Identification of the Transferrin Receptor as a Novel Immunoglobulin (Ig)A1 Receptor and Its Enhanced Expression on Mesangial Cells in IgA Nephropathy

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Abstract

The biological functions of immunoglobulin (Ig)A antibodies depend primarily on their interaction with cell surface receptors. Four IgA receptors are presently characterized. The FcαRI (CD89) expressed by myeloid cells selectively binds IgA1 and IgA2 antibodies, whereas the poly-IgR, Fcα/μR, and asialoglycoprotein receptors bind other ligands in addition to IgA. IgA binding by mesangial cells, epithelial cells, and proliferating lymphocytes is also well documented, but the nature of the IgA receptors on these cells remains elusive. A monoclonal antibody (A24) is described here that specifically blocks IgA binding to epithelial and B lymphocyte cell lines. Both the A24 antibody and IgA1 myelomas bind a cell surface protein that is identified as the transferrin receptor (CD71). The transferrin receptor selectively binds IgA1 antibodies, monomeric better than polymeric forms, and the IgA1 binding is inhibitable by transferrin. Transferrin receptor expression is upregulated on cultured mesangial cells as well as on glomerular mesangial cells in patients with IgA nephropathy. The characterization of transferrin receptor as a novel IgA1 receptor on renal mesangial cells suggests its potential involvement in the pathogenesis of IgA nephropathy.

Key words: transferrin receptor • IgA • Fc receptor • mesangial cells • IgA nephropathy

Introduction

IgA Abs constitute the second most abundant class of Abs in the systemic circulation and the major Ig class at the mucosal surfaces (1). IgA-producing plasma cells in many human tissues, such as the bone marrow, produce IgA1 in a monomeric form that constitutes the predominant type of IgA in serum. In contrast, a predominance of IgA2-producing plasma cells in the mucosal compartment accounts for the IgA2 predominance in external secretions. Secretory IgA (SIgA),* composed of IgA1 and IgA2 polymers which also contain the J chain (1), is transported across epithelial cells into mucosal secretions. The importance of SIgA in host defense is well established, but the functions of serum IgA Abs are still incompletely understood (2).

Most of the known IgA Ab-mediated functions require an interaction with the IgA receptors, four of which have been identified in humans. These include the IgA Fc receptor (FcαRI; CD89) that is expressed on myeloid cells, including monocyte/macrophages, neutrophils, eosinophils, and dendritic cells (3–6), the polymeric-Ig receptor (poly-IgR) that transports polymeric IgA (and IgM) across mucosal epithelial cells (7), the Fcα/μR on B cells and macrophages that also binds both IgA and IgM (8), and the hepatocyte asialoglycoprotein receptor (ASGP-R) that binds IgA via its O-linked moieties in addition to asialoglycoprotein (9).

IgA binding that may not involve the previously defined receptors has been described on human mesangial cells (10–14), epithelial cells (15), and dividing T and B lymphocytes

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**Abbreviations used in this paper:* CEF, chicken embryo fibroblast; IgAN, IgA nephropathy; SIgA, secretory IgA; TfR, transferrin receptor.

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(16, 17). Mesangial region deposition of IgA1 and IgA1–immune complexes is the distinguishing characteristic of primary IgA nephropathy (IgAN [18, 19]). The IgA1 deposits in IgAN are attributed to the expression of an IgA receptor, but the molecular nature and function of this receptor remain unknown. In this study, we used an mAb that selectively inhibits IgA1 binding to characterize a candidate IgA1-binding molecule as a dimer of 90-kD chains. This molecule surprisingly proved to be the transferrin receptor (TfR or CD71) in experiments that indicate the TfR selectively binds IgA1 Abs, preferentially in monomeric form. Other studies using primary cell cultures indicate that the TfR is responsible for the IgA1 binding to mesangial cells and an immunohistochemical analysis indicates that TfR expression is upregulated in the renal mesangium in patients with IgAN.

Materials and Methods

Subjects. Renal biopsy tissues from five brazilian patients (three males and two females) with a clinical and renal immunopathologic diagnosis of primary IgAN were studied. They were symptomatic for more than 1 yr with hematuria and mild proteinuria. IgAN was diagnosed on immunofluorescence analysis by the presence of predominant IgA1 deposits in the glomerular mesangium associated with focal or diffuse mesangial cell proliferation. Normal kidney tissue was obtained from an intact pole of kidney removed for a circumscribed tumor. In each instance, informed consent was obtained from the donors for the use of tissue samples for experimental purposes.

Abs and Reagents. mAbs specific for the FcαRI included the A3, A59, A62, and A77 (all γ1κ) mAbs (20) and the My43 (μκ), which was provided by Dr. L. Shen (Dartmouth Medical School, Lebanon, NH; reference 21). A panel of anti-TfR (CD71) mAbs was purchased, including OKT9 (Ortho Diagnostic Systems Inc.), YJD1.2.2 (γ1κ; Immunotech/Beckman Coulter), M-A712 (γ2aκ; BD PharMingen), and DF1513 (γ1κ; Sigma-Aldrich). Control Abs included mouse IgG1κ and IgG2a mAbs of irrelevant specificity (Becton Dickinson). Human myeloma IgA1 proteins (001, Ret, Dou, and Via) and F(ab′)$_2$ fragments of goat anti-Id 001 Abs were prepared as described (22). Monomeric and polymeric fractions of IgA1κ (mIgA1 and pIgA1) were separated by gel filtration on Sephacryl S300 columns (Amersham Pharmacia Biotech; >98% pure) and biotinylated as described (22). Human serum IgG was purified by ammonium sulfate precipitation and DEAE ion exchange chromatography (20). Human SIgA was purified from defatted colostrum by affinity chromatography on CNBr-Sepharose-4B bound to goat anti–human IgA as described (23). SIgA contained both IgA1 and IgA2 molecules as evaluated by ELISA using anti-IgA1 and anti-IgA2 mAbs (18). Unconjugated goat polyclonal Abs specific for mouse Ig and human IgA were purchased from Southern Biotechnology Associates, Inc. and extensively adsorbed with human monoclonal Ig as described (22). IgA2λ (IgA2m(2); Fel) was provided by Dr. J. Mestecky (University of Alabama at Birmingham; reference 24). A second IgA2κ (IgA2m(2); Bel) was a gift from Dr. P. Aucouturier (Necker Hospital, Paris, France; reference 24). Apo and holo forms of Tf were purchased from GIBCO BRL and Sigma-Aldrich, respectively. Proteins were quantified by the BCA method according to the manufacturer's instructions (Pierce Chemical Co.).

Generation of the A24 mAb. A24 (γ2bκ) was produced as described previously (20). Briefly, a Balb/c mouse was hyperimmunized with IgA-binding proteins derived from U937 cells before the fusion of regional lymph node cells with the Ag8.653 mouse myeloma cell line. The supernatants were screened for Ab activity by immunofluorescence and immunoprecipitation assays.

Cells. Human mesangial cells were isolated as described previously (25) from the cortex of normal human cadaver kidneys unsuitable for kidney transplantation. Briefly, intact glomeruli were collected from cortical homogenates by serial sieving. The isolated glomeruli were digested with collagenase (type IV, 750 U/ml; Roche) and then seeded in culture flasks. After the outgrowth of mesangial cells, the glomeruli were removed by washing and the cells were cultured in RPMI 1640, supplemented with glutamine (2 mmol/liter), 5 μg/ml insulin, 20% FCS (Life Technologies), 7 mM glucose, 50 U/ml penicillin, and 50 μg/ml streptomycin in an atmosphere of 7% CO_2. Cells were passaged using 0.25% trypsin and 0.5% ethylenediaminetetra-acetic acid. The glomerular mesangial cells were stellate or fusiform in appearance, grew in multilayers, and stained for α-smooth muscle actin and myosin by direct immunofluorescence. These cells were negative for cytokeratin, factor VIII, and common leukocyte antigen, excluding contamination of epithelial cells, endothelial cells, lymphocytes/monocytes, and fibroblasts. Studies were performed between passages 4 to 7, when the cells retained all of the morphologic and immunofluorescent features described above. Established cell lines included U937, Daudi, Jurkat, and HT29 that were obtained from American Type Culture Collection. Cells were stimulated with PMA (50 ng/ml) over a 18-h period in some experiments.

Expression of Human TfR in Chicken Embryo Fibroblasts. Cells were obtained from SPAFAS as primary chicken embryo fibroblasts (CEFs; c/o Line 22) and grown in DMEM supplemented with 1% chicken serum and 1% FCS, 2% (vol/vol) tryptose phosphate broth, 2 mM L-glutamine, penicillin, and streptomycin. CEFs were transfected with 30 μg of retroviral construct DNA/10-cm tissue culture plate of 40% confluent cells using the polybrene-dimethyl sulfoxide method (26). Stable expression of wild-type TfR was achieved using a helper-independent retroviral vector, BH-RCAS, derived from the Rous sarcoma virus (27). 1–2 wk after transfection, the CEFs stably expressed wild-type TfR on their surface as a result of infection by recombinant virus (28).

Immunofluorescence Analysis. Cells (0.5×10^6) were preincubated with 10 μl of human IgG (10 mg/ml) for 15 min on ice to mask FcγR before incubation with 10 μl of test mAb (0.1 mg/ml) for 30 min at 0°C in PBS containing 1% BSA and 0.1% sodium azide. After two washes, FITC-labeled goat Abs to mouse Ig (0.1 mg/ml) were used as the developing reagent. IgA binding was examined using an indirect immunofluorescence assay in which cells preincubated with human IgG were incubated with 10 μl of biotin-labeled IgA (0.2 mg/ml) for 1 h on ice before washing and incubation with the PE-labeled streptavidin (Southern Biotechnology Associates, Inc.) developing reagent (22). For inhibition studies, cells were preincubated with A24 (1 to 0.01 mg/ml) or My43 (200 μl hybridoma culture supernantant) for 30 min prior to biotinylated IgA addition. Immunofluorescent cells were analyzed by flow cytometry (FACSCalibur™; Becton Dickinson).

Cell Surface Iodination, Immunoprecipitation, and Immunoblotting. Viable cells (3×10^7) were surface labeled with Na^{125}I (1 mCi; Amersham Pharmacia Biotech) by the lactoperoxidase method. After washing, cells were lysed in 0.5% NP-40 in PBS containing protease inhibitors as described previously (3). To deplete FcγR, cell lysates were precleared seven times with human IgG-coupled Sepharose 4B beads (20 μg). For detection of IgA-binding molecules, absorbed lysates were incubated with IgA coupled to

Sepharose 4B beads, or with 10 μg of pIgA1κ plus 20 μl of Sepharose 4B coupled with F(ab′)$_2$ anti-Id Ab for 2 h at 4°C (3). For immunoprecipitation of the CD71 and CD89 proteins, absorbed lysates were incubated with the corresponding mAb (5 μg) plus 5 μl of goat anti–mouse Ig-coupled beads (2 mg/ml) for 2 h at 4°C. Proteins were resolved by SDS-10% PAGE analysis. For immunoblotting, the immunoadsorbed proteins were separated by SDS-PAGE and electrophoretically transferred onto a nitrocellulose Hybond-C (Amersham Pharmacia Biotech) filter at 30 mA for 18–20 h. Blots were incubated in blocking buffer (25 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl containing 3% BSA, and 0.1% Tween 20) and then incubated with an anti-CD71 mAb (0.4 μg/ml) for 2 h at room temperature. Horseradish peroxidase (HRP)-conjugated goat anti–mouse IgG was used as the developing reagent. Filters were assessed using the enhanced chemiluminescence detection system (ECL; Amersham Pharmacia Biotech).

Immunohistochemistry. Frozen 3–4-μm tissue sections were adhered to microscope slides, fixed in acetone for 10 min at 4°C, and then allowed to air-dry for at least 1 h. Slides were washed in PBS before the avidin/biotin blocking steps. Slides were loaded with normal horse serum (Vector Laboratories) for 30 min to block nonspecific sites, before incubation with the primary Ab (mouse mAb) for 60 min in a humid chamber (Vector Laboratories) followed by addition of the biotinylated anti–mouse Ig (Jackson ImmunoResearch Laboratories), then the avidin/biotin-alkaline phosphatase complex (ABC-AP reagent; Vector Laboratories) and, finally, the substrate. Hematoxylin (1 min) and blueing steps were then completed before microscopic assessment.

Results

Identification of a Novel IgA Receptor. The U937 monocytoid cell line has been used previously to identify the Fcα receptor I (CD89; reference 3). To identify other possible IgA1 binding molecules, the U937 cells were iodinated, solubilized in NP-40 buffer, and the cell lysates adsorbed with mIgA1 directly coupled to Sepharose beads. This led to the identification under reducing SDS-PAGE conditions of a 90-kD cell surface protein in addition to the 55–75-kD FcαRI (Fig. 1 A). No binding was observed with control IgG-coupled beads. The amount of 90-kD IgA-binding protein was less when it was harvested by adsorption to pIgA1κ complexed to anti-Id Abs (Fig. 1 B), whereas the FcαRI/CD89 molecules harvested by this method were increased as expected (3, 20). Anti-CD89, anti-Id, and irrelevant IgG mAbs did not bind the 90-kD protein (Fig. 1 B). Immunoadsorption assays using the Daudi B cell line, which

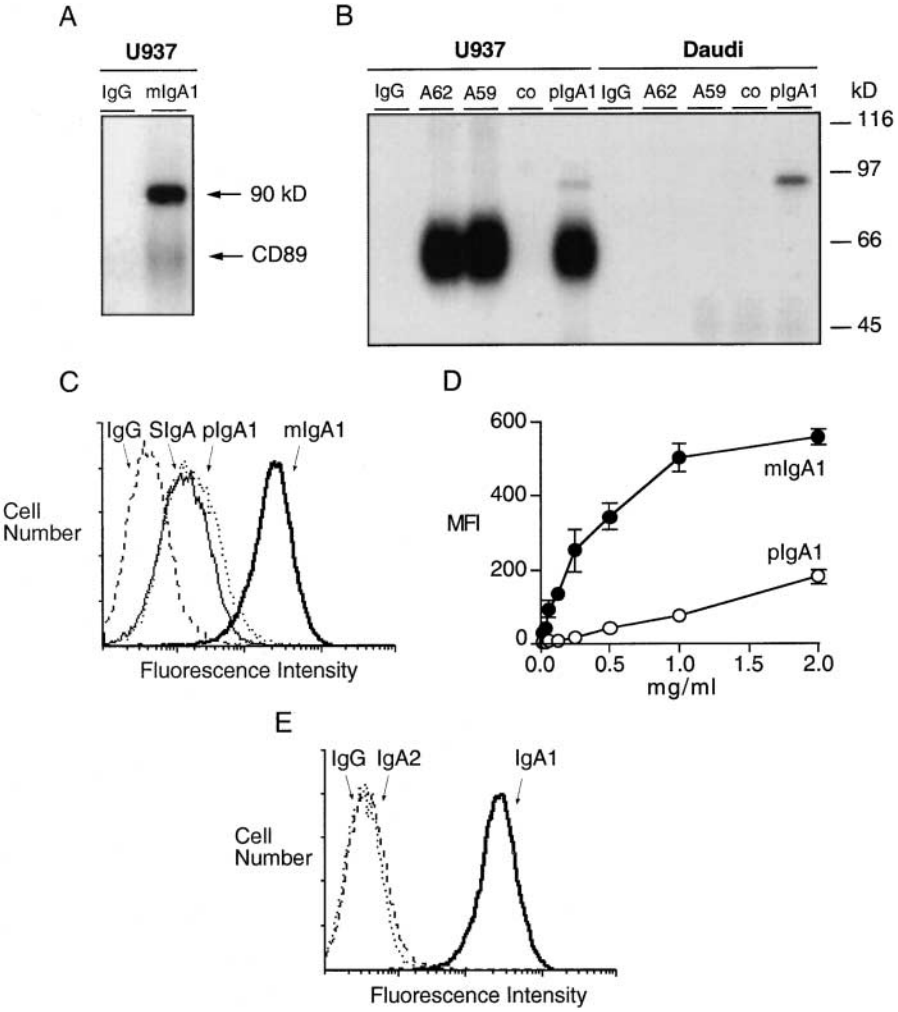

Figure 1. Identification of a novel IgA1 receptor. (A) Monomeric (m) IgA1 (001)-coupled beads precipitates a 90-kD protein from ^{125}I-radiolabeled U937 cells solubilized with 0.5% NP-40. FcγR-cleared cell lysates were incubated with IgG- or IgA1 beads and the eluates were analyzed by SDS-10% PAGE under reducing conditions before analysis by autoradiography. (B) The 90-kD protein also binds polymeric (p) IgA1 and is expressed both by U937 monocytoid cells and Daudi B cells. Cell surface proteins (^{125}I radiolabeled) were immunoprecipitated from cell lysates by either anti-CD89 (A62 and A59) mAbs or by pIgA1 (001) plus F(ab′)$_2$ fragments of anti-Id 001 (co) coupled to beads before analysis on SDS-10% PAGE under reducing conditions. (C) Comparative immunofluorescence analyses of mIgA1, pIgA1, and secretory (S) IgA (IgA1/IgA2 mixture) binding to Daudi cells. The IgA preparations were biotinylated and PE-conjugated streptavidin was used as the developing reagent. (D) IgA1 binding is concentration dependent. Daudi cells were incubated with twofold serial dilutions of biotinylated mIgA1 or pIgA1 followed by PE-conjugated streptavidin. Mean fluorescence intensities (MFI) of mIgA1 or pIgA1 were plotted after subtracting values of each corresponding concentration of biotinylated IgG. (E) Failures of IgA2 (Bel) to bind to Daudi cells.

does not express CD89, indicated that the 90-kD protein is the major IgA receptor on these cells (Fig. 1 B, right). Under the same conditions, we could not detect the recently described 70-kD Fcα/μR (8). As Daudi cells primarily express the 90-kD IgA1 receptor, we used these to compare the binding of different IgA forms and complexes. In an immunofluorescence analysis, mIgA1 was found to bind more efficiently to Daudi cells than did pIgA1 and SIgA Abs (Fig. 1 C). The mIgA1 binding was concentration dependent with maximal labeling intensity at concentrations of 1 mg/ml, whereas saturation was not evident with pIgA1 (Fig. 1 D). Remarkably, IgA2 binding was not observed (Fig. 1 E).

Characterization of the 90-kD IgA1-binding Protein as TfR. In an initial attempt to determine the molecular nature of the 90-kD IgA1-binding molecule, we employed the A24 mAb that was generated against IgA-binding proteins derived from U937 cells. The A24 mAb was found to specifically recognize the 90-kD IgA1-binding molecule expressed on the U937 cell surface (Fig. 2 A, lane 4). Immunodepletion experiments indicated that 90-kD protein has no apparent physical association with the previously defined CD89 FcαRI (Fig. 2 A). Like the IgA1-binding molecule (data not shown), the A24-binding molecule migrated as a 180-kD homodimer under nonreducing conditions (Fig. 2 B). As these results suggested the transferrin receptor (TfR/CD71) as a candidate for the novel IgA1 receptor, we examined the reactivity of known anti-TfR (CD71) mAbs with the 90-kD cell surface protein isolated on the basis of its binding to either IgA1 or the A24 mAb (Fig. 2 C). The 90-kD molecules on Daudi B cells that were bound by mIgA1 and the A24 mAb were recognized by the YDJ1.2.2 anti-CD71 mAb, thereby identifying these as components of the TfR. Notably, an IgA2λ (Fel) myeloma did not bind the TfR. Another anti-TfR mAb, OKT9 (O9), also depleted the A24-binding protein from U937 lysates, thereby confirming that the A24 mAb recognizes the TfR (Fig. 2 D).

To verify the identity of the new IgA-R as the TfR, we took advantage of the fact that chicken TfR displays only 53% homology with the human TfR extracellular domain (29). Accordingly, CEFs did not bind human IgA1, whereas human IgA1 was bound by CEF transfectants expressing the human TfR as well as by the Jurkat human T cell line used as positive control (Fig. 3). The TfR expression was monitored in these experiments by staining with the A24 anti-TfR mAb.

To exclude the possibility that the IgA1 binding to TfR was mediated by transferrin, we cultured Daudi and Jurkat cells in RPMI in the presence or absence of 10% FCS for 1 h before incubation with IgA1. Cells cultured in serum free media bound more IgA1 than cells cultured with FCS, thereby indicating that Tf does not mediate the interaction between TfR and IgA1, but rather may inhibit the IgA1 binding (data not shown).

To determine whether the A24 mAb recognizes the TfR on primary hematopoietic cells, we examined its immunofluorescence reactivity with immature hematopoietic cells in fetal liver and bone marrow samples. As anticipated, the A24 mAb decorated the surface of mononuclear hematopoietic cells (~60%) in these hematopoietic tissues, whereas the A24 mAb did not stain the nondividing populations of mononuclear and polymorphonuclear cells in adult blood samples.

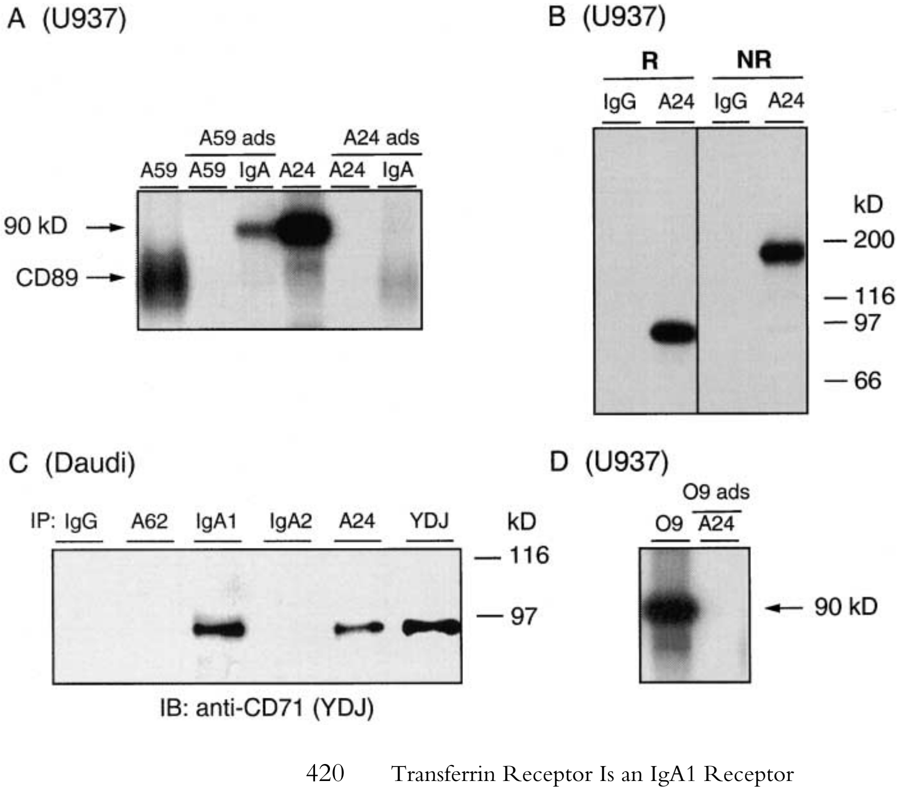

Figure 2. Characterization of the IgA1 receptor as the TfR. (A) Immunodepletion experiments indicate that the 90-kD and CD89 IgA binding molecules are nonassociated proteins, and that the A24 mAb recognizes the 90-kD I-IgA-binding protein. NP-40 solubilized ^{125}I-surface proteins were adsorbed with either anti-CD89 (A59 ads) or A24 (A24 ads) before isolation on A24- or IgA1-coupled Sepharose beads and analysis of the eluates on SDS-10% PAGE under reducing conditions. (B) The A24 mAb recognizes a 180-kD dimeric molecule. Surface proteins (^{125}I radiolabeled) from cell lysates were immunoprecipitated by A24 or an irrelevant IgG1κ plus goat anti–mouse Ig-coupled beads, and analyzed by 5 to 15% SDS-PAGE under reducing (R) and nonreducing (NR) conditions. (C) Anti-CD71 immunoblotting identifies the IgA1 receptor as TfR. Proteins adsorbed from cell lysates by immobilized IgA1 (Ret), IgA2 (Fel), A24 mAb, the YDJ1.2.2 mAb, were analyzed by immunoblotting with the YDJ1.2.2 anti-CD71 (YDJ) mAb plus HRP-conjugated goat anti–mouse Ig and developed by enhanced chemiluminescence method. (D) Immunodepletion of the 90-kD IgA1 binding protein by the anti-CD71 OKT9 (O9) mAb confirms the TfR nature of the A24 reactive protein.

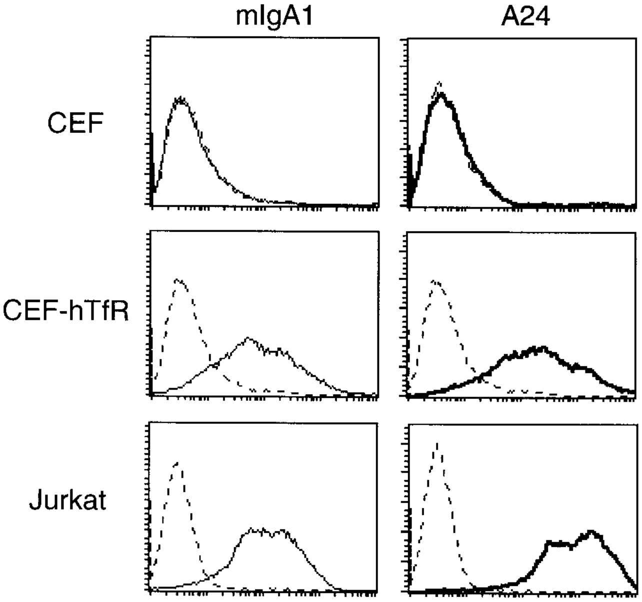

Figure 3. Identification of the TfR as an IgA1 receptor. Immunofluorescence analysis of CEFs either lacking or expressing the human TfR and Jurkat T cells was performed using monomeric IgA1 (thin line) and A24 mAb (heavy line). Cells preincubated with human IgG to mask FcγR were labeled with biotinylated human IgA1 or A24 followed by incubation with PE-conjugated streptavidin as a developing reagent. Biotinylated human IgG was used as a negative control of binding (dashed line).

The A24 mAb and Tf Block the IgA1 Binding to TfR. To further characterize the IgA1-binding specificity of TfR, Daudi cells were preincubated with either the A24 mAb or Tf as inhibitors. The A24 mAb inhibited mIgA1 binding in a dose-dependent manner, reaching >90% inhibition at an A24 concentration of 1 mg/ml, whereas the A62 anti-CD89 FcR mAb had no effect (Fig. 4 A); My43 had no effect as well (data not shown). Similar results were obtained with two additional myeloma IgA1κ monomeric proteins (001 and Via; data not shown). Competition assays were also performed using three commercially available anti-CD71 mAb and our A24 mAb. The A24 was the only anti-TfR mAb tested that significantly inhibited mIgA1 binding by the Daudi B cells in this assessment (Fig. 4 B). To determine whether IgA1 binding competed with the Tf-binding sites of TfR, we preincubated cells with purified transferrin before the addition of mIgA1 (Fig. 4 C). The apo and holo forms of Tf (unsaturated or Fe^{2+}-saturated, respectively) were used in these experiments. A dose-dependent inhibition was obtained with the holo Tf, thereby confirming the specificity of the IgA–TfR interaction and suggesting that the IgA1 binding site is located in the proximity of the Tf binding site on TfR. A lower level of inhibition was observed with the Fe^{2+} unsaturated apo form of Tf (43% maximal inhibition; data not shown).

Inversely Regulated TfR and CD89 Expression during Cellular Differentiation Correlates with Differential Monomeric and Polymeric IgA1 Binding. It has been previously demonstrated that PMA treatment induces TfR downregulation (30) in parallel with upregulation of CD89 expression (3). We therefore used IgA binding cell lines that do or do not express CD89 (FcαRI) to evaluate the binding properties of polymeric and monomeric IgA1 to TfR and CD89, before and after the induction of cellular differentiation by PMA treatment. The monocytic cell line, U937, that expresses low levels of CD89 under unstimulated conditions, bound mIgA1 better than pIgA1 and the IgA1 binding was effi-

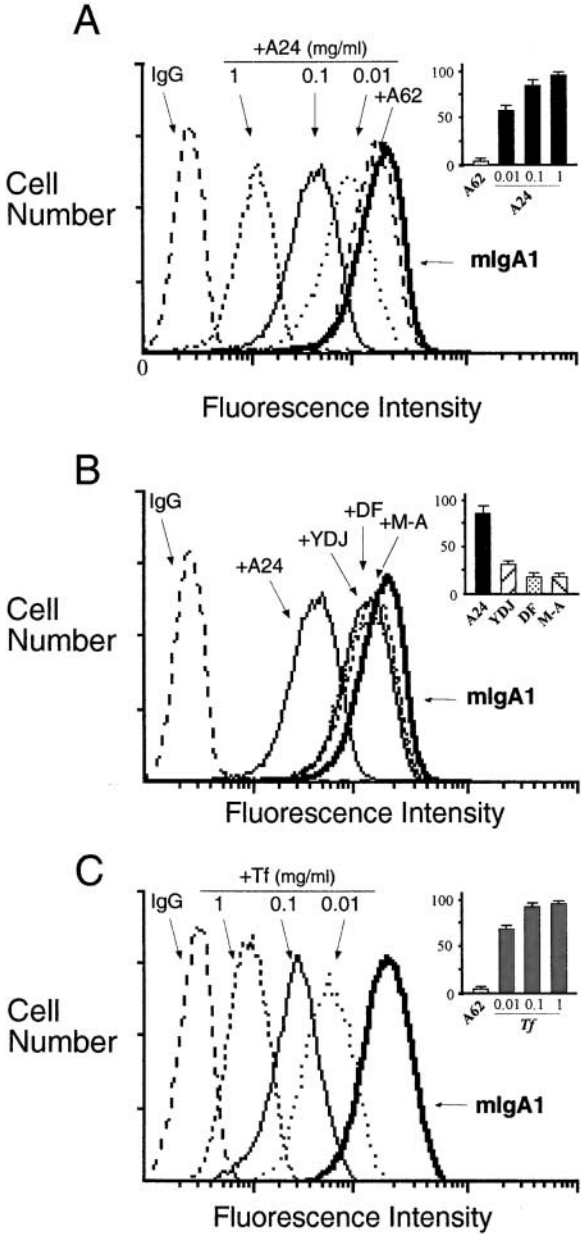

Figure 4. Immunofluorescence analysis of the ability of the A24 mAb and transferrin to block IgA1 binding by TfR on Daudi B cells. In each panel the heavy dark line indicates the uninhibited IgA1 binding histogram. (A) A24 mAb blocks IgA1 (Ret) binding to Daudi cells in a dose-dependent manner. (B) The anti-CD71 mAbs, YDJ1.2.2, DF1513, and M-A712, are inefficient inhibitors of IgA binding (<30% inhibition at 0.1 mg/ml concentration). (C) Holo-Tf blocks the IgA1–TfR interaction in a dose-dependent manner. Top right panels indicate the percent inhibition of mIgA1 binding by the anti-CD71 mAb and by holo-Tf (mean ± SD; $n = 6$).

ciently inhibited by the A24 anti-TfR mAb (>80% inhibition), whereas minimal inhibition (>10%) was observed with the My43 anti-CD89 mAb that blocks the IgA binding site of FcαRI/CD89 (Fig. 4). PMA-induced differentiation of U937 cells resulted in diminished TfR expression and mIgA1 binding that was inhibitable by the A24 anti-TfR mAb. On the other hand, the PMA-induced monocyte/macrophage differentiation led to enhanced CD89 expression and enhanced pIgA1 binding that was specifically blocked by the My43 Ab (>70%; Fig. 5). By contrast, the HT29 epithelial cell line (15), which does not express CD89 even after PMA treatment, bound mIgA1 much more efficiently than pIgA1 (Fig. 5), and the A24 anti-TfR mAb inhibited the IgA1 binding, therefore confirming that TfR mediates this mIgA1 binding. The correlation observed between the downregulation of TfR expression and of mIgA1 binding after PMA treatment confirmed that TfR is the primary IgA1 receptor on the HT29 cells. The above data also indicate that mIgA1 is preferentially bound by the TfR, whereas pIgA1 is preferentially bound by FcαRI/CD89.

IgA1 Binding by Cultured Human Mesangial Cells Is Mediated by the TfR, Which Is Overexpressed in Patients with IgAN. The presence of an uncharacterized IgA-binding protein on cultured human mesangial cells has been reported by several groups of investigators (11–14). We therefore examined the possibility that the IgA receptor expressed on cultured mesangial cells could be the TfR. This analysis indicated that A24-reactive protein is indeed expressed on cultured mesangial cells (Fig. 6 A). In lysates of mesangial cells, both mIgA1 and A24 identified the 90-kD TfR chains that are recognized by the YDJ1.2.2 anti-CD71 mAb (Fig. 6 B). Cell surface mIgA1 binding was specifically inhibited (>70%) by A24 and by Tf (Fig. 6 C). To evaluate TfR expression on primary mesangial cells, immunohistochemical staining of kidney tissue sections was performed using the anti-CD71 mAbs. In situ TfR expression was detected on mesangial cells from patients with IgAN, but not in normal glomeruli. Fig. 6, D and E, illustrates the TfR expression in mesangial areas in renal kidney biopsy tissues from two IgAN patients examined with the OKT-9 and M-A712 anti-CD71 mAbs, and an irrelevant IgG1 mAb as a control. This observation was confirmed in renal biopsies from three additional IgAN patients (not shown). These results indicate enhanced TfR expression by mesangial cells in patients with IgAN.

Discussion

The novel IgA1 receptor, characterized in this study as a disulfide-linked homodimeric molecule of M_r 180,000, was unexpectedly identified as the transferrin receptor (TfR/CD71; reference 31). IgA1 represents a third ligand for the TfR, previously recognized TfR ligands being Tf itself (31)

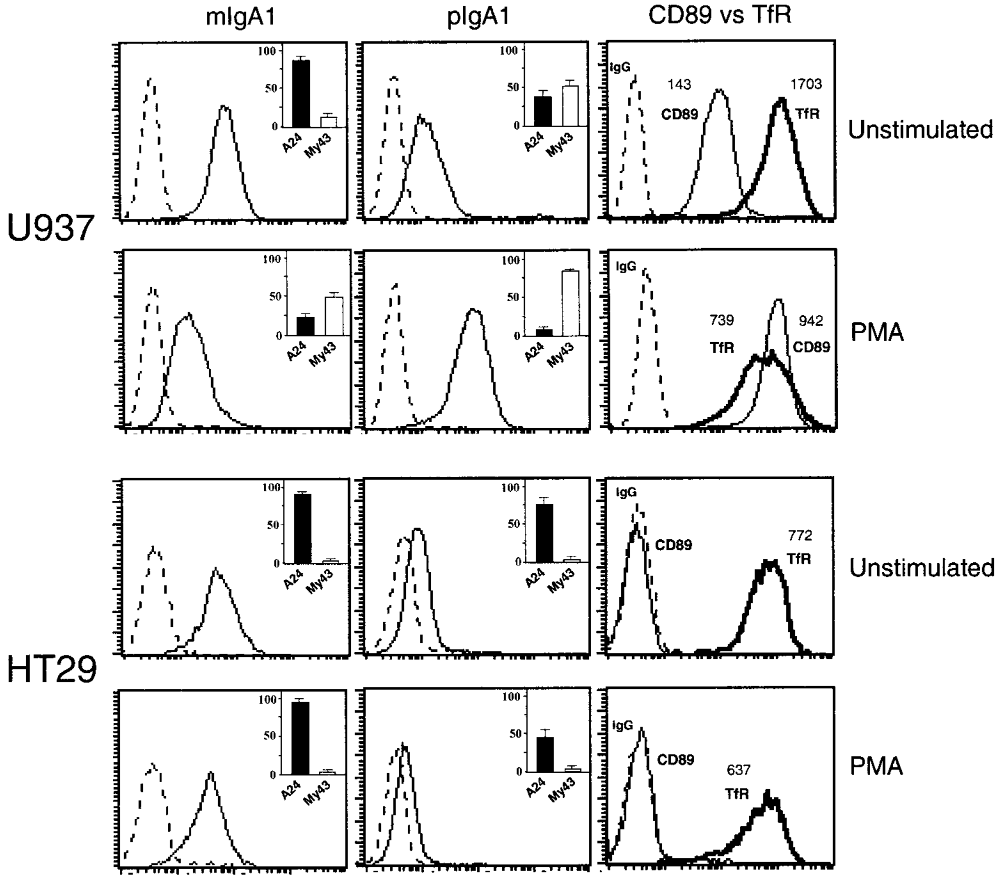

Figure 5. Comparative analysis of mIgA1 and pIgA1 binding to TfR and FcαRI (CD89) on cells that differentially express these IgA receptors. The IgA binding specificity of the two types of receptors, TfR and FcαRI, was assessed using the ligand blocking mAbs, A24 and My43. Cells were stimulated or not with PMA (10^{-7} M) for 18 h before staining with biotinylated mIgA1 (Ret) or pIgA1 (Ret) and PE-conjugated streptavidin as the developing reagent (solid lines). Biotinylated IgG (dashed lines) were used as a negative control. The percent inhibition of IgA1 binding by the A24 and My43 mAbs is shown in bar graph inserts. TfR and CD89 expression were evaluated by biotinylated A24 (heavy line) and A77 (thin line) in the third column of panels (numbers indicate the mean fluorescence intensity).

and the hemochromatosis protein (32). Analysis of the TfR–IgA interaction was facilitated by the observation that cells of the Daudi B cell line and the HT29 intestinal epithelial cell line express the TfR/CD71 but do not express the FcαRI/CD89, and that the A24 anti-CD71 mAb uniquely blocks IgA binding. Using these reagents and model cell lines, these studies show that TfR selectively binds IgA1 Abs, whereas the FcαRI binds both IgA1 and IgA2 Abs. The specificity of the IgA1–TfR interaction was verified in experiments using CEFs transfected with the human TfR cDNA. The IgA1 binding by TfR differs from that of FcαRI in that the TfR preferentially binds mIgA1 over pIgA1 and SIgA. IgA1 binding by these two types of IgA receptors was also shown to depend on the cellular differentiation status. TfR expression was predictably downregulated in cells that exit the cell cycle to undergo differentiation. This differential usage of IgA1 receptors was demonstrated using PMA as an inducer of U937 cell differentiation that results in the upregulation of CD89 and downregulation of TfR expression (3, 30). In untreated U937 monocytoid cells, therefore, TfR was the primary receptor for mIgA1, whereas FcαRI/CD89 was responsible for most of the pIgA1 binding in PMA-treated U937 cells that undergo macrophage differentiation. Conversely, in the cell types that do not express the FcαRI, such as the Daudi B cells and HT29 epithelial cells, the TfR remained the primary mIgA1 receptor even after PMA treatment.

The remarkably selective TfR affinity for the IgA1 subclass of Abs suggests that TfR/IgA1 binding is influenced by the unique IgA1 hinge region where three to five O-linked carbohydrates moieties are located, as the primary difference between IgA1 and IgA2 subclasses is a 13 amino acid deletion in the IgA2 hinge region. The IgA2 Abs consequently have fewer O-linked glycosylation sites than IgA1 Abs (1, 2), which could possibly explain the lack of IgA2 binding by TfR. N-glycans are unlikely to be involved in the IgA1 binding to TfR, as IgA1 Abs have twofold less N-linked carbohydrate sites in their CH domains than IgA2 Abs. In this regard, it is noteworthy that N-glycans confer preferential IgA2 binding to ASGP-R and promote its rapid clearance from the circulation by the liver (33).

The TfR/IgA1 interaction on the HT29 epithelial cell line provides a reasonable explanation for the ability of these cells to bind IgA (15). However, it has also been reported that HT29 cells express the polymeric Ig receptor that binds to polymeric IgA and IgM (34). Nevertheless,

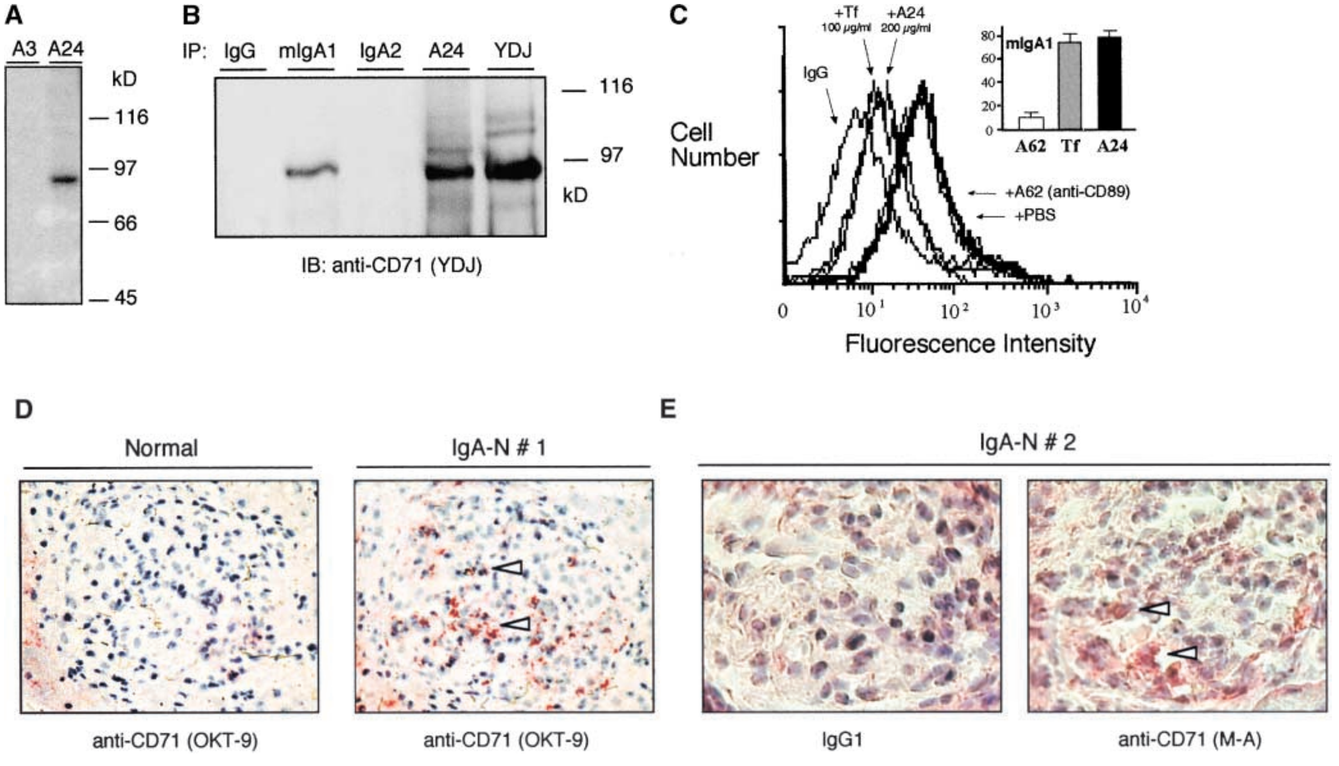

Figure 6. Identification of IgA1 binding by TfR on cultured human mesangial cells and TfR overexpression by mesangial cells in renal biopsies from IgAN patients. (A) TfR expression by cultured human mesangial cells (5th passage) is identified using the A24 anti-TfR mAb. Immunoprecipitation of TfR 90-kD chains from the ^{125}I-labeled mesangial cell lysates and analyzed in 10% SDS-PAGE under reducing conditions. (B) mIgA1 (Ret) but not IgA2 (Fel), bind a mesangial cell protein of 90 kD that is recognized by immunoblotting with the YDJ1.2.2 anti-CD71 mAb. Proteins in the cell lysates were separated by 10% SDS-PAGE under reducing conditions. (C) A24 and Tf blocked biotinylated mIgA1 (Ret) binding to cultured mesangial cells by >70%. Biotinylated IgG was used as negative control. (D) Detection of TfR in mesangial cell areas in renal biopsy material from a patient with IgAN (Pat 1), but not in normal renal tissue. Immunohistochemical analysis of TfR expression was performed using the OKT-9 anti-CD71 mAb. (E) Detection of TfR expression in mesangial cell areas in renal biopsy material from a second patient with IgAN (Pat 2) using the M-A712 anti-CD71 mAb. An IgG1 (IgG1) mAb of irrelevant specificity was used as negative control.

under our conditions, HT29 cells preferentially bind monomeric IgA1 over polymeric IgA1. TfR expression by primary epithelial cells has also been described (35), and our preliminary studies indicate that cryptic epithelial cells from normal human small intestine are stained by the A24 Ab (unpublished data). In view of the fact that the polyIgR cannot bind mIgA, it is also notable that mIgA has been found in the mucosal and glandular secretions of J chain–deficient mice (36). It seems reasonable, therefore, to propose that the TfR–IgA1 interaction on epithelial cells may provide an alternative pathway for mIgA transport into the mucosal secretions.

IgAN, also called Berger's disease, is the most common form of glomerulonephritis and is a principal cause of renal failure worldwide. Typical clinical features include haematuria and proteinuria. Kidney biopsies obtained from IgAN patients reveal mesangial deposits of IgA and proliferation of the glomerular mesangium. These mesangial deposits of IgA1 and IgA1-immune complexes constitute a diagnostic hallmark of primary and secondary IgAN in humans (18, 19). Our data point to potential roles for two types of IgA receptors in the pathogenesis of IgAN. In a previous study (37) we found that FcαRI may be involved in the development of IgAN through the formation of IgA complexes by shedding of soluble FcαRIα chain complexed with its IgA Ab ligand. This possibility was supported by the finding that transgenic mice expressing human FcαRI spontaneously develop IgAN which is dependent on the presence of IgA. It has also been suggested that the glomerular IgA1 deposition in these disorders could be mediated via a specific IgA receptor on mesangial cells (10–14). However, this possibility has not been confirmed in efforts to demonstrate FcαRI expression by human mesangial cells using anti-CD89 Abs (11–14, 38). The results of this study instead indicate that cultured human mesangial cells express TfR as the primary IgA1 cell surface receptor. Moreover, our immunohistochemical analysis indicates that glomerular mesangial cell expression of TfR is consistently enhanced in patients with IgAN. The proliferative state of the mesangial cells in IgAN patients theoretically could account for the enhanced TfR expression. Alternatively, the IgA1 Ab deposition could enhance mesangial cell proliferation (39). Although it is presently unclear which of these constitutes the primary event, our observations suggest that the upregulation of TfR expression on mesangial cells could explain the selective mesangial IgA1 deposition in patients with IgAN. The ability of TfR to bind pIgA1, albeit less well than mIgA1, is also consistent with the deposition of both polymeric and monomeric forms of IgA1 observed in renal biopsies from IgAN patients (40). Previous reports of preferential pIgA1 binding by human mesangial cells (12–14) are not so easily explained by our analysis of the TfR/IgA1 binding characteristics. This issue clearly deserves further study, along with analysis of the influence that aberrant glycosylation of IgA1 and IgA1-immune complexes in IgAN patients (41) may have on the IgA1/TfR interaction. The elucidation of a mesangial TfR–IgA1 interaction as a basis for mesangial IgA1 deposition thus opens a new avenue for study of the pathogenesis and treatment of IgAN.

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