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Cell Adhesion Molecules, Leukocyte Trafficking, and Strategies to Reduce Leukocyte Infiltration

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Cell Adhesion Molecules, Leukocyte Trafficking, and Strategies to Reduce Leukocyte Infiltration

Abstract
Leukocyte-endothelial cell interactions are mediated by various cell adhesion molecules. These interactions are important for leukocyte extravasation and trafficking in all domestic animal species. An initial slowing of leukocytes on the vascular endothelium is mediated by selectins. This event is followed by (1) activation of β₂ integrins after leukocyte exposure to cytokines and proinflammatory mediators, (2) adherence of leukocyte β₂ integrins to vascular endothelial ligands (eg, intercellular adhesion molecule-1 [ICAM-1]), (3) extravasation of leukocytes into tissues through tight junctions of endothelial cells mediated by platelet and endothelial cell adhesion molecule-1 (PECAM-1), and (4) perivascular migration through the extracellular matrix via β₁ integrins. Inhibiting excessive leukocyte egress and subsequent free radical-mediated damage caused by leukocyte components may attenuate or eliminate tissue damage. Several methods have been used to modify leukocyte infiltration in various animal models. These methods include nonspecific inhibition of pro-inflammatory mediators and adhesion molecules by nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, inhibition of cytokines and cytokine receptors, and inhibition of specific types of cell adhesion molecules, with inhibitors such as peptides and antibodies to β₂ integrins, and inhibitors of selectins, ICAMs, and vascular cell adhesion molecule-1 (VCAM-1). By understanding the cellular and molecular events in leukocyte-endothelial cell interactions, therapeutic strategies are being developed in several animal models and diseases in domestic animal species. Such therapies may have clinical benefit in the future to overcome tissue damage induced by excessive leukocyte infiltration.

Keywords
Inflammation, Integrins, Intercellular adhesion molecule-1 (ICAM-1), Neutrophils, Selectins

Disciplines
Veterinary Pathology and Pathobiology

Comments

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Leukocyte infiltration is vital to both innate and active immunity. This process is an important host defense mechanism against infectious agents, foreign antigens, and neoplastic cells. It also occurs as a physiologic response in cell injury due to ischemia and reperfusion, toxins, and metabolic disturbances. When leukocyte infiltration is in excess, however, the release of products (proteases and free radicals [eg, O₂⁻, NO]) can result in extensive tissue damage and subsequent fibrosis. Reducing leukocyte infiltration may have special importance for clinical therapies and has been the focus of studies of several diseases of humans such as asthma, arthritis, reperfusion injuries after cerebral hemorrhage, and myocardial infarctions. In veterinary medicine, many additional diseases are associated with excessive leukocyte infiltration (eg, laminitis, gastric dilatation/volvulus in dogs, mastitis, reperfusion injury after tissue ischemia in recumbent cattle and horses, and Mannheimia [Pasteurella] haemolytica pneumonia), and strategies to reduce leukocyte infiltration have been developed as a therapeutic approach.

The purpose of this article was to review the structure, expression, and function of the various cell adhesion molecules, with special attention to studies in domestic animal species. The molecular events in leukocyte-endothelial cell interactions and current therapeutic approaches that target adhesion molecules also are reviewed.

Adhesion Molecules Regulating Leukocyte Trafficking

Leukocyte adherence to the vascular endothelium is mediated by a sequence of binding between adhesion molecules expressed on leukocytes and adhesion molecules expressed by the intraluminal and intercellular membranes of vascular endothelial cells. Neutrophils, for example, express selectins that adhere transiently to selectin receptors on the endothelial cell surface (Fig 1). During vasodilation, intravascular hydrostatic pressure reduces neutrophil flow through the vessel and allows additional selectin-selectin receptor binding events that eventually slow neutrophil circulation in a tethering-like fashion. Upon activation, neutrophils shed some selectin molecules and greatly enhance expression of β₂ integrins that bind intercellular adhesion molecules (ICAMs) expressed on activated endothelial cells. Neutrophils migrate through the intercellular junctions of endothelial cells, and this process is partly mediated by other adhesion molecules (eg, PECAM-1), and extravascular migration of neutrophils utilizes additional adhesion molecules (eg, β₁ integrins).

The importance of these molecules is underscored by the severity of disease that can occur when even one family of adhesion molecules is lacking or nonfunctional. In Irish Setter dogs and Holstein cattle, functional expression of the β₂ integrins by leukocytes and transmigration by neutrophils through the vascular endothelium into sites of infection can be markedly impaired. Affected animals often develop severe infections of mucosal surfaces and die.
Leukocyte Cell Adhesion Molecules and Trafficking

**Fig 1.** Phases of leukocyte infiltration from the vascular space into sites of infection and various adhesion molecules involved and modulation of these adhesion molecules are outlined in each phase. In the 1st phase, tethering occurs via L- and P-selectin, followed by selectin-mediated rolling in which cells gradually slow down. These phases can be inhibited by dexamethasone (DXM), antisense oligonucleotides (ASOs), monoclonal antibodies (mAbs), selectin mimetic (TBC), and sialyl Lewis x analog (sLex). Tight adhesion between the expressed β2 integrin can be inhibited by DXM, mAbs, synthetic peptide analogs (SPs), and NPC 15199 (N-[fluorenyl-9-methoxycarbonyl-L-leucine]). Intercellular adhesion molecule-1 (ICAM-1) can be inhibited by mAbs and ASOs and occurs after activation by cytokines (eg, interleukin-1 [IL-1] and tumor necrosis factor [TNF]) and pro-inflammatory mediators (eg, lipopolysaccharide [LPS]). The actions of these cytokines and pro-inflammatory mediators can be inhibited by mAbs, cytokine, nonsteroidal anti-inflammatory drugs (NSAIDs), receptor antagonists (RAnt.), and CD14 receptor antagonists (CD14R Ant.). Finally, egress of leukocytes occurs via platelet and endothelial cell adhesion molecule-1 (PECAM-1) and binding to extracellular matrix proteins via β1 integrins and other integrins.

Shortly after birth. Cattle affected by bovine leukocyte adhesion deficiency (BLAD) often die of alimentary tract infections or pneumonia. Similar defects in β2 integrin expression occur in humans (leukocyte adhesion deficiency-1, [LAD-1]); however, the clinical course of LAD-1 in humans varies depending on the location of the mutation in the β2 integrin molecule. Clinical manifestations in LAD-1 and other adhesion deficiencies of humans often include recurrent mucosal infections, as in affected cattle and dogs. Other rare adhesion molecule deficiencies in humans include impaired β2 integrin conformational change (LAD-1 variant), impaired expression of selectin receptor expression (leukocyte adhesion deficiency-2, [LAD-2]), and excessive proteolytic cleavage of 1 type of selectin (E-selectin).3–7

**Selectins**

Initial leukocyte rolling on vascular endothelium is mediated by selectins. Selectins (L-, E-, and P-selectins) are heavily glycosylated membrane proteins.7 Structurally, selectins contain an N-terminal lectin domain, an epidermal growth factor (EGF)-type domain, several complement regulatory repeats, and a short cytoplasmic domain. The terminal lectin domains of selectins bind fucosylated tetrasaccharides of selectin receptors (sialyl Lewis x [sLe x]) in the presence of calcium (Ca²⁺). Two types of selectin deficiencies are reported in humans: LAD type II (impaired fucosylated tetrasaccharide production) and enhanced cleavage of E-selectin. Although transgenic mice deficient in selectins or selectin ligands have impaired leukocyte adherence, no reports of naturally occurring selectin deficiencies in animals have been reported.

L-selectin (CD62L, or leukocyte endothelial cell adhesion molecule-1 [LECAM-1]) is a glycoprotein that is constitutively expressed and functional on all leukocytes.1,6–9 L-selectin is located on the tips of microvillus-like projections of the leukocyte surface and interacts reversibly with its endothelial cell ligand. L-selectin vascular endothelial cell ligands include glycoprotein cell adhesion molecule-1 (GlyCAM-1), mucosal addressin cell adhesion molecule-1 (MAdCAM-1), cell surface carbohydrate structures that possess a specific sLe x structure, and CD34.10,11 L-selectin undergoes a conformational change during cellular activation by pro-inflammatory substances such as C5a and leukotriene B4. Activation of L-selectin increases binding affinity to its ligand.12,13 After L-selectin mediates slow rolling of leukocytes on the vascular endothelium, proteases induce shedding of L-selectin from the leukocyte surface.

Leukocytes of humans and animals (eg, baboon, cat, dog, rabbit, mouse, sheep, and pig) express L-selectin,14–24 and L-selectin is structurally conserved among bovine, human, and murine species.9,25 Expression of L-selectin by bovine γδ T cells is much higher than that of bovine αβ T and B cells.26–28 The significance of this high concentration of expression remains to be determined, but calves have a relatively high number of γδ T cells, and selectin adherence may be very critical to the activity of these cells and immune surveillance.

A soluble form of L-selectin has been found in human plasma and may have a role in preventing leukocyte-vascular endothelium interaction, thus regulating the extent of leukocyte infiltration.29,30 Selectin-mediated leukocyte rolling on vascular endothelium also can be inhibited by antibodies to leukocyte L-selectin32 and synthetic mimetics.
P-selectin (CD62P, originally identified as a platelet granule membrane protein-40 or GMP-40) is stored preformed in the α granules of platelets and Weibel-Palade bodies of endothelial cells. P-selectin is mobilized from storage granules to the cell surface after activation (eg, by lipopolysaccharide [LPS], tumor necrosis factor alpha [TNF-α], interleukin-1 [IL-1], oxygen radicals, histamine, thrombin, and interferon gamma [IFN-γ]) that causes phosphorylation of tyrosine, threonine, and serine residues in its cytoplasmic tail. This process occurs by fusion of secretory granules with the plasma membrane. Increased P-selectin expression serves to slow leukocyte rolling. P-selectin binds to the aminoterminal region of its ligands, P-selectin glycoprotein ligand-1 (PSGL-1), and sLeα on endothelial cells and platelets and GlyCAM-1 on endothelial cells. The interaction of the P-selectin cytoplasmic domain with clathrin-coated pits enhances leukocyte adhesion. After its transient appearance on the cell surface, P-selectin is internalized for lysosomal degradation and may be recycled to facilitate egress of other leukocytes entering the area.

P-selectin has been studied in humans and many animals (dog, cat, cattle, and sheep). In the dog, P-selectin expression on endothelial cells is increased in various skin diseases, and in both lambs and cats, inhibition of P-selectin protects the myocardium from postischemic reperfusion injury. A soluble form of P-selectin has been found in the circulation of healthy humans. The blood concentrations of soluble P-selectin are high in many disease conditions (eg, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, chronic obstructive pulmonary disease, myocardial infarction, hypercholesterolemia, and rheumatoid arthritis). As with soluble L-selectin, the activity of the soluble form of P-selectin may be downregulated, and its concentration may be useful in monitoring disease severity. Increases in soluble adhesion molecules may be associated with extensive and possibly excessive leukocyte infiltration or recurrence of an otherwise stable condition.

E-selectin (CD62E), previously known as endothelial leukocyte adhesion molecule-1 (ELAM-1), is synthesized and expressed by endothelial cells. E-selectin expression is induced by cytokines (eg, IL-1 and TNF-α) and other pro-inflammatory molecules (eg, LPS) in acute and chronic inflammatory responses. E-selectin expression peaks about 4–6 hours after initiation of inflammation and declines to basal concentrations after 24–48 hours. Sialylated and fucosylated molecules such as sLeα and sLeα oligosaccharides and E-selectin ligand-1 (ESL-1) on leukocytes are the ligands for E-selectin. Increased binding of E-selectin to its ligand on leukocytes is regulated both by the number of molecules expressed on the endothelial cells and by conformational changes that occur with cellular activation. Nuclear factor-κ B (NF-κ B) is involved in E-selectin activation. After expression, E-selectin is internalized by endocytosis and delivered to lysosomes for degradation (Table 1).

**Integrins**

Integrins are required for firm leukocyte-endothelial cell adherence. They also act as cell surface receptors that mediate cell-cell interactions and attachment to the extracellular matrix, and they require divalent cations (eg, Ca2+ and Mg2+) for adherence to their ligands. Integrins are glycoprotein heterodimers composed of noncovalently linked α and β transmembrane subunits. Eight β subunits and 16 α subunits have been identified. Different combinations of the α and β units result in a wide variety of adherence specificity by the heterodimers. The different subfamilies that can be defined by their β subunit on leukocytes include β1 through β8 integrins (Table 1).

The β1 integrins share a common β subunit (CD29) and were originally termed very late activation (VLA) integrins. These VLA integrins are composed of at least 6 members (VLA-1, -2, -3, -4, -5, and -6). The β1 integrins are expressed by leukocytes and are important for leukocyte infiltration into perivascular stroma. β1 integrins generally mediate cell-extracellular matrix interactions (eg, with collagen, fibronectin, laminin, and vitronectin). Specialized regions of fibronectin peptides (eg, arginine-glycine-aspartate [RGD]) and other extracellular matrix proteins are ligands for β1 integrins. Several cytoplasmic domains of the β1 integrin subunit interact with cytoskeletal actin filaments via α-actinin. Such interactions of integrins with the leukocyte cytoskeleton are important for chemotaxis of leukocytes across the perivascular adventitia and extracellular matrix.

VLA-4 (the αβ2 integrin) is expressed by both B and T lymphocytes, basophils, eosinophils, monocytes, and macrophages and, to a minor degree, by neutrophils. VLA-4 is an important ligand for VCAM-1 as well as extracellular matrix components such as fibronectin, vitronectin, and collagen. VLA-4 binds to specific domains on fibronectin such as the connecting segment-1 (CS-1) and the fibronectin RGD domain. VLA-4/VCAM-1 adherence is especially active in monocyte transendothelial migration and plays a role in cattle with BLAD and in humans with LAD type 1. In both BLAD and LAD type 1, the numbers of neutrophils in blood are greatly increased because of their inability to adhere to the vascular endothelium. Although neutrophils lack or have minimal expression of VLA-4, other leukocytes express VLA-4 and presumably utilize VLA-4/VCAM-1 binding as well as selectins to pass across the vascular endothelium.

The αβ2 integrin is a major fibronectin receptor that is regulated by divalent cations (eg, Mg2+ and Ca2+). The αβ2 integrin plays a role in repair of airway epithelium after injury. Also, it has been found that the IpaB, IpaC, and IpaD proteins of *Shigella flexneri* can interact with the αβ2 integrin and may facilitate leukocyte trafficking in the alimentary tract.

The β1 integrin subfamily consists of 4 distinct α chains (CD11a, CD11b, CD11c, and CD11d) and a common β chain (CD18). The structure of CD18 is highly conserved among human, avian, murine, and bovine species. Numerous cytokines such as TNF-α, IL-1, interleukin-8 (IL-8), and other pro-inflammatory molecules, such as platelet-activating factor (PAF), leukotrienes, and LPS, increase β2 integrin expression. Leukocyte function antigen-1 (LFA-1; αβ2; CD11a/CD18) is expressed by all leukocytes and interacts with ICAM-1 and ICAM-2 on vascular endothelial cells. ICAM-4 and ICAM-5 also bind to LFA-1. Mac-1...
Table 1. Summary of adhesion molecules.

<table>
<thead>
<tr>
<th>Common name</th>
<th>CD</th>
<th>Expressed on</th>
<th>Binds</th>
<th>Expressed on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2 integrins:</td>
<td>(CD29);</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLA-1</td>
<td>(CD49a);</td>
<td>ec</td>
<td>Collagen</td>
<td>ECM</td>
</tr>
<tr>
<td>VLA-2</td>
<td>(CD49b);</td>
<td>ec</td>
<td>Collagen</td>
<td>ECM</td>
</tr>
<tr>
<td>VLA-3</td>
<td>(CD49c);</td>
<td>ec</td>
<td>Fibronectin</td>
<td>ECM</td>
</tr>
<tr>
<td>VLA-4</td>
<td>(CD49d);</td>
<td>L</td>
<td>VCAM-1</td>
<td>ec</td>
</tr>
<tr>
<td>VLA-5</td>
<td>(CD49e);</td>
<td>ec</td>
<td>Fibronectin</td>
<td>ECM</td>
</tr>
<tr>
<td>VLA-6</td>
<td>(CD49f);</td>
<td>ec</td>
<td>Laminin</td>
<td>ECM</td>
</tr>
<tr>
<td>β1 integrins:</td>
<td>(CD18);</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LFA-1</td>
<td>(CD11a);</td>
<td>L</td>
<td>ICAM-1</td>
<td>ec</td>
</tr>
<tr>
<td>Mac-1</td>
<td>(CD11b);</td>
<td>L</td>
<td>ICAM-1</td>
<td>ec, L</td>
</tr>
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<td>gp150,95</td>
<td>(CD11c);</td>
<td>L</td>
<td>ICAM-1</td>
<td>ec</td>
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<td>αβ2 integrins:</td>
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<td>MQ, CD8 T cells</td>
<td>ICAM-3</td>
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<td>β2 integrins:</td>
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<td>β1 integrins:</td>
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<tr>
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</tr>
<tr>
<td>Selectins</td>
<td></td>
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<tr>
<td>L-selectin</td>
<td>(CD62L);</td>
<td>L</td>
<td>GlyCAM-1, CD34, MAdCAM-1</td>
<td>ec</td>
</tr>
<tr>
<td>E-selectin</td>
<td>(CD62E);</td>
<td>ec</td>
<td>ESL-1, sLeα</td>
<td>L</td>
</tr>
<tr>
<td>P-selectin</td>
<td>(CD62P);</td>
<td>ec, platelets</td>
<td>PSGL-1, GlyCAM-1, sLeα</td>
<td>ec, P</td>
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<td>Immunoglobulin superfamily</td>
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<td></td>
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<td>ICAM-1</td>
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<td>ec, epithelia, SC</td>
<td>β2-integrins</td>
<td>L</td>
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<td>(CD102);</td>
<td>ec, Lympho</td>
<td>β2-integrins</td>
<td>L</td>
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<td>ICAM-3</td>
<td>(CD50);</td>
<td>ec, Lympho, PMN, M</td>
<td>β2-integrins</td>
<td>L</td>
</tr>
<tr>
<td>ICAM-4</td>
<td></td>
<td>Red blood cells</td>
<td>β2-integrins</td>
<td>L</td>
</tr>
<tr>
<td>ICAM-5</td>
<td></td>
<td>Subset of neurons</td>
<td>β2-integrins</td>
<td>L</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>(CD106);</td>
<td>ec</td>
<td>VLA-4 (L except PMN)</td>
<td>L</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>(CD31);</td>
<td>ec, L</td>
<td>PECAM-1</td>
<td>ec, L</td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td>(CD11d);</td>
<td>ec (mucosal)</td>
<td>L-selectin</td>
<td>PMN, Lympho</td>
</tr>
</tbody>
</table>

CD, cluster determinant; ec, endothelial cells; ESL-1, E-selectin ligand; ECM, extracellular matrix; GlyCAM-1, glycoprotein cell adhesion molecule-1; L, leukocytes; M, monocytes; MAdCAM-1, mucosal addressin cell adhesion molecule-1; MQ, macrophages; PMN, polymorphonuclear cells (neutrophils); PSGL-1, P-selectin glycoprotein ligand; Lympho, lymphocytes; SC, Schwann cells; sLeα, sialyl lewis α; see text for definition of adhesion molecule abbreviations.

*a This table is not comprehensive.

(αβ2; CD11b/CD18) and gp150,95 (αβ2; CD11c/CD18) are expressed by macrophages and granulocytes and also bind ICAM-1 but not ICAM-3.68 CD11d/CD18 was 1st described in the dog and is expressed constitutively by macrophages, including synovial macrophages and splenic red pulp macrophages. CD11d/CD18 expression by leukocytes is restricted to CD8+ lymphocytes in the splenic red pulp in dogs.66,67 The canine CD11d/CD18 (αβ2) heterodimer on leukocytes has homology to human αβ2.66 In contrast with other β2 integrins, the αβ2 integrin binds only to ICAM-3.66,68 Although the αβ2 integrin has not been described in cattle, bovine ICAM-3 has been sequenced from a mammary gland cDNA library (Table 1).69

Deficiency in β2 integrin expression in Holstein calves is due to a single-point mutation (adenine→guanine) at position 128, resulting in a single amino acid change (aspartic acid→glycine) in the β subunit (CD18) of the β2 integrin.69,70,71 This defect occurs in a highly conserved extracellular region (aa 96-389). A 2nd, silent mutation also has been detected in cattle with BLAD and does not alter the amino acid sequence. In Irish Setters with canine LAD (CLAD), a single missense mutation results in a G to C
conversion at nucleotide 107 of the cDNA sequence, resulting in a serine residue that replaces a highly conserved cysteine residue. Although cattle with BLAD, dogs with CLAD, and humans with LAD-type 1 have high numbers of neutrophils in the blood, these neutrophils are characterized by impaired passage across vascular endothelium and numerous other functional abnormalities related to inadequate membrane adherence. Affected cattle develop severe gingivitis, tooth loss, oral ulcers, enteric ulcers, cutaneous ulcers and abscesses that lack exudate formation, and pneumonia. The hallmark lesion histologically is a sparse infiltration of neutrophils into an ulcerated mucosal surface despite high numbers of neutrophils within the vascular lumens of submucosal blood vessels. No effective treatments are available for calves and Irish Setters, but one of the authors (MEK) has observed 2 cattle with BLAD that survived into adulthood with intensive medical care. In humans, LAD type 1 varies in severity, depending on the location of the CD18 mutation. People with severe phenotype (expressing <1% of functional β2 integrin) often are susceptible to severe infections, whereas those with moderate phenotype (3–10% expression) can live to adulthood.37 In the lung, β2 integrins mediate neutrophil infiltration into various microanatomic regions. Initial neutrophil infiltration into bronchi and bronchioles requires functional β2 integrins in cattle.72 However, CD18-deficient neutrophils in calves with BLAD can readily infiltrate pulmonary alveoli after intrabronchial deposition of M haemolytica,73 which suggests that additional adhesion molecules mediate neutrophil infiltration into alveoli. Recent work in a rat model suggests that sequestration of neutrophils in pulmonary alveolar capillaries involves adherence of several different adhesion molecules, including 2 β2 integrins (αMβ2 [VLA-4] and αβ2 [VLA-5]), β2 integrins, and selectins.74 Stiffening of neutrophils by actin polymerization is a physical change that further enhances neutrophil sequestration in alveolar capillaries.75 The type of inculom is an important factor for neutrophil migration into the lung. Escherichia coli induces a CD18-dependent type of neutrophil migration, whereas Staphylococcus aureus induces a CD18-independent type of migration.75,76 In addition, the duration of pneumonia also affects β2 integrin-mediated infiltration into the lung. In a rabbit model, a CD18-dependent mechanism was observed in acute pulmonary inflammation, and a CD18-independent mechanism was identified in chronic inflammation.77 The ability of β2 integrins to mediate neutrophil infiltration into precise regions of the lung may make it possible to site direct neutrophil infiltration into a location of interest (eg, bronchi and bronchioles). Such a therapeutic approach, if refined, may allow better treatment of tracheitis and bronchitis.

The β2 integrins (CD61) include αMβ2 and αβ2. The β2 integrins generally mediate adherence to extracellular matrix ligands and clotting factors. β2 integrins recognize fibronectin, fibrinogen, vitronectin, and von Willebrand factor (vWF).

The αMβ2 integrin is expressed by platelets and has a major role in platelet aggregation, which results from fibrinogen or vWF binding to αMβ2 complexes.80 Mutations in the α or β subunits of the αMβ2 integrin may lead to increased susceptibility to bleeding as described in Glanzmann thrombasthenia, a heterozygous disease in humans.79 Heterozygous defects result either in diminished function or impaired expression of the protein on platelets. In dogs, Glanzmann thrombasthenia has been reported in the Great Pyrenees and Basset Hound breeds.80,81 These molecules are important targets for antithrombotic treatments of myocardial infarction, stroke, phlebitis, and reperfusion injury.

The αβ2 integrin is expressed on endothelial cells, monocytes, macrophages, and vascular smooth muscle cells. It mediates endothelial cell adhesion and proliferation and also has a role in angiogenesis.82 In a rabbit model of angioplasty, iliac artery intimal hyperplasia was reduced by αβ2 integrin inhibitors.83 Inhibition of integrins that mediate angiogenesis in neoplasms is an important area of cancer research,84,85 whereas enhancement of angiogenic integrins may be beneficial for wound healing, as in diabetic patients with ulcers, for example.

The αMβ2 integrin is a laminin and kalinin receptor expressed by various cell types (eg, epithelial cells, endothelial cells, and Schwann cells).86-88 The αMβ2 integrin may play a role in epithelial integrity by forming a link between the plasma membrane and the basement membrane.80,89 Loss of cellular adherence to laminin and adjacent cells leads to enhanced cellular invasion by neoplastic cells. The αβ2 integrin may be important in tumor progression by facilitating a phosphatidylinositol 3-kinase (PI 3 K)-dependent invasion via cooperative interaction between the αβ2 integrin and erb B-2.91

The αβ2 integrin is expressed in high concentration by a subset of lymphocytes that are home to gut-associated lymphoid tissue (GALT).92,93 It is constitutively expressed by peripheral blood B lymphocytes but is absent on resident B cells in tissues and is weakly expressed by resting peripheral blood T lymphocytes. The αβ2 integrin also is expressed by human endothelial cells in vitro and in vivo.94 It also binds and mediates cell attachment to MadCAM-1, VCAM-1, and fibronectin.95 MadCAM-1 binding is important in lymphocyte trafficking to Peyer’s patches and intestinal mucosa. The αβ2 integrin also mediates lymphocyte binding to E-cadherin for adherence of endothelial cells.96 Modulation of lymphocyte homing may be beneficial in reducing the severity of autoimmune diseases such as lupus erythematosus or diseases characterized by excessive cell-mediated immunity (eg, inflammatory bowel disease).

Immunoglobulin Superfamily of Adhesion Molecules

The immunoglobulin superfamily adhesion molecules are involved in leukocyte-endothelial cell interactions. These proteins include ICAMs, VCAM-1, and PECAM-1. ICAMs are members of the immunoglobulin superfamily of proteins and include ICAM-1, ICAM-2, ICAM-3, ICAM-4, and ICAM-5. ICAMs are expressed by endothelial cells and bind to β2 integrins, LFA-1, and Mac-1.97,98 ICAM-1 (CD54) is an 80–115-kd surface glycoprotein that is composed of 5 immunoglobulin domains, a transmembrane domain, and a short cytoplasmic sequence. It is constitutively expressed at low concentration on resting endothelium. ICAM-1 also is expressed by Schwann cells and

mann
plays a role in the pathogenesis of inflammation in the peripheral nervous system. Neutrophils and macrophages express ICAM-1 in acute *M. haemolytica* pneumonia in calves. Many epithelial cells (eg, keratinocytes) and other leukocytes (eg, antigen-presenting cells) express ICAM-1. This expression is important in leukocyte epithelial and mucosal infiltration. ICAM-1 expression increases after activation by cytokines such as IL-8, IL-1, IFN-γ, TNF-α, and other pro-inflammatory substances such as PAF and LPS. ICAM-3 is a highly glycosylated protein with 5 Ig domains and a molecular weight of 130 kD. ICAM-3 has been derived from a bovine mammary gland cDNA library, ICAM-3 may play a role in the innate defense of the mammary gland.

ICAM-4 and ICAM-5 are recently discovered and less characterized adhesion molecules of the immunoglobulin superfamily that bind the CD11a/CD18 integrin. ICAM-4 (originally named Landsteiner-Wiener [LW] glycoprotein) has 2 Ig domains and is expressed on red blood cells. It binds CD11a/CD18 on neutrophils and lymphocytes. The LW antigen is a red cell membrane glycoprotein that requires divalent cations (eg, Mg²⁺) for its reactivity.

ICAM-5 (telencephalin) has 9 Ig domains and a molecular weight of 130 kD. It is a glycoprotein that is expressed by a subset of neurons (somato-dendritic) within the telencephalon of the mammalian brain. ICAM-5 can bind to CD11a/CD18 expressed by T lymphocytes. ICAM-5 immunoreactivity is decreased in Alzheimer’s patients. A soluble form of ICAM-5 is increased in patients with acute encephalitis and has potential in monitoring severity of disease.

VCAM-1 (CD106) is another member of the immunoglobulin gene superfamily and has 5 Ig domains. VCAM-1 is expressed at high concentration after LPS administration and cytokine activation (eg, IL-1 and TNF-α) on all vascular endothelial cells. This expression 1st appears 4–6 hours after stimulation and reaches maximal concentration at 12–18 hours. VCAM-1 interacts with VLA-4 on different leukocytes (monocytes, lymphocytes, and eosinophils but only minimally, if at all, with neutrophils) and is up-regulated by endothelial cells in subacute and chronic stages of inflammation and in atherosclerotic lesions. VCAM-1 requires divalent cations (eg, Ca²⁺ and Mg²⁺) for adherence to its ligand. The soluble form of VCAM-1 (sVCAM-1) has angiogenic and chemotactic activity for endothelial cells and chemotactic activity for monocytes in rheumatoid arthritis. VCAM-1 also induces T cell infiltration during synovial inflammation by an IL-2-dependent mechanism. sVCAM-1 is up-regulated in several disease states (eg, myocardial infarction, type 2 diabetes mellitus, primary antiphospholipid syndrome, and rheumatoid arthritis).

PECAM-1 (CD31) is an important 130-kd transmembrane glycoprotein immunoglobulin adhesion molecule that plays a role in leukocyte transendothelial migration. PECAM-1 is highly expressed at the intercellular junction of endothelial cells and is integral to passage of infiltrating leukocytes between endothelial cells. PECAM-1 appears to bind to PECAM-1 expressed on transmigrating leukocytes, and this type of adhesion event is critical to leukocyte egress through tight junctions. PECAM-1 has 6 Ig domains and is expressed constitutively by endothelial cells, platelets, natural killer cells, leukocytes, and certain T cell subsets. The cytoplasmic domain has numerous sites for phosphorylation of serine, threonine, and tyrosine residues and mediates inhibition of T cell receptor signaling.

As with other adhesion molecules, PECAM-1 requires divalent cations (eg, Ca²⁺ and Mg²⁺) for binding to its ligand. Bovine PECAM-1 has been sequenced, and its distribution and cellular expression have been determined by immunohistochemistry.
Therapeutic Implications of Leukocyte Trafficking

Although leukocyte infiltration into sites of infection is an important part of host defense, this infiltration, when excessive, can cause tissue damage. This event is central to the pathogenesis of various human and animal diseases. Neutrophil infiltration during posts ischemia reperfusion can cause excessive injury to the brain after infarcts or cerebral hemorrhage and to the heart after infarcts.\textsuperscript{145,146} In veterinary medicine, various diseases are characterized by extensive neutrophil infiltration associated with tissue damage (eg, reperfusion injury [gastric dilatation volvulus in dogs, prolonged recumbency, and colic], and \textit{M haemolytica} pneumonia). Platelet aggregation in laminitis, lymphocyte infiltration in autoimmune diseases, and macrophage infiltration in granulomas also are conditions that require leukocyte adherence for development. Therefore, inhibiting excessive leukocyte egress, aggregation, and their subsequent free radical-, enzymatic-, or cytokine-mediated damage may reduce tissue damage. Several methods have been used to modify the inflammatory response or leukocyte infiltration and decrease tissue damage. Inhibition of prostaglandin, leukotriene, and cytokine production reduces both the activation of inflammatory cells and endothelial cells and reduces adhesion molecule expression. Other therapies such as antibodies, peptide fragments, synthetic mimetics, and antisense oligonucleotides target adhesion molecules more specifically.

Modulation of Cell Adhesion Molecules and Leukocyte Trafficking

Glucocorticoids and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Arachidonic acids (AAs) are biologically active lipids derived from cell membrane phospholipids upon cell activation (eg, membrane phospholipase \textit{A}$_{2}$). AA metabolites include cyclooxygenase products (prostaglandins and thromboxanes) and lipoxygenase products (leukotrienes and lipoxins), both of which activate leukocytes and enhance adhesion molecule expression. Most NSAIDs target cyclooxygenase or lipoxygenase activities responsible for prostaglandin and leukotriene formation. Most, if not all, effects of NSAIDs on adhesion molecules likely occur indirectly through inhibition of these pathways, which, in turn, reduce the stimuli for adhesion molecule expression. Zileuton is a specific 5-lipoxygenase inhibitor that markedly reduces leukotriene B4 production.\textsuperscript{147} Other NSAIDs (eg, aspirin, flunixin meglumine, and ibuprofen) inhibit prostaglandin production, adhesion molecule expression, and leukocyte infiltration. More specifically, aspirin (a cyclooxygenase-1 \textit{[COX-1] inhibitor}) inhibits transcription factor kappa \textit{B},\textsuperscript{148-151} In a sheep model of skin inflammation, it has been found that flunixin meglumine is a potent cyclooxygenase inhibitor.\textsuperscript{152} Proxicyam (COX-1 inhibitor) inhibits \textit{p} integrin activation by interfering with conformational changes that occur on activation.\textsuperscript{148,153}

Glucocorticoids such as cortisol and dexamethasone reduce the production of both prostaglandins and leukotrienes by inhibition of phospholipase \textit{A}$_{2}$. This activity modulates leukocyte trafficking by reduction of chemotactic factors and limitation of leukocyte and endothelial cell activation. Cortisol and dexamethasone also reduce L-selectin expression by bovine neutrophils that normally cause leukocytosis.\textsuperscript{154} Similar effects of glucocorticoids have been demonstrated with human L-selectin expression by neutrophils.\textsuperscript{155} In one study, dexamethasone caused release of bovine \textit{yB} \textit{T} cells from peripheral blood.\textsuperscript{156}

Inhibition of Cytokines to Reduce Cell Adhesion Molecule Expression

Monoclonal Antibodies to Pro-Inflammatory Cytokines.

Monoclonal antibodies have been used to inhibit leukocyte infiltration and suppress inflammation. Anti–TNF-\textalpha monoclonal antibodies (anti–TNF-\textalpha-mAbs) markedly reduced IL-1\textbeta and IL-6 production during \textit{E coli} bacteremia\textsuperscript{157} and resulted in an overall reduction of leukocyte infiltration. Anti–TNF-\textalpha-mAbs have been used to treat the inflammatory infiltrates in arthritides and have been found to be an effective therapy because of their effectiveness in reducing IL-1\textbeta, IL-6, sCD14, and sCAM-1.\textsuperscript{158,159} Monoclonal antibodies against IL-6 prevented lethal effects and leukocyte activity of \textit{E coli} in a mouse model of septic shock.\textsuperscript{160} Other cytokine antagonists (eg, anti–IL-5-mAbs) also have been used to prevent leukocyte infiltration into airway mucosa.\textsuperscript{161,162} In addition to monoclonal antibodies, low-molecular-compound molecules have been used as anti-inflammatory compounds. For example a low-molecular-weight fraction isolated from milk of dairy cows that have been hyperimmunized with a multivalent bacterial vaccine has been evaluated.\textsuperscript{163}

Inhibition of Cytokine Receptors.

Inhibition of the various pro-inflammatory cytokines (eg, IL-1 and TNF-\alpha) and inflammatory mediators has been used as a therapeutic strategy. Pro-inflammatory cytokine receptor antagonists have been tested for their anti-inflammatory effects. IL-1 receptor (IL-1R) antagonists have been shown to reduce the inflammatory response in animal models of contact hypersensitivity, autoimmune encephalomyelitis, and arthritis.\textsuperscript{164-167} TNF-\alpha antagonists and soluble TNF-\alpha receptor administration have protective effects in endotoxic shock.\textsuperscript{168,169} In addition, inhibitory cytokines such as IL-4, soluble IL-4 receptor, soluble IL-2 receptor, \gamma IFN, and IL-10 have been demonstrated to inhibit cytokine production.\textsuperscript{170,171}

Other Methods of Cytokine Inhibition.

IL-10 and IL-4 have been used to inhibit TNF-\alpha production and subsequent lethal effects in endotoxemia and reperfusion injury.\textsuperscript{2,172-174} Other cytokine production inhibitors also have been used, such as pentoxifylline (IL-6, IL-1\textbeta, TNF-\alpha, and IL-8 inhibitor), \textit{beta}-estradiol (inhibits IL-6 production by osteoblasts), recombinant soluble CD14 (CD14 receptor antagonist), and adenosine (inhibits TNF-\alpha production).\textsuperscript{175-182} Antisense oligonucleotides (ASOs) have been used experimentally to inhibit inflammatory responses, including phospholipase \textit{A}$_{2}$ activity.\textsuperscript{183}

Inhibition of Integrins with Synthetic Peptides and Antibodies

\textit{VLA-4} and CD18 (\textit{beta}, Integrin). Inhibition of tight adhesion is another method to attenuate leukocyte infiltration.
Many studies have used monoclonal antibodies against integrins to reduce infiltration in experimental models. In a guinea pig asthma model, CD18 blockade decreased eosinophil in bronchoalveolar lavage fluid, and mAbs to CD18 reduced lesions of *E. coli* O157:H7 enteritis in a rabbit model. Monoclonal antibodies directed against VLA-4 reduced subendothelial infiltration of mononuclear cells, and reduction in leukocyte accumulation occurred after treatment with a VLA-4 mAb in experimental autoimmune encephalomyelitis (used as a model for multiple sclerosis). These types of therapies demonstrate precision in reducing leukocyte infiltration by inhibition of specific components of adhesion molecules but presently are not practical because of the expense of generating such antibodies and other potential problems (e.g., hypersensitivity reactions).

Some integrins bind the extracellular matrix, and fragments of extracellular matrix proteins have been used to inhibit leukocyte adherence. In a rabbit model, inhibition of the VLA-4 integrin binding to the CS-1 domain of fibronectin by a synthetic CS-1 peptide reduced arteriopathy by reducing lymphocyte infiltration into blood vessel walls. Synthetic fibronectin peptides synthesized from several fibronectin domains were tested in vivo and found to suppress arthritis by inhibition of extracellular matrix binding to integrins. Synthetic peptides that block VLA-4 binding to the CS-1 peptide or heparin-binding protein reduced leukocyte binding to extracellular matrix proteins and were effective in attenuating ischemia-reperfusion tissue damage. Differences associated with large-scale production of peptide fragments and hypersensitivity reactions limit the availability of these products clinically.

Leumidins such as NPC 15199 (N-(fluorenyl-9-methoxy carbonyl-L-leucine) and NPC 15669 (N-9H-(2,7-dimethyllumifluorenyl-9-methoxy carbonyl-L-leucine)) are anti-inflammatory compounds that reduce neutrophil infiltration by inhibiting Mac-1 expression on neutrophils in vivo and T lymphocyte activation in vitro. NPC 15199 has been shown to attenuate ileitis in a guinea pig model, and NPC 15669 markedly reduced neutrophil infiltration in an acetic acid-induced model of colitis in rats.

### Inhibition of Selectins

Inhibition of the 1st steps in leukocyte trafficking may reduce leukocyte tight adhesion, infiltration, and subsequent associated tissue damage. Various therapeutic agents have been used to block this step. Rolling can be inhibited by antibodies to leukocyte L-selectin. L-selectin inhibition with mAbs decreases ischemia-reperfusion injury. Mulligan et al. demonstrated that anti-L-selectin mAbs reduced immune complex-induced pulmonary injury. Cortisol and dexamethasone reduce the activity of phospholipase A2 and dexamethasone down-regulates circulating and endothelial concentration of ICAM-1 and VCAM-1, and inhibits rhinovirus-induced ICAM-1 up-regulation in the lung. ASOs have been used to inhibit ICAM-1 and VCAM-1 expression. ASOs also have been effective in reducing activity of ICAM-1 and VCAM-1. Inhibition of ICAM-1 expression by ASOs after acute ischemia in renal reperfusion injury and renal failure has been evaluated. Also, ICAM-1 ASOs have been used to prevent rejection in cardiac allografts. In a mouse model of delayed-type hypersensitivity, ICAM-1 ASOs reduced ICAM-1 mRNA expression.

In summary, leukocyte adhesion molecules are vital for health and play important roles in disease prevention. Their molecular characterization and functional activities are becoming better understood. This review has highlighted the features of some leukocyte adhesion molecules of veterinary importance. Taking advantage of these properties in the development of therapeutic strategies for diseases of
veterinary importance will require an understanding of these molecules and an insight to disease pathogenesis. Deficiencies of leukocyte adhesion molecules can lead to serious health problems and even death. However, excessive tissue infiltration by leukocytes often leads to tissue damage. Methods to alter alternate tissue leukocyte infiltration by means of compounds that regulate expression or activity of leukocyte adhesion molecules still are developing and have potential benefits as adjuncts to current clinical therapies.

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