Tracking leukocyte migration

*in vivo*

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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 $\rightarrow$ lymphatic vessels
- Antigen presentation in lymph nodes
• 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
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

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 $\sim 100$ um deep
  - 1 shortwave excitation photon
  - 1 longer wave emission photon

- Multi-photon excitation $\sim 1$ mm
  - 2 longwave photons excitation
  - 1 shorter wave photon emission
*In vivo* confocal microscopy: Rapid image acquisition

Point scan ~ 10 mins

Spinning disc ~ 10 seconds

Resonance point scan ~ 30 seconds

Colom et al. Leukotriene B₄-neutrophil elastase axis drives neutrophil reverse transendothelial cell migration in vivo. Immunity. (In press)
Role of pericytes in neutrophil extravasation


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
In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation.
Kreisel et al, PNAS (2010)
Intravascular Danger Signals Guide Neutrophils to Sites of Sterile Inflammation.
McDonald et al, Science (2010)
Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus.

Devi et al, Nat Med (2013)
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)
Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection.
Abtin et al, Nat Imm (2014)
Myocardium

Endoscopic Time-Lapse Imaging of Immune Cells in Infarced Mouse Hearts

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

- Altered mechanisms of inflammation?
- Therapeutic opportunity?
Normal vasculature
Chronic ischemia and angiogenesis

Frequency of vessel diameters

Total vessel number

Vessel diameter (µm)

Sham

Chronic ischemia
Neutrophil recruitment in chronic ischemia tissues

Endothelium - Anti-PECAM-1
Neutrophils - LysM-GFP
Chronic inflammation in ischemic tissues

**Endothelium - Anti-PECAM-1**

**Monocytes/macrophages – CX3CR1-GFP**

**Sham**

7 days chronic ischemia

**CX3CR1-GFP^ve cells**

**GR1 (Ly6C) expression**

**Relative mRNA expression**

- **IL-1β**
- **CCL3**
- **CCL5**
- **CXCL2**
- **TNFα**
Peripheral arterial disease & femoral artery occlusion

- Non-healing sores and ulcers
- Possible amputation
- Intermittent exercise induced ischemia/hypoxia/pain (claudication)
- Capillary proliferation
- Elevated CX3CR1-GFP cells
- Elevated LPS stimulated neutrophil recruitment
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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