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α8 Integrin in glomerular mesangial cells and in experimental glomerulonephritis

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Background. Mesangial cell (MC) proliferation and extracellular matrix accumulation are typical responses of renal glomeruli to injury. Extracellular matrix components are known to affect MC behavior, which is mediated primarily via integrin receptors of the β1 family. In addition to α1, α3, α5, and α6 chains of β1 integrins, recent studies have shown the α8 chain to be expressed in glomeruli and renal vasculature. α8β1 can serve as a receptor for fibronectin, which is abundant in the mesangium. We investigated the glomerular expression pattern of the α8 chain in renal tissues of mouse, rat, and humans as well as in cultured MCs. In addition, the regulation of α8 expression in MCs was studied in culture and in nephritic rats.

Methods. The expression of α8 protein in kidney tissue and cultured MCs was investigated by immunohistochemistry, immunocytochemistry, and Western blotting. The effects of TGF-β1 on α8 mRNA levels in MCs were studied by Northern blot analysis. In addition, time course studies of glomerular abundance and localization of α8 were performed in rats with mesangioproliferative anti-Thy1.1 nephritis.

Results. In tissue sections of normal human, rat, and mouse kidney, we found strong immunohistochemical staining for α8 in the mesangium and in the media of renal arterioles. Double staining for α8 and Thy1.1, a surface antigen of rat MCs, showed α8 to be specifically expressed in MCs but not in glomerular endothelial and epithelial cells. In anti-Thy1.1 nephritis of rats, the glomerular abundance of α8 protein was reduced in the early mesangiolytic phase but was increased greatly with subsequent MC proliferation, peaking at day 6 of disease. At later stages of this reversible form of nephritis, the number of MCs and the extent mesangial α8 staining declined to control levels. Cell culture experiments revealed that freshly plated MCs organize α8 into focal contacts within one hour after attachment to fibronectin and vitronectin substrata, showing colocalization with focal contact proteins vinculin and talin. Stimulation of MCs with transforming growth factor-β1 led to increases of α8 mRNA and protein levels.

Conclusions. These results show that in human, rat, and mouse glomeruli, α8 integrin is strongly and exclusively expressed in MCs. Gene expression of α8 is regulated in cultured MCs, and α8 protein abundance is regulated *in vivo* and in MC culture. It is currently unclear what functional properties this integrin receptor protein has with regard to MC anchorage to extracellular matrix and modulation of the MC phenotype in normal and diseased glomeruli. However, in view of its abundance in the mesangium, α8β1 integrin could be an important MC receptor of matrix ligands and may play a role in the embryology, physiology, and pathophysiology of the glomerular capillary tuft.

Key words: cell proliferation, glomerular injury, extracellular matrix, matrix ligands.

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The accumulation of extracellular matrix (ECM) in the glomerulus is a prominent feature of progressive glomerular diseases seen in patients and in experimental animals. This process may eventually lead to end-stage renal failure. Mesangial cells (MCs) are an important source of glomerular ECM under normal and nephritic conditions. ECM synthesis by MCs is regulated by soluble factors and nondiffusible components of the ECM [1]. ECM molecules can exert their effects on MCs either indirectly due to their potential to sequester and release soluble cytokines and growth factors or directly via specific ECM receptors. Among the latter, protein receptors of the β1 integrin family are predominant. They have been shown to mediate various cell–matrix interactions. These can serve mechanical and structural purposes, for example, by connecting the MC cytoskeleton with the ECM [2]. Moreover, integrin receptors have been shown to transduce specific biochemical signals to the cell upon binding of an ECM ligand [3]. Thus, integrins play an important role in regulating cell function and behavior not only by influencing cell attachment to and movement across the ECM, but also by affecting cell shape, polarity, differentiation and survival [1–3].

Integrins are heterodimeric transmembrane glycoproteins, each consisting of two noncovalently linked chains, the α and β subunits. There are at least 15 α chains and 8 β chains known to date [4]. Investigation of integrin patterns of MCs in healthy glomeruli and in cell culture revealed the presence of α1, α2, α3 A, α5, α6B, αv, β1 and β3 integrin chains [1, 5, 6]. More recently, a novel member of the β1 integrin family, α8β1, has been described to be expressed by neuroneal and vascular smooth muscle cells [7]. In the kidney, α8 chain expression was also noted in glomeruli and renal arterioles [6, 7]. Ligand proteins which bind to α8β1 include fibronectin, vitronectin, tenascin C, and osteopontin [8, 9]. Homozygous knockout mice for the α8 chain have severe defects in kidney development, either having no kidneys and dying neonatally or being born with only one kidney or a rudimentary kidney [10], while heterozygous animals carrying one mutated α8 locus do not display an altered phenotype. During development, α8β1 integrin is induced in mesenchymal cells at the border of the branching ureter epithelium [10]. Thus, it is possible that lack of α8 disturbs interactions between these two tissue compartments and that α8 is important for normal kidney morphogenesis at the stage of growth of the ureteric bud into the metanephric mesenchyme.

It is presently unclear whether or not α8 also plays a role in postnatal renal development and in the biology of normal adult kidneys. Early immunohistochemical studies of kidney sections have shown that staining for α8 protein localized in a mesangial pattern [6]. The objective of our current study was to analyze the expression and regulation of α8 integrin in human, rat and mouse glomeruli as well as in cultured MCs on the mRNA and protein levels. Furthermore, we examined changes of the α8 expression pattern in the course of a rat model of mesangioproliferative glomerulonephritis. The results confirm that α8 integrin protein, besides its presence in the arteriolar vessel walls, is strongly expressed in the human, rat and mouse glomerular mesangium. In nephritic glomeruli of rats, the abundance of α8 protein was greatly increased, correlating with the degree of MC hyperplasia. Plated onto fibronectin and vitronectin substrata, adherent MCs organized α8 integrin into focal contacts. Our findings indicate that MCs strongly express and utilize α8 to interact with ECM components, and that MC expression of α8 is regulated in culture and *in vivo.*

METHODS

Induction of anti-Thy1.1 nephritis in rats

Inbred male Sprague-Dawley rats (150 to 200 g) were obtained from Charles River Deutschland (Sulzfeld, Germany). The monoclonal antibody against Thy1.1 (ER4) is described by Bagchus et al [11], and was a gift from Dr E. de Heer, University of Leiden, The Netherlands. Anti-Thy1.1 nephritis was induced by a single intravenous injection of 1 mg/kg body weight anti-Thy1.1 antibody into the tail vein. Groups of four animals were sacrificed on days 2, 6 and 12 after induction of nephritis and renal tissue was obtained for further preparation. Control animals were injected isotonic saline into the tail vein and sacrificed six days after injection. The kidneys were decapsulated; portions were immediately snap-frozen in liquid nitrogen, or fixed in methyl-Carnoy solution (60% methanol, 30% chloroform and 10% glacial acetic acid).

Human and mouse kidney tissue

Human kidney tissue was taken from unused donor kidneys. Mouse kidneys were obtained from C57/black6 mice purchased from Charles River Deutschland, or from α8 null mice [10].The tissue was fixed as described above.

Cell culture

Rat MCs were isolated and cultured as described [12]. MCs showed a typical vascular smooth muscle cell-like morphology and positive immunostaining for Thy1.1, smooth muscle cell α-actin and myosin. MCs were grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Eggenstein, Germany) containing 10% FCS, 5 μg/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (Sigma, Deisenhofen, Germany) in a 95% air-5% CO2 humified atmosphere at 37°C. MCs were used for experiments in passages 7 to 15. MCs were serum starved for two days. Human recombinant transforming growth factor (TGF-β1) was obtained from Life Technologies and used in a concentration of 5 ng/ml for stimulation.

Northern blot analysis

RNA of MCs was extracted using the single step method by Chomczynski [13] with Tri-reagent (MRC Inc., Cincinnati, OH, USA). Total RNA was size fractioned on a 1% agarose formaldehyde gel and transferred onto Hybond nylon membranes (Amersham Buchler, Braunschweig, Germany). The blots were baked at 80°C for two hours, prehybridized with 5 Denhardt's solution, 5× SSC, 50% formamide, 50 mM Na_3PO_4, 0.1% SDS, 0.25 mg/ml salmon sperm DNA at 40°C for two hours. The 527 bp cDNA hybridization probe was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from rat MCs (sense primer, 5′-GCT GCC TCC CCA GAG GAY ACC; antisense primer, 5′-GTC AGY TGT TCC CTG TCK GTC AT). These degenerate primers were derived from the mouse and human α8 sequences; Genbank accession numbers are AF 041409 for mouse and L 36531 for human [8]. Location of the sense primer is 2507–2527 in the mouse and 2527–2547 in the human α8 sequence; location of the antisense primer is

3011–3033 in the mouse and 3031–3053 in the human α8 sequence. The amplified PCR product from rat MCs was sequenced and submitted to Genbank (accession number, AF 148797). Nucleotide sequence homology to the corresponding mouse α8 integrin fragment was 99%. The probe was labeled with [α-^{32}P]dCTP using a random-primed labeling kit (Boehringer, Mannheim, Germany). The blots were hybridized in prehybridization solution containing 2×10^6 cpm/ml of probe at 40°C overnight. The blots were washed twice for 15 minutes with 2 × standard sodium citrate (SSC) containing 0.1% sodium dodecyl sulfate (SDS) and then 30 minutes with 0.1 × SSC containing 0.1% SDS at 40°C. Blots were exposed to Kodak X-ray films with intensifying screens at −80°C for eight hours.

Antibodies

The rabbit antibody to mouse α8 integrin (cross reacting with rat) was prepared as described before [9] and diluted 1:50. The rabbit antibody to human β1 integrin, cross-reacting with rat and mouse, was a gift from Dr. Johannson (Uppsala, Sweden). Rabbit antihuman αv, cross reacting with rat, was purchased from Biomol (Hamburg, Germany). Rabbit antirat fibronectin was from Life Technologies. The mouse monoclonal antibody to the mesangial cell marker Thy-1 (1:250) was obtained from Serotec (Biozol, Eching, Germany). Mouse monoclonal antihuman vinculin, cross reacting with rat, was from Sigma (Deissenhofen, Germany).

Immunohistochemistry

After overnight fixation in methyl-Carnoy solution, tissues were dehydrated by bathing in increasing concentrations of methanol, followed by 100% iso-propanol. After embedding in paraffin, 4 μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). After deparaffinization, endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 20 minutes at room temperature. Staining reactions were carried out as described previously [14]. Briefly, sections were layered with the primary antibody, and incubated at 4°C overnight. After addition of the secondary antibody (dilution 1:500; biotin-conjugated, goat antirabbit immunoglobulin G), the sections were incubated with avidin-biotinylated horseradish peroxidase complex (Vectastain DAB kit, Vector Lab, Burlingame, CA, USA) and exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 as a source of peroxidase substrate. Each slide was counterstained with hematoxylin. As negative controls, equimolar concentrations of preimmune rabbit immunoglobulin G or an irrelevant secondary antibody were used. For localization of α8 integrin, 5 μm cryostat kidney sections were air dried for 10 minutes, fixed in cold acetone for 10 minutes, washed twice in TBS/1% BSA and incubated with 100% FCS for 30 minutes at 37°C. Double-immunofluorescence was performed for α8 integrin and Thy-1. Primary antibodies were applied simultaneously overnight at 4°C. After washing, sections were incubated with secondary antibodies, CY2 labeled goat antimouse immunoglobulin G and CY3 labeled goat antirabbit immunoglobulin G, both from Dianova (Hamburg, Germany) at the same time for two hours. Washed sections were then covered with Tris-buffered Mowiol, pH 8.6 (Hoechst, Frankfurt a.M., Germany).

Cell adhesion assay and immunocytochemistry

Mesangial cells were harvested with trypsin-ethylenediaminetetraacetate (EDTA), washed and seeded (5000 cells/well) on glass eight-well chamber slides coated with 50 μg/ml fibronectin and subsequently blocked with 2% bovine serum albumin (BSA). The cells were allowed to adhere for different time periods. Then, supernates were removed, adherent cells were rinsed with PBS for three times and fixed in 3% paraformaldehyde for 20 minutes. After blockade of free aldehyde groups with 50 mM ammonium chloride, cells were permeabilized by 1% Triton X-100 and nonspecific binding was blocked using 100% fetal calf serum (FCS). Cells were incubated with the primary antibodies overnight, followed by a CY3 labeled goat antirabbit immunoglobulin G (Dianova) as secondary antibody and embedding in Mowiol.

Western blot analysis

Cellular protein was extracted using Tri-reagent (MRC Inc.). Protein concentration was determined using a protein assay kit (Pierce, Rockford, IL, USA). Protein samples containing 20 μg total protein were denatured by boiling for five minutes and separated on a 15% denaturing SDS-PAGE gel. After electrophoresis, the gels were electroblotted onto PVDF membranes (Pall Filtron, Karlstein, Germany), blocked with 3% BSA/TBS/0.2% NaN_3/0.1% Tween 20 overnight and incubated with the primary antibody for two hours. α8 integrin protein was visualized with a secondary horseradish peroxidase-conjugated antirabbit IgG antibody, using the enhanced chemiluminescence (ECL) system according to the manufacturer's instructions (Amersham, Braunschweig, Germany).

RESULTS

Integrin α8 is expressed in the glomerular mesangium of human, mouse and rat kidneys

In normal adult human kidney tissue, immunohistochemical staining for α8 showed a strictly mesangial distribution in glomeruli. Staining for β1 revealed a comparable pattern (Fig. 1A, B). In normal mouse kidney sections, α8 immunofluorescence also showed a mesangial pattern (Fig. 1C). To ensure specificity of the α8

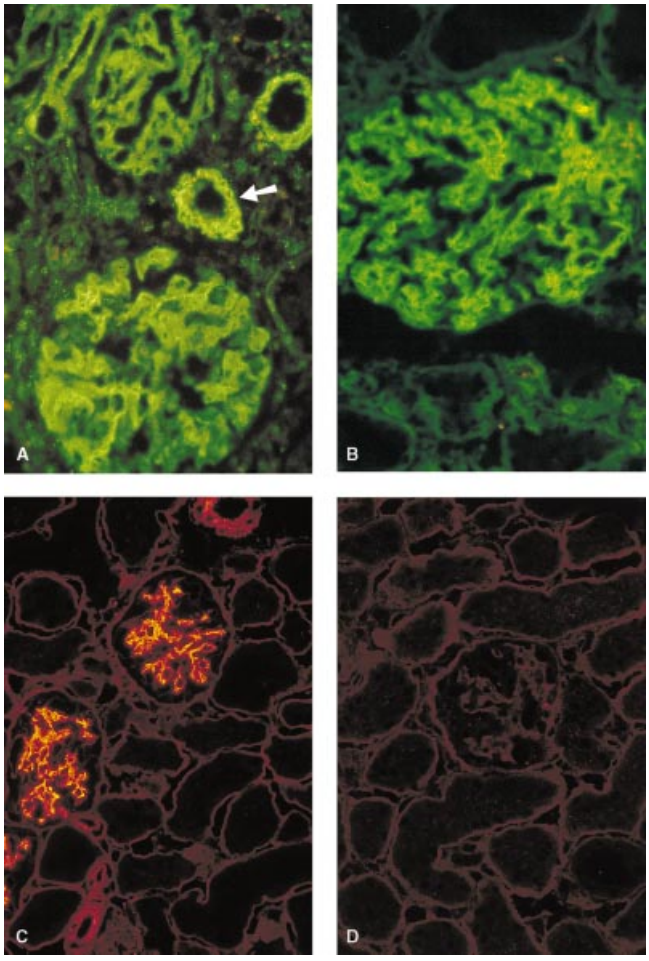
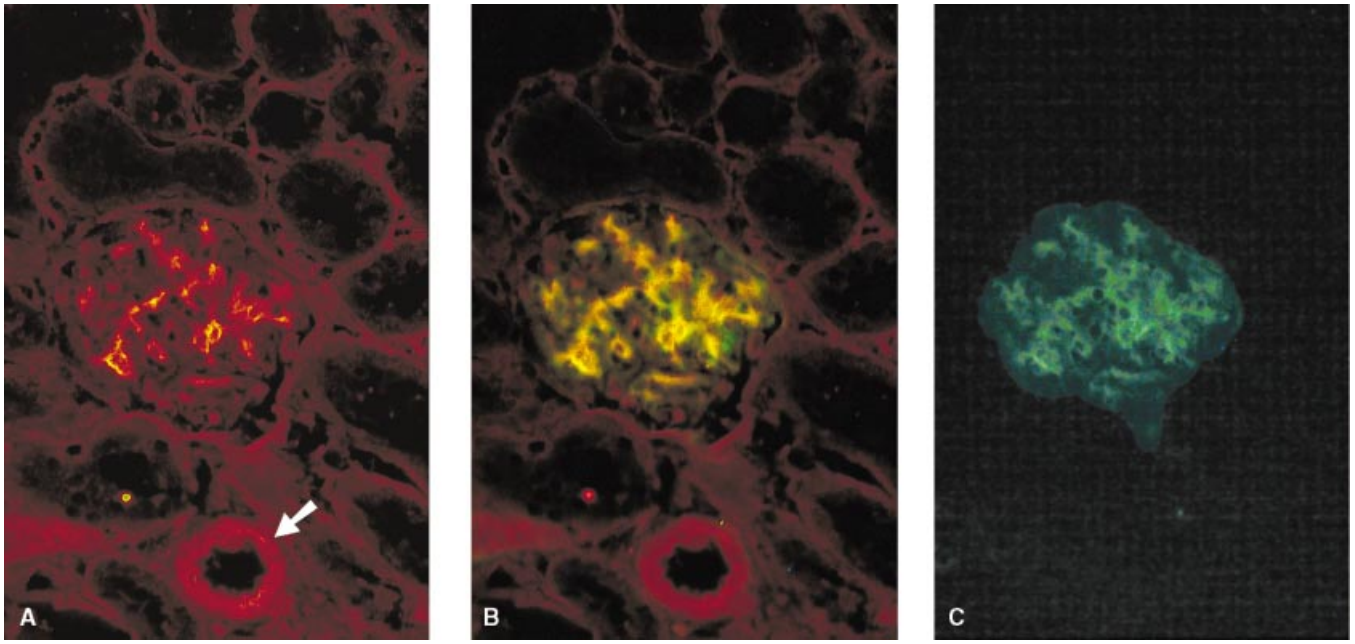


Fig. 1. Immunofluorescence detection of the α8 integrin in kidney sections of human and mouse. FITC detection of α8 (*A*) or β1 (*B*) in human kidney sections. CY3 detection of α8 in normal mouse kidney (*C*) and in a kidney from a α8 knockout mouse exhibiting a regular histological structure (*D*). White arrow indicates vascular staining of α8 integrin (magnification ×400).

Fig. 2. Immunofluorescence detection of α8 in rat glomeruli. In rat kidney sections, double staining was simultaneously performed for α8 (*A*; red) and Thy1.1 (*C*; green), as a mesangial cell (MC) marker. Using a fluorescence double filter, colocalization of both antigens could be detected in yellow color (*B*). White arrow indicates vascular staining of α8 integrin (magnification ×400).



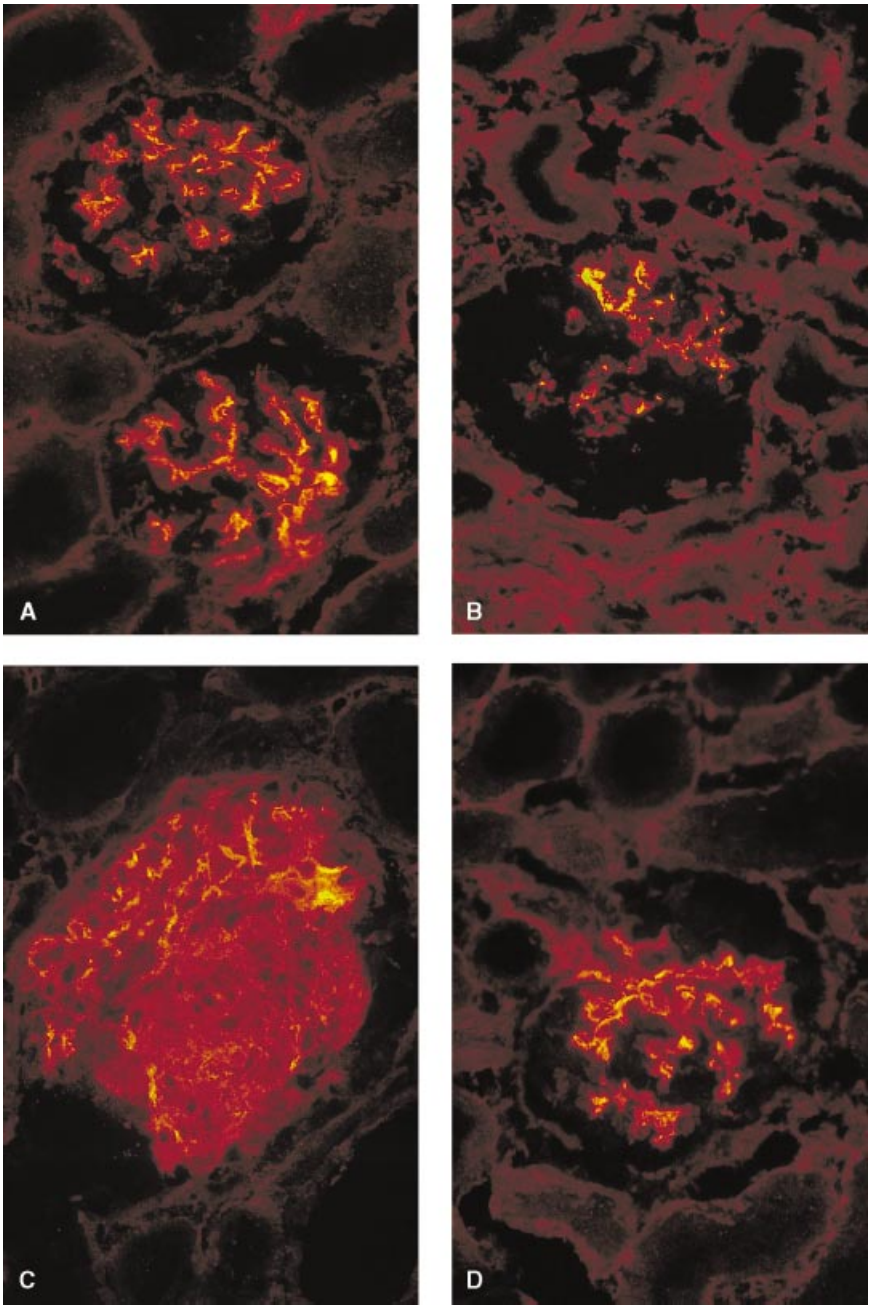


Fig. 3. Time course of α8 expression in anti-Thy1.1 nephritis in rats. Immunofluorescence microscopy studies for α8 were performed for three time points: isotonic saline-injected controls (*A*), day 2 (*B*), day 6 (*C*) and day 12 (*D*) of disease ($N = 5$ animals at each time point). Strong increase of immunoreactivity in mesangial areas was detected during the course of disease, peaking at day 6 (magnification ×400).

antibody, renal sections from α8 homozygous knockout mice with one kidney of regular macroscopic appearance were examined. No positive immune staining could be detected in these kidneys (Fig. 1D). Results comparable to findings in human and mouse were obtained in rat kidney. Double-immunofluorescence in renal sections of rats showed that the MC-specific anti-Thy1.1 antigen colocalizes with α8-positive cells (Fig. 2). This finding indicates that in rat glomeruli, α8 is expressed exclusively by MCs. Positive staining for α8 was also noted in the media of renal arterioles and arteries in all species investigated (Figs. 1 and 2).

Anti-Thy1.1 nephritis: Changes of mesangial α8 expression

In a rat model of mesangioproliferative glomerulonephritis, induced by the application of anti-Thy1.1 antibody, drastic changes of glomerular α8 abundance were observed (Fig. 3). Compared to the prominent mesangial staining pattern for α8 in controls, at day 2 of nephritis, glomerular immunoreactivity for α8 was slightly diminished or unchanged (Fig. 3B), depending on the degree of mesangiolysis. At day 6 of nephritis, mesangial hypercellularity peaked and α8 immunoreactivity was most

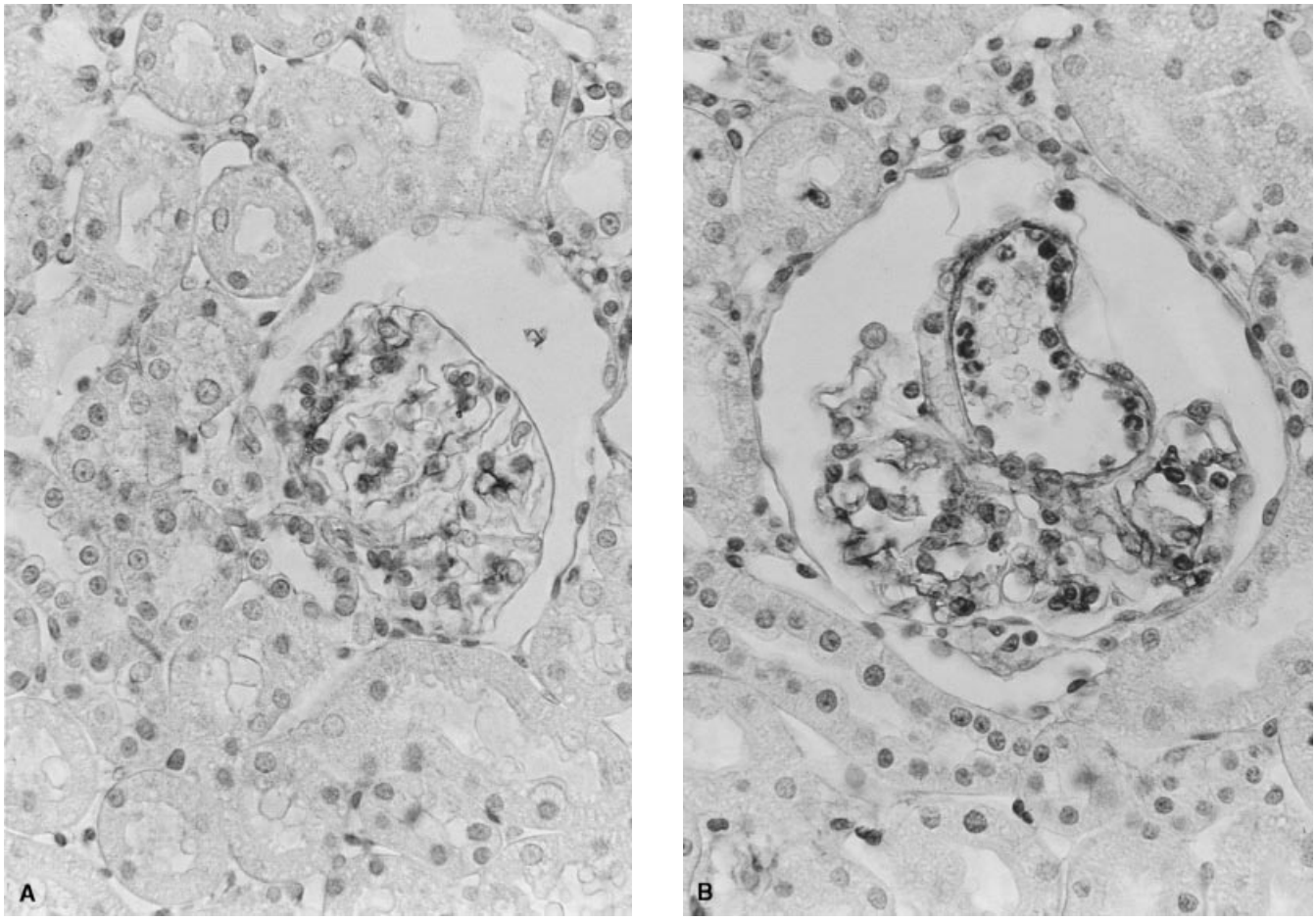


Fig. 4. Time course of fibronectin expression in anti-Thy1.1 nephritis in rats. Immunohistochemical staining for fibronectin was performed for three time points: isotonic saline-injected controls (*A*), day 2 (*B*), day 6 (*C*) and day 12 (*D*) of disease ($N = 5$ animals at each time point). Strong increase of immunoreactivity in mesangial areas was detected during the course of disease, peaking at day 6 (magnification ×400).

abundant and intense (Fig. 3C). Staining for α8 was detected not only close to nuclei of MCs, but also in the widened mesangial matrix. At day 12, during glomerular repair, staining for α8 was less impressive (Fig. 3D), and by day 56, when mesangial hypercellularity had resolved, glomerular expression of α8 had returned to control levels (not shown).

As a potential ligand for α8, fibronectin was examined immunohistochemically in the course of anti-Thy1.1 nephritis. Glomerular staining for fibronectin also correlated with MC hypercellularity, revealing an analogous pattern as seen for α8-staining (Fig. 4).

αv Integrin can also serve as a receptor for fibronectin. We found immune staining for αv in MCs in culture (not shown). To investigate, whether the upregulation of α8 is a unique feature of α8 among fibronectin receptors during anti-Thy1.1 nephritis, we also examined glomerular expression patterns of αv. In glomeruli of normal rat kidneys, αv was predominantly expressed by podocytes (Fig. 5). Weaker staining was detectable in mesangial areas. However, after onset of anti-Thy1.1 nephritis, αv was strongly up-regulated in MCs, peaking at day 6 and declining with the resolution of MC proliferation (Fig. 5).

Organization of α8 integrin into focal contacts in rat MCs

After seeding MCs onto culture plates coated with fibronectin, cell adhesion was detectable after 10 to 20 minutes. At that time, focal contacts were clearly visible in about 20% of attached cells, as determined by immunocytochemistry for vinculin. β1 integrin was present in focal contacts of 30% of the cells. In firmly attached cells, α8 integrin was found to be organized in focal contacts at about one hour in 10% of adherent cells. Three hours after plating onto fibronectin, α8 was detectable in focal contacts in about 50% of all attached cells (Fig. 6A), while all MCs displaying focal contacts were positive for β1. Double staining with vinculin showed that all α8-containing focal contacts stained positively for vinculin (Fig. 6B). However, more focal contacts per

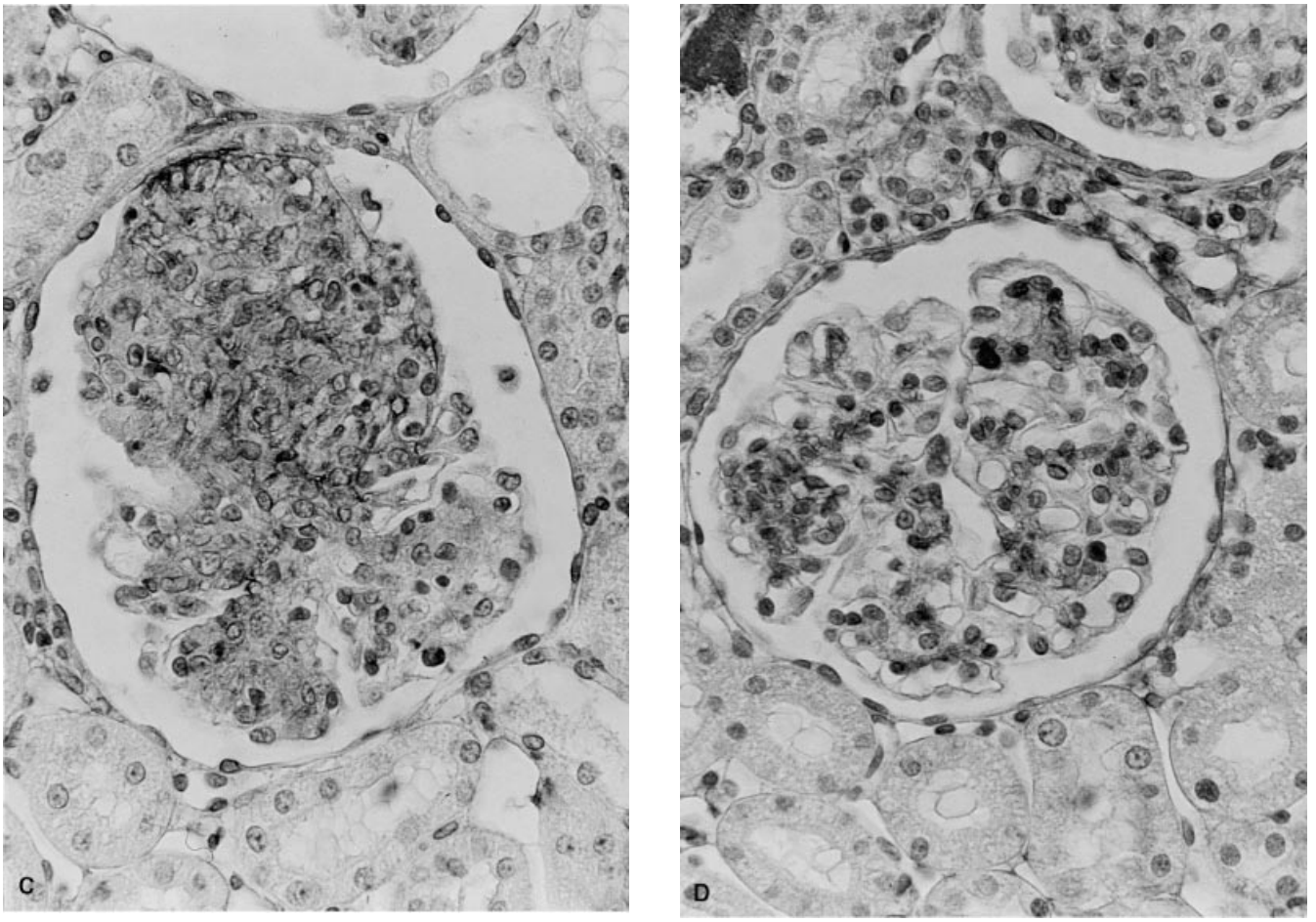


Fig. 4. (Continued)

cell strongly expressed vinculin and β1 integrin (Fig. 6C) than α8, with vinculin showing a pattern comparable to β1 integrin staining (Fig. 6D). When individual MCs detached from the substratum, they left so-called "footprints" behind which are plasma membrane patches sticking firmly to the underlying substratum or shed integrins. These "footprints" stained positively for α8 (Fig. 7).

Using vitronectin as substratum, α8 was also organized in focal contacts, with focal contact formation similarly abundant as seen on fibronectin (data not shown). The immunocytochemical results for vinculin and β1 integrin also showed higher percentages of positive cells as compared to α8.

TGF-β1 enhances expression of α8 integrin mRNA and protein in cultured MCs

To further examine the regulation of α8 integrin, MCs were incubated with 5 ng/ml TGF-β1 for time periods of 4, 8, 16 and 24 hours. TGF-β1 led to induction of α8 integrin on the mRNA and protein levels. Northern blot analysis of total RNA revealed that α8 mRNA was up-regulated after stimulating MCs with TGF-β1 up to threefold at 24 hours as compared to controls (Fig. 8). By Western blot analysis, a maximum of a threefold increase in α8 protein levels was observed in TGF-β1-stimulated MCs after 24 hours (Fig. 9).

DISCUSSION

The results of the present study demonstrate that α8 integrin, besides its localization in the renal vessel walls, is strongly and specifically expressed in the mesangium of human, rat and mouse glomeruli. In the anti-Thy1.1 rat model of glomerulonephritis, mesangial α8 is strongly albeit transiently up-regulated, paralleling the development of MC hyperplasia. The peak of α8 expression in this model correlated with the increase of fibronectin deposition, as well as with the upregulation of the αv integrin. Studies of MCs in culture confirmed that α8 integrin was organized into focal contacts of MCs when

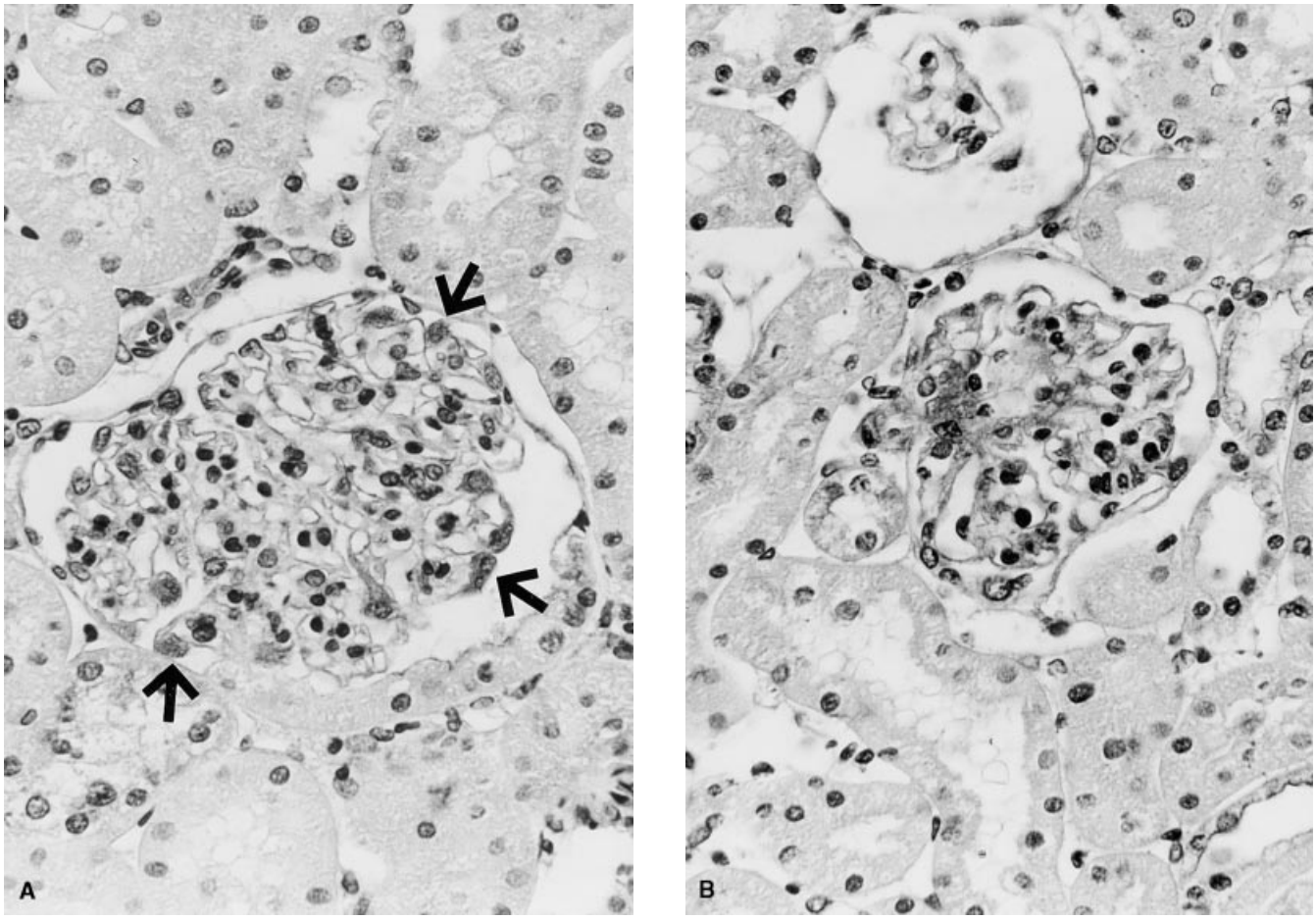


Fig. 5. Time course of αv expression in anti-Thy1.1 nephritis in rats. Immunostaining for αv was performed for three time points: isotonic saline-injected controls (*A*), day 2 (*B*), day 6 (*C*) and day 12 (*D*) of disease ($N = 5$ animals at each time point). Basal expression was noted in podocytes (arrows) and weakly in MCs; strong increases of immunoreactivity was detected in mesangial areas during the course of disease, peaking at day 6 (magnification ×400).

plated onto fibronectin and vitronectin. TGF-β1 stimulated the expression of α8 at the mRNA and protein levels in cultured MCs.

The strictly mesangial localization in glomeruli of the three species investigated suggests that α8 can be used as a MC marker in mouse and human glomeruli, where other exclusive markers for MCs are presently lacking. Several other integrins, such as α1, α2, α3 A, α5, α6B, αv, β1 and β3 integrin chains [5, 6] were shown to be expressed by MCs *in vitro* and *in vivo*, but in the glomerulus none of them localized selectively to MCs. In preliminary *in vivo* studies, we found that α8 integrin chains expressed on MCs may possibly serve as a target for anti-α8 antibodies to induce experimental mesangial immune injury in mice (Hartner et al, unpublished observations). Such a model could be useful because a reliable model of murine mesangioproliferative glomerulonephritis is presently lacking. While α8 expression in smooth muscle cells of the renal vasculature could pose a problem for such an approach, these cells are protected from circulating antibodies by the endothelium and its basement membrane, whereas glomerular mesangial cells are more directly exposed.

Muller et al have shown that the α8 integrin subunit is of critical importance for normal kidney development [10], similar to the reported function of the α3 chain [15]. Knockout mice for these integrin chains exhibit profound defects in kidney morphogenesis [10, 15]. Such findings raise the question of whether or not α8 could be important not only in normal kidney development but also in tissue remodeling after glomerular injury. For this reason, we investigated the regulation of α8 in the anti-Thy1.1 nephritis, which is a model with initial mesangiolysis and subsequent transient MC proliferation and matrix deposition. In analogy to wound healing, these changes are serving reconstitution of the glomerular capillary tuft, with eventual complete healing. Correlating with MC hypercellularity, α8 immunostaining was strongly enhanced during the course of glomerulonephritis and returned to control level after resolution of the

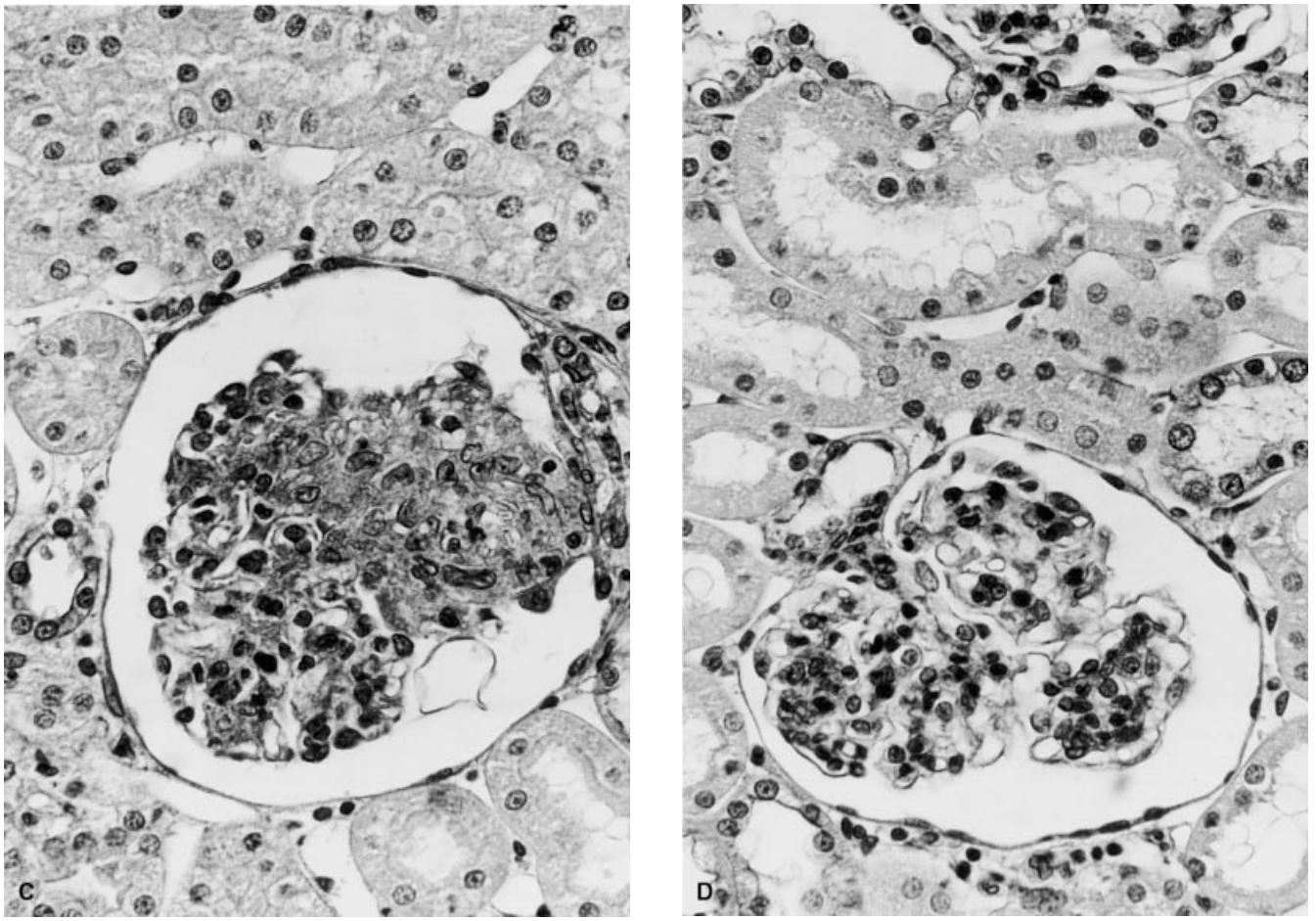


Fig. 5. (Continued)

glomerular injury. At day 6, when mesangial hypercellularity and ECM deposition peaked, α8 staining was confined not only to MCs, but acellular mesangial areas of excess matrix deposition were also positive for α8. Positive α8 staining of ECM could reflect the α8 integrin chains that were shed from the MC surface. A similar phenomenon was observed and discussed for another cell matrix adhesion receptor CD44 [16]. Another finding for MCs cultured on fibronectin seems to support this hypothesis. Detaching cells left "footprints" of focal contacts staining positively for the α8 integrin chain on the matrix surface. This is most likely due to shed MC membrane patches containing integrins and integrin-associated molecules.

Thus far, only one integrin heterodimer containing the α8 chain has been described, which is α8β1. Of the known ligands of α8β1, fibronectin is abundant in normal and diseased glomeruli. Other α8β1 ligands, such as tenascin C and vitronectin, are up-regulated in glomerulonephritis [1]; while another α8β1 ligand, osteopontin, has been found to be synthesized and secreted by proliferating rat MCs in culture [17], it has not been detected in the mesangium of normal or altered glomeruli (Hartner and Pröls, unpublished observations) [18]. Recent findings by Denda et al have shown that tenascin C is not a ligand for α8β1 in its native form, as deduced from binding studies using a receptor alkaline phosphatase binding assay [19]. In contrast, α8β1 bound to tenascin fragments exposing the RGD site [19]. *In vivo*, cryptic RGD sites of tenascin C could become accessible to integrin binding, for example, after exposure to metalloproteases or mechanical bending of the tenascin molecule, as suggested by Denda et al [19].

Our immunohistochemical studies showed that the αv integrin, another receptor of vitronectin and fibronectin, was localized mostly to podocytes and to a lesser extent to MCs in normal rat glomeruli. This observation supports the results of Roy-Chaudhury et al in human glomeruli [20]. In anti-Thy1.1 nephritis we found that mesangial expression of αv was altered in parallel to α8. Expression of other β1 integrins (α1β1 and α5β1) is also enhanced in human and rat glomerulonephritis [21, 22],

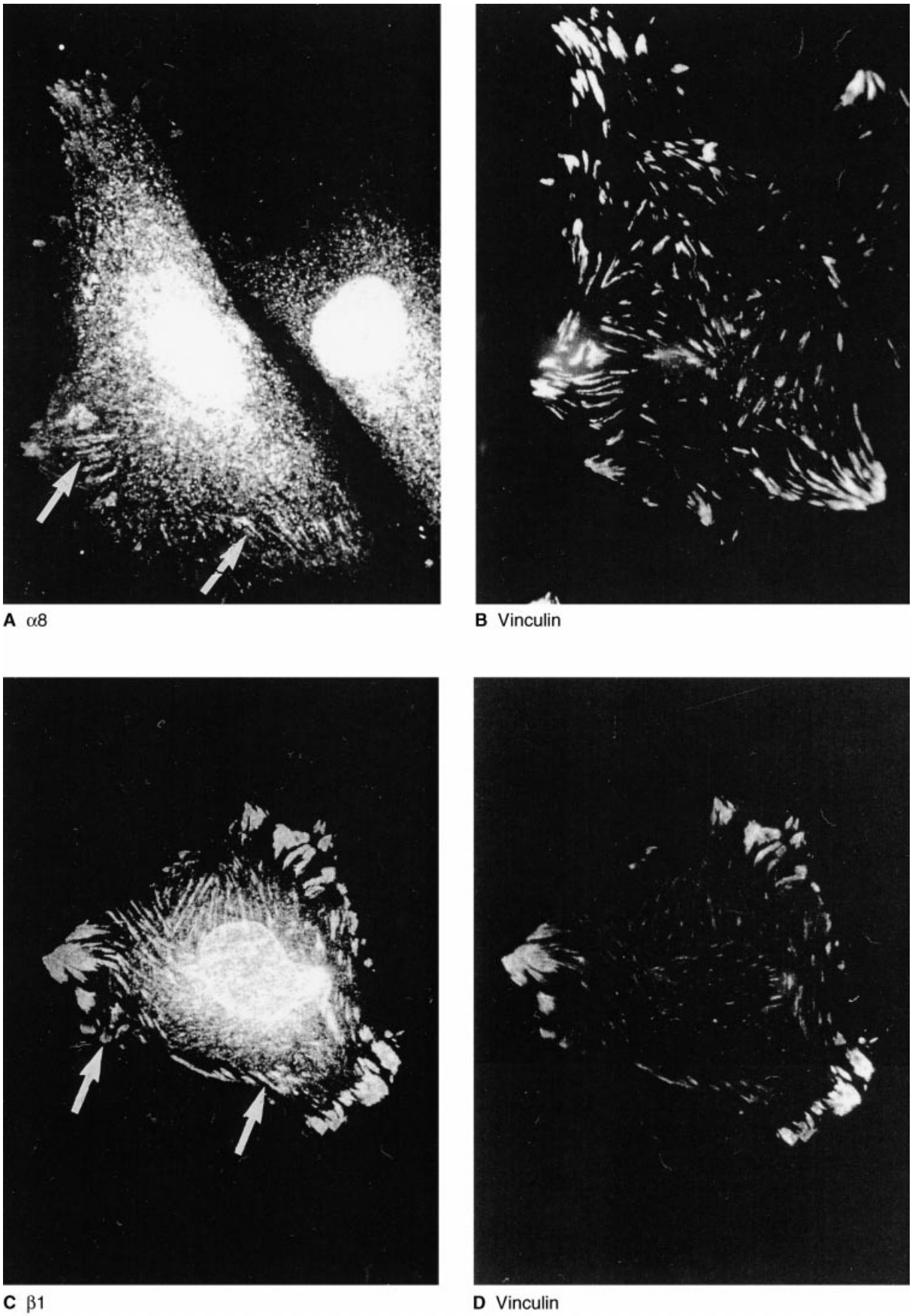


Fig. 6. Immunocytochemical detection of α8 and β1 in focal contacts (white arrows) of cultured mesangial cells (MCs). Cells were allowed to attach and spread for 3 hours on fibronectin. Then, MCs were fixed and simultaneously stained for α8 (*A*) or β1 (*C*) and vinculin (*B* and *D*; magnification ×1000).

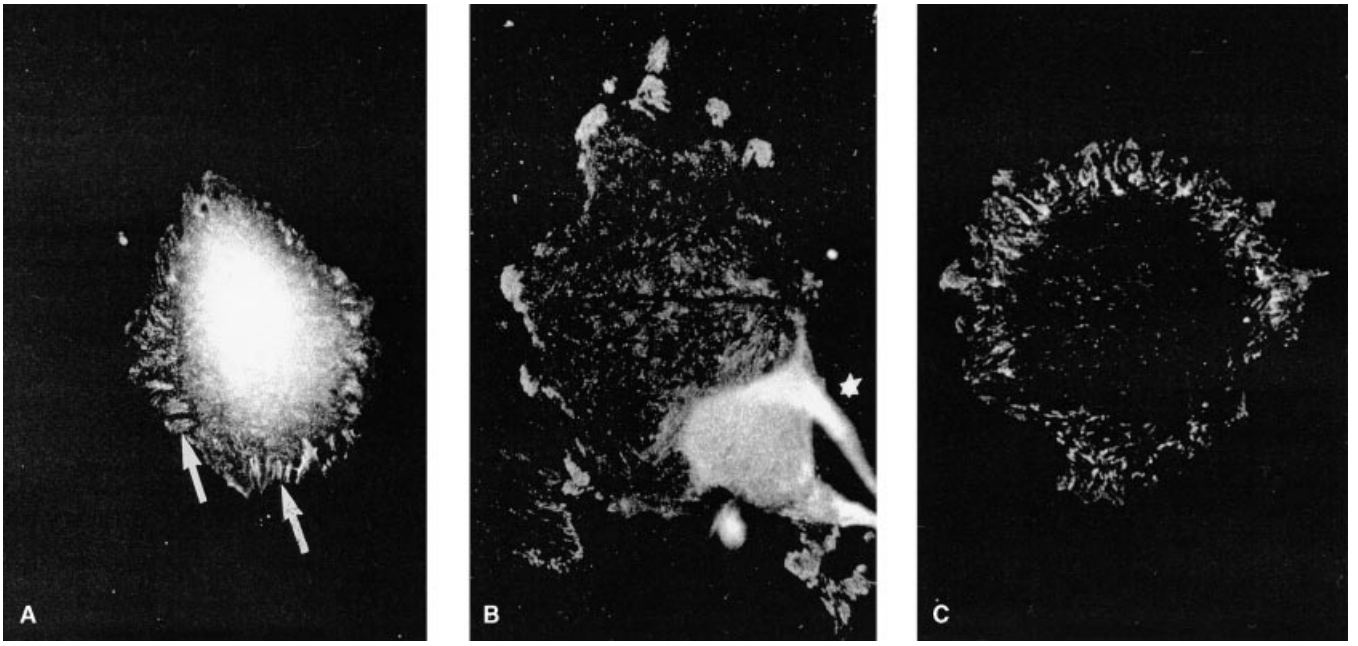


Fig. 7. Immunocytochemical detection of α8 integrin in detaching mesangial cells (MCs). MCs detaching from the matrix surface (*) were stained for α8 (*B*). Immunoreactivity was also detected in "footprints" of MCs (C) that had detached. (*A*) Adherent MC stained for α8. Focal contacts are indicated with white arrows (magnification ×1000).

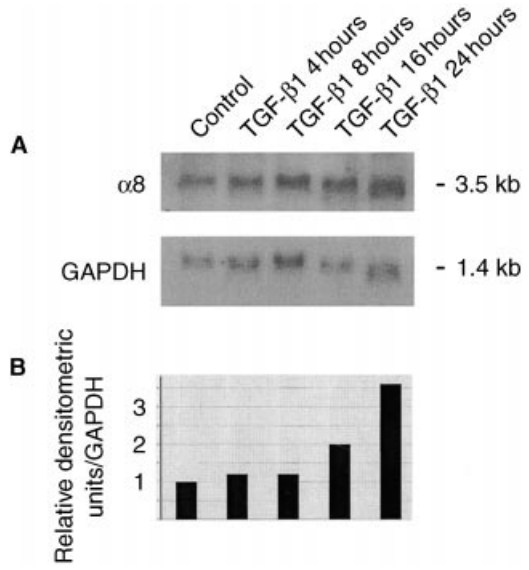


Fig. 8. Northern blot for α8 from mesangial cell (MC) RNA (*A***).** MCs were stimulated with 5 ng/ml TGF-β1 for the time periods indicated. Northern blot was probed for α8 or GAPDH to control for RNA loading (*A*). Expression levels for α8 are quantitated by densitometric analysis and expressed as relative densitometric units corrected for expression of GAPDH (*B*). Expression of α8 under control conditions was defined as 1 densitometric unit. The results shown are representative for 3 independent experiments.

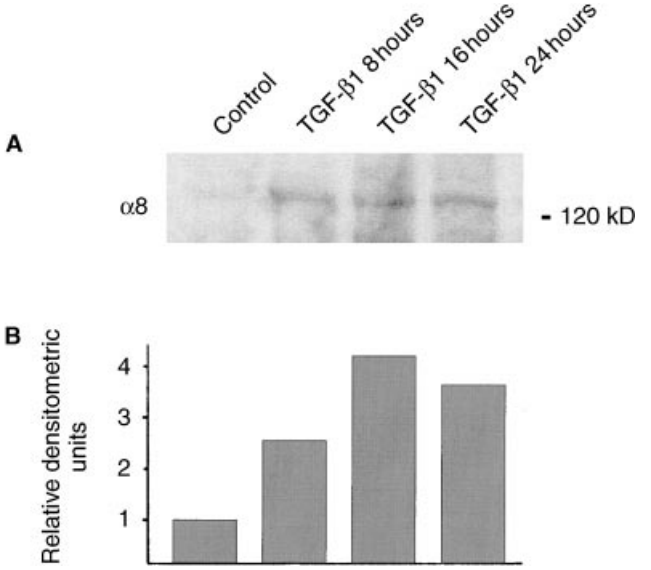


Fig. 9. Western blot for α8 from mesangial cell (MC)-derived protein. MCs were stimulated with TGF-β1 for the time periods indicated, lyzed and protein was extracted. An approximately 120 kDa signal was detected by enhanced chemiluminescence. Densitometric evaluation of α8 protein expression is expressed as relative densitometric units. Protein abundance at control conditions was defined as 1 densitometric unit. The results shown are representative for 3 independent experiments.

suggesting that these integrins could promote cell adhesion to matrix proteins known to accumulate in inflammatory glomerular injury, such as collagen type I or fibronectin [23]. Likewise, our results indicate that α8β1 and αv integrins and their matrix ligands are up-regulated in experimental glomerulonephritis. The parallel induction of β1 integrins and TGF-β1 in glomerulonephritis and the ability of exogenously applied TGF-β1

to enhance β1 integrin protein expression in glomerular cells of normal animals are consistent with the notion that TGF-β1 contributes to the up-regulation of integrins in glomerular disease [22]. Our finding of TGF-β1-augmented expression of α8 in MCs further supports this concept.

MCs organized α8 chains into focal contacts when plated onto fibronectin. However, more focal contacts per cell contained vinculin than α8. The antibody to β1 integrin also stained more focal contacts per cell than antibody to α8. Furthermore, only 50% of MCs that had attached to fibronectin matrix and formed focal contacts, were positive for α8. This indicates that other fibronectin-binding β1 integrins, such as α5, also organized within focal contacts under the assay conditions applied. This was confirmed by immunocytochemistry for α5 (not shown). On vitronectin substratum, α8 localized within focal contacts in about 55% of attached MCs. This is a result similar to the one observed on fibronectin, but the number of focal contacts per cell was not as numerous as on fibronectin (not shown). In addition, the numbers of focal contacts can change considerably among various assays, an observation previously described by Muller et al [24] with α8-transfected K562 cells. The reason for this variability was discussed by Muller et al [24] as a possible difference in vitronectin preparations, giving rise to cryptic fragments with accessible RGD sites in some preparations and nonaccessible RGD sites in others.

The finding of marked yet transient up-regulation of α8 integrin in anti-Thy1.1 glomerulonephritis is in keeping with the interpretation that α8 may be involved in glomerular reconstitution after injury. However, from available data it cannot be concluded that the observed changes in mesangial α8 expression are an epiphenomenon reflecting the activation of MCs during the course of inflammation, or whether or not the increased α8 expression is relevant for restoration processes in the mesangium. When plated onto fibronectin, α8-transfected K562 cells showed attachment and spreading, in contrast to attachment and nonspreading in nontransfected α8-negative K562 cells [24]. Untransfected K562 cells did not contain the α8 chain and attached to fibronectin via α5β1 [24]. Future studies will have to clarify whether up-regulated MC expression of α8 integrin subsequent to mesangiolysis enables MCs to repopulate the scaffold of the glomerular capillary tuft and thus contribute to the repair of glomeruli.

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