



THE UNIVERSITY OF ARIZONA HEALTH SCIENCES
Asthma & Airway Disease
Research Center



Arizona Center for the
Biology of Complex Diseases

BIO5™

Environmental Modulation of Allergic Inflammation: The Amish Paradigm

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Arizona Center for the Biology of Complex Diseases (ABCD)

Department of Cellular and Molecular Medicine

The Bio5 Institute

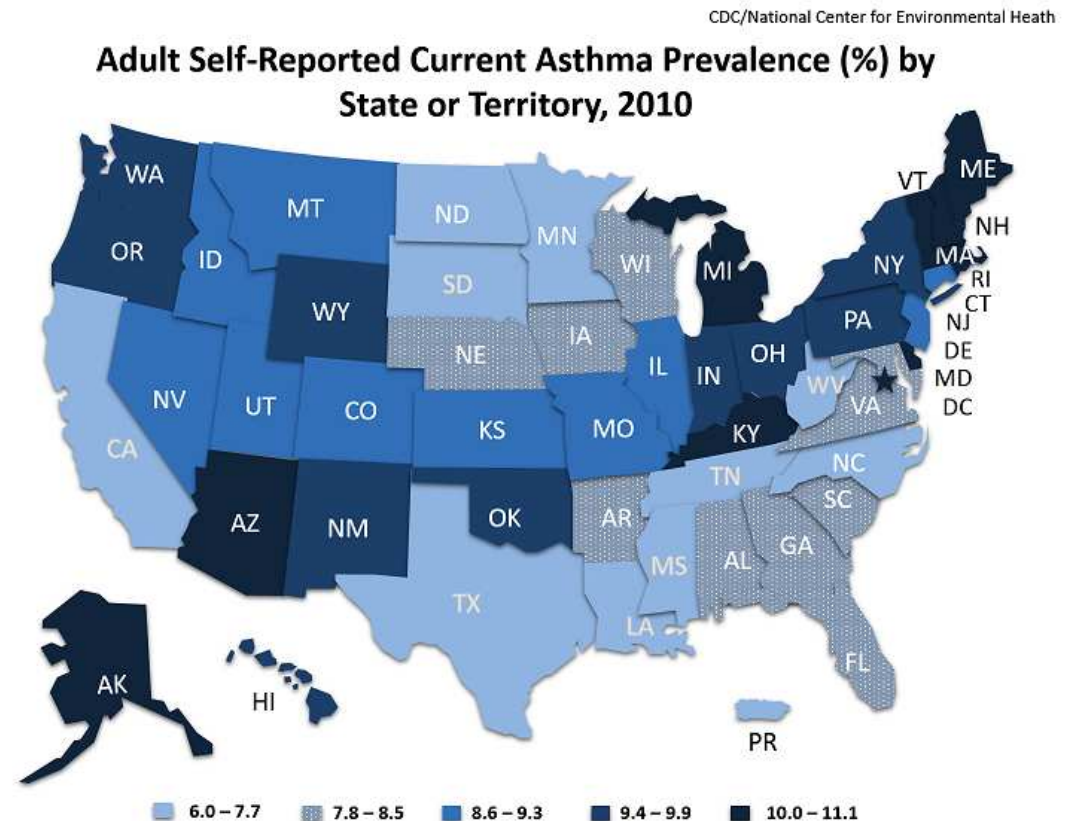
University of Arizona

A Couple of Premises

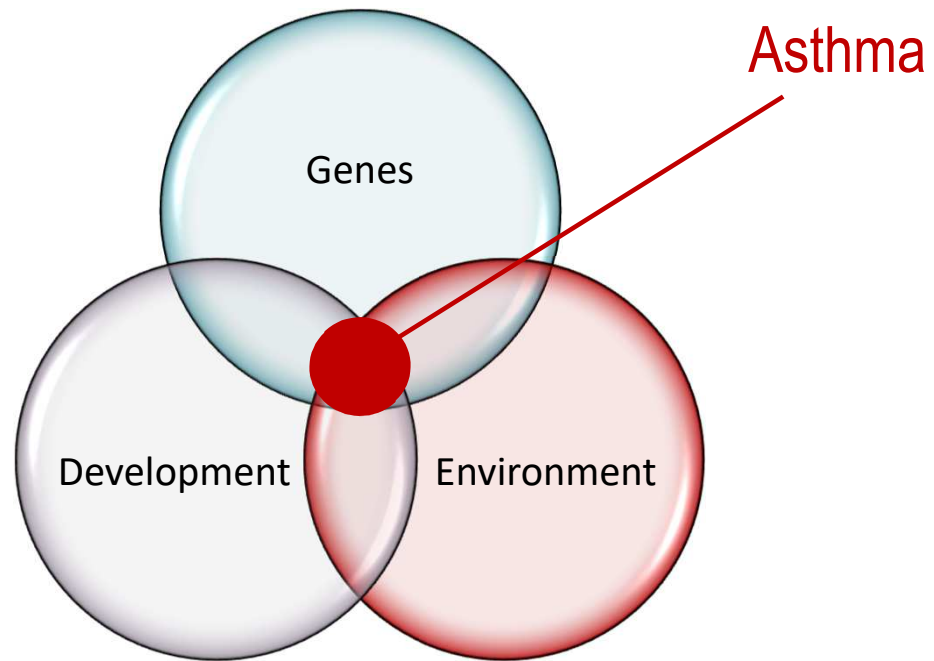
Asthma affects > 200 million people world-wide; in the US, 25.7 million people, including 7.0 million children under 18 (CDC).

The economic burden of asthma exceeds that of tuberculosis and HIV/AIDS combined.

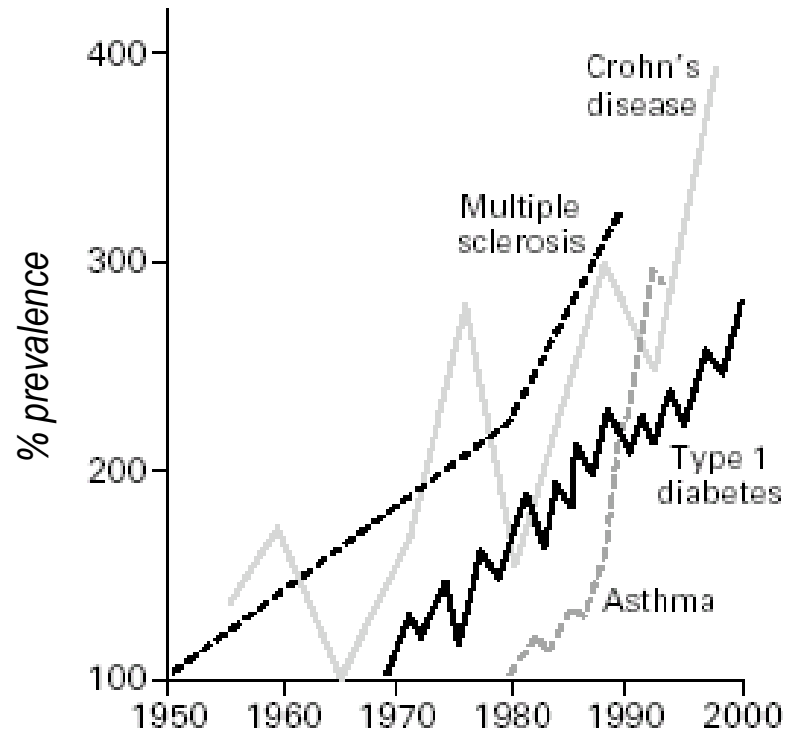
Currently, asthma can be treated but not cured.



Asthma as a complex disease



Asthma as an environmental disease



N Engl J Med, Vol. 347, No. 12 • September 19, 2002 •

Asthma and allergy are (to a large extent) environmental diseases.

*Environmental exposures are involved in the recent **increase** in asthma prevalence*

BUT: Can the environment teach us about asthma protection?



Courtesy of Erika von Mutius

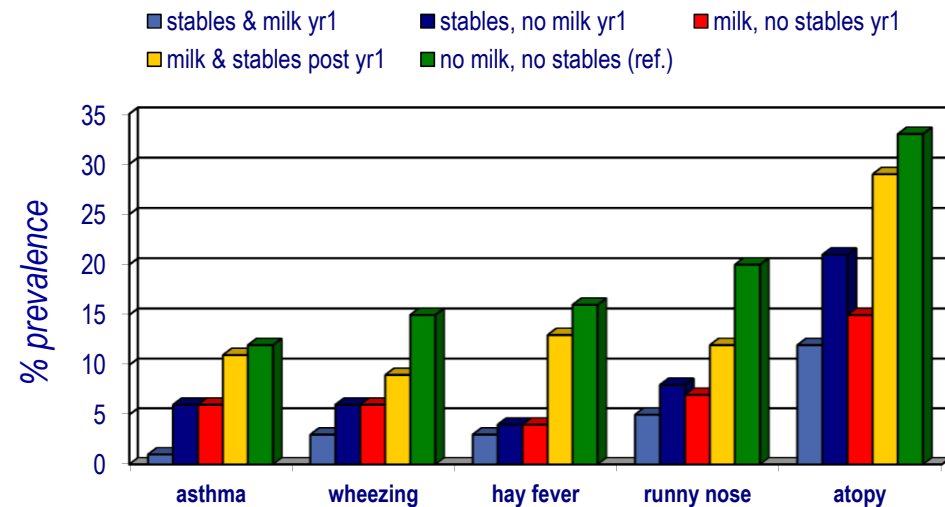
- Cows
- Hay
- Unpasteurized milk

How it all began: Farming in Alpine Europe

Lancet 2001; 358: 1129–33

Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey

Josef Riedler, Charlotte Braun-Fahrlander, Waltraud Eder, Mynda Schreuer, Marco Waser, Soyoun Maisch, David Carr, Rudi Schierl, Dennis Nowak, Erika von Mutius, and the ALEX Study Team*



Farm living: effects on childhood asthma and allergy

Erika von Mutius and Donata Vercelli

What is the impact of farm exposure on the immune response?

What are the relevant protective exposures?

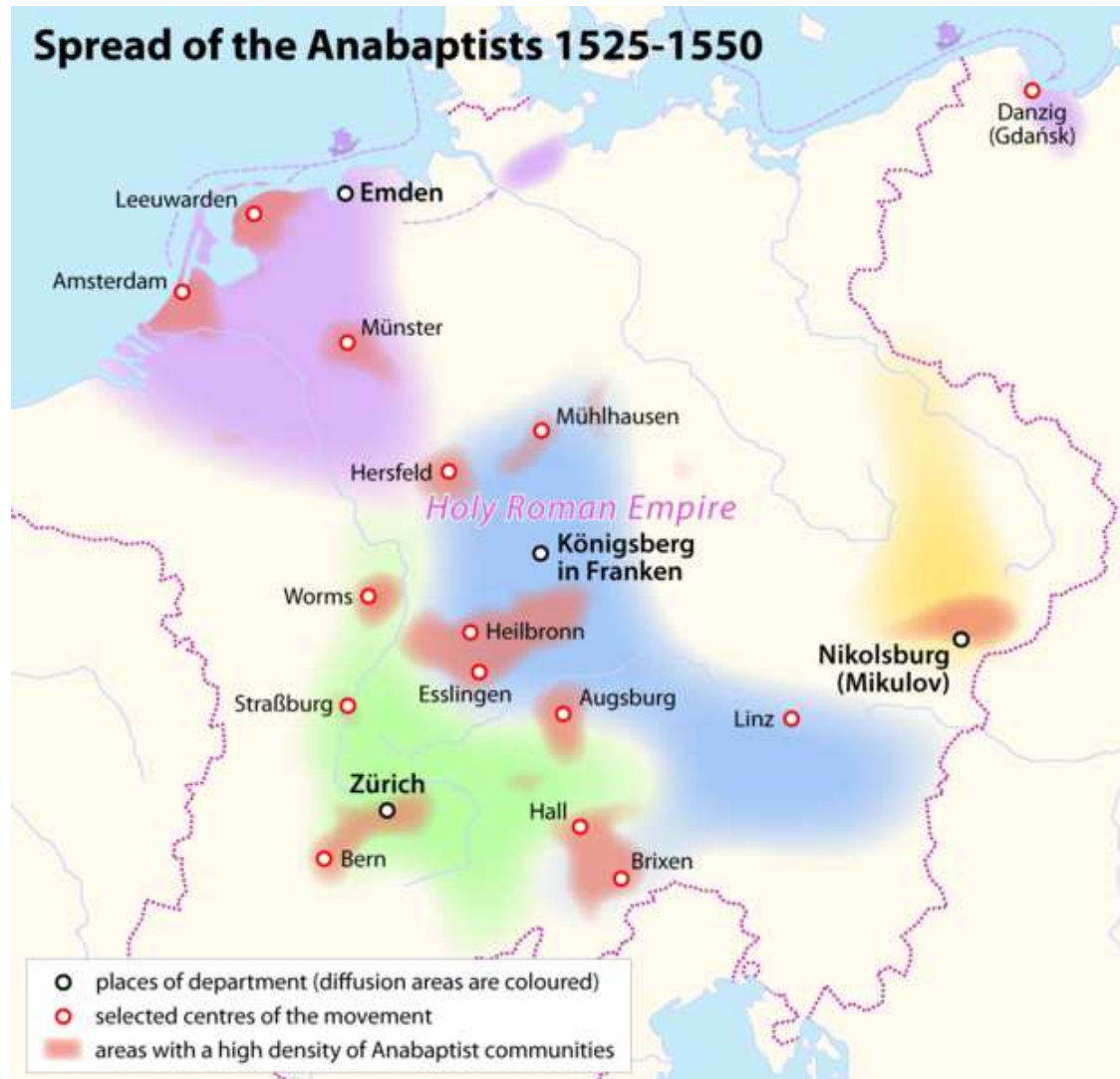
What are the mechanisms underlying protection?

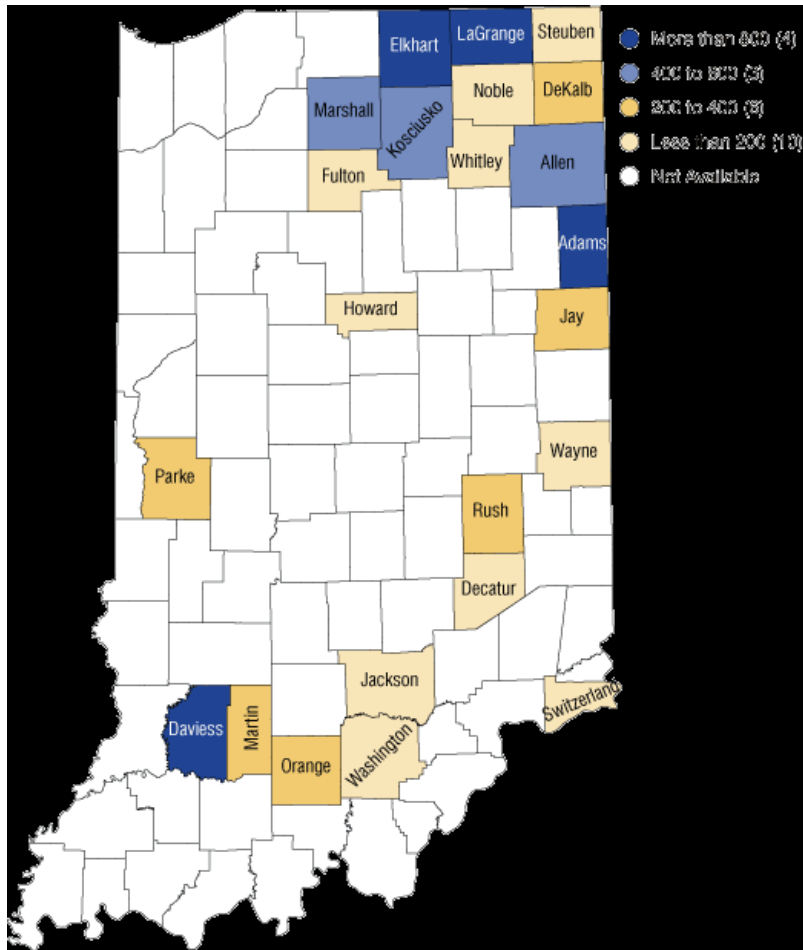
nature
REVIEWS
december 2010 volume 10 no. 12
www.nature.com/reviews

IMMUNOLOGY



Spread of the Anabaptists 1525-1550



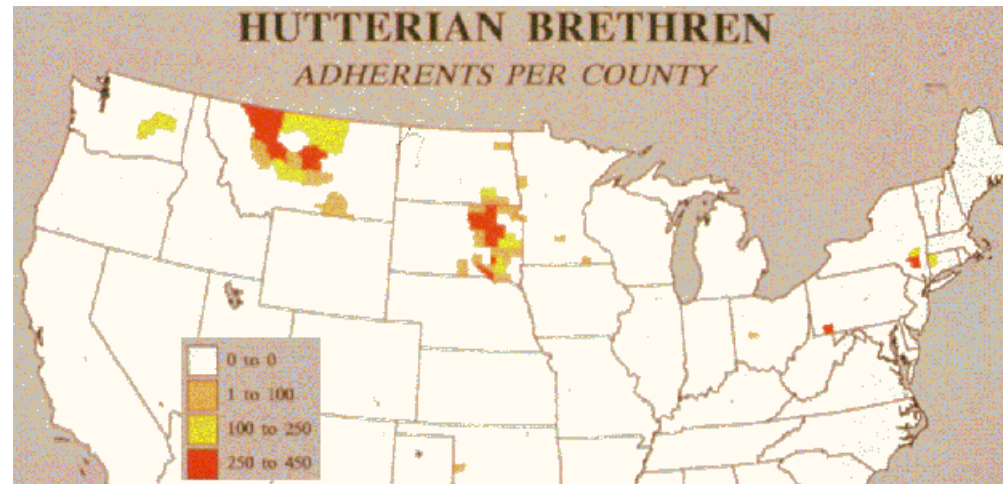


Indiana Amish

The model: human populations

German-speaking Anabaptists migrated to North America and became....

South Dakota Hutterites





The **NEW ENGLAND**
JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 4, 2016 VOL. 375 NO. 5

Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children

Michelle M. Stein, B.S., Cara L. Hrusch, Ph.D., Justyna Gozdz, B.A., Catherine Igartua, B.S., Vadim Pivniouk, Ph.D., Sean E. Murray, B.S., Julie G. Ledford, Ph.D., Mauricius Marques dos Santos, B.S., Rebecca L. Anderson, M.S., Nervana Metwali, Ph.D., Julia W. Neilson, Ph.D., Raina M. Maier, Ph.D., Jack A. Gilbert, Ph.D., Mark Holbreich, M.D., Peter S. Thorne, Ph.D., Fernando D. Martinez, M.D., Erika von Mutius, M.D., Donata Vercelli, M.D., Carole Ober, Ph.D., and Anne I. Sperling, Ph.D.

- Large sibship sizes
- Childhood vaccinations
- Diets rich in salt, fat and raw milk
- Long nursing durations
- Minimal exposure to tobacco smoke, air pollution
- No indoor pets or carpets
- Taboos against TV, radio, internet





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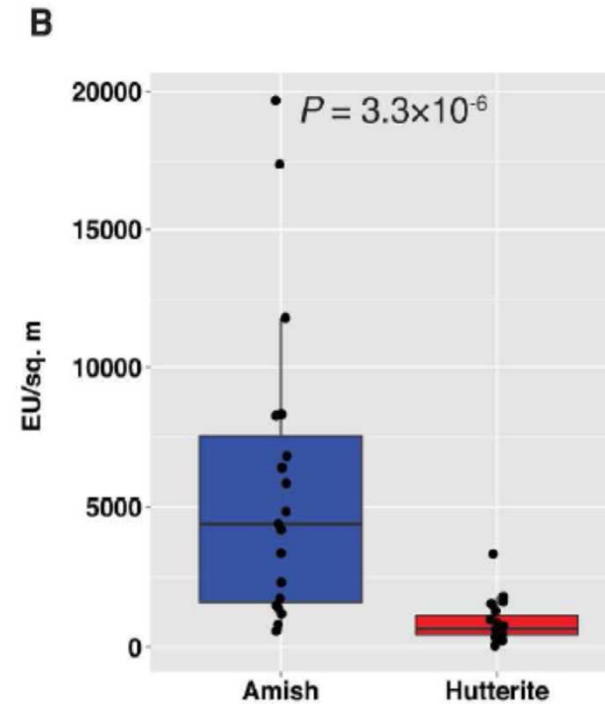
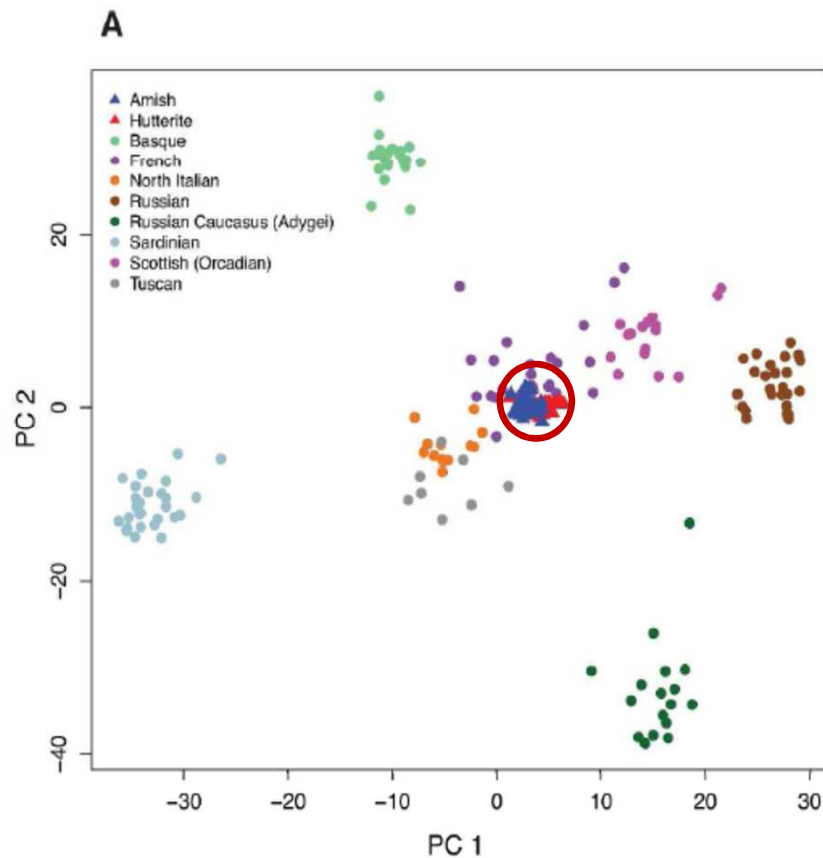
Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children

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	Prevalence of Asthma	Prevalence of Allergic Sensitization
Amish ¹	5.2%	7.2%
Hutterites ²	21.3%	33.3%
¹ Holbreich et al. 2012; <i>JACI</i> 29:1671 ² Motika et al. 2011; <i>JACI</i> 128:774		

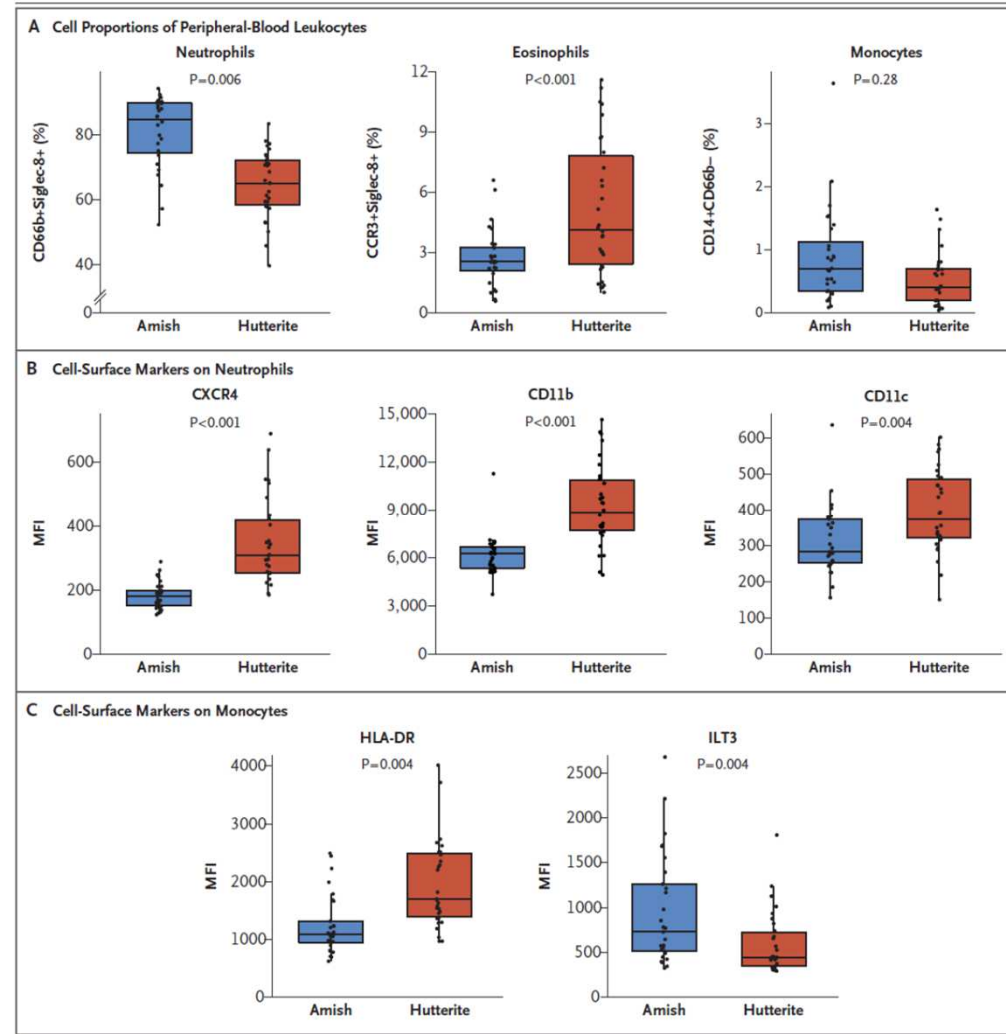


Amish and Hutterites: Comparable genetics, distinct environments



Stein et al., NEJM 2016

Immune profiles in Amish and Hutterite children

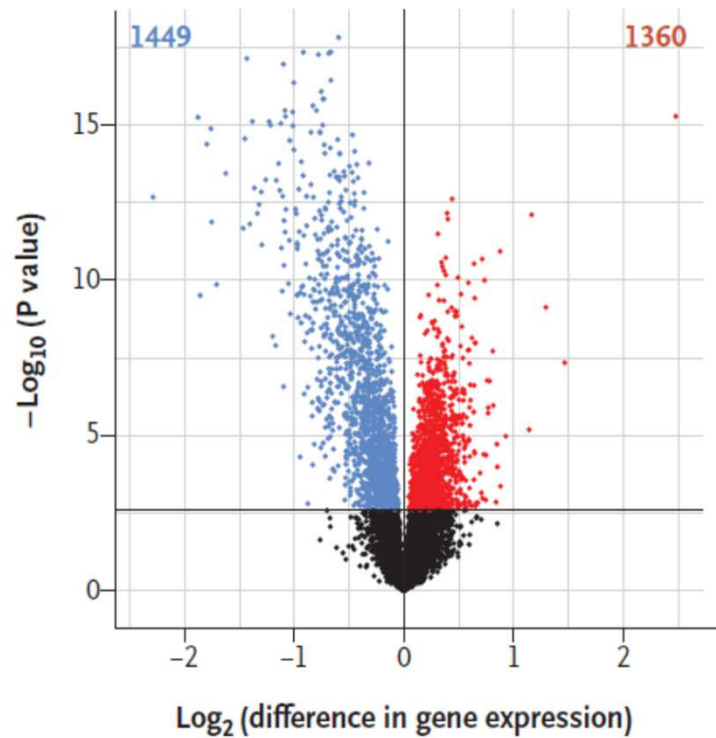


No differences in Treg cell (CD3⁺CD4⁺FoxP3⁺CD127⁻) proportions

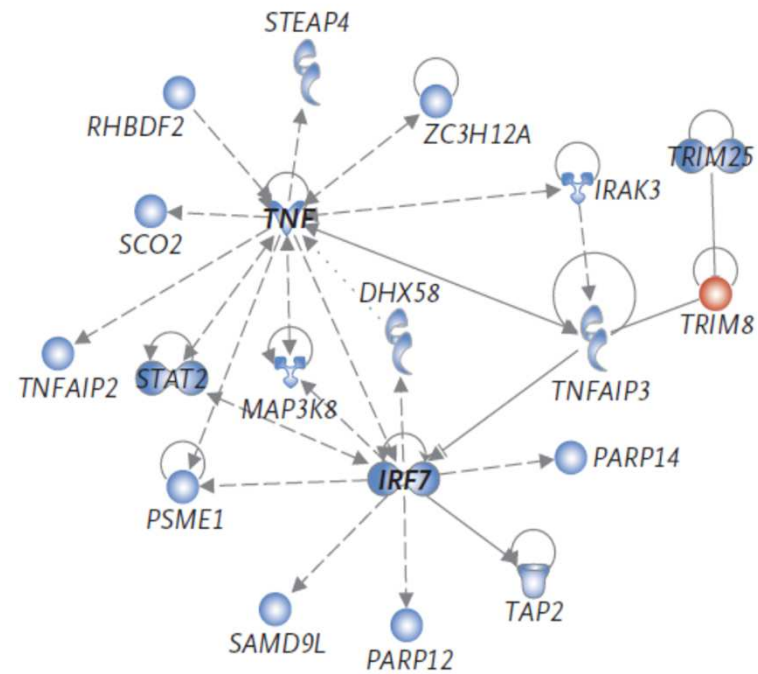
Stein et al., NEJM 2016

Immune profiles in Amish and Hutterite children

A



B



Stein et al., NEJM 2016

Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children

Marked differences in asthma prevalence despite similar genetic ancestries and lifestyles:

Relative to the Hutterites, the Amish show exceedingly low asthma rates and distinct immune profiles suggestive of profound effects on innate immunity

The environment in a tube in a mouse: Comparing indoor dust from Amish and Hutterite homes



Electrostatic dust collectors



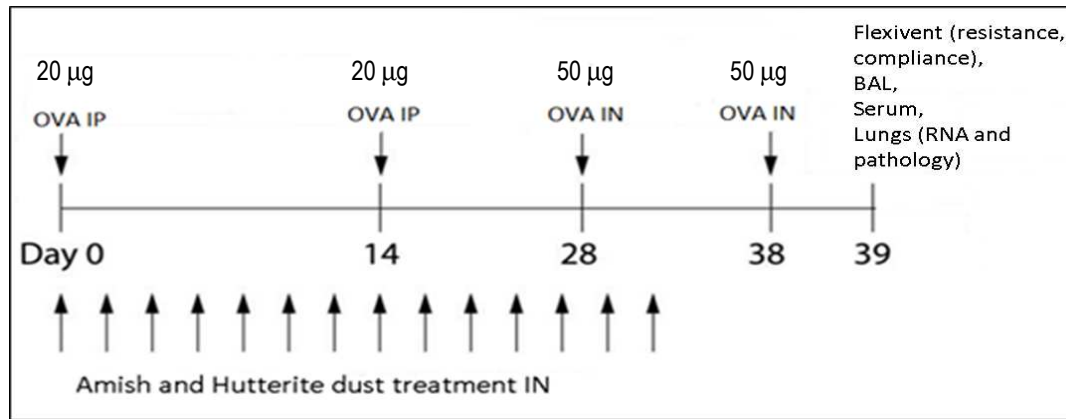
Place in children's bedrooms for 30 days



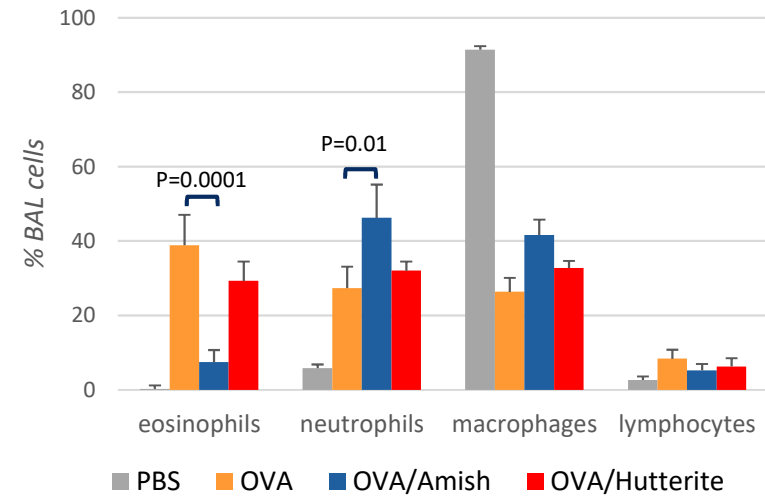
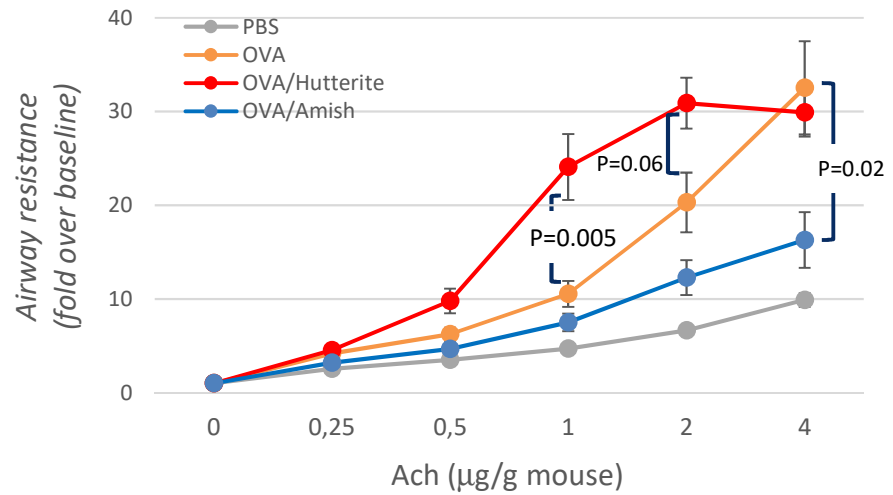
Extract dust with water



Administer dust extracts i.n. to allergen-treated mice

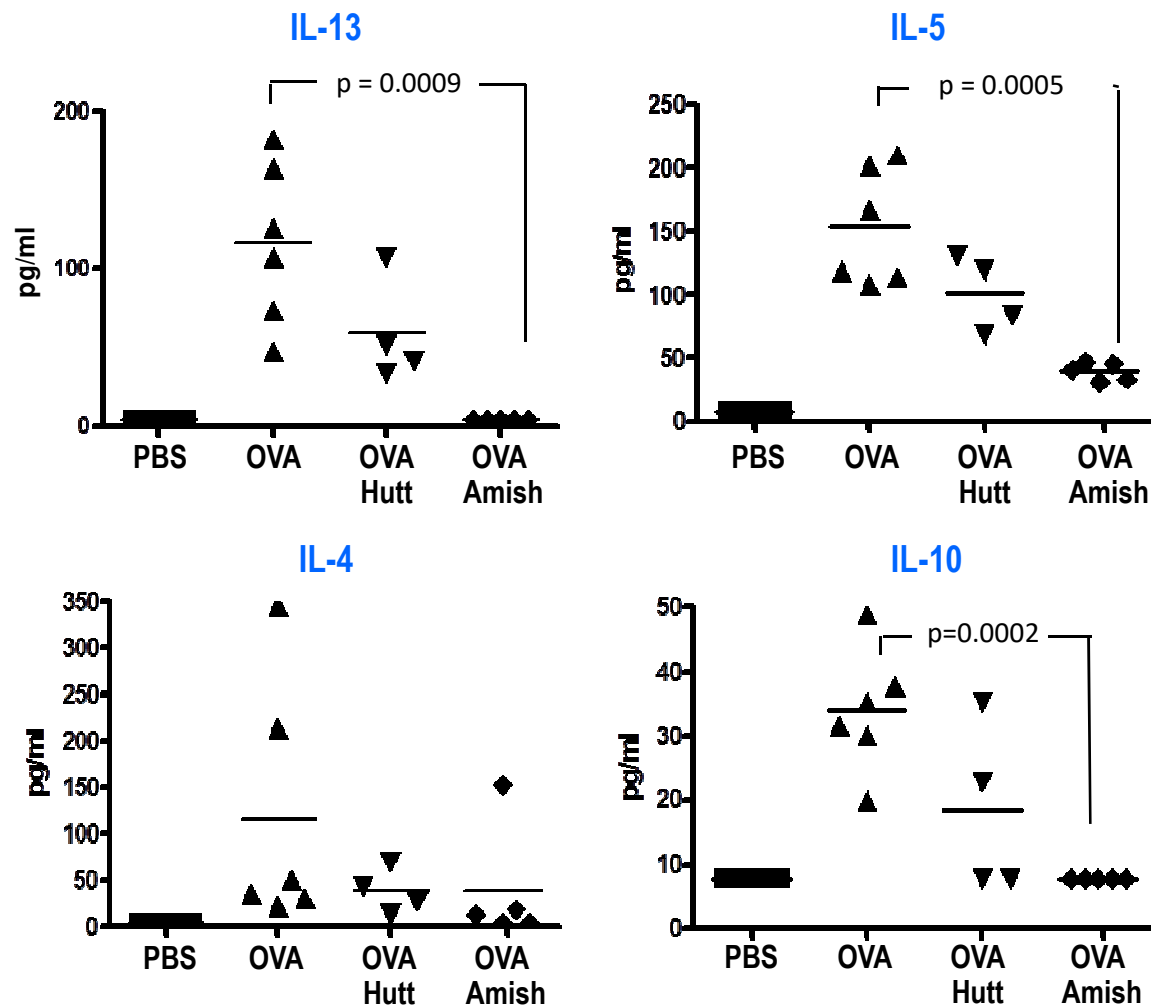


Strong suppression of OVA-induced airway hyperresponsiveness and Th2 inflammation in Amish but not Hutterite mice

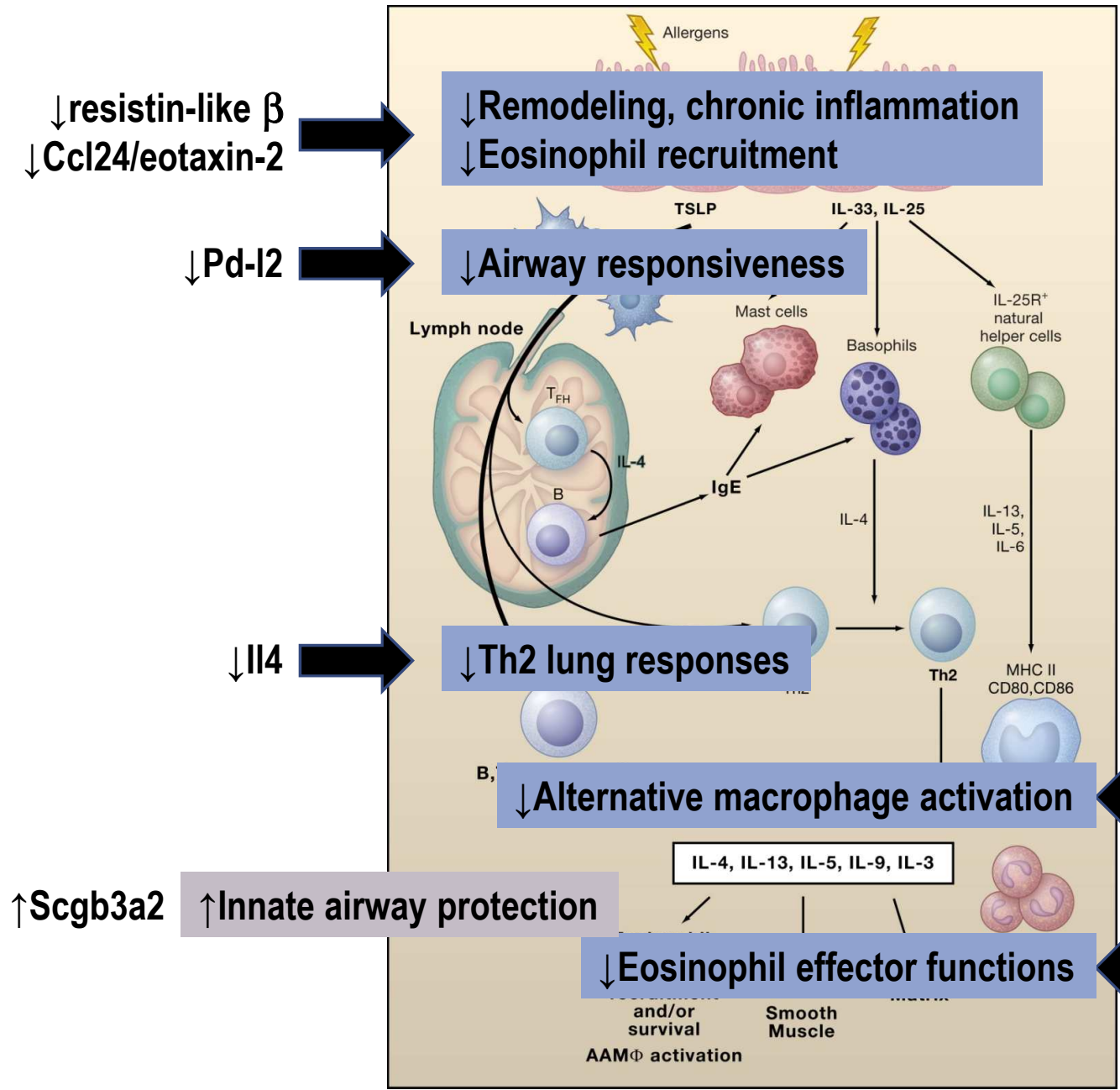


Stein et al., NEJM 2016

Strong suppression of OVA-induced BAL Th2 cytokines in Amish BALB/c mice



Profound suppression of OVA-induced lung gene expression in Amish mice



↓ resistin-like β
↓ Ccl24/eotaxin-2

↓ Pd-I2

↓ Il4

↑ Scgb3a2 ↑ Innate airway protection

↓ Remodeling, chronic inflammation
↓ Eosinophil recruitment

↓ Airway responsiveness

↓ Th2 lung responses

↓ Alternative macrophage activation

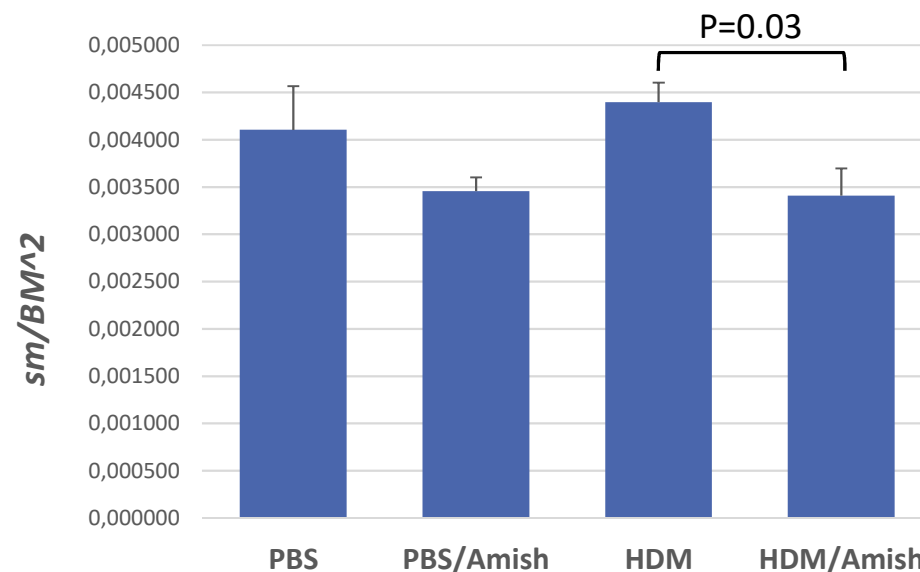
↓ Eosinophil effector functions

↓ Chi3I4/YKL40
↓ Arg1
↓ CCL7/MCP-3
↓ Cd163

↓ Ear6

Lung gene expression profiling: A smooth muscle inhibition signature in mice exposed to Amish dust extracts

gene	gene name	adj.P.Val	FoldChange
Dmpk	dystrophia myotonica protein kinase	1.07E-13	0.57
Podnl1	podocan-like 1	1.61E-13	1.82
Tpm2	tropomyosin 2	1.86E-12	0.46
Acta2	alpha smooth muscle actin	1.93E-12	0.53
Des	desmin	1.93E-12	0.53



with RC Lantz and BT Chau

→ *The **environment** is sufficient to confer protection against asthma and allergy among the Amish*

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Farm Children

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Antibiotics?

Gut microbiota?

Diesel powered tractors?

????????????????

CORRESPONDENCE



Innate Immunity and Asthma Risk

N ENGL J MED 375;19 NEJM.ORG NOVEMBER 10, 2016

Justyna Gozdz, B.A.

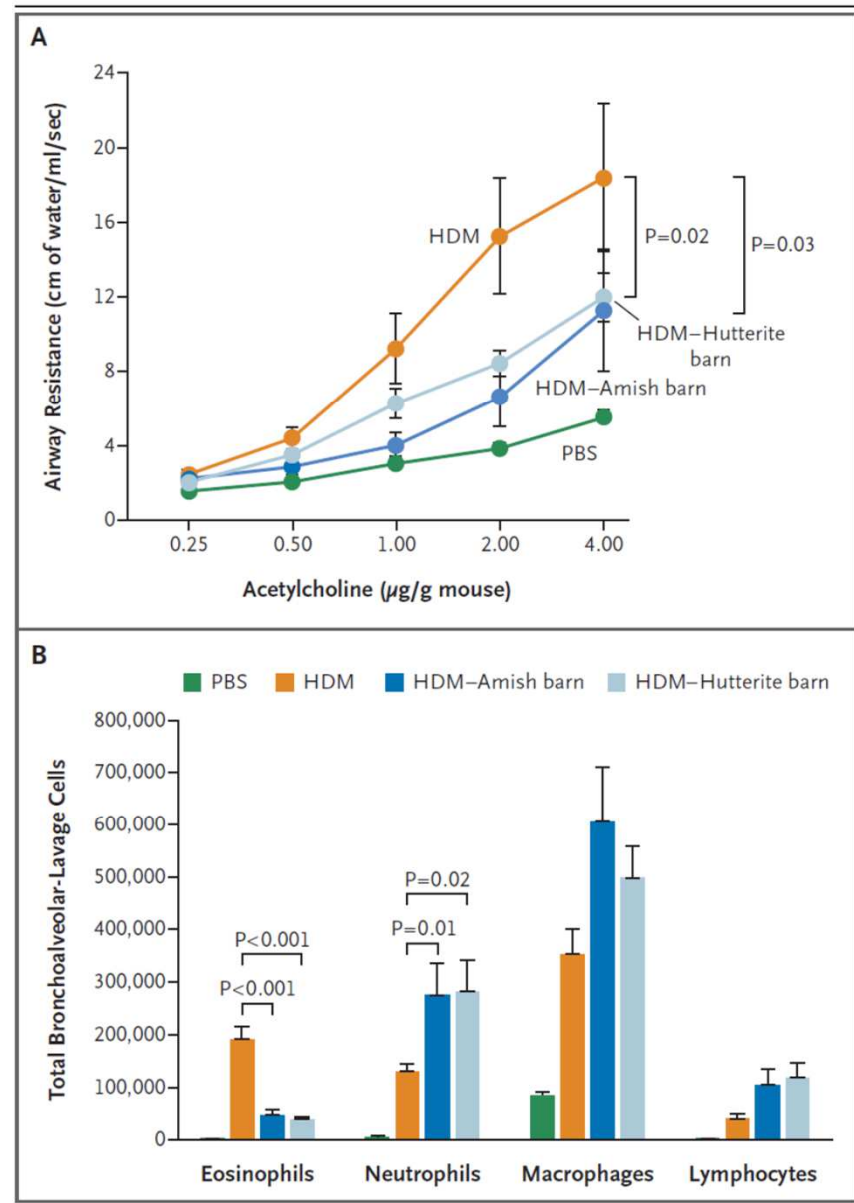
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University of Arizona



Amish and Hutterite Farms

Amish women and children are directly exposed to barn animals and their products.

Single family Amish farm



Hutterite women and children are not directly exposed to barn animals or their products.

Communal Hutterite farm (colony)

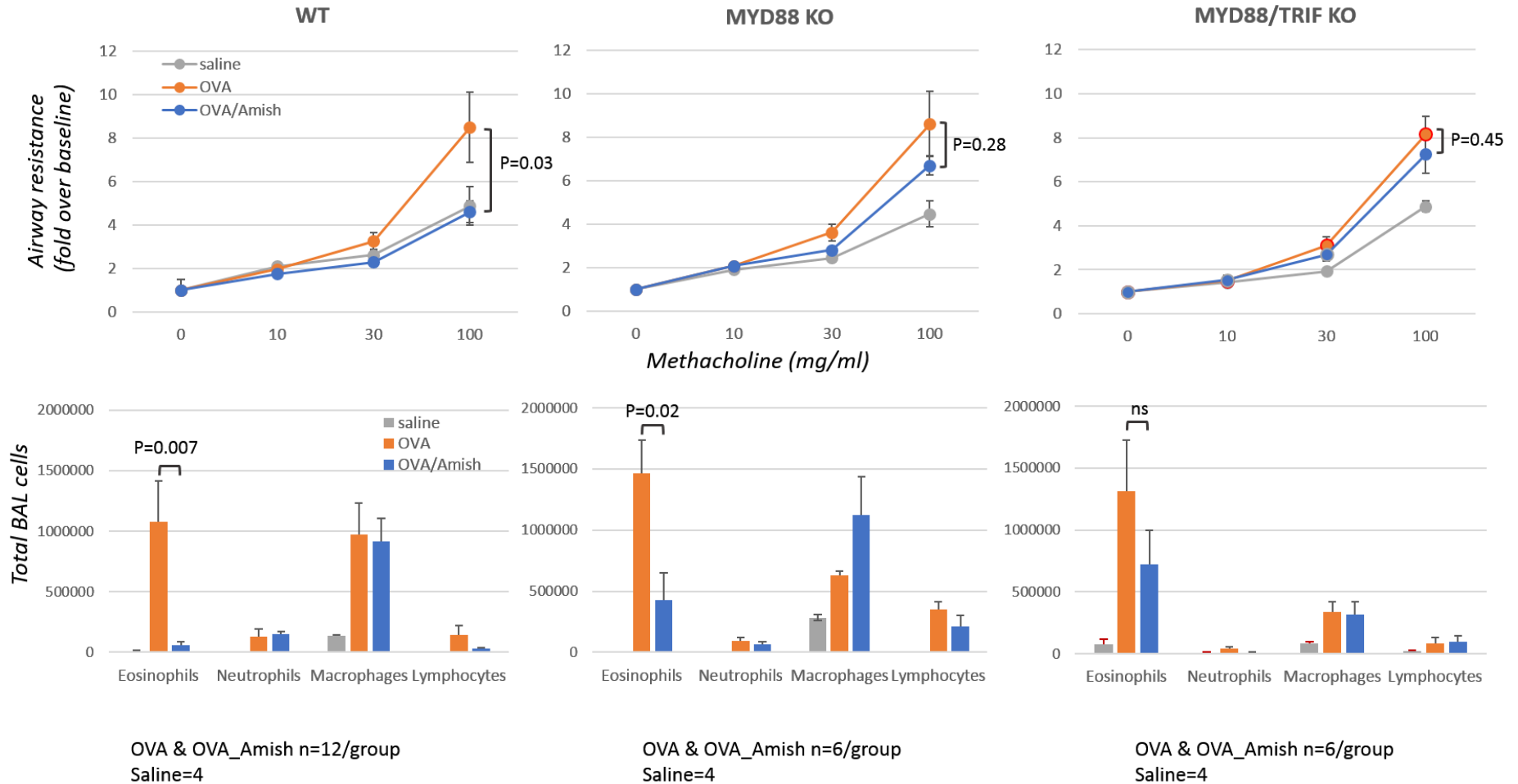


The environment is sufficient to confer protection against asthma and allergy among the Amish

Does innate immunity play a role in asthma protection?

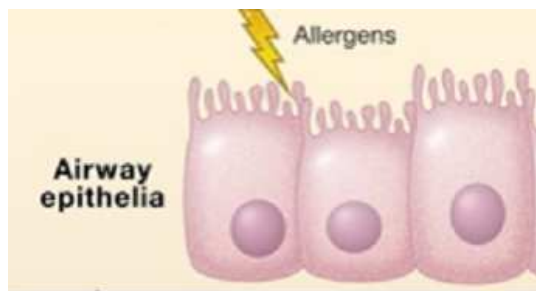
Inhibition of AHR and airway eosinophilia by Amish dust extracts is MyD88- and TRIF-dependent

Stein et al, NEJM 2016



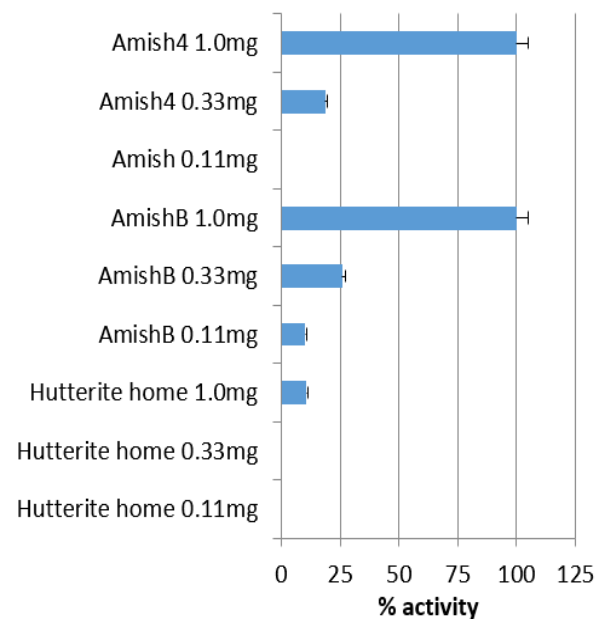
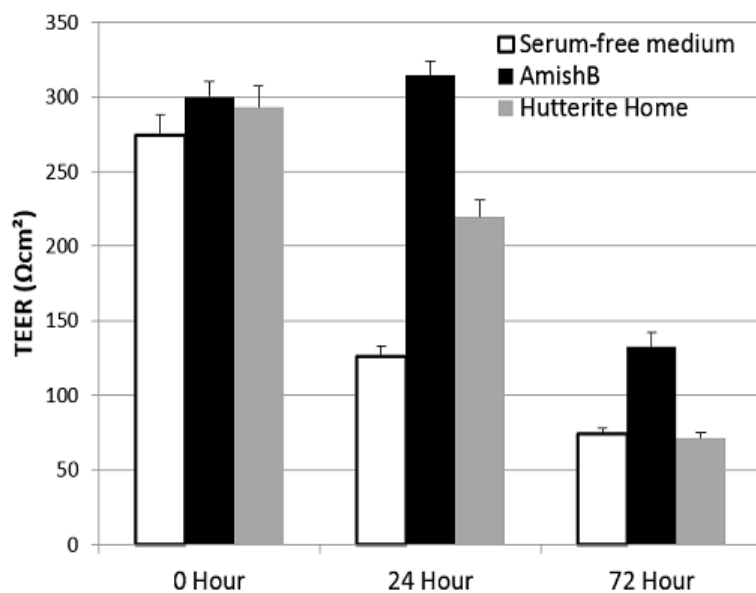
Amish dust extracts enhance barrier function and suppress inflammatory cytokine secretion in human 16HBE14o- epithelial cells

Dayna Anderson, 2017

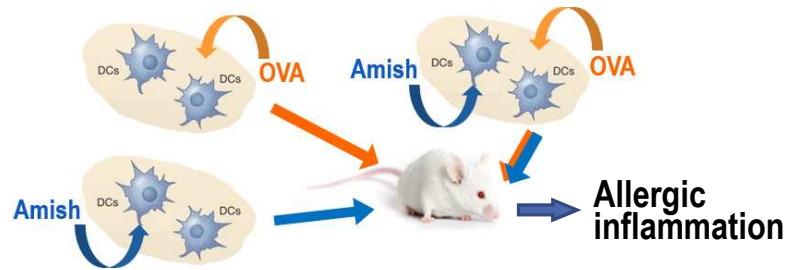


- Barrier function
- Pro-inflammatory IL-6

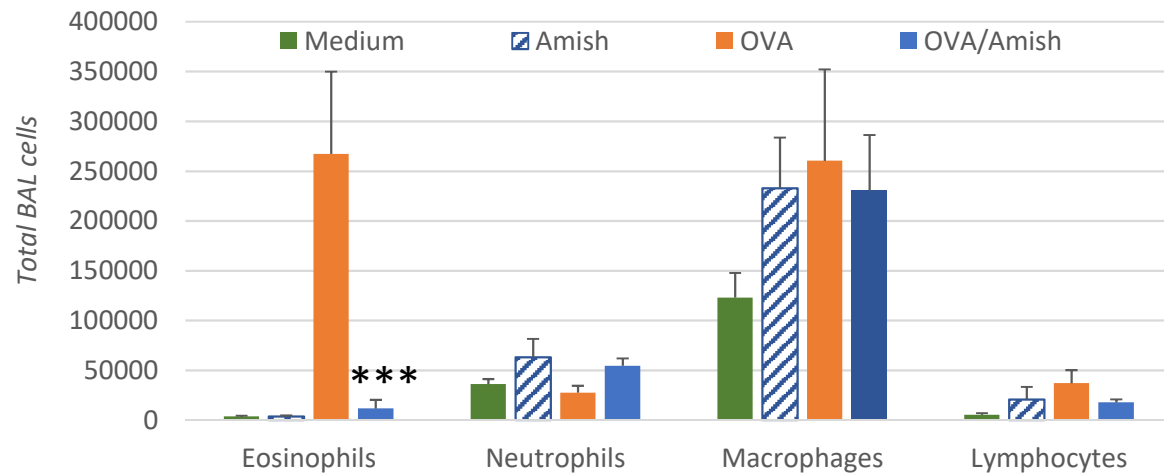
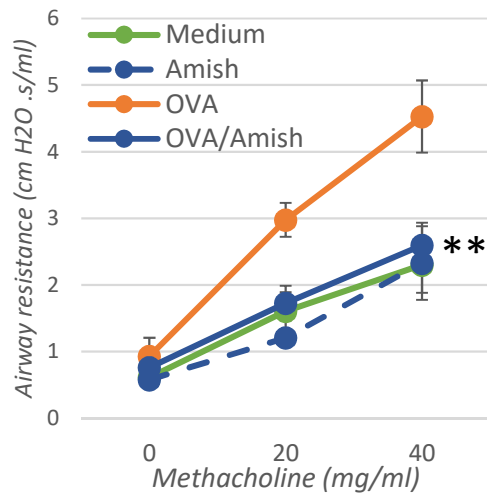
Is the airway epithelium a target of Amish dust extracts?



Airway transfer of Amish dust extract-treated dendritic cells is sufficient to protect mice from OVA-induced allergic inflammation



Is airway transfer of DCs pulsed with Amish dust extracts sufficient to confer protection from OVA-induced allergic inflammation?



** p=0.01 *** p=0.001

What we know so far

The environment is sufficient to confer protection against asthma and allergy among the Amish

Innate immunity plays a central role in asthma protection

The airway epithelium is a target of Amish dust

Amish dust-treated dendritic cells are sufficient to confer asthma protection



The Team

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Mark Holbreich

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Marblehead, MA, June 2013