The Role of Lymphatic Growth and Remodeling in Chronic Lung Disease

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Lymphatic vessel
Lymph vessel contraction

Lymphatic System – the Immunovascular Network

- Network of blind-ended lymph capillaries, precollectors, collecting vessels, afferent and efferent lymph ducts and lymphoid organs such as nodes, tonsils and Peyer’s patches.
- Open-ended input, one-way transport system for fluid, macromolecules, lipids and immune agents.
Immune function of the dermal lymphatics

The Lymphatic Continuum

- Vasculature of cancer and cancer metastasis
- Autoimmune disease
- Chronic infection and inflammation
- Organ transplantation
- Coronary artery disease and CHF
- Obesity, metabolic syndrome and DM
### Pathway Analysis for genes up in Lymphedema vs. Normal

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Gene Category</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>GO Molecular Function</td>
<td>defense/immunity protein activity</td>
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<tr>
<td>GO Molecular Function</td>
<td>extracellular matrix structural constituent conferring tensile strength</td>
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<tr>
<td>GO Molecular Function</td>
<td>extracellular matrix structural constituent</td>
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<td>GO Molecular Function</td>
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<td>GO Molecular Function</td>
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<td>GO Biological Process</td>
<td>immune response</td>
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<td>GO Biological Process</td>
<td>response to stress</td>
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<td>GO Biological Process</td>
<td>response to biotic stimulus</td>
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<tr>
<td>GO Biological Process</td>
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<td>GO Cellular Component</td>
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### Pathway Analysis for genes up in Normal vs. Lymphedema

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<th>Annotation</th>
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<td>GO Biological Process</td>
<td>lipid metabolism</td>
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</table>
Expression pathways invoked in human lymphedema

- IL-6 signaling
- Cytokine network
- T cell receptor signaling
- VEGF signaling
- Antigen processing and presentation
- EGF signaling
- Adipocytokine signaling
- Visceral Fat deposits and metabolic syndrome
- mTOR signaling
LYMPHATIC DYSFUNCTION

INTERSTITIAL EDEMA

IMMUNE DYSFUNCTION

RECURRENT INFECTION

CHRONIC INFLAMMATION

PROGRESSIVE FIBROSIS

ADIPOSE DEPOSITION
Lymphatics in the lung

- Trachea
- Bronchus
- Lymph node
- Subpleural lymphatics
- Intralobular lymphatics
- Alveoli
- Paravascular lymphatics
- Pulmonary arteriole
- Pulmonary venule
Lymphatics in the lung

- Lymphatics in the lung accompany the major airways and respiratory bronchioles
- They are also present near the intralobular arterioles and small veins
- There is a network of subpleural lymphatics, which are distributed beneath pulmonary pleura
- Under physiological conditions, lymphatic vessels generally do not extend to the distal alveolar spaces
# Imaging lymphatics in lung

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<th>Cell type</th>
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<tr>
<td>Lymphatic endothelial Cells</td>
<td>EGFP in Prox1-EGFP mice</td>
<td>Chicken</td>
<td>Aves</td>
<td>GFP 1020</td>
<td><em>Overall best marker</em> Strong signal. Marks cytoplasm and nucleus</td>
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<td>Prox1</td>
<td>Rabbit</td>
<td>AngioBio R&amp;D</td>
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<td>Goat</td>
<td>Systems</td>
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<td>VEGFR-3</td>
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<td>R&amp;D Systems</td>
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<td>Neuropilin-2</td>
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<td>Podoplanin</td>
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<td><em>Not recommended for the lungs.</em> Expressed by lung and tracheal epithelial cells.</td>
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<td>LYVE-1</td>
<td>Rabbit</td>
<td>AngioBio R&amp;D</td>
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<td><em>Not recommended for the lungs.</em> Strongly expressed by blood vessels in lung [18], but not in trachea.</td>
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<td>Blood vessel endothelial cells</td>
<td>Pecam1 (CD31)</td>
<td>Armenian hamster Rat</td>
<td>Thermo BioLegend</td>
<td>MA3105 102502</td>
<td>Clone 2H8 Clone MEC13.3 Weaker expression also on lymphatic EC</td>
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<td>Endothelial cells</td>
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<td>R&amp;D Systems</td>
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<td>Weaker expression also on lymphatic EC</td>
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<td>High endothelial venules (HEV)</td>
<td></td>
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<td></td>
<td>553863</td>
<td>Found in bronchoalveolar lymphoid tissue (BALT)</td>
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<td>Smooth muscle cells</td>
<td>α-smooth muscle actin</td>
<td>Mouse</td>
<td>Sigma Sigma</td>
<td>F3777 (FITC) C6198 (Cy3)</td>
<td>Marks large airways and blood vessels</td>
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<td>Pericytes</td>
<td>NG2</td>
<td>Rabbit</td>
<td>Millipore</td>
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</table>
Lymphatics in lung development

• Humans:
  – Increased saccular dilation in pulmonary lymphangiectasia

• Animals:
  – Increased dilated lymphatics ~ pulmonary lymphangiectasia from embryonic VEGF-C overexpression
  – Perinatal morbidity and decreased lymphatic vessel density from deletion of VEGF-C, CCBE-1 or VEGFR-3
Lymphatics in asthma

- **Humans:**
  - Decreased lymphatic vessel density

- **Animals:**
  - Decreased lymphatic vessel density with blockade of VEGFR-3, TNF-α blockade
  - Decreased lymphatic vessel density with dexamethasone administration (decreasing TNF-α and IL-1β), Th2 secretion of IL-4 and IL-13 through the JAK1 and STAT6 pathways, Th1 secretion of IFN-γ
Lymphatics in asthma

• Data gathered from patients with asthma support diminished lymphangiogenesis.

• Postmortem tissue analysis demonstrated lymphatics in close proximity to the mucosal surface of both large and small airways

• Smooth muscle proliferation and fibrotic changes are seen
Lymphatics in asthma

- Despite elevated levels of VEGF-C and VEGF-D, lymphatic density is diminished compared with control subjects.
- This suggests that, on balance, anti-lymphangiogenic factors prevail in asthma.
Lymphatics in COPD

• Very little is known about the role lymphatics play in the pathogenesis of COPD

• Examination of human COPD lung tissue identified increased lymphatic density (LYVE-1 and D2-40 staining)
  – Despite this clinical evidence, little is known about the role of lymphatic vessels in COPD pathogenesis and how modulation of lymphangiogenesis might affect progression of disease.
Lymphatics in COPD

- Another study evaluated lung tissue in COPD, smokers without COPD, and nonsmoker control subjects
  - the most pronounced increase in lymphatic formation within the alveolar parenchyma of patients with stage IV disease
  - lymphangiogenesis appeared to also occur in the bronchiolar and arterial walls of patients with severe disease as compared with healthy control subjects
  - the changes were proportional to the level of surrounding tissue remodeling
  - expressions of chemokine (C-C motif) ligand 21 and lymphatic and lymphatic chemokine scavenger receptor D6 were up-regulated, suggesting enhanced lymphatic transport of activated immune cells in COPD
Lymphatics in COPD

• Despite the clinical evidence, little is known about the role of lymphatic vessels in COPD pathogenesis or

• How modulation of lymphangiogenesis might affect progression of disease.
Lymphatics in COPD

• Despite the clinical evidence, little is known about the role of lymphatic vessels in COPD pathogenesis or
• How modulation of lymphangiogenesis might affect progression of disease.
Lymphatics in interstitial lung disease

• Humans:
  – *Increased* lymphatic vessel density

• Animals:
  – *Newly formed lymphatic vessels after* radiation exposure *regress* with subsequent development of pulmonary fibrosis
  – *Unchanged* lymphatic vessel density with bleomycin exposure, but with evidence of *impaired* lymphatic vessel function
Lymphatics in ILD

• In IPF lung tissue sections, increased alveolar lymphangiogenesis and lymphatic density correlate with worsening disease severity

• Increasing lymphatic density was associated with increased organizing and fibrotic collagen as well as progressive physiologic dysfunction (decreased forced vital capacity and carbon monoxide diffusing capacity)
Lymphatics in experimental ILD

- In a mouse model of radiation-induced lung fibrosis, lymphatic vessel density decreased 1 week after ionizing radiation and preceded the development of fibrosis at week 16, suggesting potential roles for the lymphatic system in disease pathogenesis.

- In the bleomycin-induced fibrosis model that PDGFR-β+ mural cells block lymphatic vessels from draining macromolecules, especially hyaluronan, a critical molecule in lung injury and repair.
Lymphatics in experimental ILD

• These two models of experimental fibrosis suggest that impairment of lung lymphatics could be key to the development of fibrosis.

• The apparent discrepancy in lymphatic changes between human disease and animal models is not completely understood
  – could potentially be explained by the change of lymphatic function.
Lymphatics in experimental ILD

- It is possible that, even though lymphatic density is increased in human disease, the newly formed lymphatics are not functional.
- Further studies are needed to completely characterize the contribution of the lymphatic vasculature to fibrotic lung diseases.
Lymphatics in lung transplant

- **Humans:**
  - *Increased* lymphatic vessel density in the setting of acute rejection

- **Animals:**
  - VEGF-C induced lymphangiogenesis-*attenuates* allograft rejection through improved hyaluronan clearance
Lymphatic remodeling in chronic lung infection

Am J Pathol 2012 Jun; 180(6): 2561–2575
Lymphatic remodeling in chronic lung infection
What can be learned from this model?

• In infected mice **not treated** with dexamethasone, capillaries enlarged into venules expressing leukocyte adhesion molecules and sprouting angiogenesis and lymphangiogenesis occurred.

• Inflammatory cytokines TNF and IL-1 increased.
What can be learned from this model?

• Concurrent dexamethasone treatment largely prevented the remodeling of blood vessels and lymphatics

• Dexamethasone also significantly reduced cytokine expression, bacterial burden, and leukocyte influx into airways and lungs over 4 weeks of infection
What can be learned from this model?

• In contrast, when infection was allowed to proceed untreated for 2 weeks and then was treated with dexamethasone for 4 weeks, most blood vessel changes reversed but lymphangiogenesis did not, suggesting that different survival mechanisms apply.

• Furthermore, dexamethasone significantly reduced the bacterial burden and influx of lymphocytes but not of neutrophils or macrophages or cytokine expression.
What can be learned from this model?

• These findings show that lymphatic remodeling is more resistant than blood vessel remodeling to corticosteroid-induced reversal.

• It is possible that lymphatic remodeling that persists (after the initial inflammatory response has resolved) may influence subsequent inflammatory episodes in clinical situations.
Conclusions
Mechanisms Driving Lymphangiogenesis in Lung Diseases

• Postnatal lymphatic development occurs primarily through sprouting of new vessels from existing lymphatic vessels

• The stimuli necessary to drive lymphangiogenesis are mostly well characterized in animal models of persistent airway and lung inflammation, where cells from the bronchus-associated lymphoid tissue generate VEGF-C and VEGF-D, leading to enhanced lymphangiogenesis
Mechanisms Driving Lymphangiogenesis in Lung Diseases

• In addition, other chemokines and cytokines have been shown to contribute to lymphangiogenesis stimulation (e.g. TNF-α and IL1-β), or inhibition (e.g. IL4 and IL13).

• Another mechanism that could potentially drive lymphangiogenesis could be resident or circulating lymphatic endothelial progenitor cells.
Mechanisms Driving Lymphangiogenesis in Lung Diseases

- There is evidence to suggest that circulating progenitor LECs could contribute to postnatal lymphangiogenesis during health and disease.
- Although many surface markers have been used to identify these progenitor cells, their direct contribution to lymphangiogenesis in experimental models of lung disease has, to date, not been shown.
Future Directions
• In animal models of chronic airway inflammation (*M. pulmonis*), TB, and lymphangiectasia, induction of lymphangiogenesis is irreversible, in marked contrast to the reversible angiogenesis response.

• Whether the **irreversibility** of newly formed lymphatic vessels contributes to disease pathogenesis or the persistence of disease is a subject that requires intense investigation.
• Noninvasive means of assessing lymphatic function in normal and diseased lungs are nonexistent.

• Further strategies using transit time ultrasonic flow meters might help us understand the lymphatic function in a more direct manner.
• Advances in intravital microscopy and introduction of reporter mice with fluorescent lung lymphatics have made it possible to directly monitor lymphatic in the lung parenchyma

• Developing **less invasive** methods to image lung lymphatics will constitute a major advance in the field
• High-throughput screens of existing drugs and small molecules to identify targets that modulate lymphangiogenesis could accelerate translational applications of preclinical findings.