Lung Disease in Pediatrics: is it all in the Genes?

Jay K. Kolls, M.D.
Chair, Department of Genetics
LSU Health Sciences Center
New Orleans, LA
Children’s Hospital of Pittsburgh
Pathogenesis of Cystic Fibrosis

Boucher et al. Advanced Drug Delivery Reviews, 203
Why do some patients with CF do worse than other?
• Outcomes are better at CF centers
• There is huge variation in lung function outcomes from center to center
• Centers now share data online
• Center staff visit high performing centers to develop best practices
Are there other reasons why some patients with CF with the same mutation do worse than others?

- **Modifier genes – lung disease**
  - $Tgfb1$
  - $Irfd1$ - neutrophil function

- **Modifier genes – liver disease**
  - $Tgfb1$
  - $Serpina1 Z$ allele
Alpha-1 antitrypsin deficiency
Why do some patients with CF do worse than other?

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- **Allergic Bronchopulmonary Aspergillosis?**
ABPA

- Asthmatics 0.5-1%
- Cystic Fibrosis 4-15%
  - Highly associated with atopy
- In CF classic ABPA can be defined as
  - 1. Acute or subacute clinical deterioration not attributable to another etiology.
  - 2. Total serum IgE concentration of 1000 IU/mL
  - 3. Immediate cutaneous reactivity to Af or in vitro demonstration of IgE antibody to Af.
  - 4. One of the following: (a) precipitins to Af or in vitro demonstration of IgG antibody to Af; or (b) new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.
ABPA -a TH2 Disease?

Antonio J. and Janet Palumbo CF Center

450 CF Patients

Af negative 250

Af positive 200

35 with ABPA

165 with Af exposure
Figure 5

Aspergillus life form
- dextin-1
- APC / Macrophage
  - upregulation of B7S1 or insufficient CD80/86
  - TNF-α
  - IL-1β
  - CXCL 1, 2, 5, 6

Early PMN responses

Anergy

Th2
- GATA-3
- IL-4, IL-5

Treg
- FOXP3
- IL-10
- TGF-β1

Spin 2,880 x g 20 minutes
Remove PBMCs
Separate CD4+ and CD14+
using magnetic beads

CD4+
CD4+ Th0

Pulse with antigen

Aspf1, Zymosan, RC, HKSC, Asp extract

Luminex, Intracellular IL-4, IL-10, TGF-beta, GATA-3

Luminex, Intracellular IL-4, IL-10, TGF-beta, GATA-3

FACS for CD80, CD86, B7s1, HLA-DR
Luminex
CD4+ T-cells

- CD40
- CD40L
- CD4
- CD28
- CTLA4
- TCR
- MHC
- Ag
- B7.1
- B7.2
- OX40
- OX40L

IL-4

TH2
STAT 6

IL-4
IL-5
IL-13
Blockade of OX40L blocks Th2 cytokine production in CD4+ T-cells
Anergy versus tolerance

Mucosal Immunology advance online publication 17 February 2010.
10.1038/mi.2010.4
Af colonized CF patients have increased Tregs
ABPA patients are vitamin D deficient

<table>
<thead>
<tr>
<th></th>
<th>ABPA Positive</th>
<th>ABPA Negative</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin D (ng/mL)</strong></td>
<td>22.04 ± 1.999</td>
<td>36.56 ± 5.021</td>
<td>0.0201</td>
</tr>
<tr>
<td><strong>Vitamin D w/ IL5 conc &gt;1300 pg/mL</strong></td>
<td>18.22 ± 2.160</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Vitamin D w/ IL5 conc &lt;1300 pg/mL</strong></td>
<td>22.45 ± 2.175</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Vitamin A (µg/dL)</strong></td>
<td>50.93 ± 2.955</td>
<td>53.27 ± 4.188</td>
<td>0.989</td>
</tr>
<tr>
<td><strong>Vitamin E (µg/mL)</strong></td>
<td>9.754 ± 0.9482</td>
<td>10.13 ± 1.092</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Vitamin D increases TGFβ1 expression on Tregs

C
Af extract pulsed DCs

D
Af extract pulsed DCs + 1, 25 OH-vitamin D3
CD4+ T-cells

- APC
- MHC
- Ag
- CD40
- CD80
- B7.2
- B7.1
- TCR
- CD4
- CD28
- CTLA4
- CD40L
- OX40
- OX40L
- Vitamin D3
- TGFβ
- IL-4
- IL-5
- IL-13
- STAT 6
- STAT 4
- Foxp3
- TGFβ
- IL-10
- IL-13

TH2

β36
Why do some patients with CF do worse than other?

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• Modifier genes – liver disease
  - Tgfb1
  - Serpina1 Z allele

• Allergic Bronchopulmonary Aspergillosis
  - Vitamin D – alleles?
What can animal models teach us about CF?

- **Cftr -/- mice**
  - 100% rate of meconium ileus
  - Essentially no lung disease

- **Cftr Δ F058 knock-in mice**
  - Meconium ileus
  - +/- lung disease
Why don’t CF mice get lung disease

- **Anatomy**
  - Lack of sub-mucosal glands
  - Murine airway is 80% Clara cells/20% ciliated cells

- **Electrophysiology**
  - Lack of increased ENaC activity
  - Other Cl- channels
Pathogenesis of Cystic Fibrosis

Boucher et al. Advanced Drug Delivery Reviews, 203
**Normal**

- ENaC
- CFTR and alternative Cl⁻ channels
- Na⁺ absorption
- Anion secretion

**β-ENaC transgenic mice/cystic fibrosis**

- ENaC
- Alternative Cl⁻ channels
- Na⁺ hyper-absorption
- Reduced anion secretion in CF

**Pathways**

- PCL homeostasis
- Mucus clearance
- Neutrophils
- Elastase
- Mucus cell granule

- GAGs
- Chemokines, growth factors

- PCL depletion
- Mucus stasis, inflammation, goblet cell hyperplasia
Dubin et al. Inflammation Research, 2007
What about Pigs, Ferrets, or zebrafish?

Cystic Fibrosis Pigs Develop Lung Disease and Exhibit Defective Bacterial Eradication at Birth

David A. Stoltz,1 David K. Meyerholz,2 Alejandro A. Pezzulo,1 Shyam Ramachandran,3 Mark P. Rogan,1 Greg J. Davis,1 Robert A. Hanfland,4 Chris Wohlford-Lenane,3 Cassie L. Dohrn,2 Jennifer A. Bartlett,3 George A. Nelson IV,1 Eugene H. Chang,5 Peter J. Taft,1 Paula S. Ludwig,1 Mira Estin,1 Emma E. Hornick,1 Janice L. Launspach,1 Melissa Samuel,6 Tatiana Rokhлина,1 Philip H. Karp,1,7 Lynda S. Ostedgaard,1 Aliye Uc,3 Timothy D. Starner,3 Alexander R. Horswill,8 Kim A. Brogden,9 Randall S. Prather,6 Sandra S. Richter,2 Joel Shilyansky,4 Paul B. McCray Jr.,3 Joseph Zabner,1 Michael J. Welsh

(Published 28 April 2010; Volume 2 Issue 29a31)
Ferrets

A

B

C

D

JCI, Sept 2010
What have we learned from these new models?

- Meconium ileus has 100% penetrance in both null and Δ F508 pigs
- There is no evidence of infection or inflammation immediately after birth
- There are reduced numbers of sub mucosal glands in CF pigs and Ferrets
  - Is CF a developmental disease?

- Although genotype can predict pancreatic phenotype, lung phenotype is more variable
Cellular Responses in Asthmatics

Lukacs, NW. Nature Reviews Immunology 2006, 1:108.
Helper T Cells and Asthma

• Classically Known as $T_{H2}$
• Elevated IL-4, 5, 13, IgE, IgG₁
• Pathology Associated with Cytokine Signaling
• Eosinophil Component

What About $T_{H17}$ and Asthma?

• IL-17 Levels are Elevated in Asthma and Correlate to Severity (Bullens et al. Resp Res 7:135, 2006)
• Anti-IL-17 Worsened Ova-induced Inflammation in Mice (Hellings et al. Red 28:42, 2003)
Neutrophils and Asthma

• Approx 50% of Asthma is non-eosinophilic phenotype (Douwes Thorax 57:643, 2002)

• Neutrophilia is increased in non-eosinophilic asthma (Gibson Chest 119:1329, 2001)

• Neutrophilic inflammation correlates with decreased improvement in FEV₁ and Mch responsiveness after Glucocorticoids (Green Thorax 57:875, 2002)

(Tsokos Virchows Arch 441:494, 2002)

Sudden Onset Fatal Asthma

• Neutrophil products IL-8/KC, LTB4, MMP9 elevated in asthma (Wenzel Clin Exper All Rev 1:89, 2001)


• Neutrophils are inherently steroid insensitive (Cox Am Assoc Immunol 154:4719, 1995; Schleimer J Pharmacol Exp Therap 250:598, 1989)
How does one study asthma in pre-clinical models

• Antigen sensitization and challenge
  - Ova
  - Cockroach
  - HDM
  - House Dust extract
  - Aspergillus spp.

• Issues
  - IP versus airway
  - In utero?
Polarization of T_H cells *in vitro*

**T_H0 Cell**
- 5 µM OVA_{323-339}
- 20 U/ml IL-2
- 5 ng/ml IL-4
- 10 µg/ml anti-IFN-γ

**T_H2 Cell**
- High IL-4, IL-5, IL-13, IL-10

**T_H17 Cell**
- 5 µM OVA_{323-339}
- 20 ng/ml IL-6
- 10 ng/ml IL-23
- 1 ng/ml TGF-β
- 10 µg/ml anti-IL-4
- 10 µg/ml anti-IFN-γ

**DO11.10 Mice**
- 10 ng/ml IL-10

**+ irradiated splenocytes (APC)**

(Mangan et al. Nature 441:231, 2006)
Cytokine Stimulation by Polarized $T_H$ Cells

*In vitro* restimulation –
- After 6 Days Culture
- CD3/CD28 Beads + IL-2
- 48 hr. Stimulation
Th17 Cytokine Production is Steroid-Resistant

**IL-5**

- Control
- Dex
- CD3/28
- 1 μM
- 0.5 μM
- 0.1 μM

**IL-13**

- Control
- Dex
- CD3/28
- 1 μM
- 0.5 μM
- 0.1 μM

**IL-17**

- Control
- Dex
- CD3/28
- 1 μM
- 0.5 μM
- 0.1 μM

**IL-22**

- Control
- Dex
- CD3/28
- 1 μM
- 0.5 μM
- 0.1 μM

48hr restimulation
T<sub>H</sub> Adoptive Transfer Ovalbumin Challenge Model

Day -1
- OVA Challenge i.t.
  - 50 µg/mouse

Day 0
- Cell Transfer i.v.
  - 1.0 x 10<sup>6</sup> cells/mouse
- Dexamethasone i.p.
  - 2.5 mg/kg

Day 1
- OVA Challenge i.t.
  - 50 µg/day/mouse

Day 2

Day 3

Day 4
- Sacrifice—24-hours
**T_H17 Induced Cytokines are Steroid-Resistant in vivo**

IL-5

- TH2
- TH2 Dex
- TH17
- TH17 Dex

IL-13

- TH2
- TH2 Dex
- TH17
- TH17 Dex

G-CSF

- TH2
- TH2 Dex
- TH17
- TH17 Dex

KC

- TH2
- TH2 Dex
- TH17
- TH17 Dex

Lung Homogenate
$T_{H17}$ Induced Neutrophil Recruitment is Steroid-Resistant
T_{H17} Induced Mucus Production is Steroid-Resistant
Assessing AHR *in vivo* in Mice - Flexivent

- Tracheotomize & Cannulate mice
- Ventilate at 200 breaths/min, 0.25ml
- Mch Dose Response – 20min
- Procedure 45-60min/mouse
Flexivent Measures Relationship Between Pressure and Volume

12.5 Mch

50 Mch
$T_{H2}$ Induced Airway Hyperresponsiveness is Inhibited by Steroids

- **Rn** = Airway Resistance
- **G** = Tissue Resistance
- **H** = Tissue Elastance
**T\textsubscript{H}17** Induced Airway Hyperresponsiveness is Steroid-Resistant

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**Control**  
- \( \text{T}_{\text{H}17} \)  
- \( \text{T}_{\text{H}17} + \text{Dex} \)

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**Rn**

---

**PBS**

---

**G**

---

**H**

---

**Mch**

---

**% Increase over Baseline**

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**mg/ml**

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How do steroids work

• Lymphocytes
  - Suppress effector function
  - Induce cell death/apoptosis

• Smooth muscle cells
  - Suppress cytokine signaling
  - Upregulate B2 adrenergic receptors
Mechanisms of Steroid Resistance

• Low expression of GRalpha

• Failure to translocate the GR to the nucleus
  - GRbeta

• Lack of GREs or GREs are inaccessible
  - Histone deacetylases
Glucocorticoid Receptor Translocation Occurs in $T_H^{17}$ Cells
What happens in severe asthma?

Receptors for Th1, Th2, Th17, Th22 effector cytokines expressed in the lung epithelium
Why RNAseq?
The epithelial transcriptome gives clues to etiologies of severe asthma
Conclusions

• Chronic lung disease phenotypes are controlled by gene-environment interactions
• Vitamin D deficiency exacerbates Th2 diseases such as ABPA and asthma by inhibiting Treg development
• Th17 cells may cause some cases of steroid resistant asthma
• Epithelial transcriptomics may advance personalized medicine for Asthma
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