RESEARCH MADE REAL

Advances in Managing Allergen Exposure in Homes, Schools and the Workplace

Martin D Chapman, PhD

#### **Conflict of Interest Statement**

This presentation will be evidence based.

**Disclosures:** 

In relation to this presentation, I declare the following real or perceived conflicts of interest:

Financial interest, Co-owner of the InBio companies Research support, NIH NIAID R01 Al077653-12 Research support, European Union iFAAM project

## **Allergen Exposure and Asthma**



> Regular aerobic exercise

#### Inner-City Asthma Studies 1991 - 2001

Cockroach allergen



#### Rosenstreich et al, NEJM 1997

#### Mouse allergen



Phipatanakul et al, JACI 2000

## **Dose Response Relationship between Sensitization and Exposure to Cockroach**

Eggleston JACI 1998



## **Cat and Dog Allergen Exposure**

Gergen JACIP 2018

- Is ubiquitous
  - occurs in homes without pets (1-10µg/g)
  - occurs in schools, daycare centers, offices and other public places.
  - is passively transferred on clothing
  - Accounts for ~350,000 hospital ER visits/year
- In schools:
  - airborne cat allergen increased 5-fold in classes with cat owners
  - indirect cat exposure increases the risk of asthma exacerbation (9-fold)



## Anatomy of an Allergen

House Dust Mite, feces, Der p 1 major allergen







Courtesy of Dr. Euan Tovey



## National Allergen Survey of Allergen Exposure in U.S. Homes

Arbes, Zeldin NIEHS JACI 2004

• 831 housing units surveyed in 75 locations across the US

 $\bullet$  Estimated that 23% (~22 million) of U.S. housing units contain high levels (>10µg/g) of Group 1 mite allergen

• Cat and dog allergens present in all homes

 $\bullet$  Levels of mouse allergen associated with sensitization (>1.6µg/g) found in 22% of homes

Studies conducted by National Institute of Environmental Health Sciences and US Dept. Housing and Urban Development



ZOCH ECHRS II Study JACI 2006





#### Neighborhood Asthma Study, NYC

Olmedo, Perzanowski JACI 2011

- Compared high asthma prevalence with low asthma prevalence neighborhoods
- HAPN homes had higher cockroach, mouse and cat \* allergen levels and sensitization than LAPN homes
- $\diamond$   $\uparrow$  allergen exposure increased the probability of becoming allergic (cockroach, dust mite, cat)
- Cockroach allergen exposure contributes to higher asthma prevalence in some NYC neighborhoods



2.6% to <6.0%

6.0% to <9.0% 9.0% to <11.4%

11.4% to 14.7% 14.7% to 18.5%

Airports, Landfill

Parks

#### **Allergen Avoidance – What Works?**

- "Allergen free conditions" (e.g high altitude)
- Encasing mattresses, pillows (6µ fabric)
- Washing bedding, cats, dogs
- Reducing humidity
- Removing carpets
- Acaricides/tannic acid/chemicals
- Insecticides, gel baits (cockroach)

## **Clinical Efficacy of Interventions**

#### Selected Studies

Multi-component interventions for dust mite and cockroach: Inner-City Asthma Studies



#### Morgan et al, NEJM 2004

## Single intervention: mattress covers for children with asthma



#### **Current Recommendations**

J Allergy Clin Immunol 2020

Working group report

#### 2020 Focused Updates to the Asthma Management Guidelines: A Report from the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group



Expert Panel Working Group of the National Heart, Lung, and Blood Institute (NHLBI) administered and coordinated National Asthma Education and Prevention Program Coordinating Committee (NAEPPCC)\* Bethesda, Md

- Recommends multi-component allergen-specific mitigation interventions
- Integrated pest management for cockroaches and rodents
- Mattress covers to be part of a multi-component approach for dust mites

## Environmental exposure conditions that 'predictably' result in allergic (IgE) responses

INDOOR AND OUTDOOR ALLERGENS

Route:	Inhalation (genetically predisposed host)							
Antigen:	Soluble 10-50 kDa protein							
Particle Size:	2-50 microns							
Dose (In dust):	Mite, 1-50µg/g*							
	Cat, 0.5-25µg/g*							
	Roach, 0.1-16µg/g*							
Dose (Airborne):	10-200 ng/m <sup>3</sup>							
Annual exposure:	1-100µg							

#### Scientific Accountability -Challenging Scientific Status Quo

## Move Fast and Measure Things!





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Editor Philippe Eigenmann Geneva, Switzerland A12 – Molecular exposure: Systematic approaches, clinical significance, and harmonization

Martin D Chapman, Adnan Custovic, Clare E N Mills, Thomas Holzhauser

**MOLECULAR ALLERGOLOGY USER'S GUIDE 2.0** 

## Assessment of Allergen Exposure in the Home, Workplace, Schools, Public Buildings

Portnoy et al, AAAAI/ACAAI Practice Parameter, Ann Allergy Asthma Immunol, 2013 Gold et al, NIAID, NIEHS, NHLBI, and MCAN Workshop Report JACI 2017

Collect "reservoir" dust samples from bedding, carpets, furnishings

Collect air samples for animal allergens

Send to lab for analysis by ELISA or MARIA®

• provides most accurate exposure measurement

Test on site using rapid lateral flow test

• "yes/no" answer



#### Sample Collection Devices

ENVIRONMENTAL DUST AND AIR SAMPLES



Electrostatic Dust Collector



Membrane Filter Cassette



DUSTREAM® dust collector





**AirAnswers®** 

## Allergen-Antibody Structures

X-RAY CRYSTALLOGRAPHY POMÉS, CHRUSZCZ 2021

- Mouse monoclonal antibody pairs binding to separate sites on allergen molecules
- Confirms suitability for use in immunoassays
- Determines precise location of amino acid residues involved in antibody binding
- Enables molecular engineering to suit specific applications



## Multiplex ARray for Indoor Allergens (MARIA)

InBio antibodies on the Luminex xMAP system

- Measures 8-12 indoor allergens at once
  - Der p 1, Fel d 1, Can f 1, Bla g 2 etc
- Sensitive and specific assay for environmental testing (house dust and air samples)
- Used in CDC, NIEHS, NIAID studies of allergen exposure in asthma
- Validated by international multi-center ring trial
- Gold standard for environmental exposure assessment



## MARIA Case Study: National Health Examination and Nutrition Survey (NHANES 2005-6)

Paivi Salo, Darryl Zeldin, NIEHS, Research Triangle Park, NC

A survey of allergen exposure in US homes that were representative of the general US population

- A mixed bed and bedroom floor dust sample was obtained from ~7,000 homes.
- Samples were analyzed using a MARIA 9-plex array for Der p 1, Der f 1, Mite Group 2, Fel d 1, Can f 1, Mus m 1, Rat n 1, Bla g 2, Alt a 1.

#### Over 56,000 data points:

 >90% of homes had detectable levels of 3 allergens, usually Fel d 1, Can f 1, Mus m 1, Der p 1, or Der f 1.

- 15.8% had detectable levels of 7 or more allergens.
- Other variables: race/ethnicity, poverty index ratio, urbanization, regional variation, climate factors

## MARIA Case Study: The Schools Inner-City Asthma Studies (SICAS), 2008-13

Wanda Phipatanakul, Children's Hospital, Harvard Medical School, Boston, MA

A study of allergen exposure in 37 inner-city elementary schools in the northeastern US.

- ~1,100 dust/air/table wipe samples were collected from schools and children's homes.
- Samples were by MARIA 9-plex array for Der p 1, Der f 1, MiteGroup 2, Fel d 1, Can f 1, Mus m 1, Rat n 1, Bla g 2, Alt a 1 and for endotoxin.

#### Over 11,000 data points:

- Mus m 1 was the most common allergen found in schools and homes
- Higher Mus m 1 levels found in settled dust from schools (which was highly correlated with airborne Mus m 1)
- School-wide IPM program or HEPA filter purifiers in the classrooms did not significantly reduce symptom-days with asthma (JAMA 2021).

### **Molecular Approach to Food Allergy**

CHALLENGING THE STATUS QUO

- Purified food allergen proteins
- Precise measurement of specific food allergens proteins:
  - ≻ ELISA 2.0
  - MARIA® for Foods Multiplex technology for simultaneous allergen measurements
- > Mass spectrometry for detecting multiple allergens.
- > IgE monoclonal antibodies from food-allergic patients.

## Food Allergen Proteins: The 'active ingredients' that cause allergic reactions









## Peanut: Ara h 1, Ara h 2, Ara h 3, Ara h 6

- Milk: Bos d 4, Bos d 5, Bos d 11
- Egg: Gal d 1, Gal d 2
- Shellfish: Tropomyosin
- Fish: Cyp c 1

Examples

- ➢ Hazelnut: Cor a 1, Cor a 9, Cor a 14
- Cashew: Ana o 3
- Sesame: Ses i 1
- Wheat: Tri a 19

Arah 2 Gal d 1 Bosd 5

Gly m 5

WHO/IUIS Allergen Nomenclature Database: <u>www.allergen.org</u> Pomés et al, JACI 2015; 136:29-37; Goodman, Chapman, Slater JACIP, 2020

#### MARIA<sup>®</sup> for Foods Control Curves

#### **17-PLEX ARRAY**









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#### **MARIA for Foods**

- Provides simultaneous detection of major food allergens
- Measures food allergens ('active ingredients') with sensitivity, accuracy and precision
- > All components (antibodies, allergen references) are standardized
- Can be used in tandem with mass spectrometry

#### **Applications:**

- Screening for allergen contaminants, validation of allergen control processes
- > Formulation of immunotherapy products and food challenge materials
- Environmental food allergen detection in homes and schools- Maciag et al, SICAS study JACIP 2021
- Risk assessment and establishing risk thresholds based on actual allergen doses and exposures

## Apollo

A Novel Air Sampling Device

- High volume air sampler (>400L/min)
- Quiet operation (<40 decibels)
- Proprietary allergen capture filter that offers improved efficacy over IOM samplers
- Tested for detection of a wide range of indoor allergens and food allergens
- Long-duration sampling without saturation, suitable for longitudinal exposure studies
- Currently in clinical trials



## Apollo vs IOM Sampling



• Apollo and IOM samplers were run side-by-side for 12 hours in the same room.

	Nanogram of allergen per filter after 12 hours											
	Der p 1	Mite G2	Fel d 1	Ara h 3	Ara h 6	Gal d 2	Bos d 5	Shrimp	Cor a 9	Ana o 3	Gly m 5	
Apollo	11	5	272	97	98	206	300	1	88	13	1	
IOM	0.3	0.1	10	0.1	0.8	2.3	6.9	<lod< th=""><th><lod< th=""><th>0.3</th><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th>0.3</th><th><lod< th=""></lod<></th></lod<>	0.3	<lod< th=""></lod<>	

- ~30 >100 more allergen was captured on the Apollo sampler filter
- Shrimp, Cor a 9 and Gly m 5 were detected using Apollo, but were undetectable using IOM.

#### Apollo Sampling Reproducibility

- Apollo devices were placed in three different locations within a lounge, and sampled for 24-hours.
- Sampling as above was repeated on three separate days.



• Consistency of sampling was observed between Apollo devices over all three days tested, over a broad range of airborne allergen levels (1-100 ng/filter)

#### **Environmental Testing Suite**

An advanced facility for testing allergen products, intervention procedures and product safety

- Independent air handling, HEPA filtration and temperature-controlled suite
- Facilitates safety and efficacy testing of products and procedures for allergen and mold remediation
- Built to AHAM specifications for testing services, R&D, and product development





#### **Environmental Testing Suite**

Two suites, with dry/wet rooms to mimic rooms found in the home or office



#### **Environmental Testing Suite**

**Multiple Capabilities** 

#### **Safety Testing**

Simulated use studies to determine exposure of consumers to allergens

Particle monitoring

#### **Efficacy Testing**

Third party validation of allergen denaturing or removal claims on products: cleaning solutions, wipes, vacuum cleaners, air purifiers, etc.

#### **Aerosolization of Bacteriophages**

Optimizing MS2 bacteriophage as a representative non-enveloped virus & SARS-CoV-2 surrogate





### **Occupational Exposures in the IH Arena**

#### LABORATORY ANIMAL ALLERGY (LAA)

- Exposure to laboratory animals can cause occupational allergic respiratory diseases, including asthma.
- Allergens: Mouse, Mus m 1; Rat, Rat n 1: Guinea Pig (urinary proteins) & Rabbit. Ory c 3.
- 'Benchmarking' exposure limits in the pharmaceutical industry
  - $\sim$  <5ng m<sup>-3</sup>
  - 😑 5 50 ng m<sup>-3</sup>
  - >50 ng m<sup>-3</sup>

Mason and Willerton, AIMS Allergy and Immunology 2017

#### Enzyme Testing

Meeting an Emerging Need

#### **Increasing Use of Enzymes in Industry**

Pharmaceuticals, cleaning products, food, and textiles

#### **Enzymes are Known Respiratory Sensitizers**

Occupational monitoring is essential

Current exposure limits range from 15-60ng/m<sup>3</sup>

#### Ability to Analyze Multiple Sample Types

InBio can obtain quantifiable data from air filters, surface wipes, liquid samples, product samples, and more



#### **Occupational Exposure to Cannabis**

#### DECUYPER JACIP 2020



Can s 3 ELISA



Skypala et al, Allergy 2022 Cannabis-related allergies: overview and recommendations (Int. Cannabis Allergy Collaboration)

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#### **NIOSH Can s 3 Study**

Collaboration with Capt. Bradley King

- Determine optimal sampling and extraction protocol to measure Can s 3 as a potential marker for occupational exposure in cannabis facilities in Colorado
- Assess Can s 3 stability and recovery from spiked filter samples
- Compare Can s 3 extraction from air filters, surface samples, and dust using Can s 3 ELISA 2.0



#### New Approaches to Target Cat Allergen



- Introduced egg IgY anti-Fel d 1 antibody into cat food to reduce cats' salivary Fel d 1 levels.
- Treated cats showed 30% reduction in salivary Fel d 1 and 47% reduction in haircoat Fel d 1 compared to baseline.



- Immunized cats to induce the production of anti-Fel d 1 antibodies.
- Treated cats showed ~50% reduction in Fel d 1 levels in cat tear extracts.

Caveat: Modest reductions in Fel d 1 may not significantly alleviate patient symptoms

## CRISPR Gene Editing

A NOVEL SOLUTION TO DELETE FEL D 1 FROM CATS







### CRISPR Gene Editing of Cat Allergen, Fel d 1

**BRACKETT CRISPR J 2022** 

- Fel d 1, an immunodominant allergen, accounts for 60-90% of anti-cat IgE antibody
- Guide RNA's for CRISPR editing were designed based on Fel d 1 sequences from 50 cats
- CRISPR-CAS9 gene editing efficiencies of up to 55% were obtained using the guide RNA's in a feline cell line
- Multisequence alignments suggest that Fel d 1 gene sequences are not conserved in exotic cats
- Proof of principle that Fel d 1 is a suitable target for gene deletion

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## The CRISPR Journal



#### Allergen exposure assessment and indoor air quality

- Allergen exposure should be considered as part of IAQ investigations
- ELISA or multiplex array (MARIA) technology provide quantitative data on exposure levels
- Rapid tests can be used for on-site screening by IAQ personnel
- Multiplex technology is ideally suited for:
  - Population studies, health outcomes, public health
  - Monitoring occupational and workplace exposures
  - Contract research and product development