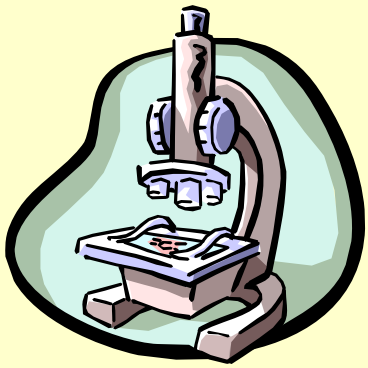
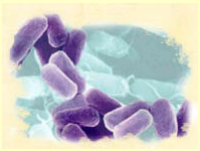
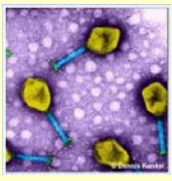
Basic Science/ Toxicology



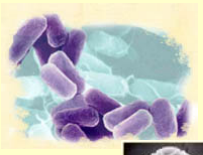


Risk factors for asthma



Examples of toxicology/basic science studies on environmental exposures leading to interventions

- Microbes



- Allergen

- Air pollution

Hygiene Hypothesis

Reduced risk of atopy:

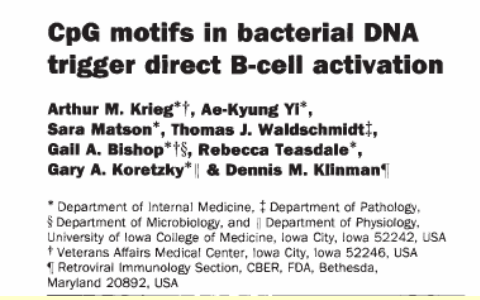
associated with increasing numbers of older siblings;
Early entry into day-care settings;
BCG vaccination;
early-life measles and enteric infections;
reduced use of antibiotic therapy;
and growing up on a farm

Hygiene Hypothesis

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

CpG

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



CpG motifs in bacterial DNA trigger direct B-cell activation

Arthur M. Krieg*†, Ae-Kyung Yi*, Sara Matson*, Thomas J. Waldschmidt‡, Gail A. Bishop*†§, Rebecca Teasdale*, Gary A. Koretzky*‖ & Dennis M. Klinman¶

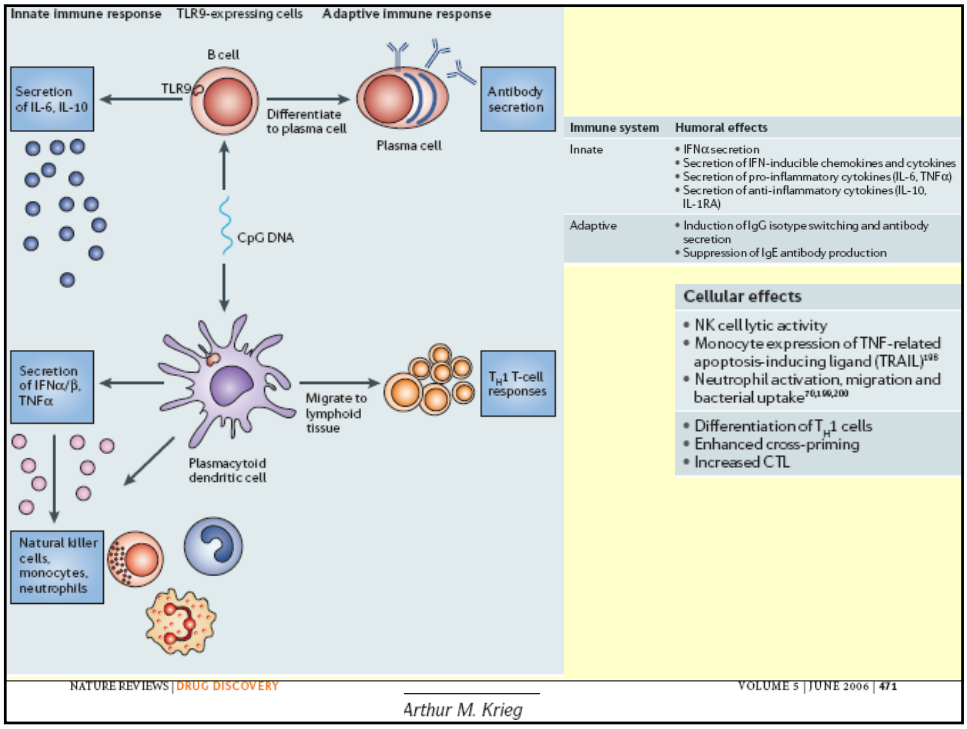
* Department of Internal Medicine, ‡ Department of Pathology, § Department of Microbiology, and ‖ Department of Physiology, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA
† Veterans Affairs Medical Center, Iowa City, Iowa 52246, USA
¶ Retroviral Immunology Section, CBER, FDA, Bethesda, Maryland 20892, USA

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 mileu
- Synthetic oligodeoxynucleotides containing CpG motifs ('CpG ODN') have been developed as TLR9 agonists



Asthma/allergy		
Monotherapy	Mouse: asthma, allergic rhinitis, conjunctivitis, allergic aspergillosis. Guinea pig: RSV sensitization Monkey: asthma. All reviewed in REF. 143	• AVE 7279 (Phase I; sanofi-aventis/Coley) • AVE 0675 (preclinical; sanofi-aventis/Coley) • 1018 ISS (Phase II; Dynavax) • IMO (preclinical; Novartis/Idera)
Vaccines	Mouse: asthma, allergy immunotherapy and atopic dermatitis	• B-class ODN 1018 ISS conjugated to protein[137,138] (Phase III; Dynavax)

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

ORIGINAL ARTICLE

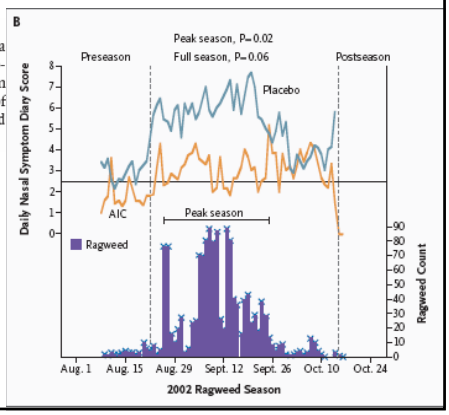
Immunotherapy with a Ragweed–Toll-Like Receptor 9 Agonist Vaccine for Allergic Rhinitis

Peter S. Creticos, M.D., John T. Schroeder, Ph.D., Robert G. Hamilton, Ph.D., Susan L. Balcer-Whaley, M.P.H., Arouna P. Khattignavong, M.D., Robert Lindblad, M.D., Henry Li, M.D., Ph.D., Robert Coffman, Ph.D., Vicki Seyfert, Ph.D., Joseph J. Eiden, M.D., Ph.D., David Broide, M.B., Ch.B., and the Immune Tolerance Network Group

N Engl J Med 2006;355:1445-55.

METHODS

We conducted a randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of Amb a 1, a ragweed-pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC) in 25 adults who were allergic to ragweed. Patients received six weekly injections of the AIC or placebo vaccine before the first ragweed season and were monitored during the next two ragweed seasons.



Racila and Kline

J ALLERGY CLIN IMMUNOL
DECEMBER 2005

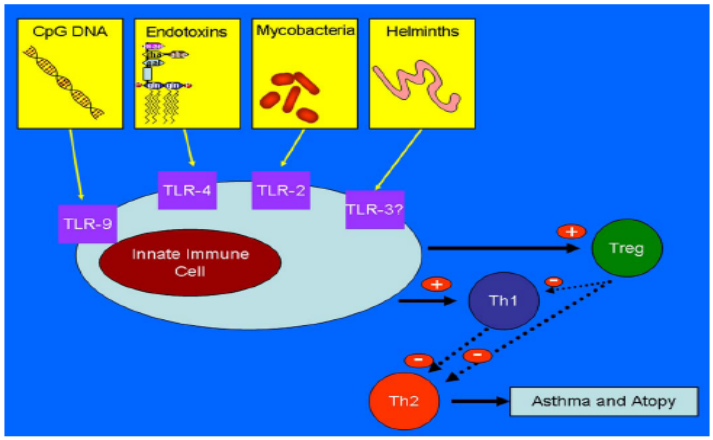
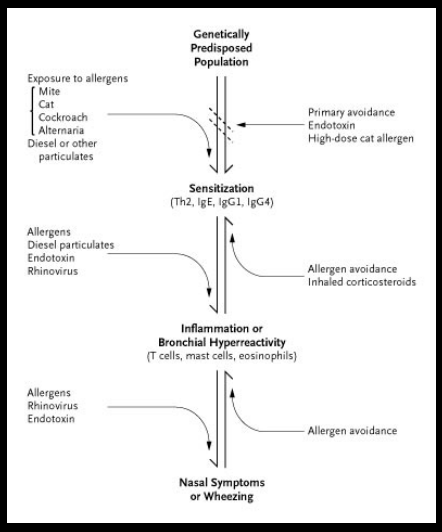


FIG 1. Microbes, parasites, and microbial products induce protection against asthma and atopic inflammation. Interactions with TLRs on innate immune cells, including DCs, alveolar macrophages, B cells, and others, are known to be an important link in these innate immune pathways that lead to enhanced T_H1, suppressed T_H2, and induced T-regulatory responses. Both T_H1 and regulatory T lymphocyte (Treg) cells can suppress T_H2 cells and their mediators.

Allergen Avoidance as a Treatment for Perennial Rhinitis and Asthma.



Platts-Mills, T. A.E. N Engl J Med 2003;349:207-208

The NEW ENGLAND JOURNAL of MEDICINE

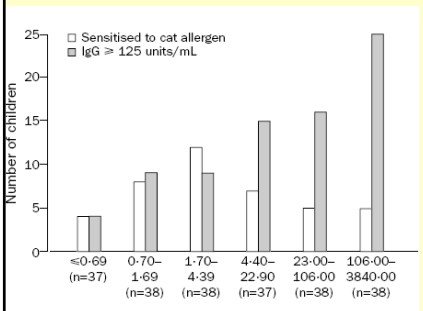


Figure 1: **Prevalence of sensitisation to cat allergens and of IgG antibody to Fel d 1 ≥125 units/mL for six equal-exposure groups for cat allergen**

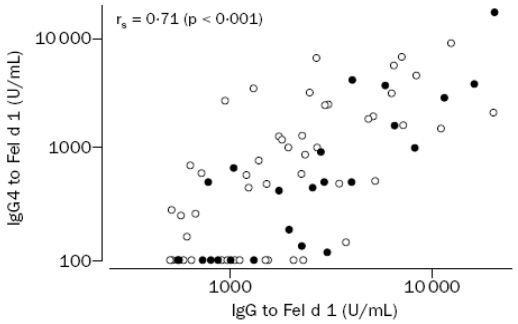
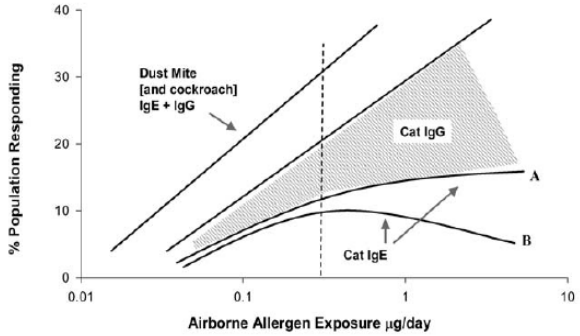


Figure 2: **Correlation between IgG and IgG4 antibodies to Fel d 1 serum samples from sensitised (closed circles) and non-sensitised (open circles) individuals**

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

	Class I: dust mite (Cockroach)	Class II: cat (Rat and dog)
Characteristics of major allergen	Der p 1 is a cysteine protease[a]	Fel d 1 is not an enzyme (but can selectively induce IL-10) (homology with CCSP[b])
Particles	Faecal: 20–40 μm in diameter	"Dander": 2–20 μm in diameter
Airborne	Only airborne transiently during and after disturbance	Allergen remains airborne
Exposure	≤10 ng/day	Up to 1 μg/day[c]
Immune response	Increased prevalence and titer of IgE Ab with increased exposure	"Tolerance" becomes more common with increased exposure, up to 20% produce IgG/IgG4 without IgE

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



The dashed line indicates the approximate value of 20 μg Fel d 1/g floor dust or the presence of a cat.

Editorial Clin Exp All April 2002, Custis et al. Clin Ex All 2003

High dose allergen stimulation of T cells induces expansion of IFN-gammaT 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

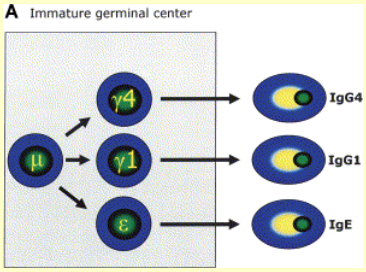
No differences in CD4+IL-4+ and CD8+IL-4+ T cell numbers

Proportion of apoptotic cells increased with allergen concentration

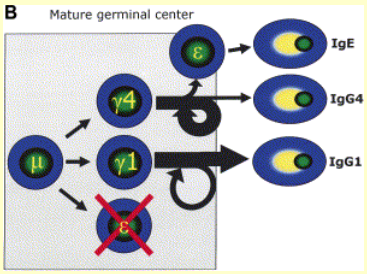
HDM-induced proliferation was decreased in high allergen concentration cultures

•Gardner et al., Int Arch Allergy Immunol. 2004 Jan;133(1):1-13.

The modified Th2 response?



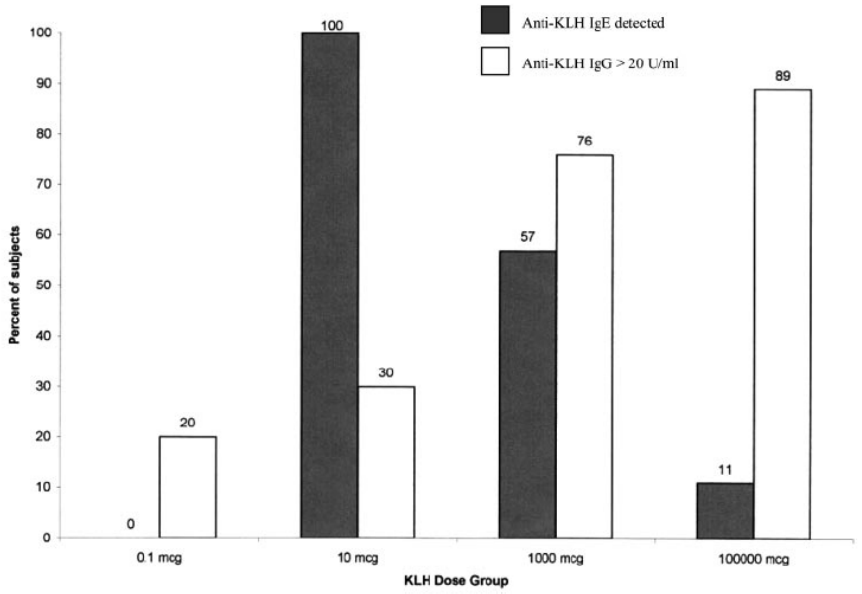
A weak T_H2 response fails to induce a mature germinal center.
Direct switching from μ to ε
Antibody response is low and nonresponsive to subsequent antigen exposure
Little clonal expansion or formation of a B memory population.
Long-lived plasma cell population without effective production of B memory cells.



A strong T_H2 response induces a mature germinal center.
Indirect switching from γ4 to ε
Survival of IgE memory cells compromised due to:

- Defective membrane form of ε chain preventing rescue
- CD23-mediated IgE suppression by FDCs that interface with B cells in the germinal center

HIGH-DOSE INDUCTION OF NASAL ALLERGY TOLERANCE



Riedl et al., *The Journal of Immunology*, 2005, 174: 7440–7445.

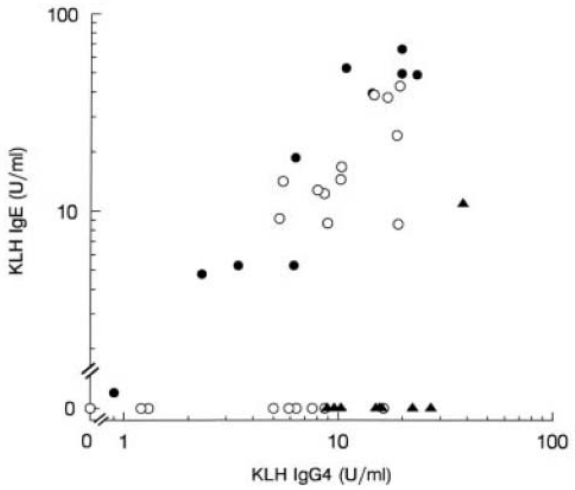
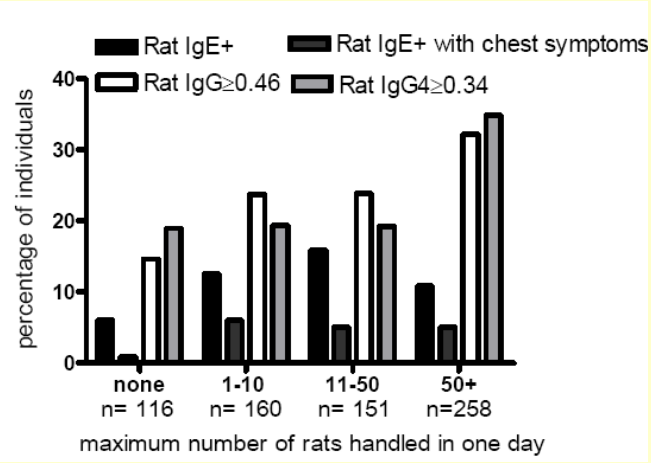


FIGURE 3. KLH-specific IgG4 and IgE correlation by dose group. Correlation between anti-KLH IgG4 and IgE Abs from day 33 nasal lavage for low-dose exposure (●), medium-dose exposure (○), and high-dose exposure (▲) subjects. Both axes on a log scale from 1 to 100 U/ml, with broken axes to include undetectable (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

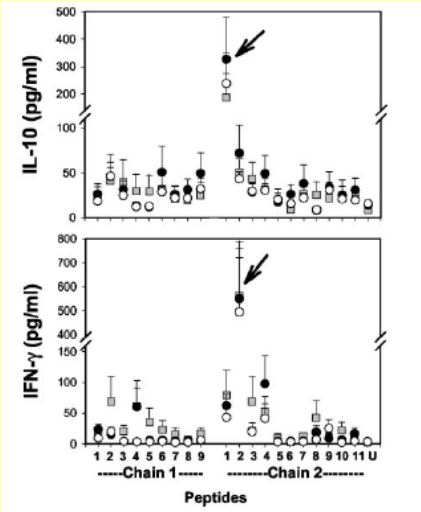
Jeal et al., Am J Respir Crit Care Med. 2006 Apr 7

Role of allergen in regulating IgE responses

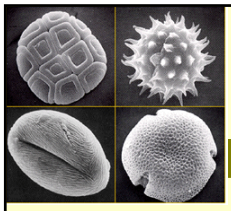
2 overlapping proteins in FelD1 induce IL-10 or IFN-γ identified

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



Reefer et al., *The Journal of Immunology*, 2004, 172: 2763–2772.



Response to Allergens

Intrinsic properties of allergen

Hypoallergenic

Allergen dose

High dose

Genetics

High risk

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.

Components of Air Pollution

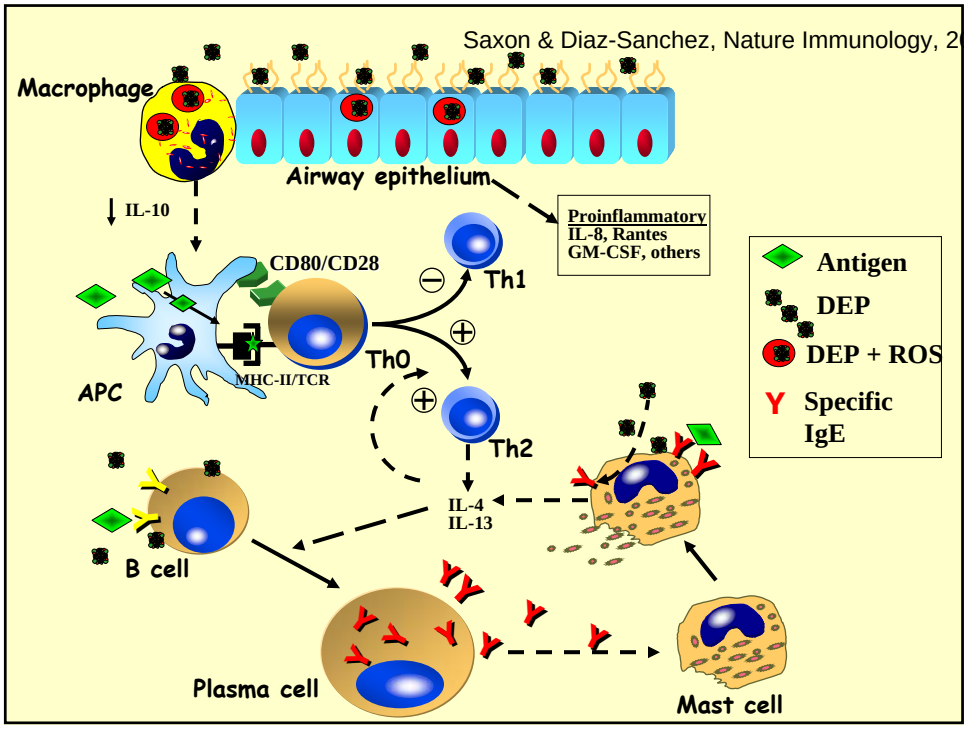
A. Primary-secondary pollutants
  (i) Primary: pollutants emitted directly into the atmosphere (eg, SO_2, some NO_x species, CO, PM)
  (ii) Secondary: pollutants that form in the air as a result of chemical reactions with other pollutants and gases (eg, ozone, NO_x, and some particulates)
B. Indoor-outdoor pollutants
  (i) Indoor pollutants
    (a) Sources: cooking and combustion, particle resuspension, building materials, air conditioning, consumer products, smoking, heating, biologic agents
    (b) Products: Combustion products (eg, tobacco and wood smoke), CO, CO_2, SVOC (eg, aldehydes, alcohols, alkanes, and ketones), microbial agents and organic dusts, radon, manmade vitreous fibers
  (ii) Outdoor pollutants
    (a) Sources: industrial, commercial, mobile, urban, regional, agricultural, natural
    (b) Products: SO_2, ozone, NO_x, CO, PM, SVOC
C. Gaseous-particulate pollutants
  (i) Gaseous: SO_2, NO_x, ozone, CO, SVOC (eg, PAH, dioxins, benzene, aldehydes, 1,3-butadiene)
  (ii) Particulate: coarse PM (2.5-10 μm; regulatory standard = PM_{10}), fine PM (0.1-2.5 μm; regulatory standard = $PM_{2.5}$); ultrafine PM (<0.1 μm; not regulated)

NO_x, Nitrogen oxides; *SVOC*, specific volatile organic compounds.

Health effects of air pollution

Editor: Jonathan A. Bernstein, MD[a]
Contributors: Neil Alexis, PhD,[b] Charles Barnes, PhD,[c] I. Leonard Bernstein, MD,[a] Jonathan A. Bernstein, MD, Andre Nel, MD, PhD,[d] David Peden, MD,[b] David Diaz-Sanchez, PhD,[d] Susan M. Tarlo, MB, BS,[e] and P. Brock Williams, PhD[c]

J Allergy Clin Immunol 2004;114:1116-23

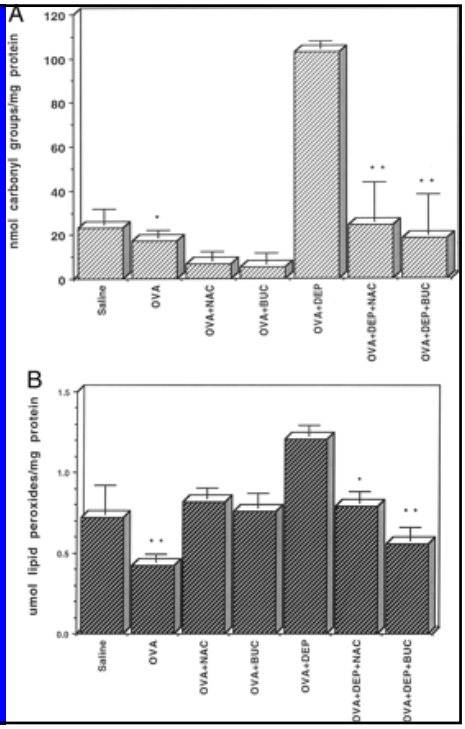


Inhaled DEP-induces Oxidative Stress in Murine Lungs

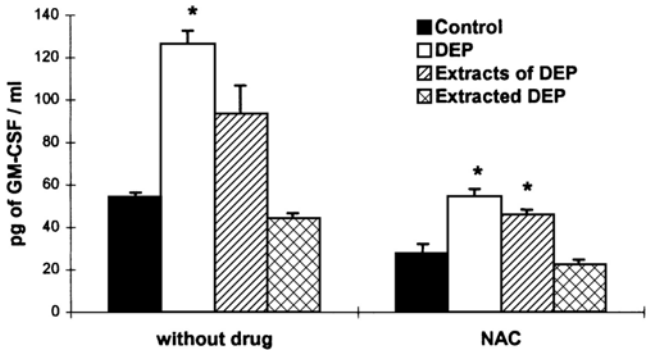
BALB/c mice exposed to saline, OVA, or OVA plus DEP by aerosolized inhalation daily for 10 days

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

Whitekus MJ et al., J. Immunol 168(5):2560-2567, 2002



Antioxidants Block DEP-induced Cytokine Release



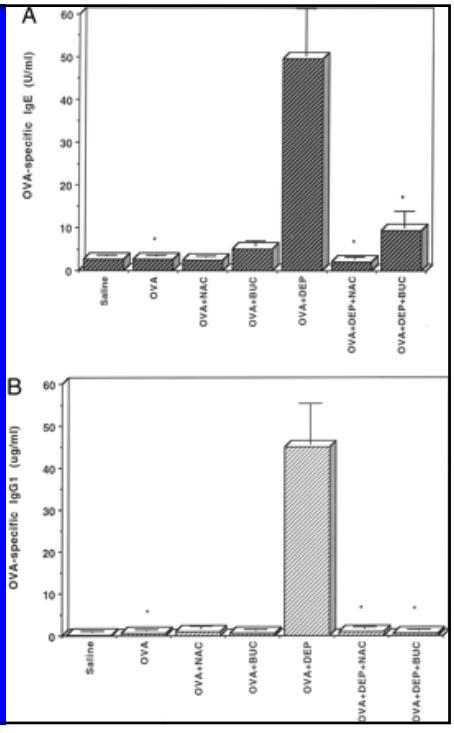
- NAC (10 mM) on GM-CSF release by human bronchial epithelial cells (16HBE14o) treated for 24 h with DEP (10 µg/cm2) and organic extracts of DEP
- **Boland S et al.,** Am J Physiol Lung Cell Mol Physiol 278:L25-32, 2000

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

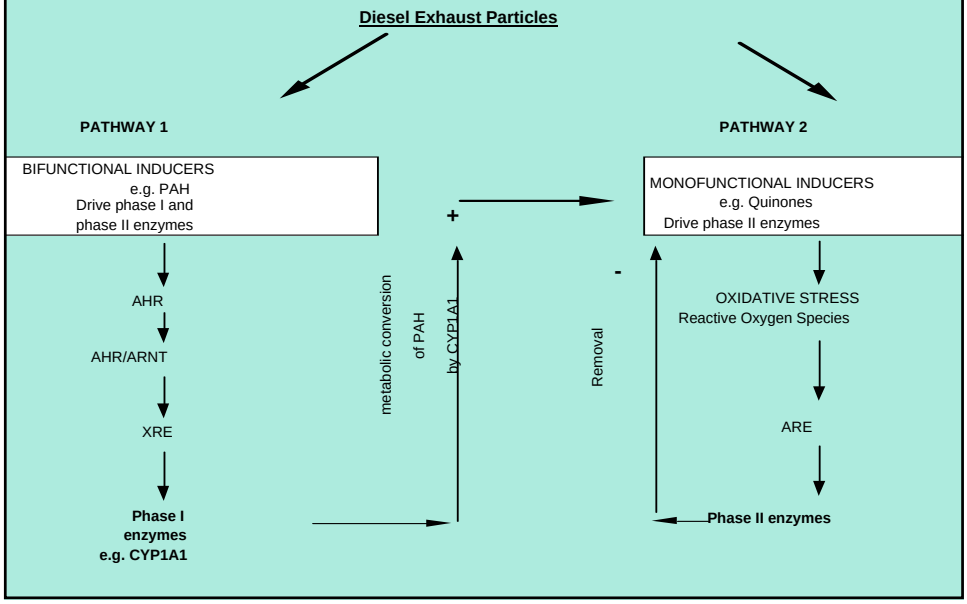
BALB/c mice exposed to saline, OVA, or OVA plus DEP by aerosolized inhalation daily for 10 days.

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

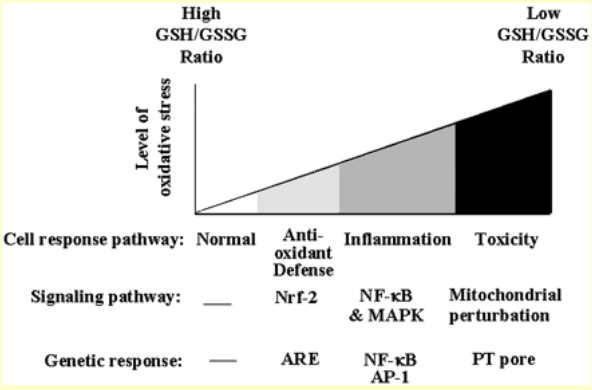
Whitekus MJ et al., J. Immunol 168(5):2560-2567, 2002.



Metabolic Pathways for Detoxification of DEP Chemicals



Hierarchical oxidative stress model in response to diesel exhaust particles



Xiao GG, Wang M, Li N, Loo JA, Nel AE. *J Biol Chem*. 2003

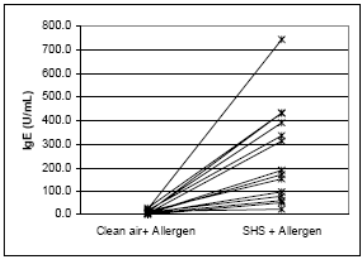
Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study

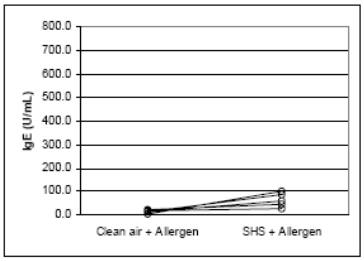
Frank D Gilliland, Yu-Fen Li, Andrew Saxon, David Diaz-Sanchez

Lancet 2004; 363: 119–25

	GSTM1		
	Null (n=14)	Present (n=5)	p
IgE			
Clean air and allergen	6·9 (2·6–24·3)	8·9 (4·3–18·8)	0·40
DEP and allergen	106·6 (8·8–534·8)	49·8 (14·2–79·4)	0·15
Difference	102·5 (1·0–510·5)	45·5 (–1·5–60·6)	0·03
Histamine			
Clean air and allergen	2·9 (1·3–5·9)	2·8 (1·9–6·7)	0·96
DEP and allergen	16·9 (2·9–27·6)	9·8 (3·1–19·0)	0·08
Difference	14·0 (–0·2–24·7)	7·4 (1·2–12·3)	0·02

Figure 1.





Legend: * *GSTM1* null o *GSTM1* present

AJRCCM Articles in Press. Published on October 5, 2006 as doi:10.1164/rccm.200509-1424OC

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.[1,2], Henry Gong, Jr., M.D.[1], David Diaz-Sanchez, Ph.D.[3]

ASTHMA

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

I Romieu, J J Sienra-Monge, M Ramírez-Aguilar, H Moreno-Macías, N I Reyes-Ruiz, B Estela del Río-Navarro, M Hernández-Avila, S J London

Thorax 2004;**59**:8–10

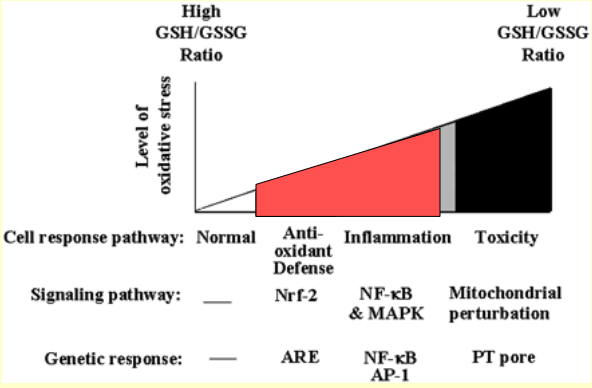
See end of article for authors' affiliations

Correspondence to: Dr I Romieu, Instituto Nacional de Salud Publica, 655 Avenida Universidad, Col. Santa Maria Ahuacatitlán, 62508 Cuernavaca, Morelos, México; iromieu@correo.insp.mx

Received 31 March 2003
Accepted 13 August 2003

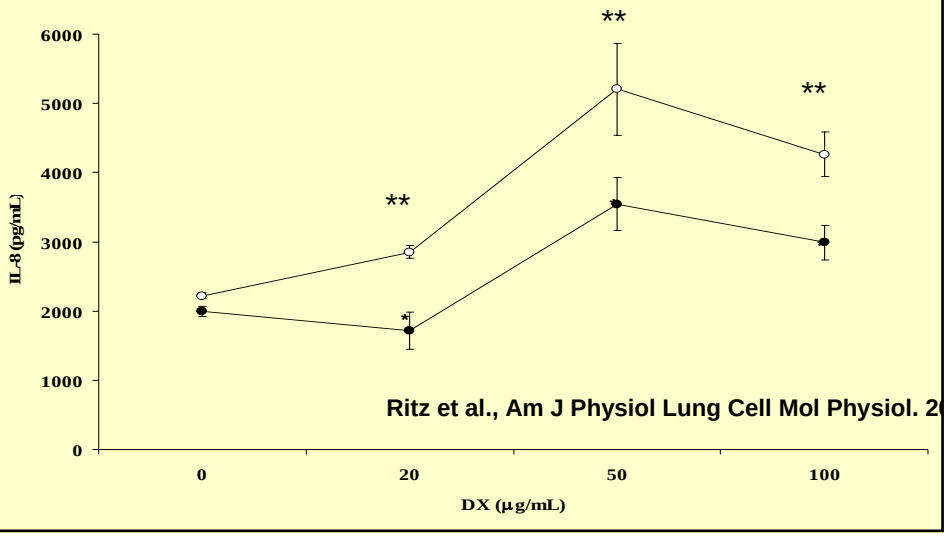
Background: We recently reported that antioxidant supplementation with vitamins C and E mitigated ozone related decline in forced expiratory flow ($FEF_{25–75}$) in 158 asthmatic children in an area with high ozone exposure in Mexico City.
Methods: A study was undertaken to determine whether deletion of glutathione S-transferase M1 (*GSTM1* null genotype), a gene involved in response to oxidative stress, influences ozone related decline in $FEF_{25–75}$ and the benefit of antioxidant supplementation.
Results: *GSTM1* null children receiving placebo had significant ozone related decrements in $FEF_{25–75}$ (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.

Hierarchical oxidative stress model in response to diesel exhaust particles



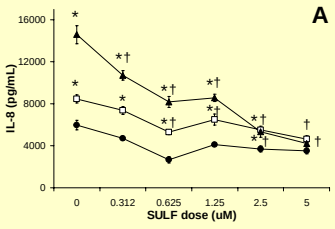
Xiao GG, Wang M, Li N, Loo JA, Nel AE. *J Biol Chem*. 2003

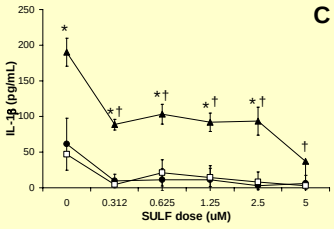
NQO1 over-expression decreases IL-8 production in DX-stimulated BEAS-2B cells

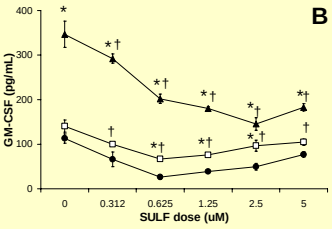


Ritz et al., Am J Physiol Lung Cell Mol Physiol. 2(

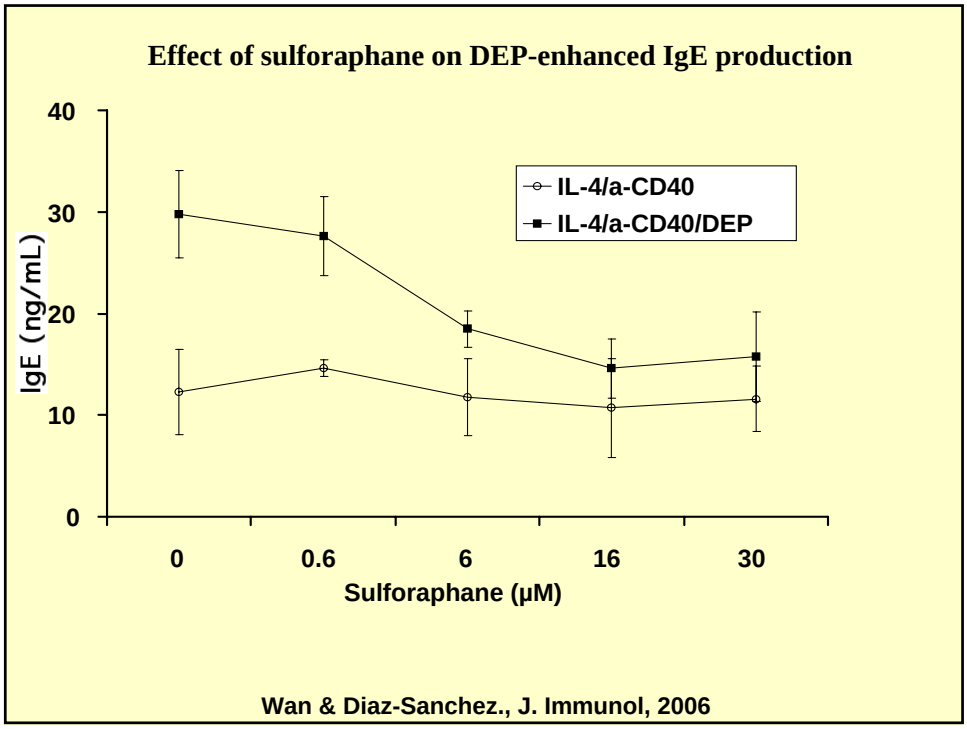
Sulforaphane inhibits DEP increased cytokine expression by NHBECs

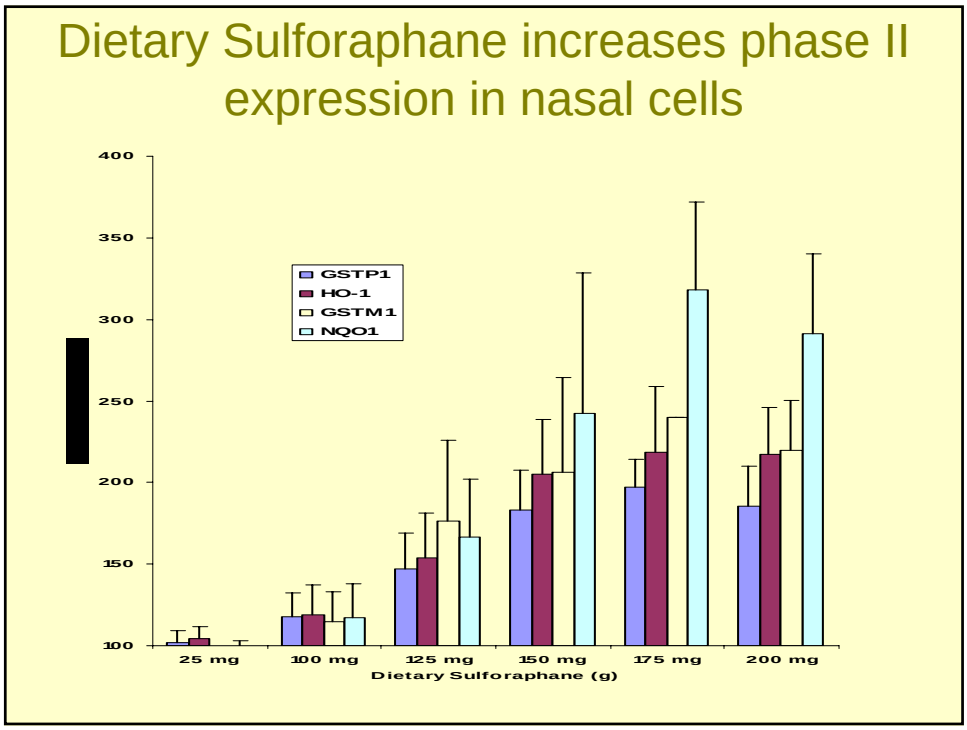


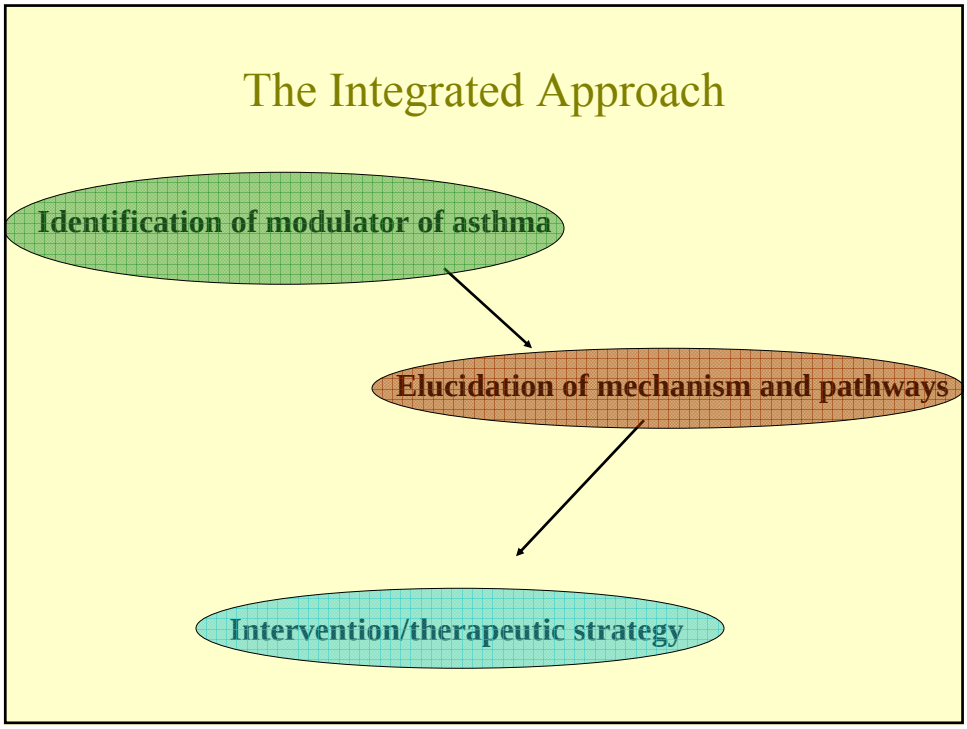




Ritz et al., AJP-Lung *2006*


Effect of sulforaphane on DEP-enhanced IgE production
IgE (ng/mL)
40
30
20
10
0
IL-4/a-CD40
IL-4/a-CD40/DEP
0
0.6
6
16
30
Sulforaphane (μM)
Wan & Diaz-Sanchez., J. Immunol, 2006


Dietary Sulforaphane increases phase II expression in nasal cells
400
350
300
250
200
150
100
GSTP1
HO-1
GSTM1
NQO1
25 mg
100 mg
125 mg
150 mg
175 mg
200 mg
Dietary Sulforaphane (g)


The Integrated Approach
Identification of modulator of asthma
Elucidation of mechanism and pathways
Intervention/therapeutic strategy