

# Canadian Perspective on Lymphatic Research

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# Disclosure slide

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I have no financial or otherwise conflict of interest to disclose.

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Crohn's and Colitis  
Foundation of Canada

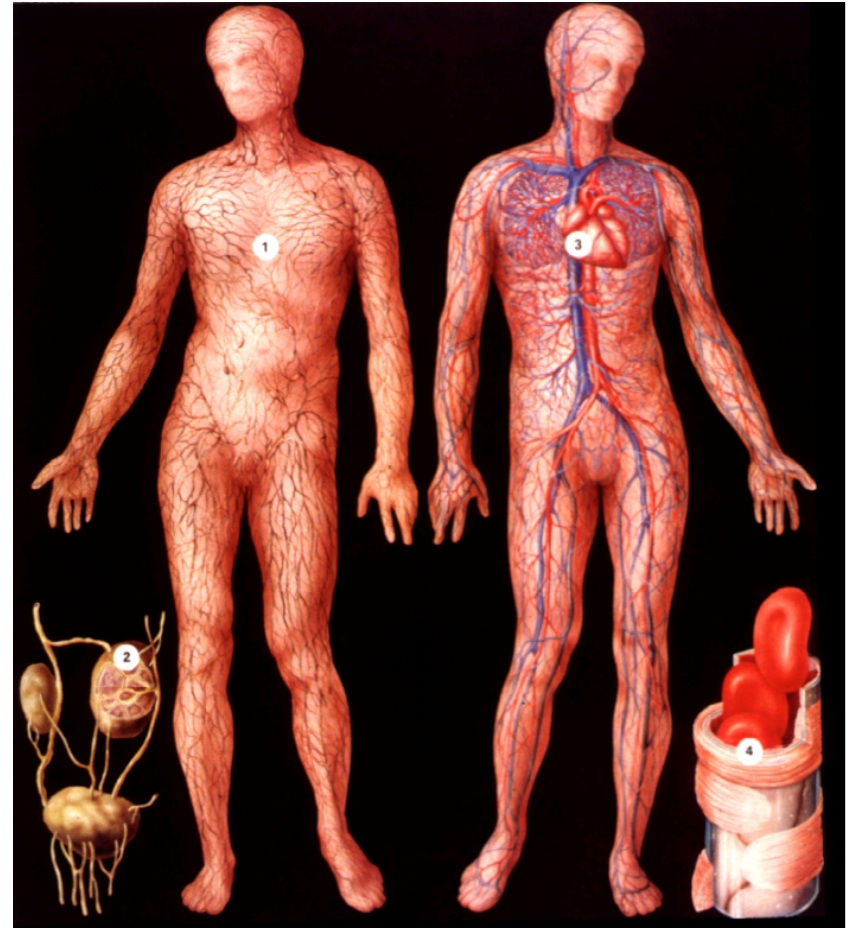
Fondation canadienne des  
maladies inflammatoires  
de l'intestin



# Lymphatic system

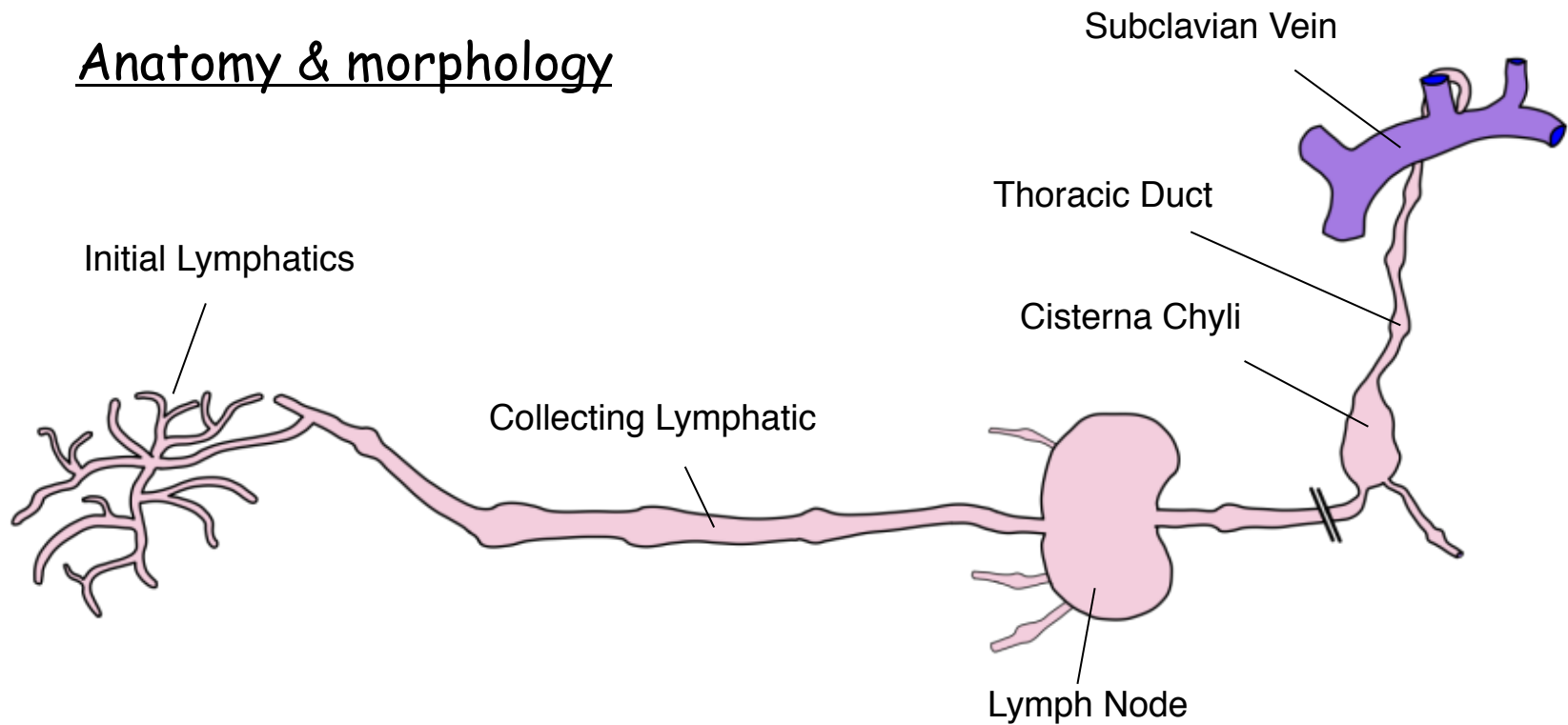
## Anatomy & morphology

- 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

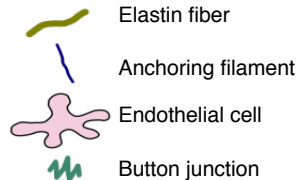
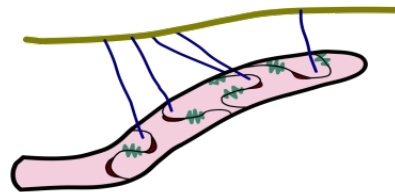
## Anatomy & morphology





# Lymphatic system

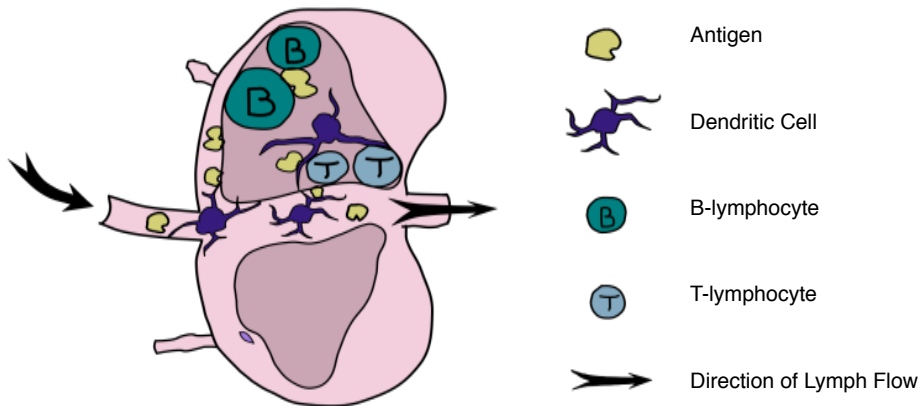
## Initial lymphatics



- = 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

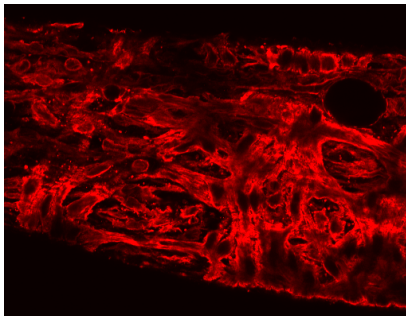
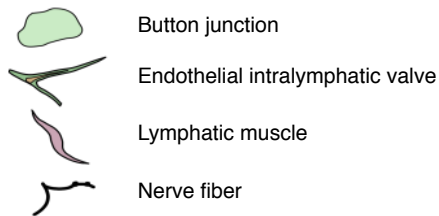
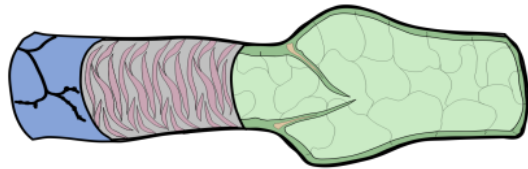
## Lymph nodes



- 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

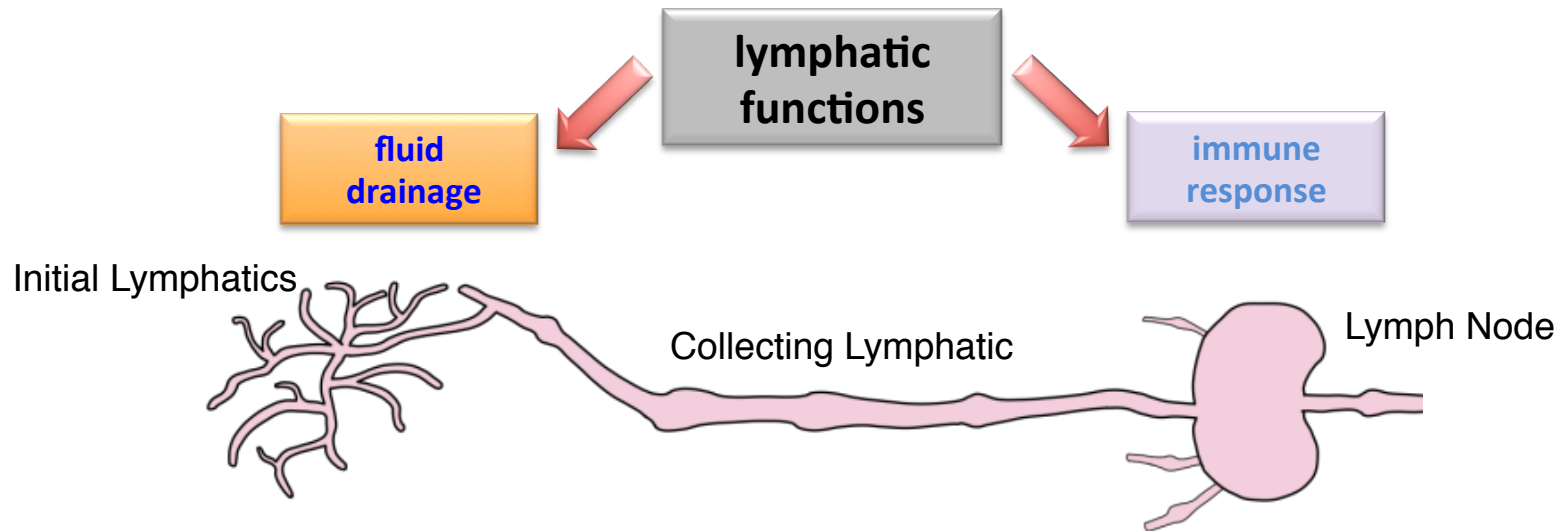
# Lymphatic system

## 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.

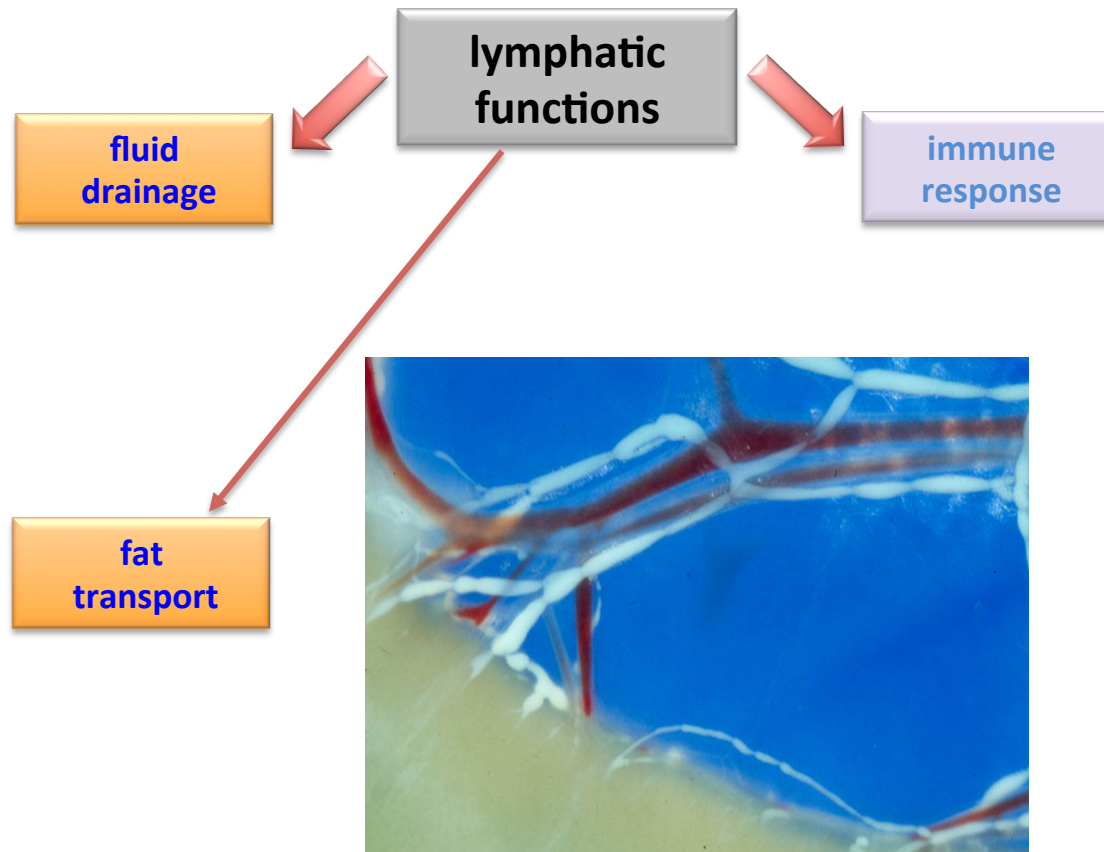
# Lymphatic functions



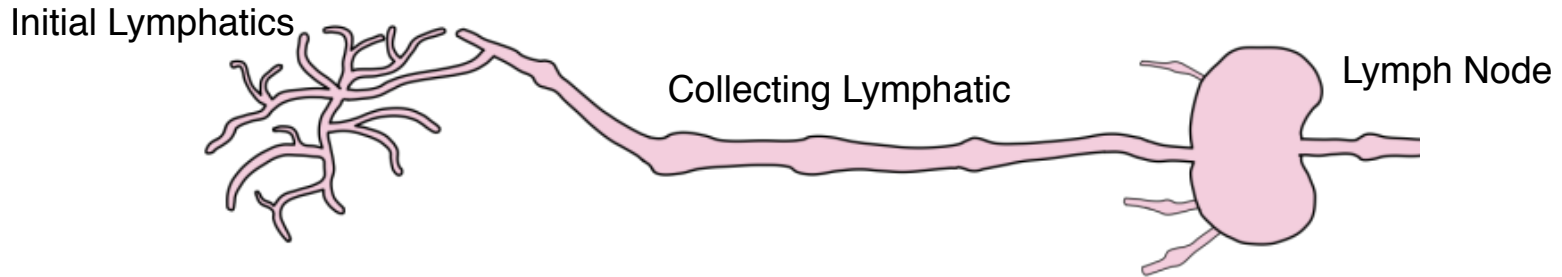
- ✓ Uptake of fluid from tissues and return of fluid back into the circulation (3-4 liters/day) --> lymph formation.
- ✓ Return of escaped proteins from interstitial fluid (up to 60%).

- ✓ Transportation route for antigens and immune cells
- ✓ Sites for interaction between antigens, antigen presenting cells and lymphocytes.

# Lymphatic functions



# How is Lymph transported ?



Passive flow of  
fluid dependent  
on fluid pressure

**Extrinsic** → by movement of the body

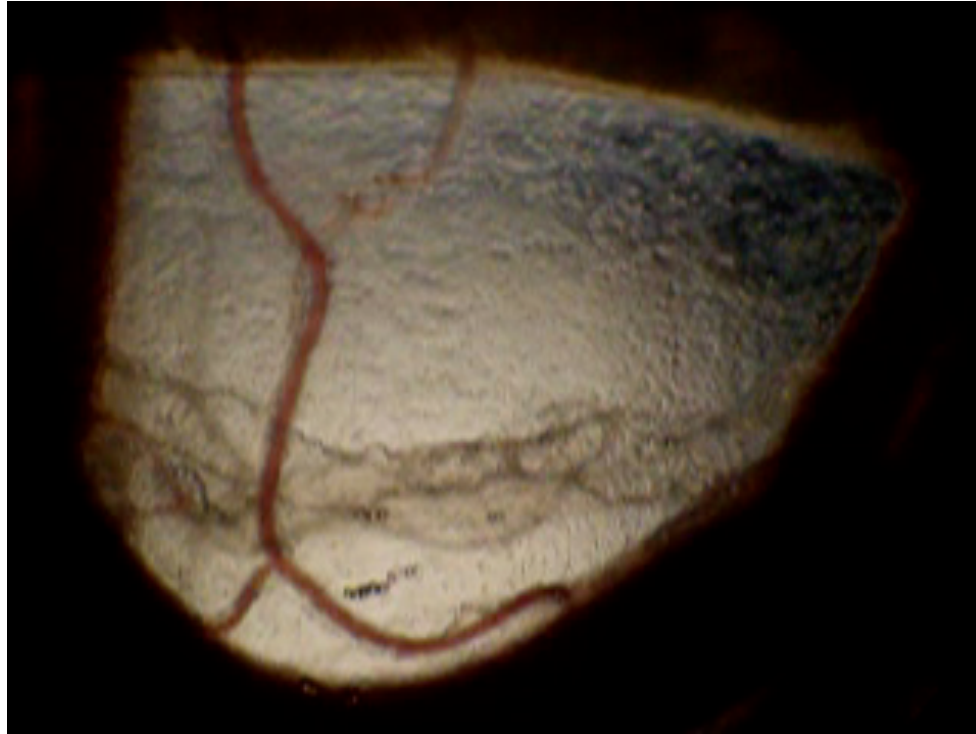
- Compression by skeletal muscles
- Respiratory movements

**Intrinsic** → contraction of smooth muscle  
cells in the vessels

→ **LYMPHATIC PUMPING**

# How is Lymph transported ?

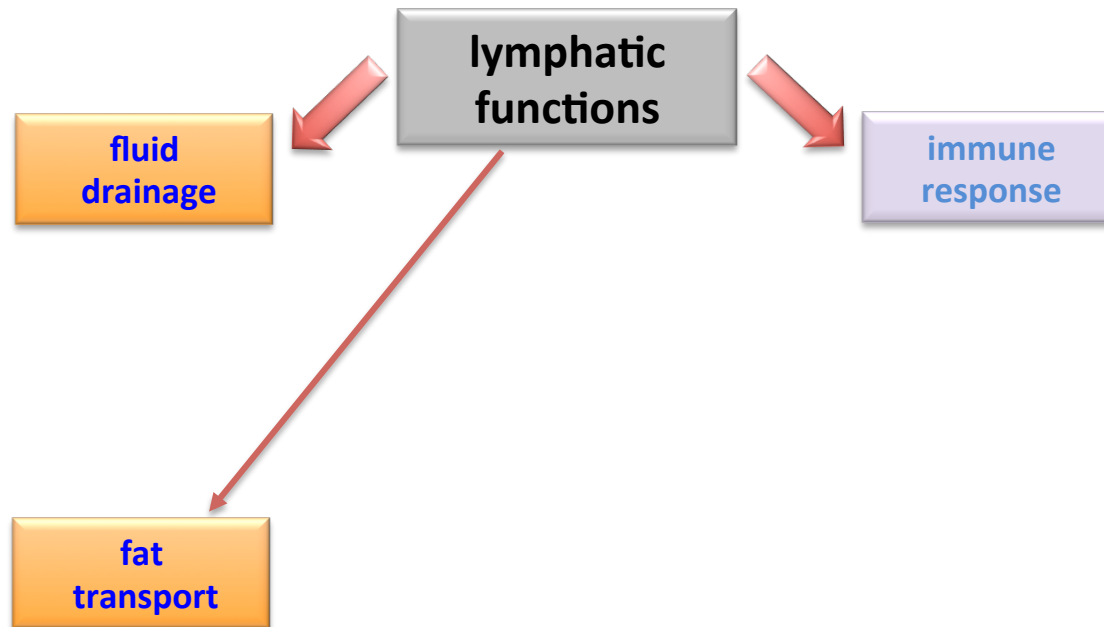
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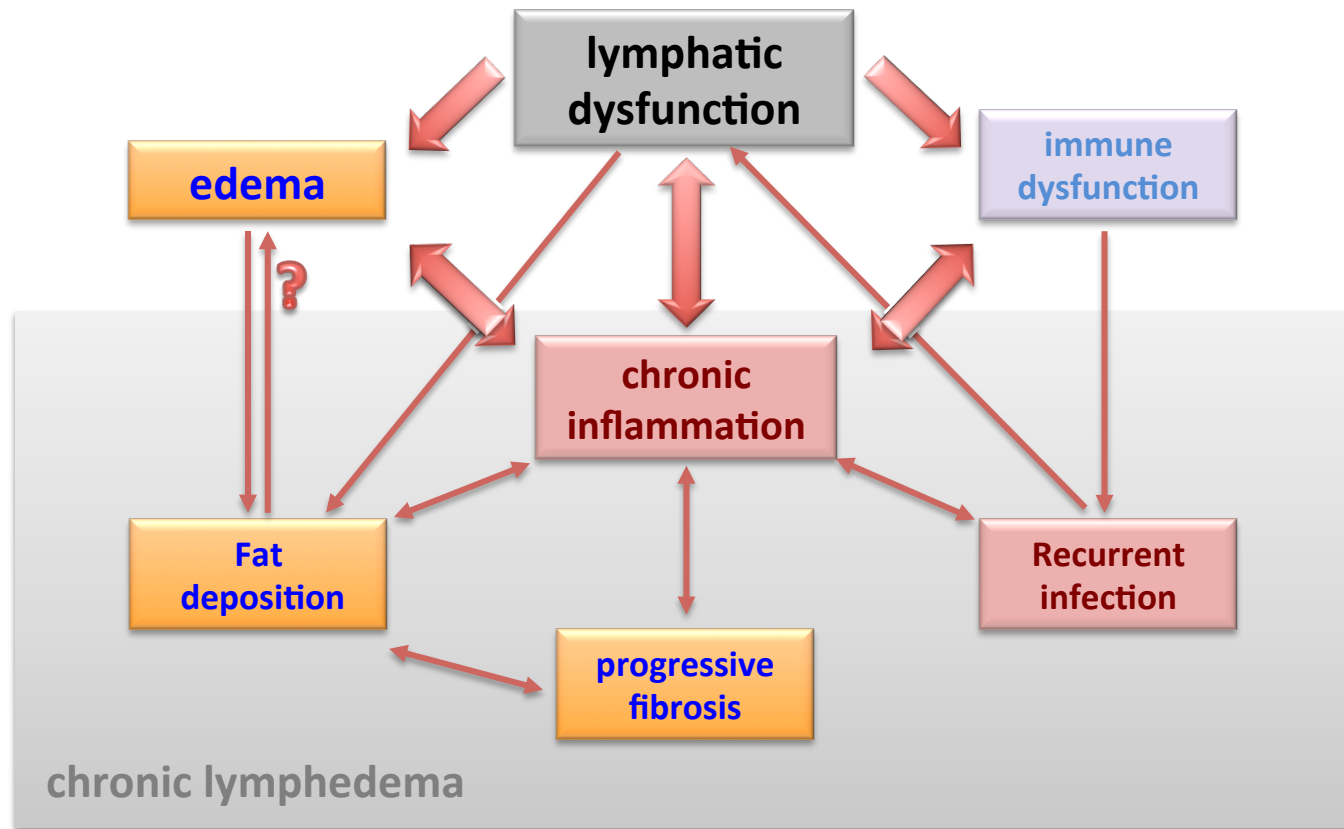


# Lymphatic functions

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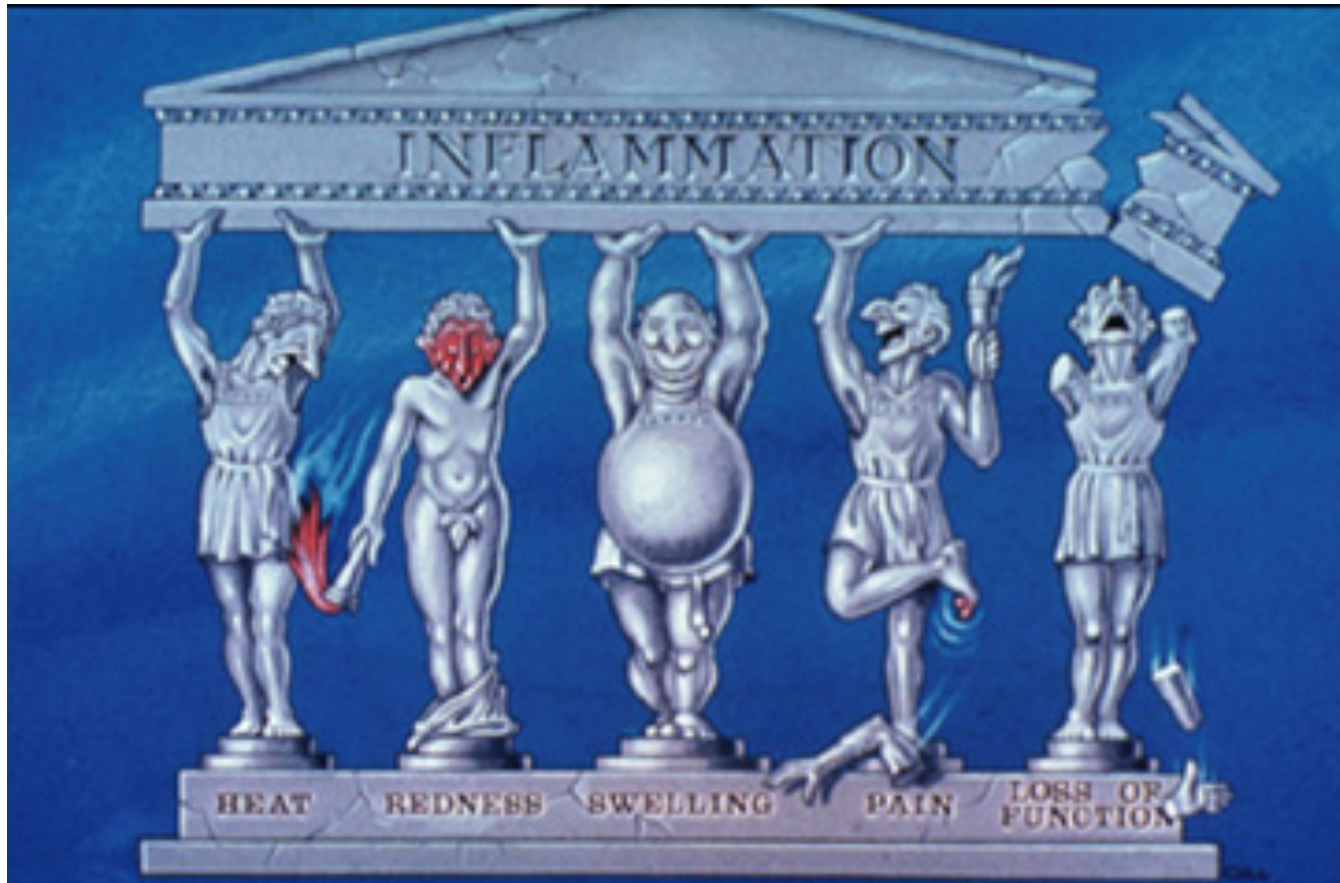
# Pathogenesis of chronic Lymphedema



Modified from Rockson. LRB 2013

# Central role of inflammation in Lymphedema

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*Cardinal signs of inflammation*

# 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 intracellular fluid volume/pressure**

- Increase in vascular permeability

=

- Vasodilation

**edema**

- Recruitment and extravasation of inflammatory cells

- Transportation of cells, antigens and debris to lymph nodes

**Initiation of immune response**

- Lymphocyte activation, differentiation, and proliferation

# How to study Lymphedema ?

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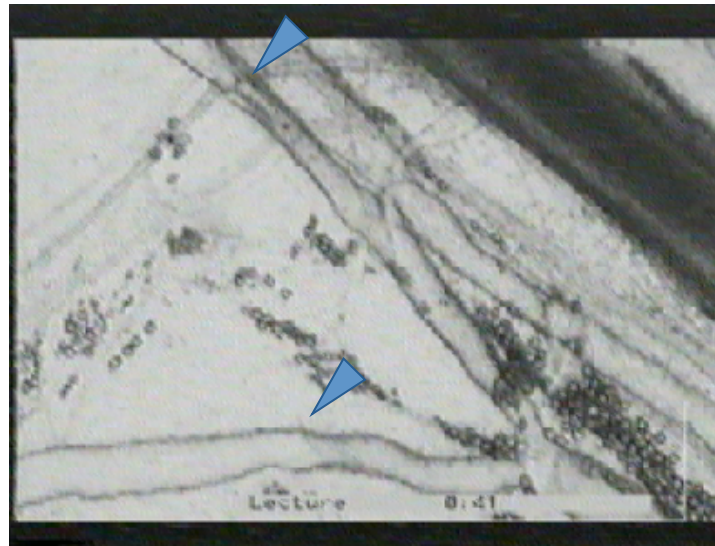
## *Our approach:*

- *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

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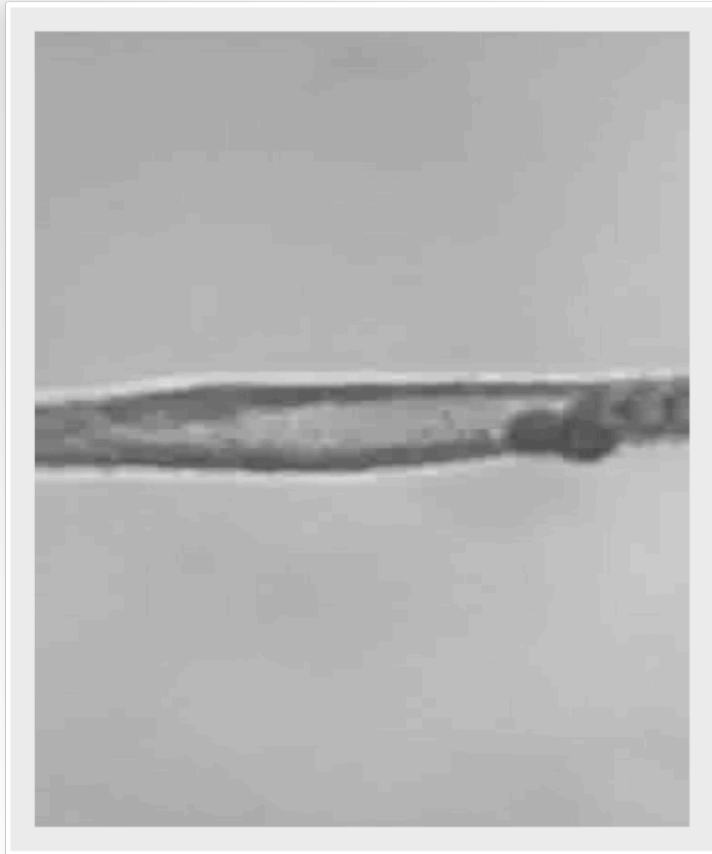
## Guinea pig mesenteric collecting lymphatics



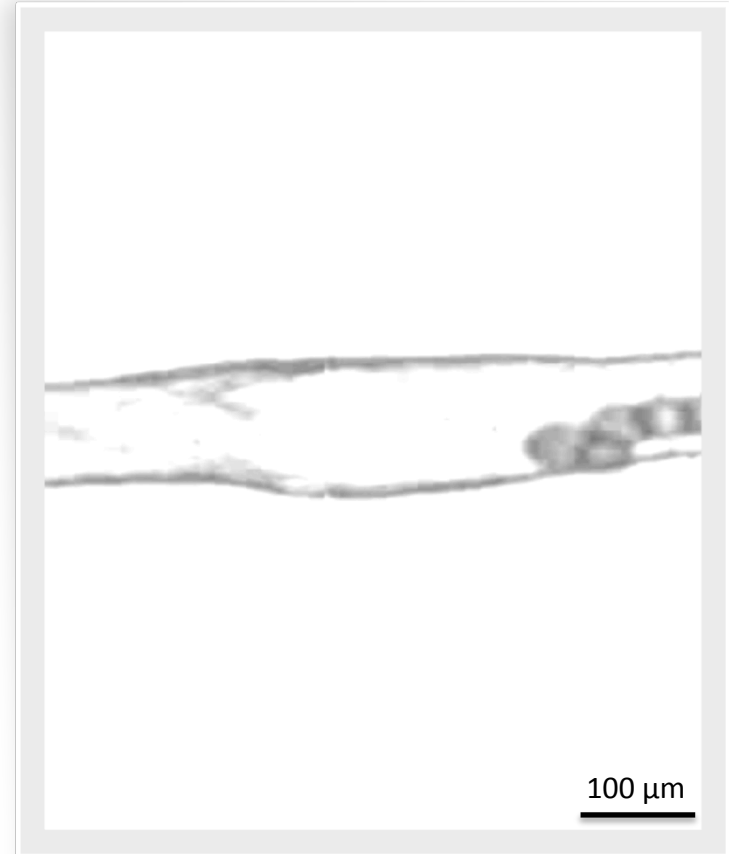
⇒ Lymph progression

# Lymphatic pumping

## Rat mesenteric collecting lymphatics



Applied Pressure: 6 cm H<sub>2</sub>O

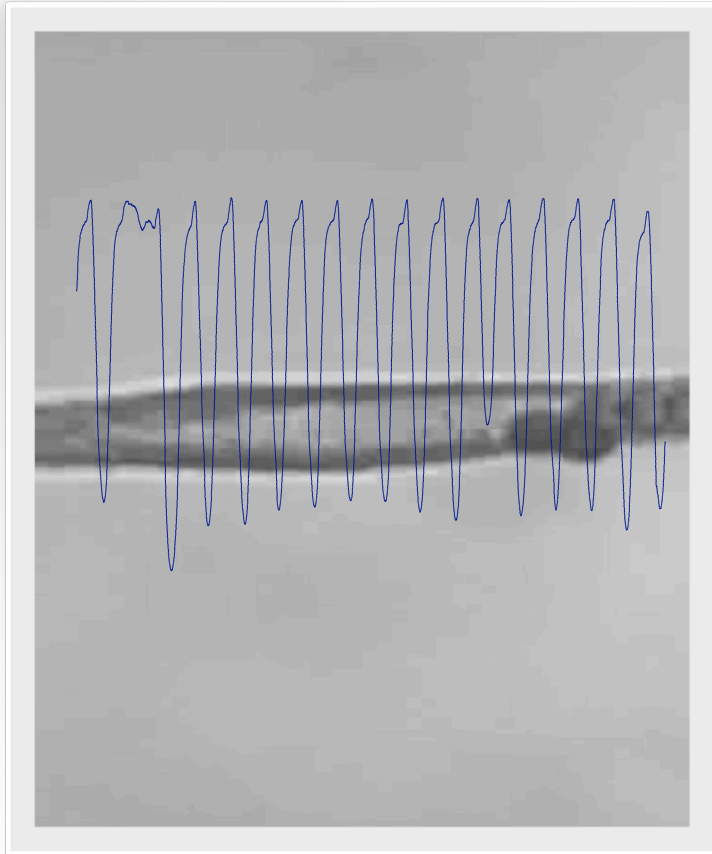


Applied Pressure: 12 cm H<sub>2</sub>O

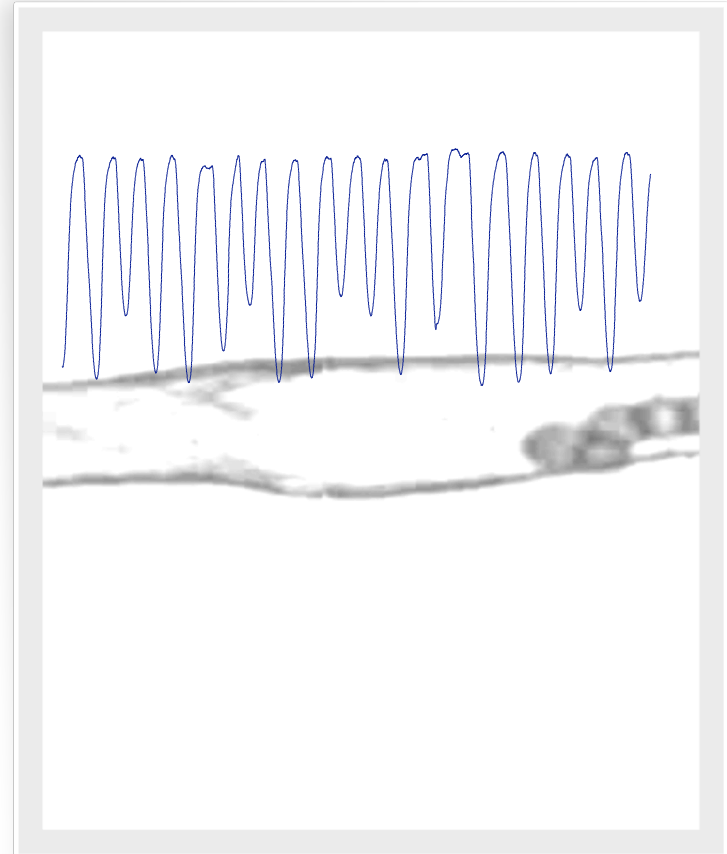


# Lymphatic pumping

## Rat mesenteric collecting lymphatics



Applied Pressure: 6 cm H<sub>2</sub>O

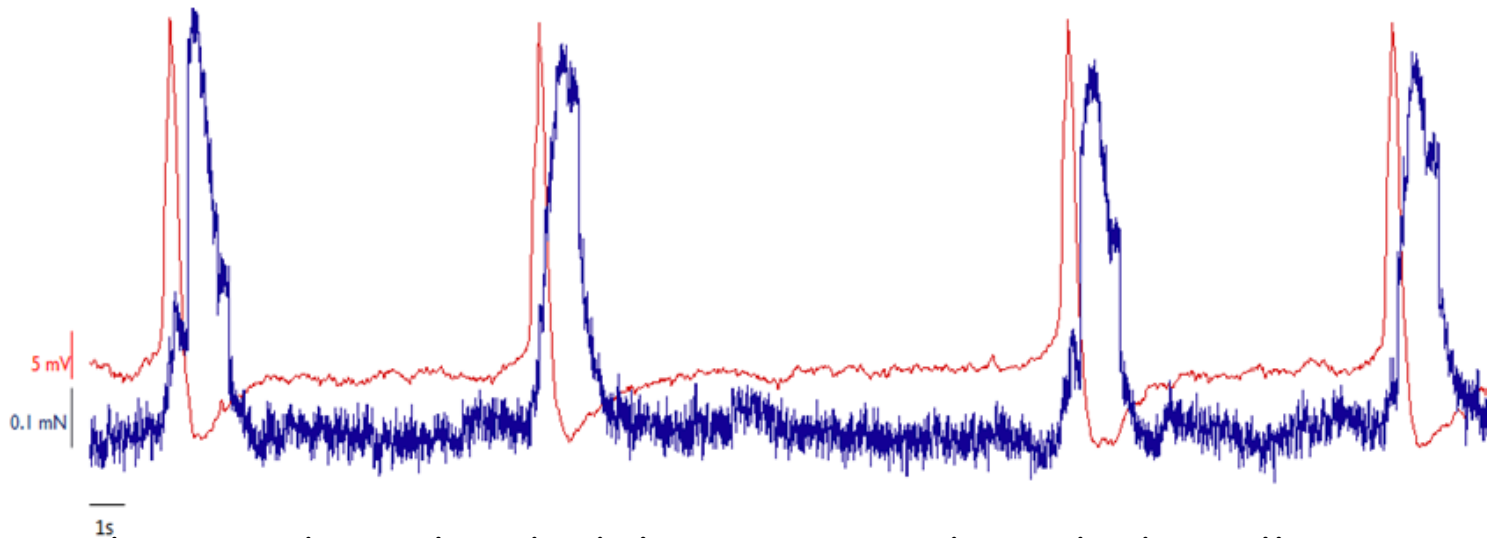


Applied Pressure: 12 cm H<sub>2</sub>O

# Lymphatic pumping

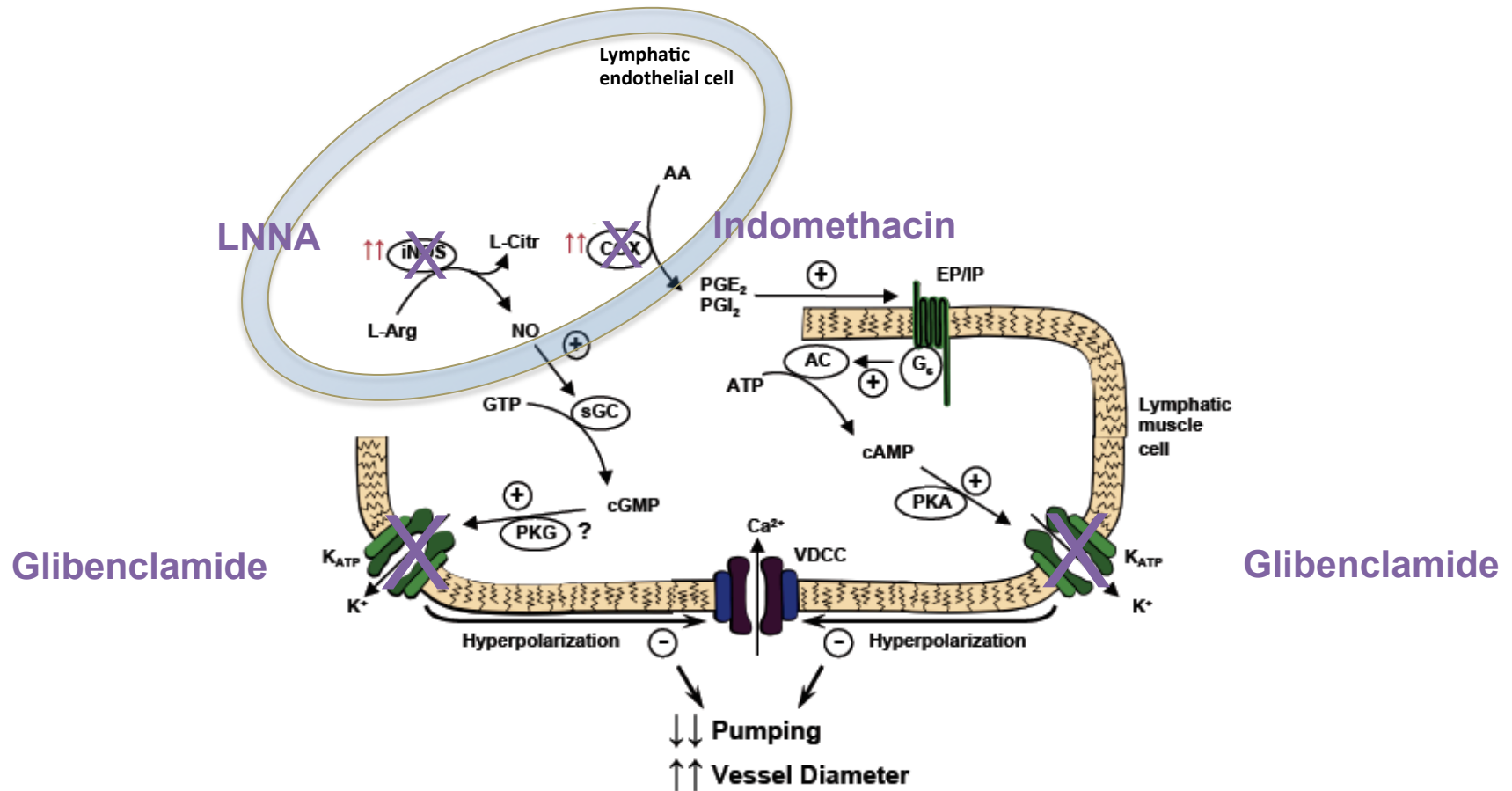
## Lymphatic contractions are generated by:

- 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



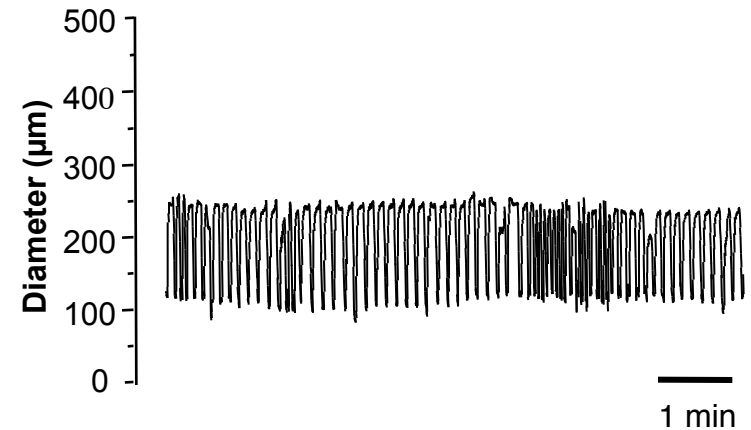
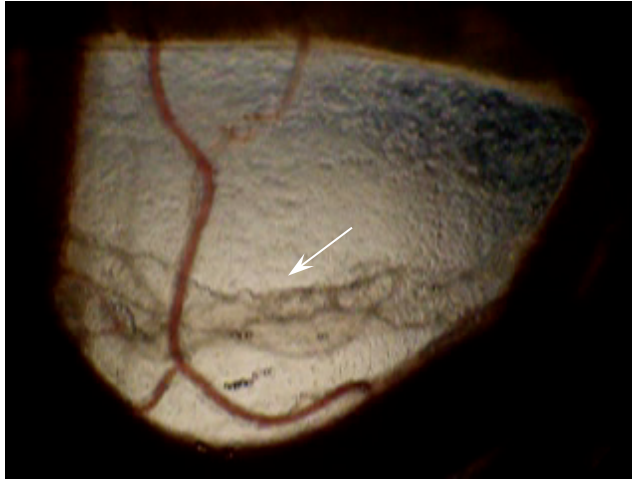
# Regulation of Lymphatic vessel pumping

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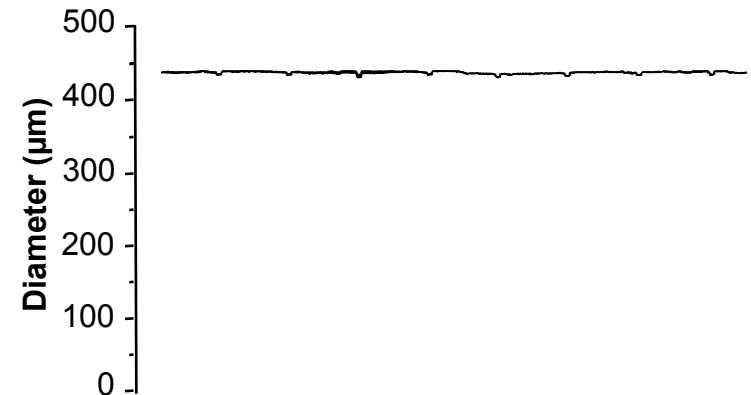
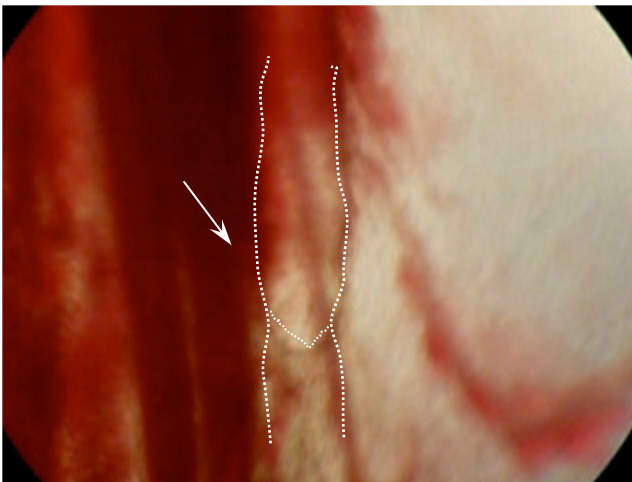
.... during inflammation

# Lymphatic pumping during intestinal inflammation

Sham-treated



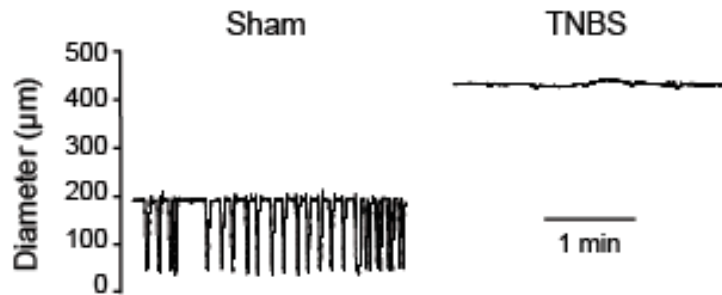
TNBS-treated



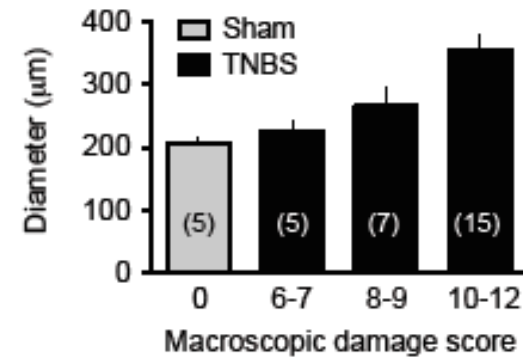
Wu *et al.*, Am. J. Physiol, 2006

# Lymphatic pumping during intestinal inflammation

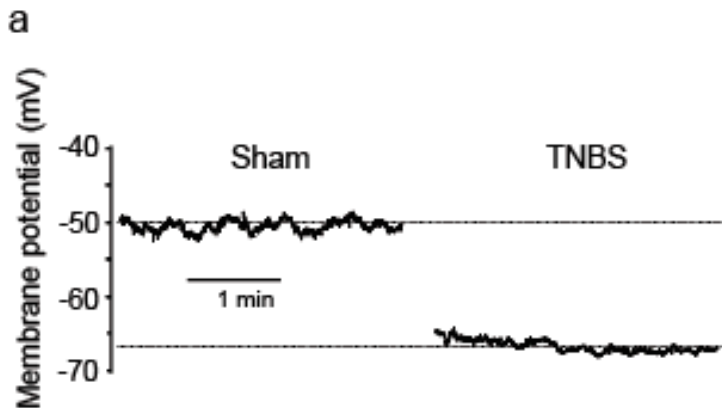
A



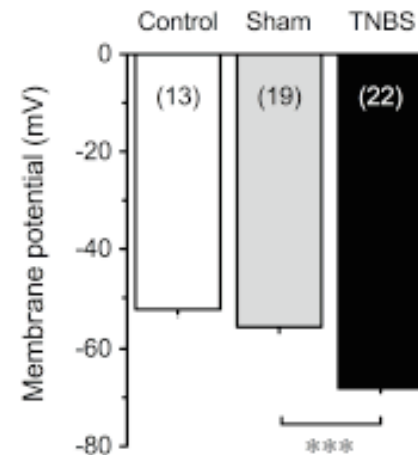
B



C



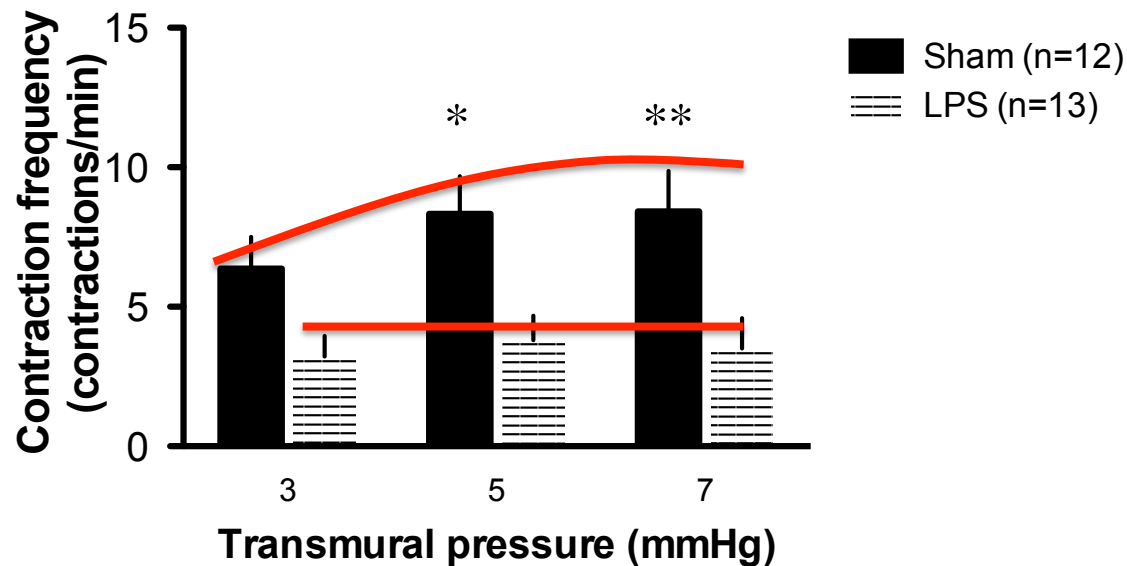
b



Wu *et al.*, 2006 *Physiol*, 2013

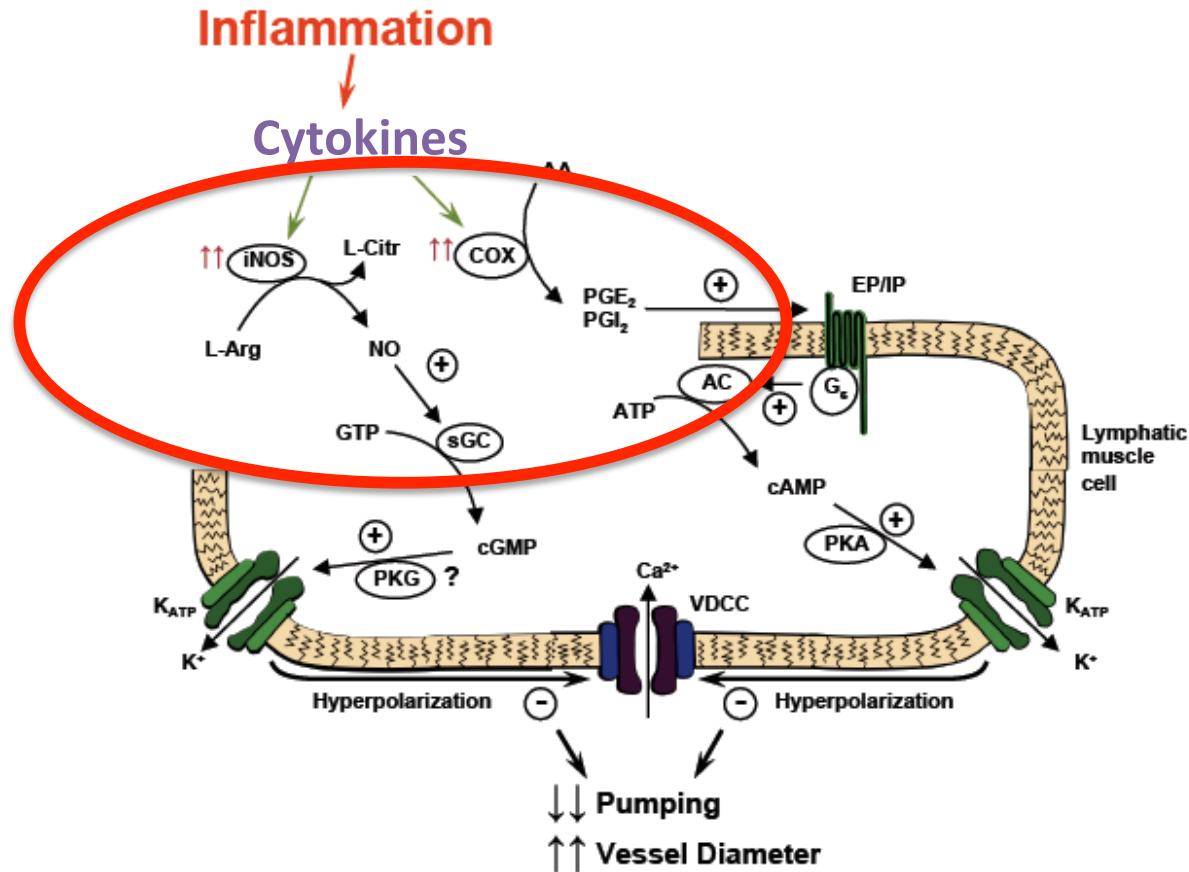
# Lymphatic pumping during inflammation

## Effect of LPS on Lymphatic contractile function

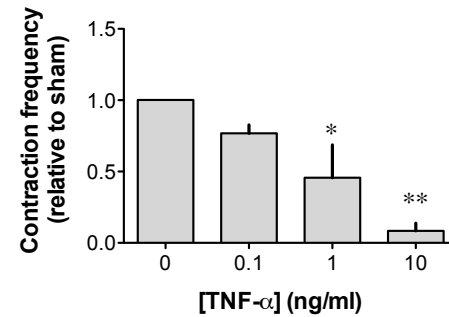
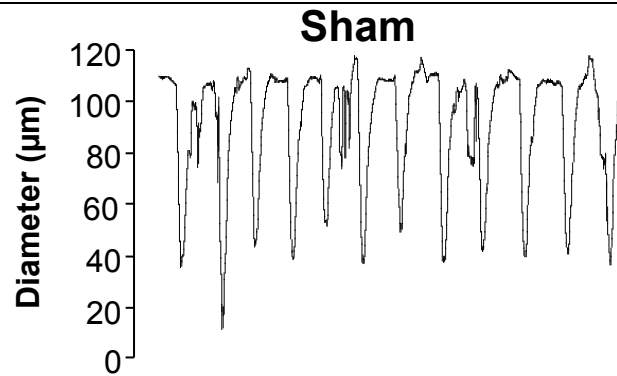




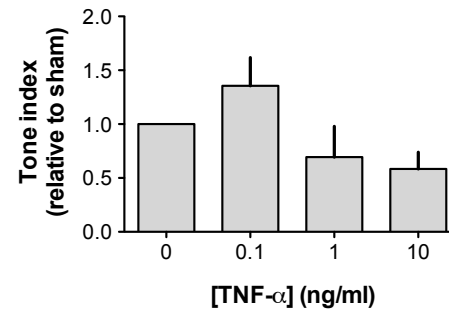
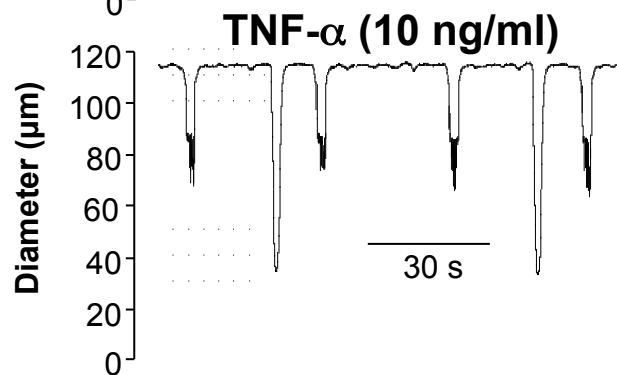
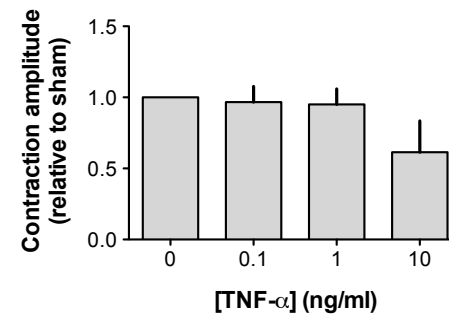
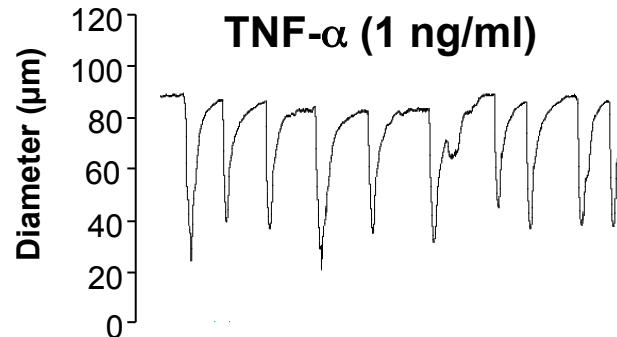
# Mechanisms of inflammation-induced lymphatic vessel contractile dysfunction



# Lymphatic contractile function during TNF- $\alpha$ treatment

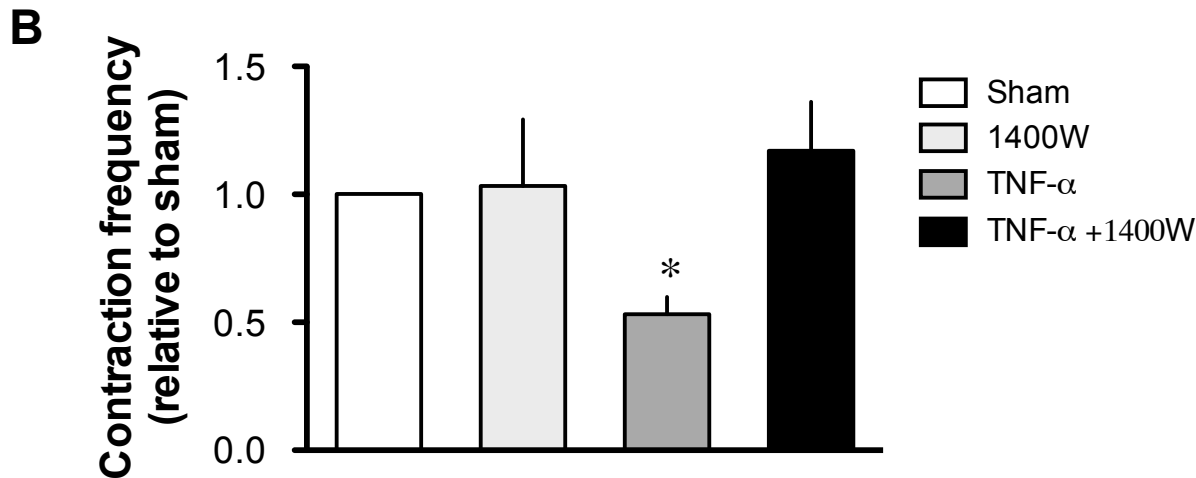
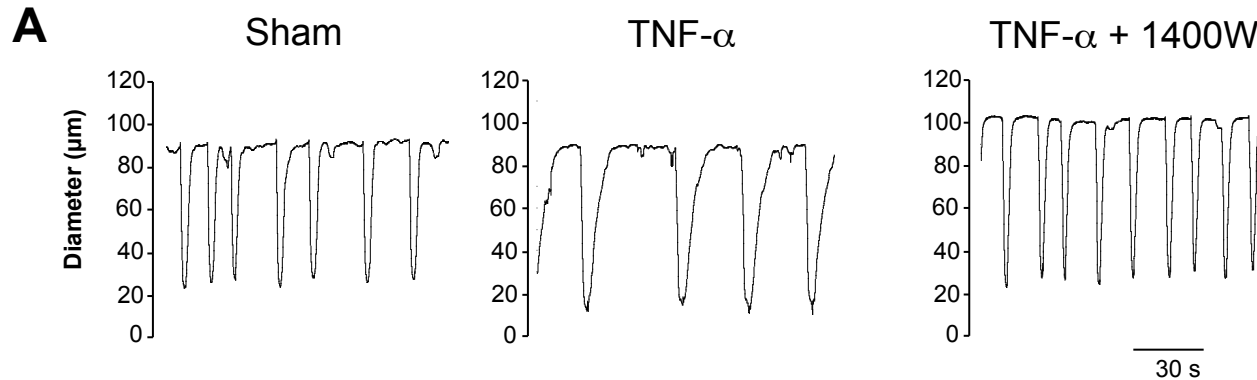


(\* $P < 0.05$ , \*\* $P < 0.01$ ; repeated measures of ANOVA/Dunnett's)



Chen et al. in preparation

# Lymphatic contractile function during TNF- $\alpha$ treatment

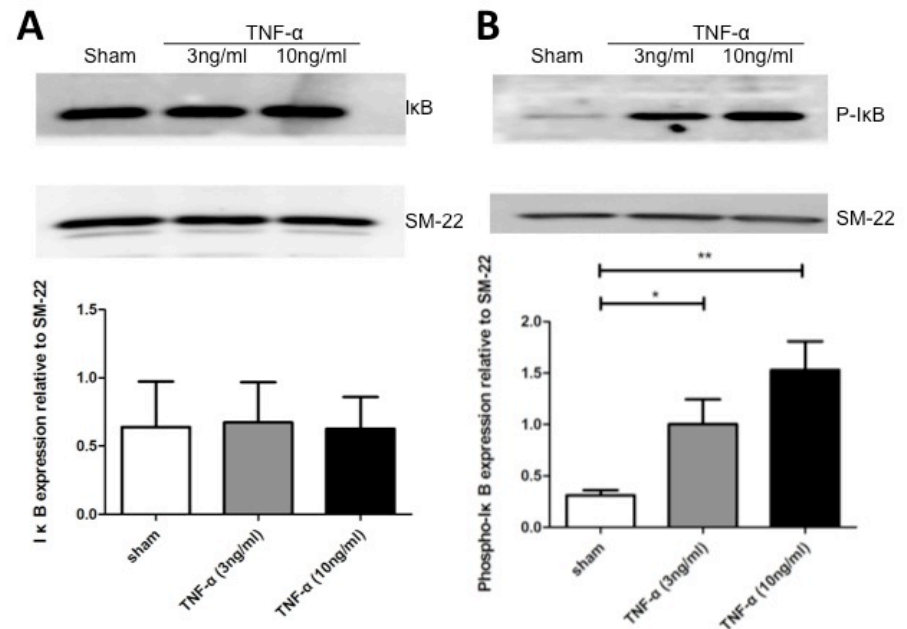
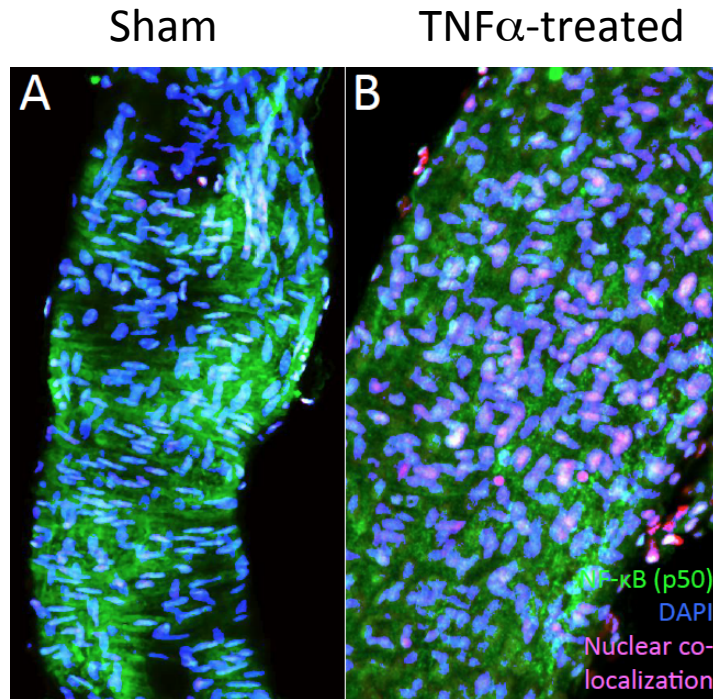


( \* $P < 0.05$  vs own sham, paired Student t-test )

Chen et al. in preparation

# Lymphatic contractile function during TNF- $\alpha$ treatment

*TNF- $\alpha$  causes NF $\kappa$ B activation in lymphatic vessels*



Chen et al. in preparation

# Summary

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- 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**

# Acknowledgements

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Lab members (past and present)

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*Stewart Lee*

*Bernhard Nausch*

*Simon Roizes*

*Jenny Khakh*

*Yingxuan Chen*

*Catharine Bowman*

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*Rusha Barua*

*Xinxing Gao*

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TAMUHSC, Temple, TX

Walter Cromer

Wei Wang

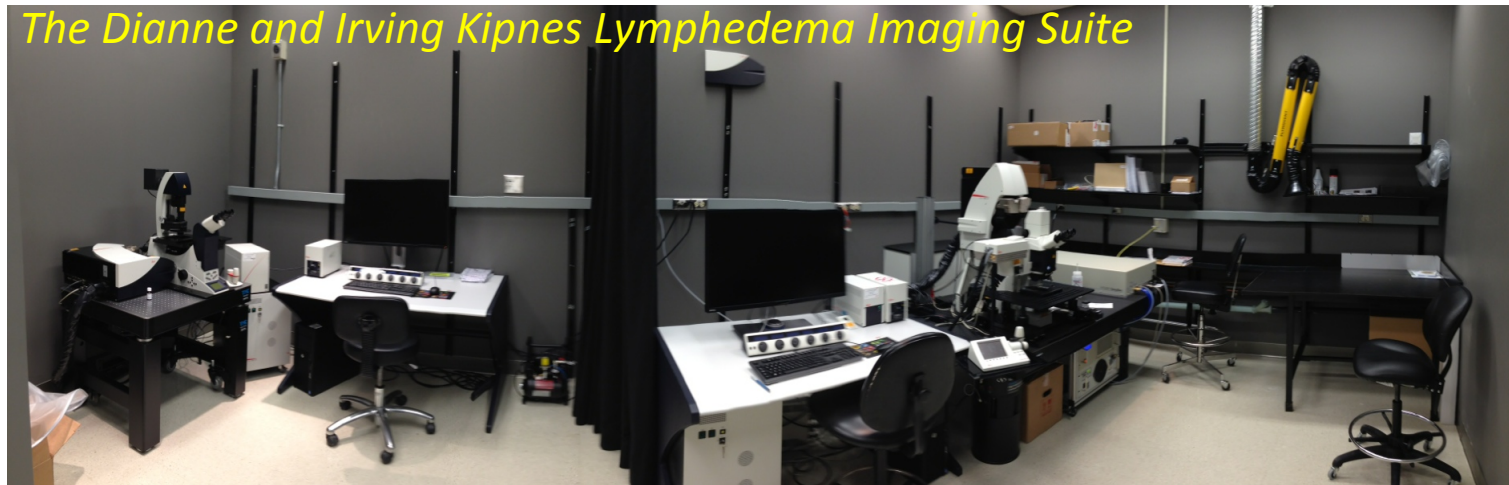
Sanjukta Chakraborty

# What's going on in Calgary?

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## *Lymphedema Research and Education Program*

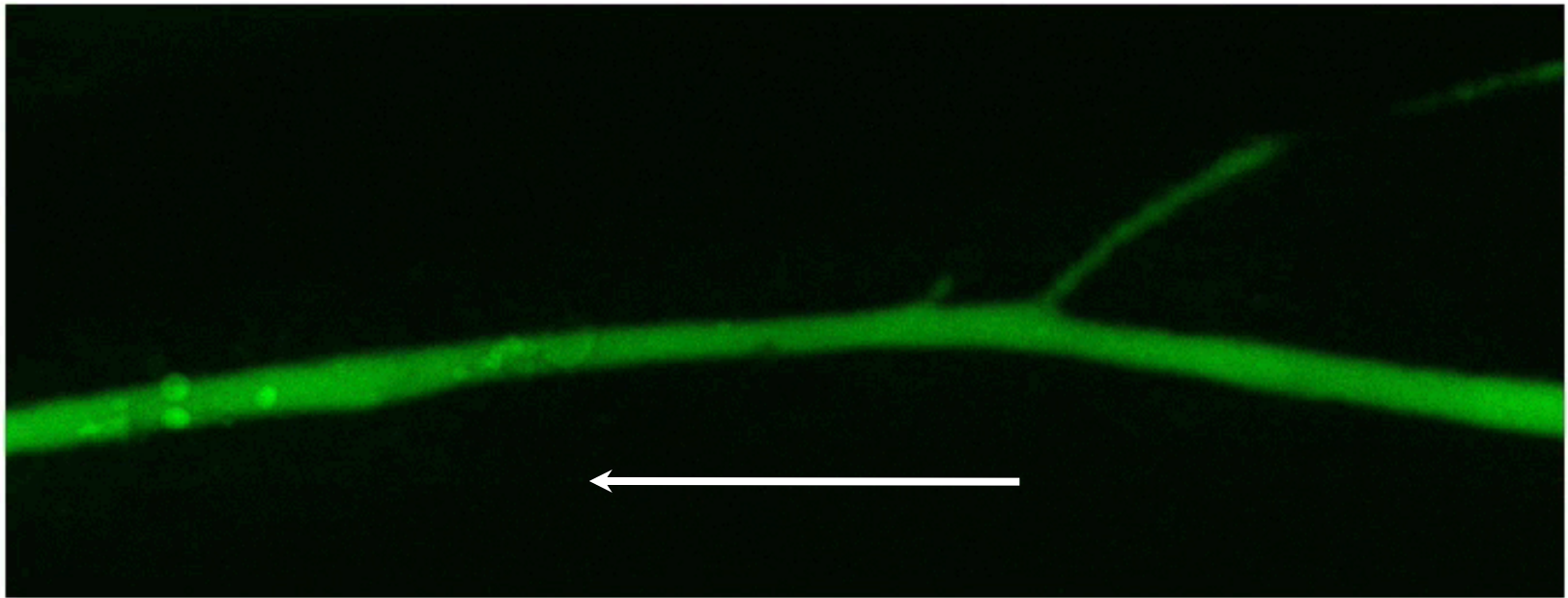
*The Dianne and Irving Kipnes Lymphedema Imaging Suite*





# Lymphatic contraction in the leg of a normal mouse

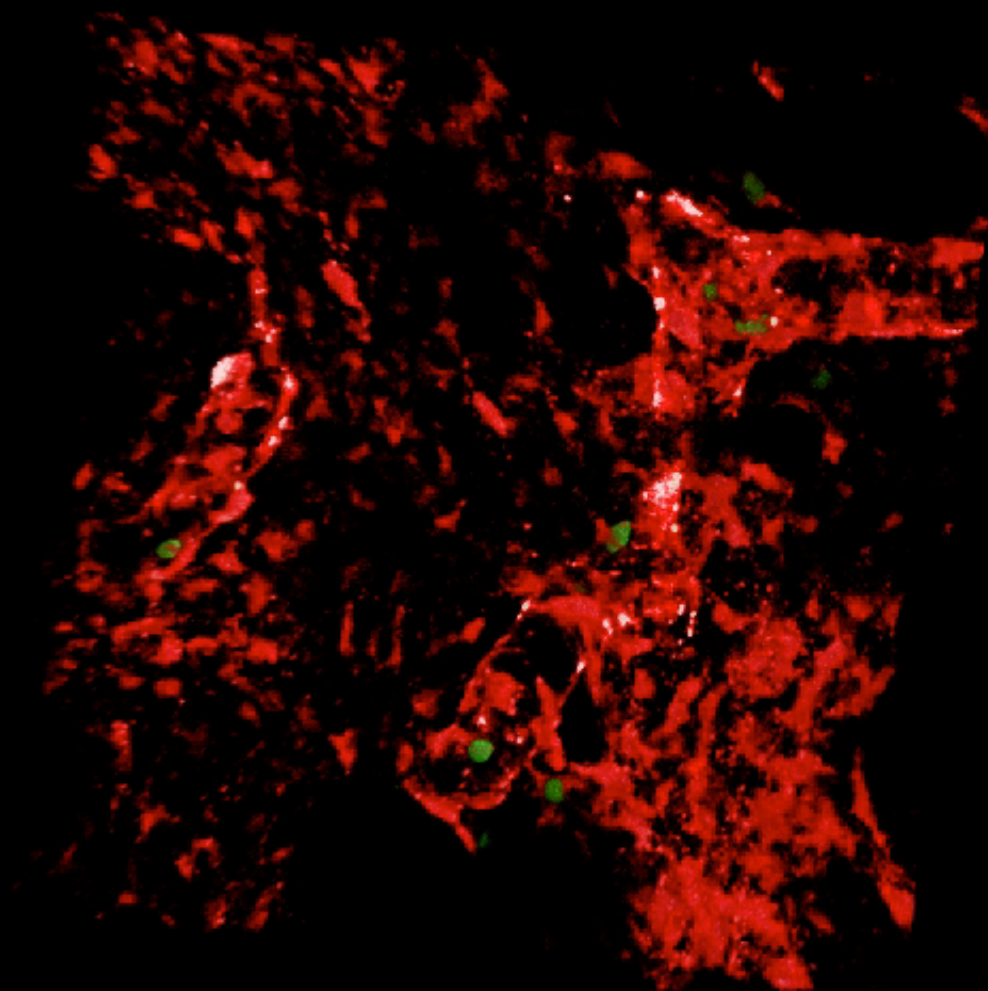
0 sec



Valve

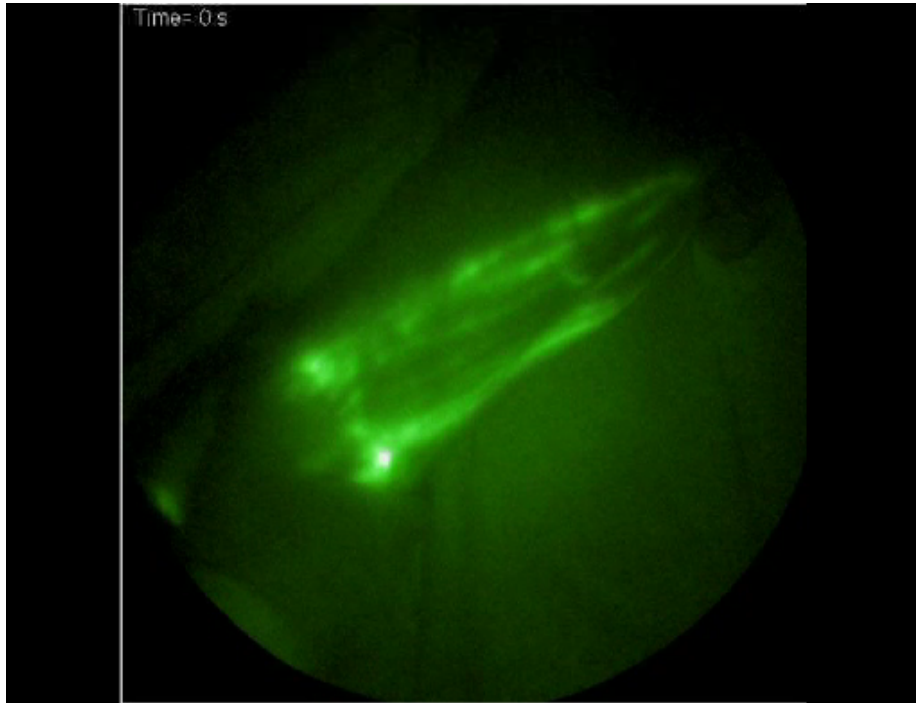
Lymphangion

250μm

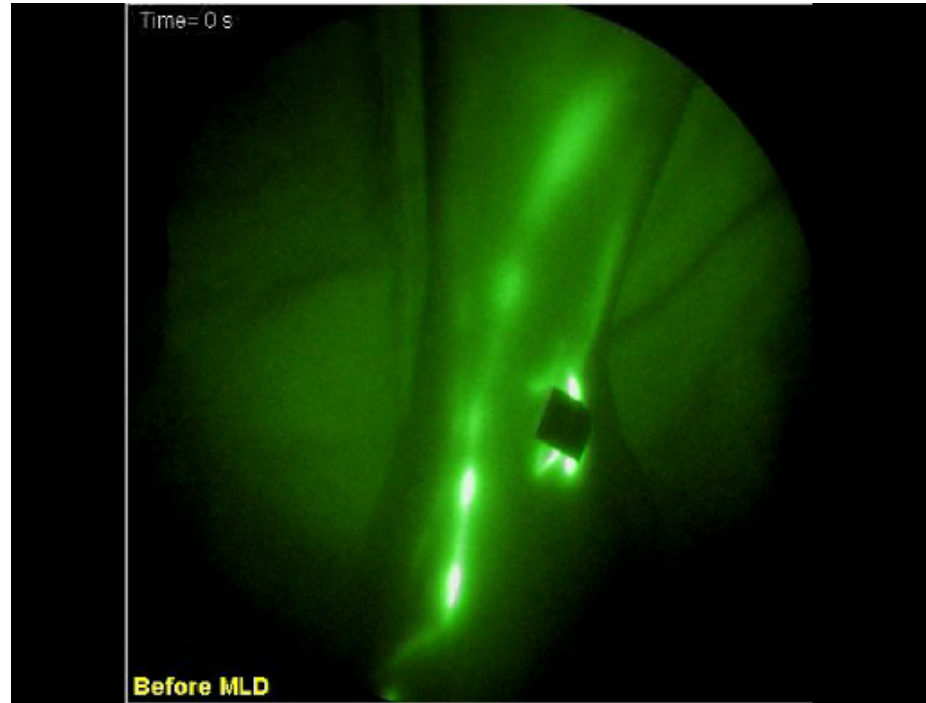


50  $\mu\text{m}$

# 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?

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## *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!

