11-2001

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

Zaher A. Radi
Iowa State University

Marcus E. Kehrli Jr.
United States Department of Agriculture

Mark R. Ackermann
Iowa State University, mackerma@iastate.edu

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 β_2 integrins after leukocyte exposure to cytokines and proinflammatory mediators, (2) adherence of leukocyte β_2 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 β_1 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 $\beta_2$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
This article is from *Journal of Veterinary Internal Medicine* 15, no. 6 (November 2001): 516–529, doi:10.1111/j.1939-1676.2001.tb01586.x.

Review

J Vet Intern Med 2001;15:516–529

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

Zaher A. Radi, Marcus E. Kehrli, Jr., and Mark R. Ackermann

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 β_2 integrins after leukocyte exposure to cytokines and pro-inflammatory mediators, (2) adherence of leukocyte β_2 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 β_1 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 β_2 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.

Key words: Inflammation; Integrins; Intercellular adhesion molecule-1 (ICAM-1); Neutrophils; Selectins.

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, O_2^-, 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 extensive 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.

From the Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA (Radi, Ackermann); and the Metabolic Diseases and Immunology Research Unit, USDA-ARS/National Animal Disease Center, Ames, IA (Kehrli). Dr Radi is presently affiliated with Virginia-Maryland Regional College of Veterinary Medicine, Department of Biomedical Sciences and Pathobiology, Blacksburg, VA. Dr Kehrli is presently affiliated with Animal Health Research, Pfizer, Inc, P.O. Box 88, 9303 South Carlisle Street, Terre Haute, IN.

Reprint requests: Mark R. Ackermann, DVM, PhD, DACVP, Department of Veterinary Pathology, 2738 Veterinary Medicine, Iowa State University, Ames, IA 50011-1250; e-mail: mackerma@iastate.edu.

Submitted October 27, 2000; Revised February 15, 2001; Accepted June 1, 2001.

0891-6640/01/1506-0002/$3.00/0

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 β_2 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, β_1 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 β_2 integrins by leukocytes and transmigration by neutrophils through the vascular endothelium into sites of infection can be markedly impaired.[1,2] Affected animals often develop severe infections of mucosal surfaces and die

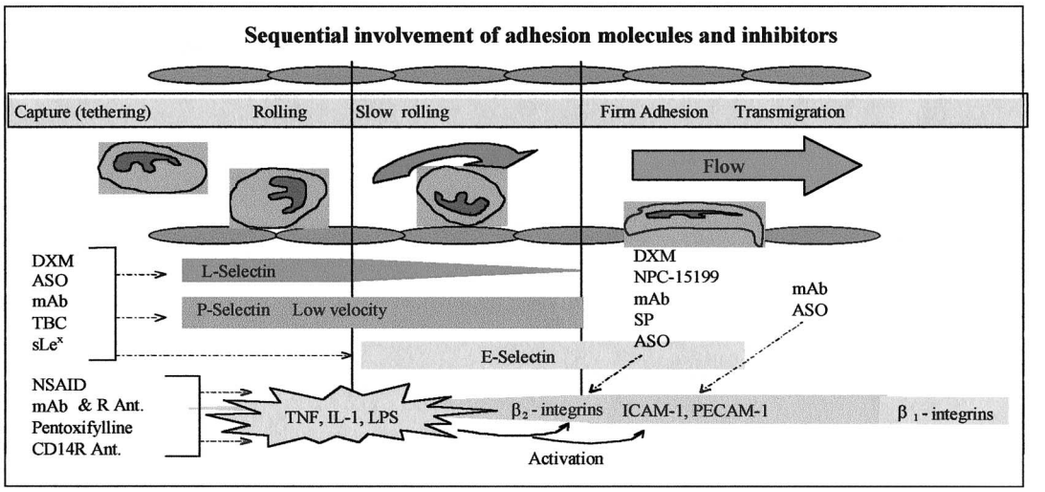

Fig 1. Phases of leukocyte infiltration from the vascular space into sites of infection and various adhesion molecules involved and modulation of these adhesion molecules are outlined in each phase. In the 1st phase, tethering occurs via L- and P-selectin, followed by selectin-mediated rolling in which 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 (sLe^x). Tight adhesion between the expressed β_2 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 β_1 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 β_2 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 β_2 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 β_2 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 (Ca^{+2}). 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.[8,9] 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, cattle, dog, cat, rabbit, mouse, sheep, and pig) express L-selectin,[8,14–24] 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.[26–29] 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.[30,31] Selectin-mediated leukocyte rolling on vascular endothelium also can be inhibited by antibodies to leukocyte L-selectin[32] 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.[33,34] 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.[35] This process occurs by fusion of secretory granules with the plasma membrane.[36] 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 sLe^x 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.[37] 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.[34,38]

P-selectin has been studied in humans and many animals (dog, cat, cattle, and sheep).[39–44] In the dog, P-selectin expression on endothelial cells is increased in various skin diseases,[45] and in both lambs and cats, inhibition of P-selectins protects the myocardium from postischemic reperfusion injury.[41,44] A soluble form of P-selectin has been found in the circulation of healthy humans.[46] 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).[46–50] 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.[51] 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 sLe^x and sLe^a oligosaccharides and E-selectin ligand-1 (ESL-1) on leukocytes are the ligands for E-selectin.[52] 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-$_K$ B (NF-$_K$ B) is involved in E-selectin activation.[53] After expression, E-selectin is internalized by endocytosis and delivered to lysosomes for degradation (Table 1).[54]

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, Ca^{+2} and Mg^{+2}) 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.[55] 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 β_1 through β_8 integrins (Table 1).

The β_1 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 β_1 integrins are expressed by leukocytes and are important for leukocyte infiltration into perivascular stroma.[56] β_1 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 β_1 integrins. Several cytoplasmic domains of the β_1 integrin subunit interact with cytoskeletal actin filaments via α-actinin.[57] Such interactions of integrins with the leukocyte cytoskeleton are important for chemotaxis of leukocytes across the perivascular adventitia and extracellular matrix.

VLA-4 (the $\alpha_4\beta_1$ 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)[58] and the fibronectin RGD domain.[59] 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 $\alpha_5\beta_1$ integrin is a major fibronectin receptor that is regulated by divalent cations (eg, Mg^{+2} and Ca^{+2}).[60] The $\alpha_5\beta_1$ integrin plays a role in repair of airway epithelium after injury.[61] Also, it has been found that the IpaB, IpaC, and IpaD proteins of *Shigella flexneri* can interact with the $\alpha_5\beta_1$ integrin and may facilitate leukocyte trafficking in the alimentary tract.[62]

The β_2 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.[63,64] 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 β_2 integrin expression. Leukocyte function antigen-1 (LFA-1; $\alpha_L\beta_2$; 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.[a]

Common name	CD	Expressed on	Binds	Expressed on
Integrins				
β_1 integrins:	(CD29):			
VLA-1	(CD49a)	ec	Collagen	ECM
VLA-2	(CD49b)	ec	Collagen	ECM
VLA-3	(CD49c)	ec	Fibronectin	ECM
VLA-4	(CD49d)	L	VCAM-1	ec
VLA-5	(CD49e)	ec	Fibronectin	ECM
VLA-6	(CD49f)	ec	Laminin	ECM
β_2 integrins:	(CD18):			
LFA-1	(CD11a)	L	ICAM-1	ec
Mac-1	(CD11b)	L	ICAM-1	ec, L
gp150,95	(CD11c)	L	ICAM-1	ec
$\alpha_d\beta_2$	(CD11d)	MQ, CD8 T cells	ICAM-3	L, ec
β_3 integrins:	(CD61):			
$\alpha_{IIb}\beta_3$		Platelets	Fibrinogen	ec
$\alpha_v\beta_3$		ec, MQ, M	Fibronectin	ECM
β_4 integrins:				
$\alpha_6\beta_4$		ec, SC	Laminin	ECM
β_5 integrins:				
$\alpha_v\beta_5$		ec, epithelia	Vitronectin	ECM
β_6 integrins:				
$\alpha_v\beta_6$		epithelia	Fibronectin	ECM
β_7 integrins:				
α_4		ec, Lympho	MAdCAM-1, VCAM-1	ec
α_e		Intraepith Lympho	E-cadherin	ec
β_8 integrins:				
$\alpha_v\beta_8$			Laminin	ECM
Immunoglobulin superfamily				
ICAM-1	(CD54)	ec, epithelia, SC PMN, MQ	β_2-integrins	L
ICAM-2	(CD102)	ec, Lympho	β_2-integrins	L
ICAM-3	(CD50)	ec, Lympho, PMN, M	β_2-integrins	L
ICAM-4		Red blood cells	β_2-integrins	L
ICAM-5		Subset of neurons	β_2-integrins	L
VCAM-1	(CD106)	ec	VLA-4	L (except PMN)
PECAM-1	(CD31)	ec, L	PECAM-1	ec, L
MAdCAM-1		ec (mucosal)	L-selectin $\alpha_4\beta_7$-integrin	PMN, Lympho
Selectins				
L-selectin	(CD62L)	L	GlyCAM-1, CD34, MAdCAM-1	ec
E-selectin	(CD62E)	ec	ESL-1, sLe^x	L
P-selectin	(CD62P)	ec, platelets	PSGL-1, GlyCAM-1, sLe^x	ec, P

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; sLe^x, sialyl lewis x; see text for definition of adhesion molecule abbreviations.

[a] This table is not comprehensive.

($\alpha_M\beta_2$; CD11b/CD18) and gp150,95 ($\alpha_X\beta_2$; CD11c/CD18) are expressed by macrophages and granulocytes and also bind ICAM-1 but not ICAM-3.[65] 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 ($\alpha_d\beta_2$) heterodimer on leukocytes has homology to human $\alpha_d\beta_2$.[66] In contrast with other β_2 integrins, the $\alpha_d\beta_2$ integrin binds only to ICAM-3.[66,68] Although the $\alpha_d\beta_2$ integrin has not been described in cattle, bovine ICAM-3 has been sequenced from a mammary gland cDNA library (Table 1).[69]

Deficiency in β_2 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 β_2 integrin.[2,70,71] 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.[1] 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.[2,70] 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 I varies in severity, depending on the location of the CD18 mutation. People with severe phenotype (expressing $<1\%$ of functional β_2 integrin) often are susceptible to severe infections, whereas those with moderate phenotype (3–10% expression) can live to adulthood.[3]

In the lung, β_2 integrins mediate neutrophil infiltration into various microanatomic regions. Initial neutrophil infiltration into bronchi and bronchioles requires functional β_2 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 β_1 integrins ($\alpha_4\beta_1$ [VLA-4] and $\alpha_5\beta_1$ [VLA-5]), β_2 integrins, and selectins.[74] Stiffening of neutrophils by actin polymerization is a physical change that further enhances neutrophil sequestration in alveolar capillaries.[74] The type of inoculum 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 β_2 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 β_2 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 β_3 integrins (CD61) include $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. The β_3 integrins generally mediate adherence to extracellular matrix ligands and clotting factors. β_3 integrins recognize fibronectin, fibrinogen, vitronectin, and von Willebrand factor (vWF).

The $\alpha_{IIb}\beta_3$ integrin is expressed by platelets and has a major role in platelet aggregation, which results from fibrinogen or vWF binding to α_{IIb}-β_3 complexes.[78] Mutations in the α or β subunits of the $\alpha_{IIb}\beta_3$ integrin may lead to increased susceptibility to bleeding as described in Glanzmann thrombasthenia, a heterozygous disease in humans.[79] Homozygous 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 antithrombolytic treatments of myocardial infarction, stroke, phlebitis, and reperfusion injury.

The $\alpha_v\beta_3$ 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 $\alpha_v\beta_3$ 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 $\alpha_6\beta_4$ integrin is a laminin and kalinin receptor expressed by various cell types (eg, epithelial cells, endothelial cells, and Schwann cells).[86–88] The $\alpha_6\beta_4$ integrin may play a role in epithelial integrity by forming a link between the plasma membrane and the basement membrane.[89,90] Loss of cellular adherence to laminin and adjacent cells leads to enhanced cellular invasion by neoplastic cells. The $\alpha_6\beta_4$ integrin may be important in tumor progression by facilitating a phosphatidylinositol 3-kinase (PI_3K)-dependent invasion via cooperative interaction between the $\alpha_6\beta_4$ integrin and *erb* B-2.[91]

The $\alpha_4\beta_7$ 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 $\alpha_4\beta_7$ 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 $\alpha_4\beta_7$ 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 β_2 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

plays a role in the pathogenesis of inflammation in the peripheral nerve.[99] Neutrophils and macrophages express ICAM-1 in acute *M haemolytica* pneumonia in calves.[100] 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.[100,101] Immunoglobulin (Ig) domain 3 of ICAM-1 mediates Mac-1 binding of the β_2 integrin.[102,103] ICAM-1 requires divalent cations (eg, Ca^{+2} and Mg^{+2}) for adherence to its ligand.[103] Increased ICAM-1 expression occurs by 4 hours and remains high at 24 hours after pro-inflammatory stimuli. ICAM-1 expression is important in leukocyte infiltration in sites of tissue injury (Fig 1) and, because of its widespread expression, ICAM-1 mediates leukocyte passage across the vascular endothelium, skin, and between mucosal epithelial cells. Mice with deficient ICAM-1 expression have impaired leukocyte migration during inflammation.[104]

Humans, dogs, mice, chimpanzees, cattle, and other species express ICAM-1.[101–103] Cysteine residues that are important for the Ig domain fold structure are highly conserved in ICAM-1 in all species.[103] Bovine ICAM-1 contains increased numbers of serine residues in the cytoplasmic tail in comparison to human, canine, or murine ICAM-1.[101] The functional significance of the increase in serine residues in bovine ICAM-1 is not known. A soluble form of ICAM-1 (sICAM-1) plays a role in neutrophil adhesion.[105] sICAM-1 is released by mononuclear and endothelial cells in patients with arthritis and release correlates with the quantity of rheumatoid factors, soluble IL-2 receptor, and disease severity.[106,107] Also, increased concentrations of sICAM-1 have been observed during myocardial infarction.[108] Increased concentrations of sICAM are therefore useful in monitoring clinical severity of myocardial infarction or recurrence of disease in such patients. Certain infectious agents, such as the human rhinovirus, can bind ICAM-1.[109] Aerosols of ICAM-1 delivered to the nasal mucosa can reduce adherence of rhinovirus and decrease the severity of clinical signs of cold.[109,110] In addition, *Plasmodium falciparum* induces binding of infected red blood cells to ICAM-1, which is crucial in development of cerebral malaria.

ICAM-2 has 2 Ig domains and is present at low concentrations on resting endothelium, T lymphocytes, and B lymphocytes. ICAM-2 expression is not up-regulated by cytokines.[111] LFA-1 of the β_2 integrin family is the predominant ligand for ICAM-2 expressed by resting endothelial cells.[111] Thus, ICAM-2 complements ICAM-1 in the binding of β_2 integrins and leukocyte adherence.

ICAM-3 is a highly glycosylated protein with 5 Ig domains that is expressed strongly on resting lymphocytes, neutrophils, and monocytes.[112] Bovine ICAM-3 mRNA is expressed predominantly by resting neutrophils and, to a lesser extent, by activated neutrophils.[101] Because of a lack of appropriate antibodies, no actual determination of ICAM-3 protein expression by bovine cells has been reported, and a specific function for ICAM-3 has not been identified in cattle. However, because leukocyte accumulation during mastitis is marked and cDNA for bovine 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.[113,114] ICAM-4 (originally named Landsteiner-Wiener [LW] glycoprotein) has 2 Ig domains and is expressed on red blood cells.[112] It binds CD11a/CD18 on neutrophils and lymphocytes.[113,114] The LW antigen is a red cell membrane glycoprotein that requires divalent cations (eg, Mg^{2+}) for its reactivity.[115]

ICAM-5 (telencephalin) has 9 Ig domains and a molecular weight of 130 kd.[116] It is a glycoprotein that is expressed by a subset of neurons (somato-dendritic) within the telencephalon of the mammalian brain.[117–121] ICAM-5 can bind to CD11a/CD18 expressed by T lymphocytes.[121,122] ICAM-5 immunoreactivity is decreased in Alzheimer's patients.[123] A soluble form of ICAM-5 is increased in patients with acute encephalitis and has potential in monitoring severity of disease.[124]

VCAM-1 (CD106) is another member of the immunoglobulin gene superfamily and has 5 Ig domains.[125] VCAM-1 is expressed at high concentration after LPS administration and cytokine activation (eg, IL-1 and TNF-α) on all vascular endothelial cells.[126–129] This expression 1st appears 4–6 hours after stimulation and reaches maximal concentration at 12–18 hours.[127] 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.[127] VCAM-1 requires divalent cations (eg, Ca^{+2} and Mg^{+2}) for adherence to its ligand.[129] The soluble form of VCAM-1 (sVCAM-1) has angiogenic and chemotactic activity for endothelial cells and chemotactic activity for monocytes in rheumatoid arthritis.[130,131] sVCAM-1 also induces T cell infiltration during synovial inflammation by an IL-2–dependent mechanism.[132] sVCAM-1 is up-regulated in several disease states (eg, myocardial infarction, type 2 diabetes mellitus, primary antiphospholipid syndrome, and rheumatoid arthritis).[131–135]

PECAM-1 (CD31) is an important 130-kd transmembrane glycoprotein immunoglobulin adhesion molecule that plays a role in leukocyte transendothelial migration.[136] 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.[137] PECAM-1 has 6 Ig domains[138–140] and is expressed constitutively by endothelial cells, platelets, natural killer cells, leukocytes, and certain T cell subsets.[136,137] The cytoplasmic domain has numerous sites for phosphorylation of serine, threonine, and tyrosine residues and mediates inhibition of T cell receptor signaling.[137] As with other adhesion molecules, PECAM-1 requires divalent cations (eg, Ca^{+2} and Mg^{+2}) for binding to its ligand.[141–143] Bovine PECAM-1 has been sequenced, and its distribution and cellular expression have been determined by immunohistochemistry.[144]

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 postischemia reperfusion can cause excessive injury to the brain after infarcts or cerebral hemorrhage and to the heart after infarcts.[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 *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 A_2). AA metabolites include cyclooxygenase products (prostaglandins and thromboxanes) and lipooxygenase products (leukotrienes and lipoxins), both of which activate leukocytes and enhance adhesion molecule expression. Most NSAIDs target cyclooxygenase or lipooxygenase 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-lipooxygenase inhibitor that markedly reduces leukotriene B4 production.[147] Other NSAIDs (eg, aspirin, flunixin meglumine, and ibuprofen) inhibit prostaglandin production, adhesion molecule expression, and leukocyte infiltration. More specifically, aspirin (a cyclooxygenase-1 [COX-1] inhibitor) inhibits transcription factor kappa B.[148–151] In a sheep model of skin inflammation, it has been found that flunixin meglumine is a potent cyclooxygenase inhibitor.[152] Piroxicam (COX-1 inhibitor) inhibits β_2 integrin activation by interfering with conformational changes that occur on activation.[148,153]

Glucocorticoids such as cortisol and dexamethasone reduce the production of both prostaglandins and leukotrienes by inhibition of phospholipase 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.[154] Similar effects of glucocorticoids have been demonstrated with human L-selectin expression by neutrophils.[155] In one study, dexamethasone caused release of bovine γδ T cells from peripheral blood.[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-α monoclonal antibodies (anti–TNF-α-mAbs) markedly reduced IL-1β and IL-6 production during *E coli* bacteremia[157] and resulted in an overall reduction of leukocyte infiltration. Anti–TNF-α-mAbs have been used to treat the inflammatory infiltrates in arthritis and have been found to be an effective therapy because of their effectiveness in reducing IL-1β, IL-6, sCD14, and sICAM-1.[158,159] Monoclonal antibodies against IL-6 prevented lethal effects and leukocyte activity of *E coli* in a mouse model of septic shock.[160] Other cytokine antagonists (eg, anti–IL-5-mAbs) also have been used to prevent leukocyte infiltration into airway mucosa.[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.[163]

Inhibition of Cytokine Receptors. Inhibition of the various pro-inflammatory cytokines (eg, IL-1 and TNF-α) 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.[164–167] TNF-α antagonists and soluble TNF-α receptor administration have protective effects in endotoxic shock.[168,169] In addition, inhibitory cytokines such as IL-4, soluble IL-4 receptor, soluble IL-2 receptor, γIFN, and IL-10 have been demonstrated to inhibit cytokine production.[170,171]

Other Methods of Cytokine Inhibition. IL-10 and IL-4 have been used to inhibit TNF-α production and subsequent lethal effects in endotoxemia and reperfusion injury.[52,172–174] Other cytokine production inhibitors also have been used, such as pentoxifylline (IL-6, IL-1β, TNF-α, and IL-8 inhibitor), 17 beta-estradiol (inhibits IL-6 production by osteoblasts), recombinant soluble CD14 (CD14 receptor antagonist), and adenosine (inhibits TNF-α production).[175–182] Antisense oligonucleotides (ASOs) have been used experimentally to inhibit inflammatory responses, including phospholipase A_2 activity.[183]

Inhibition of Integrins with Synthetic Peptides and Antibodies

VLA-4 and CD18 (β_2 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 eosinophils in bronchoalveolar lavage fluid,[184] and mAbs to CD18 reduced lesions of *E coli* O157:H7 enteritis in a rabbit model.[185] Monoclonal antibodies directed against VLA-4 reduced subendothelial infiltration of mononuclear cells,[186] and reduction in leukocyte accumulation occurred after treatment with a VLA-4 mAb in experimental autoimmune encephalomyelitis (used as a model for multiple sclerosis).[187] 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 (eg, 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.[188] 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.[189] 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.[190] Difficulties 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-methoxycarbonyl-L-leucine]) and NPC 15669 (N-9H-[2,7-dimethylfluorenyl-9-methoxycarbonyl-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.[191–194] NPC 15199 has been shown to attenuate ileitis in a guinea pig model,[195] and NPC 15669 markedly reduced neutrophil infiltration in an acetic acid-induced model of colitis in rats.[196]

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.[33] L-selectin inhibition with mAbs decreases ischemia-reperfusion injury.[19] Mulligan et al[21] demonstrated that anti–L-selectin mAbs reduced immune complex-induced pulmonary injury. Cortisol and dexamethasone reduce the activity of phospholipase A_2 and down-regulate neutrophil L-selectin expression by bovine[15,154] and human neutrophils.[155] Similarly, P- and E-selectin inhibition with mAbs has been found to reduce leukocyte infiltration.[197–199] Inhibition of P-selectin with an mAb has been found to reduce ischemia and reperfusion injury.[198] In the dog, mAbs against P-selectin inhibited neutrophil rolling on endothelial cells.[41] Monoclonal antibodies against E-selectin decrease myocardial-reperfusion injury.[197] ISIS 4730 is an ASO that inhibits E-selectin mRNA and protein by altering the mRNA splicing process.[200]

Selectin ligand (sLe^x) inhibition also may reduce leukocyte damage. In a guinea pig model of pulmonary injury, pretreatment with anti–sLe^x mAbs resulted in decreased neutrophil infiltration and reduced pulmonary injury.[201] Other investigators demonstrated selectin inhibition and attenuation of tissue injury in sepsis with sLe^x.[202,203] Ridings et al[204] demonstrated that treatment with a synthetic sLe^x analog attenuated pulmonary injury after *Pseudomonas aeruginosa* infection, as measured by improved arterial oxygenation and various parameters of inflammation. In a mouse model of neuronal injury, glycosylated sLe^x treatment protected against leukocyte and platelet infiltration and neuronal injury.[205]

A novel synthetic inhibitor of selectins, TBC1269, has been developed by glycosylation and hydrolysis. TBC1269 (1,6-bis[3-(3-carboxymethylphenyl)-4-(2-α-D-mannopyranosyloxy)phenyl]hexane) is a nonoligosaccharide selectin antagonist that contains 2 mannose and 2 carboxylates and has been tested in vitro.[206] It inhibits sLe^x-dependent binding of HL-60 cells to E-, P-, and L-selectins and has enhanced activity when compared to monomeric compounds.[206] We have found that in an in vivo calf model, TBC1269 decreases neutrophil infiltration into pulmonary alveoli and decreases protein concentration in bronchoalveolar lavage fluid during acute *M haemolytica* pneumonia.[207,208] TBC1269 may provide an important method for attenuating detrimental aspects of the acute inflammatory process without eliminating the process entirely.[207] In a sheep model of asthma, TBC1269 also markedly reduced eosinophil infiltration.[209] In this model, other adhesion molecule inhibitors such as anti–VLA-4 and the drug Zileuton have been effective in reducing asthma.[210–212]

Inhibition of ICAMs and VCAM-1

Monoclonal antibodies to ICAM-1 reduced renal leukocyte infiltration and proteinuria.[213] Wong et al[214] demonstrated reduced severity of colitis in a rat model with mAbs against ICAM-1. Leukocyte infiltration decreased after administration of anti–ICAM-1 antibodies in monkeys with renal allograft transplantation.[215] Dexamethasone down-regulates circulating and endothelial concentration of ICAM-1[216,217] and ICAM-3[218] and inhibits rhinovirus-induced ICAM-1 up-regulation in the lung.[219] ASOs have been used to inhibit ICAM-1 and VCAM-1 expression.[220] ASOs also have been effective in reducing activity of ICAM-1 and VCAM-1.[220] Inhibition of ICAM-1 expression by ASOs after acute ischemia in renal reperfusion injury and renal failure has been evaluated.[221–224] Also, ICAM-1 ASOs have been used to prevent rejection in cardiac allografts.[225] In a mouse model of delayed-type hypersensitivity, ICAM-1 ASOs reduced ICAM-1 mRNA expression.[226]

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 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.

References

1. Kijas JMH, Bauer TR Jr, Gafvert S, et al. A missense mutation in the β_2-integrin gene (ITGB2) causes canine leukocyte adhesion deficiency. Genomics 1999;61:101–107.
2. Kehrli ME Jr, Ackermann MR, Shuster DE, et al. Animal model of human disease: Bovine leukocyte adhesion deficiency: β_2 integrin deficiency in young Holstein cattle. Am J Pathol 1992;140:1489–1492.
3. Anderson DC, Springer TA. Leukocyte adhesion deficiency: An inherited defect of Mac-1, LFA-1, and p159,95 glycoproteins. Annu Rev Med 1987;35:175–221.
4. Kuijpers TW, van Lier RAW, Hamann D, et al. Leukocyte adhesion deficiency type 1 (LAD-1)/variant. A novel immunodeficiency syndrome characterized by dysfunctional β_2 integrins. J Clin Invest 1997;100:1725–1734.
5. Etzioni A, Frydman M, Pollack S, et al. Recurrent severe infections caused by a novel leukocyte adhesion deficiency. N Engl J Med 1992;327:1789–1792.
6. Delisser HM, Christofidou-Solomidou M, Sun J, et al. Loss of endothelial surface expression of E-selectin in a patient with recurrent infections. Blood 1999;94:884–894.
7. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest 1993;91: 379–387.
8. Butcher EC. Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. Cell 1991;67:1033–1036.
9. Walcheck B, White M, Kurk S, et al. Characterization of the bovine peripheral lymph node homing receptor: A lectin cell adhesion molecule (LECAM). Eur J Immunol 1992;22:469–476.
10. Lasky LA. Selectins: Interpreters of cell specific carbohydrate information during inflammation. Science 1992;258:964–969.
11. Tedder TF, Steeber DA, Chen A, Engel P. The selectins: Vascular adhesion molecules. FASEB J 1995;9:866–873.
12. Li X, Steeber DA, Tang MLK, et al. Regulation of L-selectin–mediated rolling through receptor dimerization. J Exp Med 1998;188: 1385–1390.
13. Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999;79:181–213.
14. Burton JL, Kehrli ME Jr. Regulation of neutrophil adhesion molecules and shedding of *Staphylococcus aureus* in milk of cortisol- and dexamethasone-treated cows. Am J Vet Res 1995;56:997–1006.
15. Crockett-Torabi E, Fantone JC. L-selectin stimulation of canine neutrophil initiates calcium signal secondary to tyrosine kinase activation. Am J Physiol 1997;272(3 Pt 2):H1302–H1308.
16. Kashima T, Hasegawa A, Iwata H, Inoue T. Expression of recombinant bovine L-selectin in *Escherichia coli* and insect cells. J Vet Med Sci 1999;61:251–254.
17. Kubo H, Doyle NA, Graham L, et al. L- and P-selectin and CD11/CD18 in intracapillary neutrophil sequestration in rabbit lungs. Am J Respir Crit Care Med 1997;159:267–274.
18. Ma X-L, Weyrich AS, Lefer DJ, et al. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. Circulation 1993;88:649–658.
19. Monfardini E, Burvenich C, Massart-Leen AM, et al. Effect of antibiotic induced bacterial clearance in the udder on L-selectin shedding of blood neutrophils in cows with Escherichia coli mastitis. Vet Immunol Immunopathol 1999;67:373–384.
20. Mulligan MS, Miyasaka M, Tamatani T, et al. Requirements for L-selectin in neutrophil-mediated lung injury in rats. J Immunol 1994; 152:832–840.
21. Sakurai H, Schmalstieg FC, Traber LD, et al. Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep. J Appl Physiol 1999;86:1151–1159.
22. Shailubhai K, Streeter PR, Smith CE, Jacob GS. Sulfation and sialylation requirements for a glycoform of CD34, a major endothelial ligand for L-selectin in porcine peripheral lymph nodes. Glycobiology 1997;7:305–314.
23. Tedder TF, Steeber DA, Pizcueta P. L-selectin–deficient mice have impaired leukocyte recruitment into inflammatory sites. J Exp Med 1995;181:2259–2264.
24. Tsurushita N, Fu H, Berg EL. PCR cloning of the cDNA encoding baboon L-selectin. Gene 1996;28:219–220.
25. Bosworth BT, Harp JA. Evidence for conservation of peripheral lymphocyte homing receptor between the bovine, murine and human species. Vet Immunol Immunopathol 1992;33:79–88.
26. Jutila MA, Kurk S. Analysis of bovine gamma delta T cell interactions with E-, P-, and L-selectin. Characterization of lymphocyte on lymphocyte rolling and effects of O-glycoprotease. J Immunol 1996;156:289–296.
27. Leid JG, Speer CA, Jutila MA. Comparative ultrastructure and expression of L-selectin on bovine alpha beta and gamma delta T cells. J Leukoc Biol 1998;64:104–107.
28. Walcheck B, Jutila MA. Bovine gamma delta T cells express high levels of functional peripheral lymph node homing receptor (L-selectin). Int Immunol 1994;6:81–91.
29. Wilson E, Walcheck B, Davis WC, Jutila MA. Preferential localization of bovine gamma delta T cell subsets defined by anti-T cell receptor for antigen antibodies. Immunol Lett 1998;64:39–44.
30. Steeber DA, Tang ML, Green NE, et al. Leukocyte entry into sites of inflammation requires overlapping interactions between the L-selectin and ICAM-1 pathways. J Immunol 1999;163:2176–2186.
31. Schleiffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. J Cell Biol 1992;119:229–238.
32. Von Andrian UH, Chambers JD, McEvory LM, et al. Two-step model of leukocyte endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte β2 integrins in vivo. Proc Natl Acad Sci USA 1991;88:7538–7542.
33. Geng J-G, Bevilacqua MP, Moore KL, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 1990;343:757–760.
34. McEver RP, Beckstead JH, Moore KL, et al. GMP-140, a platelet α-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989;84:92–99.
35. Fujimoto T, McEver RP. The cytoplasmic domain of P-selectin is phosphorylated on serine and threonine residues. Blood 1993;82: 1758–1766.
36. Burns AR, Bowden RA, Abe Y, et al. P-selectin mediates neutrophil adhesion to endothelial cell borders. J Leukoc Biol 1999;65: 299–306.
37. Setiadi H, Sedgewick G, Erlandsen SL, McEver RP. Interactions of the cytoplasmic domain of P-selectin with clathrin-coated pits enhance leukocyte adhesion under flow. J Cell Biol 1998;142:859–871.
38. Green SA, Setiadi H, McEver RP, Kelly RB. The cytoplasmic domain of P-selectin contains a sorting determinant that mediates rapid degradation in lysosomes. J Cell Biol 1994;124:435–448.
39. Buerke M, Weyrich AS, Zheng Z, et al. Sialyl Lewis^x-containing oligosaccharide attenuates myocardial reperfusion injury in cats. J Clin Invest 1994;93:1140–1148.
40. Dore M, Simon SI, Hughes BJ, et al. P-selectin and CD18-mediated recruitment of canine neutrophils under conditions of shear stress. Vet Pathol 1995;32:258–268.
41. Miura T, Nelson DP, Schermerhorn ML, et al. Blockade of se-

lectin-mediated leukocyte adhesion improves postischemic function in lamb hearts. Ann Thorac Surg 1996;62:1295–1300.

42. Strubel NA, Nguyen M, Kansas GS, et al. Isolation and characterization of a bovine cDNA encoding a functional homolog of human P-selectin. Biochem Biophys Res Commun 1993;192:338–344.

43. Wang Y, Paape MJ, Leino L, et al. Functional and phenotypic characterization of monoclonal antibodies to bovine L-selectin. Am J Vet Res 1997;58:1392–1401.

44. Weyrich AS, Ma XY, Lefer DJ, et al. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 1993;91:2620–2629.

45. Chenier S, Dore M. P-selectin expression in canine cutaneous inflammatory diseases and mast cell tumors. Vet Pathol 1998;35:85–93.

46. Katayama M, Handa M, Araki Y, et al. Soluble P-selectin is present in normal circulation and its plasma level is elevated in patients with thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. Br J Haematol 1993;84:702–710.

47. Davi G, Romano M, Mezzetti A, et al. Increased levels of soluble P-selectin in hypercholesterolemic patients. Circulation 1998;97: 953–957.

48. Ertenli I, Kiraz S, Arici M, et al. P-selectin as a circulating molecular marker in rheumatoid arthritis with thrombocytosis. J Rheumatol 1998;25:1054–1058.

49. Ferroni P, Basili S, Martini F, et al. Soluble P-selectin as a marker of platelet hyperactivity in patients with chronic obstructive pulmonary disease. J Investig Med 2000;48:21–27.

50. Xu DY, Zhao SP, Peng WP. Elevated plasma levels of soluble P-selectin in patients with acute myocardial infarction and unstable angina. An inverse link to lipoprotein(a). Int J Cardiol 1998;64:253–258.

51. Huber TS, Gaines GC, Welborn MB III, et al. Anticytokine therapies for acute inflammation and the systemic inflammatory response syndrome: IL-10 and ischemia/reperfusion injury as a new paradigm. Shock 2000;13:425–434.

52. Phillips ML, Nudelman E, Gaeta FCA, et al. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. Science 1990;250:1130–1132.

53. Read MA, Whitely MZ, Williams AJ, Collins T. NF-kappa B and I kappa B alpha: An inducible regulatory system in endothelial activation. J Exp Med 1994;179:503–512.

54. Subramaniam M, Koedam JA, Wagner DD. Divergent fates of P- and E-selectins after their expression on the plasma membrane. Mol Biol Cell 1993;4:791–801.

55. Berman AE, Kozlova NI. Integrins: Structure and functions. Membr Cell Biol 2000;13:207–244.

56. Shimizu Y, Newman Y, Tanaka Y, Shaw S. Lymphocyte interactions with endothelial cells. Immunol Today 1992;13:106–112.

57. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. FASEB J 1990;4:2868–2880.

58. Guan J-L, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor $\alpha_4\beta_1$. Cell 1990;60:53–61.

59. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.

60. Takamatsu Y, Simmons PJ, Levesque JP. Dual control by divalent cations and mitogenic cytokines of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrin avidity expressed by human hemopoietic cells. Cell Adhes Commun 1998; 5:349–366.

61. Herard AL, Pierrot D, Hinnrasky J, et al. Fibronectin and its $\alpha_5\beta_1$-integrin receptor are involved in the wound-repair process of airway epithelium. Am J Physiol 1996;271(5 Pt 1):L726–L733.

62. Watarai M, Funato S, Sasakawa C. Interaction of Ipa proteins of *Shigella flexneri* with $\alpha_5\beta_1$ integrin promotes entry of the bacteria into mammalian cells. J Exp Med 1996;183:991–999.

63. Bilsland CA, Springer TA. Cloning and expression of the chicken CD18 cDNA. J Leukoc Biol 1994;55:501–506.

64. Shuster DE, Bosworth BT, Kehrli ME Jr. Sequence of the bovine CD18-encoding cDNA: Comparison with the human and murine glycoproteins. Gene 1992;114:267–271.

65. Arnaout MA. Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood 1990;75:1037–1050.

66. Danilenko DM, Rossitto PV, Van der Vieren M, et al. A novel canine leukointegrin, alpha d beta 2, is expressed by specific macrophage subpopulations in tissue and a minor CD8+ lymphocyte subpopulation in peripheral blood. J Immunol 1995;155:35–44.

67. el-Gabalawy H, Canvin J, Ma GM, et al. Synovial distribution of αd/CD18, a novel leukointegrin. Comparison with other integrins and their ligands. Arthritis Rheum 1996;39:1913–1921.

68. Van der Vieren M, LE Trong M, Wood CL, et al. A novel leukointegrin, alpha d beta 2, binds preferentially to ICAM-3. Immunity 1995;3:683–690.

69. Lee EK, Kehrli ME Jr, Dietz AB, et al. Cloning and sequencing of cDNA encoding bovine intercellular adhesion molecule 3 (ICAM-3). Gene 1996;174:311–313.

70. Gilbert RO, Rebhun WC, Kim CA, et al. Clinical manifestations of leukocyte adhesion deficiency in cattle: 14 cases (1977–1991). J Am Vet Med Assoc 1993;202:445–449.

71. Shuster DE, Kehrli ME Jr, Ackermann MR, Gilbert RO. Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle. Proc Natl Acad Sci USA 1992; 89:9225–9229.

72. Ackermann MR, Brogden KA, Florance AF, Kehrli ME Jr. Induction of CD18-mediated passage of neutrophils by *Pasteurella haemolytica* in pulmonary bronchi and bronchioles. Infect Immun 1999; 67:659–663.

73. Ackermann MR, Kehrli ME Jr, Brogden KA. Passage of $CD18^-$ and $CD18^+$ bovine neutrophils into pulmonary alveoli during acute pneumonia. Vet Pathol 1996;33:639–646.

74. Burns JA, Issekutz TB, Yagita H, Issekutz AC. The β2, α4, α5 integrins and selectins mediate chemotactic factor and endotoxin-enhanced neutrophil sequestration in the lung. Am J Pathol 2001;158: 1809–1819.

75. Ramamoorthy C, Sasaki SS, Su DL, et al. CD18 adhesion blockade decreases bacterial clearance and neutrophil recruitment after intrapulmonary *E coli*, but not after *S aureus.* J Leukoc Biol 1997;61: 167–172.

76. Mizgerd JP, Horwitz BH, Quillen HC, et al. Effects of CD18 deficiency on the emigration of murine neutrophils during pneumonia. J Immunol 1999;163:995–999.

77. Kumasaka T, Doyle NA, Quinlan M, et al. Role of CD11/CD18 in neutrophil emigration during acute and recurrent *Pseudomonas aeruginosa*-induced pneumonia in rabbits. Am J Pathol 1996;148: 1297–1305.

78. Shattil SJ. Function and regulation of the β_3 integrins in hemostasis and vascular biology. Thromb Haemost 1995;74:149–155.

79. Wang R, Ambruso DR, Newman PJ. Truncation in the cytoplasmic domain of integrin β_3 subunit confers an inactivated state for the $\alpha_{IIb}\beta_3$ complex in a variant type of Glanzmann thrombasthenia. Blood 1994;84:244a.

80. Boudreaux MK, Kvam K, Dillon AR, et al. Type I Glanzmann's thrombasthenia in a Great Pyrenees dog. Vet Pathol 1996;33:503–511.

81. Patterson WR, Estry DW, Schwartz KA, et al. Absent platelet aggregation with normal fibrinogen binding in Basset Hound hereditary thrombopathy. Thromb Haemost 1989;24:1011–1015.

82. Byzova TV, Rabbani R, D'Souza SE, Plow EF. Role of integrin $\alpha_v\beta_3$ in vascular biology. Thromb Haemost 1998;80:726–734.

83. van der Zee R, Murohara T, Passeri J, et al. Reduced intimal thickening following alpha(v)beta3 blockade is associated with smooth muscle cell apoptosis. Cell Adhes Commun 1998;6:371–379.

84. Kang IC, Lee YD, Kim DS. A novel disintegrin salmosin inhibits tumor angiogenesis. Cancer Res 1999;59:3754–3760.

85. Kerr JS, Wexler RS, Mousa SA, et al. Novel small molecule

αv integrin antagonists: Comparative anti-cancer efficacy with known angiogenesis inhibitors. Anticancer Res 1999;19(2A):959–968.

86. Kajiji S, Tamura RN, Quaranta V. A novel integrin ($\alpha_E\beta_4$) from human epithelial cells suggests a fourth family of integrin adhesion receptors. EMBO J 1989;8:673–680.

87. Kennel SJ, Godfrey V, Ch'ang LY, et al. The beta 4 subunit of the integrin family is displayed on a restricted subset of endothelium in mice. J Cell Sci 1992;101(Pt 1):145–150.

88. Niessen CM, Cremona O, Daams H, et al. Expression of the integrin $\alpha_6\beta_4$ in peripheral nerves: Localization in Schwann and perineural cells and different variants of the beta 4 subunit. J Cell Sci 1994;107(Pt 2):543–552.

89. Lee EC, Lotz MM, Steele GD Jr, Mercurio AM. The integrin alpha 6 beta 4 is a laminin receptor. J Cell Biol 1992;117:671–678.

90. Falk-Marzillier J, Domanico SZ, Pelletier A, et al. Characterization of a tight molecular complex between integrin $\alpha_6\beta_4$ and laminin-5 extracellular matrix. Biochem Biophys Res Commun 1998;251: 49–55.

91. Gambaletta D, Marchetti A, Benedetti L, et al. Cooperative signaling between $\alpha_6\beta_4$ integrin and ErbB-2 receptor is required to promote phosphatidylinositol 3-kinase-dependent invasion. J Biol Chem 2000;275:10604–10610.

92. Hu MC-T, Crowe DT, Weissman IL, Holzmann B. Cloning and expression of mouse integrin βp(β_7): A functional role in Peyer's patch-specific lymphocyte homing. Proc Natl Acad Sci USA 1992;89: 8254–8258.

93. Schweighoffer T, Tanaka Y, Tidswell M, et al. Selective expression of integrin $\alpha_4\beta_7$ on a subset of human CD4+ memory T cells with hallmarks of gut-tropism. J Immunol 1993;151:717–729.

94. Brezinschek RI, Brezinschek H-P, Lazarovits AI, et al. Expression of the β_7 integrin by human endothelial cells. Am J Pathol 1996; 149:1651–1660.

95. Berlin C, Berg EL, Briskin MJ, et al. $\alpha_4\beta_7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell 1993;74:185–195.

96. Cepek KL, Shaw SK, Parker CM, et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the $\alpha^E\beta_7$ integrin. Nature 1994;372:190–193.

97. Lo SK, Everitt J, Gu J, Malik AB. Tumor necrosis factor mediates experimental pulmonary edema by ICAM-1 and CD18-dependent mechanisms. J Clin Invest 1992;89:981–988.

98. Staunton DE, Marlin SD, Stratowa C, et al. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 1988;52:925–933.

99. Constantin G, Piccio L, Bussini S, et al. Induction of adhesion molecules on human Schwann cells by proinflammatory cytokines, an immunofluorescence study. J Neurol Sci 1999;170:124–130.

100. Radi ZA, Register K, Lee E-K, et al. In situ expression of intercellular adhesion molecule-1 (ICAM-1) in calves with acute *Pasteurella haemolytica* pneumonia. Vet Pathol 1999;36:437–444.

101. Lee E, Kang SG, Kehrli ME Jr. Cloning, sequencing and analysis of cDNA encoding bovine intercellular adhesion molecule-1 (ICAM-1). Vet Immunol Immunopathol 1997;59:121–129.

102. Staunton DE, Dustin ML, Erickson HP, Springer TA. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. Cell 1990;61:243–254.

103. Manning AM, Lu HF, Kukielka GL, et al. Cloning and comparative sequence analysis of the gene encoding canine intercellular adhesion molecule-1 (ICAM-1). Gene 1995;156:291–295.

104. Sligh JE Jr, Ballatyne CM, Rich SS, et al. Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. Proc Natl Acad Sci USA 1993;90:8529–8533.

105. Ohno N, Ichikawa H, Coe L, et al. Soluble selectins and ICAM-1 modulate neutrophil endothelial adhesion and diapedesis in vitro. Inflammation 1997;21:313–324.

106. Krenn V, Schedel J, Doring A, et al. Endothelial cells are the major source of sCAM-1 in rheumatoid synovial tissue. Rheumatol Int 1997;17:17–27.

107. Shingu M, Hashimoto M, Nobunaga M, et al. Production of soluble ICAM-1 by mononuclear cells from patients with rheumatoid arthritis patients. Inflammation 1994;18:23–34.

108. Ridker PM, Hennekens CH, Roitman-Johnson B, et al. Plasma concentrations of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88–92.

109. Huguenel ED, Cohn D, Dockum DP, et al. Prevention of rhinovirus infection in chimpanzees by soluble intercellular adhesion molecule-1. Am J Respir Crit Care Med 1997;155:1206–1210.

110. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: A randomized clinical trial. J Am Med Assoc 1999;281: 1797–1804.

111. de Fougerolles AR, Stacker SA, Schwarting R, Springer TA. Characterization of the ICAM-2 and evidence for a third counter-receptor for LFA-1. J Exp Med 1991;174:253–267.

112. de Fougerolles AR, Springer TA. Intercellular adhesion molecule 3, a third adhesion counter-receptor for lymphocyte function-associated molecule 1 on resting lymphocytes. J Exp Med 1992;175: 185–190.

113. Bailly P, Tontti E, Hermand P, et al. The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leukocyte integrins. Eur J Immunol 1995;25:3316–3320.

114. Tian L, Yoshihara Y, Mizuno T, et al. The neuronal glycoprotein telencephalin is a cellular ligand for the CD11a/CD18 leukocyte integrin. J Immunol 1997;158:928–936.

115. Bloy C, Hermand P, Blanchard D, et al. Surface orientation and antigen properties of Rh and LW polypeptides of the human erythrocyte membrane. J Biol Chem 1990;265:21482–21487.

116. Arii N, Mizuguchi M, Mori K, Takashima S. Development of telencephalin in the human cerebrum. Microsc Res Tech 1999;46:18–23.

117. Benson DL, Yoshihara Y, Mori K. Polarized distribution and cell type-specific localization of telencephalin, an intercellular adhesion molecule. J Neurosci Res 1998;52:43–53.

118. Kilgannon P, Turner T, Meyer J, et al. Mapping of the ICAM-5 (telencephalin) gene, a neuronal member of the ICAM family, to a location between ICAM-1 and ICAM-3 on human chromosome 19p13.2. Genomics 1998;54:328–330.

119. Tamada A, Yoshihara Y, Mori K. Dendrite-associated cell adhesion molecule, telencephalin, promotes neurite outgrowth in mouse embryo. Neurosci Lett 1998;240:163–166.

120. Yoshihara Y, Oka S, Nemoto Y, et al. An ICAM-related neuronal glycoprotein, telencephalin, with brain segment-specific expression. Neuron 1994;12:541–553.

121. Hino H, Mori K, Yoshihara Y, et al. Reduction of telencephalin immunoreactivity in the brain of patients with Alzheimer's disease. Brain Res 1997;753:353–357.

122. Mizuno T, Yoshihara Y, Inazawa J, et al. cDNA cloning and chromosomal localization of the human telencephalin and its distinctive interaction with lymphocyte function-associated antigen-1. J Biol Chem 1997;272:1156–1163.

123. Iiino II, Mori K, Yoshihara Y, et al. Reduction of telencephalin immunoreactivity in the brain of patients with Alzheimer's disease. Brain Res 1997;753:353–357.

124. Rieckmann P, Turner T, Kligannon P, Steinhoff BJ. Telencephalin as an indicator for temporal-lobe dysfunction. Lancet 1998;352: 370–371.

125. Hession C, Tizard R, Vassallo C, et al. Cloning of an alternate form of vascular adhesion molecule-1 (VCAM1). J Biol Chem 1991; 266:6682–6685.

126. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from VLA-4/fibronectin binding site. Cell 1990;60:577–584.

127. Fries JW, Williams AJ, Atkins RC, et al. Expression of VCAM-1 and E-selectin in an in vivo model of endothelial activation. Am J Pathol 1993;143:725–737.

128. Weyrich AS, Buerke M, Albertine KH, Lefer AM. Time course of coronary vascular endothelial adhesion molecule expression during reperfusion of the ischemic feline myocardium. J Leukoc Biol 1995;57:45–55.

129. Shimizu Y, Mobley JL. Distinct cation requirements for integrin-mediated CD4+ T lymphocyte adhesion to ICAM-1, fibronectin, VCAM-1, and invasion. J Immunol 1993;151:4106–4115.

130. Koch AE, Halloran MM, Haskell CJ, et al. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. Nature 1995;376:517–519.

131. Tokuhira M, Hosaka S, Volin MV, et al. Soluble vascular cell adhesion molecule 1 mediation of monocyte chemotaxis in rheumatoid arthritis. Arthritis Rheum 2000;43:1122–1133.

132. Kitani A, Nakashima N, Izumihara T, et al. Soluble VCAM-1 induces chemotaxis of Jurkat and synovial fluid T cells bearing high affinity very late antigen-4. J Immunol 1998;161:4931–4938.

133. Gurbel PA, Serebruany VL. Soluble vascular cell adhesion molecule-1 and E-selectin in patients with acute myocardial infarction treated with thrombolytic agents. Am J Cardiol 1998;81:772–775.

134. Jager A, van Hinsberg VW, Kostense PJ, et al. Increased levels of soluble vascular cell adhesion molecule 1 are associated with risk of cardiovascular mortality in type 2 diabetes: The Hoorn study. Diabetes 2000;49:485–491.

135. Kaplanski G, Cacoub P, Faarnarier C, et al. Increased soluble vascular cell adhesion molecule 1 concentrations in patients with primary or systemic lupus erythematosus-related antiphospholipid syndrome: Correlations with the severity of thrombosis. Arthritis Rheum 2000;43:55–64.

136. Adams DH, Shaw S. Leukocyte-endothelial interactions and regulation of leukocyte migration. Lancet 1994;343:831–836.

137. Newton-Nash DK, Newman PJ. A new role for platelet-endothelial cell adhesion molecule-1 (CD31): Inhibition of TCR-mediated signal transduction. J Immunol 1999;163:682–688.

138. Muller WA. The role of PECAM-1 (CD31) in leukocyte emigration: Studies in vitro and in vivo. J Leukoc Biol 1995;57:523–528.

139. Newman PJ, Berndt MC, Gorski J, et al. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. Science 1990;247:1219–1222.

140. Xie Y, Muller WA. Molecular cloning and adhesive properties of murine platelet/endothelial cell adhesion molecule 1. Proc Natl Acad Sci USA 1993;90:5569–5573.

141. Muller WA, Weigel SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med 1993; 178:449–460.

142. Gurubhagavatula I, Amrani Y, Pratico D, et al. Engagement of human PECAM-1 (CD31) on human endothelial cells increases intracellular calcium ion concentration and stimulates prostacyclin release. J Clin Invest 1998;101:212–222.

143. Jackson DE, Loo RO, Holyst MT, Newman PJ. Identification and characterization of functional cation coordination sites in platelet endothelial cell adhesion molecule-1. Biochemistry 1997;36:9395–9404.

144. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): A novel vascular cell-cell adhesion molecule. J Cell Biol 1991;114:1059–1068.

145. Kukielka GL, Hawkins HK, Michael L, et al. Regulation of intercellular adhesion molecule-1 (ICAM-1) in ischemia and reperfused canine myocardium. J Clin Invest 1993;92:1504–1516.

146. Welbourn CR, Goldman G, Paterson IS, et al. Pathophysiology of ischemia reperfusion injury: Central role of the neutrophil. Br J Surg 1991;78:651–655.

147. Rubin P, Dube L, Braeckman R, et al. Pharmacokinetics, safety, and ability to diminish leukotriene synthesis by zileuton, an inhibitor of 5-lipooxygenase. Agents Actions Suppl 1991;35:103–116.

148. Blanco FJ, Guitian R, Moreno J, et al. Effect of anti-inflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. J Rheumatol 1999;26:1366–1373.

149. Depre M, Van Hecken A, Verbesselt R, et al. Pharmacokinetic and pharmacodynamic interaction between the lipooxygenase inhibitor MK-0591 and the cyclooxygenase inhibitor ibuprofen in man. Int J Pharmacol Res 1998;18:53–61.

150. Mihelcic D, Schleiffenbaum B, Tedder TF, et al. Inhibition of leukocyte L-selectin function with a monoclonal antibody attenuates reperfusion injury to the rabbit ear. Blood 1994;84:2322–2328.

151. Pierce JW, Read MA, Ding H, et al. Salicylates inhibit I kappa B-alpha phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. J Immunol 1996;156:3961–3969.

152. Cheng Z, Nolan AM, Mckellar QA. Measurement of cyclooxygenase inhibition in vivo: A study of two non-steroidal anti-inflammatory drugs in sheep. Inflammation 1998;22:353–366.

153. Garcia-Vicuna R, Diaz-Gonzalez F, Gonzalez-Alvaro I, et al. Prevention of cytokine-induced changes in leukocyte adhesion receptors by nonsteroidal antiinflammatory drugs from the oxicam family. Arthritis Rheum 1997;40:143–153.

154. Burton JL, Kehrli ME Jr, Kapil S, Horst RL. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: Effects of cortisol and dexamethasone. J Leukoc Biol 1995;57:317–325.

155. Filep JG, Delalandre A, Payette Y, Földes-Filep E. Glucocorticoid receptor regulates expression of L-selectin and CD11/CD18 on human neutrophils. Circulation 1997;96:295–301.

156. Burton JL, Kehrli ME Jr. Effects of dexamethasone on bovine circulating T lymphocyte populations. J Leukoc Biol 1996;59:90–99.

157. Fong Y, Tracey KJ, Moldawer LL, et al. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 beta and interleucin 6 appearance during lethal bacteremia. J Exp Med 1989;170:1627–1633.

158. Lorenz HM, Antoni C, Valerius T, et al. In vivo blockade of TNF-α by intravenous infusion of a chimeric monoclonal TNF-α antibody in patients with rheumatoid arthritis. Short term cellular and molecular effects. J Immunol 1996;156:1646–1653.

159. Lorenz HM, Grunke M, Hieronymus T, et al. In vivo blockade of tumor necrosis factor-alpha in patients with rheumatoid arthritis: Longterm effects after repeated infusion of chimeric monoclonal antibody cA2. J Rheumatol 2000;27:304–310.

160. Starnes HF Jr, Pearce MK, Tewari A, et al. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor-alpha challenge in mice. J Immunol 1990; 145:4185–4191.

161. Hammelmann E, Cieslewicz G, Schwarze J, et al. Anti-interleukin 5 but not anti-IgE prevents airway inflammation and airway hyperresponsiveness. Am J Respir Crit Care Med 1999;160:934–941.

162. Shardonofsky FR, Venzor J III, Barrios R, et al. Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. J Allergy Clin Immunol 1999;104:215–221.

163. Ormrod DJ, Miller TE. The anti-inflammatory activity of a low molecular weight component derived from the milk of hyperimmunized cows. Agents Actions 1991;32:160–166.

164. Badovinac V, Mostarica-Stojkovic M, Dinarello CA, Stosic-Grujicic S. Interleukin-1 receptor antagonist suppresses experimental autoimmune encephalomyelitis (EAE) in rats by influencing the activation and proliferation of encephalitogenic cells. J Neuroimmunol 1998;85:87–95.

165. Haak-Frendscho M, Marsters SA, Mordenti J, et al. Inhibition of TNF by a TNF receptor immunoadhesion. Comparison to an anti-TNF monoclonal antibody. J Immunol 1994;152:1347–1353.

166. Kondo S, Pastore S, Fujisawa H, et al. Interleukin-1 receptor antagonist suppresses contact hypersensitivity. J Invest Dermatol 1995; 105:334–338.

167. Pan RY, Chen SL, Xiao X, et al. Therapy and prevention of arthritis by recombinant adeno-associated virus vector with delivery

of interleukin-1 receptor antagonist. Arthritis Rheum 2000;43:289–297.

168. Ashkenazi A, Marsters SA, Capon DJ, et al. Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesion. Proc Natl Acad Sci USA 1991;88:10535–10539.

169. Mohler KM, Torrance DS, Smith CA, et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993;151:1548–1561.

170. Henderson WR Jr, Chi EY, Maliszewski CR. Soluable IL-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma. J Immunol 2000;164:1086–1095.

171. Rubin LA, Nelson DL. The soluble interleukin-2 receptor: Biology, function, and clinical application. Ann Intern Med 1990;113: 619–627.

172. Gerard C, Bruyns C, Marchant A, et al. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 1993;177:547–550.

173. Horsfall AC, Butler DM, Marinova L, et al. Suppression of collagen-induced arthritis by continuous administration of IL-4. J Immunol 1997;159:5687–5696.

174. Mulligan MS, Jones ML, Vaporciyan AA, et al. Protective effects of IL-4 and IL-10 against immune complex-induced lung injury. J Immunol 1993;151:5666–5674.

175. Frevert CW, Matute-Bello G, Skerrett SJ, et al. Effect of CD14 blockade in rabbits with *Escherichia coli* pneumonia and sepsis. J Immunol 2000;164:5439–5445.

176. Haziot A, Rong GW, Lin XY, et al. Recombinant soluble CD14 prevents mortality in mice treated with endotoxin (lipopolysaccharide). J Immunol 1995;154:6529–6532.

177. Krakauer T. Pentoxifylline inhibits ICAM-1 expression and chemokine production induced by proinflammatory cytokines in human pulmonary epithelial cells. Immunopharmacology 2000;46:253–261.

178. Lo CJ, Fu M, Lo FR, Cryer HG. Cyclooxygenase 2 (COX-2) gene activation is regulated by cyclic adenosine monophosphate. Shock 2000;13:41–45.

179. Neuner P, Klosner G, Schauer E, et al. Pentoxifylline in vivo down-regulates the release of IL-1 beta, IL-6, IL-8 and TNF-α by human peripheral blood mononuclear cells. Immunology 1994;83: 262–267.

180. Olmos JM, De Vega T, Perera L, et al. Etidronate inhibits the production of IL-6 by osteoblast-like cells. Methods Find Exp Clin Pharmacol 1999;21:519–522.

181. Parmely MJ, Zhou WW, Edwards CK III, et al. Adenosine and a related carbocyclic nucleoside analogue selectively inhibit tumor necrosis factor-alpha production and protect mice against endotoxin challenge. J Immunol 1993;151:389–396.

182. Shimizu N, Watanabe T, Arakawa T, et al. Pentoxifylline accelerates gastric ulcer healing in rats: Roles of tumor necrosis factor alpha and neutrophils during the early phase of ulcer healing. Digestion 2000;61:157–164.

183. Tibes U, Rohr SP, Scheuer W, et al. Suppression of acute experimental inflammation by antisense oligonucleotides targeting secretory phospholipase A2 (sPLA2) in vitro and in vivo experiments. Adv Exp Med Biol 1999;469:199–207.

184. Noonan TC, Gundel RH, Desai SN, et al. The effects of an anti-CD18 antibody (R15.7) in antigen-induced airway hyperresponsiveness (AH) and cell influx in guinea pigs. Agents Actions 1991;34: 211–213.

185. Elliott E, Li Z, Bell C, et al. Modulation of host response to *Escherichia coli* O157:H7 infection by anti-CD18 antibody in rabbits. Gastroenterology 1994;106:1554–1561.

186. Kling D, Fingerle J, Harlan JM, et al. Mononuclear leukocytes invade rabbit arterial intima during thickening formation via CD18- and VLA-4–dependent mechanisms and stimulate smooth muscle migration. Circ Res 1995;77:1121–1128.

187. Yednock TA, Cannon C, Fritz LC, et al. Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. Nature 1992;356:63–66.

188. Molossi S, Elices M, Arrhenius T, et al. Blockade of very late antigen-4 integrin binding to fibronectin with connecting segment-1 peptide reduces accelerated coronary arteriopathy in rabbit cardiac allografts. J Clin Invest 1995;95:2601–2610.

189. Wahl SM, Allen JB, Hines KL, et al. Synthetic fibronectin peptides suppress arthritis in rats by interrupting leukocyte adhesion and recruitment. J Clin Invest 1994;94:655–662.

190. Yanaka K, Camarata PJ, Spellman SR, et al. Neuronal protection from cerebral ischemia by synthetic fibronectin peptides to leukocyte adhesion molecules. J Cereb Blood Flow Metab 1996;16:1120–1125.

191. Bator JM, Weitzberg M, Burch RM. N-[9H-(2,7-dimethylfluorenyl-9 methoxy)carbonyl]-L-leucine, NPC 15669, prevents neutrophil adherence to endothelium and inhibits CD11b/CD18 upregulation. Immunopharmacology 1992;23:139–149.

192. Burch RM, Weitzberg M, Blok N, et al. N-(fluorenyl-9-methoxycarbonyl) amino acids, a class of antiinflammatory agents with a different mechanism of action. Proc Natl Acad Sci USA 1991;88:355–359.

193. Norohana-Blob L, Lowe VC, Muhlhauser RO, Burch RM. NPC 15669, an inhibitor of neutrophil recruitment, is efficacious in acetic acid-induced colitis in rats. Gastroenterology 1993;104:1021–1029.

194. Pou S, Gunther MR, Pou WS, et al. Effect of NPC 15669, an inhibitor of neutrophil recruitment and neutrophil-mediated inflammation, on neutrophil function in vitro. Biochem Pharmacol 1993;45: 2123–2127.

195. Miller MJ, Chotinaruemol S, Sadowska-Krowikca H, et al. Guinea pig ileitis is attenuated by the leumidin N-(fluorenyl-9-methoxycarbonyl)-leucine (NPC 15199). J Pharmacol Exp Ther 1993;266: 468–472.

196. Lowe VC, Noronha-Blob L. The leukocyte recruitment inhibitor, NPC 15669, accelerates healing in acetic acid-induced colitis. Agents Actions 1993;39:C80–C82.

197. Altavilla D, Squadrito F, Ioculano M, et al. E-selectin in the pathogenesis of experimental myocardial ischemia-reperfusion injury. Eur J Pharmacol 1994;270:45–51.

198. Moore TM, Khimenko P, Adkins WK, et al. Adhesion molecules contribute to ischemia and reperfusion-induced injury in the isolated rat lung. J Appl Physiol 1995;78:2245–2252.

199. Winn RK, Paulson JC, Harlan JM. A monoclonal antibody to P-selectin ameliorates injury associated with hemorrhagic shock in rabbits. Am J Physiol 1994;267:H2391–H2397.

200. Condon TP, Bennett CF. Altered mRNA splicing and inhibition of human E-selectin expression by an antisense oligonucleotide in human umbilical vein endothelial cells. J Biol Chem 1996;271:30398–30403.

201. Lo SK, Bevilacqua B, Malik AB. E-selectin ligands mediate tumor necrosis factor-induced neutrophil sequestration and pulmonary edema in guinea pig lungs. Circ Res 1994;75:955–960.

202. Hayashi H, Koike H, Kurata Y, et al. Protective effects of sialyl Lewis X and anti-P-selectin antibody against lipopolysaccharide-induced acute lung injury in rabbits. Eur J Pharmacol 1999;370:47–56.

203. Zimmerman BJ, Paulson JC, Arrhenius TS, et al. Thrombin receptor peptide-mediated leukocyte rolling in rat mesenteric venules: Roles of P-selectin and sialyl Lewis X. Am J Physiol 1994;267: H1049–H1053.

204. Ridings PC, Holloway S, Bloomfield GL, et al. Protective role of synthetic sialylated oligosaccharide in sepsis-induced acute lung injury. J Appl Physiol 1997;82:644–651.

205. Huang J, Kim LJ, Mealey R, et al. Neuronal protection in stroke by an sLe^x-glycosylated complement inhibitory protein. Science 1999;285:595–599.

206. Kogan TP, Dupre B, Bui H, et al. Novel synthetic inhibitors of selectin-mediated cell adhesion: Synthesis of 1,6-Bis[3-(3-carboxymethylphenyl)-4-(2-α-D-mannopyranosyloxy)phenyl]hexane (TBC1269). J Med Chem 1998;41:1099–1111.

207. Radi Z, Caverly J, Dixon R, et al. Effects of a synthetic selectin inhibitor, TBC1269, on tissue damage in acute *Mannheimia* (*Pasteurella) haemolytica* pneumonia in neonatal calves. Am J Vet Res 2001;62(1):17–22.

208. Caverly JM, Radi ZA, Andreasen CB, et al. Comparison of bronchoalveolar lavage fluid between *P. haemolytica*-inoculated calves and calves pre-treated with the selectin inhibitor, TBC1269. Am J Vet Res 2001;62:665–672.

209. Abraham WM, Ahmed A, Sabater JR, et al. Selectin blockade prevents antigen-induced late bronchial responses and airway hyperresponsiveness in allergic sheep. Am J Respir Crit Care Med 1999; 159(4 Pt 1):1205–1214.

210. Abraham WM, Gill A, Ahmed A, et al. A small-molecule, tight-binding inhibitor of the integrin alpha(4)beta(1) blocks antigen-induced airway responses and inflammation in experimental asthma in sheep. Am J Respir Crit Care Med 2000;162(2 Pt 1):603–611.

211. Scuri M, Allegra L, Abraham WM. The effects of multiple dosing with zileuton on antigen-induced responses in sheep. Pulm Pharmacol Ther 1998;11(4):277–280.

212. Abraham WM, Ahmed A, Sielczak MW, et al. Blockade of late-phase airway responses and airway hyperresponsiveness in allergic sheep with a small-molecule peptide inhibitor of VLA-4. Am J Respir Crit Care Med 1997;156(3 Pt 1):696–703.

213. Harning R, Pelletier J, Van G, et al. Monoclonal antibody to MALA-2 (ICAM-1) reduces acute autoimmune nephritis in kdkd mice. Clin Immunol Immunopathol 1992;64:129–134.

214. Wong P, Yue G, Yin K, et al. Antibodies to intercellular adhesion molecule-1 ameliorate the inflammatory response in acetic acid-induced inflammatory bowel disease. J Pharmacol Exp Ther 1995;274:475–480.

215. Cosimi AB, Conti D, Delmonico FL, et al. In vivo effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts. J Immunol 1990;144:4604–4612.

216. Aziz KE, Wakefield D. Modulation of endothelial cell expression of ICAM-1, E selectin, and VCAM-1 by beta-estradiol, progesterone, and dexamethasone. Cell Immunol 1996;167:79–85.

217. Jilma B, Blann AD, Stohlawetz P, et al. Dexamethasone lowers circulating E-selectin and ICAM-1 in healthy men. J Lab Clin Med 2000;135:270–274.

218. Juan M, Mullol J, Roca-Ferrer J, et al. Regulation of ICAM-3 and other adhesion molecule expressions on eosinophils in vitro. Effects of dexamethasone. Allergy 1999;54:1293–1298.

219. Papi A, Papadopoulos NG, Degitz K, et al. Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells. J Allergy Clin Immunol 2000;105:318–326.

220. Bennett CF, Condon TP, Grimm S, et al. Inhibition of endothelial cell adhesion molecule expression with antisense oligonucleotides. J Immunol 1994;152:3530–3540.

221. Chen W, Bennett CF, Wang ME, et al. Perfusion of kidneys with unformulated ''naked'' intercellular adhesion molecule-1 antisense oligodeoxynucleotides prevents ischemic/reperfusion injury. Transplantation 1999;68:880–887.

222. Cheng QL, Chen XM, Li F, et al. Effects of ICAM-1 antisense oligonucleotide on the tubulointerstitium in mice with unilateral ureteral obstruction. Kidney Int 2000;57:183–190.

223. Dragun D, Tullius SG, Park JK, et al. ICAM-1 antisense oligodeoxynucleotides prevent reperfusion injury and enhance immediate graft function in renal transplantation. Kidney Int 1998;54:590–602.

224. Haller H, Dragun D, Miethke A, et al. Antisense oligonucleotides for ICAM-1 attenuate reperfusion injury and renal failure in the rat. Kidney Int 1996;50:473–480.

225. Stepkowski SM, Tu Y, Condon TP, Bennett CF. Blocking of heart allograft rejection by intercellular adhesion molecule-1 antisense oligonucleotides alone or in combination with other immunosuppressive modalities. J Immunol 1994;153:5336–5346.

226. Klimuk SK, Semple SC, Nahirney PN, et al. Enhanced anti-inflammatory activity of a liposomal intercellular adhesion molecule-1 antisense oligodeoxynucleotide in an acute model of contact hypersensitivity. J Pharmacol Exp Ther 2000;292:480–488.