Integrins and glomerulonephritis

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Abstract

Glomerulosclerosis characterizes the progressive form of glomerulonephritis. Accumulation of leukocytes in both the glomerulus and the renal extravascular interstitium is a common feature in several forms of glomerulonephritis. Adhesion of cells to each other or to the extracellular matrix provides essential signals that regulate many cellular functions including cell migration, proliferation, differentiation and apoptosis. The integrin superfamily orchestrates many of these complex adhesive events through regulated interactions with a large variety of ligands.

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Introduction

The function of the kidney consists in purifying blood through glomerular filtration and tubular reabsorption and secretion. The renal glomerulus is a highly specialized structure in the kidney that ensures selective ultrafiltration of plasma so that essential proteins are retained in the blood. The glomerulus is composed of three types of cells - endothelial, epithelial and mesangial cells - and the extracellular matrix (ECM) consisting of the glomerular basement membrane (GBM) and the mesangial matrix. The GBM is a super-high molecular complex comprised of adhesive glycoproteins such as collagen, fibronectin, laminin, entactin and various heparan sulphate proteoglycans such as perlecan and agrin, which are released from the adjacent cells (1,2). The adhesion between glomerular cells and the ECM is essential for the permeability characteristics of the capillary wall allowing the filtration function. The interactions between glomerular cells and the ECM are mediated through adhesion receptors or integrins that function as a two-way conduit between the cell and the ECM. The cellular events influence how integrins bind to the ECM while those from the ECM modulate the morphology and proliferation of the cell as they function and respond to several extracellular factors (3). Integrins exhibit classic receptor behaviours such as signal transduction upon binding to the ligand, susceptibility to activation, and up- or down-regulation of receptor numbers. Integrins have long been assumed to play a role in renal morphogenesis, cell proliferation, maintenance of the renal architecture and renal regeneration.

Mesangial cell proliferation, accumulation of mesangial ECM, and glomerular visceral epithelial cell -podocyte- detachment from the GBM characterize the progressive form of glomerulonephritis (GN). The molecular pathology of progressive GN can be studied with regard to integrins that are expressed by the glomerular cells. Unregulated ECM reorganization and remodelling by mesangial cell integrins may affect the morphology and function of the glomerular mesangium. A vicious cycle may arise by which these alterations would further modify mesangial cell function to induce greater mesangial remodelling, eventually leading to podocyte detachment and collapse of glomerular capillary ultrafiltration barrier.

In addition to its interactions with the ECM, renal cells are also capable of interacting with each other and with circulating blood leukocytes. Some of the integrins mediate cell-cell interactions that play a role in renal morphogenesis as well as in the maintenance of tubule epithelial polarity, which is essential to many tubular resorptive and secretory processes, and in the tubule regrowth after acute tubular necrosis. Integrins provide the foothold for leukocytes and platelets to localize in the kidney and initiate thrombotic and inflammatory events in GN. Interactions between integrins expressed in circulating leukocytes and immunoglobulin (Ig-like) superfamily members expressed in renal cells account for the strong adhesion and spreading of leukocytes over
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the endothelium and for subsequent transmigration through the endothelial cell junctions in glomerulus and interstitium. Leukocyte integrins, which are usually in a non-adherent conformational state, can be activated by soluble factors (chemokines) or leukocyte homotypic interactions mediated by co-stimulatory molecules. Homotypic interactions between migrating cells and adjacent leukocytes create amplified migration cascades.

This chapter first reviews receptors that mediate cell-matrix interactions and their roles in the normal kidney and during glomerular injury. This review will then focus on the cell-cell interactions, which play an important role in leukocyte recruitment and function in inflammatory, necrotizing, and thrombotic diseases of the kidney.

Renal cell-matrix interactions

The major ECM receptors are the integrin family that mediate cell attachment to the ECM components of the basement membrane. Integrins are transmembrane, heterodimeric, non-covalently bound protein complexes consisting of α and β chains that link the ECM to the cytoskeleton. Both subunits have a single hydrophobic transmembrane domain with large extracellular domains of 700 to 1100 residues and cytoplasmic domains of 30 to 50 residues (Fig. 1). At least 19-α and 8-β chains have been identified thus far (4). Integrins have been grouped into families based upon shared β chains, but some α chains bind to more than one β chain, resulting in differences in their binding specificities.

Figure 1. Schematic of representative integrin α and β chains. The regions labelled "Me" are divalent metal binding motifs that may bind Ca²⁺ or Mg²⁺. A binding site for Mg²⁺ but not for Ca²⁺ has been identified in the interactive domain, which is the key adhesion motif in cell-cell interactions.
β1 integrins, the largest group of the integrin family, are composed of a β1 chain (CD29) associated with 1-12 α chains (α1-α11 and αv). β1 integrins are also known as “very late activated antigens” or VLA, because some of them appear on lymphocytes 2 to 4 weeks after antigen stimulation, numbered as VLA-1 to VLA-12 according to the α chain. They are expressed on several cell types such as leukocytes, platelets, fibroblasts, epithelial cells and endothelial cells (5). β1 integrins function predominantly in cell-ECM adhesion, but certain β1 integrins, such as α4β1 integrin, have a function in leukocyte-endothelial cell interactions.

β1 integrins connect the ECM with the cytoskeleton and provide a mechanical or physical linkage. The cytoplasmic tail of the β chain appears to be predominantly responsible for interactions with the cell cytoskeleton via binding to cell proteins. Cell-ECM interaction is an active phenomenon involving the formation of adhesion plaques where matrix proteins, matrix receptors, and actin stress fibres concentrate. The link between matrix receptors and the cytoskeleton is via adaptor proteins such as talin, vinculin, α-actinin, paxillin, and tensin that are concentrated at these sites. The combination of the α and β1 chains determines the specificity of the ligand ECM component and also the intracellular signalling properties affecting the cell behaviours such as proliferation, differentiation, survival and ECM assembly. In the kidney, concentrations of actin filaments that conforms the cytoskeleton, proteins such as talin, vinculin, α-actinin, and β1 integrins are present in podocytes at areas of attachment to the GBM (6), and in mesangial cells (5,7). Binding of matrix receptors to ECM thus serves to organize the cytoskeleton.

There are several factors which act on cellular integrin function resulting in disturbance on renal cell-ECM interaction (inside-out signalling). Activation of T lymphocytes by several signals, including interferon-γ and interleukin (IL)-1 may upregulate β1 integrin expression –the receptor number and/or affinity– leading to increase in adhesion to fibronectin, laminin and collagen (8). Fibrogenic agents such as angiotensin II (Ang II), transforming growth factor-β (TGF-β), endothelin, platelet-derived growth factor (PDGF) (9) and connective tissue growth factor (CTGF) (10) upregulate β1 integrin expression. These growth factors increase the number of integrin receptors in mesangial (11), endothelial (12) and epithelial (13) cells, matrix synthesis and adhesion, thus contributing to local ECM accumulation and sclerosis.

On the other hand, in vitro studies have shown effects of the matrix on renal cell function via β1 integrins (outside-in signalling). Glomerular mesangial cell morphology, proliferation, synthesis of matrix components, proteoglycans, growth factors and cytokines, as well as responsiveness to growth factors and cytokines are modulated by ECM components. β1 integrins
are involved in the ability to incorporate newly synthesized matrix components into the ECM. Collagen influences the synthesis; localization and organization of fibronectin produced by mesangial cells (14), which is important during mesangial sclerosis.

**Integrin distribution in the normal kidney**

β1 integrins -VLA- are present in almost all cell types and are the major group expressed in the developing and adult kidney. During development, α1β1, recognizing collagen and laminin, and α4β1, that recognizes fibronectin, are present in undifferentiated renal mesenchymal cells. Developmentally regulated expression of β1 integrins suggests a role in branching morphogenesis, where they may integrate changes in the composition of the mesenchymal ECM and coordinate cell proliferation (15).

In the adult human kidney, α1β1 and α2β1 integrins are found in mesangial, endothelial and tubular epithelial cells (5,7). α3β1 is the major ECM receptor expressed by visceral epithelial cells and, in the foot processes of podocytes, promotes anchorage to GBM (6). The expression of integrin matrix receptors in adult normal human kidney and the recognized ECM components are summarized in Table 1.

**Table 1.** Renal integrins with function predominantly in cell-matrix interactions.

<table>
<thead>
<tr>
<th>Integrins</th>
<th>Molecule*</th>
<th>Glomerulus</th>
<th>Tubule</th>
<th>Ligand Matrix Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1 Integrins*</td>
<td>VLA-1</td>
<td>Endothelium, mesangium</td>
<td>Epithelium</td>
<td>Collagen I, IV, VI, laminin</td>
</tr>
<tr>
<td>α1β1 (CD49a/CD29)</td>
<td>VLA-2</td>
<td>Endothelium, mesangium</td>
<td>Epithelium</td>
<td>Collagens I-IV, laminin, tenasin</td>
</tr>
<tr>
<td>α2β1 (CD49b/CD29)</td>
<td>VLA-3</td>
<td>Endothelium, mesangium, mesangium</td>
<td>Epithelium</td>
<td>Collagen I, laminin, fibronectin, entactin</td>
</tr>
<tr>
<td>α4β1 (CD49c/CD29)</td>
<td>VLA-5</td>
<td>Endothelium, mesangium</td>
<td>Epithelium</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>α5β1 (CD49f/CD29)</td>
<td>VLA-6</td>
<td>Mesangium</td>
<td>---</td>
<td>Fibronectin, vitronectin, tenascin, nephrinectin</td>
</tr>
<tr>
<td>α6β1 (CD49b/CD29)</td>
<td>VLA-8</td>
<td>Mesangium</td>
<td>---</td>
<td>Tenascin</td>
</tr>
<tr>
<td>α6β1 (CD49b/CD29)</td>
<td>VLA-9</td>
<td>---</td>
<td>---</td>
<td>Fibronectin, vitronectin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>β2 Integrins</th>
<th>Mesangium</th>
<th>Epithelium</th>
<th>Fibronectin, vitronectin, tenascin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α2β1 (CD51/CD61)</td>
<td>---</td>
<td>---</td>
<td>Collagen, osteopontin, thrombospondin</td>
</tr>
<tr>
<td>β3 Integrins</td>
<td>Mesangium, epithelium</td>
<td>---</td>
<td>Fibronectin, vitronectin</td>
</tr>
</tbody>
</table>

β1 Integrins* (CD49a/CD29) VLA-4 and α2β1 (CD49b/CD29) VLA-7 are not present in adult normal human kidney. * Detected by immunochemistry only in cultured renal cell.
Functions of matrix receptors in the kidney

Being receptors, $\beta_1$ integrins transmit information from the ECM into the cell, and are involved in ECM-controlled signalling events that modulate multiple adhesion-related functions and non-adhesion-dependent functions.

Adhesion-related functions of integrins include: development and organization of tissues, cell anchorage, maintenance, recruitment of cytoskeleton-associated proteins, transmission of contractile forces to cytoskeleton, and reorganization of actin filaments.

$\beta_1$ integrins can play a role in renal morphogenesis, as demonstrated in both in vitro and in vivo studies. Blockade of integrin subunits $\alpha_3$, $\alpha_6$, $\alpha_v$ and $\beta_1$ leads to a marked inhibition of branching morphogenesis from early ureteric bud and to inhibition of tubulogenesis in metanephric cultures (16). In vivo studies in knockout mice have elucidated the role of $\alpha_3$ and $\alpha_8$ (17). Thus, mice not expressing $\alpha_3$ integrin chains have decreased branching of the ureteric bud, leading to fewer tubules, cystic dilatation of tubules, and significant abnormalities of the glomerular capillary wall (19,20). The latter include lack of fusion of GBM layers due to an impaired incorporation of entactin and fibronectin into the ECM, absence of foot processes, and decreased number of capillary loops. In mice lacking $\alpha_8$, growth and branching of the ureteric bud and recruitment of mesenchymal cells into epithelial structures are defective.

In the adult kidney, a function of $\beta_1$ integrins is the maintenance of unique renal structures such as the glomerular capillary wall which selectively ensures plasma ultrafiltration. Glomerular filtration depends, at least in part, on podocyte-ECM interactions. In the glomerulus, $\beta_1$ integrins receptors are concentrated on those zones of visceral epithelial cells facing the GBM. Ultrastructural analysis identified important concentrations of vinculin and talin at the base of foot processes abutting the GBM and longitudinal cytoskeleton actin filaments. The 270-kD cytoskeletal protein talin is the link between the cytoplasmic domain of the integrin and the actomyosin contractile apparatus. Talin contains binding sites for the cytoskeletal protein vinculin which in turn binds $\alpha$-actinin, $\beta$-catenin, vinexin, paxillin and actin (6).

In addition, some $\beta_1$ integrins play a role in renal cell-cell interactions. Thus, $\alpha_2\beta_1$ (VLA-2) also localizes at intercellular borders of the tubular epithelial cells, where it may help to maintain tubular integrity and thus ensures the tubular resorptive and secretory processes (5).

Non-adhesion-dependent functions of $\beta_1$ integrins are cellular changes due to signalling initiated by cross-linking of integrins by antibodies or by ligand-integrin binding to matrix proteins or their fragments. The interaction between anti-$\beta_1$ antibodies and $\beta_1$ integrins on mesangial cells leads to intracellular signalling, as demonstrated by increased intracellular concentrations of cAMP (18). The mechanisms of signal transduction via integrins conform focal
adhesion plaques rich in tyrosine kinases and phosphoproteins (19). Among these are extracellular signal-regulated kinase (ERK), members of the focal kinase (FAK) family and mitogen-activated protein kinase (MAPK) (20,21), as well as integrin-linked kinase (ILK) (22). These metabolic mechanisms modulate the connections of the extracellular matrix via integrins to the cytoskeleton through adhesion plaque signalling, and are implicated in almost every fundamental cell activity. Integrin-mediated signalling is a complex organized cascade starting with the initial receptor engagement, followed by sequential activation of various tyrosine kinases, including lipid and protein kinase, phospholipase Cγ and small guanosine triphosphatases. ILK, a cytoplasmic component of cell-ECM interactions, is a protein serine/threonine kinase interacting with β₁ and β₃ integrins cytoplasmic domain which is critically involved in the regulation of fibronectin deposition. Activation of ERK and/or ILK induces ECM signalling that can lead to altered cell behaviour, such as production of inflammatory mediators, oxygen radicals, and increases in intracellular Ca²⁺ and pH. This results in upregulation of transcription factors, induction of expression and/or activation of other cell surface receptors, collagen gene expression, phosphorylation of specific intracellular proteins, changes in the conformation of integrin chains, cell survival, as well as assembly and disassembly of ECM (7,23) (Fig. 2).

**Figure 2.** Complex cascade of reactions that conduits to cell functions and extracellular matrix assembly following β₁ integrin activation. T = talin; P = paxillin; V = vinculin; FAK = focal adhesion kinase; PI-3K = phosphatidylinositol-3 kinase; Src = nonreceptor tyrosine kinase; MAPK = mitogen-activated protein kinase; ERK = extracellular signal regulated kinase; ILK = integrin-linked kinase; BP = binding protein.
Role of renal cell-matrix interactions in experimental glomerulonephritis

The major basic result of altered cell-matrix interactions is the perturbation of the normal adhesion of the cells to their supporting scaffolding matrix. The role of integrins in glomerular cell-GBM interactions is crucial in the maintenance of normal glomerular capillary wall permeability. Disruption of integrin-matrix interaction leads to detachment of podocytes corresponding to the critical event of foot process retraction. Areas of denuded GBM are important sites of protein traffic into the urinary space. In the development of the proteinuria, the histological feature is commonly referred to as foot process fusion or effacement, and this consists of a cytoplasmic band located outside along the GBM by electron microscopy. Podocyte effacement reflects podocyte cytoskeletal disaggregation and urinary loss of actin and vinculin from podocytes. Interference with α3β1, located along the GBM, has been shown to occur in puromycin aminonucleoside nephrosis -the counterpart of the human minimal change glomerulopathy (24)-, as well as in other non-antibody-mediated models of GN, including subtotal nephrectomy and adriamycin-induced nephrosis in which lack of α1β1 has also been reported (25,26).

It is recognized that antisera containing reactivities to β1 integrins can induce podocyte injury and proteinuria in animal models. Disturbance in podocyte-matrix interactions has been demonstrated in passive Heymann nephritis, which is the model of human membranous nephropathy. Heymann nephritis is induced by nephritogenic anti-Fx1A antibody -obtained by immunization of animals with proximal tubular brush border fraction of rat kidney- that cross-reacts with antigens on podocytes. *In vitro*, affinity chromatography of surface-labelled proteins from rat podocytes showed that antibodies to α3 or β1 integrin subunits specifically immunoprecipitated proteins from eluates of a column containing immobilized anti-Fx1A, indicating that anti-Fx1A reacts with α3β1 (27). Anti-Fx1A inhibited adhesion of podocytes to collagen I, collagen IV, laminin, and fibronectin substrata, inducing adherent cells detachment.

A role of β1 integrins has also been shown in the model of anti-GBM GN in which glomerular injury and proteinuria are induced independently of complement and leukocytes. Anti-GBM antibody has prominent reactivity with β1 integrins, and inhibits podocyte attachment to collagen I, collagen IV, laminin and fibronectin substrata *in vitro* (28). Several experimental studies using antibodies to β1 integrins have demonstrated that their binding to β1 integrins leads to proteinuria by direct interference with glomerular epithelial cells-GBM interactions or by cross-linking receptors leading to signal transduction (23).
Role of renal cell-matrix interactions in human glomerulonephritis

Abnormalities of integrin expression in several human GN support that altered cell-matrix interactions play a role in glomerular injury. The linear distribution of α₃β₁ along the glomerular capillary loop is altered in renal biopsy specimens from patients with nephrotic syndrome due to minimal changes glomerulopathy and membranous nephropathy (MN) (29,30). In stages I and II of MN, α₃β₁ distribution shows an irregular pattern, and in stage III, a segmental loss of α₃β₁ is detected. Increased vitronectin and the αᵥβ₃ receptor have been described in subepithelial deposits and in foot processes in MN (29). These observations suggest that in human and experimental MN, a disruption of the normal interaction between integrin and its ECM ligand occurs. Abnormal expression of the α₃β₁ integrin has also been noted in diabetes mellitus (31). In fact, detachment of podocytes from GBM causes urinary excretion of podocytes in patients with early diabetic nephropathy (32).

The role of matrix integrin receptors has been reported in glomerular diseases associated with mesangial expansion and proliferation. It has been shown that increases of α₁β₁ -a laminin receptor- and α₅β₁ -a fibronectin receptor- are associated to mesangial expansion, mesangial cell activation, TGF-β overexpression and proteinuria in IgA nephropathy (33). High glucose levels alter integrin expression of human mesangial cells in diabetic nephropathy, in which an increase of TGF-β plays a role in matrix accumulation (10). Moreover, CTGF, a downstream mediator of TGF-β, modulates the expression of αᵥβ₃ and α₁ integrins in cultured human mesangial cells (34), and its role in diabetic nephropathy has been demonstrated.

In glomerular injury, which is accompanied by release of inflammatory mediators such as cytokines, growth factors, proteases, and oxygen radicals, it might be expected that changes in matrix, in matrix integrin receptors or in integrin-linked signalling would lead to altered cell-matrix interaction. In addition, progressive glomerulosclerosis is accompanied by tubulointerstitial expansion and accumulation of extracellular matrix. Interstitial fibrosis is associated with increased expression of α₁β₁, α₂β₁, α₅β₁ and αᵥβ₃ integrins in interstitial fibroblasts (35).

Renal cell-matrix interactions as target in glomerulonephritis

The appropriate interaction between glomerular cells and ECM components is essential for the maintenance of normal glomerular structure and function. Disturbance of normal cell-ECM interactions influences glomerular pathology in GN and determines whether glomerular injury will
progress or subside. The critical molecules controlling the glomerular cells-ECM interactions are predominantly $\beta_1$ integrins. $\beta_1$ integrins are adhesion receptors for ECM components that induce signals for migration, proliferation, survival and ECM assembly-mediating signalling cascade in association with other receptors for soluble mediator molecules. Ang II is a potent vasoconstricting peptide which stimulates the synthesis of TGF-$\beta$ and PDGF, both of which upregulate active $\beta_1$ integrins in mesangial cells leading to fibronectin formation, collagen matrix reorganization, and development of glomerular scarring. Interventions to prevent the effects of Ang II have been developed with the use of angiotensin-converting enzyme inhibitors and Ang II receptor antagonists in both experimental and human kidney disease (36-38).

Analysis of $\beta_1$ integrins-mediated signalling regulation in the pathogenetic mechanisms of glomerulosclerosis is an important issue for future research. This type of studies may lead to the development of new therapeutic strategies for chronic progressive GN for which there is still no conclusive treatment.

**Leukocyte-renal cell interactions**

Acute and chronic inflammation of the kidney is often characterized by leukocyte infiltration. Antibody-antigen interactions and local generation of chemoattractants and cytokines initiate this process. Leukocytes migrate toward the glomerulus, where they produce additional chemoattractants and cytokines and release lysosomal enzymes and oxygen free radicals leading to glomerular injury. Leukocyte adhesion is a central event in leukocyte recruitment to inflamed glomeruli. Glomerular polymorphonuclear (PMN) leukocytes and macrophages, which are responsible for proteinuria, contribute to thrombotic and necrotizing lesions leading to glomerulosclerosis, loss of glomeruli and renal failure (Fig. 3). Interstitial leukocyte infiltration composed of macrophages and mainly T lymphocytes result in interstitial fibrosis and chronic renal failure.

Renal entrapment and migration of leukocytes within the glomerulus and interstitium of the kidney is mediated by interactions between adhesion molecules expressed on these cells and their ligands on renal cells. Selectins and carbohydrates mediate the initial interaction; while proteins of the Ig-like superfamily on activated endothelium and leukocyte integrins mediate the firm adhesion (Fig. 4). Finally, extravasation of leukocytes into the surrounding tissue takes place.

$\beta_2$ integrins -so called leukocyte integrins because of their exclusive expression on leukocytes- are adhesion receptors mediating the leukocyte-endothelial cell interactions. Leukocytes express four $\beta_2$ integrins according to different $\alpha$ chains. The latter have longer extracellular regions containing a highly conserved interactive domain which is key for adhesion in cell-cell
integrins and glomerulonephritis (Fig. 1). In addition, $\alpha_4\beta_1$ integrin also contributes to leukocyte-endothelial cell interactions.

**Figure 3.** A renal biopsy showing rapidly progressive glomerulonephritis. Macrophages (CD14) were identified among crescents by immunohistochemistry using avidin-biotin-peroxidase (Amplification x250).

**Figure 4.** Sequential steps and crucial integrin/Ig-like interactions for renal leukocyte infiltration. CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) are major factors in neutrophils migration into inflamed glomeruli. LFA-1 is also primordial in trafficking lymphocytes. CD49d/CD29 (VLA-4) and gp 150,95 are involved in monocytes-macrophages transmigration.
Integrin distribution in leukocytes

All leukocytes -granulocytes, monocytes and lymphocytes- constitutively express CD11a/CD18 or LFA-1. CD11b/CD18 or Mac-1 and CD11c/CD18 are expressed in granulocytes and monocytes but only in few lymphocytes. CD11d/CD18 is moderately expressed on some circulating leukocytes and more strongly on macrophages and dendritic cells. The ligands for leukocyte β2 integrins are the Ig-like intercellular adhesion molecules (Table 2). Other ligands for CD11b/CD18 include C3b1, fibrinogen, and factor X.

Table 2. Leukocyte integrins with function predominantly in leukocyte-renal cell interactions.

<table>
<thead>
<tr>
<th>Integrins</th>
<th>Molecule</th>
<th>Distribution</th>
<th>Ligand Immunoglobulin</th>
<th>Target Renal Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1 Integrins</td>
<td>VLA-4</td>
<td>Lymphocytes</td>
<td>VCAM-1 (CD106)</td>
<td>Endothelial</td>
</tr>
<tr>
<td>α4β1 (CD49d/CD29)</td>
<td></td>
<td>Monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2 Integrins</td>
<td>LFA-1</td>
<td>Leukocytes</td>
<td>ICAM-1 (CD54), ICAM-2 (CD102)</td>
<td>Endothelial</td>
</tr>
<tr>
<td>α4β1 (CD11a/CD18)</td>
<td></td>
<td></td>
<td>ICAM-3 (CD50)</td>
<td></td>
</tr>
<tr>
<td>α4β2 (CD11b/CD18)</td>
<td>Mac-1</td>
<td>Neutrophils</td>
<td>ICAM-1 (CD54)</td>
<td>Mesangial</td>
</tr>
<tr>
<td>α5β2 (CD11c/CD18)</td>
<td>gp150,95</td>
<td>Monocytes</td>
<td>ICAM-1 (CD54)</td>
<td>Epithelial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ICAM-3 (CD50)</td>
<td>Vascular smooth muscle</td>
</tr>
<tr>
<td>β1 Integrins</td>
<td>Neutrophils</td>
<td>PECAM-1 (CD31)</td>
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<td>Endothelial</td>
</tr>
<tr>
<td>α4β1 (CD51/CD61)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations are: VLA = very late activation. LFA = leukocyte function associated. Mac = macrophage (complement receptor C3b). gp 150,95 = complement receptor 4. VCAM = vascular cell adhesion molecule. ICAM = intercellular adhesion molecule. PECAM = platelet-endothelial cell adhesion.

Functions of cell receptors in the kidney

Leukocyte recruitment is a coordinated multistep process requiring activation of integrin adhesiveness within the vasculature by chemoattractant factors. Rolling of leukocytes on endothelium, immobilization on endothelium or firm adhesion, and migration across the endothelium into extravascular tissue follows chemotaxis.

When neutrophils are encouraged to roll and stick to endothelial cells by the selectins, they come into contact with endothelial cell-derived chemoactivators such as platelet-activating factor (PAF) or IL-8, or other chemokines processed on the mesangium such as RANTES (regulated upon activation, normal T cell expressed and secreted) and eicosanoid lipids or C3b1 (23). Activated neutrophils rapidly become polarized and securely attached to endothelial cells via β2 integrins. CD11a/CD18 is upregulated by gene transcription. In addition, CD11b/CD18 and CD11c/CD18 are present on
subcellular granules that are rapidly fused with the plasma membrane, allowing receptor translocation upon activation. Several factors provoke leukocyte adhesion to glomerular endothelial cells. These include the complement component C5a, leukotriene B4 (LTB4), cytokines such as tumor necrosis factor-α (TNF-α), chemokines such as monocyte chemoattractant protein-1 (MCP-1), and antineutrophil cytoplasmic antibodies (ANCA) (39).

The major ligands of β2 integrins are intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), members of the Ig-like superfamily. ICAM-1 antigens are mainly expressed on monocytes and lymphocytes. VCAM-1 has a more restricted distribution than ICAM-1, being expressed on macrophages and dendritic cells. Both ICAM-1 and VCAM-1 are strongly expressed by activated lymphocytes and macrophages. In the normal human kidney, ICAM-1 expression is present on peritubular and glomerular capillaries (Fig. 5), the endothelium of arterial vessels and veins, and occasionally on interstitial cells. VCAM-1 is expressed on the epithelium of the Bowman’s capsule and occasionally on tubular epithelium (Fig. 6).

Figure 5. Left panel: ICAM-1 (CD54) antigens identified in endothelial cells of glomerular and peritubular capillaries (arrows) in normal renal tissue. Tubular epithelial cells are negative for this adhesion molecule. Right panel: ICAM-1 (CD54) antigens identified in endothelium and crescent. Tubular epithelium is positive for this adhesion molecule in a renal biopsy showing rapidly progressive glomerulonephritis. This adhesion molecule was immunochemically detected by using avidin-biotin-peroxidase (Amplifications x250).
Figure 6. Left panel: VCAM-1 (CD106) antigens identified in epithelial cells of the Bowman’s capsule in normal renal tissue. Endothelial cells were negative for this molecule. Right panel: VCAM-1 (CD106) antigens identified in endothelium and crescent. Tubular epithelium was positive for this adhesion molecule in a renal biopsy showing rapidly progressive glomerulonephritis. This adhesion molecule was immunochemically detected by using avidin-biotin-peroxidase (Amplification x250).

Whereas early adhesive events in the inflammatory cascade are supported through preformed molecules, de novo biosynthesis of adhesion molecules induced by cytokines amplifies and sustains leukocyte recruitment (23). Thus, macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulate adhesion molecule biosynthesis by leukocyte precursors. With respect to endothelial cells and other renal parenchymal cells, several proinflammatory cytokines such as IL-1, TNF-α and interferon-γ induce the expression of ICAM-1 and VCAM-1 on endothelial and epithelial cells. VCAM-1 is also induced by IL-4.

Role of leukocyte-renal cell interactions in experimental glomerulonephritis

The role of leukocyte integrins and their Ig-like ligands has been demonstrated in various forms of GN using experimental models (Table 3). Among the inflammatory models, experimental anti-GBM nephritis -the counterpart of human anti-GBM or type I RPGN- has been well studied. The autoimmune anti-GBM nephritis is produced by active immunization of experimental animals with purified GBM. This is followed by the release of circulating antibodies directed to the α3 chain of type IV collagen -Goodpasture’s antigen-, which is localized as linear deposits along the glomerular capillary wall. Heterologous phase proteinuria develops within the
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Table 3. Major integrins in experimental and human glomerulonephritis (GN).

<table>
<thead>
<tr>
<th>Integrins</th>
<th>Experimental GN</th>
<th>Human GN</th>
<th>Biological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1(CD29)</td>
<td>Paromycin nephrosis</td>
<td>Minimal change glomerulopathy</td>
<td>Podocyte detachment from the GBM</td>
</tr>
<tr>
<td>VLA-3</td>
<td>Passive Heymann nephritis</td>
<td>Membranous nephropathy</td>
<td></td>
</tr>
<tr>
<td>VLA-4</td>
<td>Nephrotoxic nephritis</td>
<td>Anti-GBM GN</td>
<td>Crescent formation</td>
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<td></td>
<td>Lupus nephritis</td>
<td>Immune complex GN (Lupus, Cryoglobulinemia)</td>
<td>Glomerular macrophage infiltration</td>
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<td>VLA-5</td>
<td>Membranoproliferative GN IgA nephropathy</td>
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<td>Glomerulosclerosis</td>
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<td>β2 (CD18)</td>
<td>Nephrotoxic nephritis</td>
<td>Anti-GBM GN</td>
<td>Crescent formation</td>
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<td>LFA-1</td>
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<td>Proliferative GN (lupus, cryoglobulinemia, IgA)</td>
<td>Autologous phase proteinuria</td>
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<td>Glomerular hypercellularity</td>
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<td>Interstitial leukocyte infiltration</td>
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<td>Mac-1</td>
<td>Nephrotoxic nephritis</td>
<td>Anti-GBM GN</td>
<td>Glomerular influx of neutrophils</td>
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<td>Heterologous phase proteinuria</td>
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<tr>
<td>β3 (CD61)</td>
<td>Nephrotoxic nephritis</td>
<td>Anti-GBM GN</td>
<td>Glomerular influx of neutrophils</td>
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References: 24, 27, 28, 40-48, 29, 30, 33, 49-53, 64, 46, 48, 61-62

Abbreviations are: VLA = very late activation. LFA = leukocyte function associated. Mac = macrophage (complement receptor C3b). GBM = glomerular basement membrane.

first 24 hours, depending on the antibody and complement provoking recruitment of PMNs into the glomerular tuft, and resolves after 48 to 72 hours. Autologous phase proteinuria develops subsequently, in association with a host immune response to the heterologous antiserum, and this is characterized by recruitment of T cells to glomerular tuft and crescent formation due to infiltration of the Bowman's space with monocytes-macrophages. The increased ICAM-1 expression in glomerular endothelial cells and LFA-1 detection within glomerular cellularity two weeks after rat immunization with bovine GBM demonstrate the interaction of leukocytes with glomerular endothelium. The administration of antibodies to ICAM-1 or the α chain of LFA-1 reduces crescent formation, glomerulosclerosis, tubulointerstitial injury and the severity of renal failure (40). Anti-ICAM-1 and anti-LFA-1 antibodies do not alter either circulating leukocyte counts or titers of anti-GBM antibodies in the circulation.

The autoimmune anti-GBM nephritis model more widely used is induced by an intravenous injection of heterologous anti-GBM antiserum, known as passive nephrotoxic nephritis. Following administration of this antibody, glomerular ICAM-1 and VCAM-1 are upregulated in control rats; while animals treated with antibodies to ICAM-1, β2 integrin subunit, αM subunit of Mac-1, or α4 subunit of VLA-4 exhibit a significant reduction in glomerular
neutrophil infiltration and proteinuria. Rats receiving anti-GBM antiserum together with antibodies to ICAM-1 or to the $\alpha_2$ subunit of LFA-1 show dose-dependent reductions in urine protein excretion, associated with lower glomerular hypercellularity and crescent formation (41).

The role of humoral mediators in $\beta_2$ integrin interactions has been studied in nephrotoxic nephritis. TNF-$\alpha$ augments the pro-inflammatory action of PMNs via $\beta_2$ integrins (42). In contrast, the administration of a TNF-$\alpha$ antibody abolishes both neutrophil infiltration and proteinuria (40). In an accelerated model of nephrotoxic nephritis, produced by preimmunizing rats with rabbit IgG, followed by administration of rabbit anti-GBM antiserum, treatment with IL-1 receptor antagonist before nephritis induction results in a partial reduction in glomerular ICAM-1 and leukocyte infiltration (43).

An additional model for the study of GN has been the use of murine lupus models representing the counterpart of human immune complex-mediated GN. Murine lupus nephritis is characterized by deposition or formation of immune complexes within the glomerulus, complement activation, and glomerular inflammatory infiltration composed of PMNs and monocyte-macrophages, mediated by $\beta_2$ integrins/ICAM-1 interactions. ICAM-1 overexpression in both endothelium and mesangium occurs in autoimmune nephritis MRL-1pr mice, in accordance with increased TNF-$\alpha$ and IL-1$\beta$ (44). Accelerated lupus nephritis induced in ICAM-1 knockout mice -ICAM/Faslpr- has a marked increase in survival compared with that in control Faslpr mice (45). ICAM-1 deficiency results in moderation of glomerular disease, less vasculitis and considerably less end-stage global glomerulosclerosis via LFA-1 interaction (46,47). In murine lupus nephritis, VCAM-1 appears abnormally overexpressed in the glomerular endothelium, contributing to glomerular infiltration of monocyte-macrophages via VLA-4 interaction (48). Moreover, $\beta_3$ integrin also influences monocyte transendothelial migration, stimulating the LFA-1/ICAM-1 interactions.

Role of leukocyte-renal cell interactions in human glomerulonephritis

Immunohistochemical studies have identified different histologic patterns of leukocyte integrins and their Ig-like ligands in human GN (Fig. 7). The renovascular expression of ICAM-1 is unchanged in minimal change glomerulopathy, membranous nephropathy and focal segmental glomerulosclerosis, not usually accompanied by leukocyte infiltration, with respect to that observed in normal kidney. In general, proliferative GN characterized by leukocyte glomerular infiltration presents upregulation of ICAM-1 on the endothelium and mesangium. Glomerular expression of
ICAM-1 is particularly enhanced in crescentic GN (Fig. 5), lupus nephritis and renal vasculitis because of the severe endothelial injury and immune activation. VCAM-1 is undetected on endothelial cells from normal human kidney but appears de novo in crescentic GN (Fig. 6). A relationship between VCAM-1 expression in glomerular endothelium and pauci-immune GN has been reported, particularly in necrotizing lesions as a consequence of the inflammatory context (49). VCAM-1 is expressed in mesangial cells in GN with crescent formation, composed of monocyte-macrophages, which would reflect VLA-4/VCAM-1 interactions. ICAM-1 and VCAM-1 are well identified among the cellular crescents, due predominantly to macrophage accumulation in crescentic GN (50,51), and their expression declines when the crescents become fibrous (52,53).

In both proliferative (as described above) and mesangioproliferative types of GN, IgA nephropathy and Henoch-Schönlein purpura, ICAM-1 is abnormally expressed on the luminal surface of proximal tubule, distal convoluted tubule and collecting duct cells. Meanwhile, VCAM-1 is upregulated on proximal tubule cells. There are several mechanisms by which ICAM-1 and VCAM-1 may be induced in the tubular epithelium in GN. First, glomerular high-grade proteinuria itself may cause tubular epithelial cell injury in experimental GN models (54). Second, the association between the glomerular hypercellularity and the tubular expression of ICAM-1 observed in proliferative GN (55), and the association between normal glomeruli and the lack of tubular stain for ICAM-1 observed in non GN (56), suggest that glomerular inflammatory cytokines may reach the tubulointerstitium via blood, urine or diffusion through extravascular tissue, which is a stimulus for the
induction of adhesion molecules on tubular epithelium. Third, acute GN and chronic glomerulosclerosis may lead to downstream ischemia and hypoxia in the tubulointerstitium, both of which are potent inducers of adhesion molecules. In support of this hypothesis, a tubule epithelium expressing ICAM-1 is associated with the tubular atrophy in IgA nephropathy (57).

Fourth, the upregulation of ICAM-1 and VCAM-1 in the tubular epithelium is associated with interstitial cellular infiltration (58). In fact, tubular ICAM-1 and CD18 (the $\beta_2$ chain of LFA-1) expression is related to VCAM-1 and CD49d (the $\alpha_4$ chain of VLA-4) positivity in interstitial leukocytes (58-60). These observations support the existence of leukocyte-epithelial interactions at tubulointerstitial level in human GN.

**Leukocyte-renal cell interactions as a putative target in glomerulonephritis**

Insights into the knowledge of adhesion molecules have led to different strategies targeting inflammatory events in GN. Monoclonal antibodies to block leukocyte integrins and Ig-like interactions in the renal endothelium have been used in experimental models of crescentic GN. Different results have been obtained depending on the characteristics of the model, the species, and the timing of the intervention. Thus, anti-CD18, anti-CD11b and anti-ICAM-1 antibodies, but not anti-CD11a, markedly reduce neutrophil infiltration and proteinuria in Long-Evans rats with nephrotoxic serum nephritis, an experimental model of acute immune complex GN, but not in this model in rabbits (23). In contrast, anti-CD11a and anti-ICAM-1 antibodies (61), as well as anti-VLA-4 antibodies (62), abrogate crescent formation in nephrotoxic nephritis in Wistar-Kyoto rats, a model of anti-GBM GN, and in an experimental model of murine lupus nephritis (48).

In human GN, monoclonal antibodies against CD18 have been used in vasculitis (63), but they are of limited use in clinical practice because of their general antigenicity. Other interventions point to inhibition of cytokine bioactivity upregulating adhesion molecules. This approach has yielded promising results by the use of either molecules interfering with TNF-α biosynthesis (64) or blocking soluble TNF-α receptors (65) as treatments in proliferative crescentic GN. The dissection of the molecular events in leukocyte-endothelial cell adhesion may lead to the development of more selective inhibition of inflammatory events, avoiding the immunologic and nonimmunologic toxicity of current therapies and thus improving GN treatment.

**References**