR-mediated cell stimulation by targeting membrane TfR, soluble forms of TfR1 and 2 would inhibit TfR-mediated cell stimulation by competing with membrane TfR for IgA. Therefore, we next used TfR1 and 2 ectodomains to block pIgA1-induced cell activation. Whereas soluble FcαRI (CD89) did not significantly inhibit mesangial cell proliferation, TfR1 and TfR2 ectodomains dramatically inhibited pIgA1-induced HMC proliferation (Figure 5A). This inhibition was not due to a toxic effect of the soluble receptors, because neither spontaneous (Figure 5B) nor cytokine-induced (data not shown) proliferation of HMC was altered by incubation with either sTfR1 or sTfR2. Altogether, these data indicate that pIgA1-mediated proliferation is dependent on TfR.
Proinflammatory cytokines play a major role in the IgAN renal injury. IgA can induce mesangial cells to synthesize IL-6 and TNF-α, which act in an autocrine manner to induce mesangial cell proliferation (14). In addition, transgenic mice that produce high amounts of human IL-6 develop mesangioproliferative glomerulonephritis (24), and high urinary levels of IL-6 are associated with a bad prognosis in patients with IgAN (25).

Because TfR triggering by pIgA1 induced HMC proliferation, we examined whether IL-6 secretion by pIgA1-stimulated HMC could be dependent on TfR. For this purpose, serum-starved HMC (cultured in 0.5% FCS) were incubated with pIgA1 in the presence or absence of anti-TfR mAb A24. Under these conditions, the level of IL-6 production was approximately 350 pg/ml, a concentration that might be lower than that found under pathologic situations in the confined local mesangial environment. A24 but not the control isotype (30.9) inhibited pIgA1-induced IL-6 secretion by HMC (Figure 6A). No inhibition by A24 of cytokine-induced IL-6 production was observed (Figure 6B). Therefore, TfR engagement by pIgA1 induces IL-6 production by HMC.

IL-6 production could act as a feedback loop for mesangial cell proliferation after pIgA1-mediated activation. For further shedding light on the mechanism that leads to mesangial cell proliferation after activation initiated by IgA1, cells first were activated by pIgA1 and cell supernatant was depleted using an anti-IgA affinity column. This IgA-depleted but not mock-depleted supernatant was no longer capable to promote mesangial cell proliferation (Figure 7). Therefore, the sustained presence of pIgA1 is required to support mesangial cell proliferation, indicating a direct rather than an autocrine mechanism.

TfR Triggering by pIgA1 Induces TGF-β Secretion by HMC

In addition to cell proliferation, IgA-stimulated mesangial cells secrete profibrogenic factors such as TGF-β that act in an autocrine manner and that are important in IgAN physiopathology (26). To examine whether involvement of IgA1-dependent TfR engagement is restricted to IL-6 secretion and cell proliferation or can also...
include matrix expansion, we evaluated TGF-β production. As expected, plgA1 induced TGF-β production by mesangial cells (Figure 8). However, this production was inhibited by preincubation of mesangial cells with A24 but not with a control isotype (Figure 8). By contrast, the spontaneous secretion of TGF-β was not affected by A24. Therefore, TfR is involved in both mesangial cell proliferation and secretion of profibrogenic factors that lead to matrix expansion.

IgAN but not Normal Serum Promotes IgA-Dependent TfR Expression on HMC

Although purified plgA1 can induce TfR expression in vitro, mesangial TfR expression is not observed in biopsies of normal kidney. However, in patients with IgAN, TfR expression is found in mesangial areas and is co-localized with IgA deposits (17). Therefore, experiments were designed to compare the capacity of IgA in normal and in IgAN serum to induce TfR expression on HMC. As shown in Figure 9, A and B, serum from patients with IgAN strongly induced expression of TfR as compared with serum from normal individuals. Because IgAN is characterized by higher concentrations of serum IgA (Figure 9C) (4), we selected sera from patients with IgAN that contained IgA concentrations that were both comparable to and higher than those from normal individuals. These sera were depleted of their IgA1 content by jacalin affinity columns and were examined for their residual capacity to induce TfR expression. Only sera from patients with IgAN demonstrated an ability to induce an IgA-dependent TfR expression (Figure 9D). However, there was also a residual capacity to induce TfR expression observed in both normal and IgA-depleted IgAN sera that could be due to the presence of cytokines or other soluble factors. The difference between normal and IgAN serum in their ability to induce TfR synthesis is not due to differences in IgA concentrations. Rather, in a circulating environment, normal plgA1 is prevented from inducing TfR expression. In a pathologic situation such as IgAN, in which IgA glycosylation is altered and polymeric:monomeric IgA ratio is enhanced, serum factors are no longer able to prevent induction of TfR expression by plgA1.

Discussion

IgAN is an immune complex–mediated glomerulonephritis characterized by mesangial IgA1 deposition and mesangial cell proliferation. Patients with IgAN often have elevated IgA serum levels as well as enhanced polymeric:monomeric IgA ratio (6). Acid elution of IgAN biopsies shows that predominantly polymeric forms of IgA1 are involved in IgA deposits (27). The correlation between the presence of high molecular weight forms of IgA in serum and in renal biopsies from patients corroborates the importance of IgA complexes in the disease. The pathogenic role of Ig complexes is not restricted to IgAN.

Figure 5. Soluble TfR1 and 2 block plgA1-induced HMC proliferation. Quiescent HMC were preincubated for 15 min with or without 50 μg/ml soluble TfR1 or TfR2 (or soluble CD89 as a control) before addition or not of plgA1 (0.5 mg/ml) in the culture for another 24 h. Proliferation of the cells then was measured by [3H]thymidine incorporation. Data are means ± SD of triplicate from one of at least three separate experiments. (A) Inhibition of plgA1-induced HMC proliferation by soluble receptors. Significance over cells that were treated with plgA1 in the absence of soluble receptors is shown above the corresponding bars. (B) Absence of inhibition of spontaneous HMC proliferation by TfR ectodomains.
because they have been implicated in mesangial cell activation of several glomerulopathies such as lupus erythematosus nephritis and Henoch-Schönlein nephritis (4).

We reported previously that TfR is an IgA1 receptor (15). IgA1 binding to TfR is dependent on the IgA1 glycosylation and multimerization status (16). Indeed, increased pIgA binding is observed for IgA from patients with IgAN and for de-galactosylated pIgA1, whereas no binding is observed for monomeric IgA (16). TfR is hyperexpressed in IgAN kidney biopsies (15) and is colocalized with mesangial IgA1 deposits (17).

Here we show that pIgA1 is able to induce TfR expression in cultured quiescent HMC in a dose- and time-dependent manner. This observation is confirmed further in experiments using sera from patients with IgAN. Upregulation of TfR expression was observed at the messenger RNA and protein levels and at the membrane cell surface. To our knowledge, this is the first report that TfR expression is modulated by an Ig. Although it is well known that some Ig receptors (e.g., IgE Fc receptor I) can be upregulated by their ligands (28), IgA downregulates expression of FcαRI (29). By contrast, we show that pIgA1 alone induces TfR expression.

Proinflammatory cytokines were poor inducers of TfR expression. Other studies with the HepG2 hepatoblastoma cell line showed that TfR expression could be enhanced between 1.15- and 1.35-fold by treatment with IL-1β, IL-6, and TNF-α proinflammatory cytokines (20). Our data extend these results to HMC and demonstrate that pIgA1 alone is a novel major inducer of TfR expression. Because pIgA1 can also induce production of these cytokines by HMC, one can hypothesize an autocrine mechanism whereby cytokines that are produced after pIgA1-mediated stimulation act synergistically to increase TfR expression and cell proliferation. However, the data collected in this study do not argue in favor of such a mechanism. Indeed, induction of TfR expression is rapid (2 h). In addition, our IgA depletion experiments demonstrated that factors that were released from stimulated cells were not sufficient to sustain mesangial cell proliferation.

Because we show that pIgA1 concentrations similar to their serum physiologic concentrations can induce TfR expression, the question arises as to why TfR expression on mesangial cells is not detected in normal individuals. Here we show that sera from normal individuals do not induce significant TfR expression on mesangial cells, whereas sera from patients with IgAN induce a significant and IgA-dependent expression of this receptor. Thus, we postulate that under physiologic conditions, the presence of a large excess of serum monomeric and dimeric IgA could prevent pIgA1 binding and hence could prevent HMC activation and TfR expression by serum pIgA1. Another possibility is that in serum, the high-affinity ligand Fe-Tf present at 2 to 3 mg/ml could counteract activation by IgA.
through induction of receptor capture and stocking in recycling vesicles. Under particular pathologic conditions such as IgAN, the increased ratio of polymeric to monomeric IgA together with the presence of IgA1 immune complexes and with the higher affinity of hypogalactosylated pIgA1 for TfR (16) would shift the balance toward pIgA1-mediated HMC activation.

Mesangial cell proliferation is another feature of IgAN. It is interesting that TfR expression is enhanced in highly proliferative cells but not in low proliferative cells (30). TfR expression is upregulated in rapidly growing tissues, such as intestinal crypts, liver and epidermal cells, and activated B and T lymphocytes and erythropoietic bone marrow precursor cells (31). Because we previously reported that TfR expression is enhanced in kidney biopsies of patients with IgAN (15,17) and that mesangial IgA deposits co-localize with mesangial areas where TfR is hyperexpressed (17), we examined the ability of pIgA1 to stimulate HMC proliferation through binding to TfR. We show that pIgA1 induces HMC proliferation and that pre-incubation of mesangial cells with ectodomains of TfR1 or TfR2 or with A24 mAb abrogates pIgA1-mediated (but not cytokine-mediated) mesangial cell proliferation. These results reveal that a TfR-mediated signaling pathway activated by pIgA1 is responsible for the observed HMC proliferation.

TfR1 and TfR2 share 66% homology and significant identity (45%) (32) and can form heterodimers on the cell surface (33). Although there so far is no evidence that TfR2 protein is expressed by mesangial cells, we detected TfR2 transcripts in these cells (Moura and Monteiro, unpublished results). However, its was shown recently that TfR2 transcripts can accumulate in erythroblasts without membrane protein expression (34). Whereas TfR1 expression was confirmed by different biochemical and immunochemical methods in this study, further work will be needed to clarify whether TfR2 and/or TfR1/TfR2 heterodimers are also involved in IgA-mediated functions.

IgAN is also histochemically characterized by extracellular matrix expansion. It has been reported that IgA complexes can induce the production of mesangial matrix proteins such as fibronectin and types I and IV collagen, largely through an autocrine stimulation of TGF-β (26). We demonstrate herein that TfR is responsible for the IgA1-mediated effect, because the latter is inhibited by the anti-TfR antibody A24. Therefore, TfR seems to play a key role in the pathogenesis of IgAN by its involvement in the major features of this disease. Impairing the interaction between IgA1 and its cognate receptor could lead to new avenues for IgAN treatment.

It was reported previously that in addition to the well-known function of TfR in iron metabolism, this receptor could act alternatively as a signaling molecule. In T cells, TfR has been found to be associated with the T cell receptor (TCR) ζ chain (35), a signal transducing molecule, and TfR stimulation has been implicated in proliferation, IL-2 secretion, and protein kinase C activation (36,37). That pIgA1-induced IL-6 secretion is dependent on TfR engagement reveals an unsuspected new function for TfR. The signaling pathway responsible for this function is currently under active investigation.
Finally, our data reveal that TfR could play different roles depending on the ligand that is bound to it. Although pIgA1 and Fe-Tf share the same receptor, binding of pIgA1 and of Fe-Tf do not lead to the same cell response. Thus, pIgA1 induces TfR-mediated IL-6 secretion, whereas Fe-Tf does not. Whereas Fe-Tf promotes recycling of the receptor, pIgA1 directs TfR to endosome-like compartments (16). Finally, in contrast to Fe-Tf, pIgA1 induces TfR neosynthesis. Our study also unveils a TfR-dependent mechanism whereby pIgA1 complexes could promote the HMC proliferation that is observed in IgAN. Thus, deposited pIgA1 and pIgA1 immune complexes could initiate a process of autoamplification involving TfR hyperexpression, leading to increased pIgA1 mesangial deposition and resulting in mesangial expansion. The correlation between IgA1 altered glycosylation and the increase in both serum IgA1 levels and polymeric:monomeric IgA ratio on the one hand and enhanced TfR expression on the other hand becomes meaningful in this context. This positive feedback loop could be responsible for the chronicity of the IgA1 deposition process in IgAN, providing a rationale for new therapeutic strategies for this disease. It could explain the recurrence of IgA1 deposits in the mesangium of patients with IgAN after renal transplantation.

References

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