Leukocyte Homing and Inflammation

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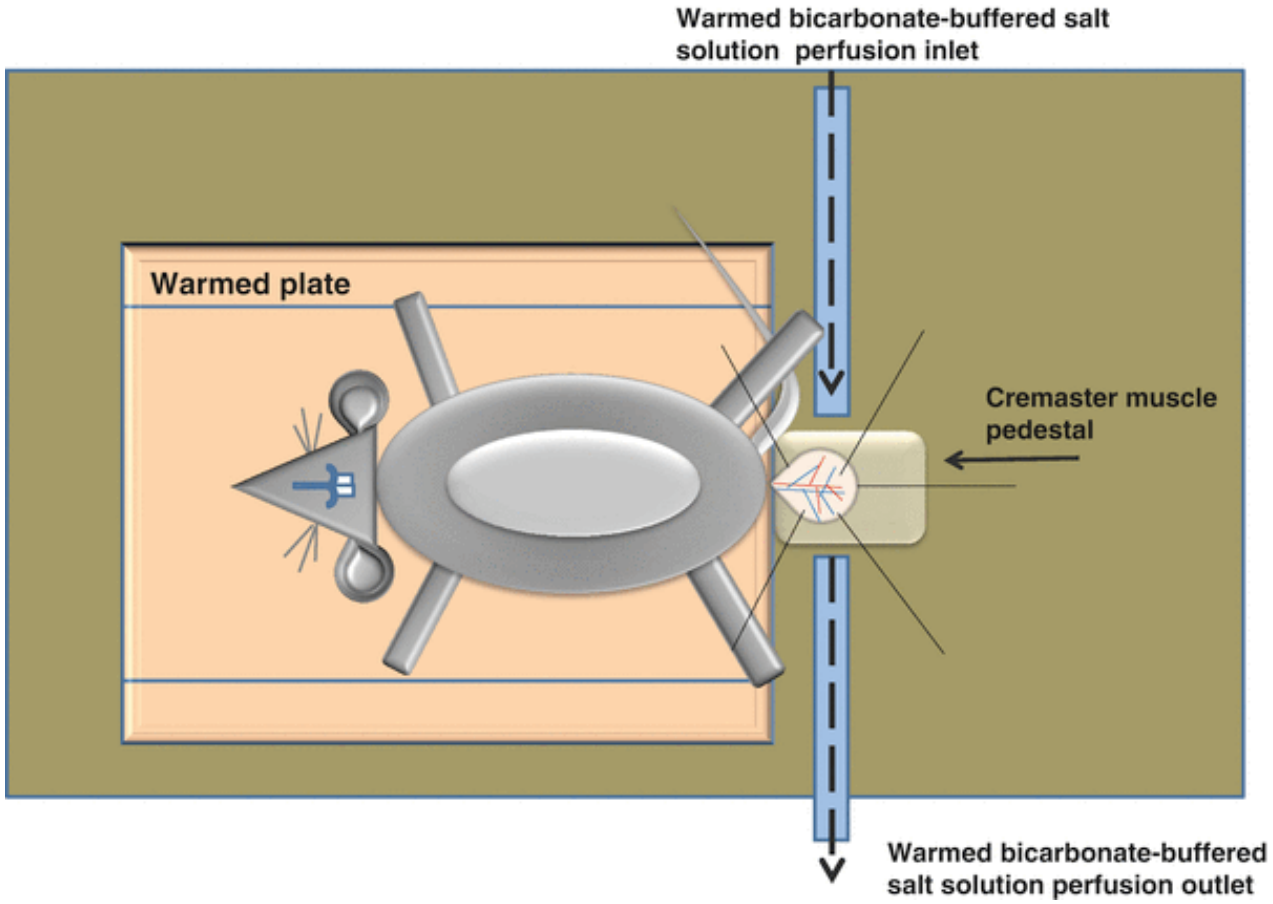
- How do cells migrate from blood into tissue?
  - the four step model
  - role of selectins, chemokines and integrins
- What controls recruitment of appropriate cell types (neutrophils, monocytes, lymphocytes) to the site of inflammation?
- How do cells exit from tissue into circulation?
- Are homing defects involved in human disease?

The science of leukocyte homing is all about movies!

Intravital imaging
in vitro slide based imaging
Two photon imaging

The science of leukocyte homing is all about movies!

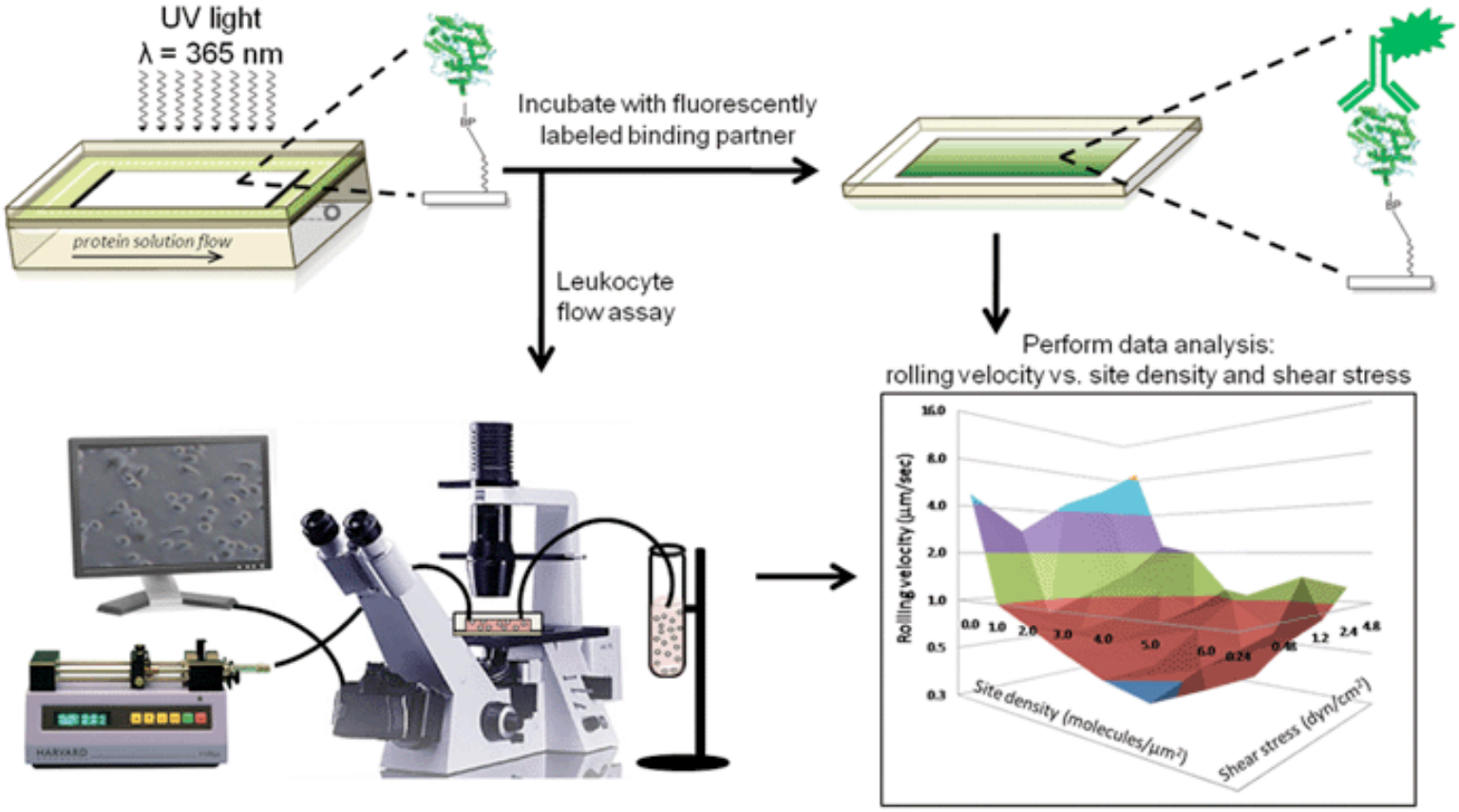
- Intravital imaging of the cremaster muscle



Rius and Sanz, Methods Mol Biol, 1339:357 (2015)

The science of leukocyte homing is all about movies!

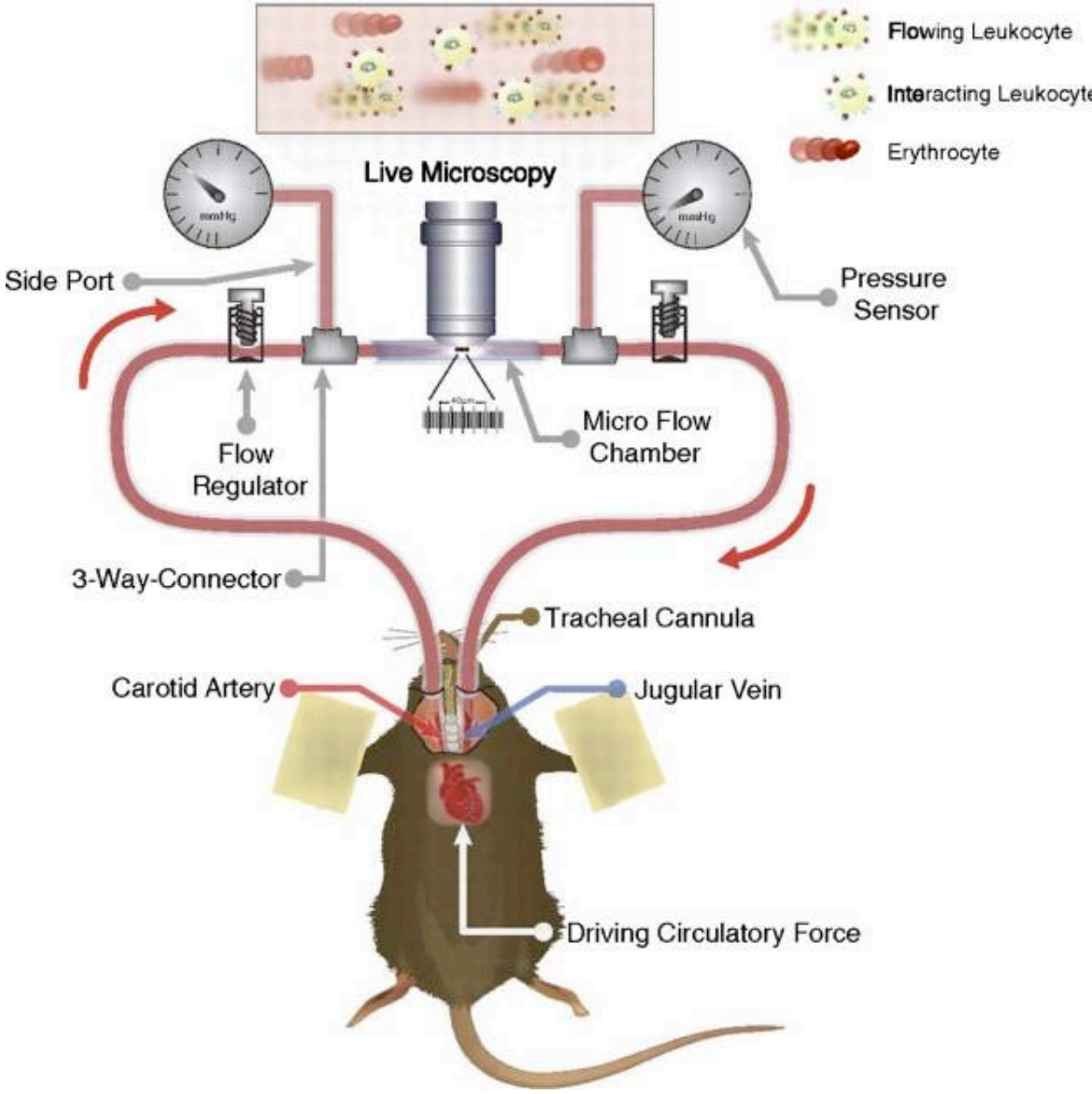
- Rolling chamber based methods – can control substrate



Herman et al, Integr Biol, 3:779 (2011)

The science of leukocyte homing is all about movies!

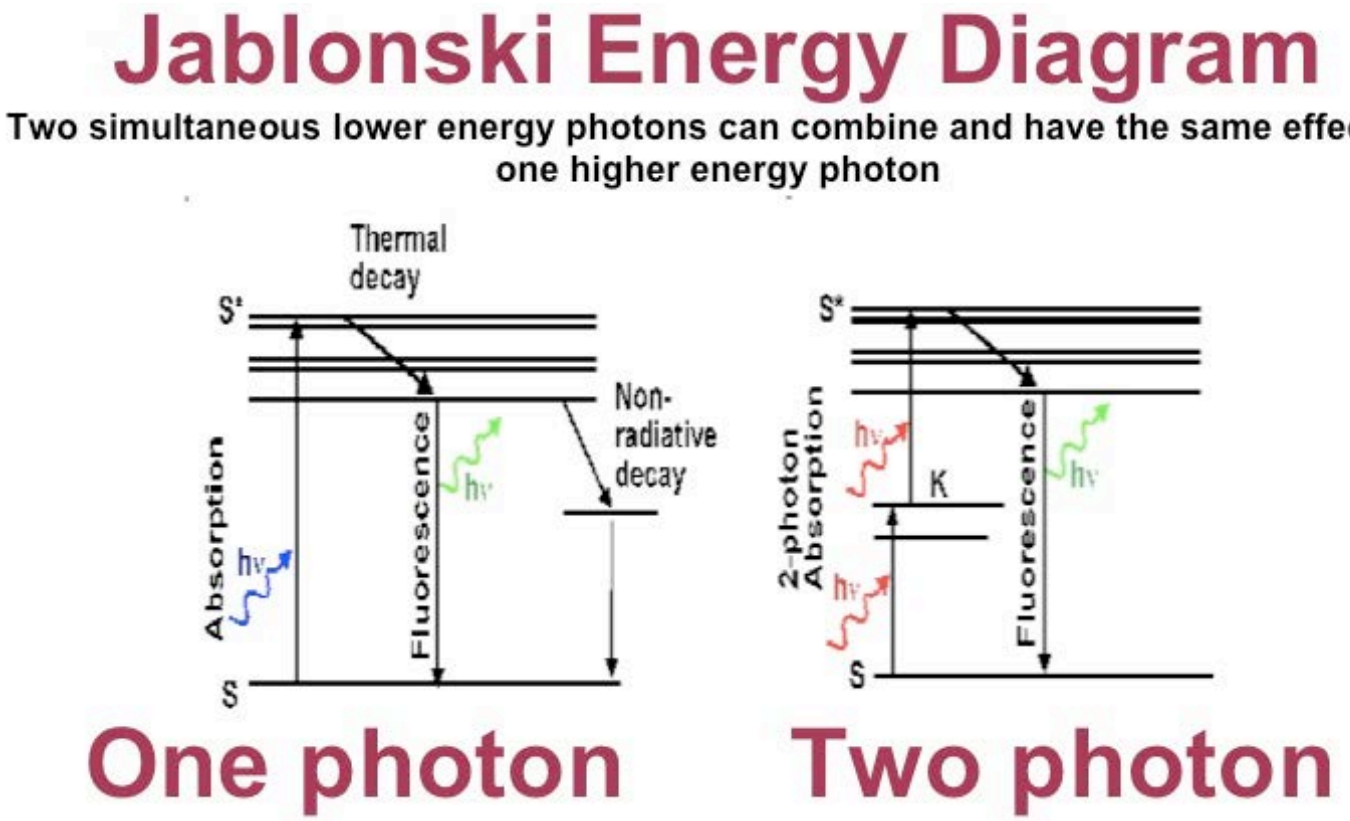
- Autoperfused chambers allow for effects of serum/protein factors and pulsatile blood flow



Feng et al. J Leuk Biol. 90:313 (2011)

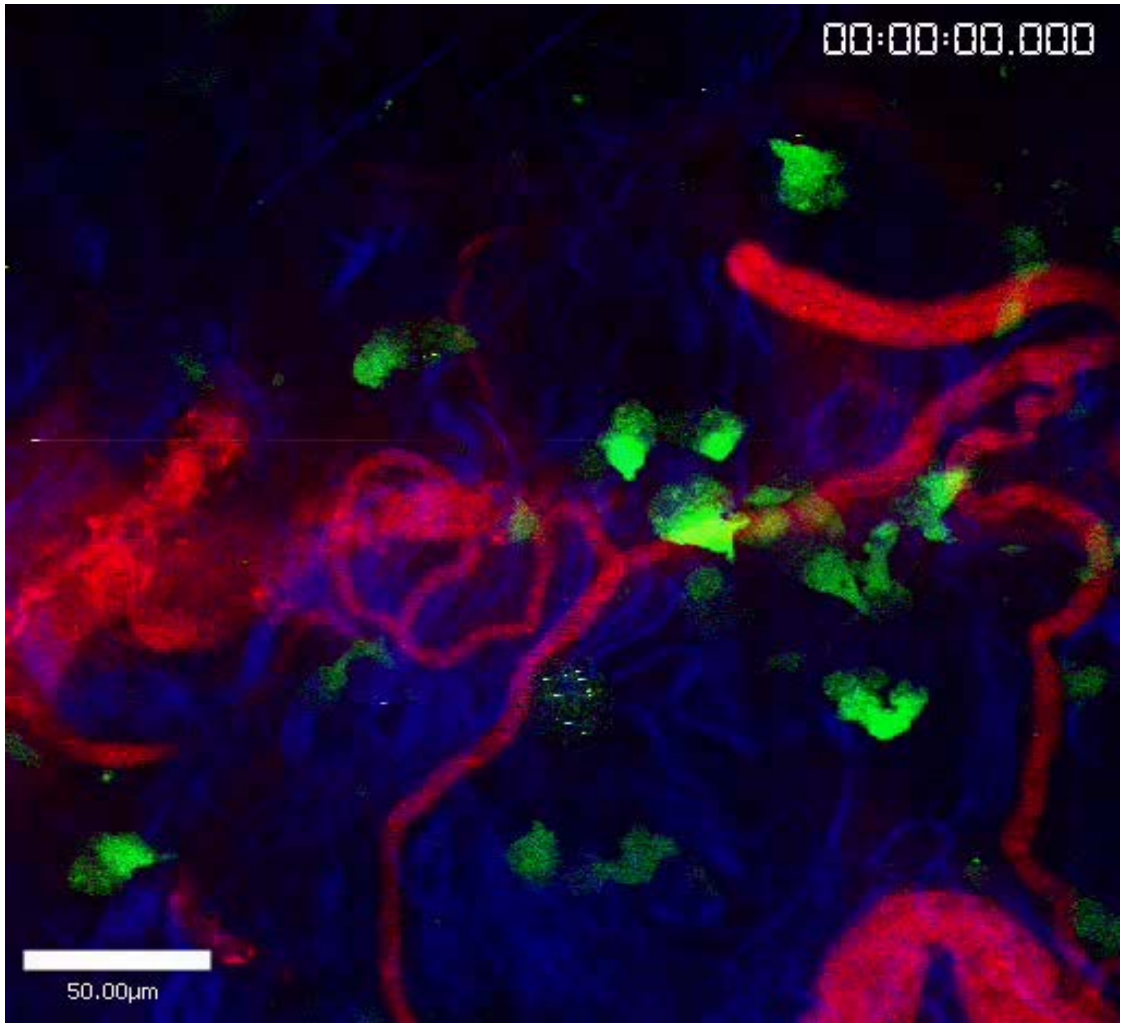
The science of leukocyte homing is all about movies!

- Two photon imaging → deeper tissue penetration
  reduced phototoxicity



The science of leukocyte homing is all about movies!

- Dermal DCs responding to skin injury
- CD11c-GFP, collagen stained blue, blood vessels are red

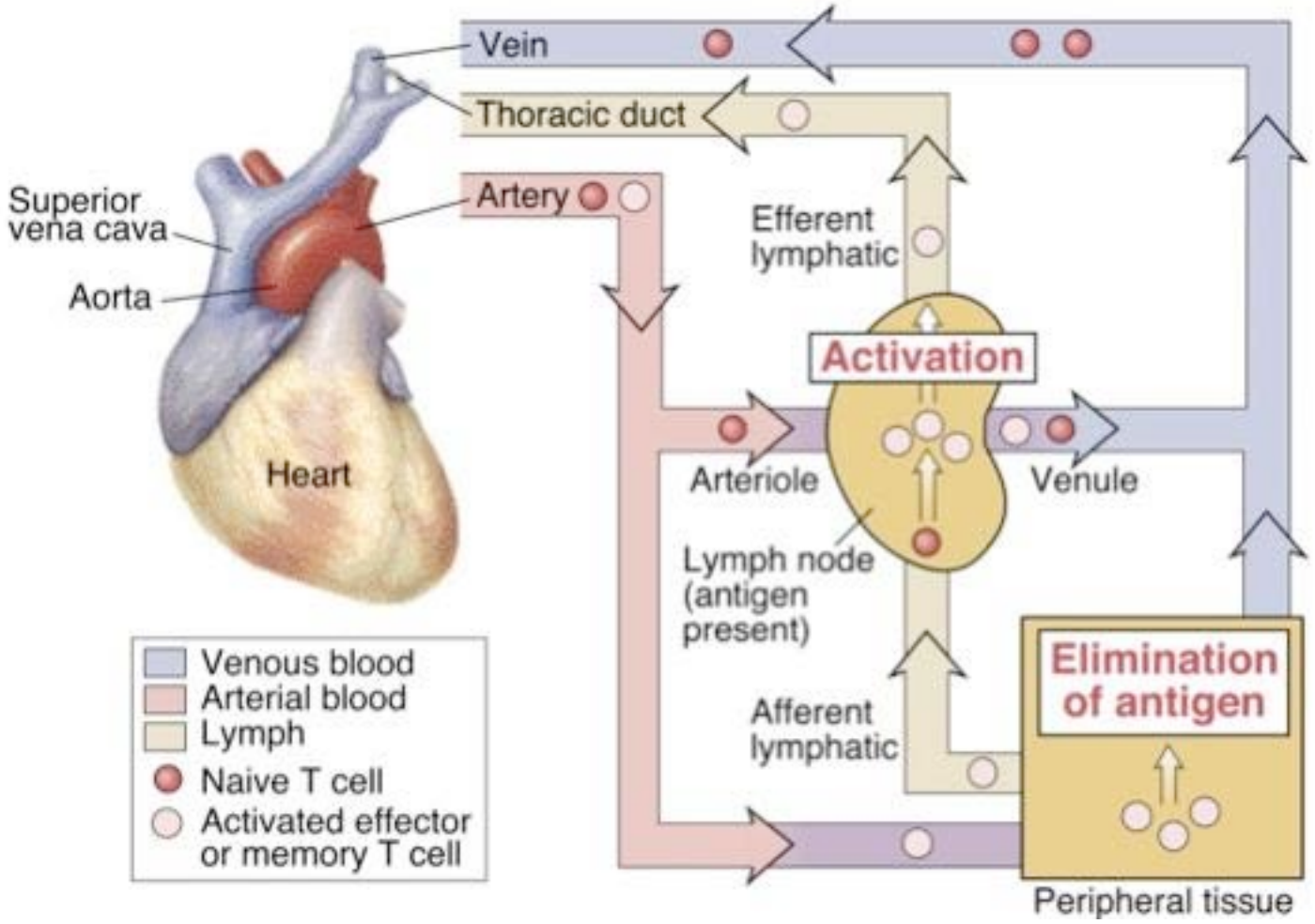


Tal et al, JEM, 208:2141 (2011)

Two major forms of leukocyte migration

- Lymphocyte homing
- Inflammation (recruitment)

Lymphocyte homing and recirculation



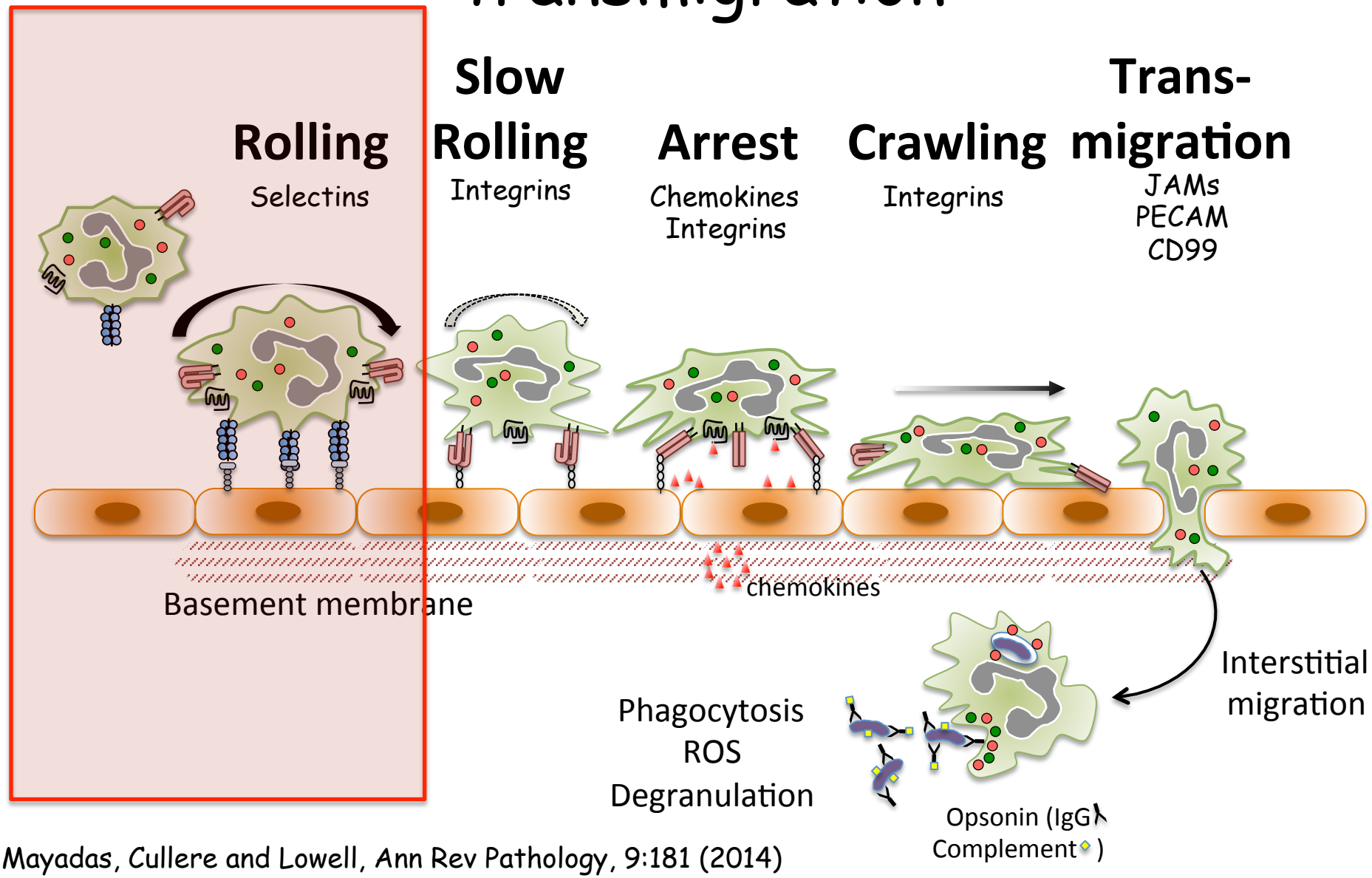
Lymphocyte Homing

- constitutive trafficking of naive T and B lymphocytes to secondary lymphoid organs
- in lymph nodes, Peyer's patches, tonsil this requires active migration across blood vessels
- entry into secondary lymphoid organs is highly selective for lymphocytes
- egress from lymphoid organs involves distinct molecular mechanisms from entry

Inflammation

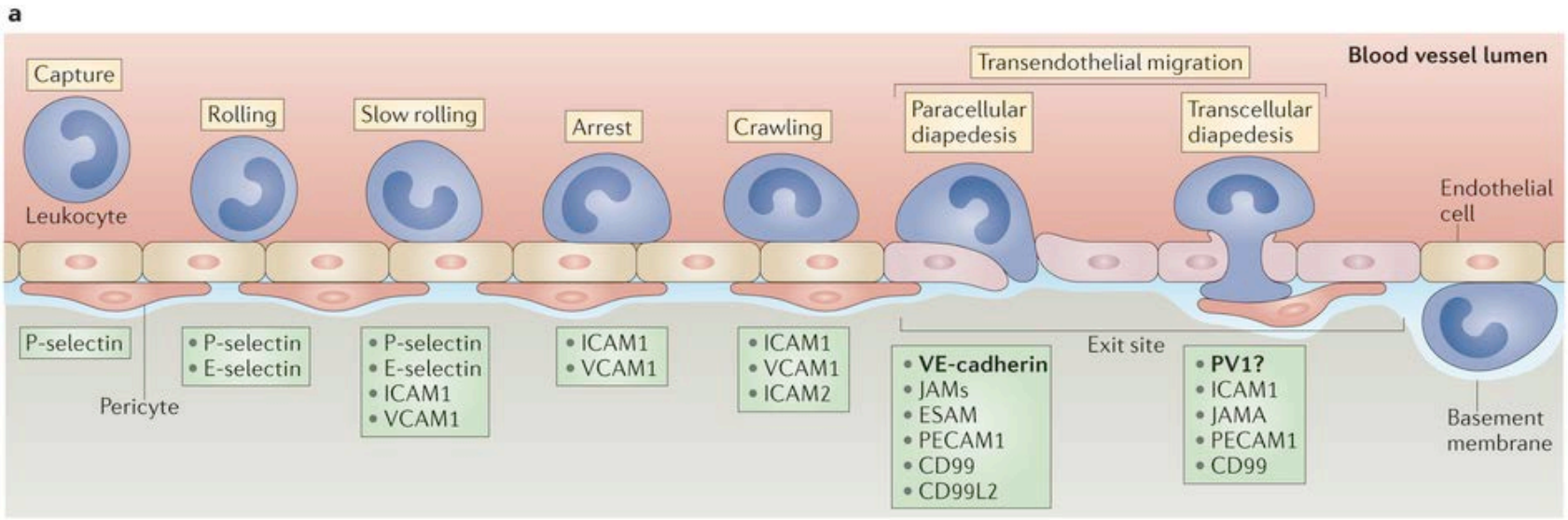
- involves local release of cytokines and chemokines by tissue cells in response to pathogen products or damage
- cytokines cause increase in vascular permeability leading to local swelling, increased entry of antibody, complement, etc.
- cytokines cause increased expression of adhesion molecules on vascular endothelium and these work together with chemokines to recruit cells - neutrophils, monocytes, NK cells and, later, effector lymphocytes

Multi-step model of leukocyte transmigration



Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

Multi-step model of leukocyte transmigration



Vestweber, Nat Rev Immunol, 15:692 (2015)

Selectins are calcium-dependent (C-type) lectins (carbohydrate binding proteins)

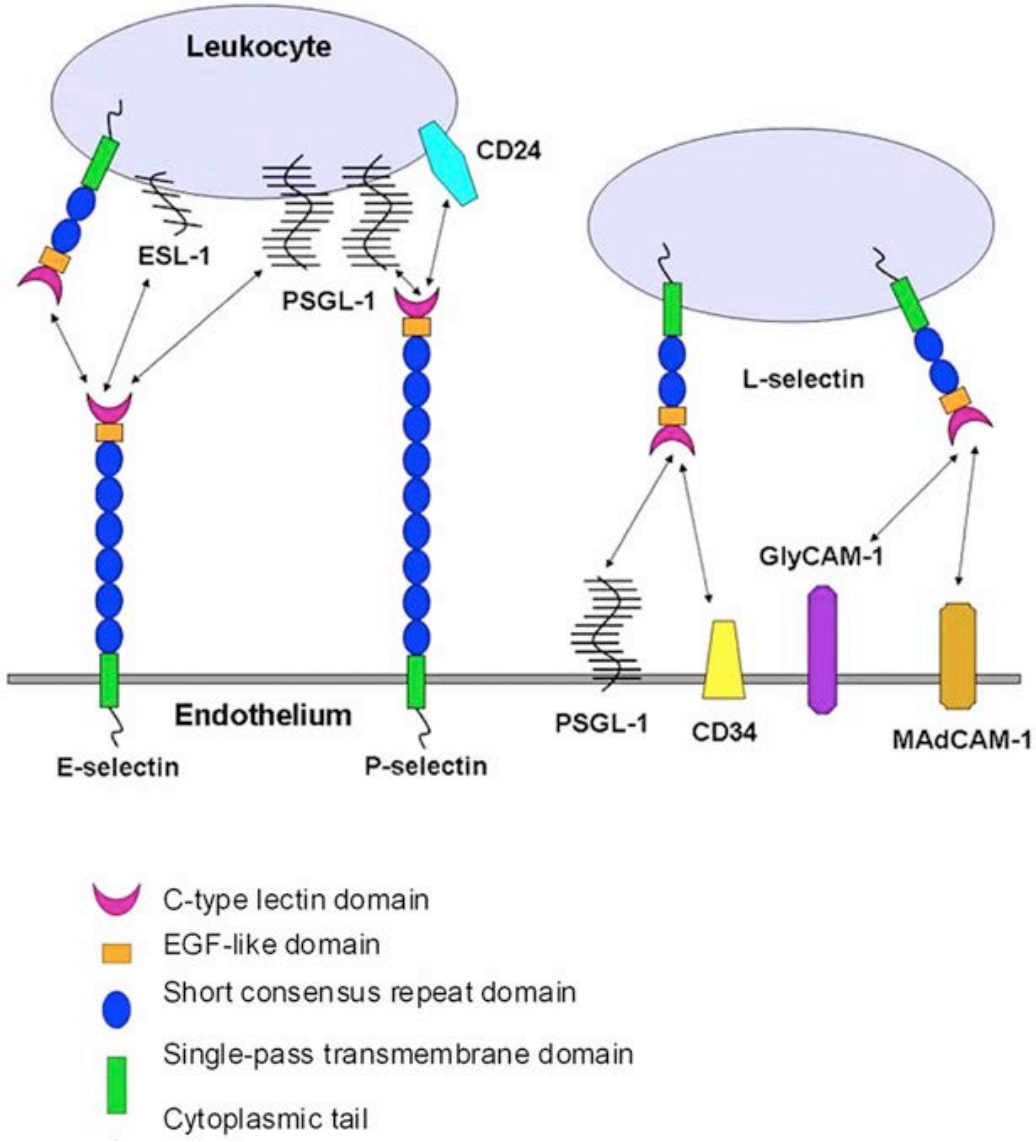
- L-selectin - entry to LNs, PPs
  - on lymphocytes (neutrophils)
  - binds specialized sulfated mucins ('peripheral node addressins' or PNAd) made by high endothelial venules (HEV)

- P-selectin - early role in entry to site of inflammation
  - in Weibel-Palade bodies in endothelial cells and α-granules of platelets
  - translocates to membrane in response to thrombin, histamine, C5a, etc
  - binds PSGL-1, a tyrosine sulfated mucin present on neutrophils, some effector T cells

- E-selectin - delayed role in entry to site of inflammation
  - cytokine inducible on endothelial cells (especially cutaneous)
  - binds carbohydrate ligand (sialyl-Le^x) on neutrophil and effector T cell glycoproteins /glycolipids

Selectins and ligands

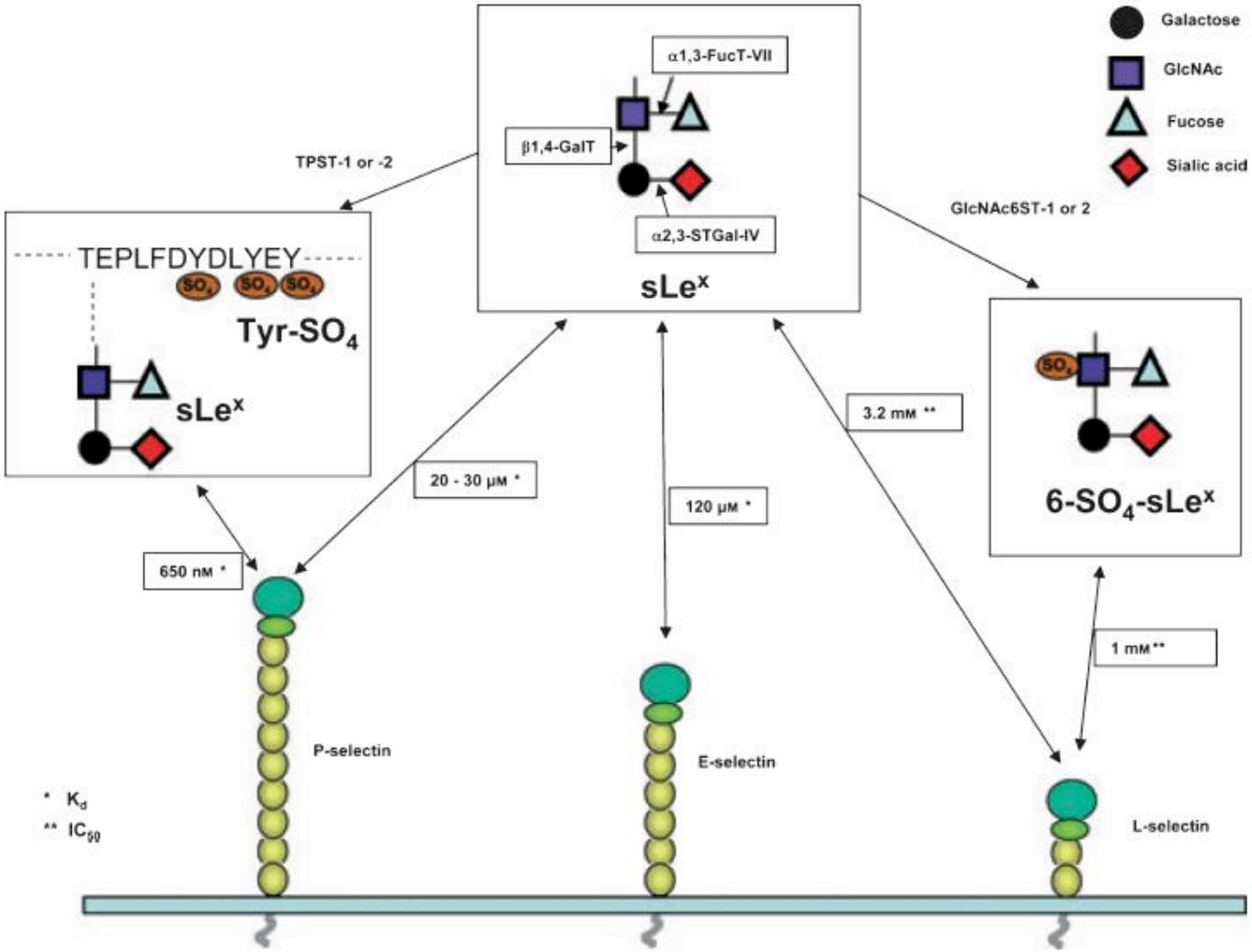
- Low affinity interactions
- Other ligands: E-selectin/CD44
- Transient interactions as a result of selectin shedding (CD62 shedding as marker of activation)



Hill, Front Bioscience, 16:3233 (2011)

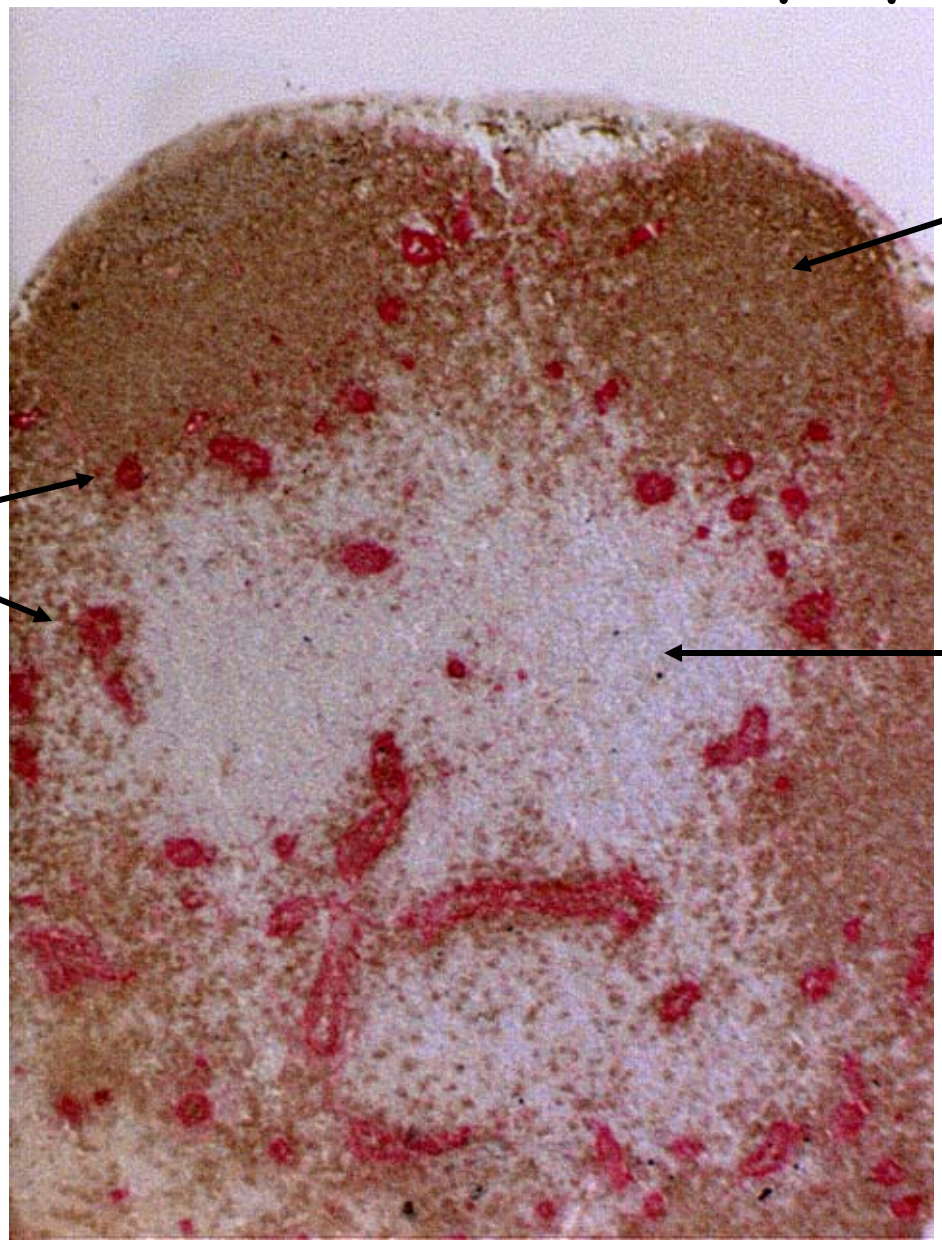
Selectins recognize sulfated carbohydrate structures on their ligands

- Sialyl Lewis X (sLex) is the core sugar structure



Sperandio et al, Immunol Rev 230:97 (2009)

Lymphocytes traverse high endothelial venules (HEVs) to enter lymph nodes



Follicle or B zone

HEV

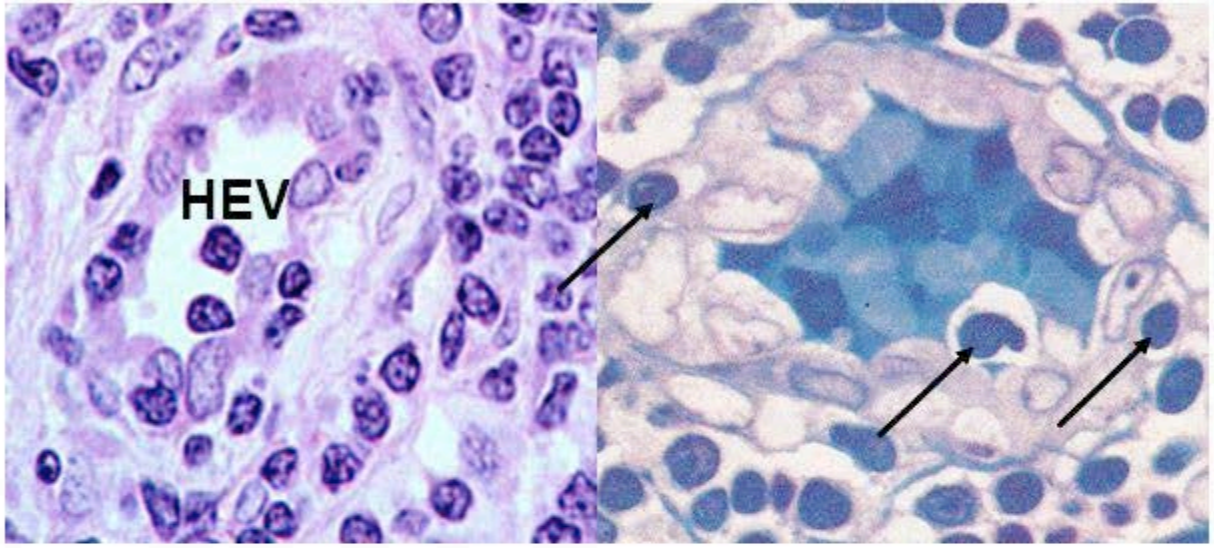
T cell area (paracortex)

LN section stained with:
B220 (pan B cell marker)
PNAd ('peripheral node address' – sulfated glycans recognized by mAb MECA79)

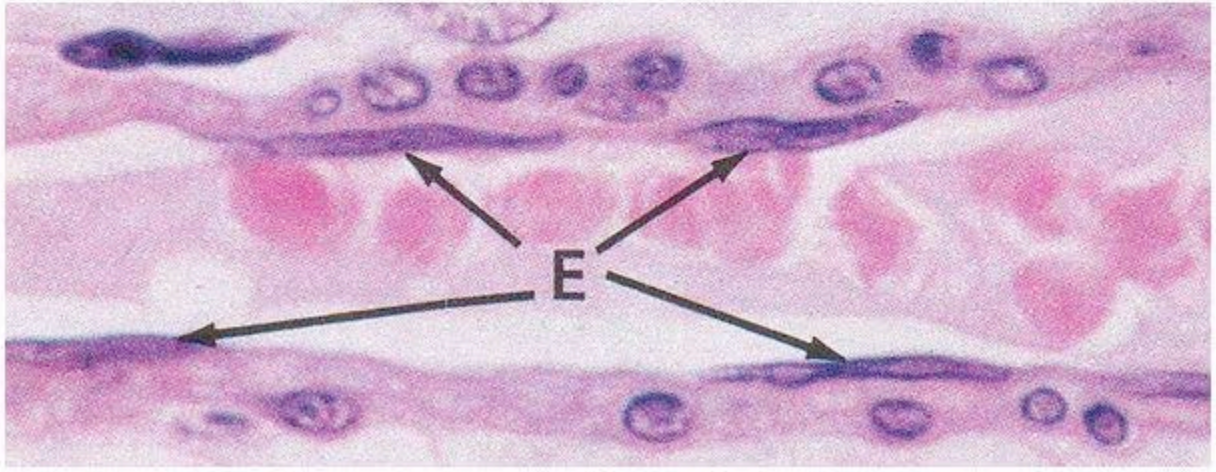
From J. Cyster

In peripheral tissues, leukocytes bind at post-capillary venules, low hydrodynamic pressure

Post capillary venules in 2° lymphoid tissue HIGH ENDOTHELIAL VENULES. Specialised to allow lymphocytes and nothing else into the lymph node



Post capillary venules in other tissues are lined by simple squamous epithelium



Key property of selectins is fast binding kinetics

- The rapid *k*on and *k*off of selectin–carbohydrate ligand interaction allows flowing leukocytes to tether and roll along endothelial cells under shear flow

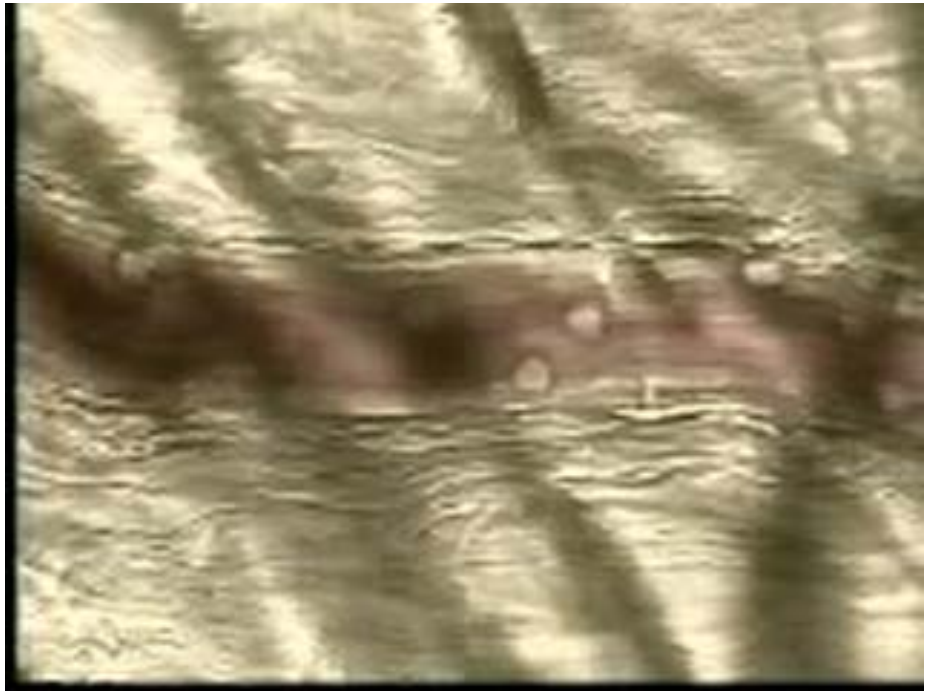
- Rolling slows down flowing leukocytes and places them in proximity to endothelial cells where chemokines are transported and expressed

- Some circumstances, integrins (VLA-4) can mediate rolling to further slow down the lymphocytes

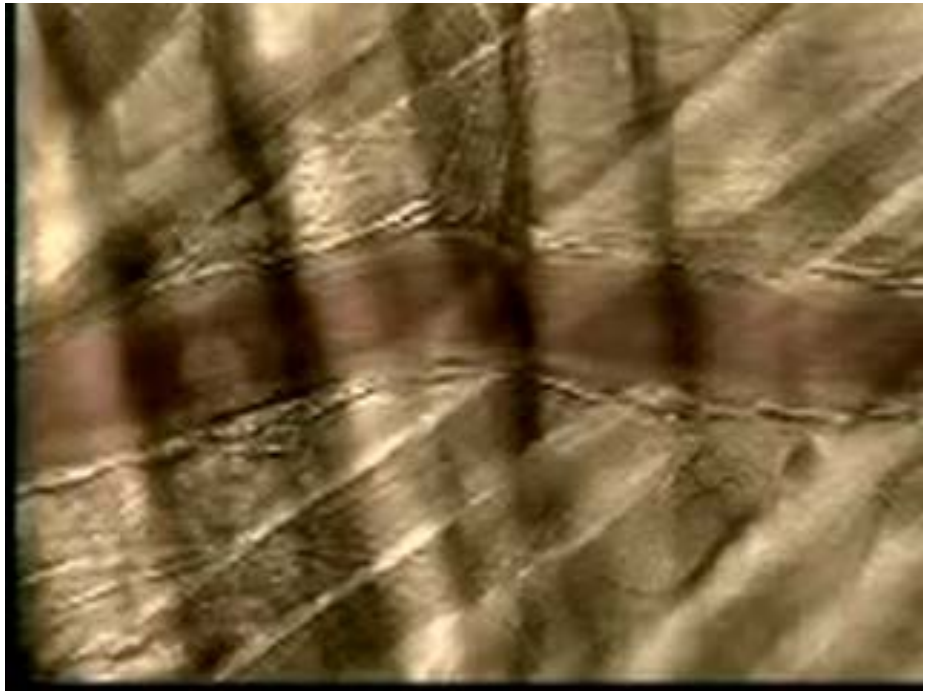
- Selectins may also have signaling function

Leukocyte rolling depends on P-selectin - PSGL1 interactions

wild-type



PSGL1-deficient

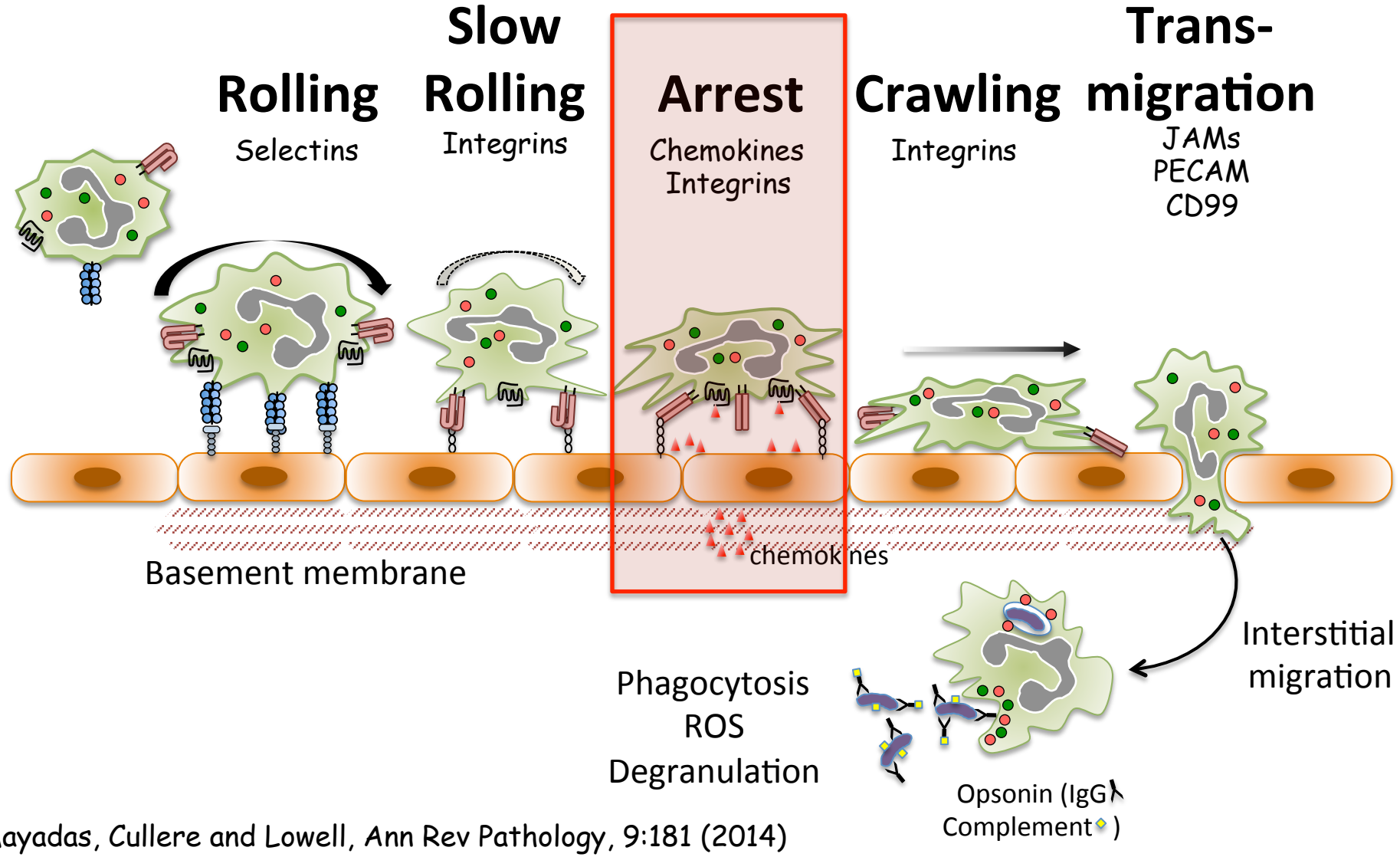


- both movies are of cremaster muscle venules within 30 minutes of surgery (selectins induced by inflammatory trauma)

PSGL-1 = P-selectin glycoprotein ligand-1

Yang et al, JEM, 190:1769 (2000)

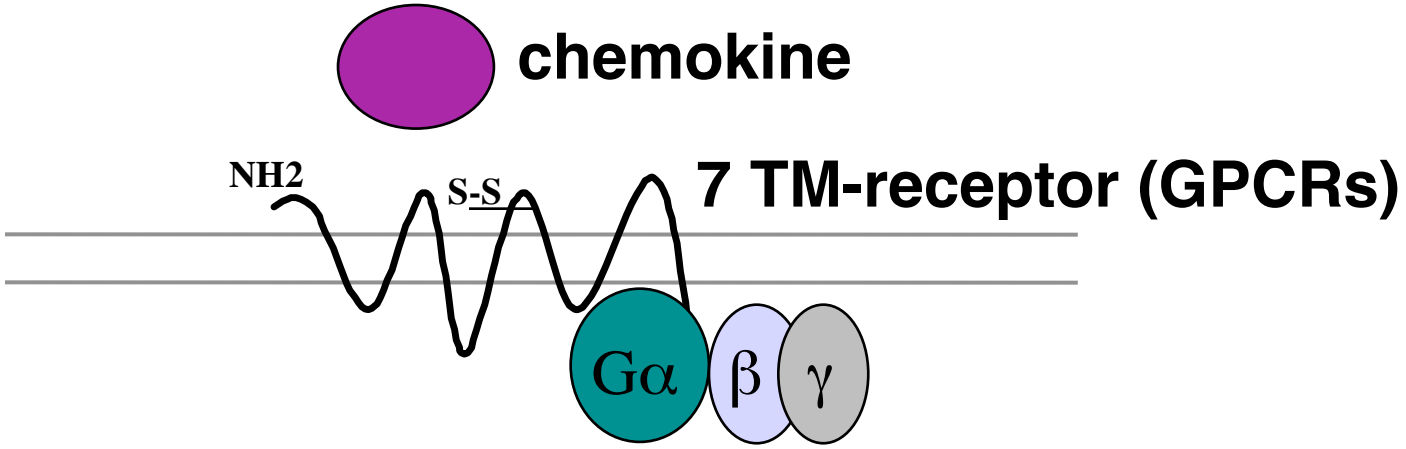
Multi-step model of leukocyte transmigration



Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

Chemokines

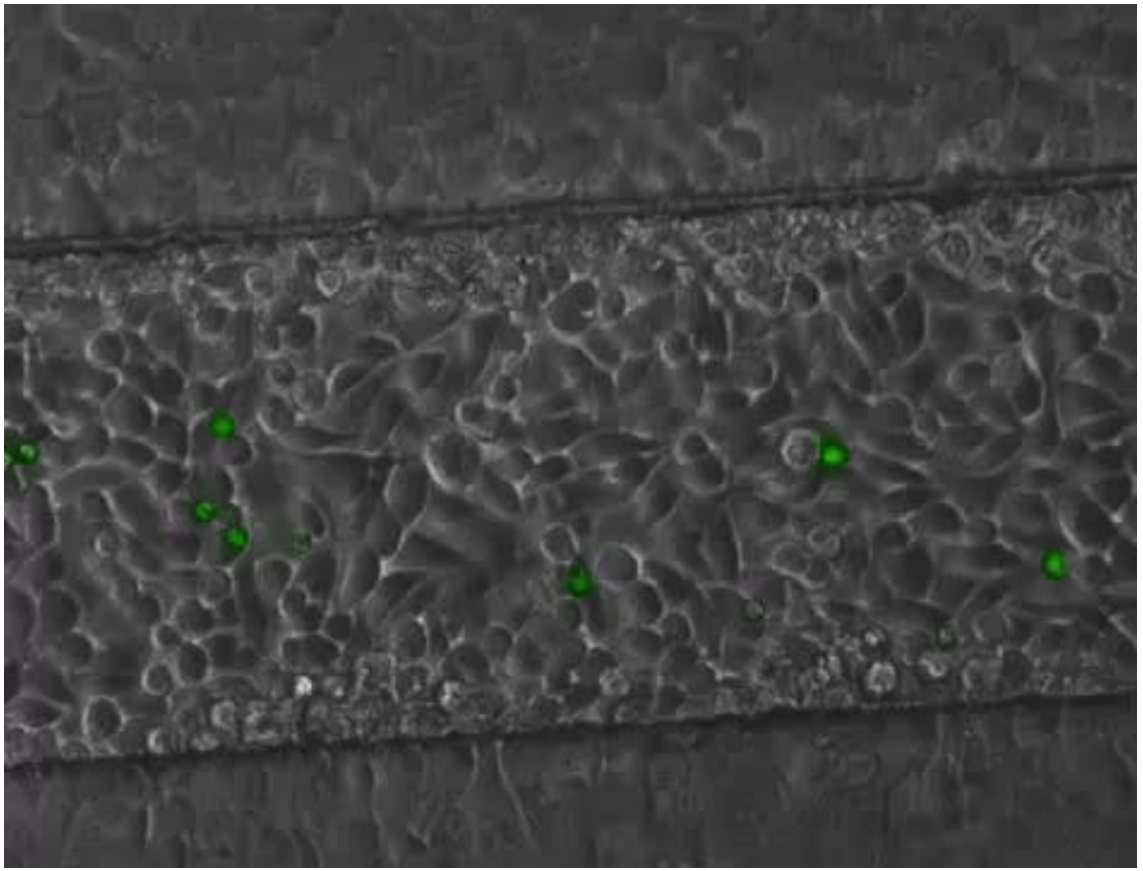
- >40, structurally related basic proteins of ~10kD
- Four families: *C*, ***CC***, ***CXC***, *CX3C*



Lymphoid chemokines – help direct the homeostatic trafficking of cells into and through peripheral lymphoid tissues (e.g. CCR7-CCL21 and CCL19; CXCR5-CXCL13)

Inflammatory chemokines – help recruit cells to sites of inflammation (e.g. CXCR2 / IL-8; CCR2 / MCP1; CCR5 / MIP1α)

Neutrophil migration/polarization in response to IL-8 is amazingly fast



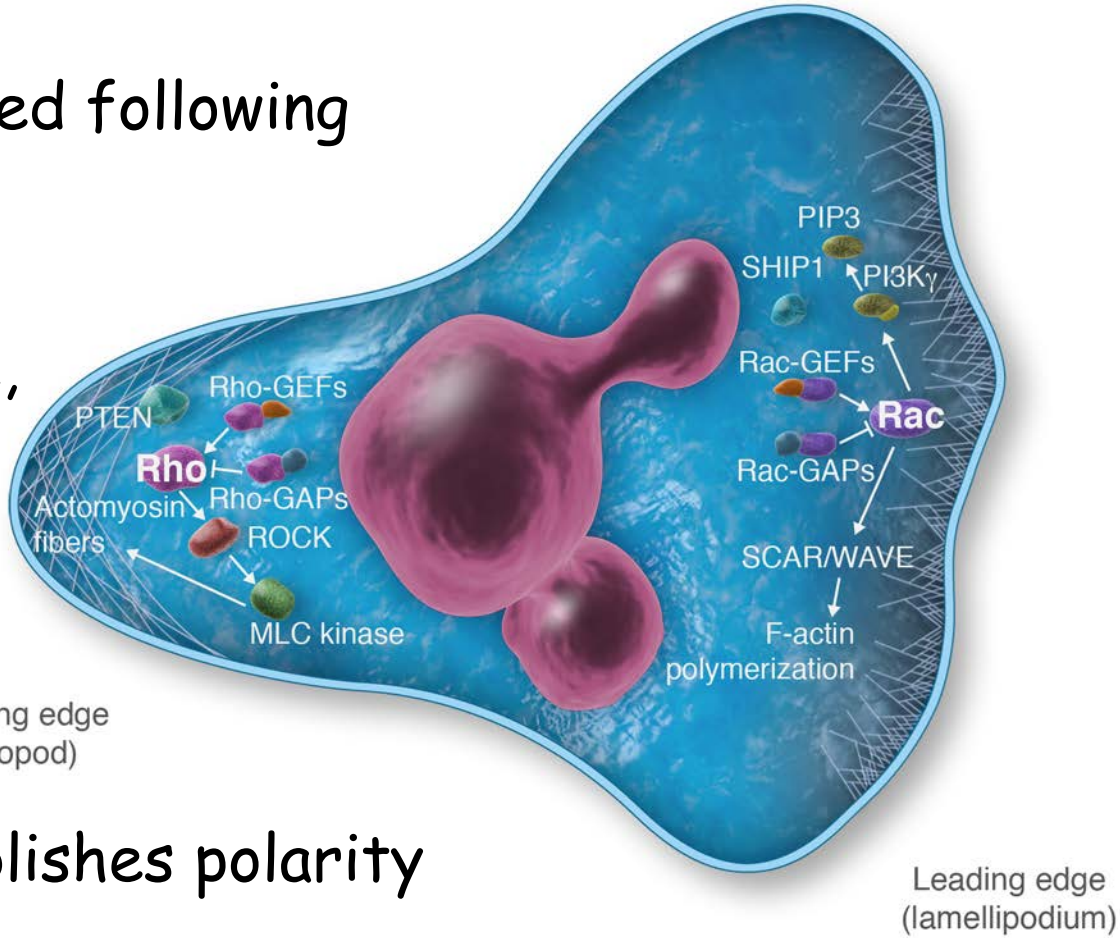
- Signaling involves rapid calcium flux

Neutrophil migration/polarization in response to IL-8 is amazingly fast

- complex signaling and cell biology to allow such rapid responses -- front end versus back end

Differential signaling in neutrophils allows them to sense chemokine gradients

- Neutrophils are polarized following chemokine stimulation
- Rac at the leading edge, Rho at the tail
- Self re-enforcing signaling plus inhibition of other pathway establishes polarity



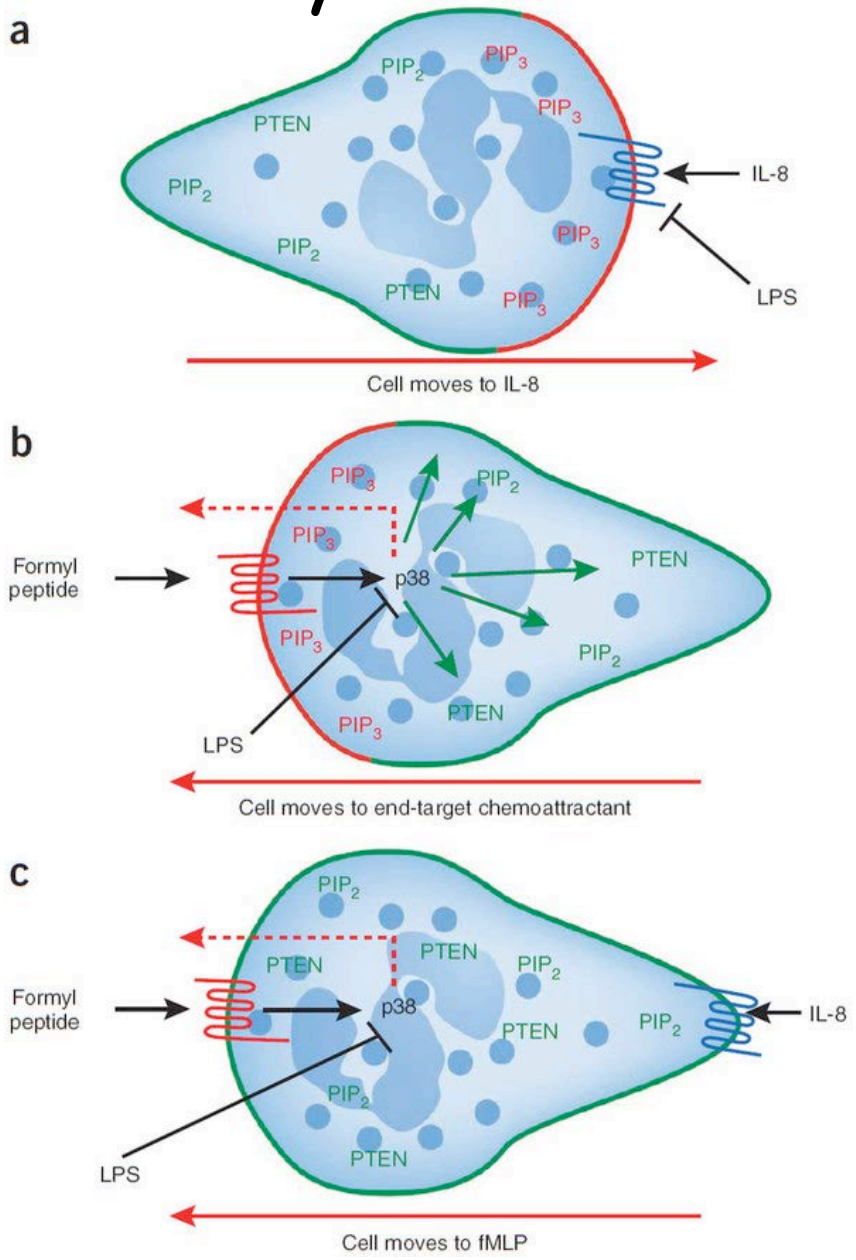
Mocsai, Walzog and Lowell, Cardiovascular Res, 107:373 (2015)

Differential signaling in neutrophils allows them to sense chemokine gradients

- Polarity increases with increasing signal strength, so as cells move up the chemokine gradient, they become more polarized (feed-forward signaling response)
- Front edge is pushed forward by actin polymerization
- Receptor de-sensitization stops the signal
- GPCRs are evenly distributed over cell surface
- Polarization is the neutrophil "compass"

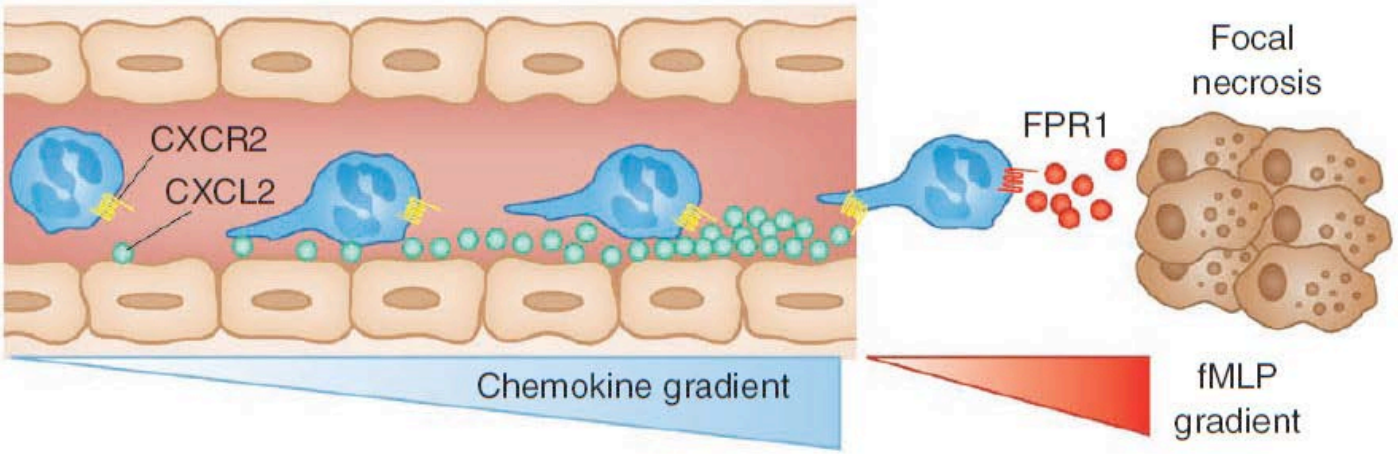
Chemokine hierarchy

- Why so many chemokines?
- Strength of signal determines directionality
- Allows fine tuning of response as well as when to arrest



Phillipson and Kubes, Nat Med, 17:1381 (2011)

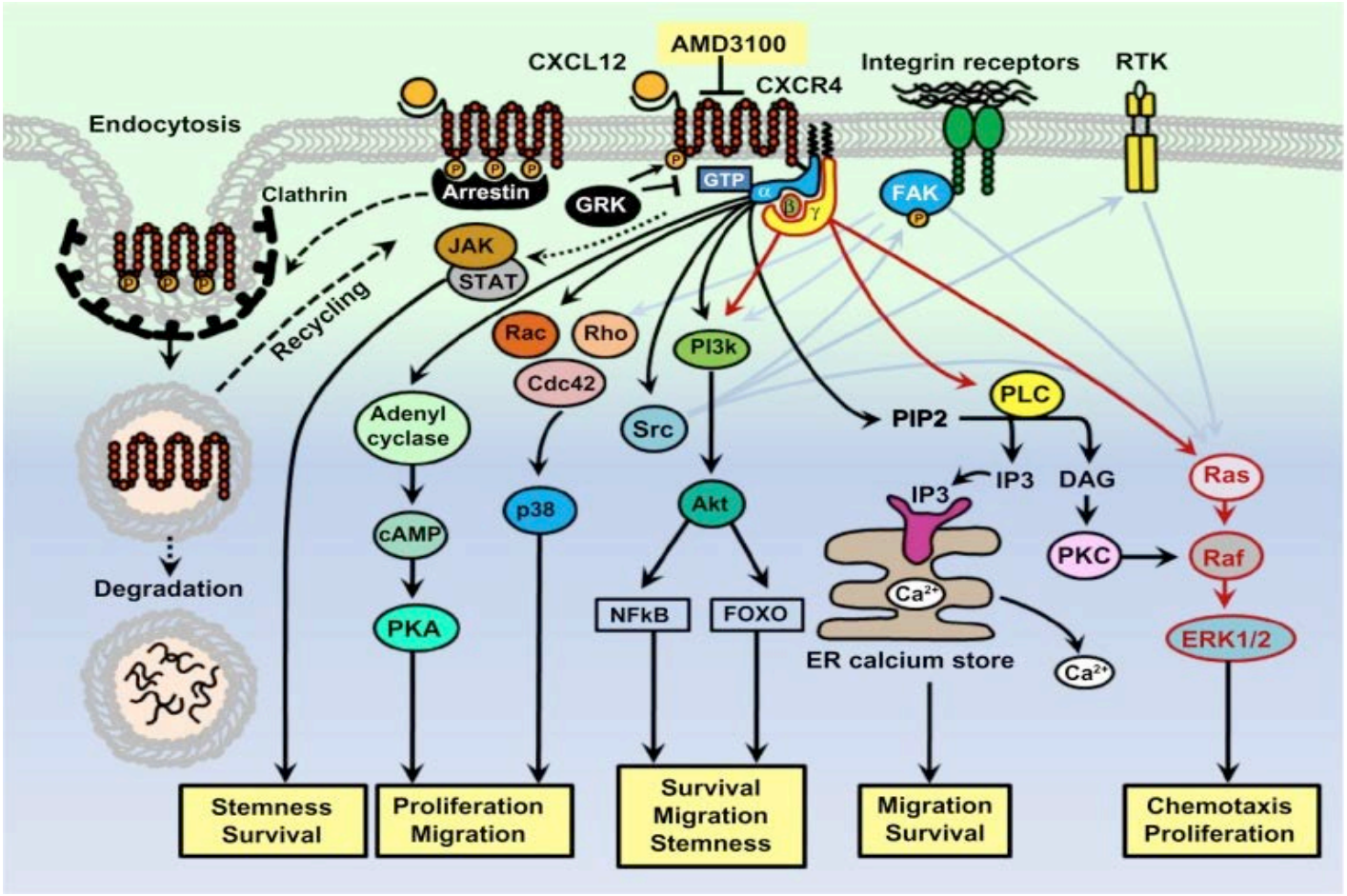
Chemokine hierarchy



- Intermediate chemokines versus end target chemokines

Phillipson and Kubes, Nat Med, 17:1381 (2011)

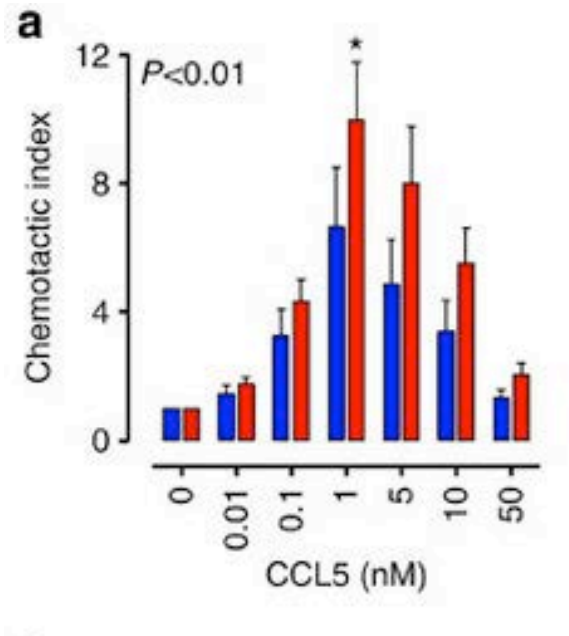
Chemokine signaling pathways



- Gα and Gβγ subunits → initiate different downstream pathways
- Ga is target of PTx, used all the time to block chemotaxis
- GRK activation critical to turn it off

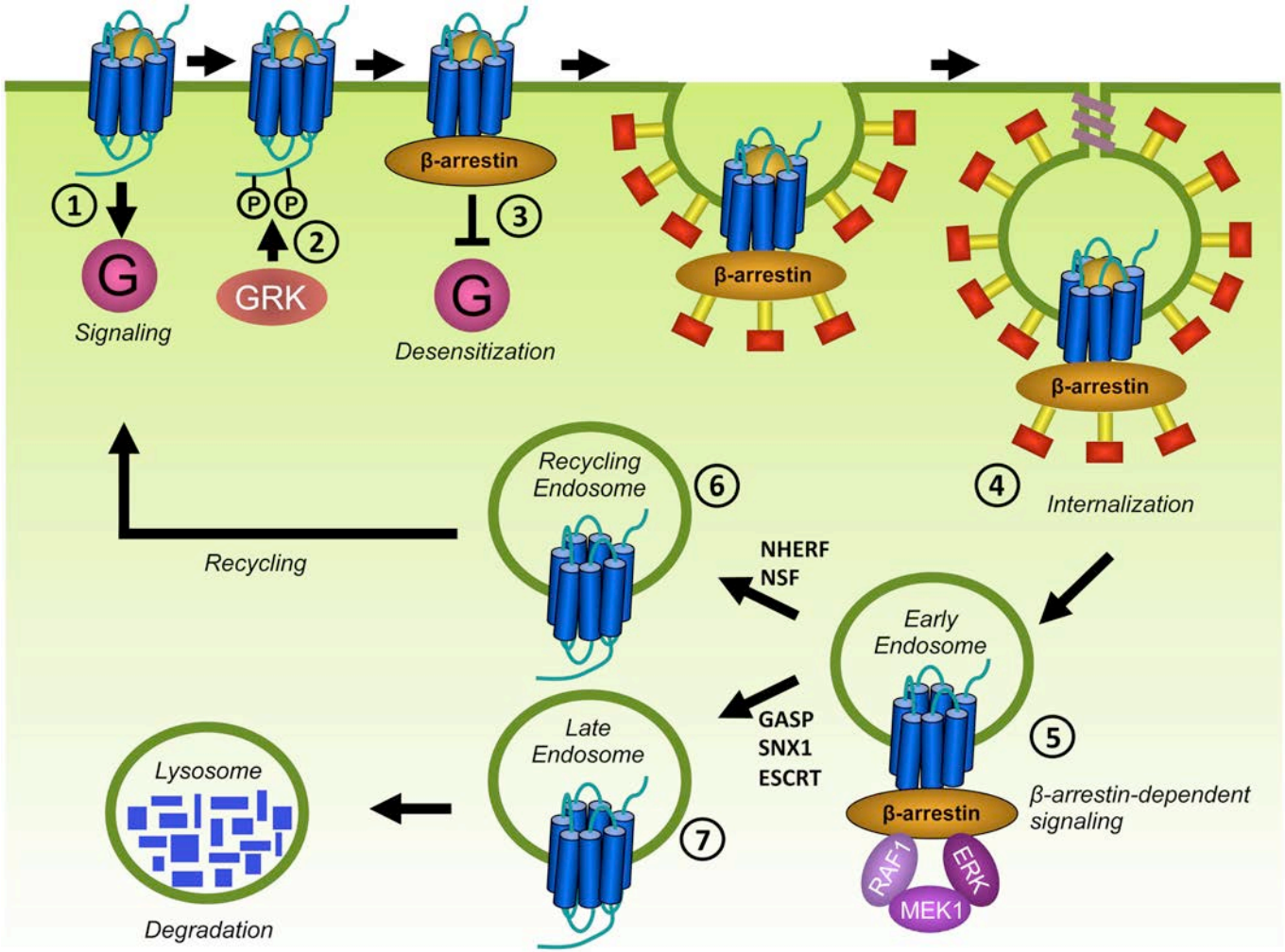
Cojoc et al, OncoTargets, 6:1347 (2013)

Chemokine responses are always bell shaped curves – high doses block migration



Patel et al, Nat Commun, 6:6614 (2015)

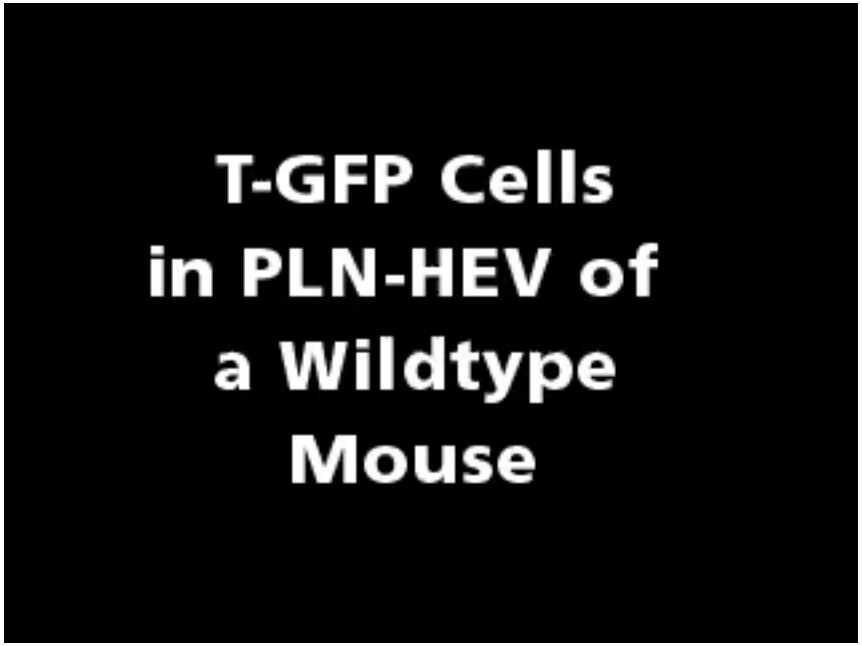
Chemokine signaling pathways receptor desensitization



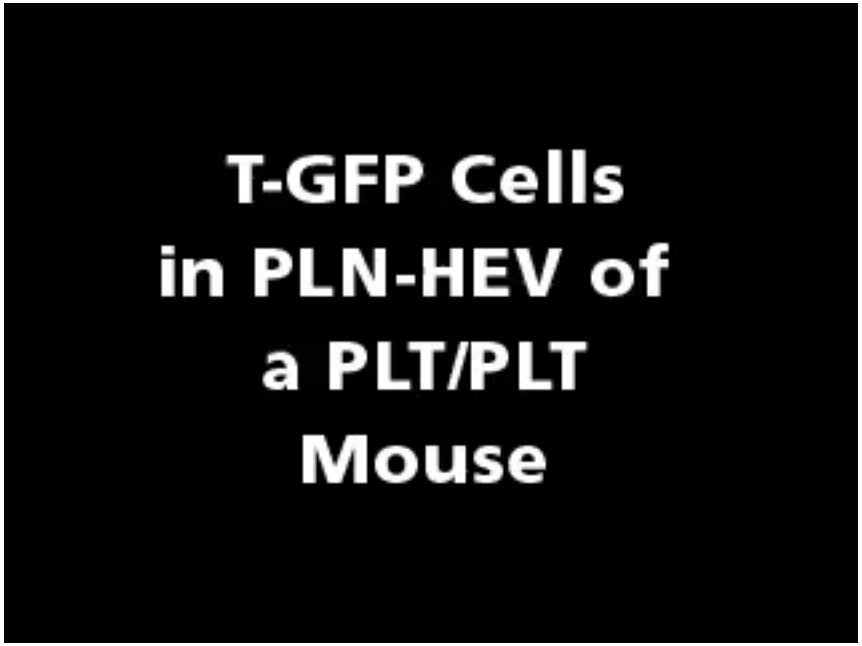
de Munnik et al, Front Pharmcol, 6:40 (2015)

Lymphocyte adhesion to HEV depends on chemokines

normal lymph node



CCL21/CCL19-deficient lymph node

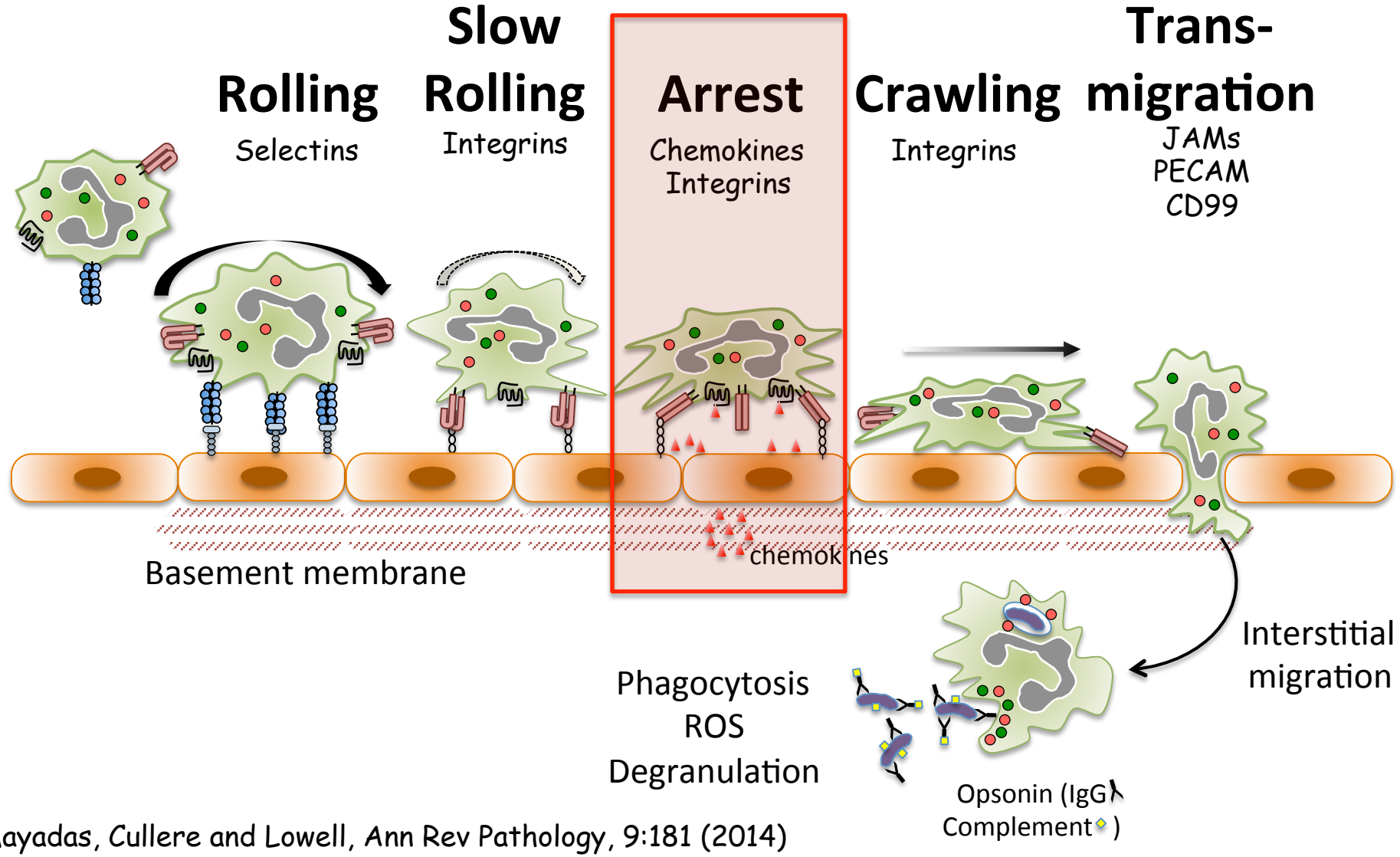


Stein et al, JEM, 191:61 (2000)

Chemokines and chemoattractants

- Chemokines produced by both inflammed endothelium as well as resident macrophages
- Inflammation is enhanced by production of chemokines by inflammatory cells (neutrophils, inflammatory monocytes)
- Chemokines often localized to endothelium by interaction with proteoglycans on cell surface
- Chemoattractants (fMLF, LTB4) vs chemokines (size and composition)

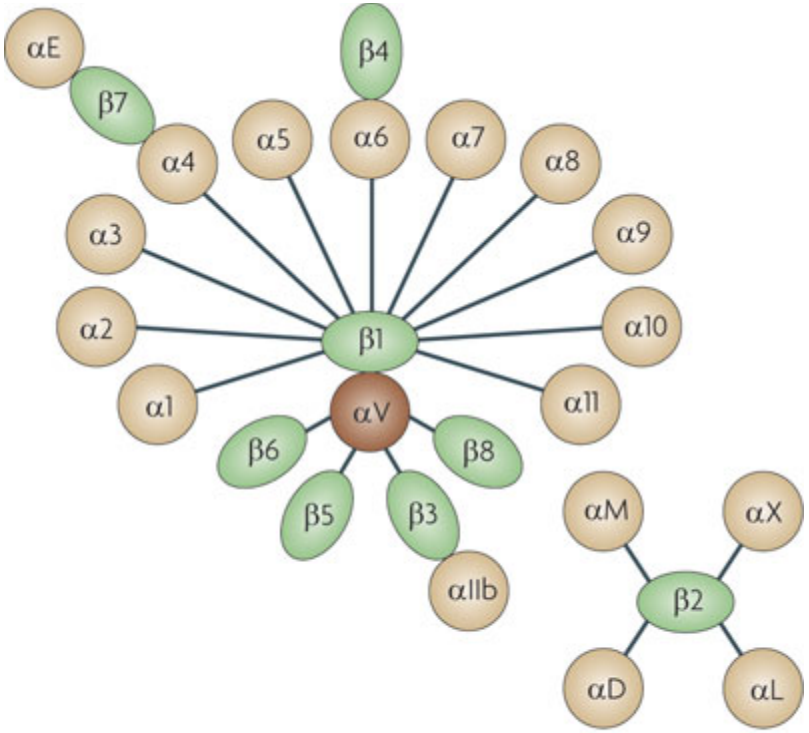
Multi-step model of leukocyte transmigration



Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

The integrin family of receptors

- dimers of α and β chains
- expressed on all nucleated cells (RBCs lack)
- classify based on β chain
- most bind ECM matrix proteins (collagen, fibronectin, etc); some have transmembrane receptors present on other cells
- bi-directional signaling properties
- involved in cell adhesion



Cox et al, Nat Rev Drug Discov, 9:804 (2010)

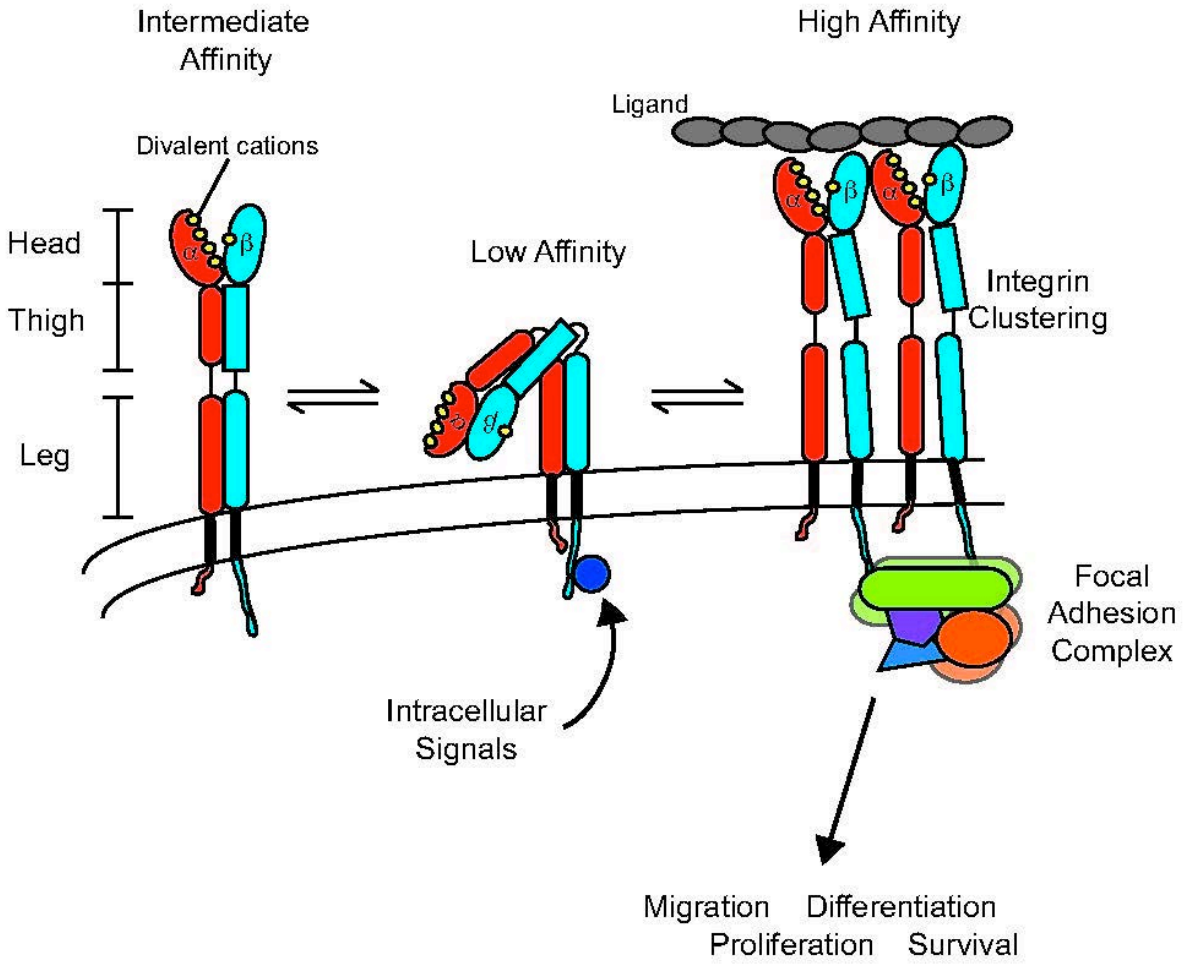
Integrins involved in leukocyte attachment to endothelium

Integrin	Ligands
αMβ2 (CD11b/CD18, Mac1)	ICAM-1, ICAM-2 (ICAM-3)
αLβ2 (CD11a/CD18, LFA1)	ICAM-1, ICAM-2 (ICAM-3)
α4β1	VCAM-1, MADCAM-1, FN
α4β7	MAdCAM-1, VCAM-1, FN

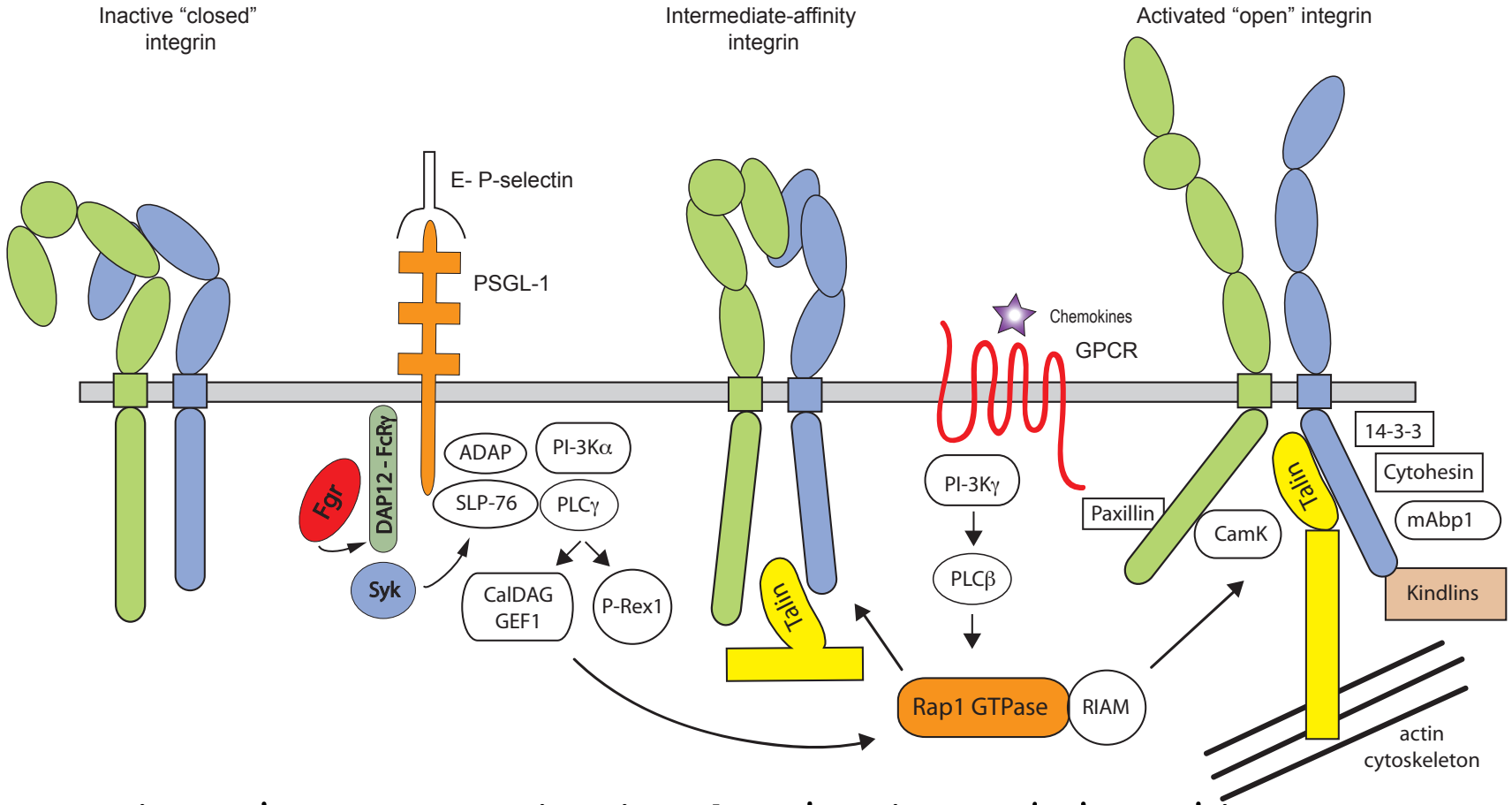
- ICAM1 and VCAM1 are constitutively expressed on lymph node HEV and are upregulated on inflammed endothelium
- MAdCAM-1 is expressed by HEV in mucosal lymphoid tissues

Bi-directional signaling for integrins: inside-out vs outside-in

- in resting cells, integrins in the bent, low affinity state
- with cell activation (via chemokines/selectins), integrins assume intermediate or high affinity state
- Allows ligand binding, integrin clustering
- leads to formation of focal adhesion (or podosome) signaling complex that activates downstream pathways



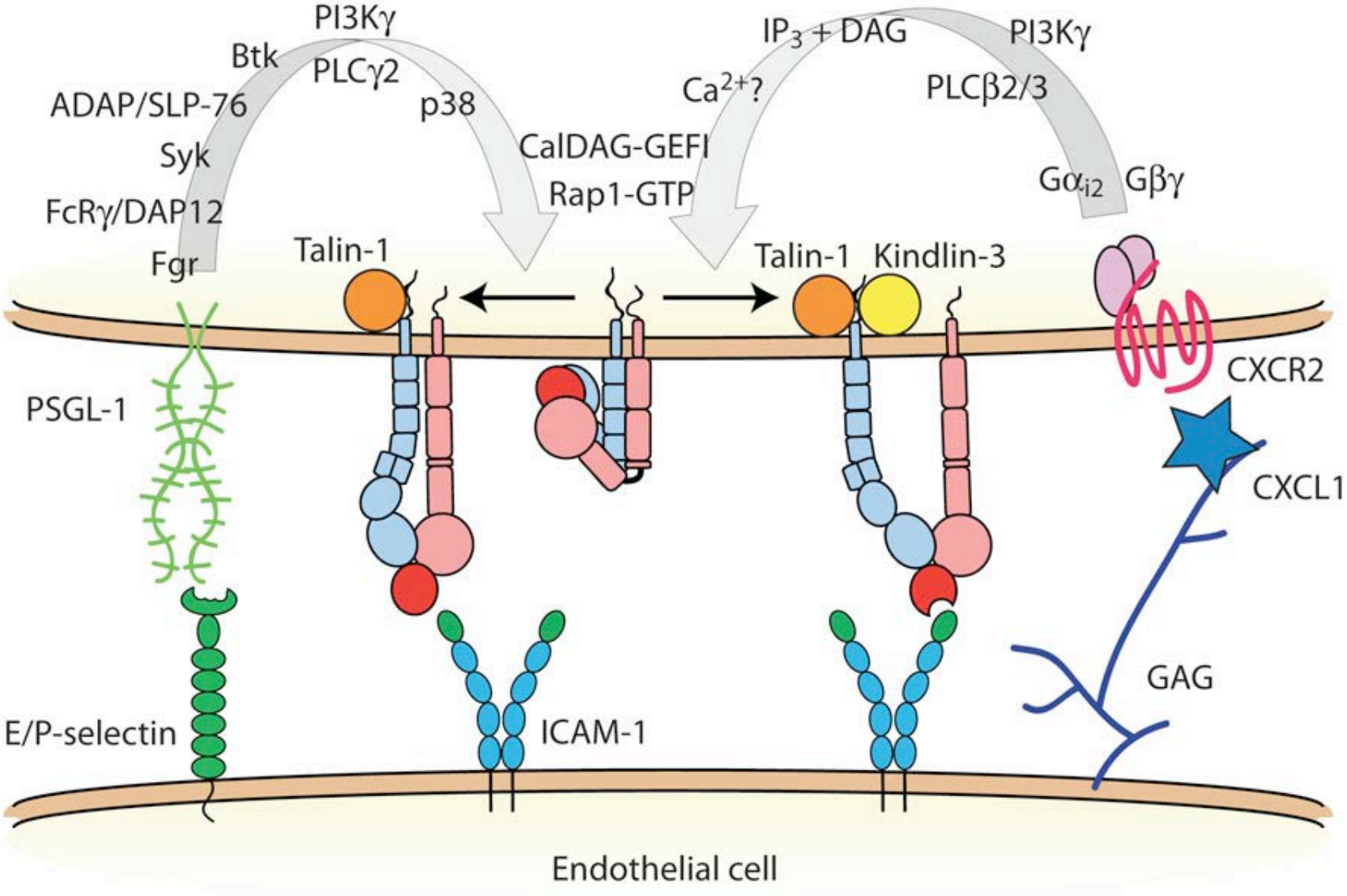
Integrin inside-out signaling leading to activation and high affinity binding



- two main pathways to activation → selectins and chemokines
- switch blade like conformational change
- link integrins to actin cytoskeleton

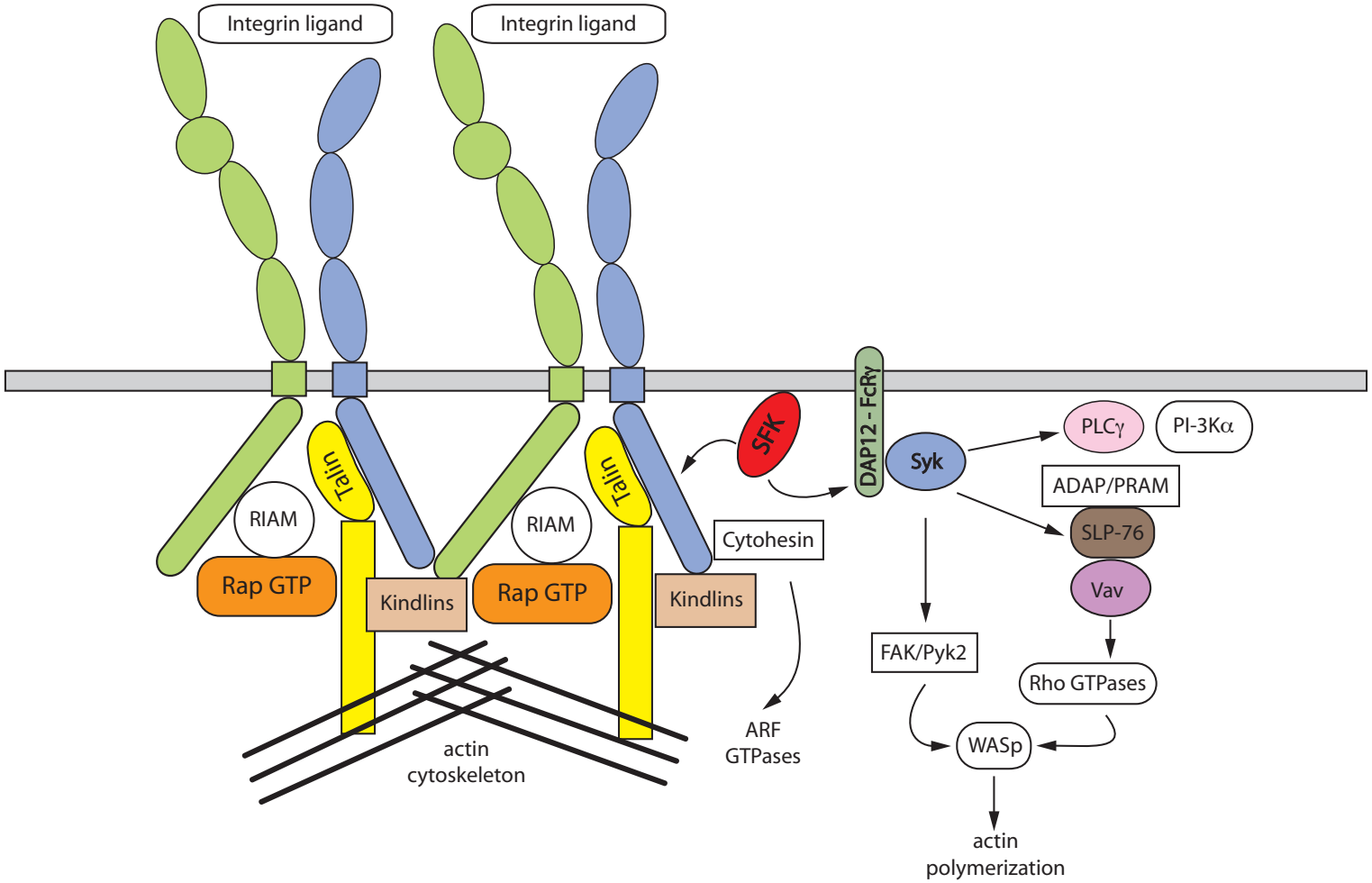
Lowell, Encyclopedia Immunobiology, 3:72 (2016)

Integrin inside-out signaling leading to activation and high affinity binding



Lefort and Ley, Front Immunol, 3:1 (2012)

Integrin outside-in signaling promotes cell activation

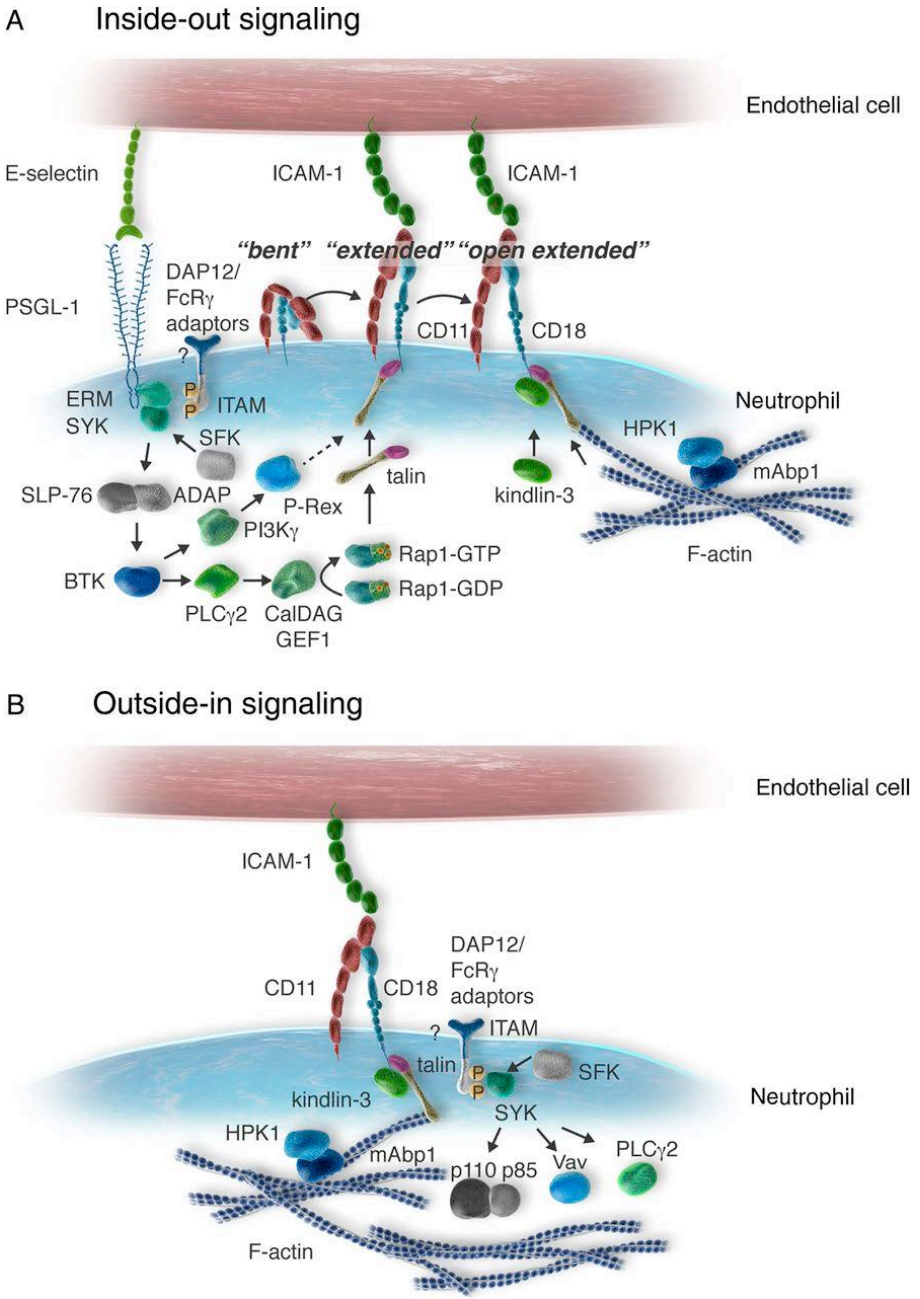


- cytoskeletal rearrangement and cell spreading → firm adhesion

Lowell, Encyclopedia Immunobiology, 3:72 (2016)

Another view of inside out versus outside in integrin signaling

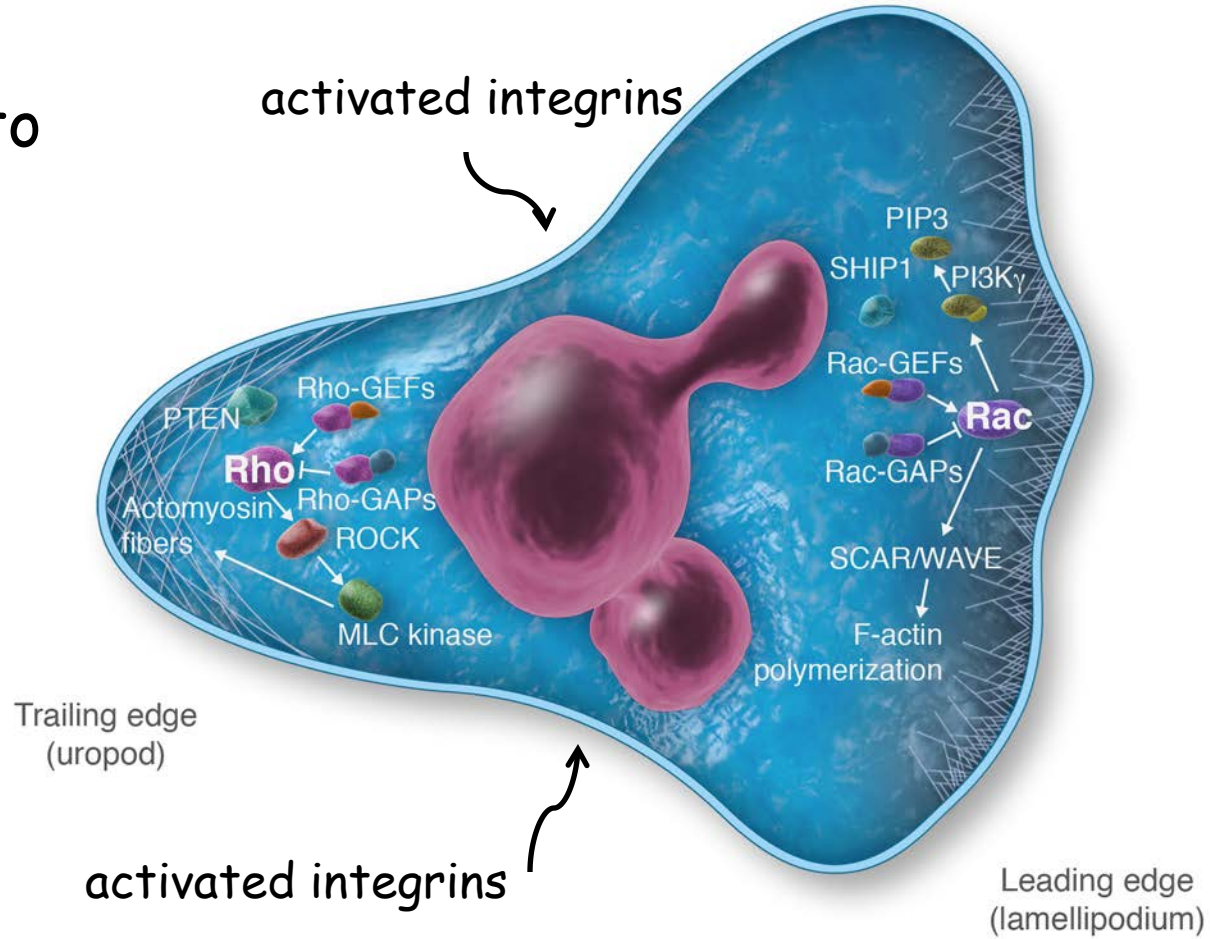
- Note that many of the same molecules involved in both pathways



Mocsai, Walzog and Lowell, Cardiovascular Res, 107:373 (2015)

Activated integrins on lateral sides of migrating leukocytes

- Allows lamellopodia to push forward
- Process of integrin deactivation at the rear of the cell → endocytosis
- Allows for next step



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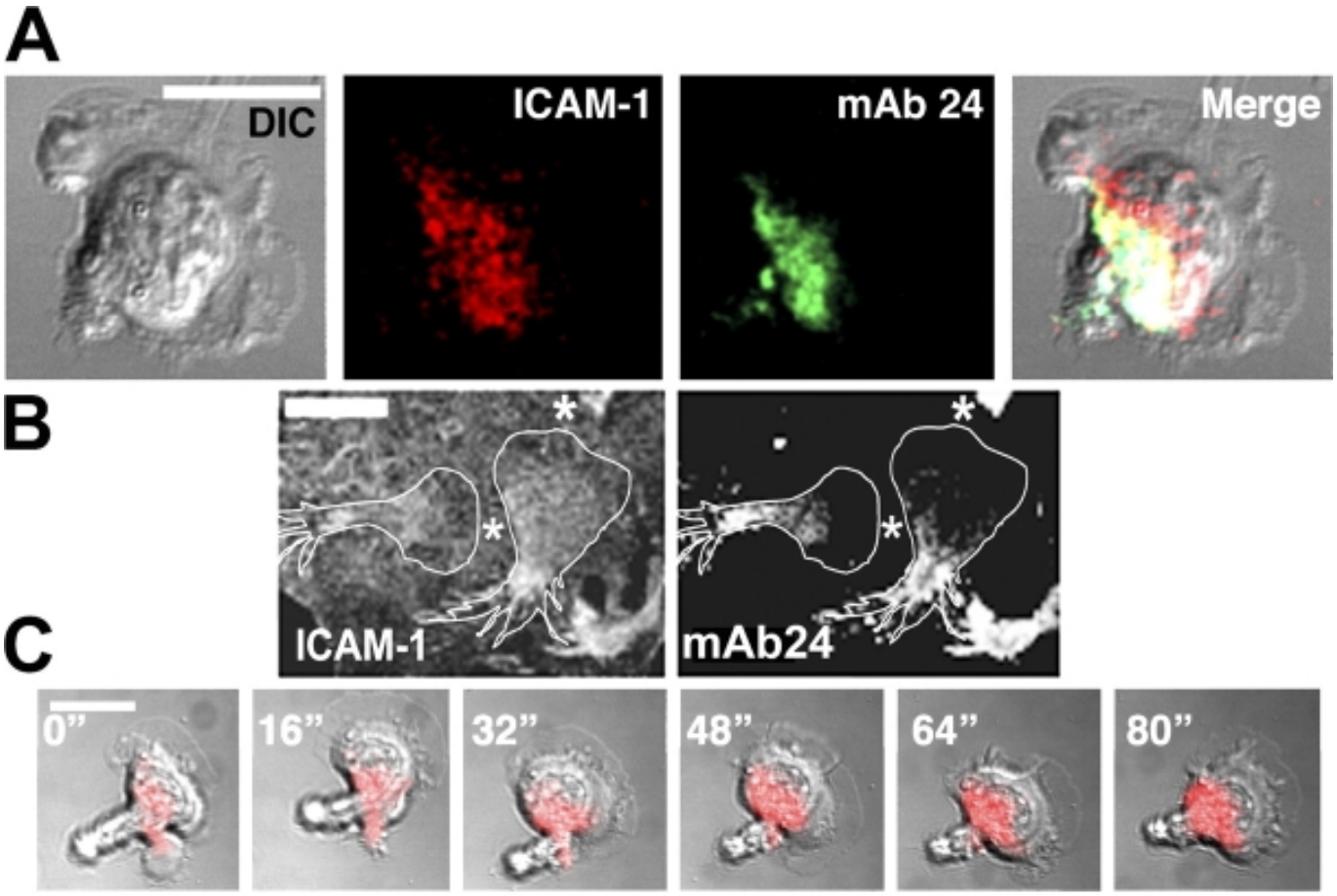

Smith et al, JCB 170:141 (2005)

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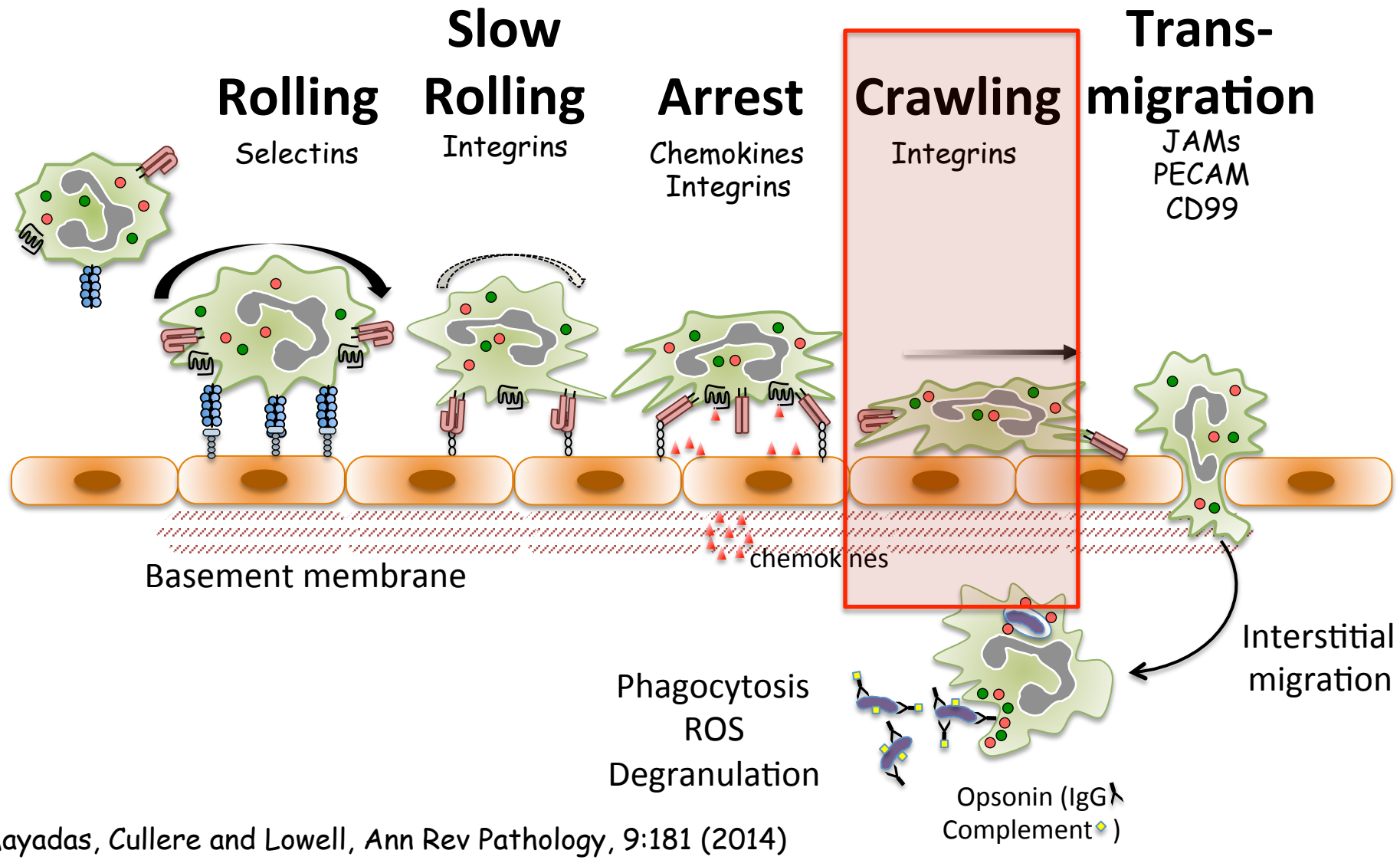
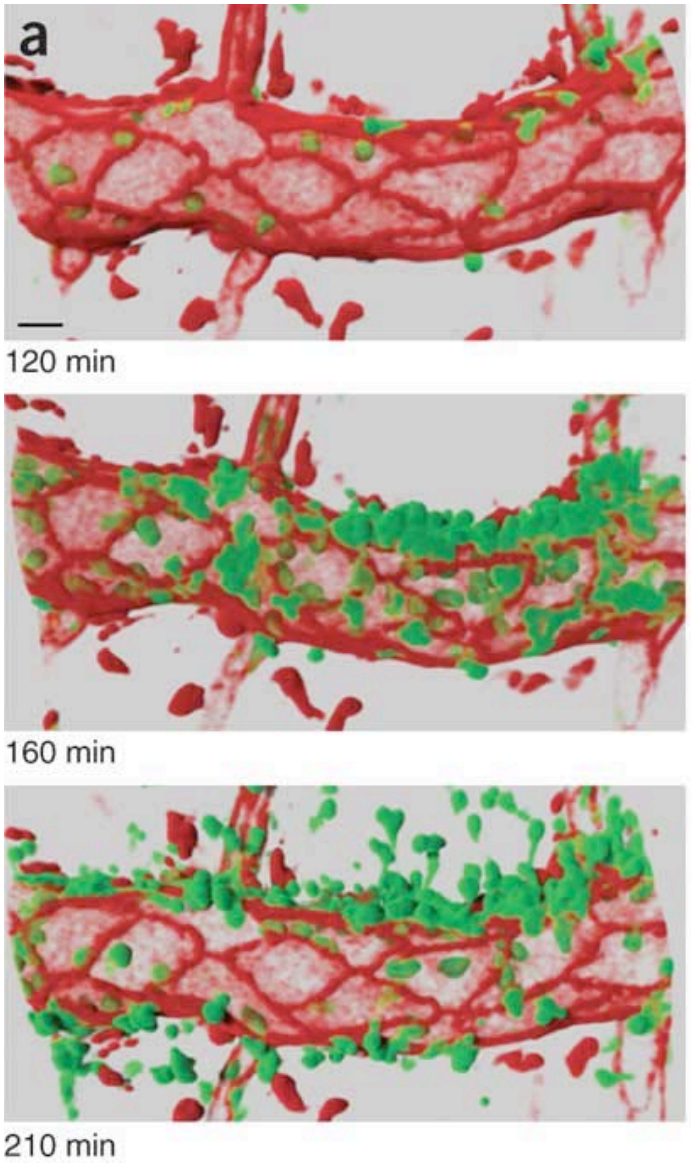


Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

Intravascular crawling

- Migration of cells to junctional regions between endothelium for easy exit from vasculature
- Dependent on Mac-1 integrin
- Slower speed than rolling
- Often against flow!



Woodfin et al, Nat Immunol, 12:761 (2011)

Intravascular crawling

Phillipson et al, JEM, 12:2569 (2006)

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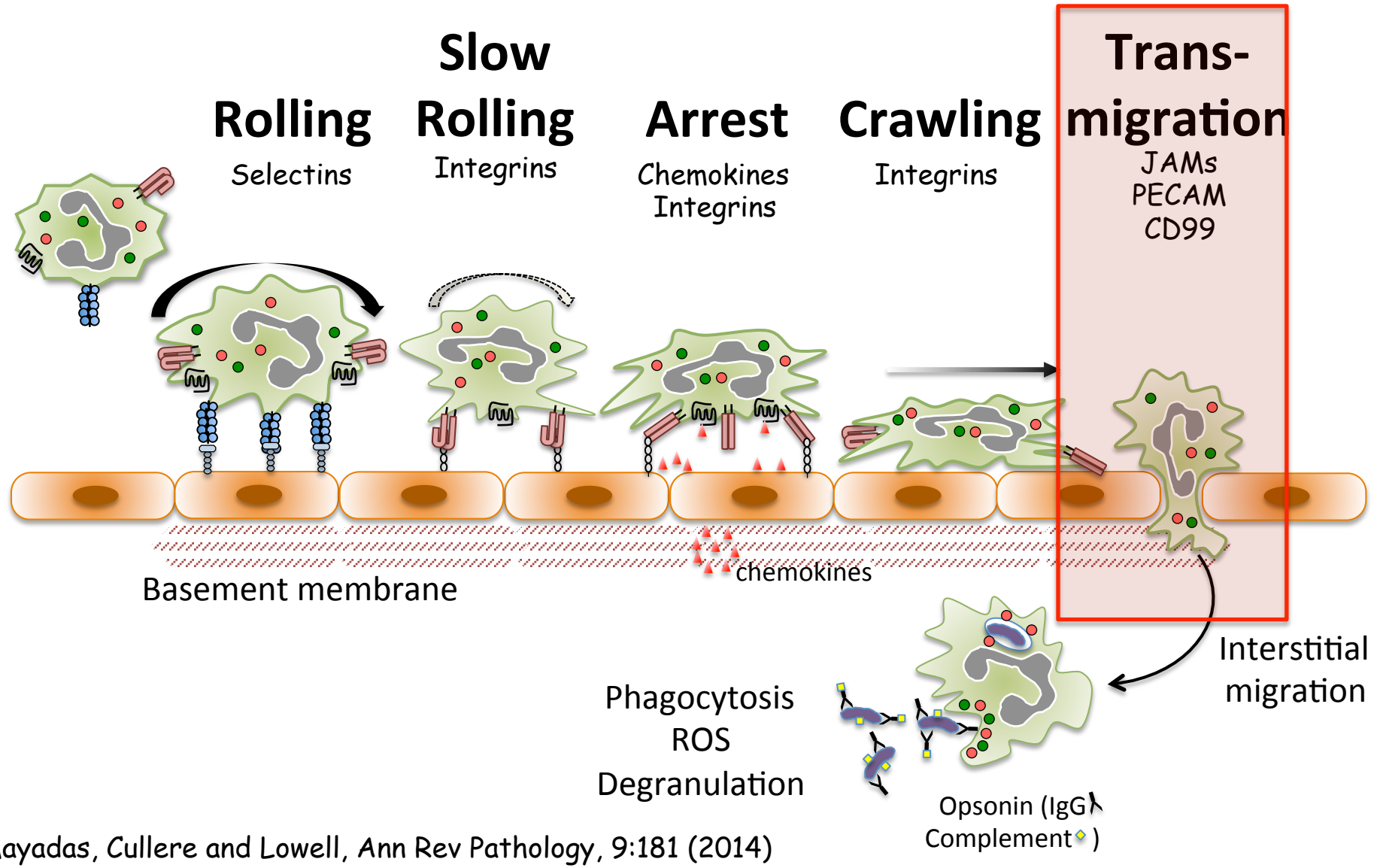
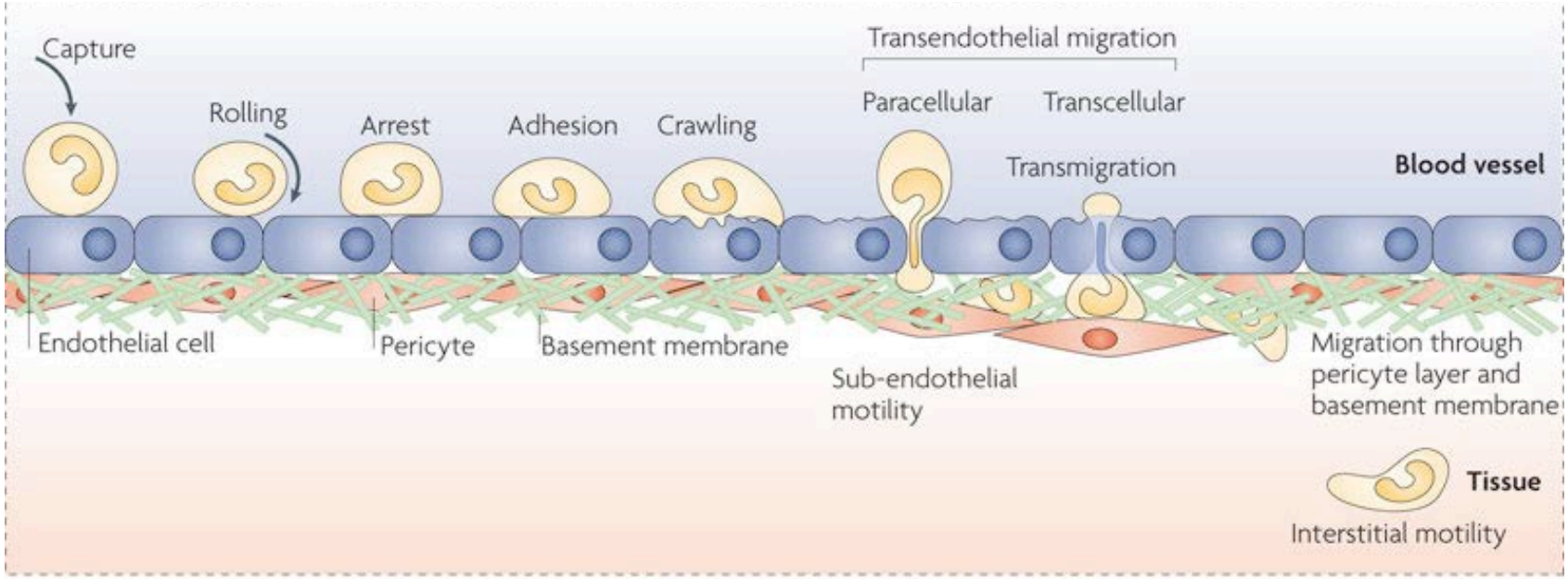


Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

Transendothelial migration

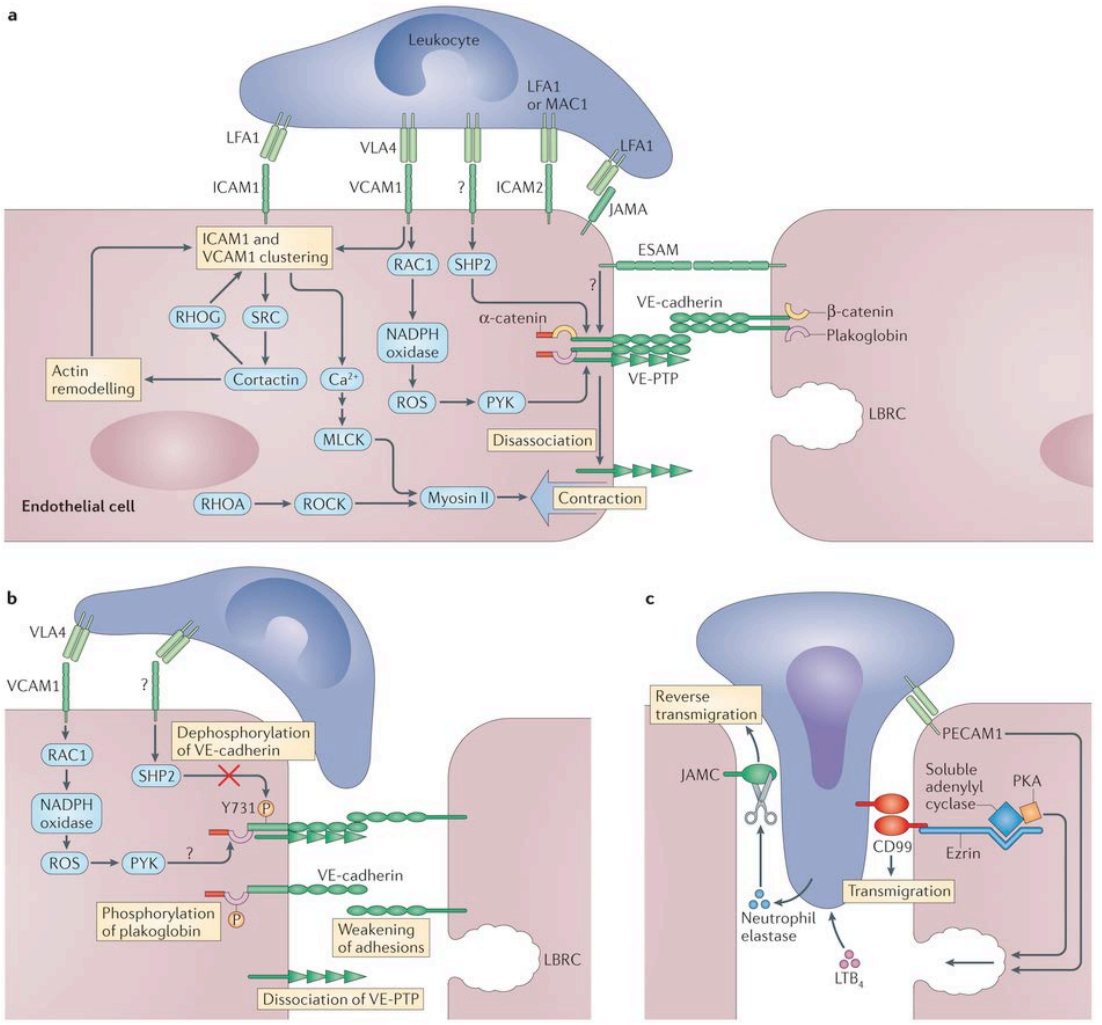


- When leukocytes crawl to the junction between endothelial cells → pass through
- Paracellular route most common, transcellular route also observed
- Migration out of the vasculature without leakage!

Noursharg et al, Nat Rev Mol Cell Biol, 11:366 (2010)

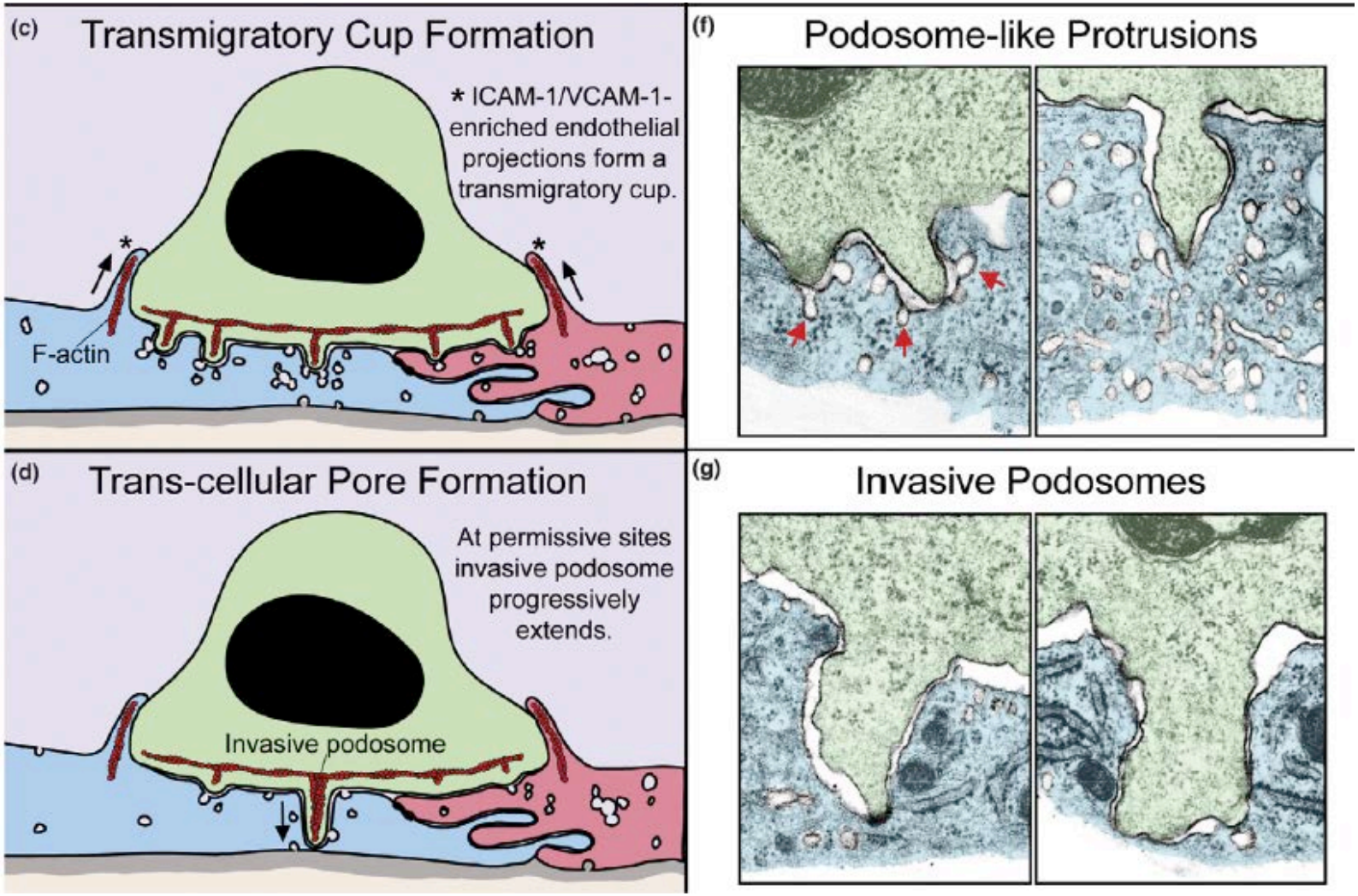
Transendothelial migration

- Integrins for crawling and transendothelial migration
- Additional molecules involved: junctional adhesion molecules (JAMs) and PECAM-1 (CD31)
- Signaling occurs into the endothelial cell to allow loosening of tight junctions
- Lateral recycling border of endothelium



Vestweber, Nat Rev Immunol, 15:692 (2015)

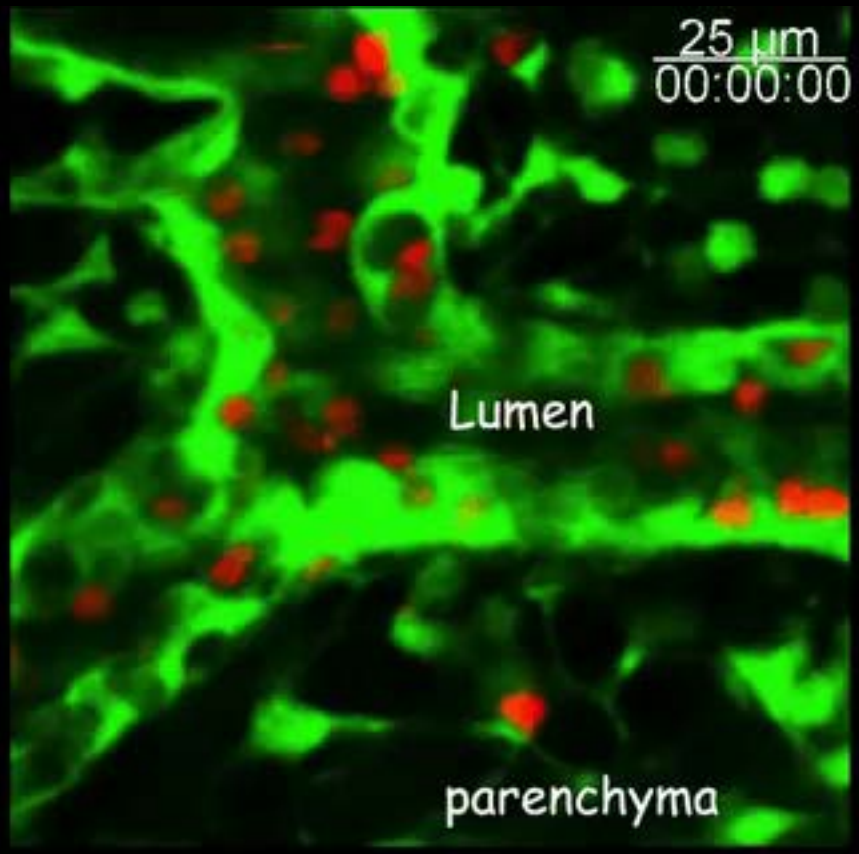
Transcellular exit from vasculature



Carman, Front Bioscience, 14:5066 (2009)

T Cells Exit HEVs in Limited Locations

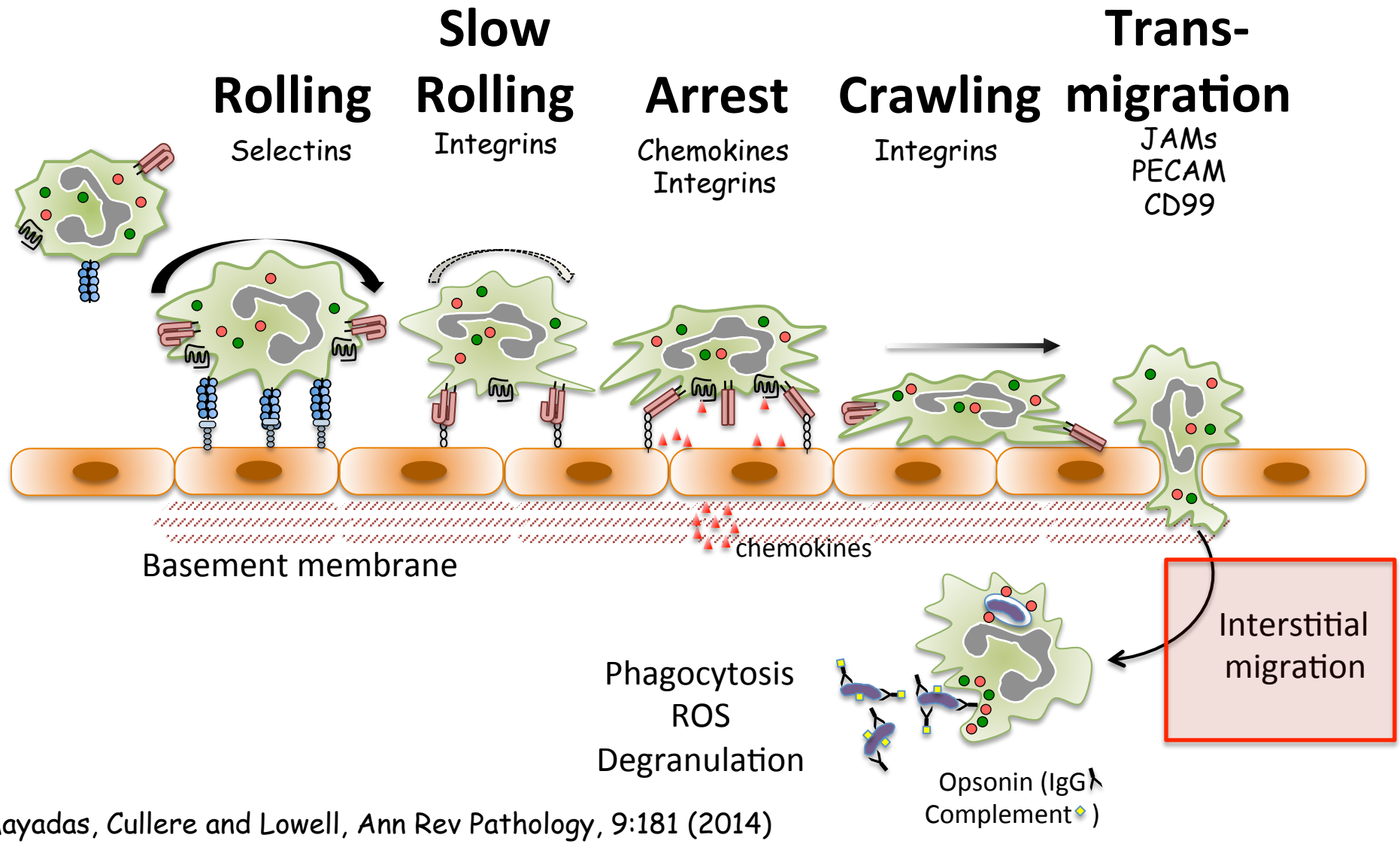
T cells exiting HEV via lucent areas that appear to be gaps in the FRC sheath (“exit ramps”).



Bajenoff et al., *Immunity* 2006

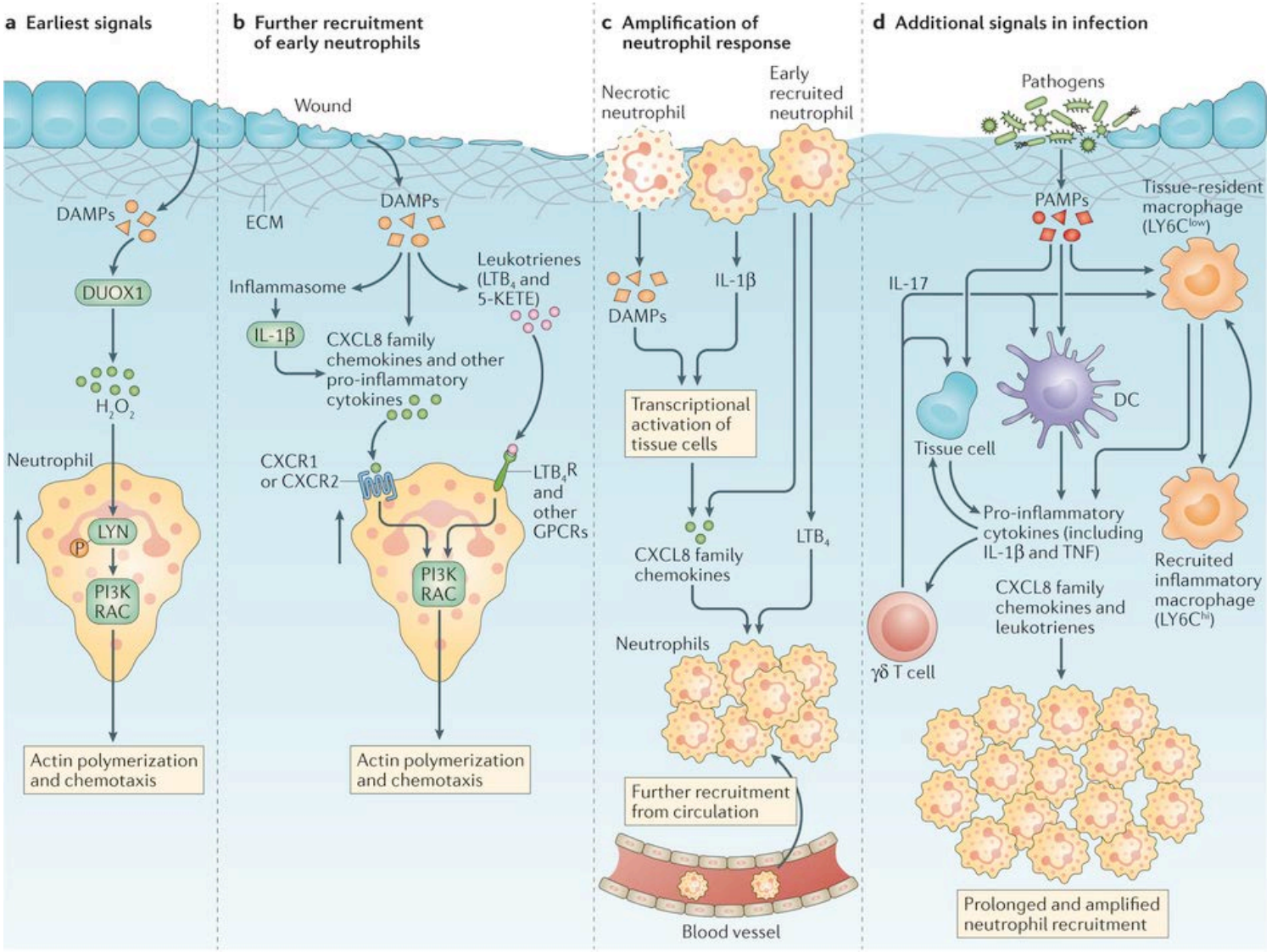
green is labeling all non-hematopoietic cells (stromal cells, endothelium etc)

Multi-step model of leukocyte transmigration



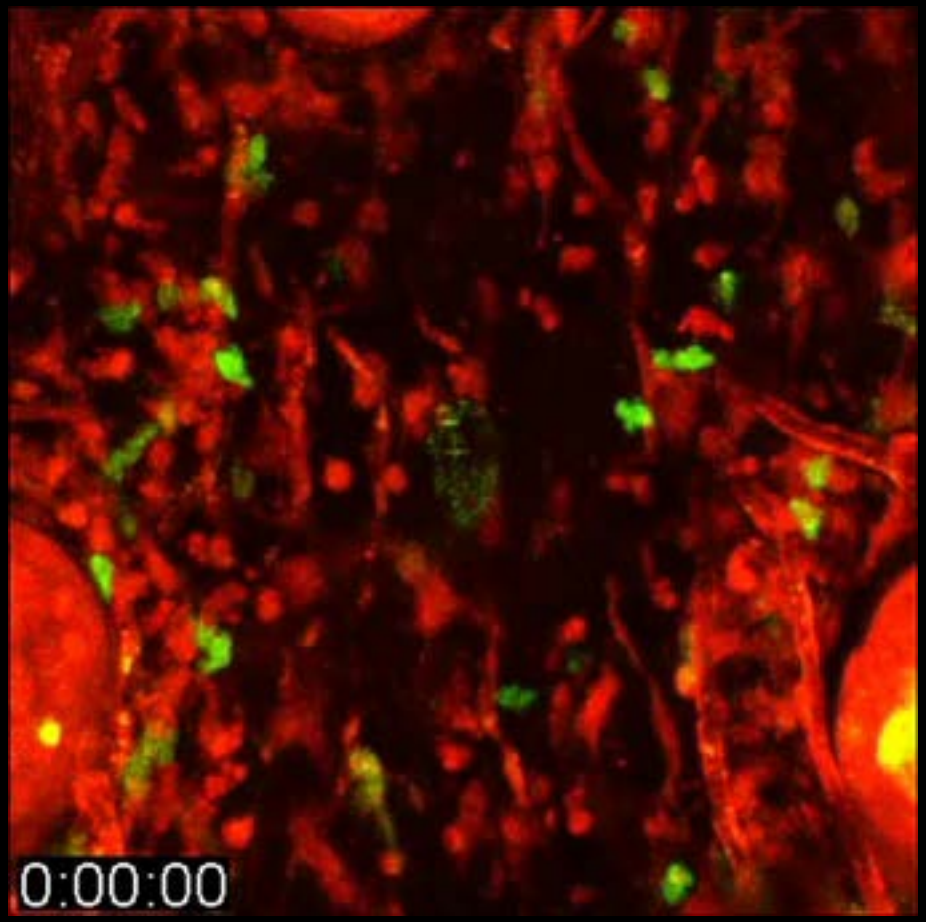
Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

Interstitial migration to infection/injury



de Oliveria et al, Nat Rev Immunol, 16:378 (2016)

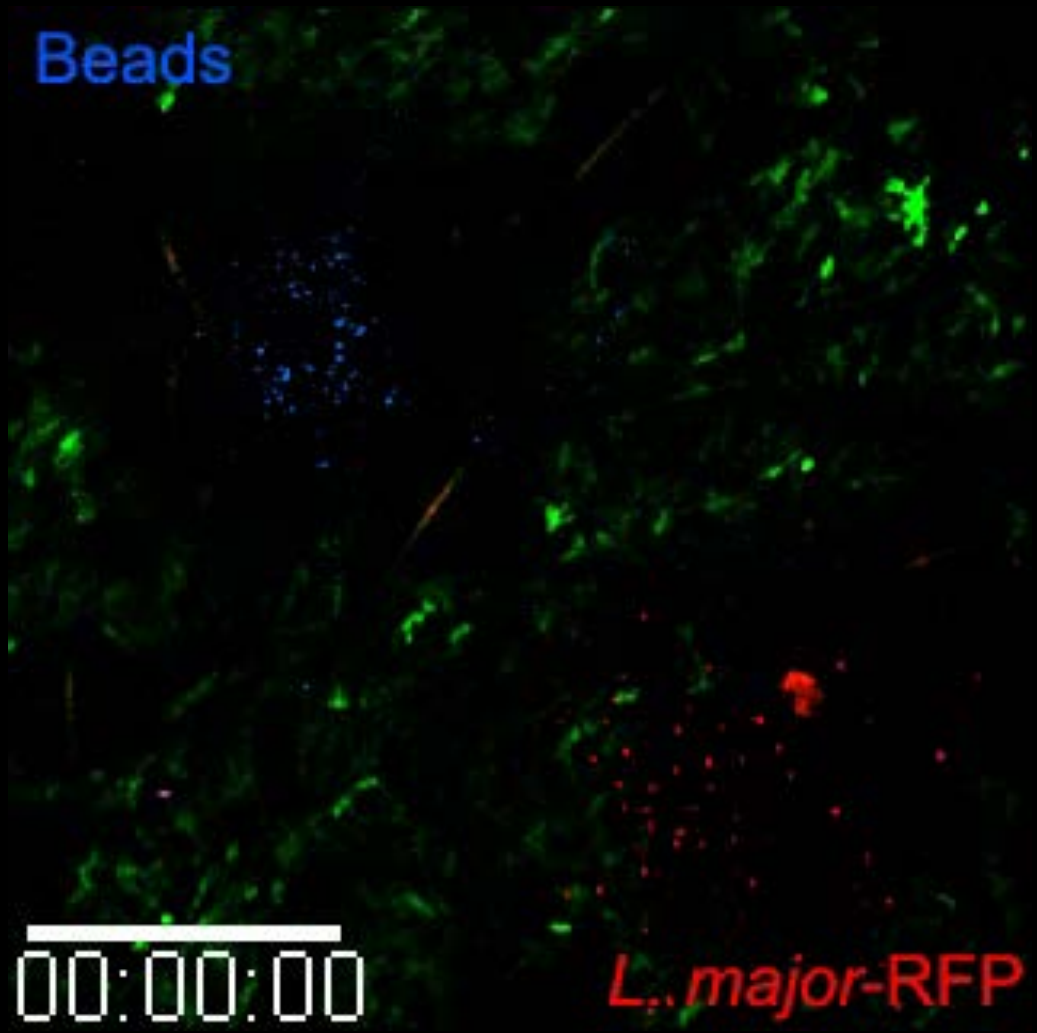
Neutrophil swarming during injury



- neutrophils in red, monocytes in green

Lammermann et al, Nature, 498:375 (2013)

Neutrophil swarming during infection



- neutrophils in green, L. major in red

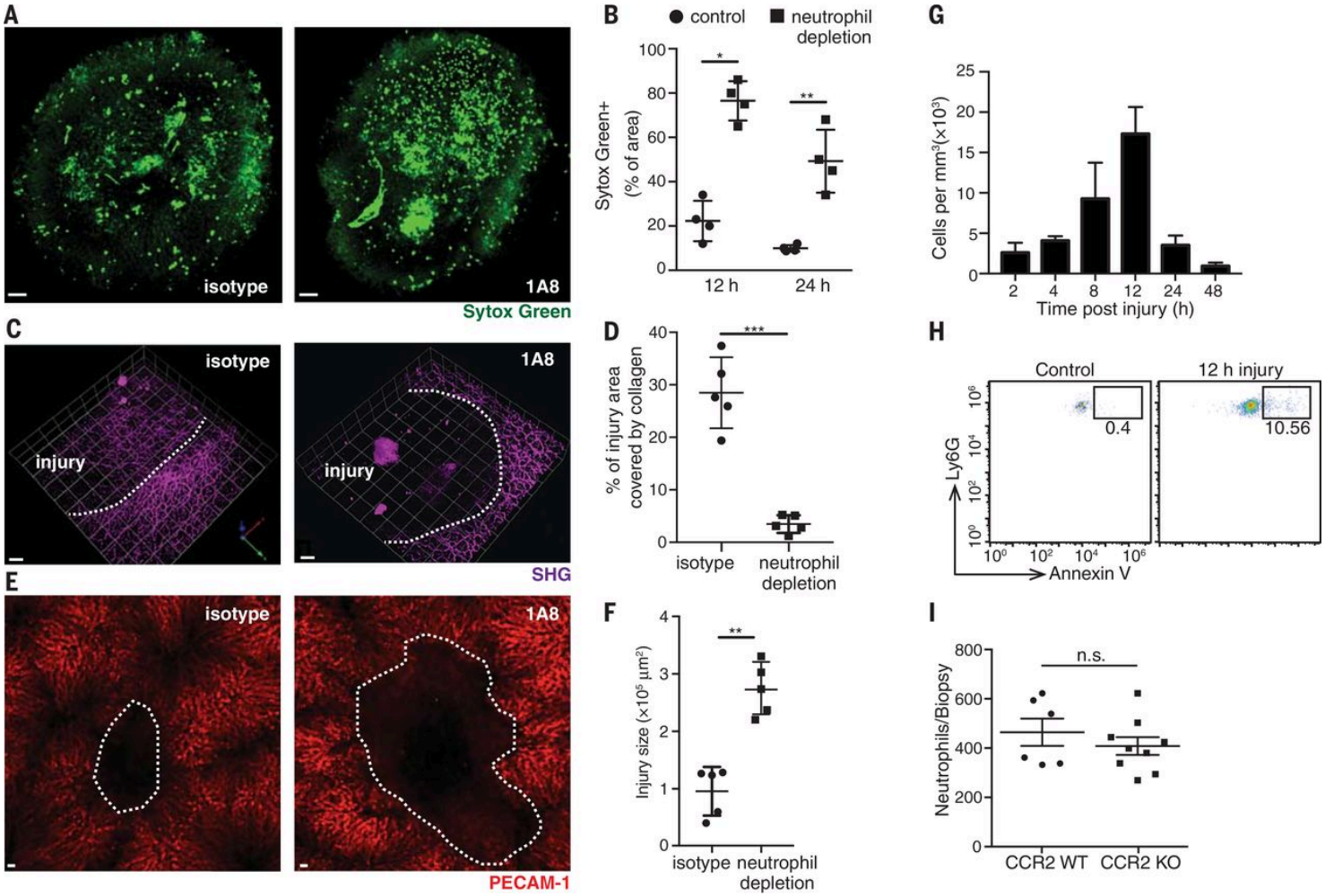
Peters et al, Science, 321:970 (2008)

Inflammatory cell recruitment

- Driven mainly by chemokines/chemoattractant gradients (LTB4)
- Requires integrins
- Leads to amplification of inflammation by additional chemokine production

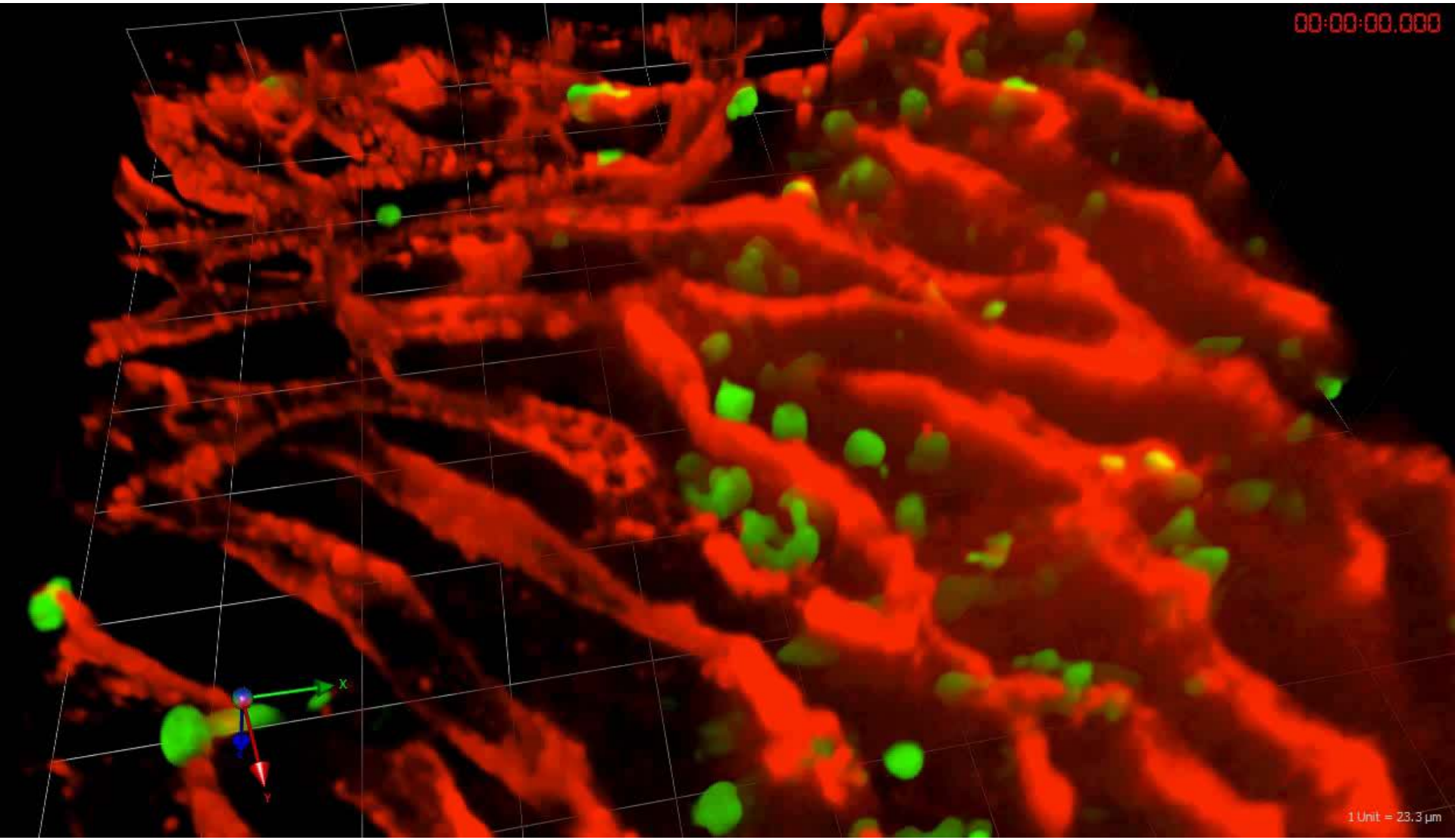
Not all neutrophil inflammation is bad
Neutrophils in tissue repair

- Sterile liver injury model
- neutrophil depletion results in BIGGER injury and impaired healing



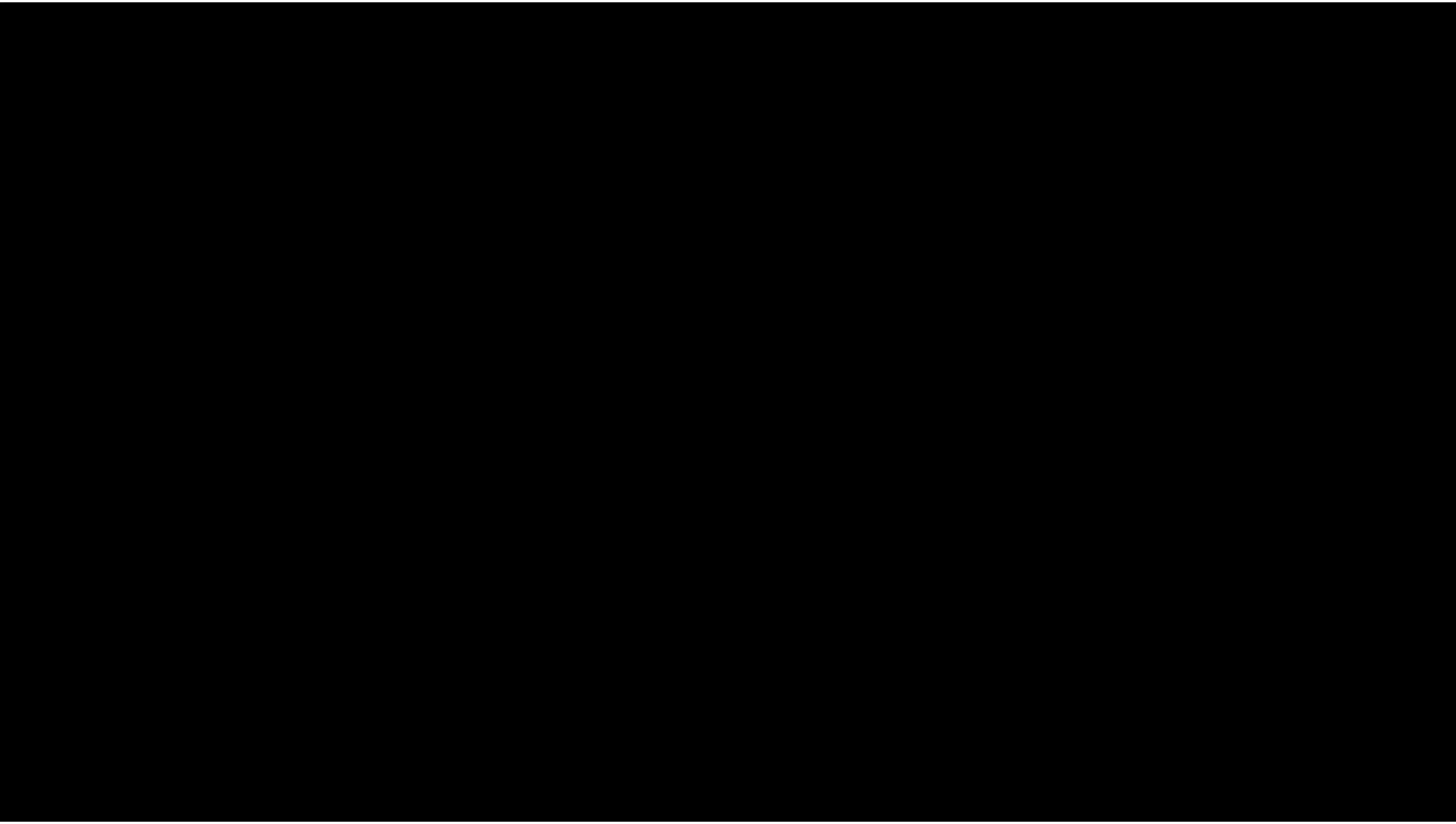
Wang et al, Science, 358:111 (2017)

Neutrophils enter border of the injury



Wang et al, Science, 358:111 (2017)

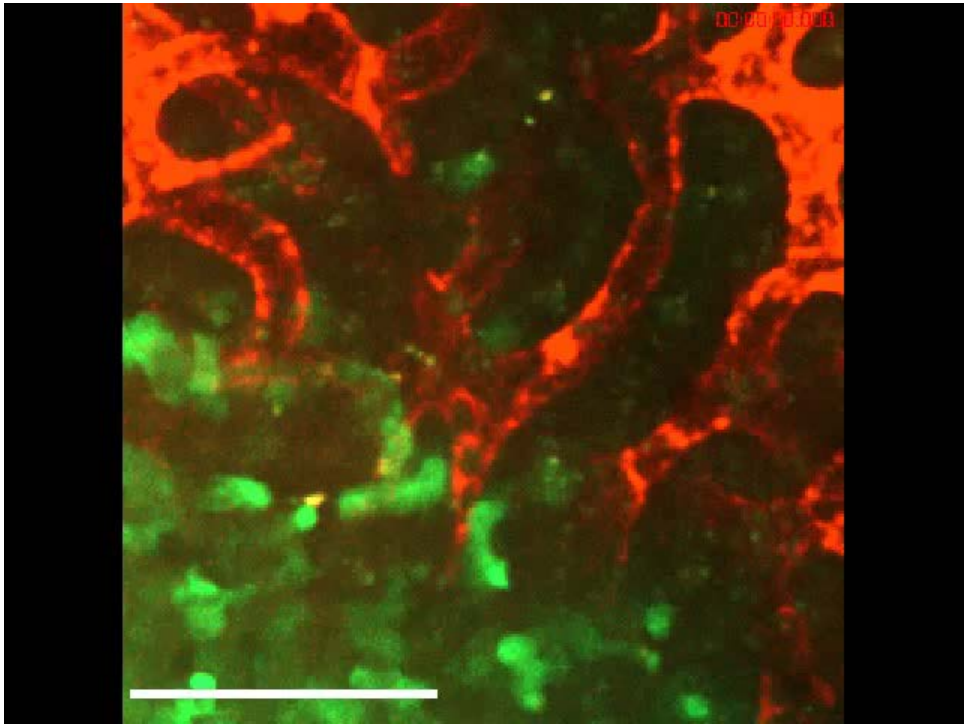
Neutrophils eat away at damaged vessels



Wang et al, Science, 358:111 (2017)

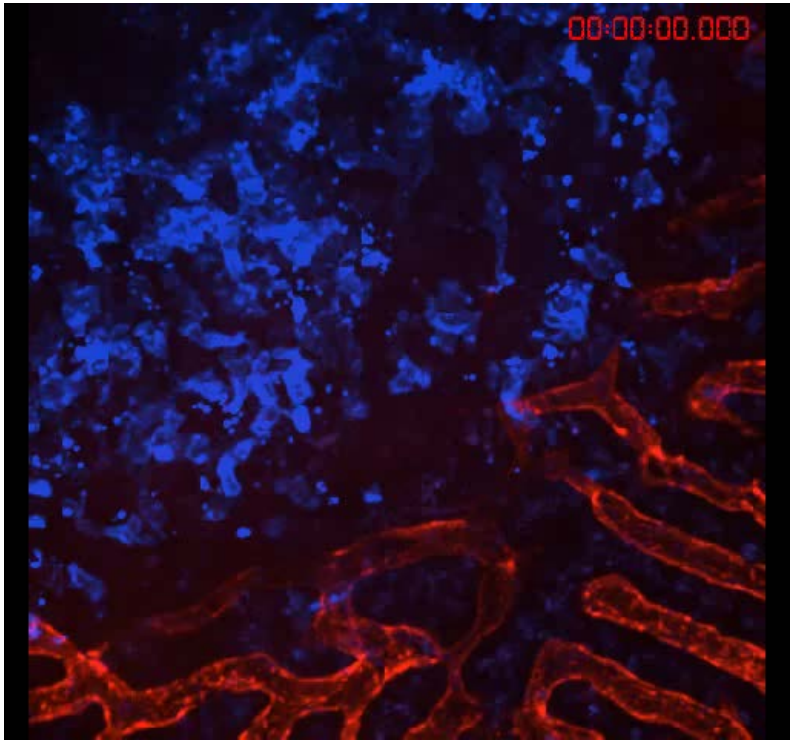
Neutrophils open up damaged vessels in protease dependent fashion

WT



neutrophils = green

Cat S$^{-/-}$



neutrophils = blue

Wang et al, Science, 358:111 (2017)

Leukocyte Adhesion Deficiency Syndromes

- LADI → absence of β2 integrin
  presents early in life (delayed separation of umbilical cord). Infections without pus. Rolling but not adhesion or migration.

- LADII → absence of fucose transferase
  failure to form sialyl-Lewis-X on selectin ligands. No rolling. Rare.

- LADIII → absence of kindlin-3
  lack of integrin activation, even though expression is normal. Rare.

Leukocyte Adhesion Deficiency Syndromes

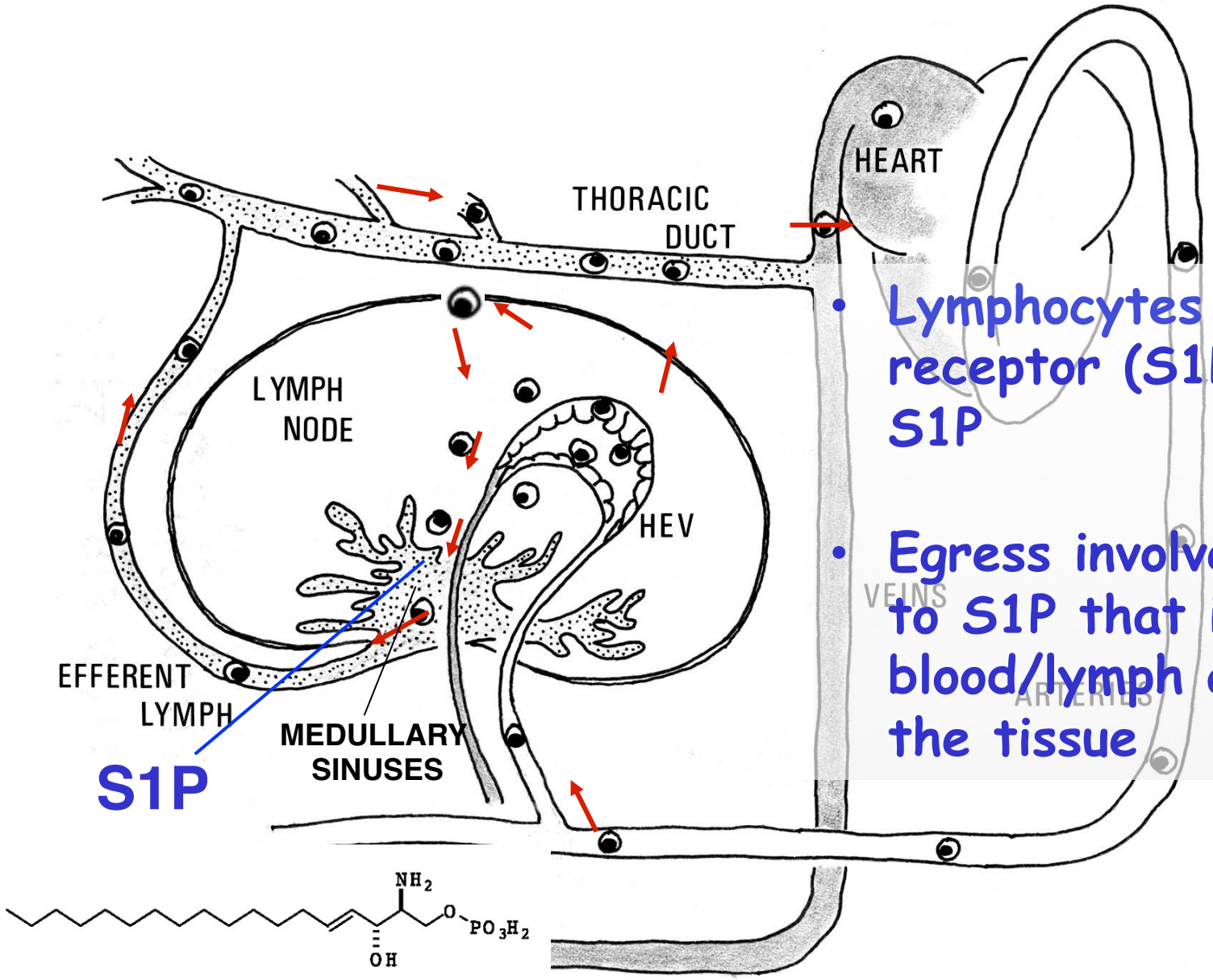
- Other types of integrin or leukocyte migration defects:

  Glanzmann's thrombasthenia → αIIb or β3 mutations. Present with bleeding.

  Wiskott-Aldrich syndrome → WASp protein mutations. Poor actin cytoskeletal rearrangment, lots of immune defects.

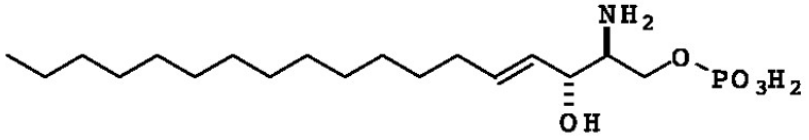
  Leukocyte hyperadhesion syndrome

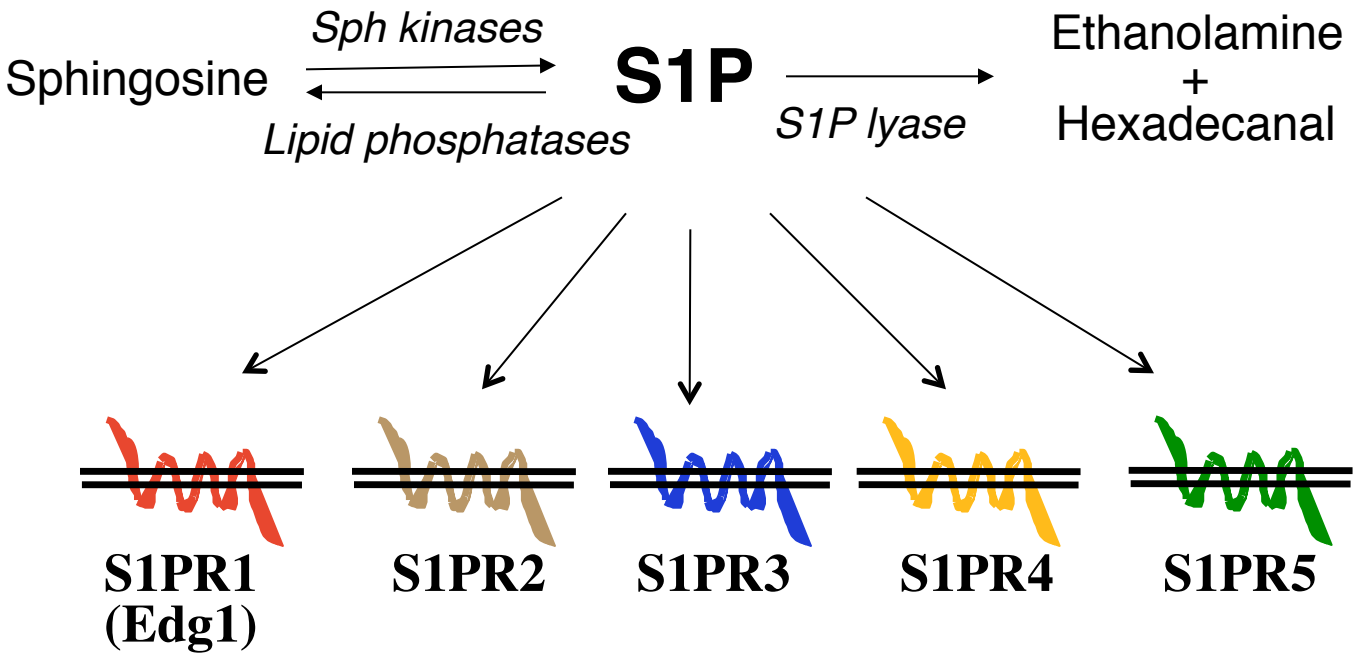
How do cells exit from lymphoid organs?



- Lymphocytes express a receptor (S1PR1) for S1P
- Egress involves migrating to S1P that is high in blood/lymph and low in the tissue

Sphingosine 1-phosphate (S1P)

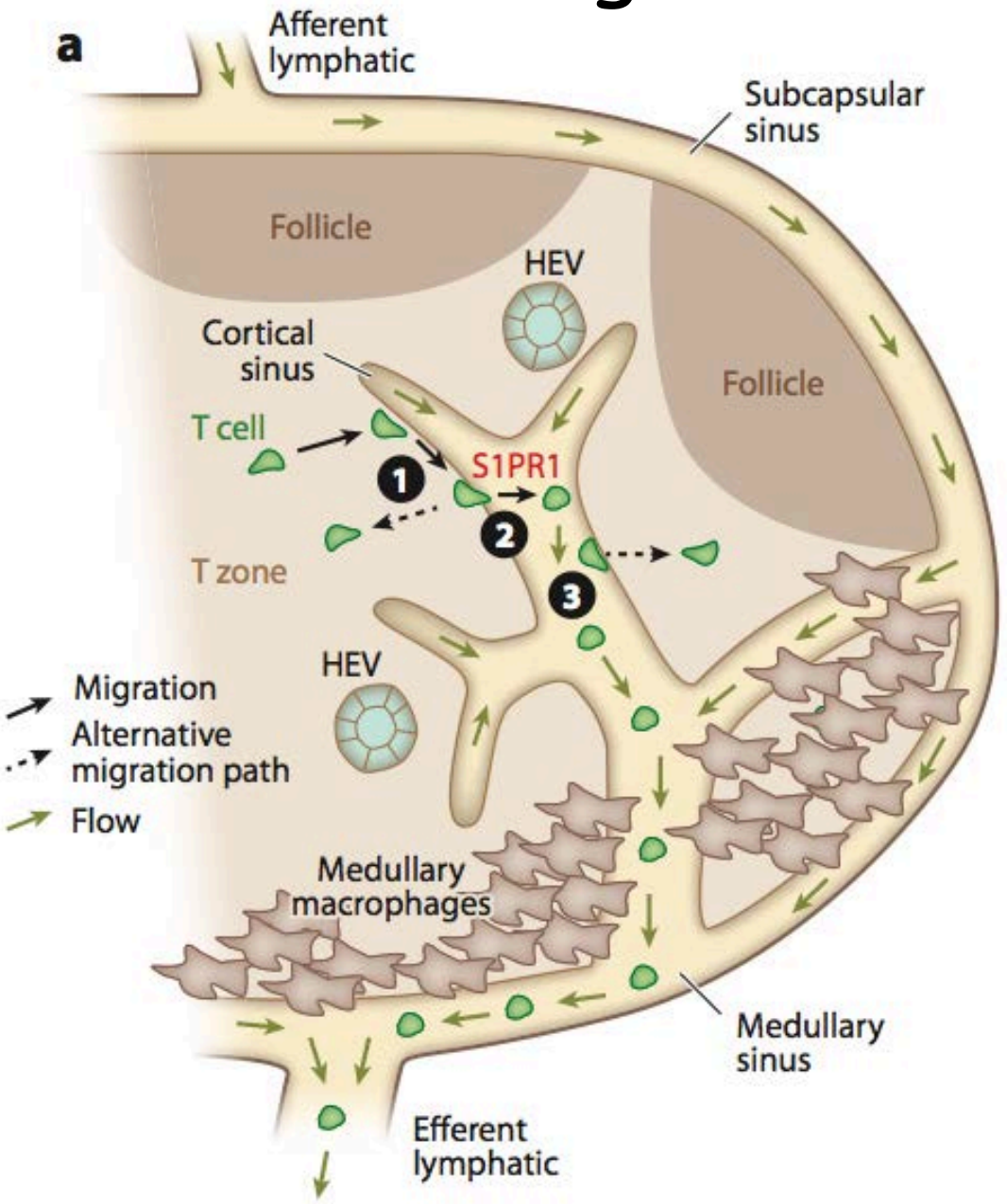




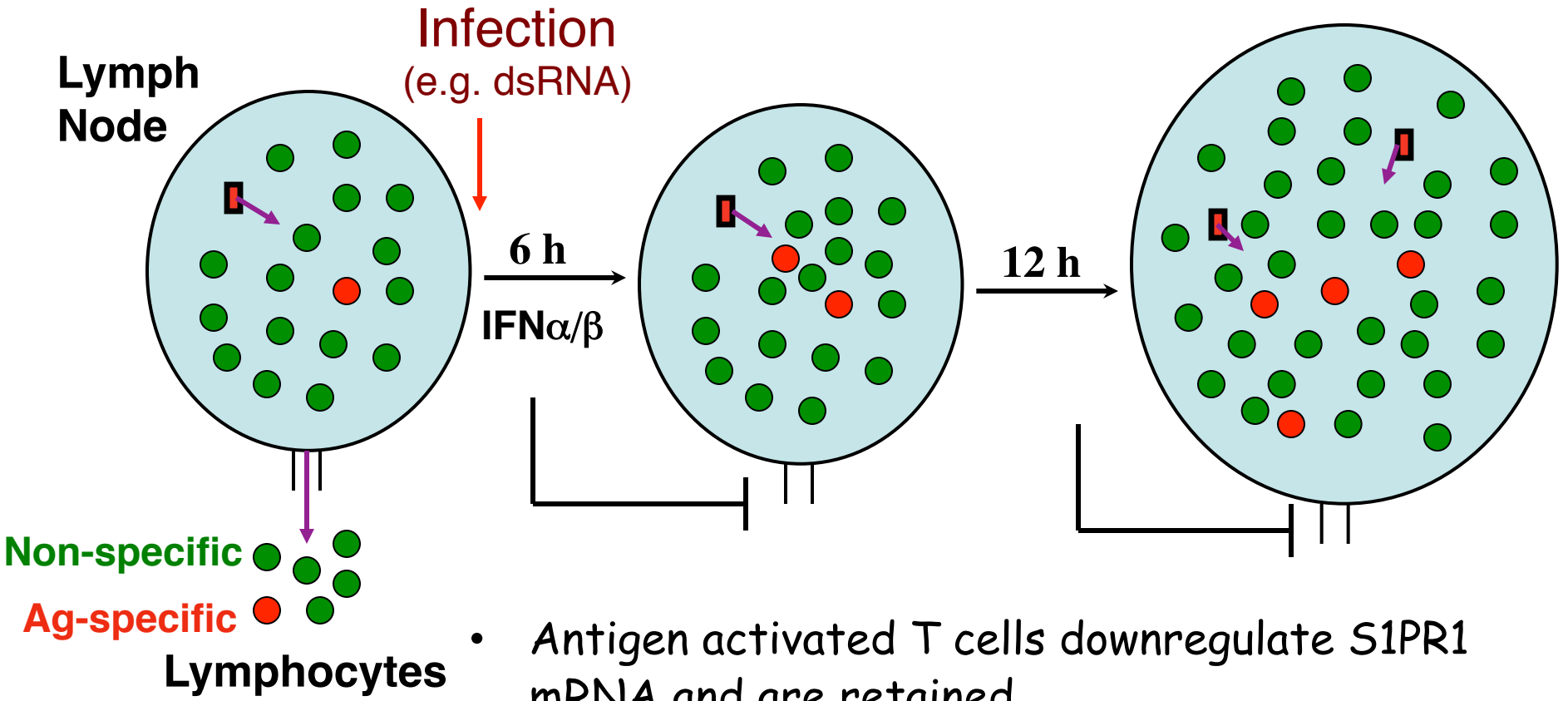
- Abundant in plasma (~1uM) and lymph (~0.1uM)
- Made intracellulary by all cell types during sphingolipid degradation but only secreted by some cell types
- Ligand for a family of G-protein coupled receptors (S1PR1-5)
- S1P receptors have roles in blood vessel and heart development

Multi-step model of T cell egress

- S1P is abundant in blood and lymph
  - RBC major source of blood S1P
  - Lymphatic endothelial cells are source of lymph S1P
- Extracellular S1P is kept low inside lymphoid tissue
- S1PR1 is a point of egress control



Innate immune stimuli cause a generalized lymph node egress shutdown

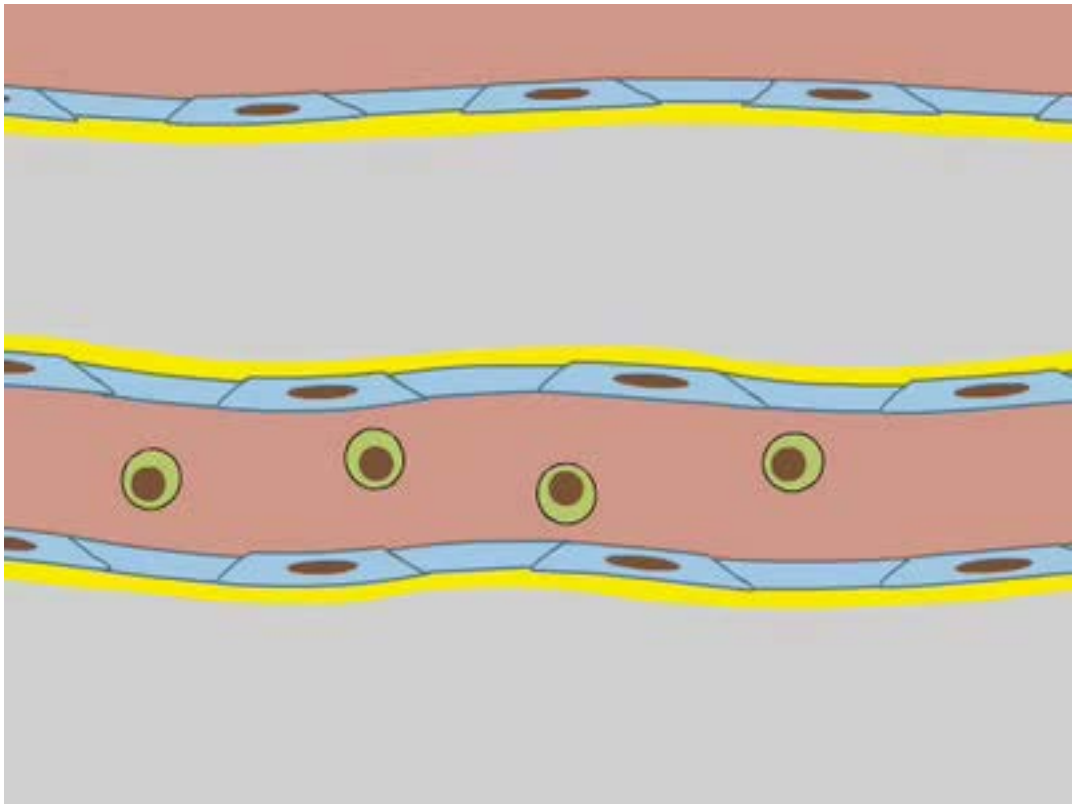


- Antigen activated T cells downregulate S1PR1 mRNA and are retained
- Retention may help ensure appropriate clonal expansion and receipt of instruction signals before exiting

Translational Impact of Homing Research

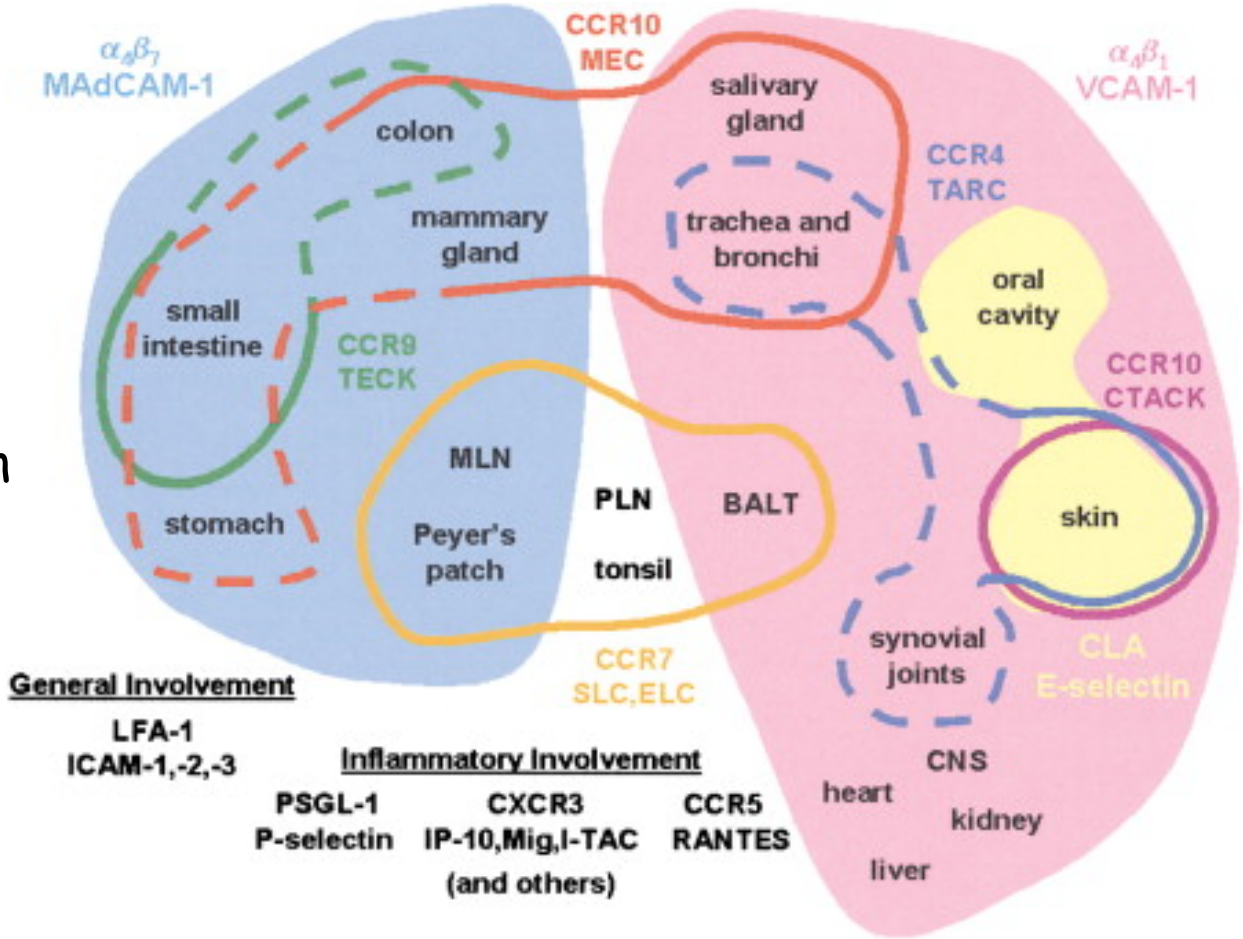
- $\alpha 4\beta 1$ blocking antibody (Natalizumab/Tysabri®) in use for treatment of Multiple Sclerosis and Crohn's disease
- $\alpha 4\beta 7$ blocking antibody (Vedolizumab/Entyvio®) approved May 2014 for ulcerative colitis and Crohn's disease
- CCR9 antagonist in phase III trial for Crohn's disease
- FTY720 (Fingolimod) first oral treatment for relapsing/remitting multiple sclerosis; in trial for ulcerative colitis
- Inhibitors of prostaglandin and leukotriene synthesis have multiple anti-inflammatory effects, including antagonizing cell recruitment
- CXCR4 antagonists used to mobilize HSC from bone marrow
- Led to advanced understanding of causes of Leukocyte Adhesion Deficiency (LAD)

The whole thing together



Tissue selective chemokine and adhesion molecule expression in the organization of the immune system

- Homing specificity provided by the selectin/ chemokine/integrin code or combination
- Same for recruitment to inflammatory sites



Kunkel and Butcher, Immunity, 16:1 (2002)