Determinants of Food Allergy Persistence and Severity
What determines sensitization?

Bert Ruiter, Wayne Shreffler

Immunology Institute and Jaffe Food Allergy Institute
Mount Sinai School of Medicine

Allergy-Immunology Research Conference
Northwestern University Feinberg School of Medicine
Why Food Allergy?

- important public health problem
- ‘clinically tractable’ phenotype presenting early in life
- paradigm of mucosal immune tolerance with highly variable expression
Variability of Both Allergen and Phenotype

- most children outgrow milk allergy, most do not outgrow peanut or shellfish allergy
- some children have much more persistent disease to the same allergen (e.g., milk)
Our Research

- IgE and Effector Cell Function and Regulation
  - Characterization of Allergen-Specific IgE/ IgG Repertoire
  - Characterization of Basophil Reactivity and Its Regulation
- Allergen-specific T cell Repertoire
- Mechanisms of Allergenicity
Outline

Background

Functional and Transcriptional Activation
- Peanut Ag Induces Functional Activation of DCs
- Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid
- Background
- Peanut Allergen-Induced RALDH2
common protein allergens are largely restricted to narrow group of protein families

relative allergenicity coupled to innate immune activation
Examples

- protease (dust mite, papain): epithelium, dendritic cells, basophils
- C-type lectin engagement (peanut, dust mite): dendritic cells
- complement activation (hymenoptera, peanut): many potential targets
Outline

Background

Functional and Transcriptional Activation
- Peanut Ag Induces Functional Activation of DCs
- Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid
- Background
- Peanut Allergen-Induced RALDH2
Method

1. 6 hours with allergen

2. Co-culture with autologous naive T cells and sAg for 5 days

3. Determine phenotype of T cells by measurement of secreted cytokine
Introduction

Background

Functional and Transcriptional Activation

Peanut Ag Induces Functional Activation of DCs

Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid

Background

Peanut Allergen-Induced RALDH2

Summary/Future

Acknowledgments
Differentiation

IL-13 (pg/ml)

IFN-γ (pg/ml)

0.01 pg/ml

0.1 pg/ml

AH1 AH2 CPE CT LPS MED T CTRL AH1 AH2 CPE CT LPS MED T CTRL AH1 AH2 CPE CT LPS MED T CTRL
Outline

Background

Functional and Transcriptional Activation
  Peanut Ag Induces Functional Activation of DCs
  Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid
  Background
  Peanut Allergen-Induced RALDH2
Microarray Expression Profiling
Microarray Expression Profiling
Transcriptional Changes Uniquely Induced by Peanut

![Transcriptional Changes Uniquely Induced by Peanut](image-url)
Food Allergy
Mechanisms

Introduction

Background

Functional and Transcriptional Activation

Peanut Ag Induces Functional Activation of DCs
Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid

Background

Peanut Allergen-Induced RALDH2

Summary/ Future

Acknowledgments

Targets Shared with LPS
Targets Uniquely Upregulated by LPS
Outline

Background

Functional and Transcriptional Activation
  Peanut Ag Induces Functional Activation of DCs
  Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid
  Background
  Peanut Allergen-Induced RALDH2
RALDH2 and Retinoic Acid

► Retinoic Acid (RA) is derived from Vitamin A by two oxidation reactions.
► The first-step conversion of vitamin A (retinol) to retinal is catalyzed by ubiquitously expressed alcohol dehydrogenases.
► RALDH2 (ALDH1A2) is the inducible and rate-limiting catalyst for the second step conversion of the aldehyde form, retinal to retinoic acid that determines cell-specific expression of RA, which can exist in two isomeric forms – ATRA and 9-cis-RA.
RALDH2 and Retinoic Acid in the Immune System

- Vitamin A deficiency has been long associated with Th2 developmental impairment and overproduction of IFN-γ.
- More recently, RA has been shown to suppress Th17 in favor of FoxP3+ Treg by antagonizing IL-6 induced RORγt.
RALDH2 and Retinoic Acid in the Immune System

- Spiegel et al. recently demonstrated that human basophils could be induced to express RA (by induction of RALDH2), which induced Th2 differentiation on co-culture with naïve T cells. *Blood 2008 vol. 112 (9) pp. 3762-71*
Food Allergy Mechanisms

Introduction

Background

Functional and Transcriptional Activation
  Peanut Ag Induces Functional Activation of DCs
  Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid
  Background
  Peanut Allergen-Induced RALDH2

Summary/ Future

Acknowledgments
Food Allergy Mechanisms

Introduction

Background

Functional and Transcriptional Activation

Peanut Ag Induces Functional Activation of DCs

Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid

Background

Peanut Allergen-Induced RALDH2

Summary/ Future

Acknowledgments

qPCR confirmation

CD1a

[Box plots showing gene expression levels for different allergens (AH1, AH2, Birch, CM, CPE, CR, CT, Cat, HDM, HSA, LPS, Soy).]
Food Allergy Mechanisms

Introduction

Background

Functional and Transcriptional Activation

Peanut Ag Induces Functional Activation of DCs

Peanut Ag Induces a Unique Transcriptional Program

RALDH2 and Retinoic Acid

Background

Peanut Allergen-Induced RALDH2

Summary/ Future

Acknowledgments

qPCR confirmation

PPARG

AH1 AH2 Birch CM CPE CR CT Cat HDM HSA LPS Soy
Reproducibility of RALDH2 Induction

![Box plot of ALDH1A2 expression](image)
Fractionation of RALDH2 Inducing Activity
Induction of Retinal Oxidation

- PN Ag Stimulation
- +

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Inhibitor

RALDH2 and Retinoic Acid

Peanut Ag Induces Functional Activation of DCs
Peanut Ag Induces a Unique Transcriptional Program

Summary/Future

Acknowledgments
Summary

- Peanut extract activates myeloid DCs to induce proliferation and differentiation of naïve T cells.
- Peanut extract induces a distinctive transcriptional program in DCs.
- A fraction of peanut extract induces the rate-limiting enzyme for the production of RA in (a subset?) of human DCs.
Future Work

▶ Demonstrate whether RA is produced in co-culture and plays a role in naïve T cell differentiation.
  ▶ RARE reporter construct in T cell line to demonstrate trans-activation.
  ▶ Substrate dose response and RAR antagonists effect on T cell differentiation.
▶ Characterize DC population that is responsive to peanut.
▶ Define and purify the fraction of peanut extract to determine its sufficiency for DC and T cell activation.
▶ Replicate RA induction by peanut allergen in murine model.
Acknowledgments

- Mount Sinai Pediatric Allergy and Immunology Institute
  - Laverne Brown, PhD
  - Jaffe Food Allergy Institute – Hugh Sampson
  - Mayer Lab
- Grant Support: NIAID, Food Allergy Initiative