Leukocyte adhesion molecules and kidney diseases

Phagocytic leukocytes, particularly neutrophils and monocytes, are important effectors of injury in many forms of glomerulonephritis, vasculitis, tubulointerstitial nephritis and allograft rejection [1–4]. Current treatment strategies for these diseases lack immunologic specificity and are frequently toxic, reflecting our relatively naive understanding of the pathophysiology of renal inflammation. Recent advances in immunopathology research have identified leukocyte adhesion as a pivotal event in inflammation that may be amenable to therapeutic intervention. Adhesion is mediated by interaction of cell surface adhesion molecules on leukocytes with cognate ligands on resident tissue cells. The leukocyte adhesion molecules are members of larger superfamilies of cell surface receptors that play critical roles in immunosurveillance, inflammation, hemostasis, wound healing, morphogenesis, maintenance of tissue architecture, atherogenesis, and tumor metastasis. This contribution (a) reviews the classification, biochemical structures and cognate ligands of the major leukocyte adhesion molecules [5–21], (b) discusses the mechanisms by which inflammatory mediators regulate leukocyte adhesion [5–14, 22–38], (c) summarizes the distribution of leukocyte adhesion molecules in normal and diseased kidneys [12–14, 39–72], (d) highlights the dynamic interplay that occurs between leukocytes and resident tissue cells during cell-cell adhesion [73–80], and (e) reviews recent studies evaluating the efficacy of monoclonal antibodies (mAb) against leukocyte adhesion molecules in the treatment of renal inflammation [81–91]. These exciting advances may antecede the development of potent and specific new agents for the treatment of inflammatory diseases of the kidney.

Overview of recruitment of phagocytic leukocytes to sites of inflammation

It is useful to review briefly normal phagocyte physiology before discussing the role of these cells in the pathogenesis of “autoimmune” renal disease. Phagocytes play a pivotal role in the initial defense against bacterial and fungal infections. The recruitment of circulating phagocytes to sites of infection involves a series of coordinated steps which include directed locomotion up concentration gradients of chemoattractants (chemotaxis), adhesion to vascular endothelial cells in the area of inflammation (margination), penetration of tight junctions, and migration between endothelial cells (diapedesis) and through basement membrane and extravascular tissue to the inflammatory focus. Phagocytes ingest (phagocytosis) and destroy foreign antigens through the actions of a variety of toxic molecules, including reactive oxygen species and proteolytic enzymes. This orchestrated response ultimately facilitates the destruction of antigens within phagocytes with relative preservation of surrounding host tissue.

The dramatic functional abnormalities that characterize acute glomerulonephritis underscore the devastating consequences of inappropriate activation of this cascade in autoimmune renal diseases. The initiating pathophysiologic event in acute glomerulonephritis is usually the intraglomerular deposition of immunoglobulin due to either trapping of circulating immune complexes or interaction of circulating immunoglobulins with resident or “planted” glomerular antigens [reviewed in 1, 2]. Immunoglobulin deposition triggers the elaboration of an array of chemoattractants by several mechanisms (Fig. 1). Many immunoglobulins fix complement and generate C5a (anaphylotoxin), a potent chemotactic peptide. Interaction of Fc regions of immunoglobulins with Fc receptors on resident and infiltrating phagocytes stimulates the latter to release chemoattractants such as interleukin-8 (IL-8), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic peptide-1 (MCP-1), tumor necrosis factor (TNF), and leukotriene B4 (LTB4). Mesangial cells also express Fc receptors and generate MCP-1 and probably other chemotactic agents by a similar mechanism [3, 4]. Most chemoattractants, in addition to stimulating chemotaxis, also provoke phagocyte adhesion to endothelial cells and diapedesis to the extravascular space. Phagocytosis is probably less efficient in autoimmune diseases because the inciting antigens are often either fixed to, or form an intrinsic part of the host tissue. As a result, reactive oxygen species, proteolytic enzymes and other cytotoxic molecules are released to the extracellular space where they disrupt renal architecture and impair function (so-called “frustrated phagocytosis”).

The critical role of adhesion in normal phagocyte trafficking was originally demonstrated in dramatic fashion in children with congenital deficiency of the CD11/CD18 family of leukocyte adhesion molecules (leukocyte adhesion deficiency (LAD) type 1) [reviewed in 7]. These unfortunate children suffer recurrent life-threatening bacterial infections characterized by systemic neutrophilia and absence of pus formation in infected tissue due to failure of neutrophils to migrate from blood to the extravascular space. Since this pioneering discovery, several other families of adhesion molecules have been characterized on leukocytes or parenchymal cells which regulate leukocyte trafficking in health and disease [reviewed in 5–14].

Classification, structural characteristics, cellular distribution, and cognate ligands of major leukocyte adhesion molecules

Recent evidence from several laboratories, derived principally from in vitro studies, suggests that phagocyte adhesion is mediated, in large part, by the coordinated interactions of four classes of adhesion molecules: selectins, carbohydrate-containing selectin ligands, integrins, and immunoglobulin-like (Ig-like)
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Fig. 1. Overview of the recruitment of phagocytic leukocytes during glomerular inflammation. Abbreviations are: TNF, tumor necrosis factor; PAF, platelet activating factor; IL-8, interleukin-8; LTB4, leukotriene B4; MCP-1, monocyte chemotactic peptide-1; C5a, fifth component of complement (anaphylotoxin).

molecules (Fig. 2) [reviewed in 5-14]. The initial attachment of phagocytes to endothelium appears to involve interaction of phagocyte or endothelial cell selectins with cognate carbohydrate-containing ligands [5, 10, 11]. Selectin-mediated adhesion is relatively resistant to shear stress, but insufficient to immobilize phagocytes on endothelium [5, 10, 11]. Rather, it causes them to roll on endothelium, where they are subject to local activation signals from endothelium and extravascular tissue (vide infra). These events facilitate activation of phagocyte integrins, immobilization of phagocytes by interaction of integrins with Ig-like molecules on endothelium, and migration to the interstitium [5-9, 12-14].

Selectins

Three selectins have been characterized and named, by agreement of most major investigators in this field [15], according to their cell of original discovery: P-selectin (platelet selectin), E-selectin (endothelial cell selectin), and L-selectin (leukocyte selectin) (Table 1). Selectins share several structural features: an N-terminal C-type lectin domain, an epidermal growth factor-like domain, several consensus repeats that share homology with complement regulatory proteins, a single transmembrane domain and a short intracellular C-terminal domain (Fig. 3) [5, 9-11]. The genes encoding P-, E-, and L-selectin have been localized to the long arm of chromosome 1 and lie in close proximity to the genes encoding complement binding proteins [10, 11]. The lectin domains of selectins share >60% homology. Selectins contain between 7 to 12 potential sites for N-linked glycosylation and it is estimated that glycosylation accounts for >30% of their mass. The biochemical structure and functional significance of these carbohydrate residues has not been elucidated. Selectins support leukocyte-endothelial cell and leukocyte-platelet adhesion (Table 1). L-selectin is constitutively expressed by most leukocytes, but not by other cell-types [5, 10, 11]. Endothelial cells of high endothelial venules of lymph nodes constitutively express ligands for L-selectin (lymphocyte homing receptors), and L-selectin plays a central role in normal recirculation of lymphocytes. Endothelial cells from other vascular beds [10, 11], including glomerular capillary endothelial cells [16], also support L-selectin-mediated adhesion after exposure to cytokines. P-selectin is expressed by endothelial cells and platelets (Table 1) [5, 10, 11]. Most P-selectin is stored in intracellular granules and mobilized to the surface upon cell activation where it supports adhesion of granulocytes, monocytes and some lymphocyte subsets. E-selectin is expressed, probably exclusively, by cytokine-activated
endothelial cells and supports adhesion of granulocytes, monocytes, some memory T-lymphocytes, and natural killer cells (Table 1) [5, 10, 11].

Carbohydrate-containing selectin ligands

Selectins bind at least three broad categories of natural or synthetic carbohydrates through their NH$_2$-lectin domain: (1) sulphated polysaccharides such as GlyCAMs (mucins); (2) oligosaccharides such as sialyl Lewis$^a$ (sLe$^a$); and (3) soluble phosphorylated mono- and polysaccharides (Table 1) [5, 10, 11]. The roles of sLe$^a$ and GlyCAM-1 as ligands for selectins have received most attention. Sialyl Lewis$^a$ is a sialylated, fucosylated cell surface tetrasaccharide (Fig. 3) that can be presented by a variety of proteins and appears to be an important ligand for both E-selectin and P-selectin. A recent report indicates that congenital deficiency of sLe$^a$, as with deficiency of CD11/CD18 integrins, is associated with defective phagocyte trafficking and impaired host defence against infection (LAD type 2) [17]. The endothelial cell ligands for L-selectin are less well-defined and have been the subject of heated debate [5, 10, 11, 18, 19]. L-selectin may present sLe$^a$ to P-selectin and E-selectin under certain experimental conditions [18]; however, this interaction probably plays a relatively minor role in L-selectin-mediated adhesion. Recent studies have identified a novel sulphated, fucosylated, and sialylated 50-kD O-glycosylated mucin GlyCAM-1 as a ligand for L-selectin on high endothelial venules of lymph nodes [19]. This ligand may regulate normal recirculation of lymphocytes through lymph nodes in vivo. The ligands which support L-selectin-mediated phagocyte adhesion to cytokine-activated endothelial cells from other vascular beds have not been characterized fully. Functional studies suggest that the glomerular ligand is a glycosylated, sialated molecule and thus shares some biochemical characteristics with those expressed by lymph nodes and large vessel endothelial cells [16].

Integrins

As discussed above, selectin-mediated adhesion facilitates the immobilization of phagocytes by interaction of phagocyte integrins with immunoglobulin-like molecules on endothelium (Fig. 2). Integrins are heterodimeric glycoproteins composed of non-covalently associated $\alpha$ and $\beta$ subunits (Fig. 3) [5–7]. Integrins mediate diverse cell-cell and cell-matrix interactions and are classified according to the structure of their $\beta$ subunits. In general, members of each class share a common $\beta$-subunit and are distinguished by their unique $\alpha$-subunits, although there are some exceptions. The integrins which appear most important in leukocyte-endothelial adhesion are the very late activation antigen-4 $\beta_1$ integrin (VLA-4) and the CD11/CD18 $\beta_2$ integrins (Fig. 3).

VLA-4 is constitutively expressed by lymphocytes, monocytes, basophils, and eosinophils, but not neutrophils (Table 1).
VLA-4 is a ligand for the inducible Ig-like vascular cell adhesion molecule-1 (VCAM-1) and also mediates cell attachment to fibronectin and possibly other matrix components. Three β2 integrins have been characterized [reviewed in 5—7]. Since these molecules are known by several different pseudonyms (Table 1), most authorities advocate the use of cluster designation (CD) nomenclature for purposes of uniformity. Accordingly, these molecules should be referred to as CD11a/CD18, CD11b/CD18 and CD11c/CD18; CD11d and CD18 being the α and β subunits, respectively (Fig. 3). CD18 is encoded by a single gene on chromosome 21 and is a 678 amino acid protein comprised of a short cytoplasmic tail which contains several potential phosphorylation sites, a highly conserved transmembrane domain, and a longer extracellular region which contains a conserved cysteine-rich region that is necessary for surface expression (Fig. 3). CD11a, CD11b, and CD11c are comprised of 1063, 1136, and 1144 amino acids, respectively, and are encoded by distinct genes in a cluster on chromosome 16. Notable structural features on CD11 subunits include short nonhomologous cytoplasmic regions containing potential phosphorylation sites, highly conserved transmembrane regions, and a longer extracellular region which contains several cation-binding repeats, and an "I" (interactive) domain which is relatively highly conserved across integrin subfamilies and may be an important adhesion domain (Fig. 3). CD11a shares 36% homology with CD11b and CD11c and is constitutively expressed by granulocytes, monocytes and lymphocytes (Table 1). In contrast, CD11b and CD11c share 63% homology and are expressed by granulocytes and monocytes, but not lymphocytes (Table 1). The major ligands for CD11a/CD18 are the Ig-like intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2). ICAM-1 is also a ligand for CD11b/CD18; however, the latter also supports phagocyte adhesion to cellular and acellular substrates by ICAM-1 independent mechanisms (Table 1). Other ligands for CD11b/CD18 include fibrinogen, clotting factors and complement fragments (C3b). The biochemical structure of the cognate ligand(s) for CD11c/CD18 and precise role of this molecule in leukocyte migration has not been defined.

Ig-like molecules

ICAM-1, ICAM-2 and VCAM-1 are members of the immunoglobulin superfamily (Fig. 3) [5, 8, 9, 13]. Other members include immunoglobulin itself, the T-cell receptor/CD3 complex, CD4, CD8, and class I and class II MHC molecules. These molecules contain one or more Ig-like regions, each consisting of a disulphide-bridged loop containing antiparallel β-pleated strands arranged into two sheets, a transmembrane domain and a short cytoplasmic tail. ICAM-1 is a glycoprotein of molecular weight 76–114 kD which contains five tandem immunoglobulin domains and, as discussed above, is a ligand for CD11a/CD18 and CD11b/CD18, but not CD11c/CD18. ICAM-1 is constitutively expressed by endothelial cells, and its expression can be induced on other cell types such as mesangial cells and epithelial cells by cytokines (vide infra). ICAM-1 is also expressed by lymphocytes and some other leukocytes. Indeed, homotypic and heterotypic lymphocyte adhesion mediated by interaction...
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Table 1. Classification, cellular distribution, ligands and target cells for major leukocyte adhesion molecules

<table>
<thead>
<tr>
<th>Major families</th>
<th>Individual members</th>
<th>Alternate nomenclature</th>
<th>Cellular distribution</th>
<th>Ligands on target cells</th>
<th>Target cells</th>
</tr>
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<tbody>
<tr>
<td>Selectins</td>
<td>L-selectin</td>
<td>LAM-1, LECCAM-1, MEL-14, LEU-8, TQ1 DREG.56</td>
<td>Leukocytes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Glycoproteins and glycolipids (sLe&lt;sup&gt;x&lt;/sup&gt;, sLe&lt;sup&gt;y&lt;/sup&gt;, GlyCAM-1, fucoidin, ?MadCAM-1, ?E- &amp; P-selectin)</td>
<td>Endothelial cells, ?other</td>
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<td></td>
<td>P-selectin</td>
<td>CD62, GMP140, PADGEM, LECCAM-3</td>
<td>Platelets&lt;sup&gt;a&lt;/sup&gt; and endothelial&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Glycoproteins and glycolipids (Le&lt;sup&gt;a&lt;/sup&gt;, sLe&lt;sup&gt;a&lt;/sup&gt;, sLe&lt;sup&gt;y&lt;/sup&gt;, fucoidin)</td>
<td>Granulocytes, monocytes T-cell subsets, some cancer cells</td>
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<td></td>
<td>E-selectin</td>
<td>ELAM-1, LECCAM-2</td>
<td>Endothelial&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Glycoproteins and glycolipids (sLe&lt;sup&gt;a&lt;/sup&gt;, sLe&lt;sup&gt;y&lt;/sup&gt;, ?L-selectin)</td>
<td>Neutrophils, monocytes, lymphocyte subsets, some cancer cells</td>
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<td></td>
<td>Carbohydrate ligands for selectins&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Sgp50, mucin-like</td>
<td>Endothelium</td>
<td>L-selectin</td>
<td>Lympocytes, ?other</td>
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<tr>
<td></td>
<td>Sulphated polysaccharides (GlyCAM-1)</td>
<td>Sgp50, mucin-like</td>
<td>Endothelium</td>
<td>L-selectin</td>
<td>Lympocytes, ?other</td>
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<td></td>
<td>Oligosaccharides (sialyl Lewis&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>sLe&lt;sup&gt;y&lt;/sup&gt;</td>
<td>Granulocytes, monocytes, lymphocytes</td>
<td>E-selectin and P-selectin</td>
<td>Endothelium and platelets</td>
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<tr>
<td>Integrins</td>
<td>VLA-4 (β-1)</td>
<td>LPAM-2</td>
<td>Monocytes&lt;sup&gt;a&lt;/sup&gt;, lymphocytes&lt;sup&gt;a&lt;/sup&gt;, eosinophils&lt;sup&gt;a&lt;/sup&gt;</td>
<td>VCAM-1, Fibronectin</td>
<td>Endothelium, epithelial, mesangial, vascular smooth muscle</td>
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<td>CD11a/CD18 (β-2)</td>
<td>LFA-1, TA-1</td>
<td>Leukocytes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ICAM-1, ICAM-2</td>
<td>Endothelial, epithelial, mesangial, vascular smooth muscle</td>
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<td>CD11b/CD18 (β-2)</td>
<td>Mac-1, Mo1, OKM1, gp160</td>
<td>Granulocytes&lt;sup&gt;a&lt;/sup&gt;, monocytes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ICAM-1, C3bi, fibrinogen, factor x other</td>
<td>Endothelial, epithelial, mesangial, vascular smooth muscle, acellular surfaces</td>
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<td></td>
<td>CD11c/CD18 (β-2)</td>
<td>p150, 95, LeuM5</td>
<td>Granulocytes&lt;sup&gt;a&lt;/sup&gt;, monocytes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not characterized</td>
<td>Endothelial, mesangial</td>
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<td></td>
<td>ICAM-1</td>
<td>CD54</td>
<td>Endothelial&lt;sup&gt;c&lt;/sup&gt;, epithelial&lt;sup&gt;c&lt;/sup&gt;, mesangial&lt;sup&gt;b&lt;/sup&gt;, smooth muscle&lt;sup&gt;b&lt;/sup&gt;, some cancer cells</td>
<td>CD11a/CD18, CD11b/CD18</td>
<td>Most leukocytes</td>
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<td>ICAM-2</td>
<td>INCAM 110</td>
<td>Endothelial&lt;sup&gt;c&lt;/sup&gt;, epithelial&lt;sup&gt;c&lt;/sup&gt;, mesangial&lt;sup&gt;b&lt;/sup&gt;, smooth muscle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CD11a/CD18, CD11b/CD18</td>
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<td>VCAM-1</td>
<td>INCAM 110</td>
<td>Endothelial&lt;sup&gt;c&lt;/sup&gt;, epithelial&lt;sup&gt;c&lt;/sup&gt;, mesangial&lt;sup&gt;b&lt;/sup&gt;, smooth muscle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>VLA-4</td>
<td>Monocytes, lymphocytes, eosinophils</td>
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<td></td>
<td>PECAM-1</td>
<td>EndoCAM, CD31</td>
<td>Endothelial&lt;sup&gt;c&lt;/sup&gt;, platelets, some leukocytes</td>
<td>PECAM-1</td>
<td>Some leukocytes, endothelial, platelets</td>
</tr>
</tbody>
</table>

<sup>a</sup> Rapid change in avidity and/or surface expression upon stimulation of cells

<sup>b</sup> Absent or expressed at low levels unless exposed to cytokines

<sup>c</sup> Constitutive expression and further induction by cytokines

<sup>d</sup> Relative contributions of these molecules to selectin-mediated adhesion have yet to be defined

<sup>e</sup> Particularly at intercellular junctions.

of ICAM-1 and CD11a/CD18 facilitates other important lymphocyte functions including antigen recognition, lymphocyte co-stimulation and cytotoxicity.

ICAM-2 is a glycoprotein of molecular weight 60 kD and is a homolog of ICAM-1 characterized by two extracellular Ig-like domains (Fig. 3). ICAM-2, like ICAM-1, is constitutively expressed by endothelial cells, lymphocytes and some other leukocytes and appears to play a role in both leukocyte trafficking and lymphocyte activation. In contrast to ICAM-1, endothelial cell levels of ICAM-2 are not influenced by cytokines. As discussed above, ICAM-2 is also a ligand for CD11a/CD18, but not other β2 integrins (Table 1).

VCAM-1 is a 110 kD glycoprotein that contains seven Ig-like domains (Fig. 3) [5, 8, 13]. A second form of VCAM-1, consisting of six Ig-like domains, is generated in some tissues by alternate splicing [20]. The former predominates in endothelial cells and the functional significance of the latter is unclear. VCAM-1 is constitutively expressed at low levels by endothelial cells and can be induced on endothelial cells and other cell-types by cytokines (vide infra). VCAM-1 supports adhesion of eosinophils, basophils, monocytes and lymphocytes, but not neutrophils, through interaction with VLA-4. Accordingly, it has been suggested that the interaction of VLA-4 and VCAM-1 may be important in the pathophysiology of allergic and delayed hypersensitivity reactions. Recent studies suggest that another Ig-like molecule PECAM-1 is expressed preferentially at tight
different mediators at sites of inflammation. Other mediators, 
mined, at least in part, by the relative concentrations of 
and the composition of leukocyte infiltrates is likely deter-
specific (such as IL-8) or monocyte-specific (such as MCP-1) 
adhesion [5—7]. Chemoattractants may be relatively neutrophil-
expression does not appear to be a prerequisite for increased 
substrates by increasing the avidity of constitutively expressed 
CD11/CD18 integrins and/or L-selectin for cognate ligands. 
Most of these mediators also provoke rapid mobilization of 
CD1 lb/CD 18 and CD1 lc/CD1 8 from intracellular 

...junctons of endothelial cells and may be a critical regulator of 
phagocyte diapedesis between endothelial cells [21].

Regulation of adhesion by chemoattractants and cytokines

The maintenance of phagocytes and endothelium in an 
inactive "anti-adhesive" phenotype is critical in preserving the 
integrity of the microvasculature in health. In disease, inflam-
mmatory mediators regulate adhesion by altering the avidity 
and/or expression of preformed molecules for cognate ligands, 
and by influencing de novo synthesis of adhesion molecules 
(Table 2). It is probable that the initial recruitment of phago-
cytes is achieved by the former mechanism and that subsequent 
adhesive interactions are modulated through the latter.

Rapid-acting stimulators of adhesion

Chemotactants (C5α, LTB4), cytokines (MCP-1, IL-8, 
GM-CSF, TNFα), membrane associated molecules (PAF, 
E-selectin) and some anti-neutrophil cytoplasmic antibodies 
(ANCA) rapidly enhance phagocyte adhesiveness for endothelial 
cells [5—7, 23], including glomerular endothelial cells [73, 
80], mesangial cells [30, 31, 76, 77], and a variety of other 
substrates by increasing the avidity of constitutively expressed 
CD11/CD18 integrins and/or L-selectin for cognate ligands. 
Most of these mediators also provoke rapid mobilization of 
preformed CD11b/CD18 and CD11c/CD18 from intracelular 
sites to the cell surface; however, a quantitative change 
in expression does not appear to be a prerequisite for increased 
adhesion [5—7]. Chemotactants may be relatively neutrophil-
specific (such as IL-8) or monocyte-specific (such as MCP-1) 
and the composition of leukocyte infiltrates is likely deter-
mained, at least in part, by the relative concentrations of 
different mediators at sites of inflammation. Other mediators, 
such as thrombin, histamine, leukotriene C4 (LTC4), reactive 
oxygen species and the membrane attack complex of comple-
ment (MAC) increase endothelial cell adhesiveness for phago-
cytes by mobilizing preformed P-selectin from Weibel-Palade 
bodies to the surface of endothelial cells [5, 10, 
11]. L-selectin and P-selectin are shed rapidly from phagocytes 
and endothelial cells, respectively, following cell activation, a 
process that may limit adhesion and facilitate diapedesis in 
vivo. An in depth discussion of the signal transduction 
events which mediate activation of phagocytes and endothelium under 
these circumstances is beyond the scope of this review. Most 
stimuli for phagocyte activation interact with stimulus-specific 
cell surface receptors and initiate a cascade of events which 
include activation of phospholipidase C through guanine nucle-
otide regulatory proteins/tyrosine kinase activity, hydrolysis of 
phosphatidylinositol bisphosphate and generation of inositol 
trisphosphate and diacylglycerol, elevation of intracellular cal-
cium concentration, and activation of protein kinase C [re-
viewed in 5, 22]. The distal signal transduction events that 
ultimately enhance the avidity of adhesion molecules for ligands 
are unknown, but probably involve phosphorylation of adhe-
sion proteins and/or cytoskeletal elements [reviewed in 5]. The 
molecular basis for mobilization of P-selectin has been studied less 
exensively, but appears to be calcium and phosphoryla-
tion-dependent.

Induction of adhesion molecule synthesis by cytokines

Prolonged exposure of phagocytes, endothelial cells, and 
other resident tissue cells to cytokines promotes adhesion by 
stimulating synthesis of adhesion molecules (Table 2) [5, 8—14]. 
Cytokines also promote extravasation of phagocytes by indu-
cing endothelial cell synthesis of IL-8, MCP-1 and PAF, and by

<table>
<thead>
<tr>
<th>Response time</th>
<th>Sites of action</th>
<th>Mechanisms</th>
<th>Molecules</th>
<th>Stimuli</th>
<th>Inhibitors</th>
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<tbody>
<tr>
<td>Seconds-minutes</td>
<td>Leukocytes</td>
<td>Increase in avidity and/or expression of preformed adhesion molecules</td>
<td>CD11/CD18, VLA-4, L-selectin</td>
<td>C5α, LTB4, PAF, IL-8, MCP-1, TNFα, bacterial wall peptides, E-selectin, ANCA</td>
<td>15-HETE, EDRF, PGL2, epinephrine, lipoxins, IL-8*</td>
</tr>
<tr>
<td>Platelets</td>
<td>Increase in avidity and/or expression of preformed adhesion molecules</td>
<td>P-selectin</td>
<td>ADP, thrombin, histamine</td>
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<tr>
<td>Endothelial</td>
<td>Increase in avidity and/or expression of preformed adhesion molecules</td>
<td>P-selectin</td>
<td>Histamine, thrombin, H2O2, MAC, LTC4</td>
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<tr>
<td>Hours-days</td>
<td>Mesangial, epithelial, smooth muscle, and cancer cells</td>
<td>De novo synthesis of adhesion molecules</td>
<td>ICAM-1, VCAM-1, E-selectin, ligands for L-selectin, ?P-selectin</td>
<td>TNFα, IL-1β, IF-γ, IL-4, endotoxin</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are: LT, leukotriene; PAF, platelet activating factor; IL, interleukin; MCP, monocyte chemotactic peptide; TNF, tumor necrosis factor; HETE, hydroxyeicosatetraenoic acid; PGL2, prostacyclin; ADP, adenosine diphosphate; H2O2, hydrogen peroxide; MAC, membrane attack complex of complement; GM-CSF, granulocyte macrophage colony-stimulating factor; IF, interferon; TGFβ, transforming growth factor beta; ANCA, anti-neutrophil cytoplasmic antibody.

* IL-8 may promote or inhibit leukocyte-endothelial cell adhesion depending on the experimental conditions.
Regulate phagocyte recruitment during inflammation. Several other cell-types [8] suggest that this compound may also induce form of nitric oxide synthetase in endothelium and cell interaction. Indeed, the recent discovery of a cytokine-compound may be a tonic inhibitor of phagocyte-endothelial vitro [25] and in vivo [26], raising the possibility that this of platelet aggregation, also attenuates neutrophil adhesion in

cells during inflammation.

The molecular basis for this differential regulation of adhe-
sion molecule expression has not been defined. Cytokine-
induced expression of ICAM-1, VCAM-1 and E-selectin is
transcriptionally regulated and a variety of potential regulatory
sequences have been identified in the 5' flanking regions of their
genes [reviewed in 5, 8]. Most cytokines interact with specific
cell surface receptors [8]. The signals which transduce receptor
binding to activation of transcription are the subject of intensive
investigation. Putative mechanisms include activation of intra-
cellular transcriptional regulatory proteins (such as NFkB) by
protein kinases, reactive oxygen species or membrane lipids,
and direct translocation of cytokine-receptor complexes to the
nucleus [5]. The elucidation of these events may suggest
strategies for inhibition of synthesis of specific adhesion mole-
cules during inflammation.

Rapid-acting inhibitors of adhesion

Given that many inflammatory diseases are self-limiting, it is
likely that endogenous compounds exist that inhibit phagocyte
adhesion. Several candidate molecules have already been iden-
tified (Table 2). Nitric oxide, a potent vasodilator and inhibitor of platelet aggregation, also attenuates neutrophil adhesion in vitro [25] and in vivo [26], raising the possibility that this compound may be a tonic inhibitor of phagocyte-endothelial cell interaction. Indeed, the recent discovery of a cytokine-inducible form of nitric oxide synthetase in endothelium and other cell-types [8] suggests that this compound may also regulate phagocyte recruitment during inflammation. Several eicosanoids also attenuate adhesion in experimental models of inflammation. These include prostacyclin (PGI₂), lipoxin A₄ (LXA₄), and 15-hydroxyeicosatetraenoic acid (15-HETE) [27–37]. PGI₂ synthesis, like nitric oxide synthesis, is generated by endothelial cells under basal conditions and in increased quantities following activation by cytokines [8]. This compound is a potent inhibitor of neutrophil-endothelial cell adhesion, probably by increasing cyclic AMP levels in neutrophils [27]. LXA₄ is generated by phagocytes alone via the sequential actions of 15- and 5-lipoxygenases on arachidonic acid [28], and during phagocyte-platelet interaction via the sequential actions of phagocyte 5-lipoxygenase and platelet 12-lipoxygenases [29]. LXA₄ is a potent inhibitor of several leukotriene-induced responses including LTD₄-induced phagocyte-endothelial cell adhesion [30, 31], LTB₄-induced chemotaxis [33], and, under certain circum-
stances, LTB₄-induced phagocyte adhesion and migration across endothelium [34]. 15-HETE is generated by a variety of cells through the actions of 15-lipoxygenases, cyclooxygenase and epoxygenases on arachidonic acid [35], and is rapidly esterified into phagocyte phospholipid pools. Esterified 15-
HETE markedly attenuates phagocyte adhesion induced by
LTB₄ and other receptor-mediated stimuli by lowering the
affinity of phagocyte cell surface receptors for their cognate
ligands [36, 37]. These observations raise the possibility that
15-HETE and the lipoxins may have anti-inflammatory activity
in vivo, and suggest that products of the 5-lipoxygenase (LT)
and 15-lipoxygenase pathways (15-HETE, LX) may exert
counter-regulatory influences on leukocyte-endothelial cell ad-
hesion. In support of this hypothesis, relatively high concentra-
tions of LXA₄ and 15-HETE have been reported in several
human diseases [35, 38], and administration of exogenous
15-HETE attenuates injury in several models of inflammation
[35, 38].

Inhibition of adhesion molecule synthesis by cytokines

Prolonged exposure of endothelial cells to certain cytokines
may also reduce their adhesiveness for leukocytes or modulate
the actions of other cytokines [reviewed in 5]. Transforming
growth factor beta (TGFβ), for example, diminishes basal
endothelial ahesiveness for phagocytes. In addition, TGFβ
blunts the pro-adhesive effects of TNFa and IL-1β on endothe-
ilum, an action that may involve inhibition of E-selectin expres-
sion. IL-4, a potent inducer of endothelial VCAM-1 expression,
attenuates ICAM-1 and E-selectin expression by these cells.
The relevance of these findings in vivo has yet to be established.
However, it seems reasonable to conclude that the profile of adhe-
sion molecules expressed by resident tissue cells during
inflammation reflects the complex interplay of pro- and anti-
adhesive cytokines, and is determined not just by the relative
concentrations of these compounds, but also by the duration of
exposure. Further characterization of the endogenous inhibi-
tors of adhesion, and the elucidation of their mechanisms of
action may prove fruitful in defining novel approaches to the
treatment of inflammation.

Renal expression of leukocyte adhesion molecules in health and
disease

ICAM-1, VCAM-1 and E-selectin in normal kidneys

ICAM-1 is normally expressed at low levels by endothelial
cells of large vessels, glomeruli, and peritubular capillaries, and
by mesangial cells, some parietal epithelial cells of Bowman’s capsule, and occasional interstitial cells (Table 3) [39—62, 81—87]. In contrast, constitutive VCAM-1 is usually found only on parietal epithelial cells of Bowman’s capsule, and occasional endothelial cells of large vessels or peritubular capillaries [59, 60, 63, 66, 67]. E-selectin is not constitutively expressed in the kidney [14, 59, 60, 69—72].

**ICAM-1 in kidney diseases**

Striking changes in ICAM-1 expression have been reported in glomerulonephritis, tubulointerstitial inflammation, and renal allograft rejection (Table 3) [39—62, 81—87]. Increased glomerular expression of ICAM-1 is a common finding in patients with active crescentic glomerulonephritis, mesangiocapillary glomerulonephritis, IgA nephropathy, Henoch-Schönlein purpura, and proliferative grades of lupus nephritis [40—45]. *De novo* expression of ICAM-1 by proximal tubules cells, particularly at the luminal membrane, and increased expression by interstitial cells are common additional findings in these diseases [40—45]. In general, the intensity of staining correlates with disease activity. Glomerular ICAM-1 may revert towards basal levels or even be reduced in patients with advanced sclerotic disease. In contrast to these proliferative forms of glomerulonephritis, glomerular and tubulointerstitial ICAM-1 levels are usually unchanged or reduced in patients with minimal change disease [41]. Variable expression of ICAM-1 has been reported in patients with focal segmental glomerulosclerosis and membranous nephropathy [41—43]; some investigators reporting increased mesangial and/or tubulointerstitial staining [41, 43], and others reporting a focal and segmental decrease in glomerular expression [42]. The reason for these disparities is unclear, but may reflect sampling at different stages in the disease process. Soluble forms of many adhesion molecules, including ICAM-1 [49, 50], circulate normally in blood. An intriguing preliminary report suggests that circulating levels of ICAM-1 are elevated in some forms of acute glomerulonephritis [50]. It will be of interest to determine the source of circulating ICAM-1 under these circumstances and whether changes in circulating levels of adhesion molecules represent another mechanism of regulation of leukocyte adhesion during inflammation.

A different pattern of expression of ICAM-1 has been reported in renal biopsy specimens from patients with allograft rejection [52—62]. Glomerular levels of ICAM-1 are usually unchanged in this setting. In contrast, there is frequently dramatic up-regulation of ICAM-1 expression on proximal tubule cells, particularly on the luminal membrane, and on some distal tubule and collecting duct cells, and infiltrating leukocytes. Tubular staining is usually most pronounced in areas of intense leukocyte infiltration. Most investigators have found it difficult to assess changes in ICAM-1 levels on vascular endothelium in rejection due to the constitutive expression of ICAM-1 at this site. Circulating levels of ICAM-1 are also increased during acute rejection, but not cyclosporine toxicity, and this parameter has been proposed as a means of differentiating these two common diagnoses [62]. ICAM-1 levels have not been reported in other forms of tubulointerstitial inflammation in humans; however, a marked increase in proximal tubule and interstitial staining has been observed in a murine model of hereditary tubulointerstitial nephritis [86].

**VCAM-1 in kidney diseases**

There are few published studies on VCAM-1 or E-selectin expression in renal inflammation (Table 3). Seron, Cameron and Haskard assessed VCAM-1 expression in normal kidneys and
50 biopsy specimens from patients with a variety of glomerulonephritides or tubulointerstitial diseases [63]. *De novo* expression of VCAM-1 was observed on proximal tubule cells in virtually all patients with vasculitis and crescentic nephritis, lupus nephritis (class II, III, IV, and V), IgA nephropathy, and acute interstitial nephritis induced by nonsteroidal anti-inflammatory drugs. VCAM-1 levels were highest in patients with vasculitis, and there was a weak positive correlation between the level of expression and the intensity of the leukocytic infiltrate. VCAM-1 expression was not observed on vascular endothelial cells in any disease, even in the presence of a marked infiltrate. Interestingly, striking proximal expression of VCAM-1 was also observed in proximal tubules of patients with diabetic nephropathy, amyloid, gouty nephropathy, minimal change disease, and membranous nephropathy; diseases that are not usually associated with leukocytic infiltration of kidney [63]. Increased renal VCAM-1 expression has also been reported in experimental nephrotoxic serum nephritis [81] and the MRL-lpr murine model of lupus nephritis [64]. The latter model is characterized by dramatic induction of VCAM-1 expression in cortical, but not medullary tubule epithelial cells. In addition, there was a marked increase in VCAM-1 expression on vascular endothelium and in the glomerular mesangium. VCAM-1 levels correlated directly with the activity of disease, as determined by proteinuria and histologic assessment, and the intensity of the associated leukocytic infiltrate [64]. Furthermore, kidney tissue sections from nephritic mice supported adhesion of T-cell and macrophage cell-lines, a process that was inhibited by monoclonal antibodies against VCAM-1 and ICAM-1. These data provided further evidence that these molecules contribute to leukocyte recruitment, and are not merely markers for cytokine release by infiltrating leukocytes. The stimulus for induction of ICAM-1 and VCAM-1 on tubular epithelium in patients with glomerulonephritis is unclear. Potential mechanisms include primary involvement of the tubulointerstitium and/or the “down-stream” actions of glomerular cytokines that reach the tubulointerstitial via blood, urine, or diffusion through extravascular tissue. In support of the former hypothesis, T-cells cloned from inflammatory renal infiltrates in experimental lupus nephritis demonstrate specific autoreactivity against renal tissue and induce ICAM-1 and MHC class II molecules on cultured tubule epithelial cells [48]. The latter process appears to be due, at least in part, to the actions of interferon-γ, as induction can be blocked by monoclonal antibody against this cytokine [48].

Several investigators have reported *de novo* expression of VCAM-1 on proximal tubule cells and occasional distal tubules in renal allograft rejection in humans [59, 60, 66–68]. VCAM-1 expression is usually focal under these circumstances (30 to 50% of tubules) and most pronounced in areas of leukocyte infiltrates. In addition, VCAM-1 tends to be expressed in a patchy distribution within individual tubules and concentrated towards the basolateral surface. Striking induction of VCAM-1 is usually observed on the endothelium of peritubular capillaries, venules, and arterioles, but not glomeruli, in areas of leukocytic infiltrates.

**E-selectin in kidney diseases**

The pattern of expression of E-selectin in renal disease is largely undefined. Cytokine-activated renal microvascular endothelial cells clearly express E-selectin *in vitro* [69, 72]. A preliminary report suggests that E-selectin is expressed *de novo* by glomerular endothelial cells in some patients with acute glomerulonephritis, lupus nephritis, and IgA nephropathy, but not in patients with focal segmental glomerulosclerosis or membranous nephropathy [51]. Induction of E-selectin on endothelial cells of interstitial venules is a frequent additional finding in patients with lupus nephritis and prominent interstitial inflammation, and in patients with IgA nephropathy during acute exacerbations [51]. Interestingly, glomerular levels of E-selectin appear to be highest in patients with high circulating levels of TNFα [51]. *De novo* expression of E-selectin on glomerular endothelium has also been reported in experimental models of septic shock [70, 71] and acute glomerulonephritis [81]. In the latter study, glomerular expression of E-selectin, ICAM-1 and VCAM-1 was mimicked by infusion of TNFα, but not IL-1β, into the renal artery of normal animals, and was attenuated in nephritic animals by prior treatment of animals with a monoclonal antibody against TNFα or soluble recombinant human TNF receptor-1 [81]. Taken together, these data suggest that TNFα may be a major stimulus for induction of adhesion molecule expression in acute glomerulonephritis.

The distribution of E-selectin on endothelium in acute rejection tends to parallel that of ICAM-1 and VCAM-1. *De novo* E-selectin expression is usually observed on endothelial cells of peritubular capillaries and large vessels, but not glomeruli, with highest levels in areas of leukocyte infiltration. However, it should be noted that induction of E-selectin is not an invariable phenomenon in either acute glomerulonephritis [51] or allograft rejection [66] in humans. These negative findings may reflect the transience of E-selectin expression (*vide supra*) and the absence of staining does not necessarily preclude an important role for this molecule in the early stages of renal inflammation. Alternatively, the absence of E-selectin may reflect extensive endothelial cell injury, as occurs frequently in necrotizing glomerulonephritis and allograft rejection. In support of the latter explanation, focal absence of PECAM-1, which is constitutively expressed by healthy renal endothelial cells, has been reported in biopsies from patients with renal allograft rejection [60].

**Other adhesion molecules**

There is little information on the distribution of ICAM-2, P-selectin, or ligands for L-selectin in renal diseases. It is also worth noting that few studies have assessed the expression of ICAM-1, VCAM-1, and E-selectin in the same disease, or correlated the expression of these molecules with the relative numbers of different types of leukocytes in inflammatory infiltrates. It is apparent from the *in vivo* and *in vitro* observations discussed above, that most resident renal cells, in their basal state or following activation with cytokines, express ligands that can support granulocyte, monocyte or lymphocyte adhesion. Yet regional differences in the composition of leukocyte infiltrates are found in many inflammatory renal diseases. Furthermore, as noted above, enhanced expression of adhesion molecules has been noted in diseases that are usually not associated with leukocyte infiltration. These observations further suggest that leukocyte recruitment is not regulated solely by the profile of adhesion molecules on invading and resident cells, but also by the complex interplay of chemoattractants and...
other soluble and cell-associated activation signals within the local inflammatory milieu.

**Adhesion facilitates dynamic interplay between phagocytes and renal parenchymal cells**

Phagocyte adhesion, in addition to facilitating phagocyte recruitment, may also promote a dynamic interplay between phagocytes and resident tissue cells that ultimately contributes to the pathophysiology of renal inflammation. Recent evidence suggests that adhesion may serve to amplify the concentrations and array of lipid mediators at sites of inflammation by facilitating the generation of eicosanoids by transcellular routes. For example, neutrophils and endothelial cells, and neutrophils and platelets effectively pool their enzymatic machinery during cell-cell interaction to generate lipoxygenase products that neither cell can generate alone (Fig. 4) [74]. Glomerular endothelial cells lack 5-lipoxygenase activity and generate little, if any, leukotrienes from arachidonic acid, but can convert neutrophil-derived LTA₄ to LTC₄ during cell-cell interaction [73, 74]. Monoclonal antibodies against CD11/CD18 integrins and L-selectin block phagocyte adhesion to glomerular endothelial cells and attenuate LTC₄ biosynthesis during concubation of phagocytes and glomerular endothelial cells [73, 74]. These observations suggest that adhesion promotes leukotriene synthesis by transcellular routes probably by approximating phagocyte and endothelial cell membranes and facilitating the transfer of the unstable lipophilic intermediate LTA₄ between cells (Fig. 4). In addition, adhesion may prime phagocyte 5-lipoxygenase pathways, as with other functional responses (vide infra), and thus promote LTA₄ generation and release by phagocytes [82].

Adhesion may also promote renal injury by priming phagocyte respiratory bursts and degranulation responses to the actions of chemoattractants and cytokines (Fig. 5) [7, 75–77]. In keeping with this hypothesis, phagocytes generate greater quantities of superoxide anion when adherent to cytokine-activated mesangial cells in vitro, and monoclonal antibodies (mAb) against CD18 attenuate adhesion, superoxide anion generation and mesangial cell injury in this setting [76]. A preliminary report suggests that CD11/CD18-mediated phagocyte adhesion to glomerular basement membrane potentiates free radical generation in a similar manner [92]. It is probable that adhesion also promotes glomerular cell injury and disruption of the filtration barrier by facilitating the release of reactive oxygen species and proteases in close proximity to glomerular cells and the glomerular basement membrane, thereby reducing the effectiveness of extracellular free radical scavengers and protease inhibitors (Fig. 5) [reviewed in 5].

Monoclonal antibodies against CD11/CD18 integrins attenuate lysis of renal tubule epithelial cells by cytotoxic T-lymphocytes in vitro [78]. In addition, anti-ICAM-1 mAb inhibit antigen presentation by cytokine-activated renal tubular cells [79] and mesangial cells in vitro [93]. These observations suggest additional mechanisms whereby adhesion may contribute to the
pathophysiology of acute allograft rejection and glomerulonephritis. Taken together, these studies highlight the importance of adhesion in promoting bidirectional flow of information between phagocytes and resident tissue cells. Furthermore, they suggest that inhibitors of adhesion may not only block recruitment of circulating phagocytes, but also attenuate potentially deleterious interactions between phagocytes and resident tissue cells at sites of inflammation.

Efficacy of monoclonal antibodies against leukocyte adhesion molecules in the treatment of renal inflammation

The efficacy of monoclonal antibodies (mAb) against different adhesion molecules as anti-inflammatory agents has been tested in experimental models of glomerulonephritis, tubulointerstitial disease, allograft rejection, and renal ischemia-reperfusion injury (Table 4) [81–91]. Mulligan et al assessed the influence of mAb against CD18, CD11a, CD11b, VLA-4, ICAM-1 and E-selectin on glomerular neutrophil accumulation and proteinuria in Long-Evans rats with nephrotoxic serum nephritis [81]. Their model is characterized by up-regulation of glomerular ICAM-1, VCAM-1 and E-selectin expression, rapid neutrophil infiltration of glomerular (maximum at 6 hr), and complement and neutrophil-dependent proteinuria. Treatment of animals with mAb against CD18, CD11b, and ICAM-1 caused a 63%, 46%, and 55% reduction, respectively, in proteinuria, and a 79%, 66%, and 54% reduction, respectively, in glomerular neutrophil counts, suggesting a role for these ligands in the pathogenesis of glomerular inflammation. In contrast, mAb against CD11a and E-selectin did not confer protection. Interestingly, mAb against VLA-4 also attenuated neutrophil recruitment and proteinuria in this model, even though this ligand does not play a direct role in neutrophil trafficking. These observations suggest that some mAb may protect via mechanisms other than direct inhibition of neutrophil adhesion (vide infra). This contention is further supported by studies by Wu et al that evaluated the influence of anti-CD11b mAb on glomerular neutrophil recruitment and proteinuria in nephrotoxic serum nephritis in Lewis rats [82]. While infusion of anti-CD11b mAb 16 hours prior to induction of disease caused a striking decrease in both proteinuria and neutrophil infiltration in their study, administration of mAb 30 minutes before induction also afforded protection without affecting glomerular neutrophil counts. In contrast to these studies in rats, two studies failed to demonstrate a protective effect of anti-CD18 mAb in nephrotoxic serum nephritis in rabbits using doses of mAb that saturate CD18 on circulating and glomerular neutrophils, inhibit neutrophil migration to other vascular beds, and have been shown previously to attenuate neutrophil-dependent tissue injury in other organs in this species (Table 4).

MAb against CD11a and ICAM-1 have been reported to attenuate injury in two studies of crescentic glomerulonephritis in Wistar-Kyoto rats [84, 85]. This model is characterized by linear deposition of rat IgG in the glomerular basement membrane, up-regulation of ICAM-1 on glomerular endothelium,
early infiltration of glomeruli by T-lymphocytes and monocytes/macrophages, and crescent formation and glomerulosclerosis. Treatment of rats with anti-CD11a and ICAM-1 prior to induction of disease and thrice weekly thereafter caused virtually complete inhibition of crescent formation and abrogation of proteinuria in both studies. Furthermore, progression of disease was retarded significantly when treatment was instituted after disease was established [85], an important consideration if such agents are to be useful therapeutic tools in humans.

The potential of ICAM-1 as a target for intervention in inflammatory renal diseases has been highlighted further in studies of tubulointerstitial nephritis and allograft rejection. Harris et al assessed the influence of anti-ICAM-1 mAb on acute autoimmune tubulointerstitial nephritis in the kkd variant of the CBA/Ca mice [86]. These animals have normal kidneys at birth, but develop progressive and ultimately lethal tubulointerstitial nephritis beginning after approximately four weeks. The disease process is associated with up-regulation of ICAM-1 expression in the renal interstitium, on infiltrating leukocytes, and on the basolateral surface of renal tubule epithelium. The administration of anti-ICAM-1 mAb to mice with established disease caused a marked reduction in leukocyte infiltration, tubular injury, and proteinuria in this model. Interestingly, however, the use of anti-ICAM-1 mAb was not associated with increased survival.

Cosimi et al demonstrated a striking delay of renal allograft rejection and increase in the survival of Cynomolgus monkeys when anti-ICAM-1 mAb was used as the sole immunosuppressive agent after transplantation (recipient survival 24.4 ± 2.4 vs. 9.2 ± 0.6 days) [87]. In this model, increased expression of ICAM-1 was observed on arterial and peritubular, but not glomerular endothelium, and on tubular epithelium and infiltrating leukocytes, as observed by other investigators (vide supra). Anti-ICAM-1 mAb caused some reduction in the intensity of the leukocyte infiltrate, tubular injury, and proteinuria in this model. Interestingly, however, the use of anti-ICAM-1 mAb was not associated with increased survival.

Table 4. Efficacy of some adhesion-blocking monoclonal antibodies in inflammatory renal diseases and ischemia-reperfusion

<table>
<thead>
<tr>
<th>Renal disease</th>
<th>Species (strain)</th>
<th>Molecule</th>
<th>Antibody</th>
<th>Preparation</th>
<th>Dose</th>
<th>Timinga</th>
<th>Protection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrotic serum nephritis</td>
<td>Rat (Long-Evans)</td>
<td>CD18</td>
<td>CL-26</td>
<td>F(ab')2</td>
<td>33 µg i.v.</td>
<td>After</td>
<td>Yes</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD11a</td>
<td>WT-1</td>
<td>Intact</td>
<td>200 µg i.v.</td>
<td>Simultaneous</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD11b</td>
<td>IB6c</td>
<td>Intact</td>
<td>300 µg i.v.</td>
<td>Not reported</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VLA-4</td>
<td>TA-2</td>
<td>Intact</td>
<td>800 µg i.v.</td>
<td>Simult + after</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICAM-1</td>
<td>IA29</td>
<td>F(ab')2</td>
<td>200 µg i.v. + i.p.</td>
<td>After</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-selectin</td>
<td>CL-3</td>
<td>F(ab')2</td>
<td>45-133 µg i.v.</td>
<td>After</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Nephrotic serum nephritis</td>
<td>Rat (Lewis)</td>
<td>CD11b</td>
<td>OX42</td>
<td>Intact</td>
<td>0.15 mg/100 g i.v.</td>
<td>Before</td>
<td>No</td>
<td>See 82</td>
</tr>
<tr>
<td>Nephrotic serum nephritis</td>
<td>Rat (Wistar)</td>
<td>ICAM-1</td>
<td>IA29</td>
<td>Intact</td>
<td>5 mg/kg i.v.</td>
<td>Before + after</td>
<td>Yes</td>
<td>83</td>
</tr>
<tr>
<td>Nephrotic serum</td>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R15.7</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Nephrotic serum nephritis</td>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R6.5</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal disease</th>
<th>Species (strain)</th>
<th>Molecule</th>
<th>Antibody</th>
<th>Preparation</th>
<th>Dose</th>
<th>Timinga</th>
<th>Protection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crescentic glomerulonephritis</td>
<td>Rat (Wistar-Kyoto)</td>
<td>CD11a</td>
<td>WT-1</td>
<td>Intact</td>
<td>1-5 mg/kg i.p.</td>
<td>Before + after</td>
<td>Yes</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICAM-1</td>
<td>IA29</td>
<td>Intact</td>
<td>1-5 mg/kg i.p.</td>
<td>Before + after</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD11a +</td>
<td>WT-1 +</td>
<td>Intact</td>
<td>1 mg/kg of both i.p.</td>
<td>Before + after</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICAM-1</td>
<td>IA29</td>
<td>Intact</td>
<td>0.5 mg of both i.v. + i.p.</td>
<td>Before + after</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Crescentic glomerulonephritis</td>
<td>Rat (Wistar-Kyoto)</td>
<td>CD11a +</td>
<td>WT-1 +</td>
<td>Intact</td>
<td>10 mg/kg/day i.p.</td>
<td>After</td>
<td>Yes</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICAM-1</td>
<td>IA29</td>
<td>Intact</td>
<td>0.01-2 mg/kg/day i.v.</td>
<td>Before + after</td>
<td>Yes</td>
<td>87</td>
</tr>
<tr>
<td>Tubulointerstitial nephritis</td>
<td>Mouse (CBA/Ca, kkd)</td>
<td>ICAM-1</td>
<td>YN1/1.7</td>
<td>Intact</td>
<td>i.v. to achieve serum levels of ~10 µg/ml</td>
<td>Before + after</td>
<td>Yes</td>
<td>88</td>
</tr>
<tr>
<td>Acute allograft rejection</td>
<td>Monkey (Cynomolgus)</td>
<td>ICAM-1</td>
<td>R6.5</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td>91</td>
</tr>
<tr>
<td>Acute allograft rejection</td>
<td>Human</td>
<td>ICAM-1</td>
<td>B1R1</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion</td>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R15.7</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion</td>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R6.5</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species (strain)</th>
<th>Molecule</th>
<th>Antibody</th>
<th>Preparation</th>
<th>Dose</th>
<th>Timinga</th>
<th>Protection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (Long-Evans)</td>
<td>CD18</td>
<td>CL-26</td>
<td>F(ab')2</td>
<td>33 µg i.v.</td>
<td>After</td>
<td>Yes</td>
<td>81</td>
</tr>
<tr>
<td>Rat (Lewis)</td>
<td>CD11b</td>
<td>OX42</td>
<td>Intact</td>
<td>0.15 mg/100 g i.v.</td>
<td>Before</td>
<td>No</td>
<td>See 82</td>
</tr>
<tr>
<td>Rat (Wistar)</td>
<td>ICAM-1</td>
<td>IA29</td>
<td>Intact</td>
<td>5 mg/kg i.v.</td>
<td>Before + after</td>
<td>Yes</td>
<td>83</td>
</tr>
<tr>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R15.7</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Rabbit (New Zealand White)</td>
<td>CD18</td>
<td>R6.5</td>
<td>Intact</td>
<td>2 mg/kg i.v.</td>
<td>Before</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

a Relative to induction of disease
b Neuringer J, Brady HR, unpublished observations
c Additive protection when compared with equivalent doses of individual mAb
d O’Meara YM, Salant DJ, Brady HR and e Tipping PG, Cornhwaite LJ, Holdsworth SR, XII International Congress of Nephrology, Jerusalem, Israel, June 1993
f Dose sufficient to saturate CD18 on circulating and glomerular leukocytes, and cause demargination of leukocytes (leukocytosis) or inhibition of chemoattractant-stimulated leukocyte migration into skin
anti-inflammatory actions without altering leukocyte trafficking. The preliminary results of a phase 1 clinical trial suggest that anti-ICAM-1 mAb may also prevent rejection in high risk human allograft recipients [88].

Neutrophils contribute to tissue injury during ischemia-reperfusion of the pulmonary, coronary, cerebral, and splanchnic circulations [5]. Anti-CD18 mAb confer protection against ischemic-reperfusion injury of these organs in many experimental models [5]. The importance of neutrophils in renal ischemic-reperfusion is less clear [89, 90] and anti-CD18 mAb did not confer functional or morphologic protection in two rabbit models studied to date (Table 4) [91; Neuringer J, Brady HR, unpublished observations]. Whether this lack of efficacy reflects a lesser role for neutrophils in ischemia-reperfusion injury in the kidney than in other organs, or involvement of other adhesion molecules is unclear. These issues clearly warrant further investigation given the prevalence of renal ischemic-reperfusion injury in clinical practice.

There are several potential explanations for the varying efficacy of adhesion-blocking mAb in renal diseases. Nephrotoxic sera may differ in composition, antigen specificity and the mechanism(s) by which they induce injury. Thus, different adhesion molecules are likely to dominate depending on the sera, species, and time of study. Antibodies vary dramatically in their ability to block adhesion depending on the epitopes with which they react and the characteristics of the antibody preparation (such as intact vs. F(ab')2). Furthermore, mAb against CD11/CD18, ICAM-1 and other adhesion molecules attenuate a variety of other important neutrophil, lymphocyte and monocyte functions such as antigen presentation, in addition to inhibiting trafficking, as discussed above. Some mAb (particularly intact mAb) provoke destruction of leukocytes in vivo. This variable may be difficult to quantify. Children with LAD types 1 and 2 have systemic neutrophilia due to failure of margination of granulocytes; thus, even "normal" neutrophil counts in mAb-treated animals may not necessarily exclude lysis of some leukocytes subsets. Given these inherent variables and the likely overlap of adhesion molecule function, it may difficult to determine the relative contributions of different molecules to renal diseases until results are accumulated using a variety of approaches (such as different mAb, soluble forms of adhesion molecules, anti-sense constructs, gene "knock-out" technology, drugs, etc.).

Role of leukocyte adhesion molecules in other disease processes

Leukocyte adhesion molecules may also play a critical role in the pathophysiology of a variety of seemingly unrelated renal disorders which include hemodialysis membrane incompatibility reactions, atherogenesis, tumor metastasis, and the pathogenesis of infections. Exposure of circulating blood to new cellulosic hemodialysis membranes frequently results in rapid activation of granulocytes, degranulation, generation of reactive oxygen species, and granulocytopenia primarily due to sequestration of granulocytes in the pulmonary vasculature ("First-use syndrome"). This granulocytopenia is usually transient and followed by rebound granulocytosis. Granulocytes isolated from patients exposed to cellulosic membranes display dramatic upregulation of CD11b/CD18 and down-regulation of L-selectin [94–97], suggesting that these molecules participate in granulocyte trafficking and activation in this setting. In keeping with this hypothesis, granulocytes from normal subjects undergo dramatic degranulation when exposed to cellulosic membranes in vitro, while degranulation is markedly attenuated with granulocytes from individuals with LAD type 1 disease [97].

Infiltration of blood vessel walls by monocytes in the area of fatty streaks is one of the earliest pathologic events in atherogenesis [98]. The mechanism by which monocytes are recruited in this setting is unclear. VCAM-1 expression is up-regulated at an early stage on endothelium overlying plaques experimental atherosclerosis, suggesting that VCAM-1 may promote monocyte infiltration in this setting [99]. Infectious organisms may usurp the function of adhesion molecules to infect host tissues. For example, rhinovirus and plasmodium falciarum-infected erythrocytes bind ICAM-1 at sites distinct from its binding site for CD11a/CD18 [100, 101] and may use these interactions to infect airway epithelium and the cerebral circulation, respectively. These observations raise the exciting possibility that it may be possible to develop antimicrobials which prevent infection by blocking the interactions between infectious organisms and adhesion molecules such as ICAM-1 without compromising immune function. Many tumors (such as colon cancer) carry a better prognosis if they are associated with a lymphocyte infiltrate. Colon cancer cells display increased expression of ICAM-1 compared to normal colonic epithelium and ICAM-1 expression appears to correlate with the degree of lymphocytic infiltration [102]. These data suggest that ICAM-1 may play an important role in immunosurveillance in this disease. The expression of adhesion molecules may be a double-edged sword; however, in cancer biology as VCAM-1, P-selectin and E-selectin support adhesion of some cancer cells in vitro (such as melanoma), suggesting a mechanism whereby circulating tumor cells may exit the circulation and seed metastases. Taken together, these observations suggest that the further elucidation of the molecular basis for leukocyte adhesion may not only shed light on basic mechanisms of inflammation, but also enhance our understanding of a variety of other biological processes and suggest novel strategies for therapeutic intervention for many common diseases.

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Note added in proof

Berg et al have provided recent evidence that MadCAM-1, a mucin, is a facultative ligand for L-selectin in mesenteric lymph nodes (Nature 366:695–697, 1994). Interestingly, MadCAM-1 contains both mucin and Ig-like residues, being a ligand for both L-selectin and α4β7 integrins. This unique adaptation to support rolling and immobilization may subserve physiologic lymphocyte homing to mesenteric lymphoid tissue. The relative contributions of other mucins (CD34 and GlyCAM-1 for L-selectin; PSGL-1 for P-selectin) to selectin-mediated adhesion
have yet to be defined (Shimizu Y and Shaw S, Nature 366:630–631, 1994). Kelly et al demonstrated a protective effect of anti-ICAM-1 mAb in renal ischemia-reperfusion, suggesting that blockade of integrin-Ig-like interactions is beneficial in some species (Proc Natl Acad Sci USA 91:812–816, 1994). Hill et al provided important evidence that ICAM-1 may direct migration and localization of interstitial leukocytes in experimental glomerulonephritis (Kidney Int 45:1–12, 1994).

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