Canadian Perspective on Lymphatic Research

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Lymphatic system

Anatomy & morphology

- Present at densities similar to those of blood vessels, through most of the body.
- One-way system, not a close-circuit.
- Composed of vessel network, lymph nodes and lymphatic organs.
Lymphatic system

Anatomy & morphology

Initial Lymphatics

Collecting Lymphatic

Lymph Node

Cisterna Chyli

Thoracic Duct

Subclavian Vein
Lymphatic system

Initial lymphatics

- Lymphatic capillaries
- Lacteals in the intestinal villi
- Blind-end tubes
- Endothelial cells only
- Anchoring filaments
- Passive flow
- Fluid and macromolecules enters through openings
Lymphatic system

Lymph nodes

- monitoring center
  - filtration of lymph (remove microorganisms and debris)
  - exposure site to antigens
  - ‘meeting room’ of T-cells to share antigen information

- housing and proliferation site for lymphocytes

- Antigen
- Dendritic Cell
- B-lymphocyte
- T-lymphocyte
- Direction of Lymph Flow
Lymphatic system

Collecting lymphatics - lymphatic pumping

- Valves & vessel segmentation
  - Small units: lymphatic chambers or lymphangions.
  - Delimited by two valves (endothelium leaflet).
  - Succession of individual pumps - “small or primitive hearts”.

- Smooth muscle cells
  - The motor unit.
  - Home of the intrinsic contractile activity.
Lymphatic functions

- Fluid drainage
- Uptake of fluid from tissues and return of fluid back into the circulation (3-4 liters/day) --> lymph formation.
- Return of escaped proteins from interstitial fluid (up to 60%).

- Immune response
- Transportation route for antigens and immune cells
- Sites for interaction between antigens, antigen presenting cells and lymphocytes.
Lymphatic functions

- Fluid drainage
- Fat transport
- Immune response
How is lymph transported?

Extrinsic
- by movement of the body
  - Compression by skeletal muscles
  - Respiratory movements

Intrinsic
- contraction of smooth muscle cells in the vessels

→ LYMPHATIC PUMPING
How is lymph transported?
Lymphatic functions

- Fluid drainage
- Fat transport

Lymphatic functions
- Immune response
Pathogenesis of chronic lymphedema

- Lymphatic dysfunction
- Edema
- Chronic inflammation
- Recurrent infection
- Progressive fibrosis
- Fat deposition

Modified from Rockson. LRB 2013
Central role of inflammation in lymphedema

**Cardinal signs of inflammation**

- Heat
- Redness
- Swelling
- Pain
- Loss of function
Lymphatic system and inflammation

- Any antigen exposure through bacteria and viruses, abrasion or injury, and infection will cause inflammation

  - Production and release of inflammatory mediators
  - Increase in vascular permeability
  - Vasodilation
  
  **Increase intracellular fluid volume/pressure**

  **edema**

  - Recruitment and extravasation of inflammatory cells
  - Transportation of cells, antigens and debris to lymph nodes
  - Lymphocyte activation, differentiation, and proliferation

  **Initiation of immune response**
How to study lymphedema?

Our approach:

• **To understand how the lymphatic system functions to move lymph – what are the mechanisms involved in lymphatic pumping?**
  – use of animal and animal vessels to assess the mechanisms involved in the initiation of the lymphatic contractions.

• **To examine how inflammation affects lymphatic pumping and its ability to drain lymph**
  – use of animal models of inflammation and assessment of lymphatic function in vivo and in vitro
Lymphatic pumping

Guinea pig mesenteric collecting lymphatics

Lymph progression
Lymphatic pumping

Rat mesenteric collecting lymphatics

Applied Pressure: 6 cm H$_2$O

Applied Pressure: 12 cm H$_2$O
Lymphatic pumping

Rat mesenteric collecting lymphatics

- Applied Pressure: 6 cm H$_2$O
- Applied Pressure: 12 cm H$_2$O
Lymphatic pumping

Lymphatic contractions are generated by:

- Electrical events – *action potentials* - occurring in the lymphatic muscle cells.

- Other ion channels, which let ions pass through the cell membrane, are involved. Opening of these channels cause enough electrical current so the action potential can fire… and the contraction occurs.
Regulation of lymphatic vessel pumping
Regulation of lymphatic vessel pumping

.... during inflammation
Lymphatic pumping during intestinal inflammation

Sham-treated

TNBS-treated

Wu et al., Am. J. Physiol, 2006
Lymphatic pumping during intestinal inflammation

A

Sham

TNBS

1 min

Diameter (µm)

B

Diameter (µm)

Macroscopic damage score

0 6-7 8-9 10-12

Sham

TNBS

(5) (5) (7) (15)

C

a

Sham

TNBS

1 min

Membrane potential (mV)

b

Membrane potential (mV)

Control Sham TNBS

(13) (19) (22)

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Wu et al., 2006 Physiol, 2013
Lymphatic pumping during inflammation

Effect of LPS on lymphatic contractile function

![Graph showing effect of LPS on lymphatic contractile function.](image)
Mechanisms of inflammation-induced lymphatic vessel contractile dysfunction
Lymphatic contractile function during TNF-α treatment

**Sham**

**TNF-α (1 ng/ml)**

**TNF-α (10 ng/ml)**

- **Contraction frequency (relative to sham)**
  - ![Graph](image)
  - **[TNF-α] (ng/ml)**
  - 0, 0.1, 1, 10
  - (*) P < 0.05, (**) P < 0.01; repeated measures of ANOVA/Dunnett’s

- **Contraction amplitude (relative to sham)**
  - ![Graph](image)
  - **[TNF-α] (ng/ml)**
  - 0, 0.1, 1, 10

- **Tone index (relative to sham)**
  - ![Graph](image)
  - **[TNF-α] (ng/ml)**
  - 0, 0.1, 1, 10

Chen et al. in preparation
Lymphatic contractile function during TNF-α treatment

A

Sham

TNF-α

TNF-α + 1400W

Diameter (µm)

30 s

B

Contraction frequency (relative to sham)

Sham

1400W

TNF-α

TNF-α + 1400W

( *P<0.05 vs own sham, paired Student t-test )

Chen et al. in preparation
Lymphatic contractile function during TNF-α treatment

**TNF-α causes NFκB activation in lymphatic vessels**

Sham  

TNFα-treated

Chen et al. in preparation

Calvin, Phoebe and Joan  
Snyder Institute  
for Chronic Diseases
Summary

- Lymphatic pumping is an activity intrinsic to the lymphatic vessel.
- It can be modulated by pressure of the lymph inside the vessel.
- ... and by mediators released by the lymphatic endothelium.
- Some of these mediators (nitric oxide and prostaglandins) inhibit pumping.
- These molecules also mediate inflammation.
- Indeed, inflammation modulates lymphatic pumping.
- In our hands inflammation leads to inhibition of pumping.
- ... and some of these effect are mediated by the same molecules nitric oxide and prostaglandins.
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UNIVERSITY OF CALGARY
INFLAMMATION RESEARCH NETWORK
Snyder Institute for Chronic Diseases
What’s going on in Calgary?

Lymphedema Research and Education Program

The Dianne and Irving Kipnes Lymphedema Imaging Suite
lymphatic contraction in the leg of a normal mouse

Valve    Lymphangion

250µm

Liao et al. PNAS 2011
Lymphatic imaging

Active lymph propulsion from arm toward axillary lymph node during MLD on a control arm.

Active lymph propulsion before and during MLD on a control foot

Tan I-Chih, et al. (2010)
What’s going on in Alberta?

Alberta Lymphedema Network (ALNET)

- Better understand the action of the lymphatic system in health and disease using
  - Animal models
  - Advanced imaging technologies
  - Novel bioassays
  - Nanotechnology
  - Metabolomics
- Translate research findings to the clinic through
  - Improved imaging
  - Diagnostics
  - Treatments
- Establish a centre in lymphatic research and lymphedema treatments
- Train future clinicians and researchers interested in lymphatic diseases
- Improve education about the lymphatic system and lymphatic diseases via a training program open to interested parties in Alberta.
Thank you!

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