Asthma Pathogenesis

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Asthma is a chronic inflammatory disease of the lung which manifest as recurrent episodes of chest tightness, breathlessness, and coughing.

Airway hyperresponsiveness - main feature

Airway inflammation - (Lymph, Eosinophils)
Elevated IgE levels - some not all asthma

Airway remodeling
  - Mucus cell hyperplasia
  - Subepithelial fibrosis
  - Smooth muscle thickening
Asthma is Clinically Heterogeneous

- Age of onset - differences between early and late onset
- Allergic vs. non-allergic (atopic or non-atopic)
- Differences in nature of immune response - mixed eosinophilic/neutrophilic inflammation
- Severity - mild, moderate, severe
Etiology of Asthma?

GENES
- atopy

ENVIRONMENT

Sensitization to Aeroallergens

Inflammation

Asthma

Triggers
- Allergens
- Virus infection
- Pollutants
- Tobacco Smoke
- Irritants
Recent studies such as those showing that IL-13 blockade does not ameliorate disease in all asthmatics have suggested that other mechanisms are also involved.
CD4+ T Cells Regulation of Immunity

- IL-12
  - DC
  - Th1
    - (IFN-γ)
    - Intracellular orgs
    - IgG antibody
    - Macrophage
  - Th17
    - (IL-17A,F, IL-21)
    - PMNs
    - autoimmunity
  - Treg
    - (IL-10, TGF-b)
    - Tolerance
    - Parasites induce
    - TSLP
    - TGF-β
    - Th2
      - (IL-4, IL-5, IL-13)
      - Extracellular org
      - Parasites
      - IgE,Eos
      - Allergic disease

- TGF-B
  - IL-6
  - IL-1B
  - IL-23

- Th2
  - IL-25
  - IL-33
  - TSLP

Protects fetus

Autoimmunity
Asthma is increasing in prevalence

Asthma in the US (1960-2002): Cases up 72%

Highest rates in children and females

African American
Inner city

CDC-Mortality and Morbidity, 2011.
Asthma Prevalence Globally

300 million people worldwide

World Map of the Prevalence of Clinical Asthma

Over 10% of Western society populations are afflicted with asthma

Allergy 2004: 59: 469–478
Increases in Many Immune-Mediated Diseases

Why is asthma increasing?

GENES
atopy

ENVIRONMENT

Sensitization to Aeroallergens

Inflammation

Asthma

Triggers
- Allergens
- Virus infection
- Pollutants
- Tobacco Smoke
- Irritants
### Hygiene Hypothesis
*(lack of microbial exposure drives disease)*

**ENVIRONMENT**

<table>
<thead>
<tr>
<th>Non-developed countries</th>
<th>Developed “westernized” countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large families</td>
<td>Small family size</td>
</tr>
<tr>
<td>Rural farm homes</td>
<td>Affluent, urban homes</td>
</tr>
<tr>
<td>Intestinal flora-aerobic bacteria</td>
<td>Intestinal flora-aerobic bacteria</td>
</tr>
<tr>
<td>Low antibiotic use</td>
<td>High antibiotic use</td>
</tr>
<tr>
<td>Poor sanitation</td>
<td>Improved sanitation</td>
</tr>
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</table>

**Increased infections**

**Reduced infections**

**Non-atopic**

**Atopic Disorders**
*(asthma, eczema, and rhinitis)*

**GENES**
Associated with Protection from Allergic Asthma in Westernized Countries...

• Residence on a farm (with animals) in childhood
  Presence of farm animals (multiple European studies)

• Presence from before birth onward of a dog in the house  
  *Peds 106:1406*

• Attendance at day care in first year of life
  *Arch Ped Adol Med 156:241*

• Exposures occurring after first year of life had no or much weaker protective effects
Potential Mechanism of Protective of Microbe Exposure

DC

- IL-12
  - Th1
    - (IFN-γ)
    - Intracellular orgs
    - IgG antibody
    - Macrophage
  - Th17
    - (IL-17A,F, IL-21)
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- TGF-B
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TSLP

Protects fetus

Th2

- IL-4, IL-5, IL-13
- Extracellular org
- Parasites
- IgE, Eos
- Allergic disease

Th1

- IFN-γ

Th17

- IL-17A,F, IL-21

Treg

- IL-10, TGF-b
- Tolerance
- Parasites induce autoimmunity

Macrophage

IL-12

TGF-β

Parasites induce autoimmunity
The specific microbes which are providing protection are not known
Changes in Intestinal Colonization  
Developed vs. Developing

• Quantitative and qualitative differences are seen in early childhood patterns of bacterial colonization between the developed and developing world

- Colonization with aerobic Gram-negative bacteria tends to occur later in westernized countries

- Once colonized, infants in developed countries tend to carry the same strains chronically; infants in developing countries tend to be colonized serially with different strains

Lifestyle changes in early childhood in the developed world may influence asthma and the microbiota.

<table>
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<tr>
<th>Incidence</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Caesarian Delivery ↑</td>
<td>↓ Bifidobacteria, Bacteroides</td>
</tr>
<tr>
<td></td>
<td>↑ C. difficile</td>
</tr>
</tbody>
</table>

↑ Antibiotic Treatment

↓ Breastfeeding

↓ Bacterial Diversity

Commensal bacteria

↑ C. difficile

C. difficile, S. aureus
Allergy is associated with a decrease in species diversity, and an increase in gut clostridia species

(Sepp E, Clin Exp Allergy, 2005).
Asthma and the lung microbiota

Phyla

- **Firmicutes** (species-Staph, Clostrida, SFB) gram positive
- **Bacteroidetes** (Soil Bacteria, Bacteroides fragilis) gram negative, anaerobic

Bronchial biopsy-adult
BAL-children

Decreased bacteroides
Increased firmicutes

(Hility, 2010).
Immune programming

- Viral and bacterial infections in infancy
- Pets
- Geohelminth infection in infancy
- Foetus/child with immature immunity
- Maternal geohelminth infection
- Rural upbringing
- Rural poverty

Helminth effect: long-lasting that remains after treatment

Active immune regulation

- Migration
- Urban poverty
- Child with mature immunity
- Geohelminth infection
- Pets

Helminth effect: disappears after treatment

Genetic predisposition + Allergen → Allergic disease

- Protective factors
- Risk factors
These studies have lead to the hypothesis that changes in the gut microflora in early life may be associated with the development of asthma.
SFB colonization and Th17 responses in C57BL/6 mice

4 week old C57BL/6
Jackson Laboratories

→
No/low SFB
Low gut Th17

4 week old C57BL/6
Taconic Farms

→
High Gut Th17

(Ivanov II, Cell, 2009)
Segmented filamentous bacteria drive a potent Th17 response

- SFB, which belong to Clostridia species has a unique lifecycle and are gut trophic (obligate intestinal anaerobe, NON-cultivable)

- SFB is associated with increased severity of mouse models of autoimmune disorders (EAE, Inflammatory bowel disease, CIA)
Is SFB associated with more severe allergic asthma and IL-17A induction?

C57BL/6 Male (4 wk old)
- Jackson laboratories (SFB free)
- Taconic Farms (SFB colonized)

![Graph showing SFB expression](image)

Interesting colonization only occurs before IgA levels develop—early life

![Microscope images](image)
Mice from Taconic (+SFB) have increased airway hyperresponsiveness and BAL neutrophilia

NO CHANGES IN EOSINOPHILS or IgE LEVELS

N=6-8; *, **, P<0.001
Enhanced AHR in Taconic mice is associated with elevated IL-17A
Does transfer of SFB drive more severe allergic asthma in mice?

Taconic

Co-housed throughout

Cohoused

Jackson

Day

-21, -1

0 14 21 28 31

IP HDM IP HDM IT HDM IT HDM

AHR, BAL, Tissue qPCR

SFB Expression x 10^5

(normalized)

Jackson

Taconic

Cohoused

Intestine

Jackson (40x)

Cohoused Jackson-3 wks (40x)
Microbiota is transferable, and correlates with more severe airway disease

No difference in Th2 cytokines

N= 8 mice; *, **, P<0.05
Transfer of SFB alone enhances AHR

N=6-8 mice, **P<0.01

No difference in BAL EOS or Th2 cytokines
SFB levels decline at 12 weeks of age, while IL-17A levels persist (maturation of IgA)
Is SFB specifically associated with more severe allergic asthma?

Feces Jackson (SFB Free) + SFB-monocolonized feces

Gavage

Feces Jackson SFB-free Jackson mice

-7 0 14 21 28 31

HDM i.p. HDM i.t SAC

Yakult Central Institute for Microbiological Research, Japan
Blocking IL-17A reduces severity in SFB-colonized mice
Regulation of Th17 Cell Differentiation

DC

IL-6
TGFB
IL-1B

Ror-γ
Stat3

Naïve CD4+ T Cell

IL-23

TH17 Cell

IL-17A
IL-17F
IL-21
IL-22
SFB colonization shifts BMDC cytokine production from a tolerogenic to a Th17-promoting pattern.

These data suggest that SFB alters the phenotype of the BM cells.
SFB-induced DC phenotype persists after SFB clearance
Can DCs from SFB colonized mice drive more severe disease in SFB free mice?

Taconic Farms (SFB positive)

- BMDDC
- GM-CSF
- LPS Pulsed

adoptive transfer
i.t. Jackson
SFB Free

Jackson Laboratories (SFB free)

HDM i.t. AHR
Transferring BMDCs from SFB colonized mice to SFB free (Jackson) mice induces more severe AHR

N=6-8, *P<0.01
How does gut SFB colonization exacerbate asthma?

SFB Clostrida colonization

Intestinal Tract

Allergen

DC

Th2

Severe Asthma

Th17

IL-23

Red blood cells
White blood cells
Platelets
Bone marrow

Lung
How does gut SFB colonization exacerbate asthma?

SFB Clostrida colonization

Intestinal Tract

Allergen

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DC

Th2

IL-23

Th17

Lung
SFB colonized mice express higher levels of SAA1 in terminal ileum

RNA seq heatmap Sunghee Oh, CCHMC
SFB-colonized mice have higher serum SAA

- SAA- is a serum apolipoprotein known as an acute phase protein. It is elevated in obesity, and CA.

- SAA instillation drives IL-17A and neutrophilic inflammation

- SAA induces IL-23 production in rheumatoid synoviocytes (Migita, 2010).
SAA1 binds to several innate immune receptors

- SAA1
- FPR11
- TLR2,4
- CD36

DC

- IL-23

Th2

Th17

Severe Asthma
Differential chromatin remodeling of *Fprl1* in BM cells at the \( \sim \) transcriptional start site

Targeted exp-holds up in whole genome analysis

No differences were observed in TLR gene activation between TAC and JAC BMDDCs

Studies underway to look at Fprl1 In vivo
Fprl1

• Fprl1 is the formyl peptide receptor like-1.

• Fprl1 is a chemoattractant for peripheral PMNs, monocytes, and T cells (JEM, 2000).

• Fprl1 mediates the chemotactic activity of SAA for human phagocytic cells (JEM, 1999).
SAA1 induction of IL-23 in TAC BMDDC is partially FPR11 dependent
• As we measured the effects of SAA1 on the whole bone marrow—the question arises as to whether different populations of cells are altered by SFB colonization
Hematopoiesis

LT-HSC → ST-HSC → MPP

CLP → T cells

CLP → MEP → B cells, NK cells

CLP → MEP → Platelets

CLP → MEP → Red blood cells

CLP → MEP → Monocytes, macrophages

CLP → MEP → Granulocytes

CMP → GMP → Granulocytes
SFB colonization drives GMP and CLP expansion in bone marrow
Hematopoiesis

LT-HSC → ST-HSC → MPP → CLP → MEP → GMP

- T cells
- B cells, NK cells
- Platelets
- Red blood cells
- Monocytes
- Macrophages
- Granulocytes
Intestinal SFB colonization exacerbates asthma

SFB Clostrida colonization

Intestinal Tract

Th17

IL-23

Conditioning to express Fprl1

SAA1

Bone Marrow

IL-23

Allergen

DC

Th2

SA

Lung

Th17

IL-23
Is SFB important in human disease?

• SFB has recently been shown to be present in the gut of humans—but only before the age of 3 (Yin et al, ISME, 2012).

• Our data suggest the possibility that early life epigenetic changes occur following SFB colonization that may confer Th17 and the severe asthma phenotype.

• These results support the observation that early life events or exposures drive changes in immune status—which can have life-long effects.
Environment

Rural living

GI
Balanced microbial community composition

TGFB/IL-10

Lung
Treg

Tolerance

Genes

High Fat Diet

Maternal Atopy
Cesarean birth
Antibiotics

Intestinal Dysbiosis

Bone marrow precursors
IL-23

DC

Autoimmune Disease

Severe Asthma

Th17

Th2
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