Phenotyping the Lung

How can we reproducibly quantify the consequences of gene expression or pathologic insults in the lung?

Wayne Mitzner, wmitzner@jhu.edu
Environmental Health & Engineering
Johns Hopkins Bloomberg School of Public Health

First “published” image of lung pathology (Leonardo da Vinci, c. 1492)

Text describes the circled abscess as a, “hard callus, filled with dust and fluid and formed in small airway branches.” Although he didn’t have biologic knowledge, it is likely that he was describing a tubercle.

Lecture Organization
1. Definitions and Nomenclature
2. Anatomy
3. Quantifying the pathophysiology
4. Quantifying the histology
5. Nonlethal phenotyping

Definition of Lung Volumes

Maximal inspiratory level

Residual volume (RV)

Maximal expiratory level

Inspiratory reserve volume (IRV)

Expiratory reserve volume (ERV)

Vital capacity (VC)

Total lung capacity (TLC)

*Not determined by spirometry
Anatomy

How does the mouse lung differ from the human lung?

Mouse vs. Human Alveoli

Alveolar diameter $\approx 50 \mu m$
Number of alveoli $\approx 2,000,000$

Alveolar diameter $\approx 300 \mu m$
Number of alveoli $\approx 500,000,000$

Human & mouse lung aging

30 month old mouse
(Bar = 125 $\mu m$)

100 year old human
(Bar = 250 $\mu m$)

Structure of Human Trachea and Major Bronchi

Missing in mice: Cartilage in conducting airways beyond mainstem bronchi

Human Airway Tree

Mouse Airway Tree

Mouse airway tree has different gross structure with a zero branch angle at carina
Structure of Human Bronchi and Bronchioles

- Blood vessels
- Submucosal glands
- Cartilage

Missing in mice: Blood vessels, Submucosal glands, Cartilage

Identification of Human Airways

23 Generations in Man
- The first 16 generations make up the conducting airways
- The last 7 make up the respiratory zone, where gas exchange can take place

Maybe 13-16 generations in mice

Missing in mice: Segmental conducting airways and respiratory bronchioles

Alveolar Duct

Alveolar Duct with Capillary Wall

- alveoli (A); alveolar ducts (D); capillary (C)

Dense Capillary Meshwork in Alveolar Walls

Pulmonary Vasculatures

Missing in mice: Bronchial Circulation
Anatomy Summary:
- The lung is a very complex 3 dimensional structure designed to optimize gas exchange.
- Mouse lungs are not human lungs.
- How these anatomical differences between humans and mice manifest themselves in lung phenotyping is not always obvious.

Relevance of Mouse to Human Assessment of Lung Function and Pathology
- Nearly all human pulmonary function today is with spirometry, a voluntary expiratory maneuver, and is something of which is not possible in the mouse.
- To assess pulmonary function in the mouse, measurements are usually made of elastance, resistance, and post-mortem histology, none of which is routinely done in humans.

The only exception is measurement of the lung diffusing capacity, which can be easily measured in both mice and humans.

Thus, relating mouse functional measurements to those in humans must be done with caution.

Quantifying the Pathophysiology

Lung Mechanics

Measurements of resistance, compliance, elastance, viscoelasticity, etc.

Airway Resistance (Raw) and Elastance (E_L)

\[ Z = R_n + I \ i \ 2 \pi f + (G - iH)/(2\pi)^a \]

Where:
- \( R_n \) = Newtonian resistance (\( \approx \) Raw)
- \( I \) = Inertance
- \( H \) = Stiffness parameter (\( \approx E \))
- \( G \) = Viscoelastic parameter
- \( i = v \cdot l \)

In recent years, the most common approach to measuring mouse pulmonary function is to ventilate mice with a Flexivent ventilator.
Airway hyperresponsiveness
• A critical phenotypic characteristic of asthma
• Excessive narrowing of the airways in response to a many stimuli
• In all mouse models of asthma, airway responsiveness is measured by the response to methylcholincine (Mch)

Representative dose-response curve:
(PC200 one approach used to quantify response)

Airway responsiveness in C57BL/6 mice
(From five mouse vendors)

Male and female airway responsiveness
(in C57BL/6 mice)

Lung Elasticity
The functional effect of structural changes in lung disease can be assessed by measuring lung compliance from the pressure-volume relation

**Method on how to generate a PV curve:**

---

**Quantifying the Histology**


---

**Lung Histology:**

To do proper **quantitative** morphology one needs a way to reliably and consistently fix lung in inflated state.

- The most common method is to instill formaldehyde at a fixed pressure (25 cmH2O).
- However, it is **ESSENTIAL** to measure lung **volume** after fixation. The easiest method to measure this is by water immersion (Archimedes principle).


---

**Fixation under constant pressure**

http://www.jove.com/video/52964/

---

**Archimedes Principle:**

The weight of water displaced by a submerged object equals the volume of the object

http://www.youtube.com/watch?v=cUhmSW4d
Quantitative Evaluation of Lung Structure in Mice

**Essential procedures**
- Fix and measure fixed lung volume (V)
- Embed and cut random lung sections
- Take random pictures from random lung sections
- Measure cell or airspace densities
- Measure Alveolar Surface Area (S)
- Calculate Lm (mean airspace chord length = 4V/S)

How to "randomly" sample pictures from the histologic sections

- Random sampling of blocks generally not done
- Use Systematic Uniform Random Sampling (SURS) to pick regions of different sections
- Get ≈20 fields (@ 20x), from at least 3 regions and measure ≈100 – 200 points and intercepts

Where do you take the pictures?

Example of transverse cut of normal mouse left lung

Example of transverse cut of emphysematous mouse left lung

Example of Systematic Uniform Random Sampling

Initial field at arbitrary location outside of lung tissue

Note: This sampling procedure should be used for all quantitative histology, not just for lung sections.
Parenchymal fraction (Fp) = \( \frac{P_{\text{par}}}{P_{\text{total}}} \)
Parenchymal volume (Vp) = \( F_p \cdot V_{l} \)
Parenchymal tissue fraction = \( \frac{P_{\text{tissue}}}{P_{\text{total}}} \)
Parenchymal alveolar fraction = \( \frac{P_{\text{alveolus}}}{P_{\text{total}}} \)

Alveolar surface area (S) = \( \frac{4(V_p \cdot I_{\text{alv}})}{(d \cdot P_{\text{air}})} \)

where \( d \) = length of a single test line
and \( I_{\text{alv}} \) = number of intercepts with alveolar septal walls
and \( P_{\text{air}} \) = number of points hitting air spaces in alveoli and ducts

\[ L_m = \frac{4V_p}{S} \]

---

### Comparison of Lung Structure in Two Mouse Strains

<table>
<thead>
<tr>
<th>C3H/HeJ</th>
<th>C57BL/6J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lm (( \mu \text{m} ))</td>
<td>Lm (( \mu \text{m} ))</td>
</tr>
<tr>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Lung Volume (mL)

<table>
<thead>
<tr>
<th>C3H/HeJ</th>
<th>C57BL/6J</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>400</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>1400</td>
<td></td>
</tr>
</tbody>
</table>

Alveolar Surface Area (cm\(^2\))

- **Emphysema** is characterized by an irreversible loss of surface area.
- Measurement of an increase compliance or an increased \( L_m \) **do not** by themselves prove emphysema. One needs to show a loss of alveolar surface area.
**Diffusing Capacity of the Lung**

- Experimental measurement of the ultimate reason we have lungs, i.e., for gas exchange.
- This is the only lung functional measurement that is routinely done in humans and can easily be done in mice.
- It is a very sensitive method that can be used to monitor and follow pathologic changes in lung function.

**Measurement of DLCO**

DLCO (mL/sec/mmHg CO) = rate of uptake of CO/ mmHg CO

In this measurement, the CO uptake is compared to that of an inert gas, that has negligible absorption into the blood. In the mouse we use neon as this inert gas.


---

**Experimental Studies**

We measured lung lung diffusing capacity with many experimental lung pathologies in the mouse:

- Fibrosis with bleomycin
- Emphysema with elastase
- PR8 influenza
- Fungal infection (aspergillus)
- Cystic Fibrosis
- Acute Lung Injury


Nonlethal Assessment Methods

1. Mouse intubation

2. Whole Body Plethysmography

Direct fiberoptic visualization method of intubation

20 g IV cannula (e.g., BD Insyte) with 0.5 mm fiberoptic cable (from Edmund Optics)

http://www.jove.com/video/50318/

http://www.jove.com/video/52964/
Intubation can even be used for repeated bronchoalveolar lavage


Whole Body Plethysmograph
(Can Measure Tidal Volume, Frequency, and O₂ Consumption in Conscious Mice)

Note: Whole body plethysmography cannot be used to assess airway resistance or lung elasticity.
Experimental Study: Strain Dependence of Ventilatory Response to Hypoxia

DBA/2J strain shows the greatest sensitivity and the A/J strain is the least sensitive.

Conclusion:
- There are many different ways of phenotyping healthy and diseased lungs.
- Therefore, one needs to carefully consider what information is desired to best quantify the particular pathology being studied.

Questions?

Contact: Wayne Mitzner, Tel: 410-614-5446, wmitzner@jhu.edu