Lymphatic Endothelial and Smooth Muscle Cell Contribution to Lymphatic Vasoreactivity and Fluid Clearance

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Lymphatic Function - Transport

- The lymphatic system has critical roles in fluid & protein homeostasis, edema resolution, lipid uptake, and immune cell trafficking.
- All of these tasks REQUIRE the movement of substances from the tissue spaces, into and through the network of lymphatics, to the lymph nodes & finally into the venous blood.
- Thus the PRINCIPAL FUNCTION of the system of lymphatic vessels is TRANSPORT.

Hydrodynamics of Lymph Transport

- Contrary to popular belief, in most tissues and instances, the fluid pressure gradients along the lymphatic network from the initial lymphatics to the central veins normally opposes “passive drainage”.

Mean pressures in different vascular compartments in the dog.

- Femoral lymphatic: 0.7 cm H₂O
- Cardiac lymphatic: 3.8 cm H₂O
- Mesenteric lymphatic: 4.7 cm H₂O
- Hepatic lymphatic: 4.5 cm H₂O
- Thoracic duct: 6.6 cm H₂O
- Right lymphatic duct: 2.8 cm H₂O
- Jugular vein: 7.6 cm H₂O

Confounding Influence of Gravity on Lymph Pressure and Transport in Man

- The potential hydrostatic pressure gradient from head to the great veins is ~ +30 cm H₂O - favors passive lymph flow.
- The potential hydrostatic pressure gradient from foot lymphatics to great veins of the neck ~ -150 cm H₂O - opposes passive lymph flow.
Overcoming the Troublesome Physics of Lymph Transport

- Since humans and other mammals do not have the specialized lymphatic hearts that lower vertebrates do, they use a series of pumps and valves to overcome these pressure gradient and move lymph from the tissue spaces to the veins.

- Without the actions of the lymph pumps and valves, the proper function of the lymphatic system, i.e. transport, cannot occur.

- Thus to study the function/dysfunction of the lymphatic system, one must always consider the functions of the pumps and valves.

Valves of the Rat Mesenteric Lymphatic

- Rat mesenteric lymphatic (~100 μm resting diameter) was isolated, cannulated, pressurized and labeled with the fluorescent dye Cell-Tracker Green for live cell imaging. The lymphatic was imaged 3D using multiphoton techniques and the vessel reconstructed.

  - Note the unique valve structure, shape and size and how this may affect lymph hydrodynamics!

Multiphoton Imaging of Lymphatic with Valve.

- Minimize lymph backflow.

- Reduce gravitational influence by breaking the hydrostatic column.

- Allow sequential buildup (lymphangion by lymphangion) of lymph pressure to overcome opposing pressure gradients.

- Anatomical site of shear-sensor/nitric oxide production/contraction regulator?

Functional Characteristics of the Lymphatic Valves
Lymph Pumps: Provide Energy to Overcome Pressure Gradients

- Extrinsic pumps - relies on the cyclical compression and expansion of lymphatics by surrounding extrinsic tissue forces, e.g. heart, skeletal muscle, gut wall and lungs.

- Intrinsic pumps - relies on the intrinsic rapid/phasic contractions of the lymphatic muscle, e.g. skin, connective tissues, peritoneum, gastro-intestinal tract, kidney.

Modulation of the Intrinsic Lymph Pump by Physical Factors

- Increased lymph pressure/stretch of the muscular lymphatics activates the intrinsic lymph pump.

- Increased lymph flow/shear in the muscular lymphatics can activate or inhibit the intrinsic lymph pump depending on the pattern and magnitude of the shear.

Activation of Lymphatic Pumping by Stretch.

Low pressure 1 cm H$_2$O - High pressure 5 cm H$_2$O

Inhibition of Lymphatic Pumping by Imposed High/Steady Shear.

No imposed flow - High imposed flow
These Unique Lymphatic Contractile Characteristics Rely on the Interaction of Muscle and Endothelium

Mechanisms of Imposed Flow-Dependent Inhibition of Lymphatic Contractile Activity

Effects of flow-steady flow are blocked by:
- Endothelial inhibition
- NO blockade
- Guanylate cyclase inhibition
- cGMP inhibition (partial)

Effects of high-flow are NOT blocked by:
- Prostanoid inhibition
- K-channel blockade

In Situ Lymph Flow Velocity and Shear Stress Due to the Pump

- Using hi-speed intravital videomicroscopy to track lymphocyte flux, we conducted the first measurement of lymph flow in spontaneously pumping interlymphatics in situ:
  - Mean diameter of 90 μm with phasic contractions of ~40%
  - Lymphocyte density ~300-35,000 cells/μL
  - Lymphocyte flux ~300-6,000 cells/minute
  - Average lymph velocity ~0.9 mm/s with peaks of 2-4 mm/s
  - Lymph velocity is ~180° out of phase with the phasic diameter changes
  - Average lymph flow is ~14 μL/hour with periods of flow reversal preceding valve closure
  - Average lymph shear ~0.4-0.6 dynes/cm² with peaks of ~10 dynes/cm²

Phasic Lymphatic Contractions Produce NO oscillations associated with shear

- NO depends on the phasic contractile cycle as well as basal changes in lymph flow.
- NO measured at the valves was ~60-100% higher than the segment without valves.
- NO declined in the interstitial space away from the lymphatic wall.
- NO on lymphatic was similar or slightly higher than that on adjacent arteriole.

G. Bohlen in review

B. Dixon et al. (2006): Microcirculation
eNOS Expression in Rat Mesenteric Lymphatic

The phasic shear patterns of the intrinsic pump modulates pumping and increases the efficiency of the pump via NO-dependent mechanisms.

O. Gasheva et al. (2006): J. Phys

Decreases basal tone 30-50%
Decreases frequency
Enhances diastolic filling (lusitropy)
Increases contraction amplitude
Increases ejection fraction

Development of Long-term Rat Vessel Culture for Studies of Lymphatic Pumping

- Allows us to use an animal model with extensive background on lymphatic contractile function from both in situ and ex vivo studies.
- Can utilize transfection techniques to modulate specific target proteins (upregulate or downregulate) in the specific lymphatic vessel/cell of interest.
- Less concern of embryonic fatality or whole animal gene compensation as in mouse knockouts.
- Can use controlled application of pharmaceuticals, chemokines, cytokines, lymphokines etc.
- Develop chronic models of lymphatic dysfunction in the isolated vessels.
- Develop models of lymphatic remodeling to altered physical conditions.

Phasic Shear Enhances the Intrinsic Lymphatic Pump Efficiency

- NO generated by the phasic shear patterns in the intrinsic pump
- Decreases basal tone 30-50%
- Decreases frequency
- Enhances diastolic filling (lusitropy)
- Increases contraction amplitude
- Increases ejection fraction

- The phasic shear patterns of the intrinsic pump modulates pumping and increases the efficiency of the pump via NO-dependent mechanisms.

Development of Model of Altered Lymphatic Function Associated with Chronic Exposure to High Lymph Pressures in Rat Vessel Culture

A. Gasheva, W. Wang, D. Zawieja, unpublished
Lymphatic - targeted Gene Therapy
Experimental Tool and Potential Therapy

Lymphatic endothelium transfected with GFP expressing Adenovirus

Lymphatic muscle transfected with GFP expressing Adenovirus

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