Basic Science/ Toxicology

Risk factors for asthma
Examples of toxicology/basic science studies on environmental exposures leading to interventions

- Microbes
- Allergen
- Air pollution

Hygiene Hypothesis

Reduced risk of atopy:
- associated with increasing numbers of older siblings;
- Early entry into day-care settings;
- BCG vaccination;
- early-life measles and enteric infections;
- reduced use of antibiotic therapy;
- and growing up on a farm

Hygiene Hypothesis
- induction by microbes of IFN-g
- other manifestations of TH1-type immunity,
CpG

- unmethylated cytosine-guanine dinucleotides in prokaryotic DNA
- suppressed and methylated in eukaryotic DNA
- Induce murine B cells to proliferate and secrete Ig

Toll-like receptors (TLRs)

- Highly conserved family of innate immune receptors for molecules present in pathogens but not in the host
- TLRs specific for extracellular pathogens such as LPS are expressed at the cell surface
- TLRs specific for intracellular pathogens are expressed within innate immune cells, and are specific for nucleic acids
TLR-9

• Receptor for CpG motifs in viral/bacterial DNA
• Expressed only on plasmacytoid dendritic cells and B cells in humans
• Binding with CpG DNA leads to Th1 cytokine milieu
• Synthetic oligodeoxynucleotides containing CpG motifs ('CpG ODN') have been developed as TLR9 agonists
Phase 1 trial: inhaled 1018 ISS in 54 healthy subjects. Well tolerated at escalating doses. Measurable increases in the expression of cytokines.

Phase 2a trial 1018 ISS in 30 mild asthmatics. Four weekly doses of either 1018 ISS or placebo. Substantial and statistically significant pharmacological activity induction of genes associated with a reprogrammed immune response.

Dynavax.com

---

**Immunotherapy with a Ragweed–Toll-Like Receptor 9 Agonist Vaccine for Allergic Rhinitis**

Peter S. Creticos, M.D., John T. Schroeder, Ph.D., Robert G. Hamilton, Ph.D., Susan L. Balcer-Wiley, M.P.H., Andrea P. Khattak-Meena, M.D., Robert Lindblad, M.D., Harvey Li, M.D., Ph.D., Robert Coffman, Ph.D., Vicki Seyfert, Ph.D., Joseph J. Eiden, M.D., Ph.D., David Bodek, M.B., Ch.B., and the Immune Tolerance Network Group


**Methods**

We conducted a randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of AEC1, a ragweed polyclonal antisera, conjugated to a plasmid containing a synthetic immunogen consisting of DNA (AEC1) with 2S in adults who were allergic to ragweed. Patients received six weekly injections of either the vaccine or placebo vaccine before the first ragweed season and were measured during the next two ragweed seasons.
Allergen Avoidance as a Treatment for Perennial Rhinitis and Asthma.

Figure 1: Prevalence of sensitisation to cat allergens and of IgG antibody to Fel d 1 (≥125 units/mL) for six equal-exposure groups for cat allergens.

Figure 2: Correlation between IgG and IgG4 antibodies to Fel d 1 serum samples from sensitised (closed circles) and non-sensitised (open circles) individuals.

Platts-Mills et al., *Lancet* 2001; 357: 752–71

Contrast Between Exposure to Dust Mite or Cat Allergens and the Relevant Immune Responses

The dashed line indicates the approximate value of 22 μg Fel d 1/g floor dust or the presence of a cat.
High dose allergen stimulation of T cells induces expansion of IFN-gamma+ T cells, apoptosis of CD4+IL-4+ T cells and T cell anergy.

HDM-allergic donor PBMC cultured for 14 days with different concentrations of HDM extract (1, 10 and 100 ug/ml)

Increased CD4+IFN-gamma+ and CD8+IFN-gamma+ T cell numbers were observed in high allergen concentration cultures compared with low concentration

No differences in CD4+IL-4+ and CD8+IL-4+ T cell numbers

Proportion of apoptotic cells increased with allergen concentration

HDM-induced proliferation was decreased in high allergen concentration cultures


The modified Th2 response?

A weak T_{H2} response fails to induce a mature germinal center.
Direct switching from \( \mu \) to \( \varepsilon \)
Antibody response is low and nonresponsive to subsequent antigen exposure
Little clonal expansion or formation of a B memory population.
Long-lived plasma cell population without effective production of B memory cells.

A strong T_{H2} response induces a mature germinal center.
Indirect switching from \( \gamma \) to \( \varepsilon \)
Survival of IgE memory cells compromised due to:
  - Defective membrane form of \( \varepsilon \) chain preventing rescue
  - CD23-mediated IgE suppression by FDCs that interface with B cells in the germinal center
FIGURE 3. KLH-specific IgG4 and IgE correlation by dose group. Correlation between anti-KLH IgG4 and IgE Abs from day 33 nasal lavage for low-dose exposure (●), medium-dose exposure (○), and high-dose exposure (▲) subjects. Both axes on a log scale from 1 to 100 U/ml, with broken axes to include undetectable (0) levels.
Modified Th2 Responses At High Dose Exposures To Allergen; Using An Occupational Model.

+689 employees exposed to rats at work on six pharmaceutical sites across the UK
+ blood sample and questionnaire

Jeal et al., Am J Respir Crit Care Med. 2006 Apr 7

Role of allergen in regulating IgE responses

2 overlapping proteins in FelD1 induce IL-10 or IFN-γ identified

Epitopes important for regulation of the immune response to cat allergen
1. Enhanced induction of IL-10 and IFN-γ by these epitopes in T cell cultures from tolerant (IgGhigh IgEneg) subjects
2. Increased T cell reactivity to these epitopes in cat-allergic patients receiving conventional IT;
3. Failure of these epitopes to induce either T cell proliferation or IFN-γ in cultures from atopic patients with high IgE to cat

Prophylaxis of atopy and asthma in children

Objectives: To reduce the frequency of allergic sensitization and expression of allergic disease (in particular atopic asthma) amongst children at high genetic risk of asthma/atopy.

Patrick G. Holt, University of Western Australia, Perth AUS

Peter Sly, University of Western Australia, Perth, AUS

Bengt Bjorksten, Karolinska Institute, Stockholm, SWE

Ulrich Wahn, Berlin Humboldt University, Germany

Richard Loh, Princess Margaret Hospital for Children, Perth, AUS

Immune Tolerance Network
Prophylaxis of atopy and asthma in children

Approach:
• 200 children aged between 18 and 30 months
• history of AD or food allergy but without sensitization to inhalants
• mother or father or sibling history of atopy (AD, allergic rhinitis or asthma)
• sublingual drops, containing either allergen (house dust mite, timothy grass & cat) or placebo daily for 12 months
• followed for three years after finishing treatment.

• Outcomes: 50% reduction in IgE and Th2 responses to allergens given and 50% reduction in asthma.
Inhaled DEP-induces Oxidative Stress in Murine Lungs

BALB/c mice exposed to saline, OVA, or OVA plus DEP by aerosolized inhalation daily for 10 days

Assays for carbonyl protein content (A) and lipid hydroperoxides (B) in the lung homogenates

Antioxidants Block DEP-induced Cytokine Release

- NAC (10 mM) on GM-CSF release by human bronchial epithelial cells (16HBE14o) treated for 24 h with DEP (10 µg/cm²) and organic extracts of DEP

Pretreatment With Anti-oxidant Blocks DEP-enhanced Allergic Antibody Responses

BALB/c mice exposed to saline, OVA, or OVA plus DEP by aerosolized inhalation daily for 10 days.

NAC or BUC given by i.P. Injection 0.5 h before inhalation on every exposure day.

Metabolic Pathways for Detoxification of DEP Chemicals

**PATHWAY 1**

**BIFUNCTIONAL INDUCERS**
- e.g. PAH
  - Drive phase I and phase II enzymes
  - AHR
  - AHR/ARNT
  - XRE
  - Phase I enzymes
    - e.g. CYP1A1

**PATHWAY 2**

**MONOFUNCTIONAL INDUCERS**
- e.g. Quinones
  - Drive phase II enzymes
  - OXIDATIVE STRESS
    - Reactive Oxygen Species
    - ARE
    - Phase II enzymes

Hierarchical oxidative stress model in response to diesel exhaust particles

Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study

Frank D Gilliland, Yu-Fen Li, Andrew Saxon, David Diaz-Sanchez

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>GSTM1 Null (n=14)</th>
<th>GSTM1 Present (n=5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>6.9 (2.6–24.3)</td>
<td>8.9 (4.3–18.8)</td>
<td>0.40</td>
</tr>
<tr>
<td>DEP and allergen</td>
<td>106.6 (8.8–534.8)</td>
<td>49.8 (14.2–79.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Difference</td>
<td>102.5 (1.0–510.5)</td>
<td>45.5 (1.5–60.6)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>2.9 (1.3–5.9)</td>
<td>2.8 (1.9–6.7)</td>
<td>0.96</td>
</tr>
<tr>
<td>DEP and allergen</td>
<td>16.9 (2.9–27.6)</td>
<td>9.8 (3.1–19.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Difference</td>
<td>14.0 (0.2–24.7)</td>
<td>7.4 (1.2–12.3)</td>
<td>0.02</td>
</tr>
</tbody>
</table>


**Figure 1**

AHRCCM Articles in Press. Published on October 5, 2006 as doi:10.1184/compmedsci.20506.14240C

**Glutathione-S-Transferase M1 and P1 Prevent Aggravation of Allergic Responses by Secondhand Smoke**

Frank D. Gilliland, M.D., Ph.D.,1 Yu-Fen Li, Ph.D.,1 Harry Gong, Jr., M.D.,1 David Diaz-Sanchez, Ph.D.1
Hierarchical oxidative stress model in response to diesel exhaust particles


ASTHMA

Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City


Background: We recently reported that antioxidant supplementation with vitamins C and E mitigated ozone related decline in forced expiratory flow (FEF25-75) in 158 asthmatic children in an area with high ozone exposure in Mexico City.

Methods: A study was undertaken to determine whether deletion of glutathione S-transferase M1 (GSTM1) null genotype, a gene involved in response to oxidative stress, influences ozone related decline in FEF25-75 and the benefit of antioxidant supplementation.

Results: GSTM1 null children receiving placebo had significant ozone related decrements in FEF25-75 (percentage change per 50 ppb of ozone 2.9 [95% CI –5.2 to –0.6], p=0.01); GSTM1 positive children did not. Conversely, the effect of antioxidants was stronger in children with the GSTM1 null genotype.

Conclusions: Asthmatic children with a genetic deficiency of GSTM1 may be more susceptible to the deleterious effects of ozone on the small airways and might derive greater benefit from antioxidant supplementation.
NQO1 over-expression decreases IL-8 production in DX-stimulated BEAS-2B cells

Ritz et al., Am J Physiol Lung Cell Mol Physiol. 2006

Sulforaphane inhibits DEP increased cytokine expression by NHBECs

Ritz et al., AJP-Lung 2006
Effect of sulforaphane on DEP-enhanced IgE production

Wan & Diaz-Sanchez., J. Immunol, 2006

Dietary Sulforaphane increases phase II expression in nasal cells
The Integrated Approach

Identification of modulator of asthma

Elucidation of mechanism and pathways

Intervention/therapeutic strategy