Biochemical heterogeneity of human asthma

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As iron sharpens iron, so one person sharpens another.

Solomon, Proverbs 27
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6. Gilead: *SAB*
Overview

1. Human asthma is highly heterogeneous.

2. Biochemical asthma classification may inform therapy: two examples.
   a. Low airway pH can be diagnosed by challenge test and treated.
   b. Increased airway nitrosothiol breakdown can be diagnosed by challenge test and treated.
Asthma is reversible bronchoconstriction associated with an array of different immune responses in the lung.
If you study a large cohort of humans, you can obscure population differences:

Adding salmeterol to inhaled corticosteroids benefits asthma patients

Fish, JE, et al., *Chest* 120:423, 2001
Yet salmeterol alone may be harmful for many patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>28-week study</th>
<th>28-week study plus 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory-related deaths and life-threatening experiences</td>
<td>1.39 (0.91–2.13)</td>
<td>1.16 (0.78–1.72)</td>
</tr>
<tr>
<td>Asthma-related deaths</td>
<td>4.33 (1.24–15.21)</td>
<td>2.50 (0.97–6.44)</td>
</tr>
<tr>
<td>Asthma-related deaths or life-threatening experiences</td>
<td>1.68 (0.99–2.85)</td>
<td>1.52 (0.92–2.52)</td>
</tr>
<tr>
<td>All-cause deaths</td>
<td>1.31 (0.83–2.08)</td>
<td>1.04 (0.70–1.55)</td>
</tr>
</tbody>
</table>

Lurie and Wolfe *Lancet* 366:9493, 2005

Knowing the patient is as important as knowing the literature: History, phenotype (genotype)

Targeted therapy can be based on genotype and/or phenotype: One phenotype is gender
Menstrual asthma/
eosinophil progenitor cells

Allergic/Th2

High GSNO reductase
Low pH/
low glutaminase
Low superoxide
dismutase
High VEGF
High arginase

Male

Female

Older

Younger

Tryptase/chymase positive
mast cells
Chronic infection

Smokers

Mucous hypersecretion

High leukotriene
High arginase
Low lipoxin
High ADMA

High YKL-40
Low superoxide
dismutase
High GSNO reductase

Chronic infection

Smokers

Mucous hypersecretion

High leukotriene
High arginase
Low lipoxin
High ADMA

High YKL-40
Low superoxide
dismutase
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Overview

1. Human asthma is highly heterogeneous.

2. Biochemical asthma classification may inform therapy: two examples.
   a. Low airway pH can be diagnosed by challenge test and treated.
   b. Increased airway nitrosothiol breakdown can be diagnosed by challenge test and treated.
It has been proposed that biomarkers can be useful to personalize asthma care: for example, exhaled NO and nitrite levels tend to be increased in asthma.

Further, breath condensate pH tends to be low in asthma, particularly during an exacerbation.

Liu et al., *Chest* 139:328, 2011
But these “biomarkers” can interfere with one another: biomarkers tend not to be simple

\[ \text{NO}_2^- + H^+ \rightarrow \text{HONO (pKa } \sim 3.6) \rightarrow \text{NO} \]
In fact, airway acid contributes to asthma pathophysiology, but has complex interactions and can be hard to detect.
Buffer challenge testing identifies asthma patients with airway acidification who can be candidates for individualized treatment

- Exhaled breath condensate pH is low in a subpopulation of stable severe asthma patients with a specific clinical phenotype (Liu et al, Chest)
- EBC pH is not the best test, however: clinically, buffer challenge is better

Liu et al., *Chest* 139:328, 2011
Gaston et al., *JACI* 118:817, 2006
An example of how this individualized approach can work:

- In a stable asthma patient:
  - **Suspect low airway pH:** An adult patient with high BMI, low FE_{NO}, GER symptoms, and low serum eosinophils (Chest 139:328, 2011).
  - **Test:** Buffer challenge or EBC (JACI 118:817, 2006)
  - **Treat:** Inhaled buffer

- In a steroid refractory asthma: consider testing, regardless of phenotype.

- Hypotheses: specific treatment
  - reduces symptoms;
  - is steroid sparing.
Overview

1. Human asthma is highly heterogeneous.
2. Biochemical asthma classification may inform therapy:
   a. Low airway pH can be diagnosed by challenge test and treated.
   b. Rapid airway nitrosothiol breakdown can be diagnosed by challenge test and treated.
“NO” is often the wrong answer, biochemically.

SNO synthesis from environmental nitrogen oxides (lung, gut, blood) and SNO synthases.

Compartmental Cellular S-nitrosothiol (RS⁻-NO⁺) stores [

\[ \text{NOS} \quad \text{Import} \quad \text{Export} \]

\[ \text{O}_2^- \quad \text{Cytotoxic effects} \]

\[ \text{H}^+ \quad \text{GC} \quad \text{cGMP} \quad \text{cGMP-dependent effects} \]

\[ \text{e}^- \quad \text{Re-uptake} \quad \text{Fusion and export} \]

\[ \text{Transnitrosation} \quad \text{Localization} \quad \text{Degradation} \]

Intracellular cGMP-independent effects

Annual number of citations

Year


0 50 100 150 200 250 300
Example: inducible NOS activation causes S-nitrosylation and activation of Cyclooxygenase 2

S-Nitrosoglutathione causes human airway smooth muscle relaxation

Gaston et al., Proc Natl Aced Sci USA 90:10957, 1993
Again, the mechanisms do not involve the NO-cGMP pathway.

Evangelista et al. PLoS ONE 5:e11209, 2010

Whalen et al., Cell 129:511, 2007
Bronchodilator GSNO levels are low in the airways of children with severe asthma

S-Nitrosoglutathione reductase-deficient mice are protected from experimental asthma

Que et al. *Science*, 308:1618, 2005
However, only a subpopulation of human asthma patients has increased GSNO reductase activity in the airway: These patients have a specific clinical phenotype.

Marozkina, et al., submitted
GSNO reductase expression in the human airways is also anatomically heterogeneous

Teague et al., Unpublished results
Biochemical challenge testing measures GSNO reductase activity to identify individuals who are rapid metabolizers.
Another example of how individualized asthma therapy can work:

• In a stable asthma patient:
  – *Suspect increased airway GSNOR activity* in: a patients with low AQLQ symptom score, and good beta-2 responsiveness, despite normal FEV1.
  – Test with ethyl nitrite challenge.
  – Treat with GSNOR inhibitor (if non-smoker).

• In steroid-refractory asthma, consider testing, regardless of phenotype.

• Hypotheses: specific treatment
  – reduces symptoms;
  – is steroid sparing.
Summary

1. Human asthma is highly heterogeneous.

2. Individuals who would respond to specific therapies can be identified inexpensively and noninvasively by challenge testing.

3. This personalized approach will have many applications in asthma.

4. This approach can prevent morbidity and mortality associated with toxic and/or ineffective therapies, particularly in severe asthma.
Nitric oxide synthase isoforms, which produce nitrite, are expressed in the lung; iNOS is increased by inflammation.

Low pH is associated with other markers of airway inflammation

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>COPD</th>
<th>Bronchiectasis</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_s )</td>
<td>( p ) Value</td>
<td>( r_s )</td>
<td>( p ) Value</td>
</tr>
<tr>
<td>( H_2O_2 )</td>
<td>-0.71</td>
<td>&lt; 0.0001*</td>
<td>-0.74</td>
<td>0.0002*</td>
</tr>
<tr>
<td>8-Isoprostane</td>
<td>-0.55</td>
<td>0.0002*</td>
<td>-0.28</td>
<td>0.21</td>
</tr>
<tr>
<td>Total ( NO_2/NO_3 )</td>
<td>-0.81</td>
<td>&lt; 0.0001*</td>
<td>0.13</td>
<td>0.57</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.75</td>
<td>&lt; 0.0001*</td>
<td>0.66</td>
<td>0.001*</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>-0.74</td>
<td>&lt; 0.0001*</td>
<td>0.33</td>
<td>0.14</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-0.37</td>
<td>0.1</td>
<td>-0.66</td>
<td>0.001*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.2</td>
<td>0.21</td>
<td>0.14</td>
<td>0.51</td>
</tr>
<tr>
<td>( FEV_1 )</td>
<td>0.81</td>
<td>&lt; 0.0001*</td>
<td>0.67</td>
<td>0.0009*</td>
</tr>
</tbody>
</table>

Kostikas et al, Am J Respir Crit Care Med 165:1364, 2002
Endothelial NOS and GSNO reductase regulate beta-2 receptor trafficking and tachyphylaxis

Whalen et al., *Cell* 129:511, 2007
Lung nitrite, produced by iNOS, is increased in asthma
Inhaled GSNO decreases airway levels of several cytokines in a mouse model of asthma

An NADH-dependent SNO catabolic protein prevents guinea-pig airway smooth muscle relaxation

Fang, et al., AJP Lung 279:L716, 2000
Henderson and Gaston TIMMS 11:482, 2005
Suspect the diagnosis;  
Test for the diagnosis;  
Treat

<table>
<thead>
<tr>
<th>Airway biochemistry</th>
<th>Phenotype biomarker</th>
<th>Potential targeted therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased SOD activity [38]</td>
<td>Decreased $FE_{NO}^4$</td>
<td>Anti-oxidant and/or SOD–mimetic</td>
</tr>
<tr>
<td>Increased arginase activity [80,81]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased ADMA level [86] and/or decreased DDAH activity [84]</td>
<td>Decreased SOD activity [38]</td>
<td></td>
</tr>
<tr>
<td>Increased iNOS activity [15]</td>
<td>Increased $FE_{NO}^4$ [10–19,49,87]</td>
<td>NOS inhibitor</td>
</tr>
<tr>
<td>Decreased glutaminase activity [75]</td>
<td>Decreased EBC pH$^+$ or decreased $FE_{NO}^4$ on buffer challenge [78,79]</td>
<td>Glutamine and/or inhaled buffer</td>
</tr>
<tr>
<td>Other causes of decreased airway pH [34]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased activity of GSNO reductase or other GSNO catabolic enzyme [48,50–52]</td>
<td>Decreased airway GSNO or increased $FE_{NO}^4$ on GSNO challenge [88]; or increased EBC formic acid [77].</td>
<td>$S$–nitrosylating agent and/or GSNO–$R$ inhibitor</td>
</tr>
<tr>
<td>Increased eosinophil peroxidase [20]</td>
<td>Increased urinary bromotyrosine [20]</td>
<td>Increased corticosteroids</td>
</tr>
</tbody>
</table>

Is there no balm in Gilead?  
Is there no physician there?  

Jeremiah
Infection lowers airway pH


n = 152 subjects; 4105 samples
S-Nitrosothiols are produced by nitric oxide synthases

Gow, *JBC* 277:9637, 2002
Citations regarding S-Nitrosothiols in medicine, 1989-2009
Additional examples

An asthma subpopulation has decreased circulating SOD activity

Masini E, et al. FREE RADICAL BIOL MED. 2005;39:520-31
Understanding of the immunological complexity of asthma has increased with advances in immunology.
Pharmacogenomic determinants of response to leukotriene modifiers and to inhaled corticosteroids


Murine models do not consistently recapitulate human asthma

Airway S-nitrosothiol biochemistry can be reported as exhaled NO
Breath condensate pH measures lung inflammation in children

Corticosteroids increase EBC pH

Microaspiration in an otherwise normal MVA victim

EBC pH is low in an intubated child with asthma

Nosocomial RSV after subglottic stenosis repair

Walsh et al., *Respir Care* 51:1129, 2006

Hunt et al. *AJRCCM* 161:694, 2000
Current acetaminophen use was associated with “severe” childhood asthma

<table>
<thead>
<tr>
<th></th>
<th>Adjusted* (all children)</th>
<th>Adjusted† (children with complete covariate data)</th>
<th>Multivariate analysis‡ (children with complete covariate data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol use for fever in first year</td>
<td>1.82 (1.70–1.95)</td>
<td>1.82 (1.65–2.00)</td>
<td>1.43 (1.30–1.58)</td>
</tr>
<tr>
<td>Current paracetamol use§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium vs none</td>
<td>1.31 (1.19–1.44)</td>
<td>1.44 (1.26–1.66)</td>
<td>1.33 (1.15–1.53)</td>
</tr>
<tr>
<td>High vs none</td>
<td>3.92 (3.56–4.32)</td>
<td>4.23 (3.65–4.91)</td>
<td>3.54 (3.05–4.11)</td>
</tr>
</tbody>
</table>

Data are OR (95% CI). *Adjusted for sex, region of the world, language, and gross national income. For analysis of paracetamol use for fever in first year of life, 192,391 children were included from 68 countries in 29 countries. For analysis of current paracetamol use, 203,361 children were included from 72 centres in 31 countries. †Adjusted for sex, region of the world, language, and gross national income. Analysis was restricted to the centres included in the multivariate analyses. ‡Multivariate analysis included centres with at least 70% data available for all covariates. Children who had a missing value for any of the covariates were removed. For analysis of paracetamol use for fever in the first year of life, 103,284 children were included from 46 centres in 20 countries. For analysis of current paracetamol use, 103,268 children were included from 46 centres in 20 countries. §Paracetamol use was referred as high if it happened once or more per month in the past 12 months; medium if it happened once or more in the past 12 months; and none if it never happened in the past 12 months. Severe asthma symptoms were: sleep disturbed by wheezing one or more nights per week; wheezing severe enough to limit speech; or four or more attacks of wheezing in the past 12 months.

Table 3: Association between paracetamol use and severe asthma symptoms in children aged 6–7 years

How can we help manage asthma?

• Tell practitioners when to suspect a relevant subphenotype
• In addition to those above, define the phenotypes of patients with
  – Increased arginase activity (test with serum level?)
  – Decreased SOD activity (test with serum level?)
  – Decreased lipoxin levels (test with EBC or sputum?)
  – Goblet cell metaplasia (test with CT?)
  – Abnormal YLK 40, P-selectin and/or protein von Willebrand (test with EBC or sputum?)
  – Increased chymase positive mast cells (test with CT, EBC or sputum or EBC?)
  – We have an idea about what a TH2-high and an ABPA phenotype look like, but we can study the biochemistry longitudinally and develop treatments.
Nitrite and S-nitrosothiol flux are increased in the asthmatic airway

\[ \text{NO}_2^- + \text{H}^+ \rightarrow \text{HONO} \quad (\text{pKa} \sim 3.6) \rightarrow \text{NO} \]

\[ \text{RSH} \rightarrow \text{RSNO} + \text{H}_2\text{O} \]

Hunt, *AJRCCM* 161:694, 2000

FEMO is higher and more variable in children with severe asthma than in those with mild or moderate asthma.

Hyperpolarized noble gas imaging shows focal bronchoconstriction in asthma