Expression of β1-Integrins on Activated Mesangial Cells in Human Glomerulonephritis

TAKASHI KUHARA, SHOJI KAGAMI, and YASUHIRO KURODA
Department of Pediatrics, School of Medicine, The University of Tokushima, Tokushima, Japan.

Abstract. β1-integrins, a family of cell-surface receptors, mediate cell-matrix interactions that play a critical role in tissue development and tissue remodeling after injury. In this study, to clarify the importance of β1-integrins in human glomerulonephritis (GN), the relationship among the glomerular expression of β1-integrins, their ligand matrix components, α-smooth muscle actin (α-SM actin) as a marker of activated mesangial cells (MC), transforming growth factor-β (TGF-β), and glomerularcellularity in two normal kidneys, ten minimal change nephrotic syndrome, 23 immunoglobulin A (IgA) GN, 13 lupus GN, and four membranous GN kidneys were studied. Immunostaining was performed on frozen sections, using monoclonal anti-α-SM actin antibody and polyclonal antibodies against fibronectin, collagen type IV, laminin, each subunit of all β1 (collagen/laminin receptor), α5β1 (fibronectin receptor) and TGF-β. Quantitation of staining indicated that the glomerular expression of α1β1 and α5β1 integrins correlated with the mesangial amounts of their ligands, collagen type IV, laminin and fibronectin (P < 0.01), α-SM actin (P < 0.01), and TGF-β (P < 0.01). In addition, a correlation was observed between an increased expression of α1β1 and α5β1 integrins and the degree of glomerular cell proliferation (P < 0.01). Double immunostaining showed that activated MC expressing α-SM actin strongly expressed α1β1 and α5β1 integrins, and these MC phenotypic alterations paralleled the level of glomerular TGF-β staining (P < 0.01). In conclusion, enhanced expression of β1-integrins by activated MC may contribute to the pathological mesangial remodeling characterized by MC proliferation and matrix deposition in human GN. Increased glomerular TGF-β appears to be involved in these MC phenotypic changes. (J Am Soc Nephrol 8: 1679–1687, 1997)

Mesangial remodeling after glomerular injury is a complex process that involves interactions between cells, extracellular matrix (ECM) components, and growth factors. A strict remodeling program seems to be required to reestablish the epithelial architecture, and uncontrolled mesangial remodeling including massive ECM deposition, constitutional fibronectin, vitronectin and tenascin, is expressed on MC. Changes in ECM components, and persistent mesangial cell proliferation may lead to glomerular sclerosis and result in a loss of renal function (1,2).

β1-integrins are a family of heterodimeric receptors consisting of noncovalently associated α and β subunits that primarily mediate cell-matrix interaction. Studies have demonstrated that β1-integrins critically participate in the control of various cell behaviors, such as cell migration, growth, apoptosis, and matrix assembly, and thereby affect tissue remodeling (3). Importantly, these integrins are good biological targets for transforming growth factor-β (TGF-β), which is a key mediator in the development of glomerulosclerosis (4,5).

Several β1-integrin receptors have been detected in human kidney glomeruli (6–9). α1β1 and α2β1, which are receptors for laminin and collagen types I and IV, are found on glomerular endothelial and mesangial cells (MC). α3β1, which is a receptor for laminin, collagens, fibronectin, and epiligrin, has been detected weakly in endothelial cells and MC and strongly in epithelial cells. α5β1, which is a fibronectin receptor, is present weakly on endothelial cells and MC. α8β1, which is a receptor for fibronectin, vitronectin and tenasin, is expressed on MC. Recent immunohistological studies in human glomerulonephritis...
phritis (GN) have reported that α5β1 and αvβ3 (a vitronectin receptor) coordinately increased with their ligand matrix components—such as fibronectin and vitronectin—in expanded mesangium, suggesting that integrin receptors play a role in cell-ECM interaction in GN (10,11). In addition, our previous study using a rat model of acute mesangial proliferative GN showed that MC which express α-smooth muscle actin (α-SM actin) simultaneously exhibit a higher expression of α1β1 and α5β1 integrins in expanded mesangium where the amounts of both their ligand ECM components and TGF-β increase, which suggests that phenotypically changed MC with increased β1-integrin expression participate in the pathological mesangial matrix remodeling in GN (12). On the other hand, Johnson et al., using the same rat GN model, reported that the appearance of MC with α-SM actin is associated with mesangial proliferative changes and proposed that this phenotype reflects activated C in proliferative GN (13). Thus under pathological conditions such as GN, rat MC undergo phenotypic changes concomitant with enhanced expression of β1-integrins, and this MC phenotype seems to contribute to the development of mesangial proliferative GN. However, the significance of the MC phenotype based on the expression of both β1-integrin and α-SM actin in human GN has not been investigated.

To evaluate the role of β1-integrins in human mesangial proliferative GN, we examined the relationship among glomerular changes in β1-integrins, α-SM actin, glomerular cellularity, and matrix components as integrin ligands. In addition, we also examined the association between the glomerular expression of β1-integrins and their strong modulator, TGF-β.

Figure 2. Expression of α1β1 and α5β1 integrins, α-SM actin, and TGF-β in normal human glomeruli. Glomeruli were stained with antibodies to the β1 subunit (A), the α1 subunit (B), α5β1 integrin (C), α-smooth muscle (α-SM) actin (D), and transforming growth factor-β (TGF-β) (E). Both the β1 subunit and α5β1 integrin are present along the capillary walls and in the mesangium (A and C), and the α1 subunit is weakly positive in the mesangium (B). α-SM actin and TGF-β are barely detectable in most of the glomeruli (D and E). (Magnification, ×300).
Materials and Methods

Kidney Specimens

Histologically normal portions of kidney tissues obtained from two patients with renal tumors were used as normal kidney tissue, and 50 tissue specimens obtained by renal biopsy from patients with renal diseases were used for this study. The diagnosis was based on studies of the tissue by light, electron, and immunofluorescence microscopy according to the classification of Churg et al. (14). The specimens consisted of immunoglobulin A (IgA) nephritis \( (n = 23) \), pure mesangial lupus GN (WHO class II) \( (n = 5) \), diffuse lupus GN (WHO class IV) \( (n = 8) \), minimal change nephrotic syndrome \( (n = 10) \), and primary membranous GN \( (n = 4) \).

Glomerular Cell Proliferation Grading

All of the glomeruli in each section (usually six to 30) were examined by light microscopy. The number of cells in at least four equatorially cut glomeruli in each section stained with periodic acid-Schiff stain was counted, and the average number of cells was used as an indicator of glomerular cell proliferation, as previously described (15).

Antibodies

Rabbit antiserum that was reactive with the cytoplasmic domains of \( \alpha_1 \)- and \( \beta_1 \)-integrin subunits was a kind gift from Dr. K. Iwamoto (University of Osaka, Osaka, Japan). A rabbit polyclonal antiserum to \( \alpha_5 \beta_1 \) was purchased from Telios (San Diego, CA). A mouse monoclonal antibody to \( \alpha \)-SM actin (1A4) was purchased from Sigma Chemical Company (St. Louis, MI) and was used to detect activated human MC (13,16). A rabbit polyclonal antibody to TGF-\( \beta \) was purchased from King Brewing Company (Kakogawa City, Japan). Rabbit antiserum against laminin, fibronectin, and collagen type IV were kind gifts from Dr. T. Nakamura (Yamanashi Medical School, Figure 3. Glomerular expression of \( \alpha_1 \beta_1 \) and \( \alpha_5 \beta_1 \) integrins, \( \alpha \)-SM actin, and TGF-\( \beta \) in immunoglobulin A (IgA) nephritis with moderate mesangial proliferation. Glomeruli were stained with antibodies to the \( \beta_1 \) subunit (A), the \( \alpha_1 \) subunit (B), \( \alpha_5 \beta_1 \) integrin (C), \( \alpha \)-SM actin (D), and TGF-\( \beta \) (E). Staining of the \( \beta_1 \) subunit and \( \alpha_5 \beta_1 \) integrins is increased along the capillary walls and in the mesangium (A and C). Increased staining of the \( \alpha_1 \) subunit is confined to the expanded mesangial area (B). \( \alpha \)-SM actin is strongly positive in mesangial lesions and TGF-\( \beta \) expression is increased at the same site (D and E). (Magnification, \( \times 300 \)).
Figure 4. Double immunostaining with antibodies to the β1 subunit (A), the α1 subunit (C), and α-SM actin (B and D) in diffuse lupus GN. The expression of α1β1 integrin and α-SM actin was colocalized in the expanded mesangium. (Magnification, ×300).

Yamanashi, Japan) (17) and were used to examine the associated distribution of β1-integrins with their ligand ECM components.

Immunofluorescence Microscopy

Indirect immunofluorescent staining of 3-μm cryostat sections was performed as described previously (12), using the following primary antibodies: anti-α1, β1 integrin subunit antiserum, anti-α5β1 integrin antiserum, anti-laminin, fibronectin and collagen type IV serum, TGF-β antibody, and anti-α-SM actin antibody. Fluorescein isothiocyanate-coupled donkey anti-rabbit IgG antibody and donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used as the secondary antibodies. In a double-staining experiment for β1-integrins and α-SM actin, sections incubated with anti-α1, β1 integrin subunit, or anti-α5β1 integrin antiserum were further incubated with anti-α-SM actin antibody and then with fluorescein isothiocyanate–coupled donkey anti-mouse antibody and tetramethylrhodamine isothiocyanate-coupled donkey anti-rabbit antibody (Jackson ImmunoResearch Laboratories, Inc.). Negative controls included omission of either of the primary antibodies, in which no staining was noted.

To evaluate the glomerular accumulation of laminin, fibronectin, and collagen type IV, as well as the glomerular expression of integrins, α-SM actin, and TGF-β, glomerular staining with each anti-matrix component, anti-integrins, anti-α-SM actin, and anti-TGF-β antibodies was graded (0 to 4+) as follows: 0, diffuse, very weak or absent mesangial staining; 1+, diffuse, weak mesangial staining with 1% to 25% focally increased mesangial staining; 2+, 25% to 50% of glomerular tuft demonstrating strong mesangial staining; 3+, 50% to 75% of glomerular tuft demonstrating strong mesangial staining; and 4+, 75% or more of the glomerular tuft strongly stained.

Human Mesangial Cell Culture and Immunoprecipitation Analysis of β1-Integrin

To confirm the specificity of the anti-integrin antibodies used here and to examine the molecular characteristics of integrins expressed by MC, we performed immunoprecipitation analysis of 35S-labeled MC. In brief, human MC were obtained from intact glomeruli of a patient with renal trauma by the graded sieving technique and were characterized as described previously (18). Cultured MC were labeled with [35S]methionine for 18 hours. Labeled MC were washed on ice with buffer containing 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and 25 mM Tris-HCl, pH 7.4 (washing buffer) and then solubilized with buffer containing 100 mM n-octyl-β-D-glucopyranoside (Sigma) in washing buffer (solubilization buffer). The insoluble materials were removed by centrifugation at 12,000 × g for 10 minutes at 4°C. The supernatants were pretreated by incubation with normal rabbit serum and protein A-Sepharose (Sigma) and then immunoprecipitated with anti-integrin subunit antiserum for 12 hours at 4°C. Immune complexes were recovered by binding to A-Sepharose. Beads were washed four times with solubilization buffer and the immunoprecipitates were analyzed by electrophoresis as described previously (12).

Statistical Analyses

Statistical significance (defined as P < 0.05) was evaluated using Pearson correlation coefficients or Spearman correlation coefficients.

Results

Expression of β1-Integrins in Normal Human Kidney and Cultured Mesangial Cells

We first examined the characteristics of β1-integrins expressed by human MC, using cultured human MC. Examina-
Figure 5. The relationships among the expression of αβ1 and α5β1 integrins, α-SM actin, and the average number of glomerular cells, which reflects glomerular cell proliferation, in diseased kidneys, including those with minimal change nephrotic syndrome (○, n = 10), IgA nephritis (●, n = 23), lupus nephritis (Δ, n = 13), and membranous GN (□, n = 4). Significant correlations were found between the glomerular expression of αβ1 and α5β1 integrins and α-SM actin (A, B, and C) (P < 0.01). The expression of αβ1 and α5β1 integrins correlates with the increase in the number of glomerular cells regardless of the type of disease (D, E, and F) (P < 0.01).

The immunohistochemical and biochemical findings, αβ1 and α5β1 integrins are synthesized in normal human MC.

We next studied the glomerular localization of β1-integrins in normal human kidney with the specific antisera described above. Immunofluorescence staining for the β1 subunit and α5β1 integrin was observed along the capillary walls and in the mesangial region, and staining for the α1 subunit was mildly observed in the mesangial region, as has been reported in normal kidney (6,7) (Figure 2A, 2B, and 2C). α-SM actin was strongly expressed by smooth muscle cells.
in a hilar arteriole but only scarcely by cells of the glomerular tuft (Figure 2D). TGF-β was not detected in most of the glomeruli, although it was observed weakly in the mesangial region in focal glomeruli (Figure 2E).

Expression of α1-Integrins in Diseased Kidneys

In minimal change nephrotic syndrome, the staining of α1β1 and α5β1 integrins was similar to that in normal kidney.

In proliferative GN, the degree of glomerular staining for both α1β1 and α5β1 integrins depended on the severity of GN. In most cases of mild proliferative IgA nephritis and pure mesangial lupus GN, staining for these integrins in the mesangial area and along the capillary wall was slightly increased, compared with that in normal tissue, although in some cases there was no difference in staining. In moderate or severe IgA nephritis, and in diffuse lupus GN, staining was definitely increased; staining for the β1 subunit and α5β1 integrin was increased along the capillary walls and in the mesangial region, and staining for the α1 subunit was increased in the mesangial region (Figure 3, A, B, and C). On the other hand, in membranous GN kidneys that had no mesangial enlargement, mesangial staining of α1β1 and α5β1 integrins was similar to that in normal kidney.

The expression of α-SM actin, a marker of smooth muscle cells that has shown to be an inducible marker of activated MC in disease states (13,16), was markedly enhanced in the expanded mesangial region observed in mesangial proliferative GN (Figure 3D). Double immunostaining for integrins and α-SM actin indicated that MC expressing α-SM actin also strongly express α1β1 integrin (Figure 4) and α5β1 integrin (not shown). The glomerular expression of α1β1 and α5β1 integrin significantly correlated with the expression of α-SM actin and glomerular cell proliferation regardless of the type of disease (P < 0.01) (Figure 5). However, in advanced glomerular sclerotic lesions, the expression of α1β1 and α5β1 integrins and α-SM actin was extremely decreased (not shown).

Furthermore, to investigate the role of β1-integrins in the mesangial deposition of ECM components, we examined the relationship between the expression of α1β1 and α5β1 integrins and the distribution of collagen type IV, laminin, and fibronectin, which are their ligands. Increased staining for ECM components was seen primarily in the expanded mesangial region in moderate or severe IgA nephritis and in diffuse lupus GN. There was a significant correlation between the mesangial expression of α1β1 and α5β1 integrins and the amounts of their ligands (P < 0.01) (Figure 6). These results suggest that MC with enhanced expression of β1-integrins

![Figure 6](image URL)
contribute not only to MC proliferation but also to ECM deposition in human GN.

Finally, we studied the glomerular expression of TGF-β, a strong modulator of β1-integrins, in diseased kidney. Glomerular staining for TGF-β was negative or very weak in minimal change nephrotic syndrome, but the staining intensity of TGF-β increased according to the degree of mesangial proliferative changes in GN (Figure 3E). There was a significant correlation (P < 0.01) between the mesangial expression of α1β1 and α5β1 integrins and staining for TGF-β (Figure 7).

Discussion

Progressive forms of many types of GN are characterized by mesangial ECM accumulation and MC proliferation (2). Several recent reports have indicated that this pathological mesangial remodeling is due to uncontrolled interaction between MC, ECM, and growth factors, suggesting that mesangial β1-integrins that mediate MC-ECM interaction play an important role in progressive glomerular diseases (2,3,5,12).

The study presented here demonstrated that normal human MC synthesize and express α1β1 (a collagen/laminin receptor) and α5β1 integrins (a fibronectin receptor) and showed that the expression of these integrins is associated with both the level of glomerular cell proliferation and the accumulation of their ligand matrix proteins, such as fibronectin, collagen type IV, and laminin in human GN. Another histochemical study focusing on the expression of glomerular vitronectin receptor (αvβ3) or α5β1 integrin also reported a positive correlation between α5β1 integrin, its ligand fibronectin, and the level of glomerular damage in diseased human glomeruli, which supports the notion that glomerular β1-integrins are important in pathological ECM accumulation (10). Some experimental studies have indicated that β1-integrins play a substantial role in ECM remodeling and cell proliferation after tissue injury. The increased expression of α5β1 in CHO cells after transfection increases the level of fibronectin deposition (19). In turn, antibodies to α5β1 integrin inhibit fibronectin assembly and deposition in cells (20,21). With respect to the relationship between β1-integrins and cell proliferation, disintegrins, a group of molecules that contain a Arg-Gly-Asp sequence, have been shown to inhibit rat mesangial cell proliferation by blocking cell-matrix interactions (22). Thus, based on these in vitro and in vivo observations, the increased expression of β1-integrins by MC seems to participate not only in pathological matrix deposition but also in cell proliferation by increasing the interaction between MC and ECM in GN.

Double immunostaining indicated that the expression of mesangial β1-integrins is increased on activated MC with the characteristics of smooth muscle cell (+ α-SM actin). This finding is consistent with our previous observation that this MC phenotype shows an increased expression of β1-integrins in expanded mesangium of rat GN (12). Johnson, Alpers and coworkers reported that when MC activation and proliferation occur, there is a phenotypic change characterized by de novo

![Figure 7](image-url)
expression of α-SM actin, which suggests that proliferating MC have acquired the characteristics of myofibroblasts (13,16). In general, myofibroblasts have been proposed to play a role in wound contraction and in retractive phenomena of several types of fibrotic diseases (23). A recent study demonstrated the synchronous appearance and distribution of α5β1 integrin, fibronectin, vinculin, and α-SM actin in fibroblasts during tracheal wound healing after mechanical injury in rats (24). Moreover, contractile fibroblasts have been found to show increased expression of α5β1 integrin and increased deposition of fibronectin fibrils in skin wounds (25,26). The collagen gel assay using cultured fibroblast or MC demonstrated that α1β1 integrin plays a significant role in interactions between cells and the collagen matrix, which result in collagen reorganization and gel contraction (27,28). Taken together, our results suggest that activated MC (+α-SM actin) along with their increased expression of α1β1 and α5β1 integrins may contribute to wound contraction or overhealing, such as glomerular scarring in human progressive glomerular diseases.

We should stress that there is a positive relationship between the level of α1β1 and α5β1 integrins and TGF-β in nephritic glomeruli. TGF-β is a multifunctional regulator of cell growth and function and has been recognized as a key mediator for ECM accumulation leading to glomerulosclerosis. We have shown that TGF-β stimulates not only the synthesis of ECM components such as collagen and fibronectin but simultaneously increases the expression α1β1 and α5β1 integrins in both rat MC and glomeruli (5,12). It has recently been shown that TGF-β induces α-SM actin expression in granulation tissue fibroblast and in quiescent and growing cultured fibroblasts (29). Moreover, overexpression of TGF-β in rat glomeruli using expression vector directly induces α-SM actin expression by MC (30). Therefore, our current observations strongly suggest that TGF-β is a potent candidate for inducing both the increased expression of β1-integrins and the activation of MC in human GN.

In conclusion, this study suggested that β1 integrins on activated MC may play a role in the pathogenesis of abnormal mesangial remodeling observed in various types of human GN. In addition, our data also suggest that the increased expression of glomerular TGF-β induces these phenotypic changes in MC. We believe that further characterization of β1-integrins on MC may provide a better understanding of the pathogenesis of chronic progressive glomerular disease.

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