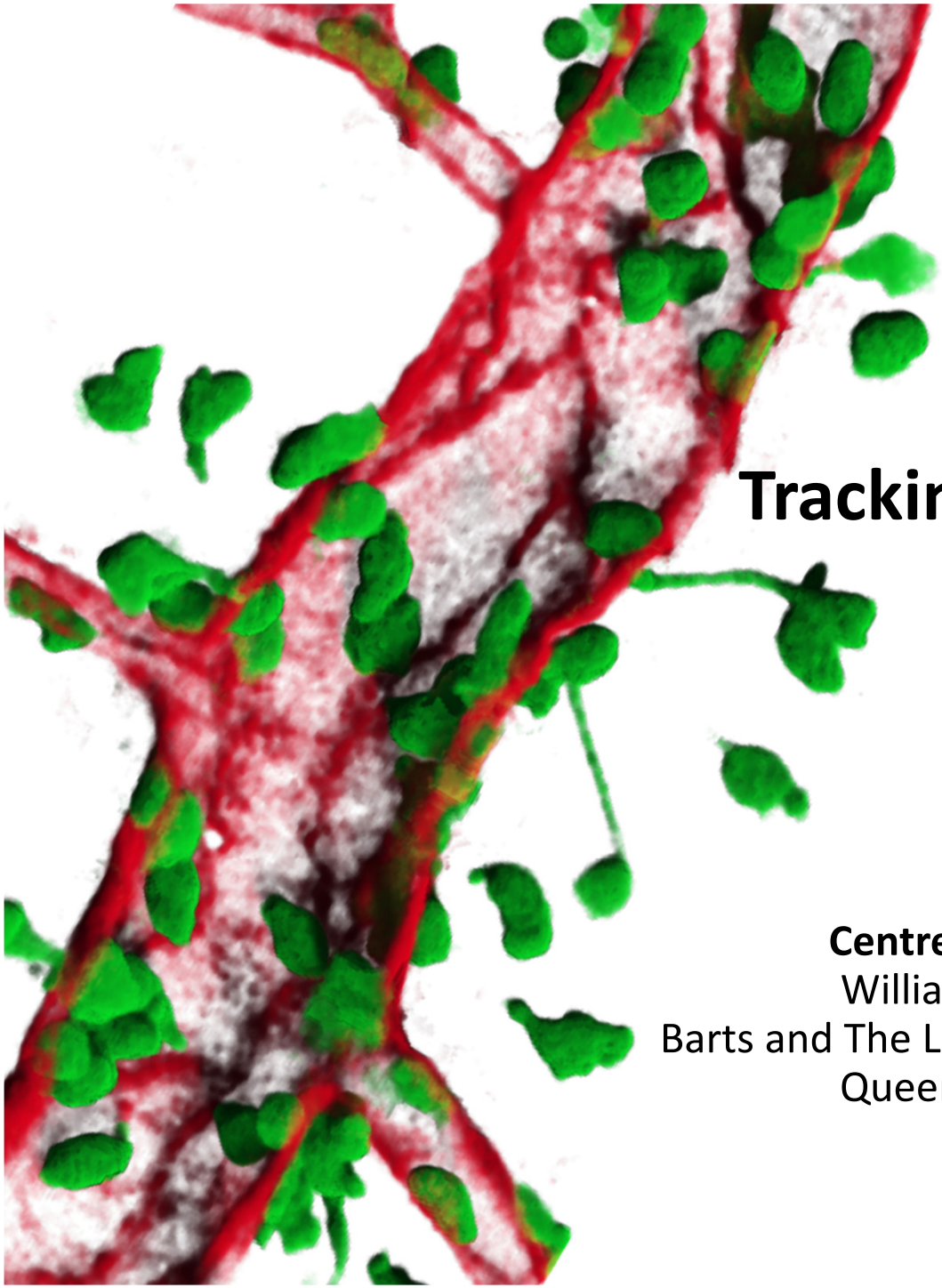


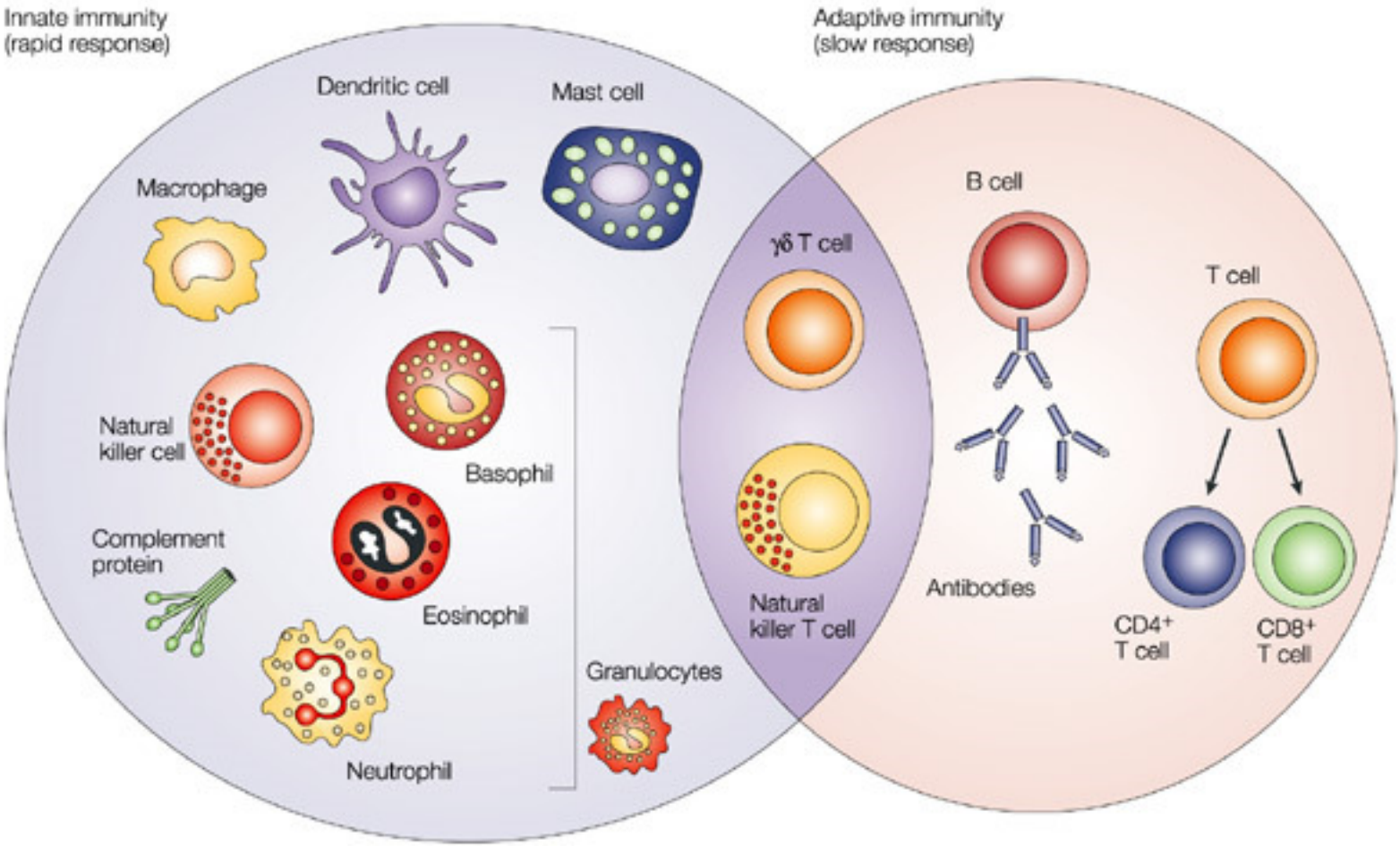
Barts and The London
School of Medicine and Dentistry

Tracking leukocyte migration *in vivo*

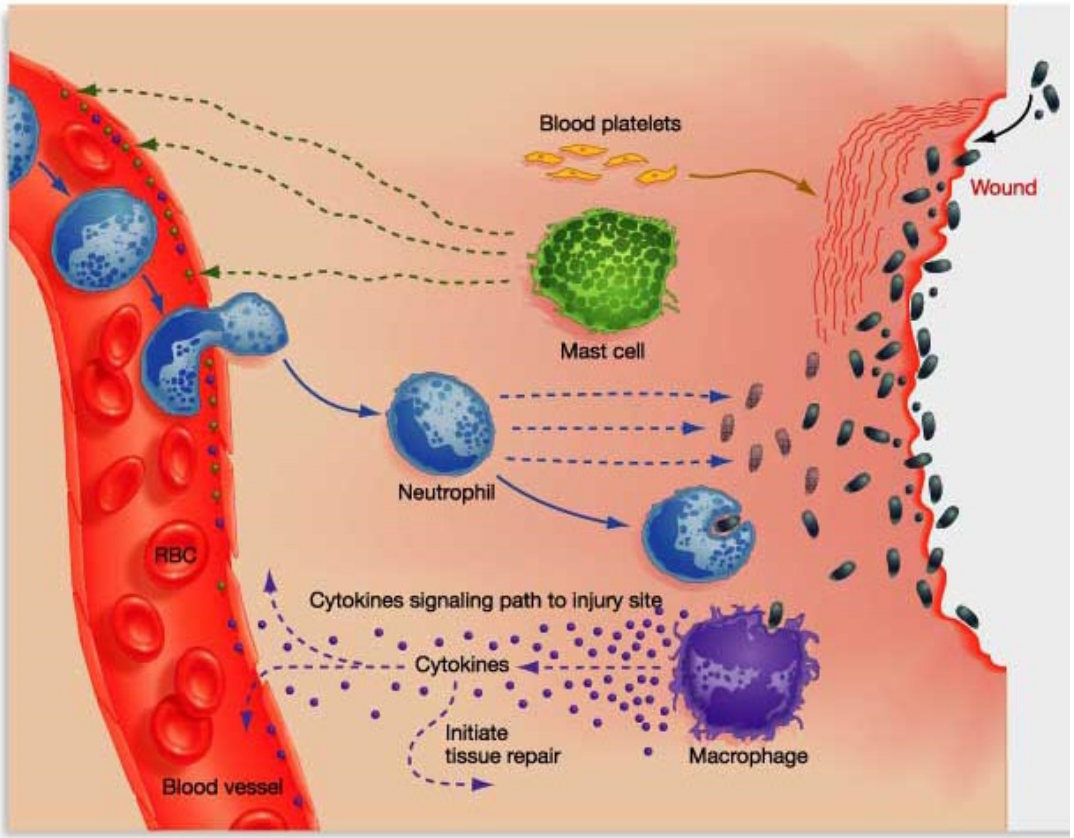
Abby Woodfin

Centre for Microvascular Research
William Harvey Research Institute
Barts and The London School of Medicine & Dentistry
Queen Mary University of London

Why track leukocytes?

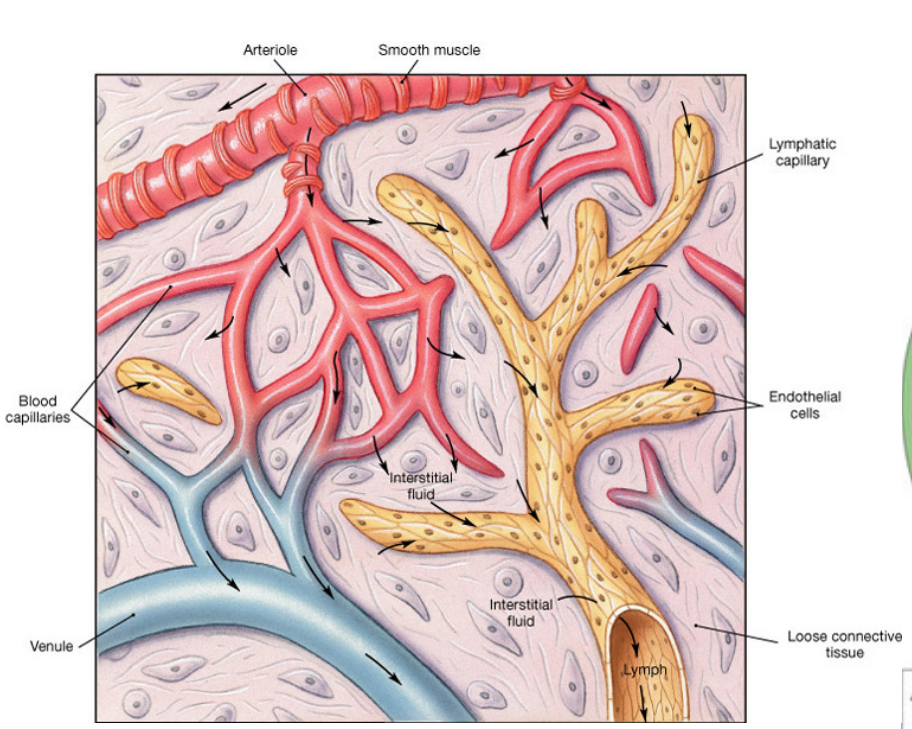


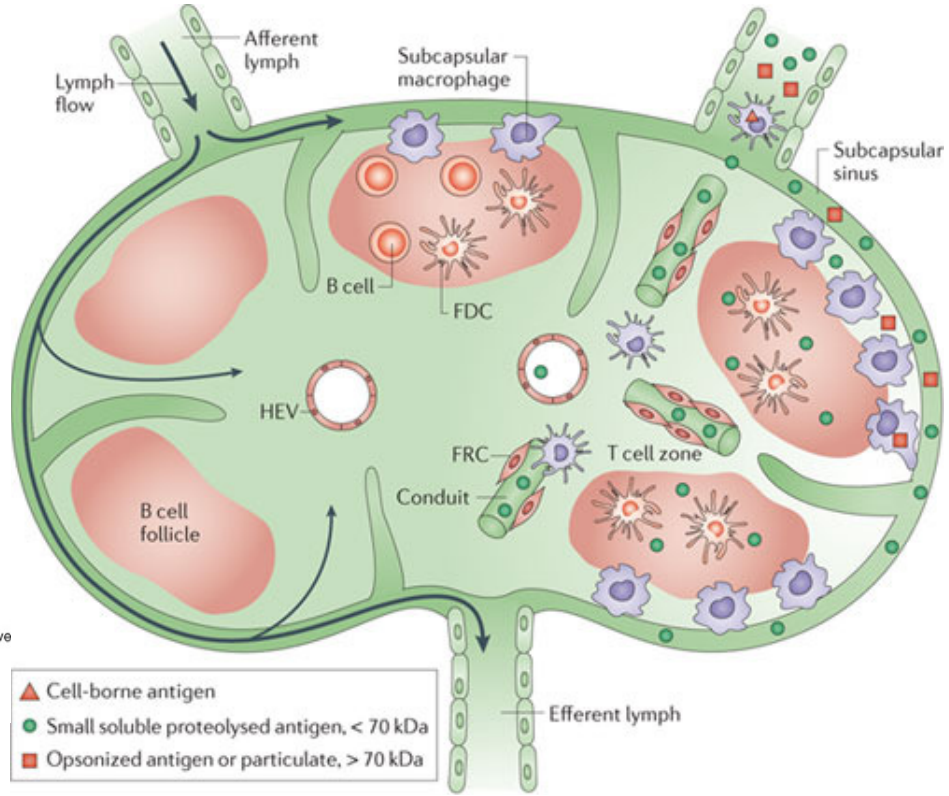
Innate immunity



- Bacteria/pathogens enter wound
- Platelet clotting
- Mast cells - increase blood flow
- Recruitment of neutrophils & monocytes – phagocytosis and secreted factors
- Macrophages mediate tissue repair
- Resolution of inflammation

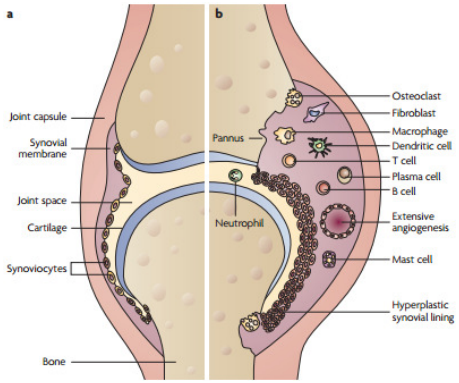
Adaptive immunity



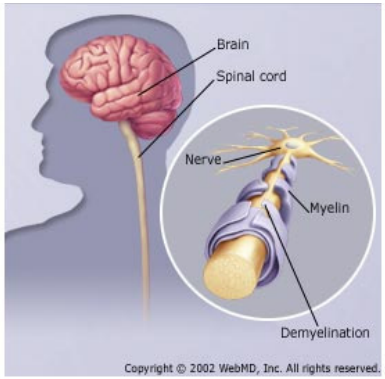


- Tissues → lymphatic vessels
- Antigen presentation in lymph nodes

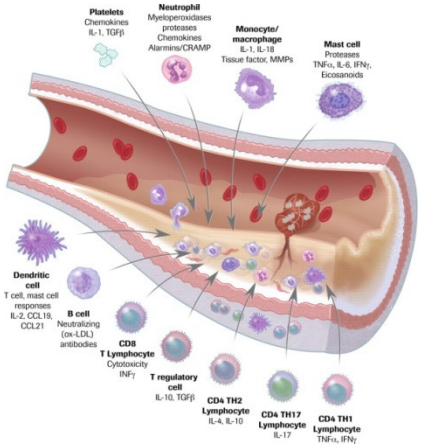
Arthritis



Multiple sclerosis

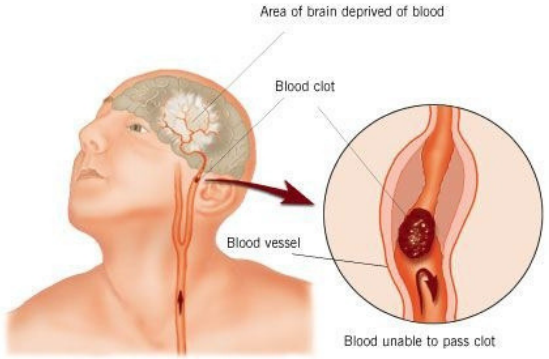


Atherosclerosis

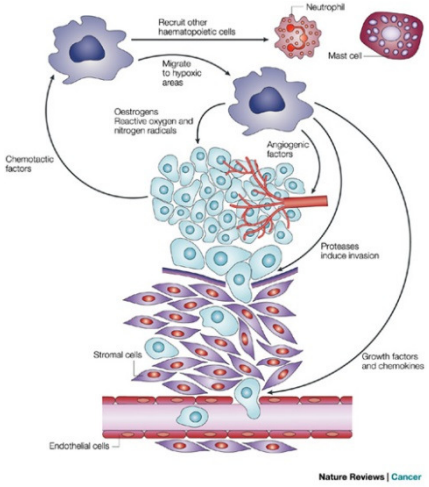


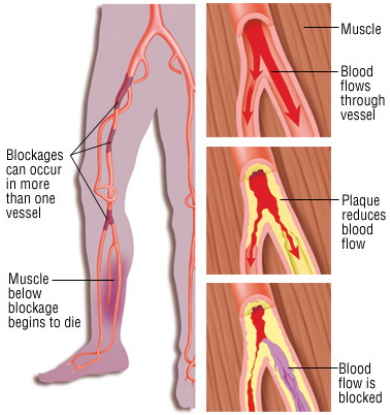
Inflammatory disorders

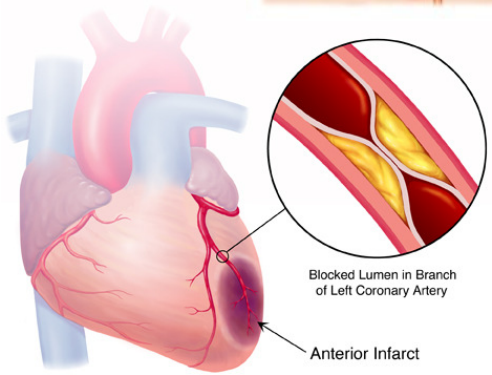
Detrimental leukocyte recruitment



Tumour growth

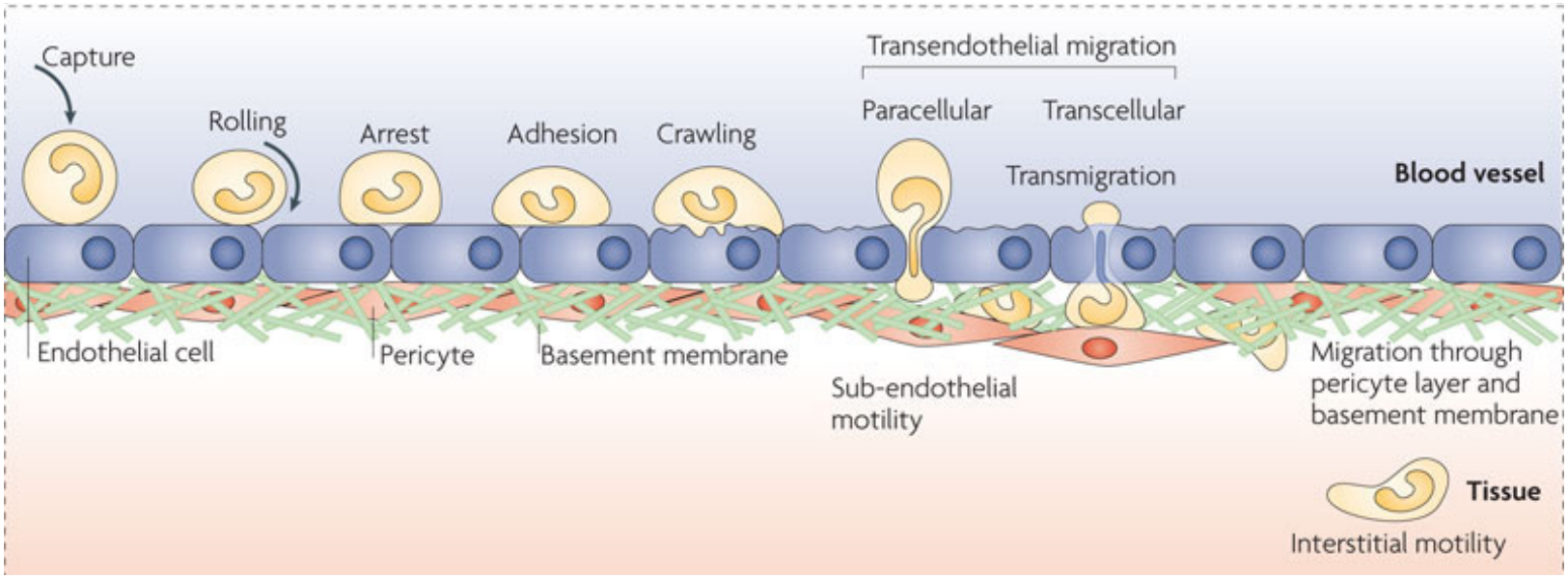




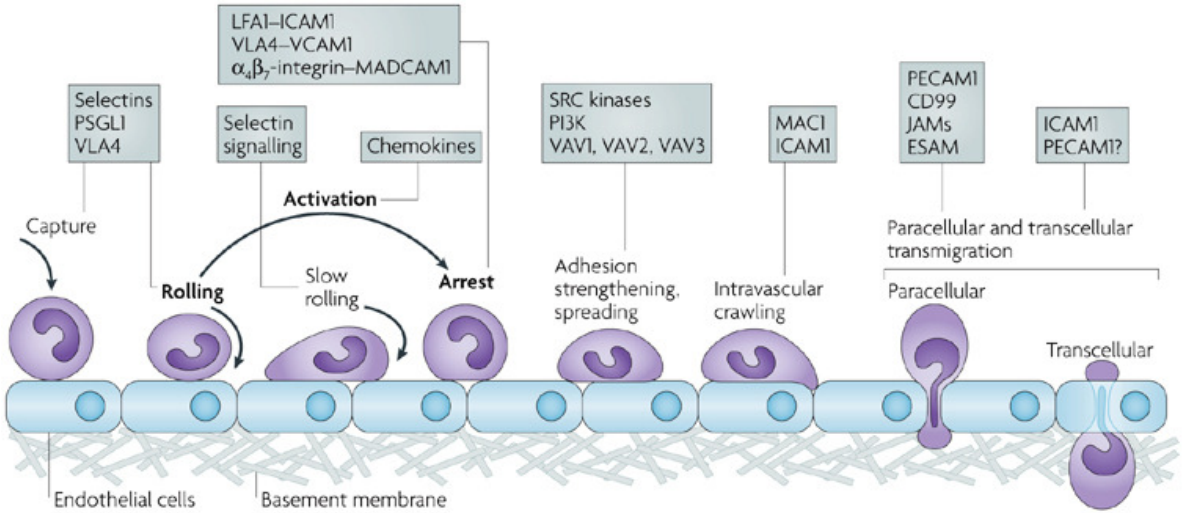


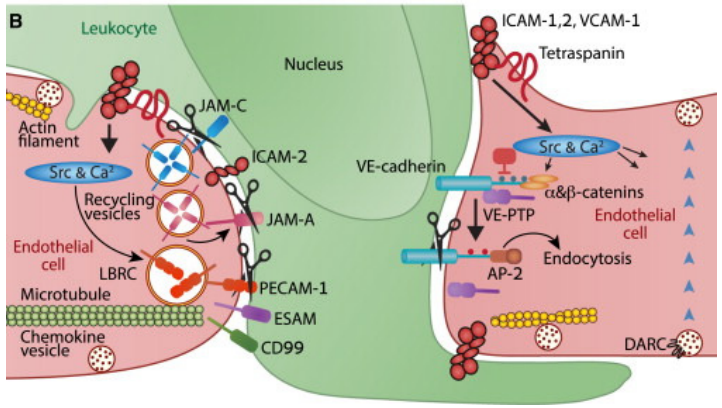
Ischemia

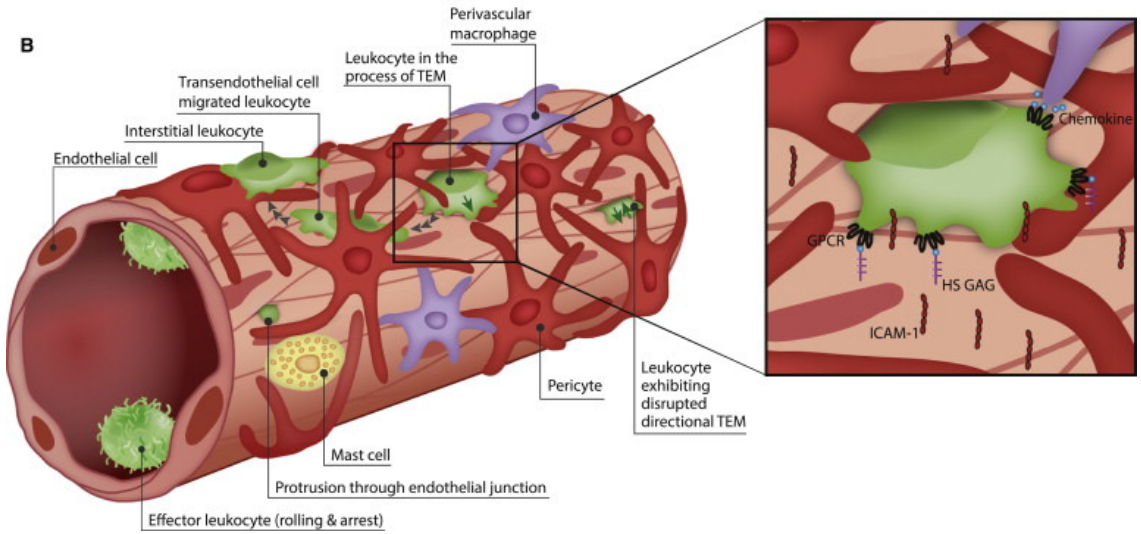
- Bone marrow → blood
- Blood vessels → tissues
- Within tissues – development, microbial defence & repair
- Tissues → lymphatic vessels
- Within lymph nodes
- From lymph nodes to the circulation



Nourshargh et al., Nat Rev Mol Cell Biol (2010)





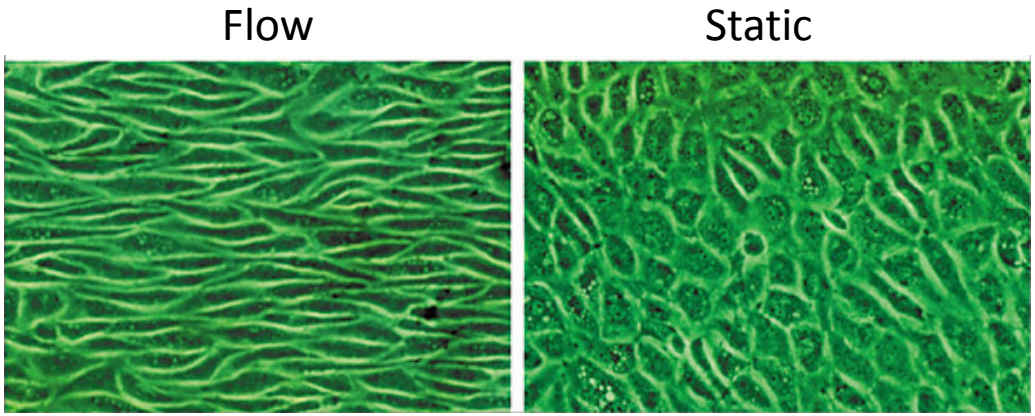


Ley *et al*. Nat Revs Imm (2007)

Nourshargh & Alon. Immunity (2014)

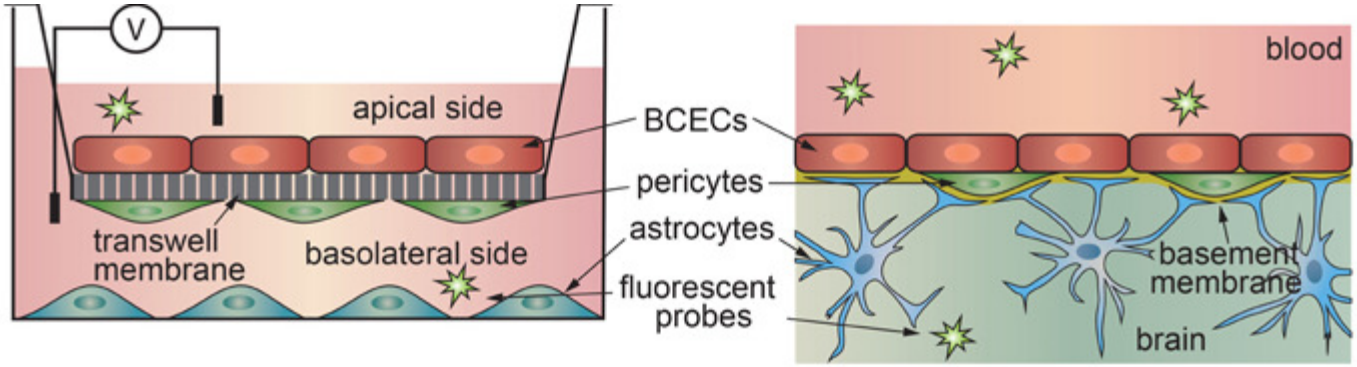
Why use *in vivo* models?

- Endothelial phenotype
  - Affected by flow
  - Origin of cells
  - Integrity of junctions

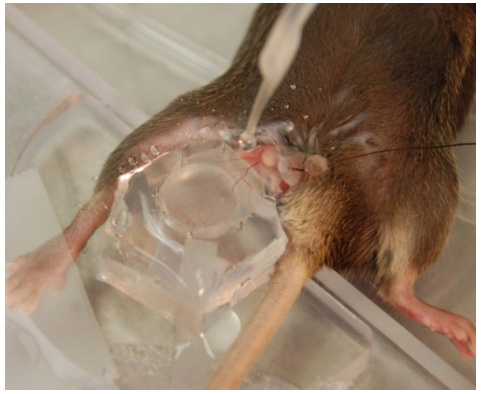


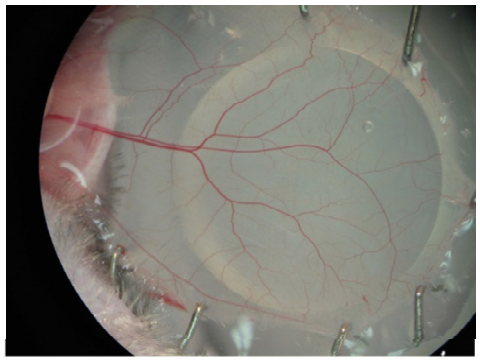
- Blood flow & sheer stress
  - Leukocyte function

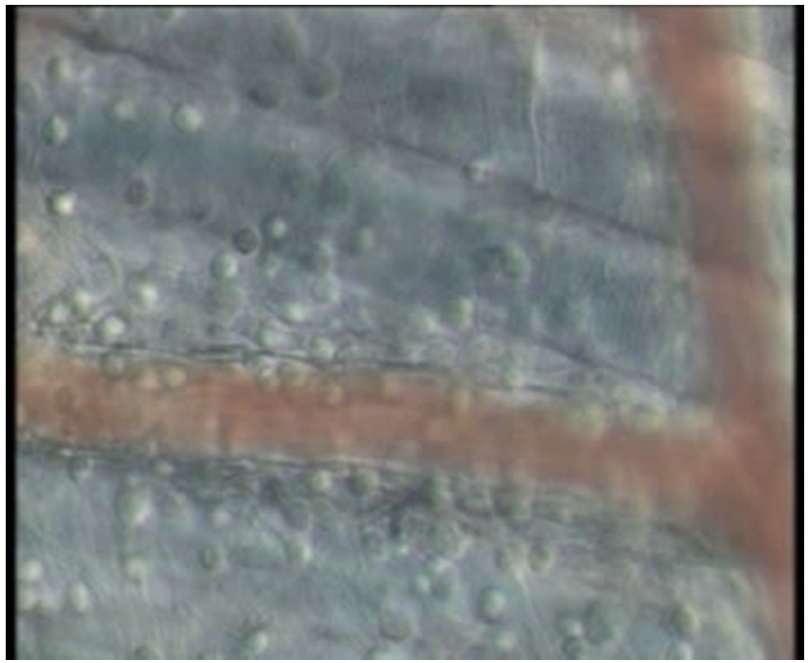
- Multiple barriers of the vessel wall
  - ECs, pericytes, basement membrane
  - Perivascular macrophages & mast cells
  - Effect of underlying substrate

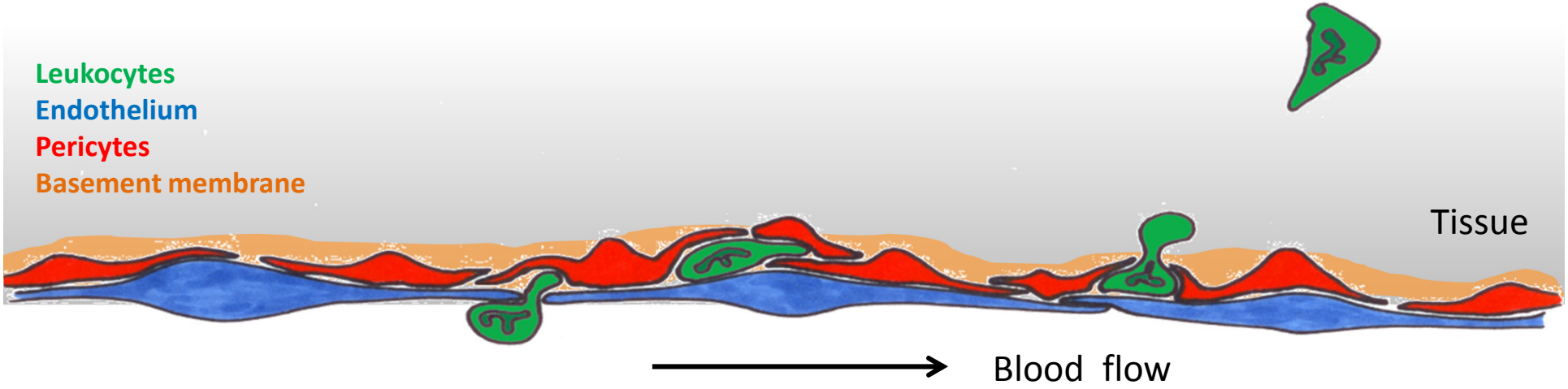


Mouse cremaster muscle



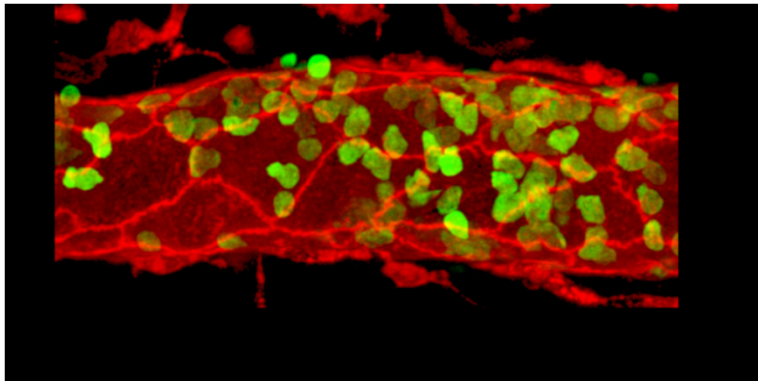




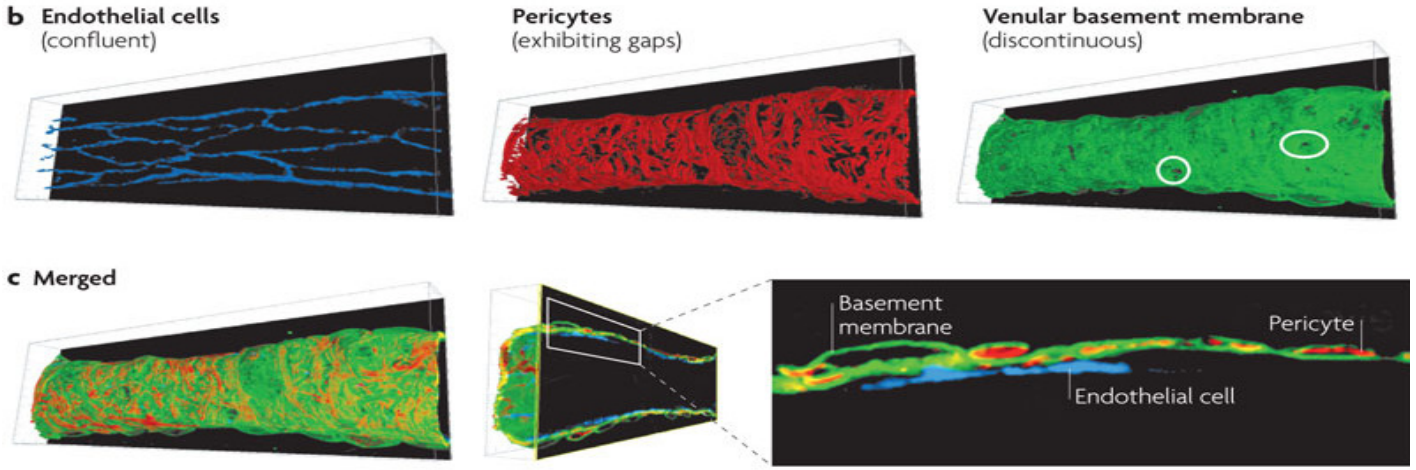


Confocal microscopy

- Fluorescent labelling of structure of interest
  - Protein specific antibodies
  - Genetic insertion of fluorescent protein
  - Intracellular dyes
- Optical sectioning



Neutrophils – anti-MRP14
Endothelium – anti-PECAM-1

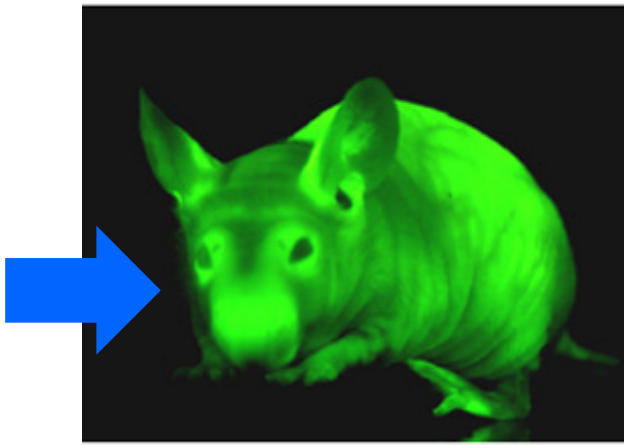


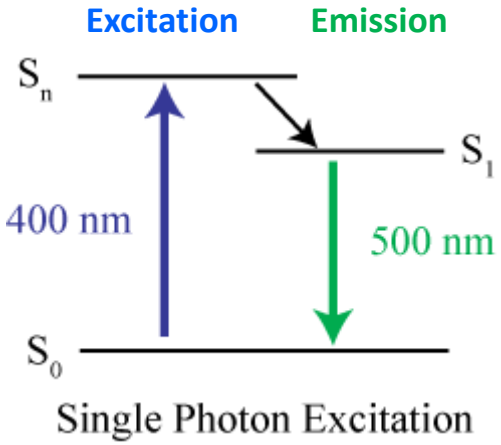
Nourshargh *et al*. Nat.Rev.Mol.Cell.Biol (2010)

In vivo confocal microscopy: labelling and tissue penetration

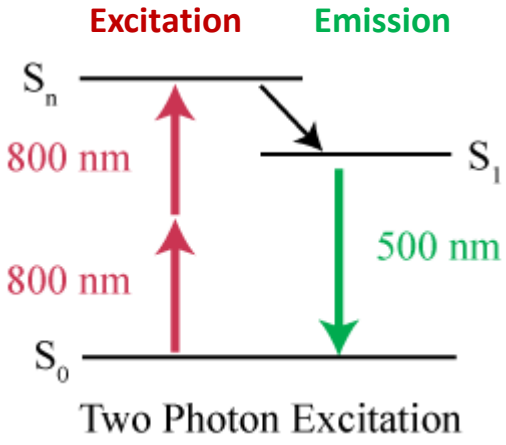
- Antibody labelling of surface antigens
  - Functional effects?
- GM animals
  - Neutrophils, monocytes, pericytes, macrophages
- Non specific markers
  - Dyes, particles, sugar binding proteins

- Single photon excitation ~ 100 um deep
  - 1 shortwave excitation photon
  - 1 longer wave emission photon
- Multi-photon excitation ~ 1 mm
  - 2 longwave photons excitation
  - 1 shorter wave photon emission





Single Photon Excitation



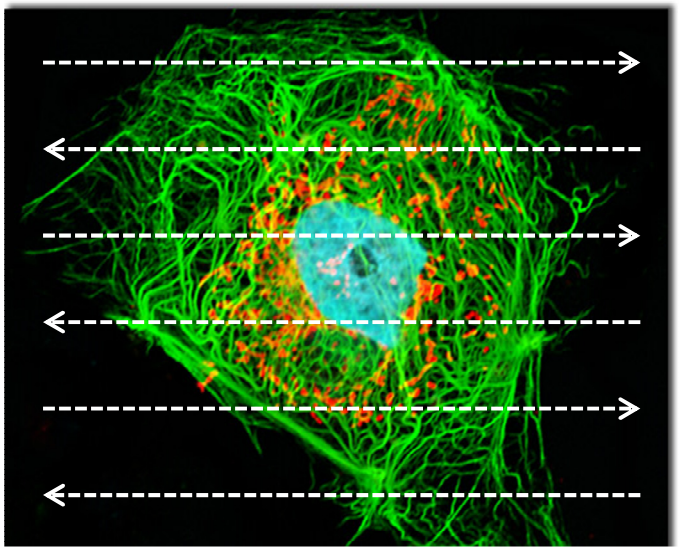
Two Photon Excitation

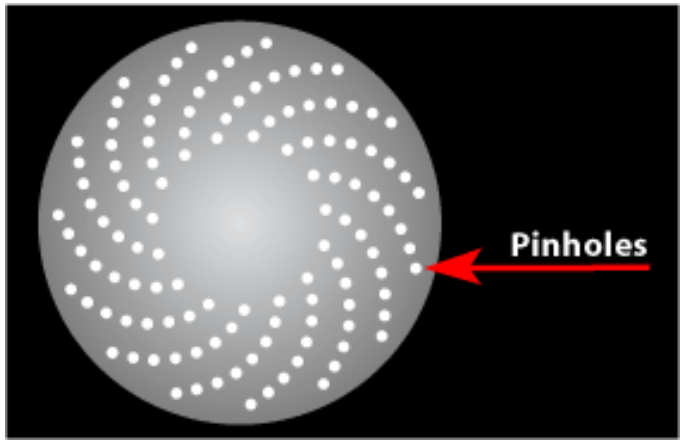
In vivo confocal microscopy: Rapid image acquisition

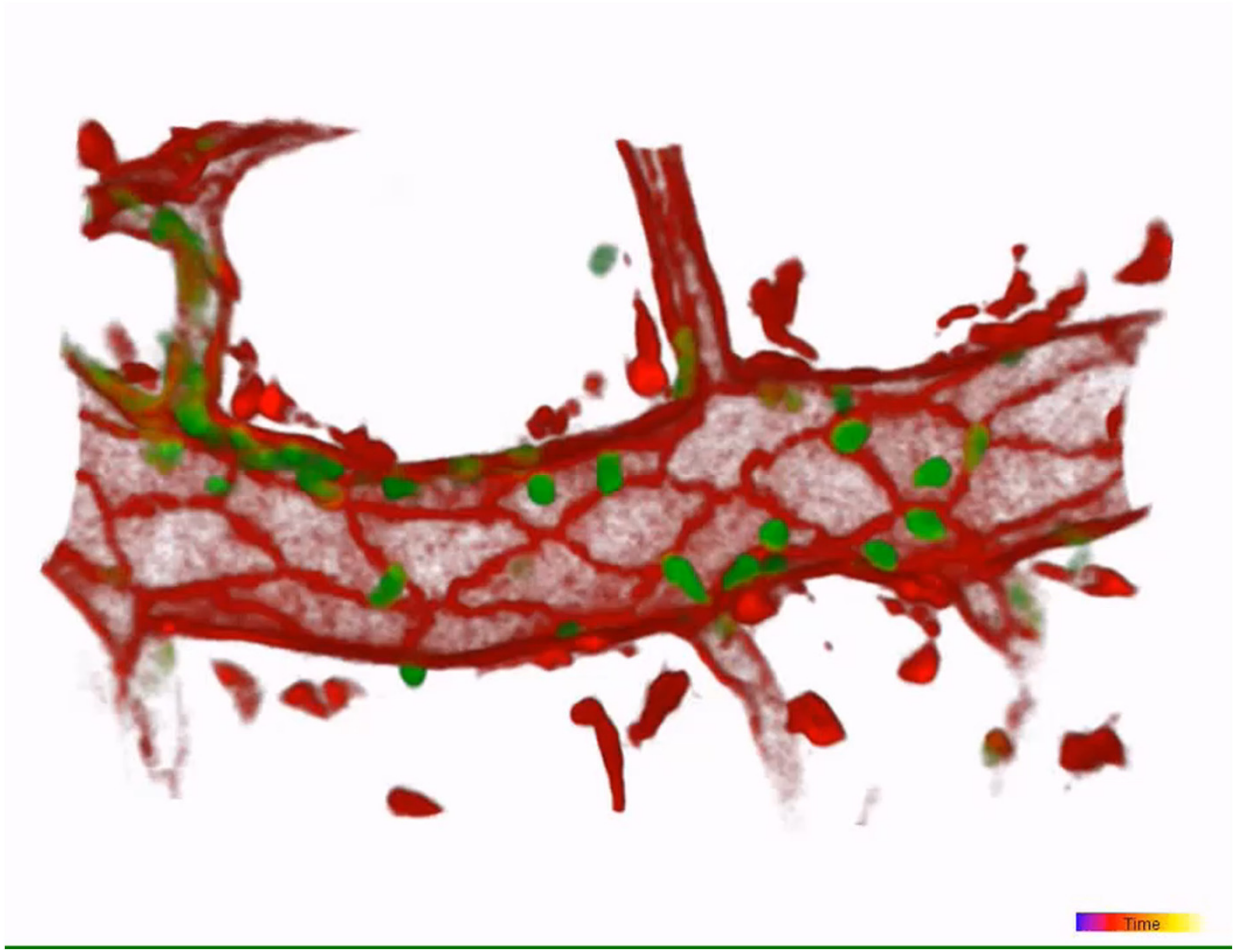
Point scan ~ 10 mins

Spinning disc ~ 10 seconds

Resonance point scan ~ 30 seconds





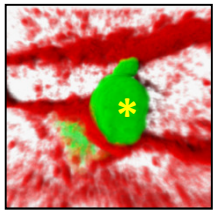


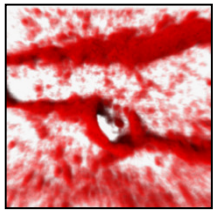
Woodfin A *et al*. *The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo*.
Nat.Immunol. (2011)

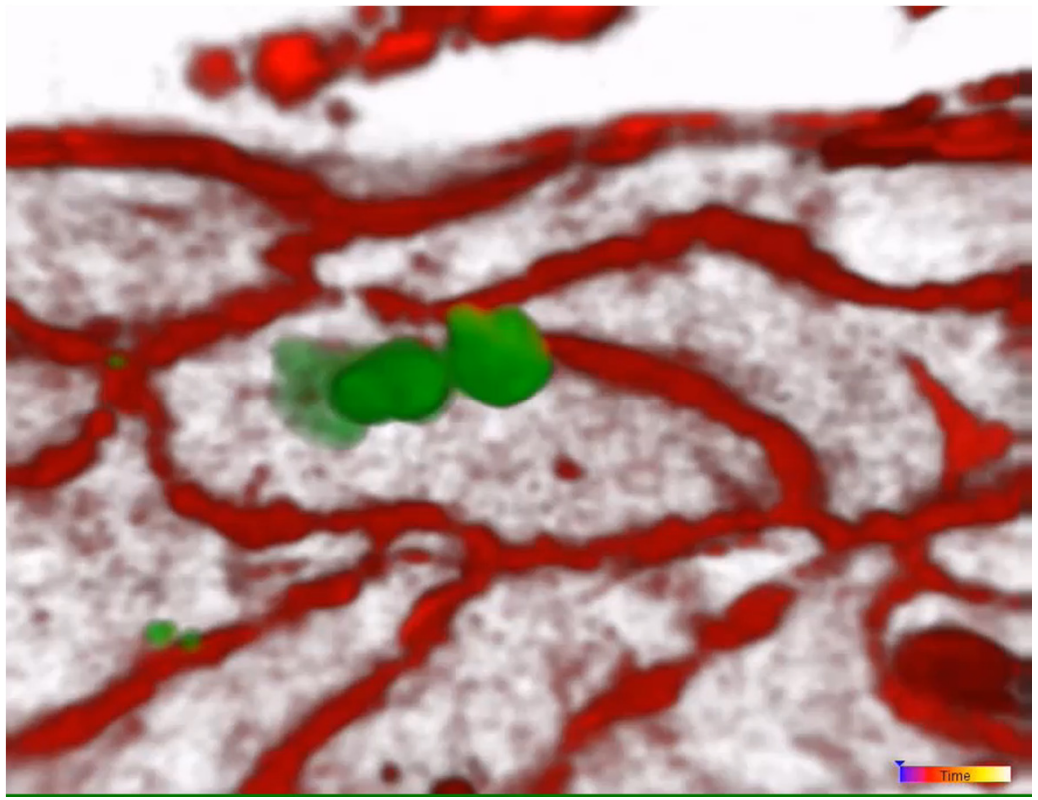
Anti-PECAM-1 - Endothelium
LysM-GFP - Neutrophils

Paracellular & Transcellular migration

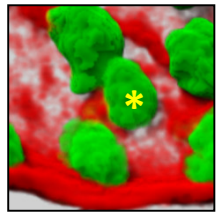
Paracellular

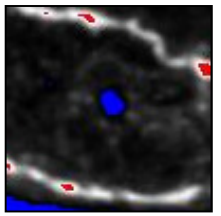




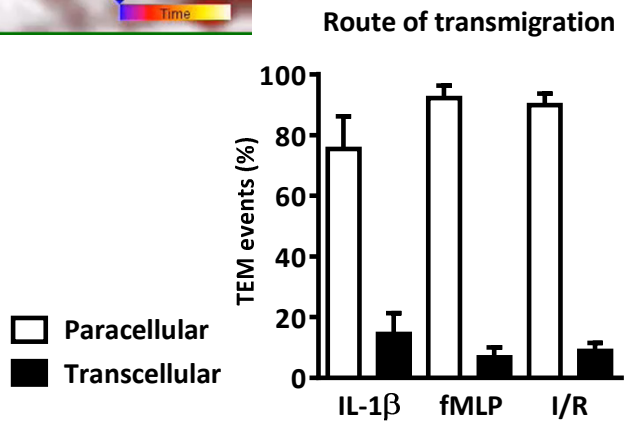


Transcellular



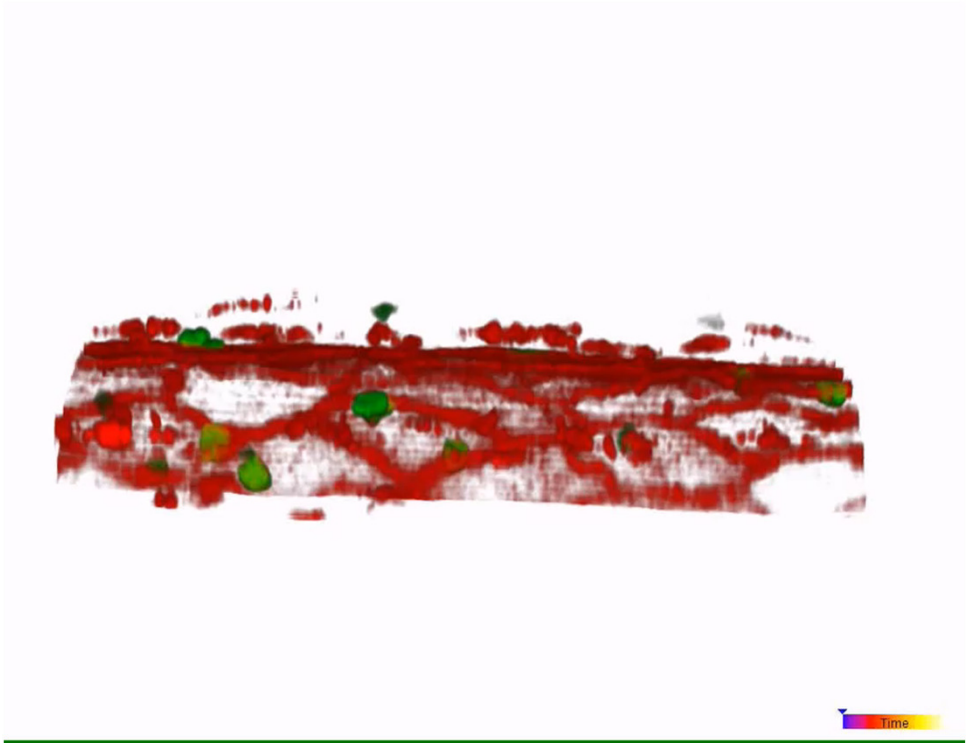


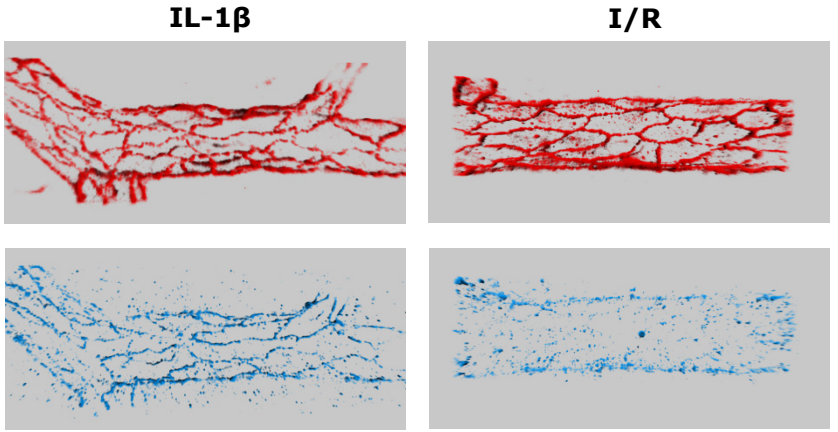
Endothelium - Anti-PECAM-1
Neutrophils - LysM-GFP



Woodfin A *et al*. *The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo*. Nat.Immunol. (2011)

Polarised paracellular migration



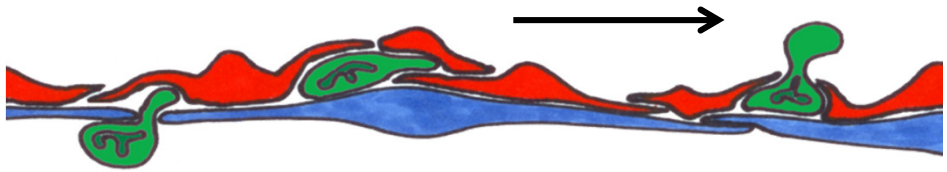


Endothelium - Anti-PECAM-1
Neutrophils - LysM-GFP

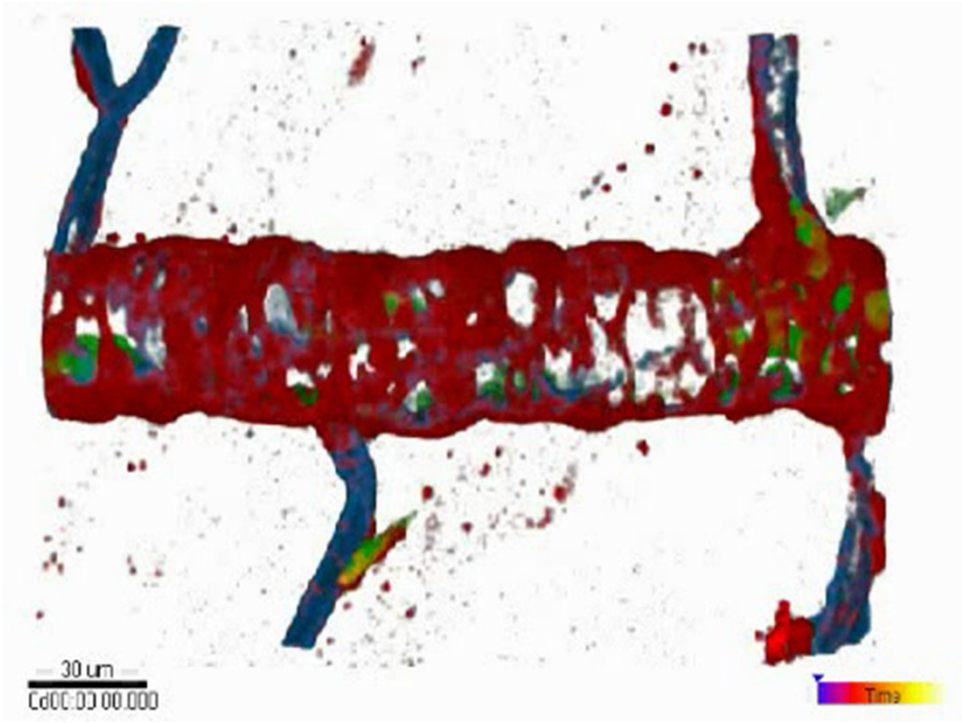
Woodfin A *et al*. *The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo*. **Nat.Immunol.** (2011)

Colom *et al. Leukotriene B_4-neutrophil elastase axis drives neutrophil reverse transendothelial cell migration in vivo*. ***Immunity.*** (In press)

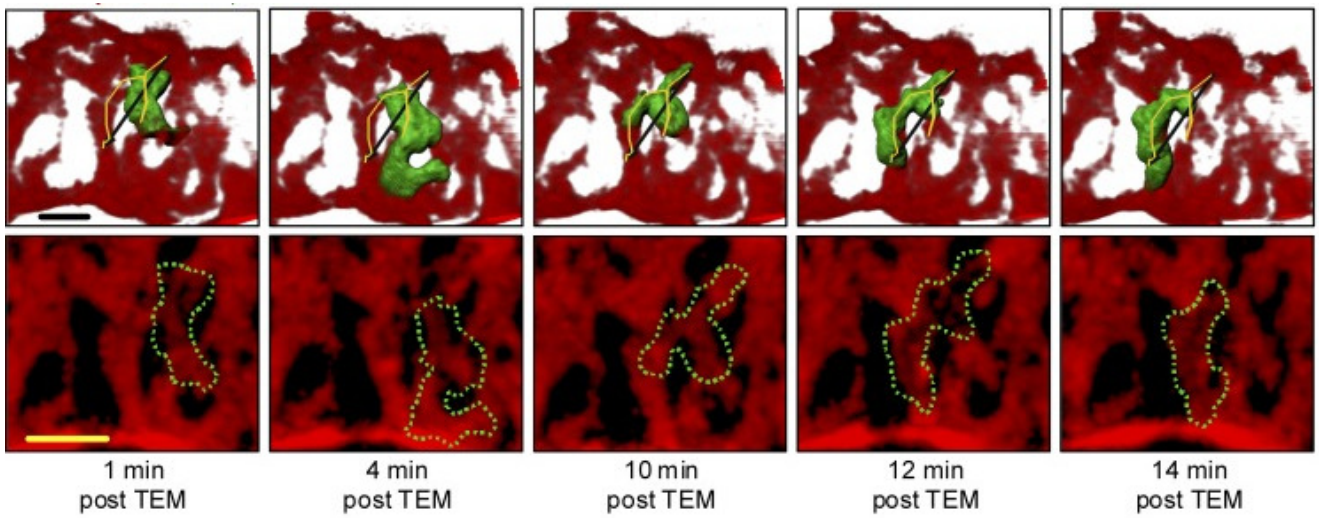
Role of pericytes in neutrophil extravasation



Probstl D & Voisin M-B *et al*. *Pericytes support neutrophil sub-endothelial cell crawling and migration through venular walls in vivo*. **J.Exp.Med** (2012)

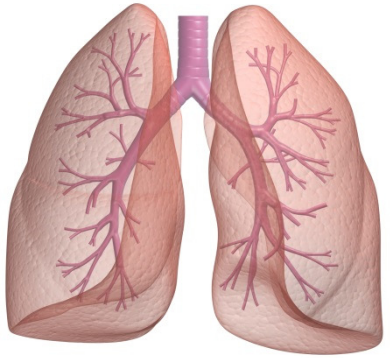


Endothelium - Anti-PECAM-1
LysM-GFP – Neutrophils
Pericytes - smooth-muscle-actin-Cherry-FP

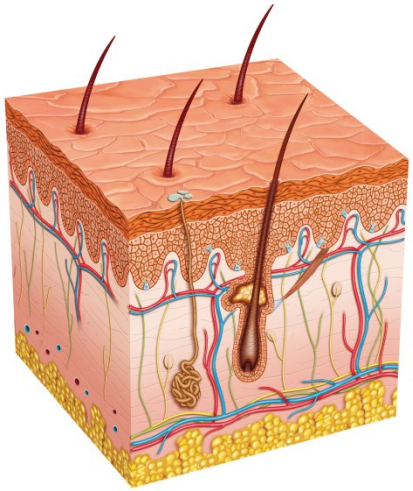


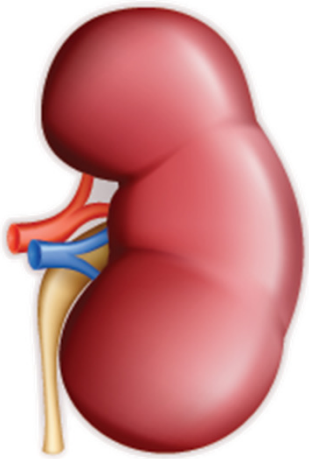
Imaging specific tissues

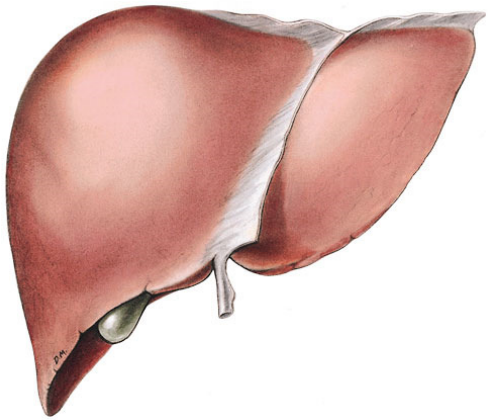
- Observation of the relevant tissues for a particular pathology.



- Sacrifice image quality
  - Depth/penetration
  - Movement
  - Labelling possibilities

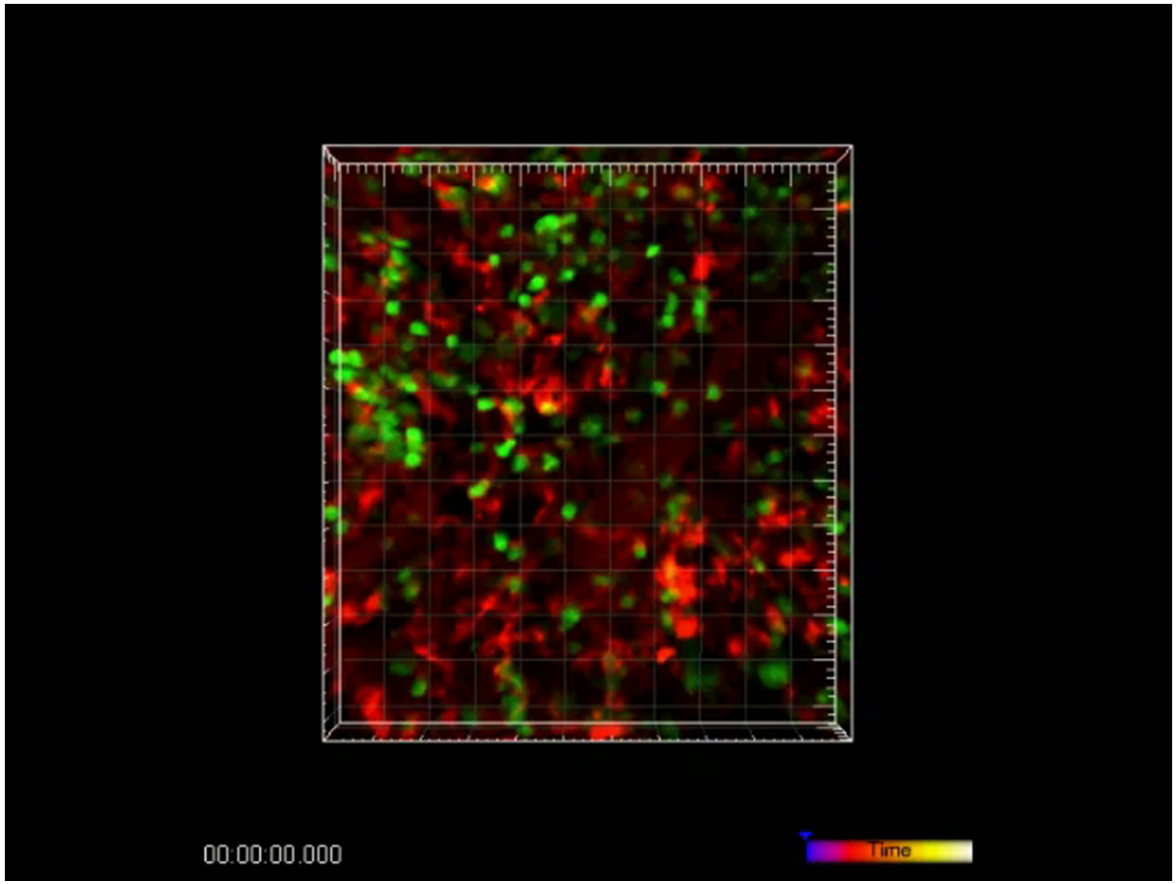






Lung

Neutrophils - LysMGFP
Blood - i.v. Q-dots

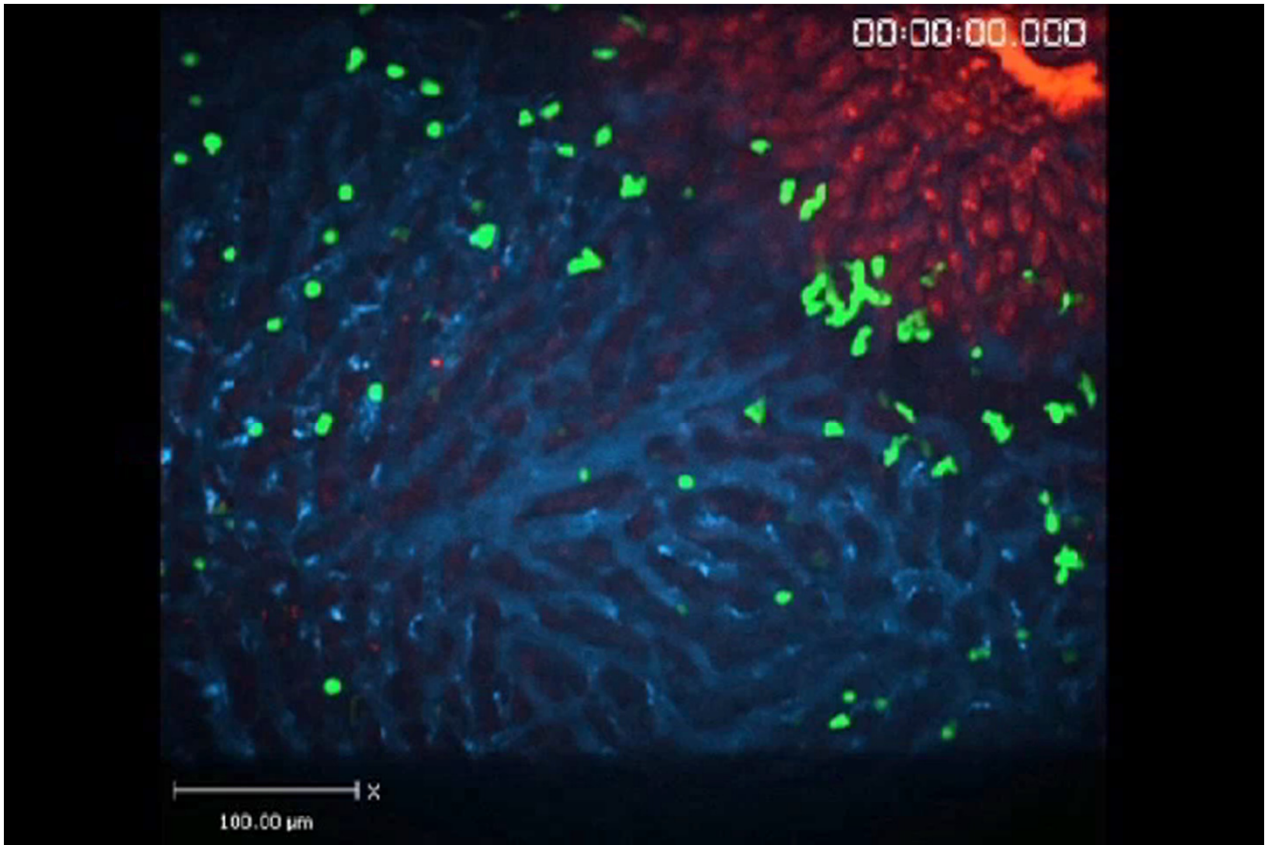


In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation.

Kreisel *et al*, PNAS (2010)

Liver

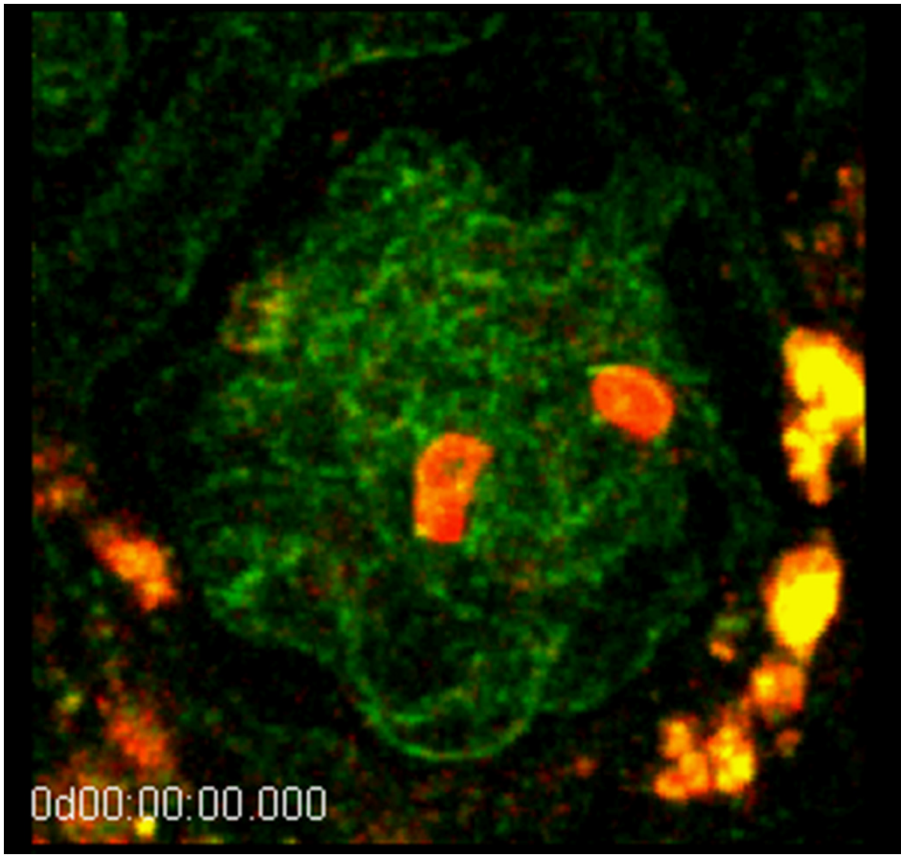
Neutrophils - LysMGFP
Blood - i.v. BSA-Alexa647
Necrosis - Propidium iodide



Intravascular Danger Signals Guide Neutrophils to Sites of Sterile Inflammation.
McDonald *et al*, Science (2010)

Kidney

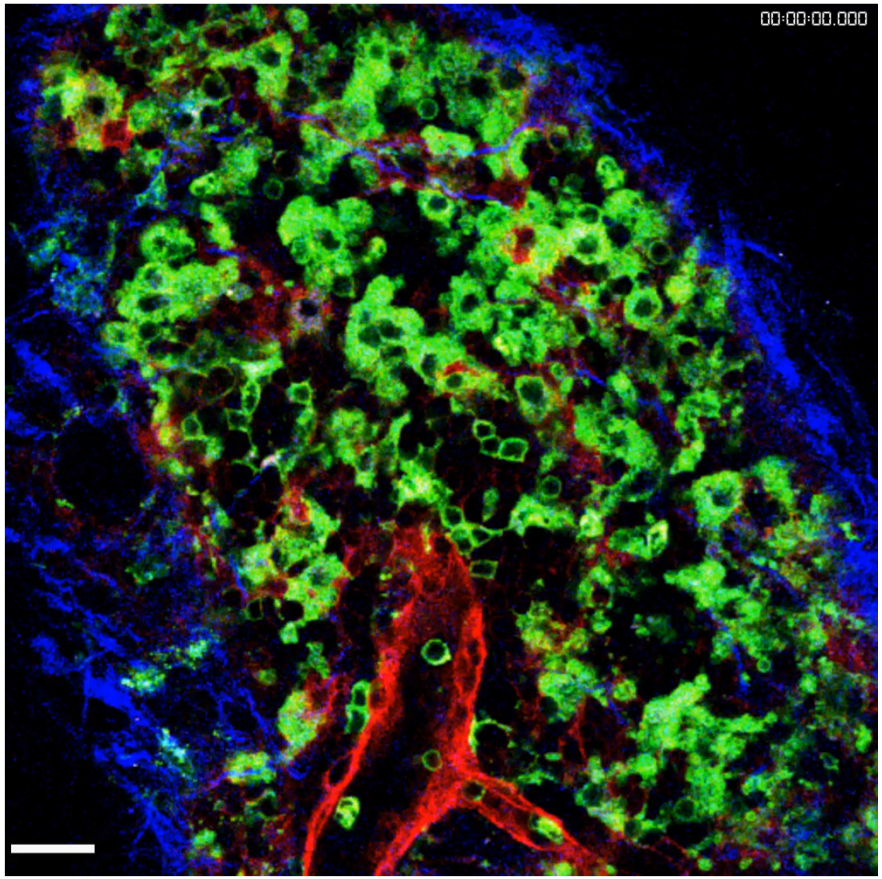
Neutrophils (anti-GR1)
Vessels (i.v. lectin-Alexa488)



Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus.
Devi *et al*, Nat Med (2013)

Lymph node

Neutrophils - LysMGFP
Vasculature - tdTom
Second harmonic

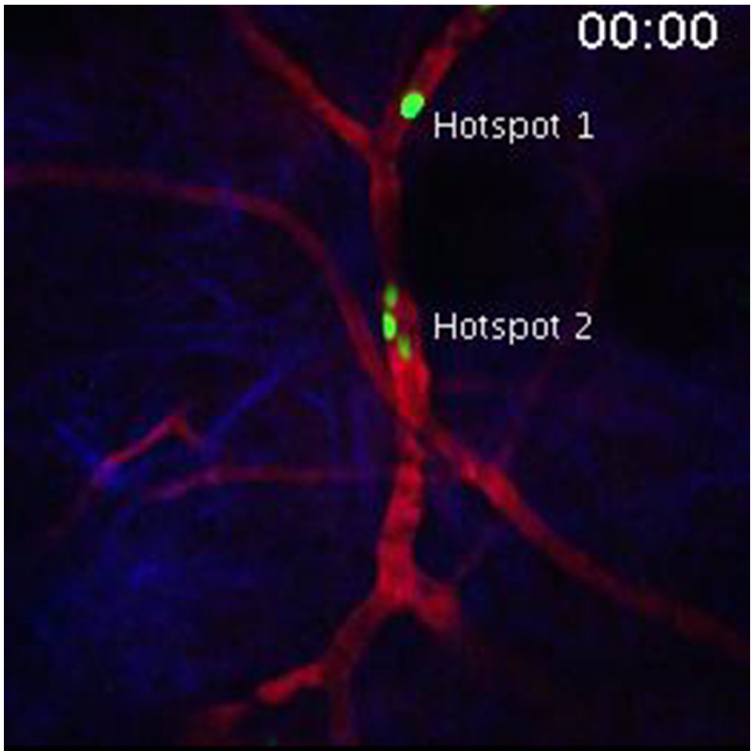


Neutrophil dynamics and migration mechanisms in the tissue draining lymph node following pulmonary *S. pneumoniae* infection.

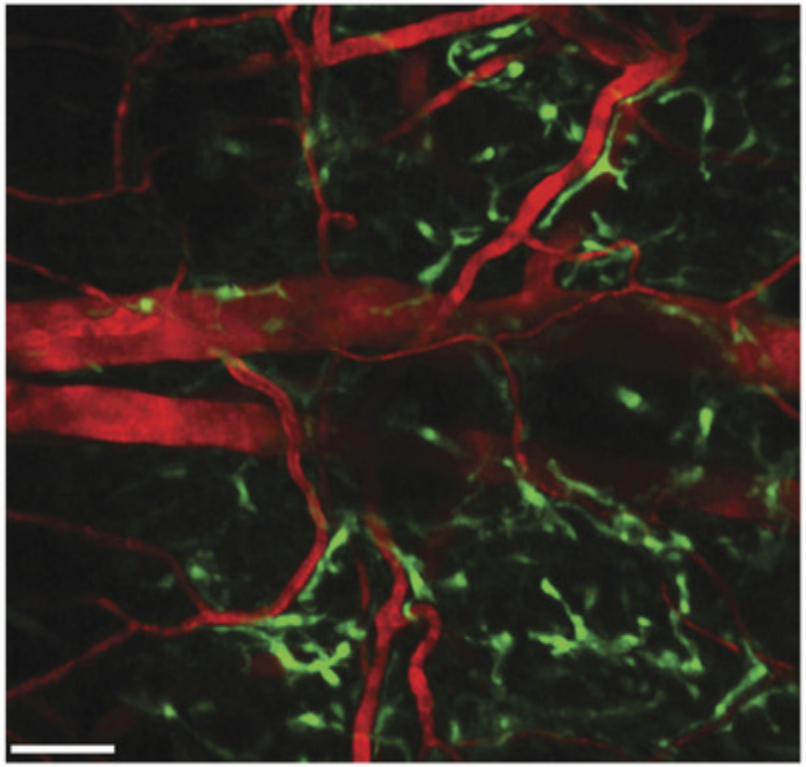
Amy Sawtell, PhD University of York (paper in preparation)

Dermis

Neutrophils - CFSE
Evans Blue-BSA
Second harmonic

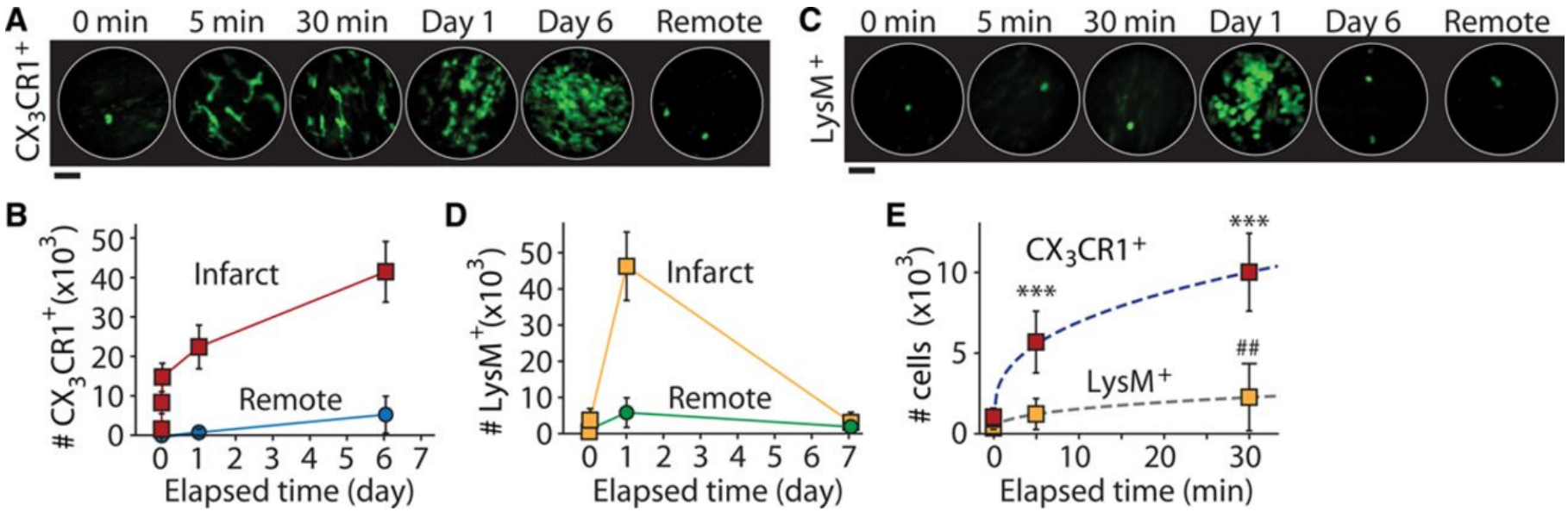


Macrophages – DPE-GFP
Evans Blue-BSA



Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection.
Abtin *et al*, Nat Imm (2014)

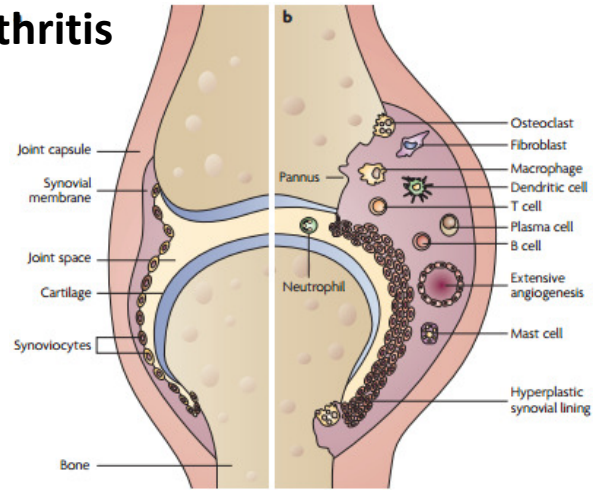
Myocardium



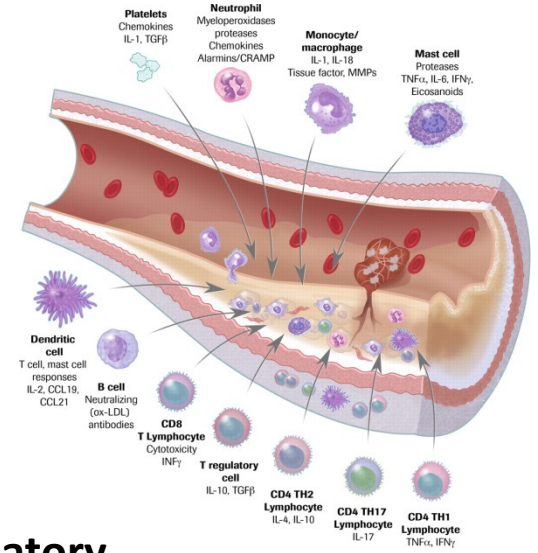
Neutrophils – LysMGFP
Monocytes/macrophages – CX3CR1-GFP

Endoscopic Time-Lapse Imaging of Immune Cells in Infarcted Mouse Hearts
Jung *et al.* Circulation Research (2013)

Arthritis



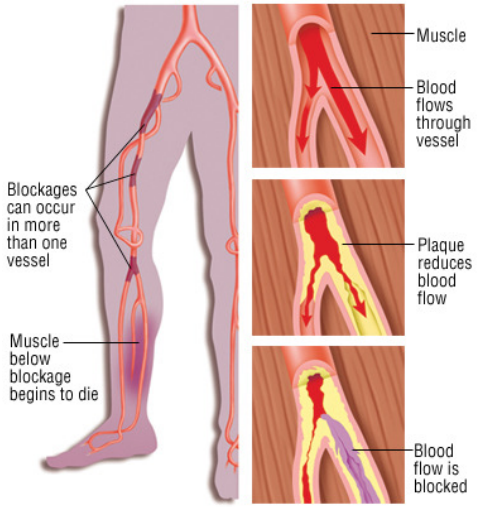
Atherosclerosis

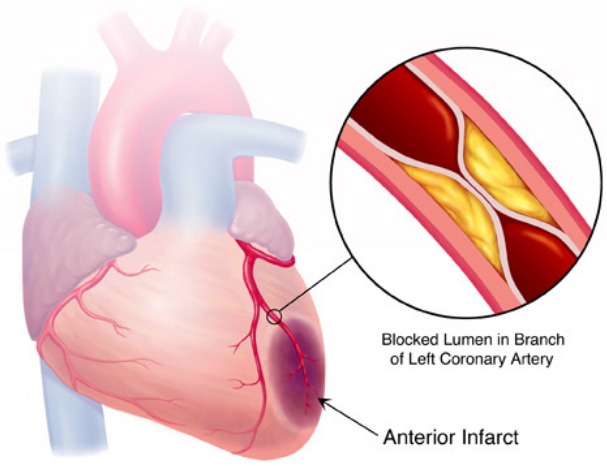


Chronic inflammatory disorders

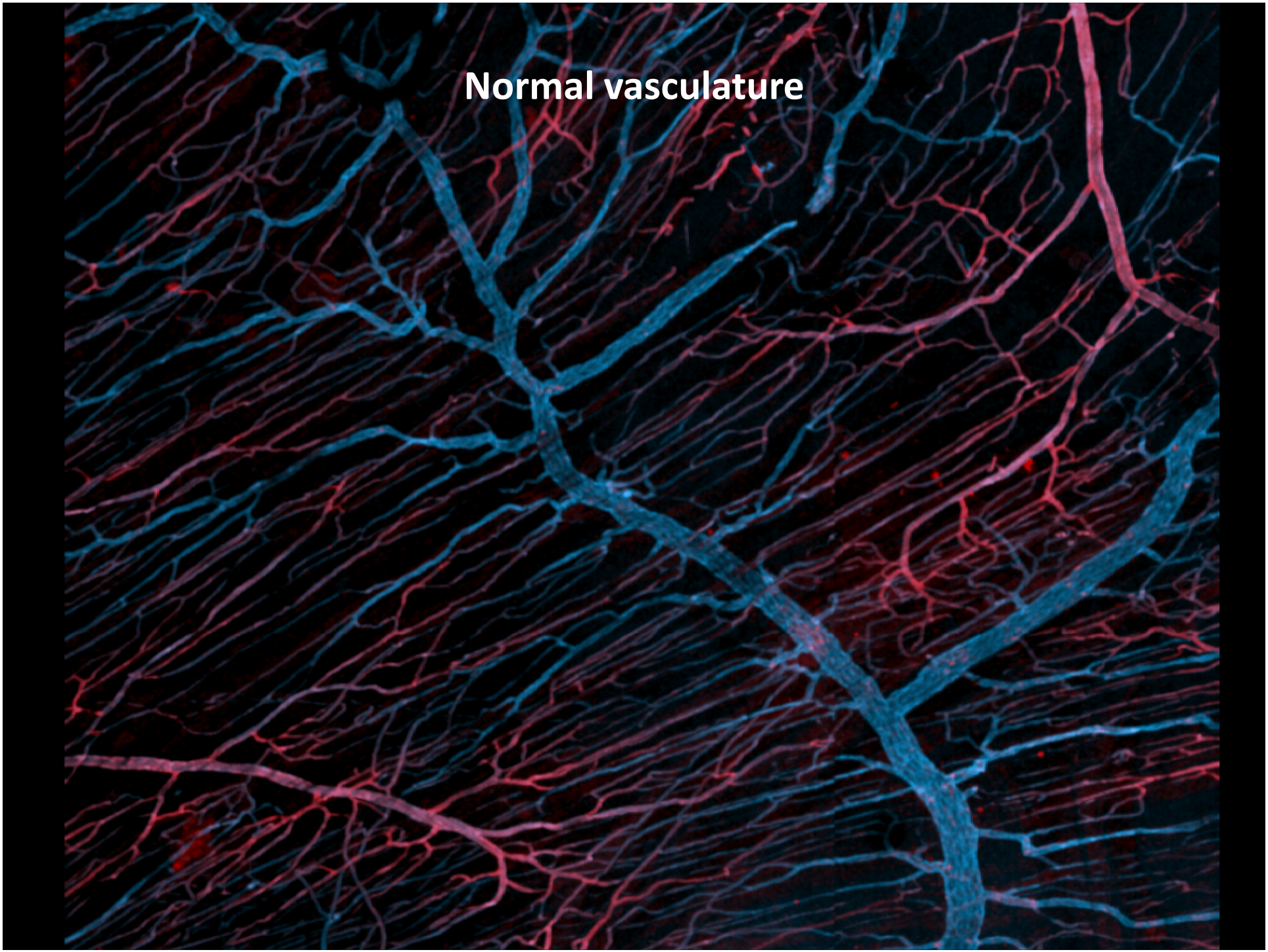
- Leukocytes
- Hypoxia & ROS
- Angiogenesis
- Edema
- Fibrosis
- Metabolic changes

- Altered mechanisms of inflammation?
- Therapeutic opportunity?

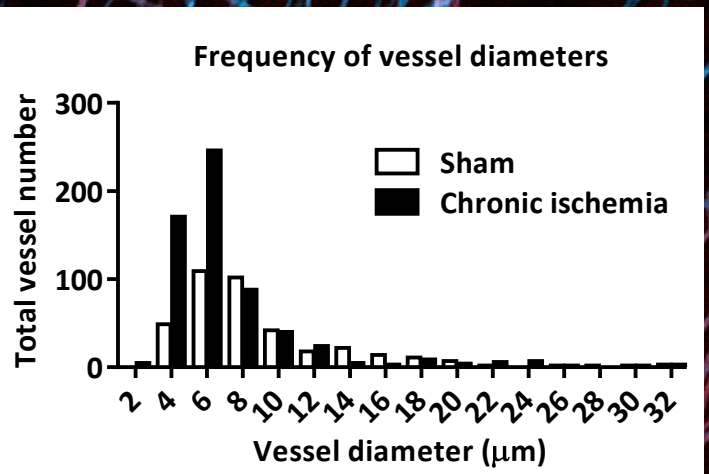




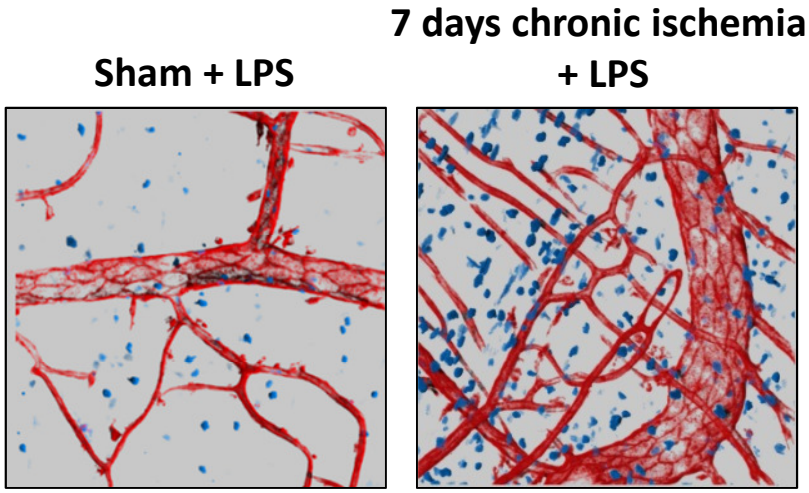
Chronic ischemia


Normal vasculature

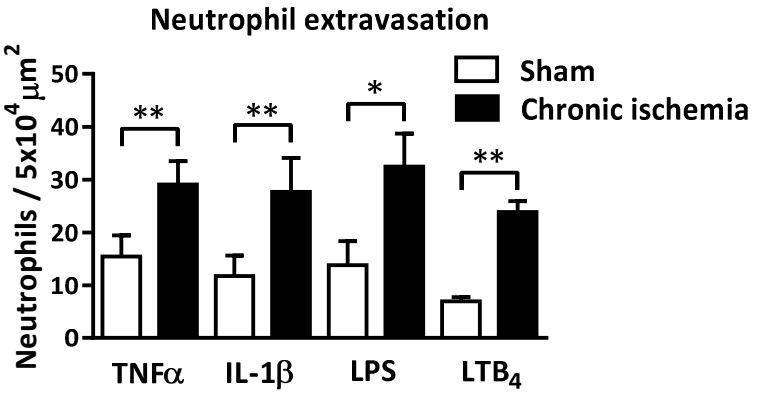
Chronic ischemia and angiogenesis

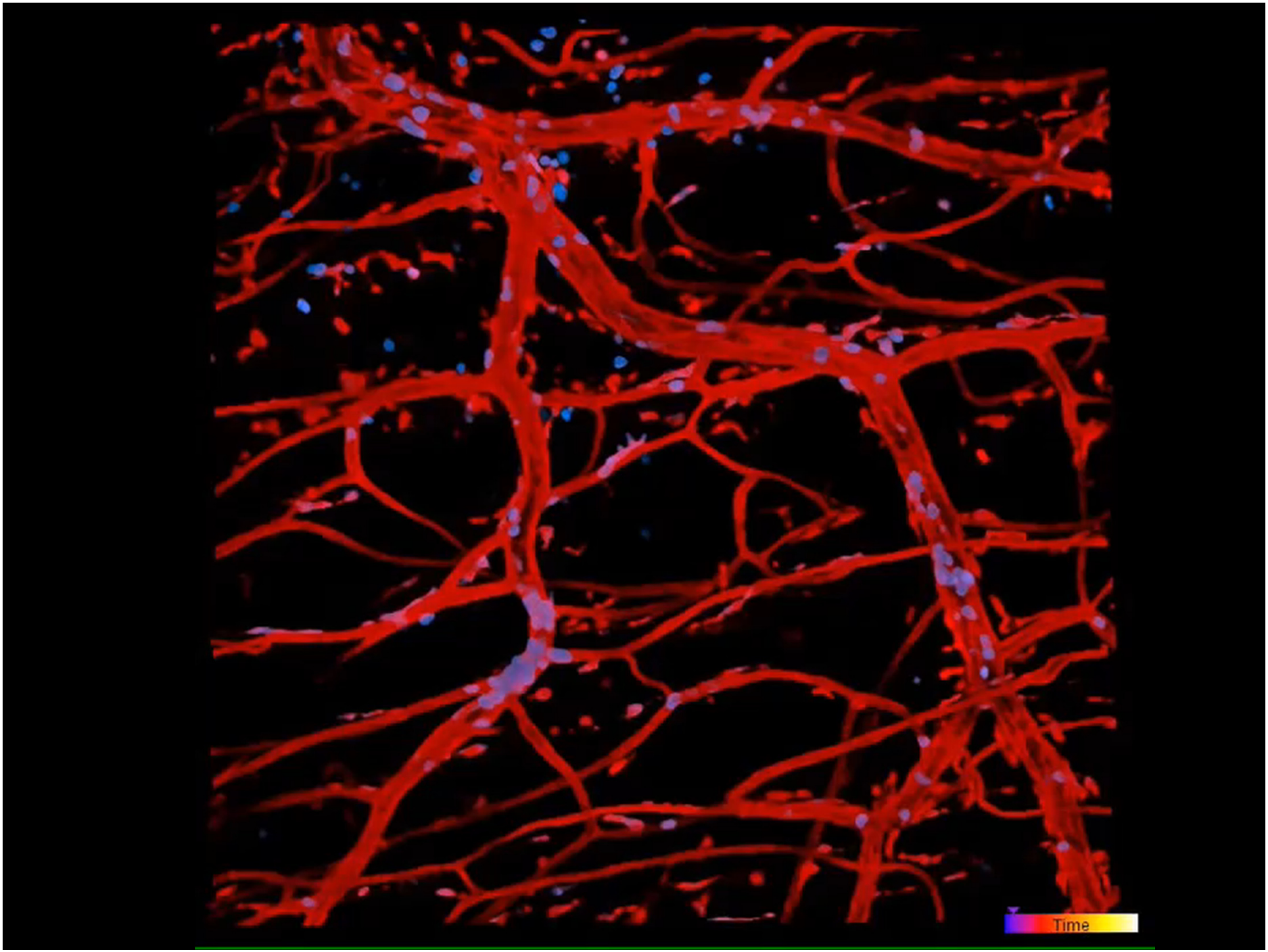


Neutrophil recruitment in chronic ischemia tissues

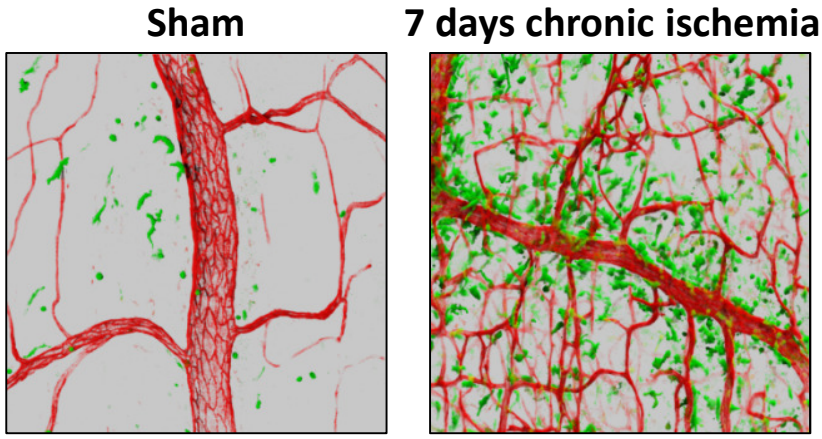


Endothelium - Anti-PECAM-1
Neutrophils - LysM-GFP



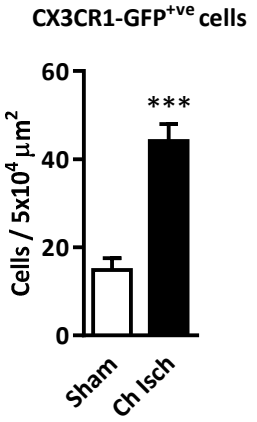

Time

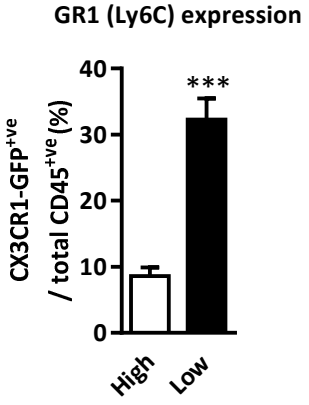
Chronic inflammation in ischemic tissues

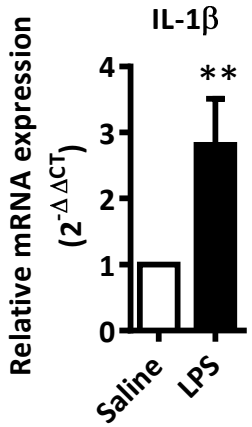


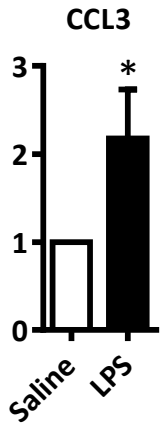
Endothelium - Anti-PECAM-1

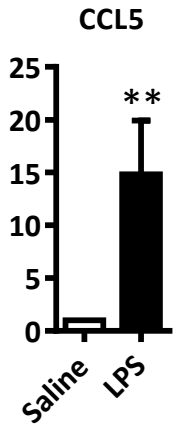
Monocytes/macrophages – CX3CR1-GFP

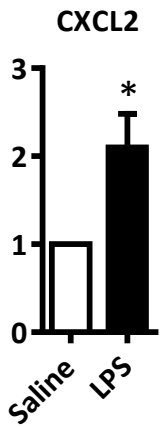


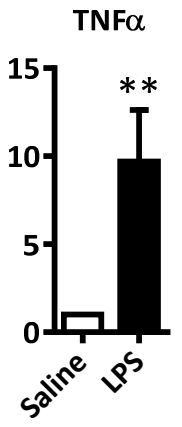




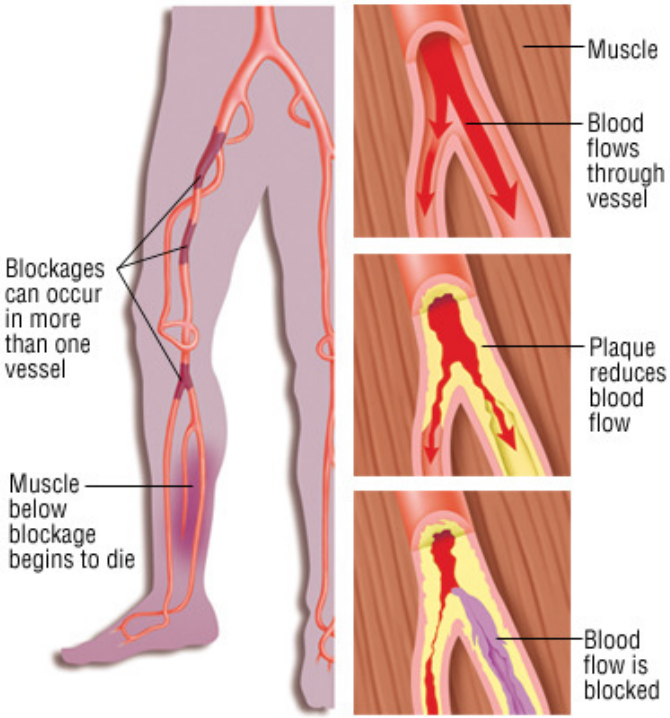


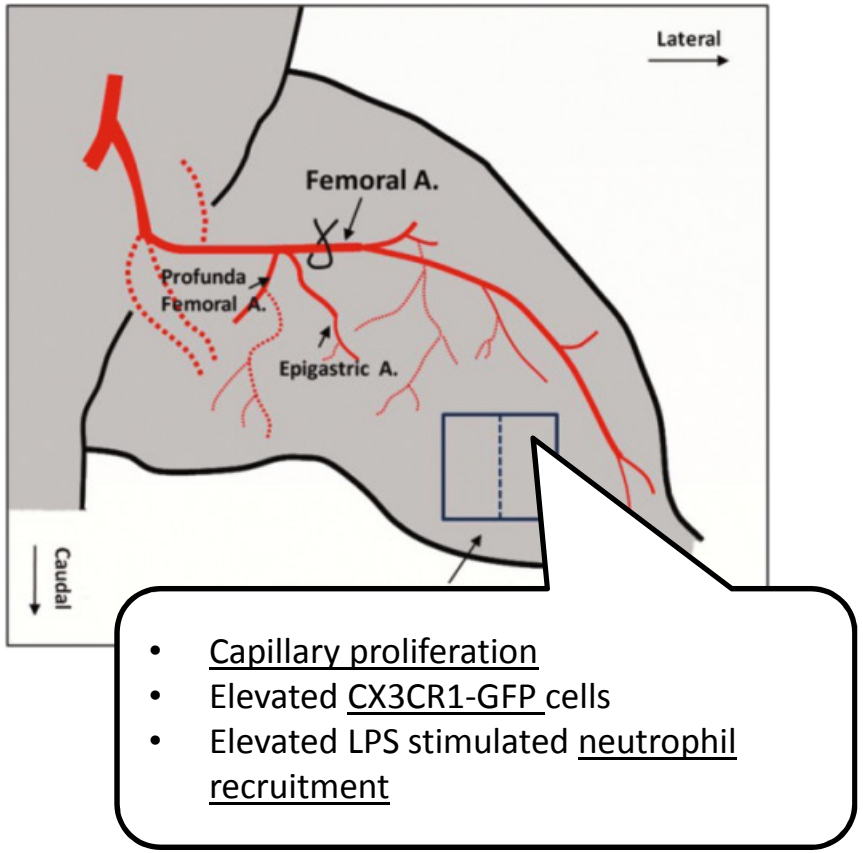






Peripheral arterial disease & femoral artery occlusion

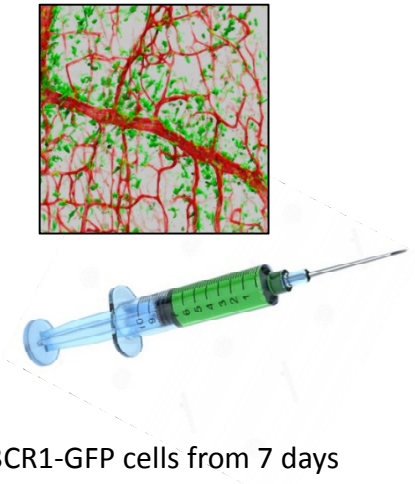




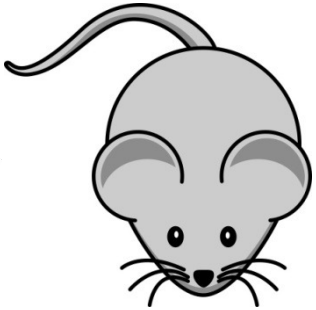
- Capillary proliferation
- Elevated CX3CR1-GFP cells
- Elevated LPS stimulated neutrophil recruitment

- Non-healing sores and ulcers
- Possible amputation
- Intermittent exercise induced ischemia/hypoxia/pain (claudication)

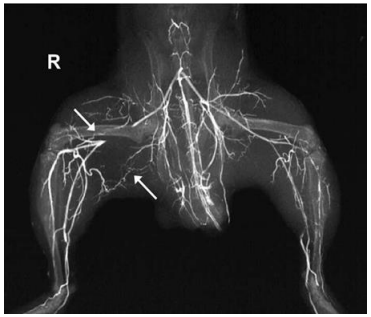
Acute ischemia reperfusion



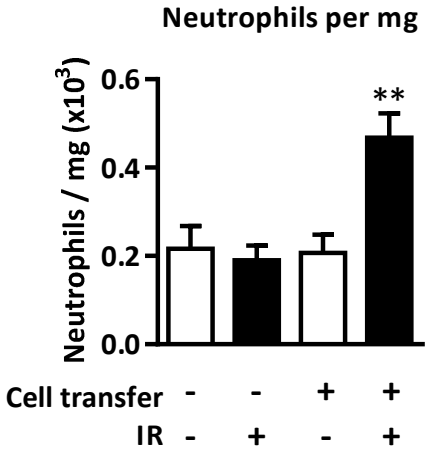
CX3CR1-GFP cells from 7 days chronic ischemia tissues



Into hind limb muscles



Acute ischemia/reperfusion (60min / 120min)



CX3CR1-GFP cells from **chronically inflamed tissues** can amplify the response to transient ischemia/hypoxia.

May be contributing to disease progression and/or severity of symptoms.

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