Leukocyte Adhesion

CVS BSc Module 2 2012 - Vascular Biology

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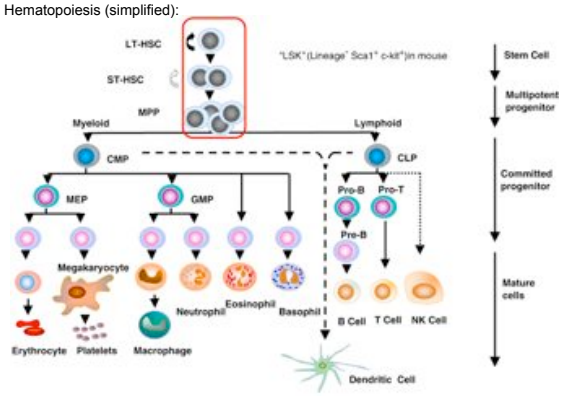
Cell adhesion is the binding of a cell to a surface, extracellular matrix or another cell using cell adhesion molecules

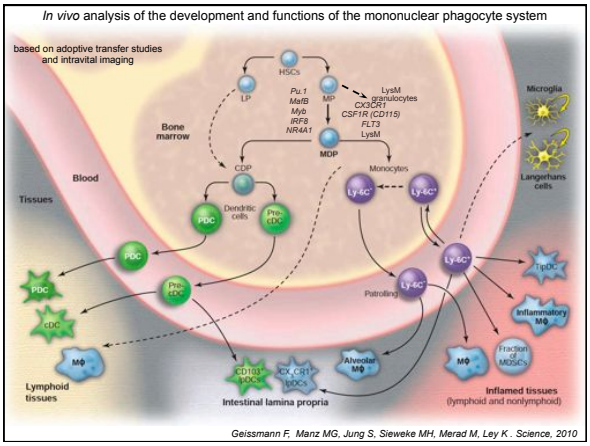
- Cell adhesion molecules (CAMs) - important molecules to selective recruitment of circulating leukocytes to sites of inflammation by promoting cell–cell and cell–matrix interactions

Hematopoiesis (simplified):



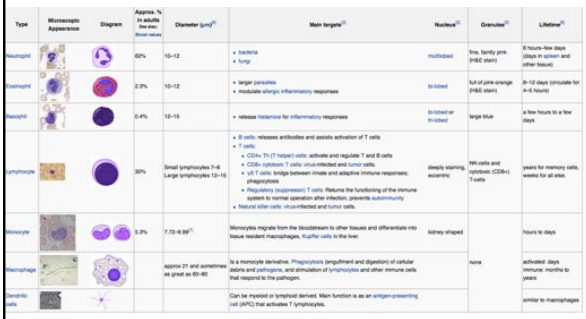
Oncogene (2005) 24, 5676–5692

In vivo analysis of the development and functions of the mononuclear phagocyte system



Geissmann F. Manz MG, Jung S, Sieweke MH, Merad M, Ley K . Science, 2010

leukocytes



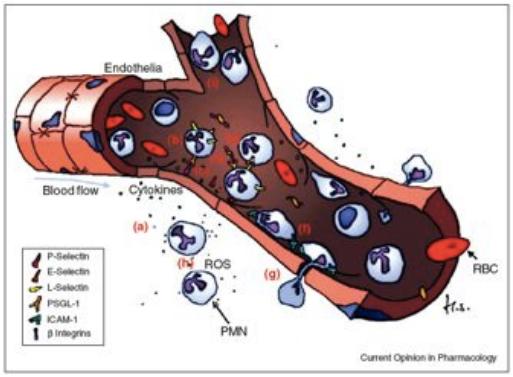
Source: http://en.wikipedia.org/wiki/White_blood_cell

What is 'CD' definition?

- Leukocyte differentiation antigen
- CD: cluster of differentiation.

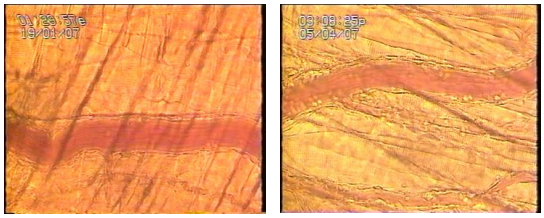
  The same differentiation antigen recognised by different monoclonal antibodies from different labs are called CD.

Adhesion to the endothelium: site of vascular recruitment of circulating leukocytes to enable effector functions (immunity & inflammation & tissue 'repair')



Current Opinion in Pharmacology 2012, 12:601–607

Endothelial inflammation induces recruitment of leukocytes



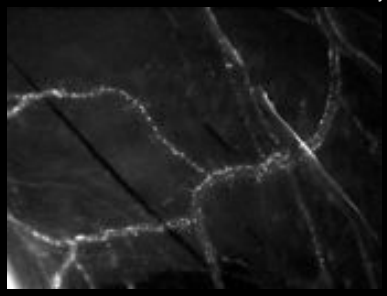
'Resting' endothelium

TNF activated endothelium

Mouse cremaster tissue (in-vivo imaging) – note: rolling leukocytes along endothelium

Woollard et al, Circ Res., 2008

Endothelial inflammation induces recruitment of leukocytes

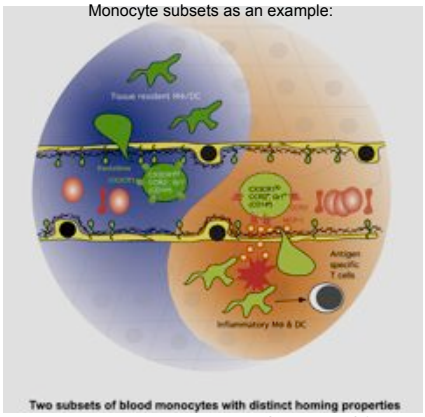


TNF activated endothelium

Mouse cremaster tissue (in-vivo imaging) – note: rolling leukocytes along endothelium

Woollard et al, Circ Res., 2008

Monocyte subsets as an example:

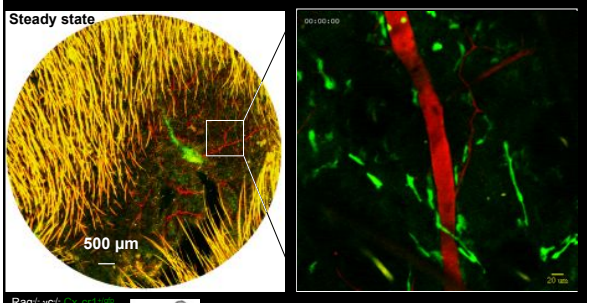


Two subsets of blood monocytes with distinct homing properties

Geissmann F. Jung S. & Littman D.R. *Immunity*, 2003

Study of monocyte functions by intravital microscopy

Aim: study Ly6C^{low} blood monocytes in the steady state
Dermal microvasculature of the ear

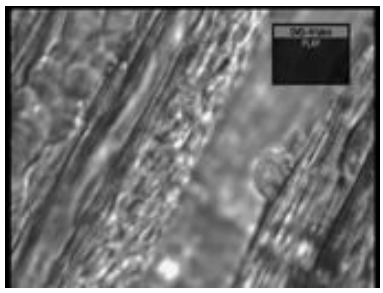


Rag$^{-/-}$ γc$^{-/-}$ Cx$_3$cr1$^{+/gfp}$
+
Fluorescent Dextran

Science, 2007

Cell Adhesion in-vivo

Examples of cell recruitment and locomotion

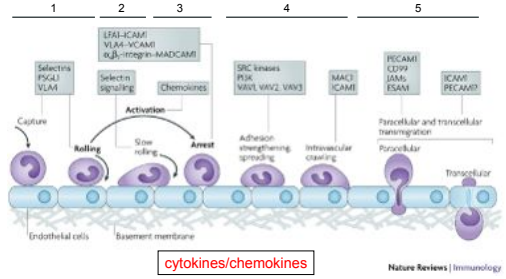


Neutrophil adhesion in-vivo after vascular injury
(note: platelet-leukocyte aggregates)

Getting to the site of inflammation: the leukocyte adhesion cascade updated

www.nature.com/reviews/immunol

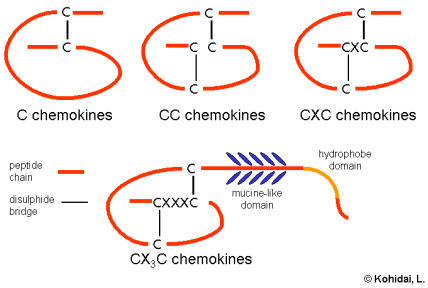
Klaus Ley, Carlo Laudanna‡, Myron I. Cybulsky§ and Sussan Nourshargh||*



cytokines/chemokines

Nature Reviews | Immunology

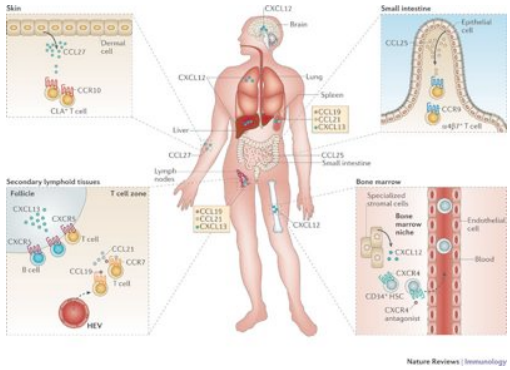
Structure of chemokine classes



© Kohidai, L.

Annu Rev Pharmacol Toxicol **42**: 2002, 469–99

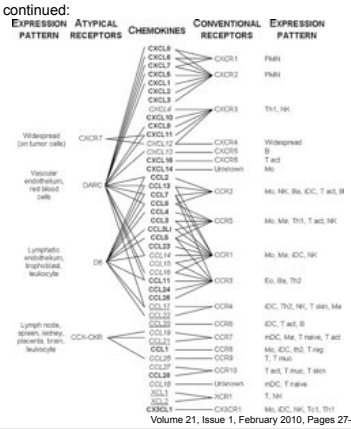
Chemokines (generalised overview):



Nature Reviews | Immunology

Nature Reviews Immunology 11, 597-606 (September 2011)

Chemokines continued:



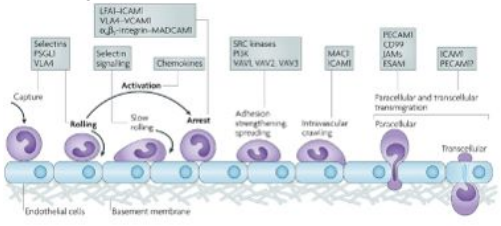
Volume 21, Issue 1, February 2010, Pages 27–39. Inflammation and Cancer

The cytokines of innate immunity

	mass (kDa)	assembly	pdb	source(s)	target(s)
IL1	17	monomer	3O4O	macrophages, endothelia, epithelia	endothelia (↑ coagulation, ↑ inflammation) hepatocytes (↑ acute phase proteins), hypothalamus (↑ fever)
IL18	17	monomer		macrophages	NK lymphocytes (↑ IFN-II γ), T lymphocytes (↑ IFN-II γ)
TNF	17	homotrimer	1TNF, 3ALQ	macrophages, T lymphocytes	endothelia (↑ coagulation, ↑ inflammation) hepatocytes (↑ acute phase proteins), neutrophils (↑ activation), hypothalamus (↑ fever)
IL6	26	homodimer	1P9M (complex)	macrophages, endothelia, T lymphocytes	hepatocytes (↑ acute phase proteins), B lymphocytes (↑ proliferation)
IL15	13	monomer		macrophages	NK lymphocytes (↑ proliferation), T lymphocytes (↑ proliferation
IL12	35/40	heterodimer	1F45 3DUH	macrophages, dendritic cells	Th1 lymphocytes (↑ differentiation), Tc lymphocytes (↑ IFN-II γ), NK lymphocytes (↑ IFN-II γ)
IL23	19/40	heterodimer		macrophages, dendritic cells	T lymphocytes (↑ IL17)
IL27	28/13	heterodimer		macrophages, dendritic cells	Th1 lymphocytes (inhibition and/or differentiation), NK lymphocytes (↑ IFN-II γ),
IL10	18	homodimer	2H24 1Y6K	macrophages, T lymphocytes	macrophages, dendritic cells (↓ IL12)
INF-I (α)	21	homodimer		macrophages	all cells (↑ viral immunity, ↑ MHC class I), NK lymphocytes (↑ activation)
INF-I (β)	25	homodimer	1AU1	fibroblasts	all cells (↑ viral immunity, ↑ MHC class I), NK lymphocytes (↑ activation)
INF-III			3OG4	under study	all cells (↑ viral immunity, ↑ MHC class I), NK lymphocytes (↑ activation)
chemo-kines	8-12	monomer		macrophages, endothelia, fibroblasts, epithelia	phagocyte (↑ migration), B lymphocytes (↑ migration), T lymphocytes (↑ migration), ↑ wound repair

‘cell adhesion cascade’

1. Capture
2. Rolling
3. Slow rolling (activation)
4. Firm adhesion
5. Transmigration



Nature Reviews | Immunology

1. Capture

Selectin

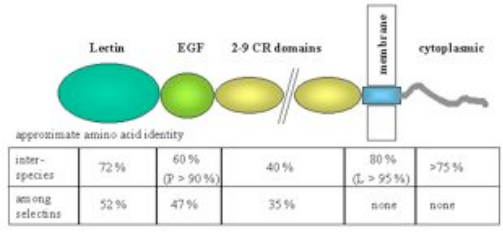
- Selectins are a family of CAMs which bind to specific sugar determinants on the surface of adjacent cells
- Selectin family
  - leukocyte-expressed L-selectin (CD62L)
  - endothelial-expressed E-selectin (CD62E)
  - P-selectin (CD62P) which is expressed by both platelets and endothelial cells

Table 1 | **Selectin expression**

Selectin	Cell/tissue	Expression pattern
L-selectin	Myeloid cells	Constitutive
	Naive T cells	Constitutive
	Effector T cells	Low/negative
	Effector memory T cells	Absent
	Central memory T cells	Re-expressed or retained
E-selectin	Skin endothelium	Constitutive
	Inflamed endothelium	Inducible in most organs
P-selectin	Choroid plexus	Constitutive
	Lung endothelium	Constitutive
	Platelets	After activation
	Platelet-derived microparticles	Constitutive
	Peritoneal macrophages	Constitutive
	Inflamed endothelium	Inducible in most organs

Klaus Ley and Kansas GS. Nat Rev Immunol 2004 (4):1-11

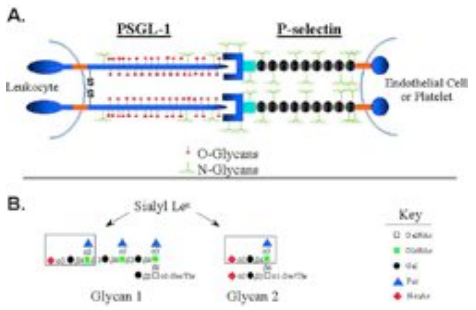
Selectin's structure:



approximate amino acid identity

inter-species	72 %	60 % (P > 90 %)	40 %	80 % (L > 95 %)	>75 %
among selectins	52 %	47 %	35 %	none	none

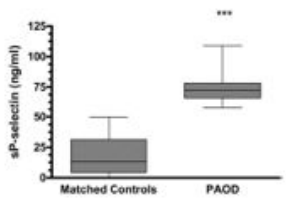
Selectin	Ligands	Distribution
E-selectin	Sialylated Lewis X and related glycans (eg. CLA1)	Endothelium activated by cytokines (IL1, TNF)
P-selectin	Sialylated Lewis X and related glycans on PSGL-1 (P-selectin glycoprotein ligand-1)	Storage granules & surface of endothelium and platelets
L-selectin	GlyCAM-1(HEV) MadCAM-1(GALT) CD 34	Lymphocytes (high expression on Naive T cell)



Braz J Med Biol Res, May 1999, Volume 32(5) 519-528

Plasma adhesion molecules (selectins) as plasma biomarkers of inflammatory disease

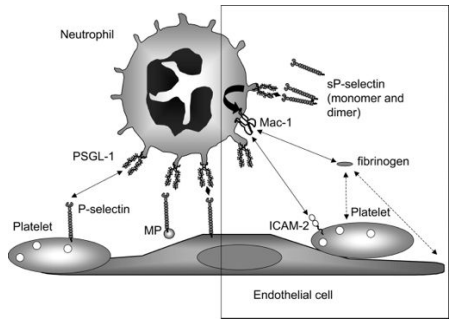
Cleaved soluble forms of endothelial selectin molecules can act as a surrogate marker of systemic inflammation



PAOD = Peripheral arterial occlusive disease

Woollard et al, Circ Res., 2006
Woollard et al, Circ Res., 2008

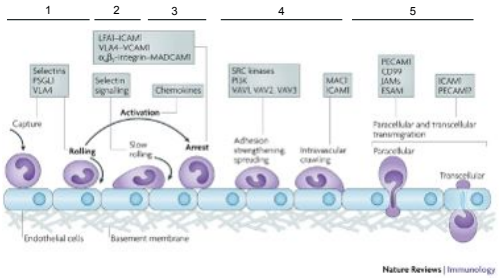
Soluble cleaved P-selectin increases leukocyte adhesion



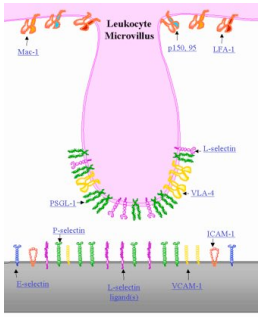
Galkina E , Ley K Circulation Research 2006;98:12-14

American Heart Association
Learn and Live

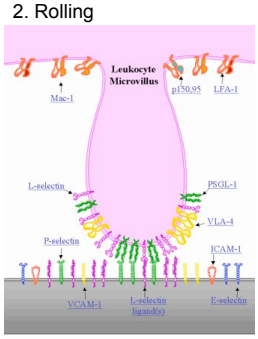
2. Rolling



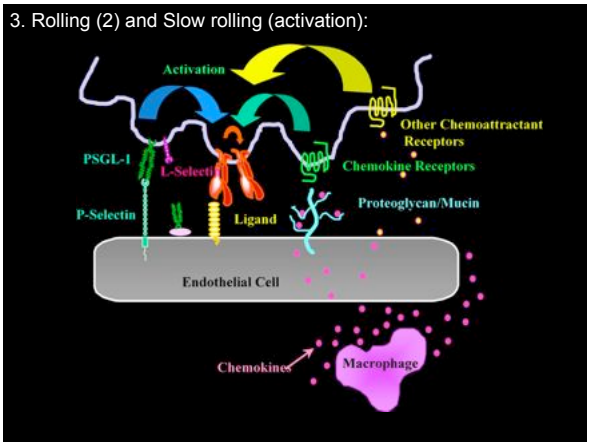
1. Capture



2. Rolling



3. Rolling (2) and Slow rolling (activation):



Integrin

- Structurally related non-covalently linked α and β heterodimeric cell adhesion receptors
- α and β subunits are type I transmembrane proteins containing large extracellular domains (700–1100 amino acids) and relatively small cytoplasmic domains (30–50 amino acids)
- In vertebrates there are 18 α subunits and 8 β subunits combining to form 24 integrins
- 4 of the β subunits are expressed on leukocytes (ie, β1, β2, β3, and β7)
- β2 and β7 expression limited to leukocytes, and β1 expression occurring on most of the body's cell types.

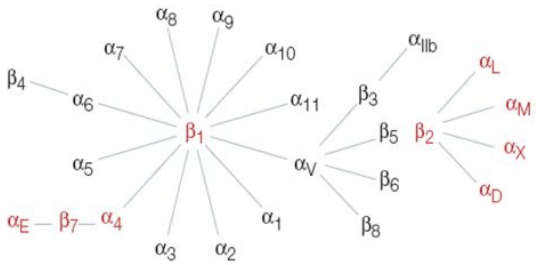
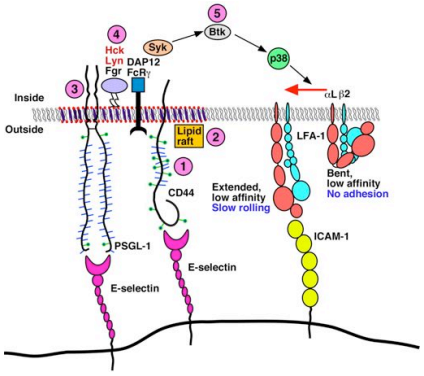


Figure 1 | **The integrin family.** The various associations that occur between the 18 types of integrin α-chain and the 8 types of integrin β-chain allow the formation of at least 24 integrins. Integrins that are expressed by leukocytes are shown in red.

Subunits		Name	Ligands/Counterreceptors	Functions
β_1	α_1	VLA-1 (CD49aCD29)	Collagens, laminin	Cell-matrix adhesion
	α_2	VLA-2 (CD49bCD29)	Collagens, laminin	Cell-matrix adhesion
	α_3	VLA-3 (CD49cCD29)	Fibronectin, collagens, laminin	Cell-matrix adhesion
	α_4	VLA-4 (CD49dCD29)	Fibronectin, VCAM-1, MadCAM-1	Cell-matrix adhesion; homing; T cell costimulation?
	α_5	VLA-5 (CD49eCD29)	Fibronectin	Cell-matrix adhesion
	α_6	VLA-6 (CD49fCD29)	Laminin	Cell-matrix adhesion
	α_7	CD49gCD29	Laminin	Cell-matrix adhesion
	α_8	CD49hCD29	?	?
	α_v		Vitronectin, fibronectin	Cell-matrix adhesion
β_2	α_L	CD11aCD18 (LFA-1)	ICAM-1, ICAM-2, ICAM-3	Leukocyte adhesion to endothelium; T cell–APC adhesion; T cell costimulation?
	α_M	CD11bCD18 (MAC-1, CR3)	iC3b, fibrinogen, factor X, ICAM-1	Leukocyte adhesion and phagocytosis; cell-matrix adhesion
	α_x	CD11cCD18 (p150,95; CR4)	iC3b; fibrinogen	Leukocyte adhesion and phagocytosis; cell-matrix adhesion
β_3	α_{IIb}		Fibrinogen, fibronectin, Willebrand factor, vitronectin, thrombospondin	Platelet adhesion and aggregation
	α_v	Vitronectin receptor (CD51CD61)	Vitronectin, fibrinogen, Willebrand factor, thrombospondin, fibronectin, osteopontin, collagen	Cell-matrix adhesion
β_4	α_6		Laminin	Cell-matrix adhesion
β_5	α_v		Vitronectin	Cell-matrix adhesion
β_6	α_v		Fibronectin	Cell-matrix adhesion
β_7	α_4	LPAM-1	Fibronectin, VCAM-1, MadCAM-1	Lymphocyte homing to mucosal lymphoid tissues
	α_E	HML-1	E-cadherin	Retention of intraepithelial T cells

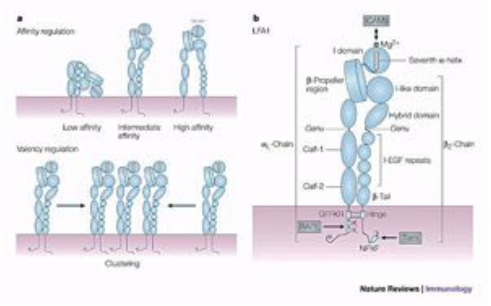
Abbreviations: APC, antigen-presenting cell; iC3b, C3b inactivated; ICAM, intercellular adhesion molecule; LFA, leukocyte function–associated antigen; MadCAM-1, mucosal addressin cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.
Adapted from Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–25, 1992. © Cell Press.

Ligand receptor 'outside-in' *vs* 'inside-out' signaling:



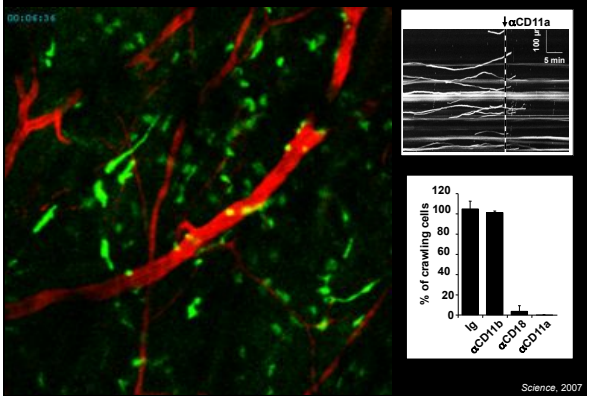
Blood July 22, 2010 vol. 116 no. 3 485-494

Integrin 'affinity' activation



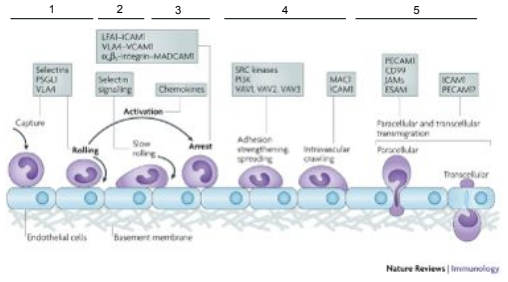
Nature Reviews Immunology: 546 | july 2005 | volume 5

Gr1^{+}/Ly6C^{low} monocytes adhere to the luminal side of the endothelium
Monocyte crawling is mediated through the β2 integrin LFA1 (CD11a/CD18)



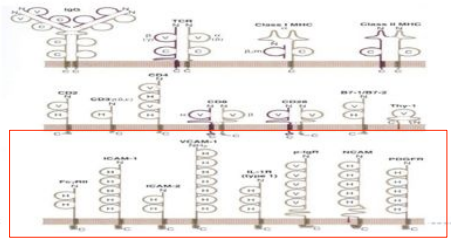
Science, 2007

4. Firm adhesion



IMMUNOGLOBULIN GENE SUPERFAMILY

- consists of more than 25 molecules
- Have a series of globular Ig-like domains, formed by disulfide bonds



Intercellular adhesion molecule (ICAM)

- ICAM-1 (CD54)
  - increase following stimulation by cytokines (IL-1, TNF-α, IFN-γ), or bacterial endotoxin
  - IFN-γ selectively induces ICAM-1 expression without affecting expression of other adhesion molecules
  - Ligands for the most N-terminal domain of ICAM-1 include LFA-1, fibrinogen, and most serotypes of rhinovirus, whereas the third domain is recognized by Mac-1
- ICAM-2 (CD102)
  - 2 Ig-like extracellular domains that possess 34% homology to the first two domains of ICAM-1
  - ligand binding site for LFA-1
  - ICAM-2 is constitutively expressed on mononuclear cells, basophils, mast cells, and platelets, and expression appears to be unaffected by cytokines

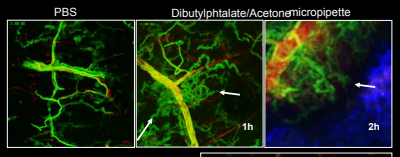
- ICAM-3 (CD50)
  - functions as an LFA-1 ligand , $\alpha_d\beta_2$ integrin
  - expressed on all leukocytes and on mast cells
  - ICAM-3 cross-linking results in calcium mobilization, tyrosine phosphorylation, enhanced adhesion, chemokine secretion, and modulation of basophil mediator release

Vascular cell adhesion molecule

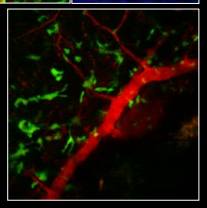
- VCAM-1 (CD106) is a cytokine-inducible endothelial cell adhesion molecule
- Expressed on macrophage, DC, astrocytes, & BM stromal cells and respiratory epithelium cell line
- Expression on umbilical vein endothelial cells induced by IL-1, TNF-α, or LPS
- Expression on endothelial cells induced by IL-4, IL-13

5. Transmigration

Tracking the fate of monocytes during inflammatory response

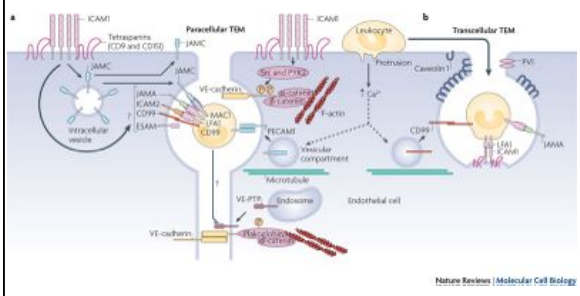


Patrolling Ly6C^{low} /Gr1-CX$_3$CR1^{high} monocytes rapidly extravasate in response to tissue damage



Science, 2007

5. Transmigration



Paracellular *vs* Transcellular migration

Platelet-endothelial cell adhesion molecule

- PECAM-1 (CD31) is a cell adhesion molecule expressed on endothelial cells and circulating leukocyte
- plays an important role in mediating neutrophil and monocyte transendothelial migration

Table 1 | **Leukocyte transendothelial cell migration**

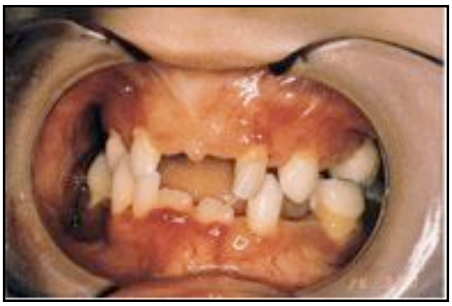
Junctional molecule	Leukocyte ligand	References
PECAM1	PECAM1	8
JAM-A	LFA1, JAM-A (?)	97
JAM-B	VLA4	7
JAM-C	MAC1	7
ICAM2	MAC1, LFA1	124,125
CD99	CD99	8
CD99L2	Unknown	126
ESAM	Unknown	92

CD99L2, CD99-related antigen; ESAM, endothelial cell-selective adhesion molecule; ICAM2, intercellular adhesion molecule 2; JAM, junctional adhesion molecule; LFA1, lymphocyte function-associated antigen 1; MAC1, macrophage receptor 1; PECAM1, platelet/endothelial-cell adhesion molecule 1.

Nature Reviews Immunology; 678, 7, 2007

What happens when adhesion goes wrong?

Leukocyte Adhesion Deficiency
autosomal recessive disorder 1:100,000

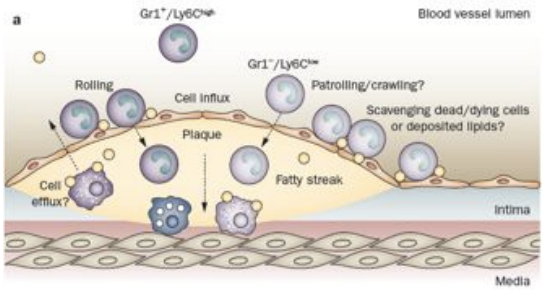


Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology
Volume 87, Issue 6, June 1999, Pages 691–694

DISEASE ASSOCIATED WITH ADHESION MOLECULE DEFICIENCY

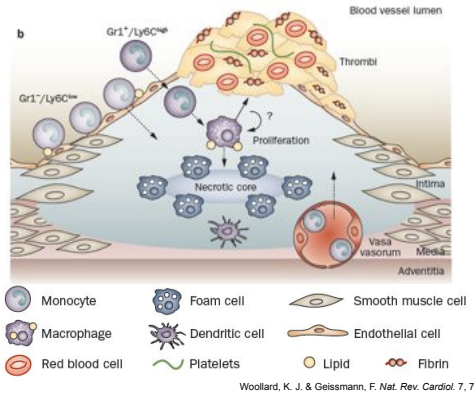
Human syndrome	Adhesion molecule defect	Clinical phenotype
LAD-I	β_2 integrin structure (mutation CD18)	Absent or near-absent expression of all β_2 integrins; blood neutrophilia with tissue neutropenia, delayed umbilical cord separation, recurrent soft tissue infections (e.g., skin, periodontal), impaired pus formation and wound healing; reduced or absent neutrophil adhesion, transendothelial migration, and chemotactic responses; normal rolling adhesion
LAD-I variants	β_2 integrin function	β_2 integrins expressed but dysfunctional; biologic consequences identical to those of LAD-I
LAD-II	GFTP gene (guanosine diphosphate-fucose transporter gene) and sLe^X	Defect in fucosylation of many structures, including sialyl-Lewis-X (sLe^X); developmental abnormalities (e.g., severe mental retardation, short stature, distinctive facial appearance). (hh) blood phenotype, impaired pus formation, pneumonia, periodontitis, and otitis; neutrophil studies, reduced or absent sLe^X expression, reduced rolling adhesion, normal firm adhesion
LAD-III	Integrin signaling (failed inside-out signaling)	LAD-III is a very rare disorder characterized by severe recurrent infections, a bleeding tendency and marked leukocytosis. Leukocytes and platelets have normal expression of CD18 (defective in LAD-I), normal expression of CD15a (defective in LAD-II), but defective integrin signaling
E-selectin	E-selectin	Case report of child with clinical presentation similar to LAD disease but whose neutrophils expressed normal levels of β_2 integrins, L-selectin, and sLe^X; staining of inflamed tissue revealed no E-selectin

‘Vulnerable’ atherosclerosis



Swirski et al. Monocyte accumulation in mouse atherogenesis is progressive and proportional to extent of disease.
Proc Natl Acad Sci USA (2006) vol. 103 (27) pp. 10340-5

Plaque rupture



Woollard, K. J. & Geissmann, F. *Nat. Rev. Cardiol.* 7, 77–86 (2010

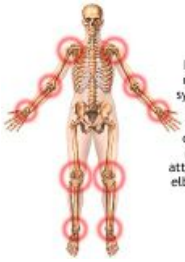
- Note: Adhesion molecules directly regulate atherosclerosis
  - P-selectin knockout mice protected from atherosclerosis
  - VCAM-1 knockout mice protected from atherosclerosis

Nat. Rev. Cardiol. 7, 77–86 (2010)
Current Pharmaceutical Design, 16. 4113-8 (2010)

Many autoimmune vasculopathies (associated with infiltration of leukocytes) - leads to accelerated atherosclerosis

Examples:
- Rheumatoid Arthritis (synovial membranes)
- Systemic Lupus Erythematosus (eg. glomerulonephritis)
- Vasculitides (eg. glomerulonephritis, lung inflammation)
- Diabetic complications (eg. retinopathy)
- …..

'when immune cells self attack'



Rheumatoid arthritis usually affects joints symmetrically (on both sides equally), may initially begin in a couple of joints only, and most frequently attacks the wrists, hands, elbows, shoulders, knees and ankles

del Rincon et al., 2001.. *Arthritis Rheum* 44:2737-2745.

Therapeutic targeting

Table 1. P-Selectin Compounds Currently in Development

Compound	Company	Phase	Indication
ABC-48 (humanised Ab)	Aeres	PC	DVT
PselmabG1 (Ab)	Selexys	PC	SCA
rPSGL (Ig)	Wyeth	I-II	Kidney transplant
P-selectin binding peptide (pep)	Astellas	PC	RA
Glysopep (pep)	Selexys	PC	DVT, AT
TBC1269 (pep)	Encysive	II	Asthma, Psoriasis
PS1697 (pep)	Wyeth	PC	AT
KF38789 (pep)	Kyowa Hakko	PC	MI, Stroke

Abbreviations: Ab = Antibody; Ig = immunoglobulin; pep = peptide sequence; PC = pre-clinical; DVT = Deep Vein Thrombosis; SCA = Sickle Cell Anemia; RA = Rheumatoid Arthritis; AT = Atherothrombosis; MI = Myocardial Infarction.
Source: www.aeresbiomedical.com; www.selexys.com; www.wyeth.com; www.astellas.com; www.encysive.com; www.kyowa.co.jp/eng/.

Woollard et al., Inflammation & Allergy - Drug Targets, 2007, Vol. 6, No. 1

Summary 1

- Leukocyte margination (recruitment, adhesion & migration) is required to deliver effector functions of bone marrow/ blood cells to sites of infection, inflammation and tissue repair.
- Cells are recruited by a 'favorable' chemotactic gradient and localised cytokine activation of cells and/or endothelium
- Cell adhesion is at least a 5 step process:
  - Capture
  - Rolling
  - Slow Rolling (activation)
  - Firm adhesion
  - Transmigration

Summary 2

1) Capture: Involves E-selectin and P-selectin (expressed on cytokine activated endothelium) binding to PSGL-1 ligands constitutively expressed on circulating leukocytes
   ① Cleaved soluble plasma selctins interfere with cell adhesion, but may result in useful biomarkers of inflammatory disease
2) Rolling involves selectin-ligand interactions and outside-in signaling
3) Slow rolling induces inside-out signaling and integrin affinity activation eg. LFA-1($\beta^2\alpha^L$):ICAM-1 and VLA-4($\beta^1\alpha^4$):VCAM-1
4) Continued activation signals induce firm adhesion via (eg.) MAC-1($\beta^2\alpha^L$):ICAM-1
5) Transmigration via transcellular or paracellular routes (JAMs, PECAM etc) enables leukocytes to enter relevant tissues and mediate target functions
   ① Targeting adhesion molecules may provide novel drug target for modulating inflammatory disease

Summary 3

- 'LAD' disables leukocytes from entering relevant organs and tissues
  » ~1:100,000 births
- 'Atherosclerosis' involves increased leukocyte recruitment to prone vascular beds and progression of 'vulnerable' atherosclerotic plaque
  » ~16-17 million deaths per year

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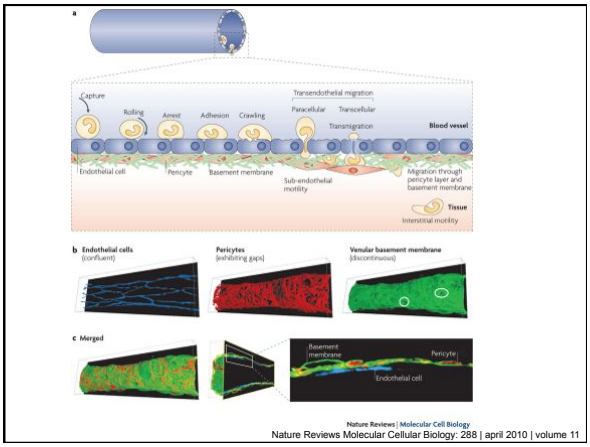
Further reading:

Nature Reviews Immunology: 678 | september 2007 | volume 7
Nature Reviews Molecular Cellular Biology: 288 | april 2010 | volume 11
Nature Reviews Molecular Cellular Biology: 366 | may 2010 | volume 11
Nature Reviews Immunology: 546 | july 2005 | volume 5



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