Anatomy & Physiology Of The Lymph Node: An Immunophotonojournalist’s POV

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PR-10-327 - The opinions presented here are the personal views of the author and are not to be construed as official or as representing the Department of Defense.
Why Immunophotojournalist?

- Photojournalists tell a story by capturing action, emotion or beauty in a photo that inspires.

- My research instrument analyzed multi-spectral photon images of dynamic biological events.

- For 30 years that “instrument” has been my eyes, and my mind enhanced by microscopy.

- My experimental designs include methods that preserve evidence of movement or involve serial sampling over time to reconstruct action.
Lymphatic System

- Parts of the immune system are all over the body, yet it functions as one organ.
- What allows this functionality?
- The blood and lymph vascular systems are the highways allowing cells to go to work.
- Lymphocytes and other cells of the immune system are constantly moving.
Lymphocytes Recirculate

- Lymphocytes were once believed to be a fountain of youth like a stream of stem cells which they resemble.
- Gowans showed that small lymphocytes are depleted if efferent lymph is drained.
- If collected, labeled and returned to the blood they reappear in efferent lymph within 18-24 hours.
Magnitude of Lymphocyte Traffic

- Traffic between tissues involves $\sim 5 \times 10^{11}$ cells per day but $< 2\%$ of these cells are in the blood at any moment.
- Lymphocytes stay in the blood for $\sim 30$ min.
- Circulating blood pool of lymphocytes is exchanged 10 to 48 times a day.
- Traffic to lymphatic tissues is 5 times faster than traffic through non-lymphatic tissues.
Lymphocytes Traffic Through Peripheral Lymphatic Tissues

- Lymphocytes recirculate from blood to tissues and back to the blood via efferent lymph.
- Antigen prompts a lymphocyte to lodge, divide and add its daughters to the recirculating pool.
- They acquire organ selectivity and often return to the type of tissue where they were first stimulated.
Skin & Regional Lymph Nodes LN Are Precise Antigen Transducers

Skin dendritic cells stained *en-face*
Skin & Regional Lymph Nodes Are Precise Antigen Transducers

Valves Direct Lymph Flow

DC in Skin Turnover and Carry Antigen to LN Via Lymphatics
LN T- & B-Cell Compartments
Carbon was used to track where antigen laden monocytes go in lymph nodes.
LN Uptake of Free Particulates

- Free particles accumulate in the subcapsular sinus, cortical sinuses, and in the medullary lymph sinuses.
- Entry of particles into the diffuse cortex is blocked.
Soluble Proteins Pass Into FRCC

While microparticles are blocked at SCS, solutes pass into FRC Conduits
Particle Traps in the LN Cortex

Migrating Dendritic Cells block and Macrophages guard the pores in the floor of the SCS.

Macrophages suspended from bridging FRC guard the cortical lymph sinuses.
Dendritic Cells

Intravital 2-photon laser-scanning microscopy
Qi et al Science 312: 1672-1676, 2006
Compare: Lymph Node 1
With:
Lymph Node 2 – What Happened?
Lymph Node Paracortex Unit
Study Lymph Node Vasculature
Lymph Node Vasculature

SCS

a-v shunts

LN Microvasculature revealed by arterial perfusion with Alcian Blue dye.
Lymph Node Vasculature

HEV

Lobular Vein
Lymph Node Vasculature
Lymphocyte Emigration in LN
Initial Contact Via Microvilli
Elaboration of Homing Cascade
Active and Polar Emigration
Orientation and Movement Out

Animation by Art Anderson
Do They Migrate Through or Between HEV Endothelial Cells?

Extracellular tracers compensate for 2 dimensional EM data.
Unidirectional Permeability of HEV

Intra-arterial HRP  Intra-lymphatic HRP
HEV is Chemotaxis Chamber

Fixative as tracer

Drawing from 1000 EMs

by Barbara Gould
Lymphocyte Chemotaxis studies

Leukocyte dialysates of < 10KDa are chemotactic for lymphocytes but they could not be further chemically purified.

Collaboration with Kouji Matsushima in the late 1980s resulted in discovery of the first 2 chemokines IL8 and MCP-1. 

Science 243:1464-1466, 1989

Chemotaxis chamber made by Don Smith RIID engineer

T-cell migration into gradient of acute phase rat serum also revealed cell-cell attraction
Chemokines are now a Field

Chemokines mediate white cell migration and activation during immune surveillance, and inflammation. They bind to G protein-coupled receptors and cause conformational changes that trigger intracellular signaling pathways involved in cell movement and activation.
Lymphocytes Take Preferred Paths Across LN High Endothelial Venules

GFP Transgenic Mice with SPARKr T Cells
Intravital 2-photon laser-scanning microscopy

Preferred Migration Channels in Wall of High Endothelial Venule

HEV Longitudinal Section. Blood flow from right to left
FRC Conducts Chemokines to HEV Wall In Conduit ‘Fiber’

Are Solutes Pushed Down FRCC?


FRC Conduit Transmits Soluble Signals & Ag From SCS TO HEV

Animation by Art Anderson
FRC Conduit Transmits Soluble Ag Also From SCS TO GC-FDC

Animation by Art Anderson
Movement of T-cells in Lymph Node Cortex. Is it Random?

Movement of T-cells Within Lymph Node Paracortex

By Intravital 2-photon laser-scanning microscopy

Not Random


Random?

LN Changes With Ag

Lymph Nodes Enlarge and Change After Exposure to Antigens
Lymphocyte Logjams in Cortical Lymphatic Sinuses of Regional LN

Traffic Related LN Changes Early after Ag Inoculation
LN Changes With Ag

HEV Endothelial Cells label with H3 Thymidine day 3-6

T Reactive Cortex During Alloantigenic Response
Lymphocyte Traffic and Immunity

- Lymphocyte traffic in, through and out of lymphatic tissues changes during an immune reaction.
- Early changes are related to cytokines.
- Late changes relate to tissue remodeling.
Germinal Center B- & T-Cells
Germinal Center cell movement

C.D.C. Allen et al. Science xpress 21 Dec 2006

Intravital 2-photon laser-scanning microscopy
Germinal Center cell movement

Immune T-cell, B-cell movement

Intravital 2-photon laser-scanning microscopy

C.D.C. Allen et al Science express 21 Dec 2006
Medullary Cords & T-Cell Areas

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Katakai et al Int Immunol 16 1133-1142 2004
Medullary Cords & T-Cell Areas

RNA Rich Plasma Cells in Medullary Cords

Med Cord Vasculature
Collaborators 1972-2007

- **Lymphocyte Chemotaxis & Recirculation at USAMRIID**
  - Norm Anderson, Bob Wylie & Barbara Gould at JHU
  - Jonathan Warren, Mike Ascher, Leo Andron, Terry Abshire, John White, Francis Shirey

- **Mechanism of Adjuvant Action at USAMRIID**
  - Ed Stephen, Sonny Crabs, Don Harrington, Jack Reynolds

- **Reovirus Molecular Pathogenesis and Mucosal Immunity at Penn**
  - Don Rubin, Alan Plotner, Mike Kornstein, John Cebra

- **Dendritic Cells and Autoimmunity at Penn**
  - David Gasser, Mike Kornstein, Bert Lisak, Arnold Levinson, Ali Naji, Clyde Barker

- **Respiratory Mucosal Immunity at USAMRIID**

- **Discovery of Chemokines IL-8 and MCP-1 USAMRIID & FCRF**
  - Joost Oppenheim, Kouji Matsushima, Ed Leonard, Chris Larsen, Claus Zachariae

- **Immunoglobulin Hypervariable Regions in Rabbit Lymphatic Tissues**
  - Rose Mage, Peter Weinstein, Richard Pospisil, Enrico Schiaffella, Harold Obiakor, Joe Dasso, D Sehgal

- **Fibroblastic Reticular Cell Conduit**
  - Norm Anderson, Alberto Degrassi, Steve Shaw, Libby Gretz, Eric Kaldjian, Klaus Ebnet, Chris Norbury, Amanda Proudfoot, Kathy Kuehl & Sergei Nedospasov’s group at FCRF.

- **Dendritic Cells, CD45 and Lymphatic Tissue Responses to Ebola or Anthrax**
  - Bill Hall, Peter Jahrling, Kelly Davis, Katherine Brittingham, Gordon Ruthel, Tanya Fuller, Rekha Panchal, Javad Aman Wil Ribot, Tim Hoover, Howard Young & Sina Bavari
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