

Glycolipid-mediated activation of allergy effector cells in alpha-gal syndrome



Onyinye I. Iweala, MD PhD

Assistant Professor of Medicine
Division of Rheumatology, Allergy, and
Immunology | UNC Food Allergy Initiative |
Thurston Arthritis Research Center



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

Alpha-Gal Syndrome : The Food Allergy Disruptor

Alpha-gal allergy challenges the current paradigm for food allergy

Conventional Food Allergies

- Rapid symptom onset (≤ 2 hours, and typically within minutes)
- IgE antibodies form against food proteins
- Initial exposure to food protein through the gut or the skin spark a rise in food allergen specific IgE levels

Alpha-Gal Allergy

- **Delayed Symptom Onset (≥ 2 hours)**
- **IgE antibodies form against a sugar**
- **In the US, bites from Lone Star Ticks are associated with elevations in alpha-gal specific and total IgE levels**

Red Meat Allergy in Alpha-Gal Syndrome: Symptoms can be inconsistent



THINKSTOCK

- Allergic reactions to alpha-gal are inconsistent, often delayed, and may not occur with every ingestion
- Variability in magnitude of allergic response depends on co-factors (exercise, alcohol), the state of the immune system (infection), the dose and form of alpha-gal
- **Lipid-rich mammalian meats are associated with more consistent delayed reactions**
- **Significant differences in lipid and fatty acid metabolism pathways between individuals with and without alpha-gal syndrome**

Project Rationale:

- **Expand our knowledge of the candidate components of mammalian meat that could mediate delayed allergic responses in AGS red meat allergy**
 - Explore glycolipids as potential food allergens
 - Is the immune machinery that handles glycolipid antigen important for the development of allergic responses to red meat in alpha-gal syndrome?

Specific Aims:

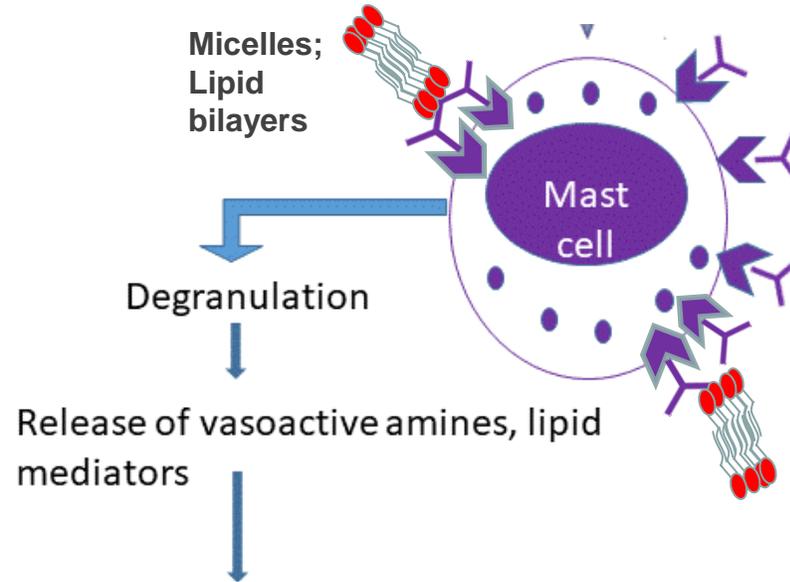
Aim 1: Evaluate candidate alpha-gal-containing components of mammalian tissue that can mediate delayed allergic responses in AGS

Working hypothesis: Alpha-gal-containing mammalian glycolipids bind alpha-gal-specific IgE displayed on allergic effector cell (i.e. mast cell and basophil) surfaces, activating these cells

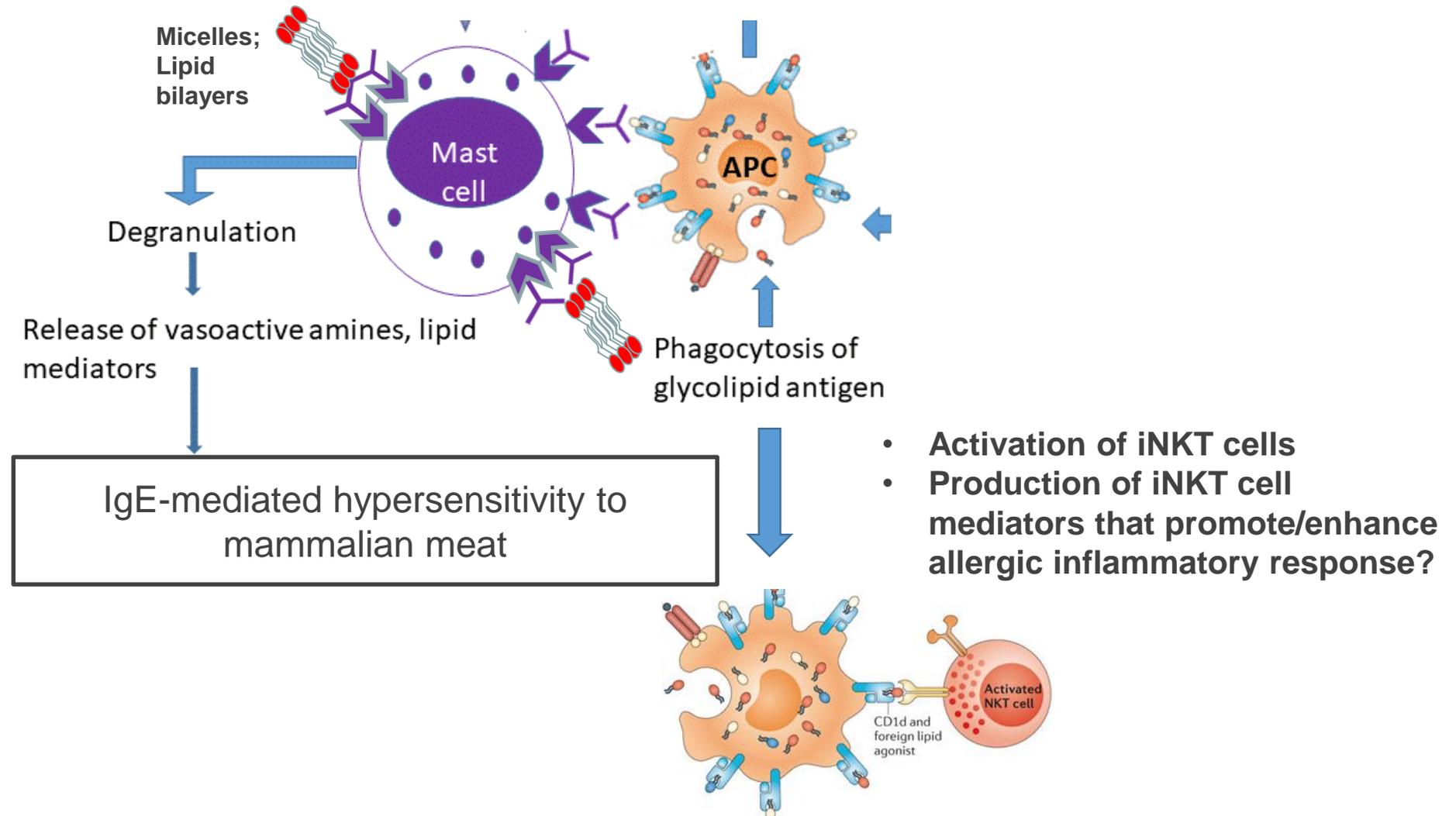
Aim 2: Identify cellular sources of type 2 cytokines critical for the generation of alpha-gal specific IgE in AGS

Working hypothesis: Unconventional T cells that recognize and respond to glycolipid antigen, specifically CD1d-restricted NKT cells, drive the type 2 cytokine environment that supports alpha-gal-specific antibody class switching to IgE, following lone star tick exposure

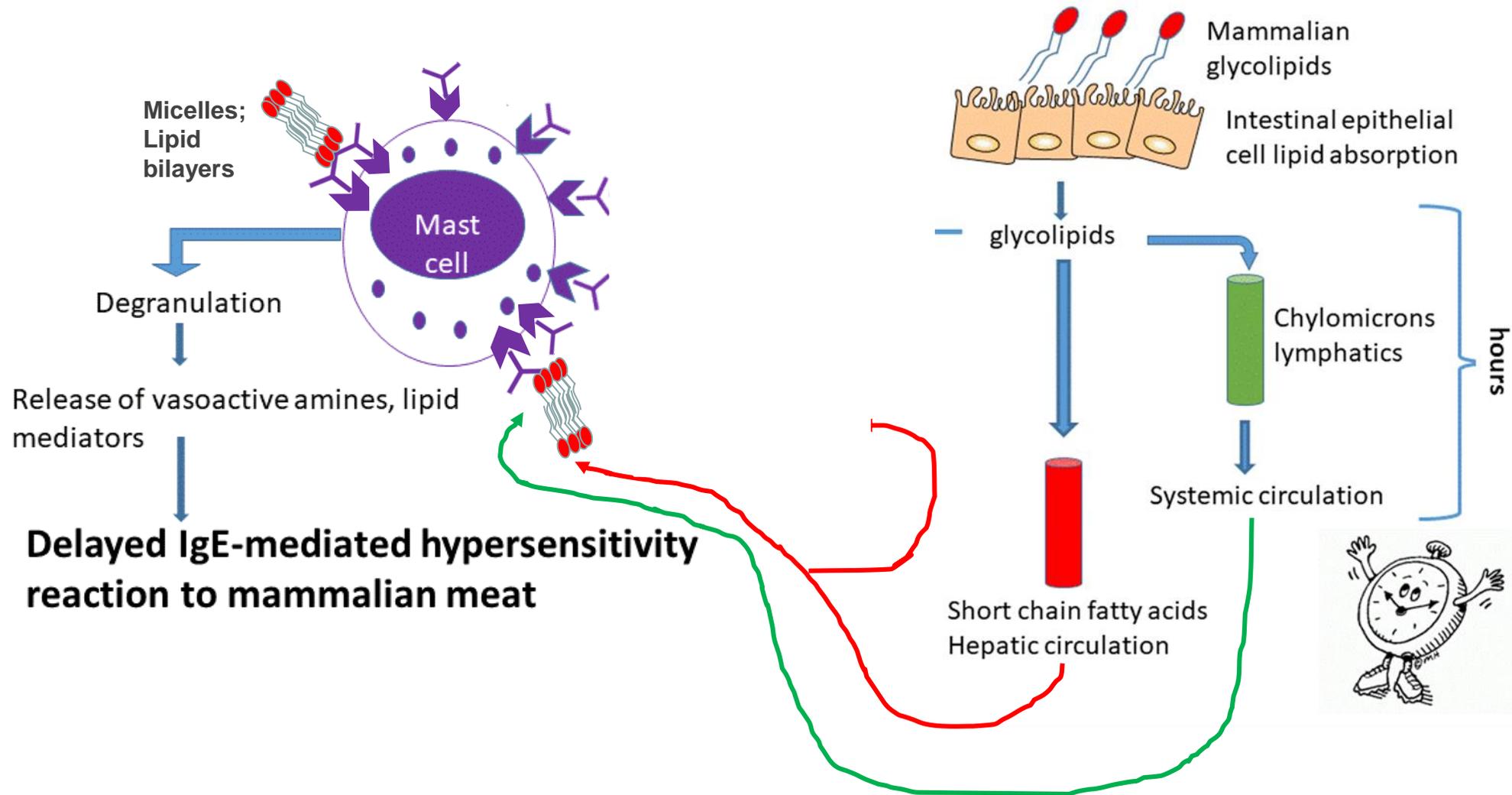
Central Hypothesis 1: Glycolipids play a role in the effector phase of alpha-gal allergy



Central Hypothesis 1: Glycolipids play a role in the effector phase of alpha-gal allergy



Central Hypothesis 2a: The hours required for lipid absorption and metabolism may explain the delayed allergic reactions in alpha-gal allergy

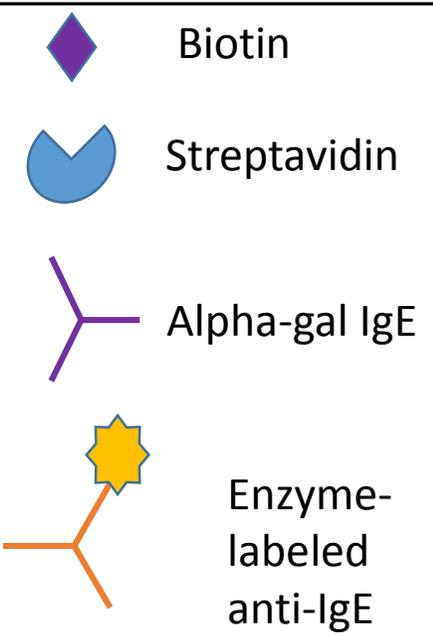
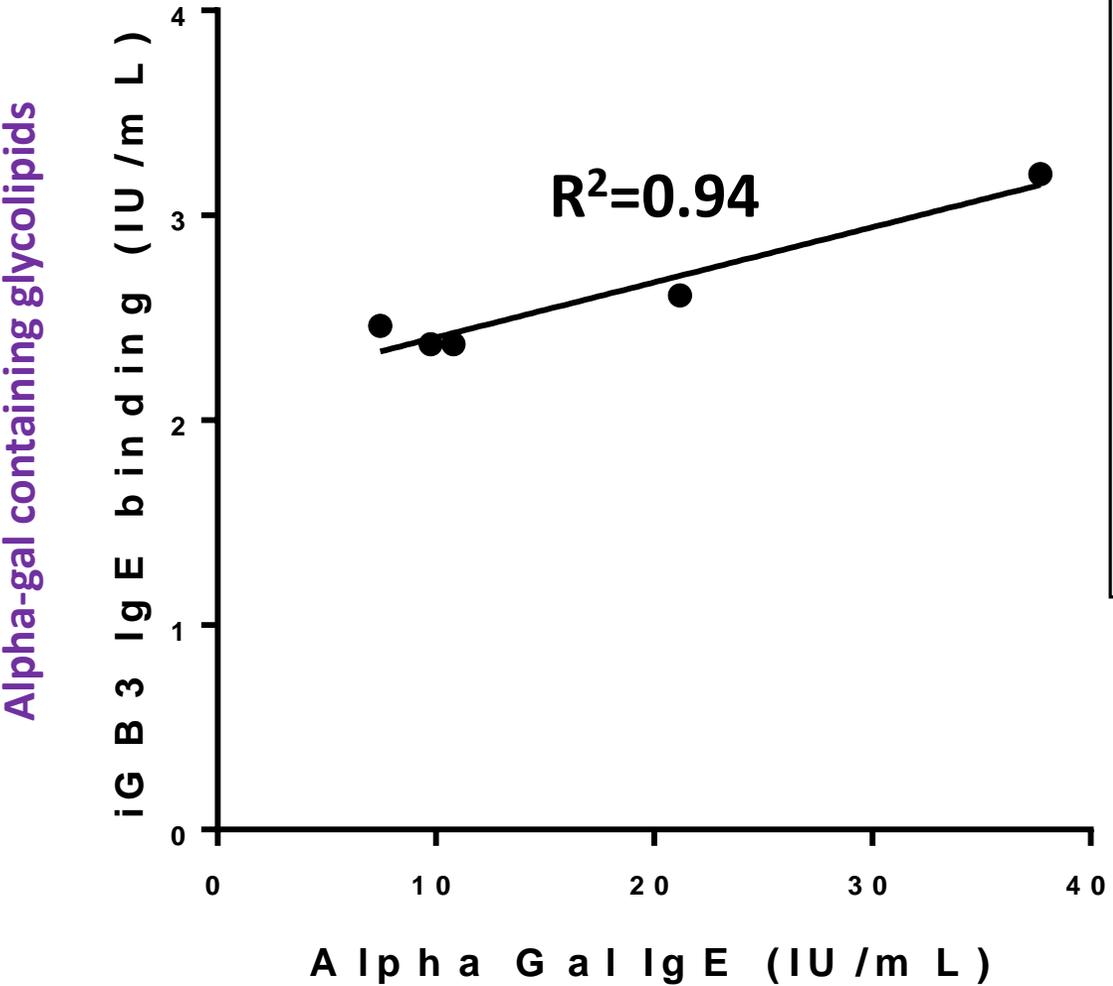


Preliminary Data

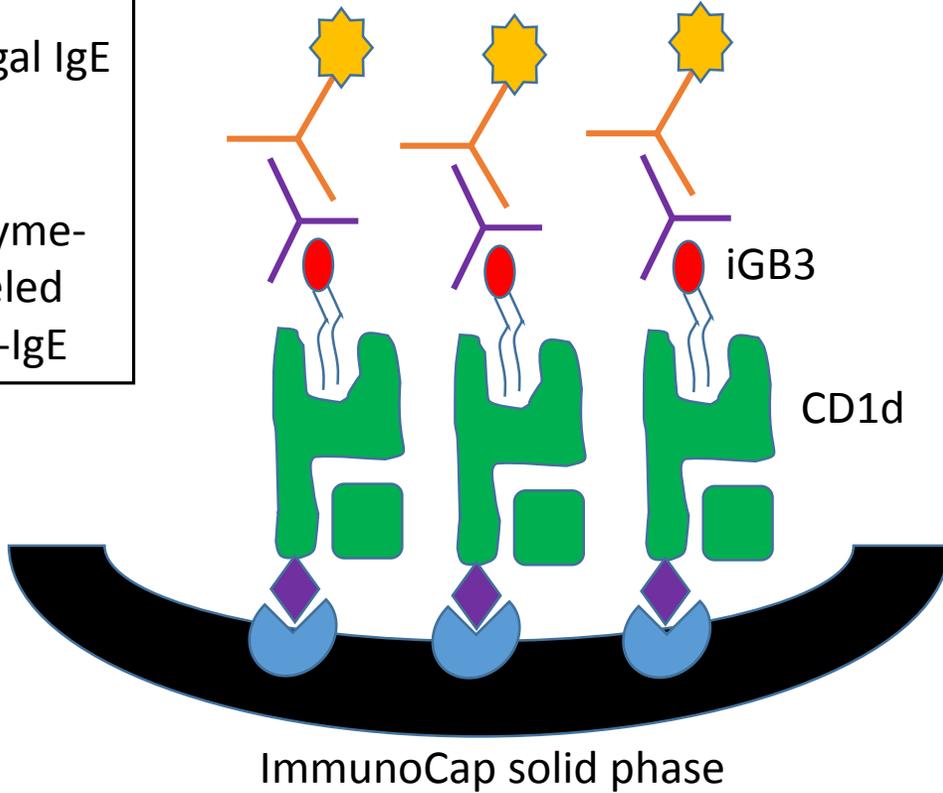
Can alpha-gal specific IgE bind to alpha-gal-containing glycolipids?

Can alpha-gal-containing glycolipids activate allergy effector cells? If so, how?

Serum IgE from alpha-gal allergic patients binds alpha-gal in both glycoproteins and glycolipids

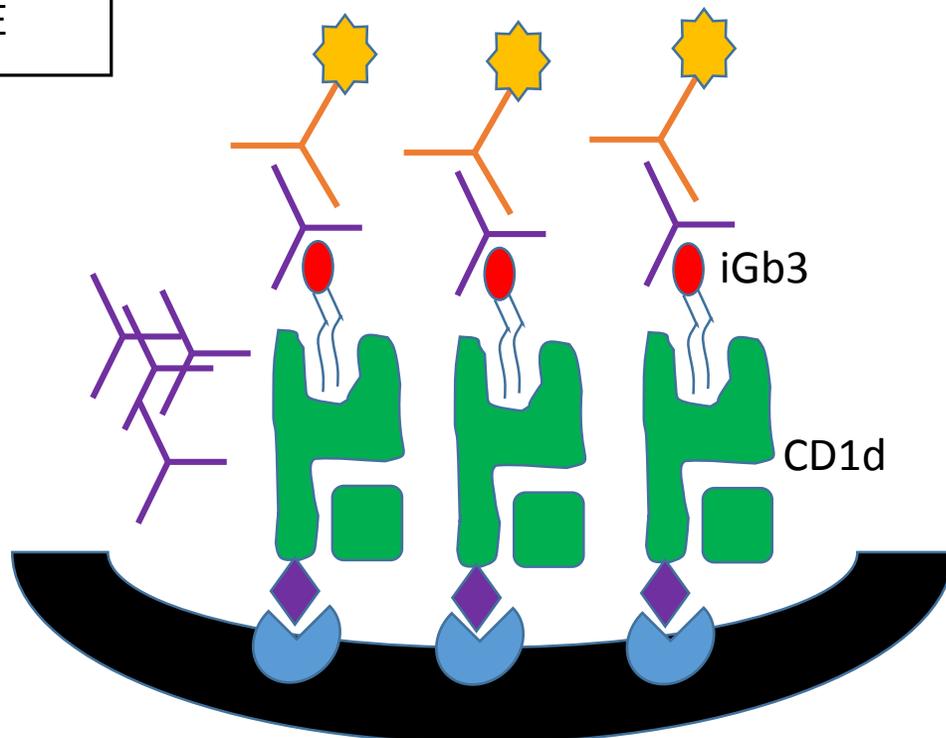
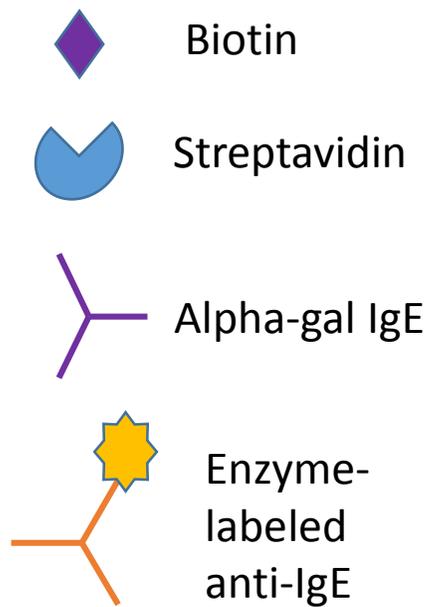


- Incubate w/developing agent
- Measure fluorescence
- Convert to IU/ml



cetuximab binding – alpha-gal containing glycoprotein

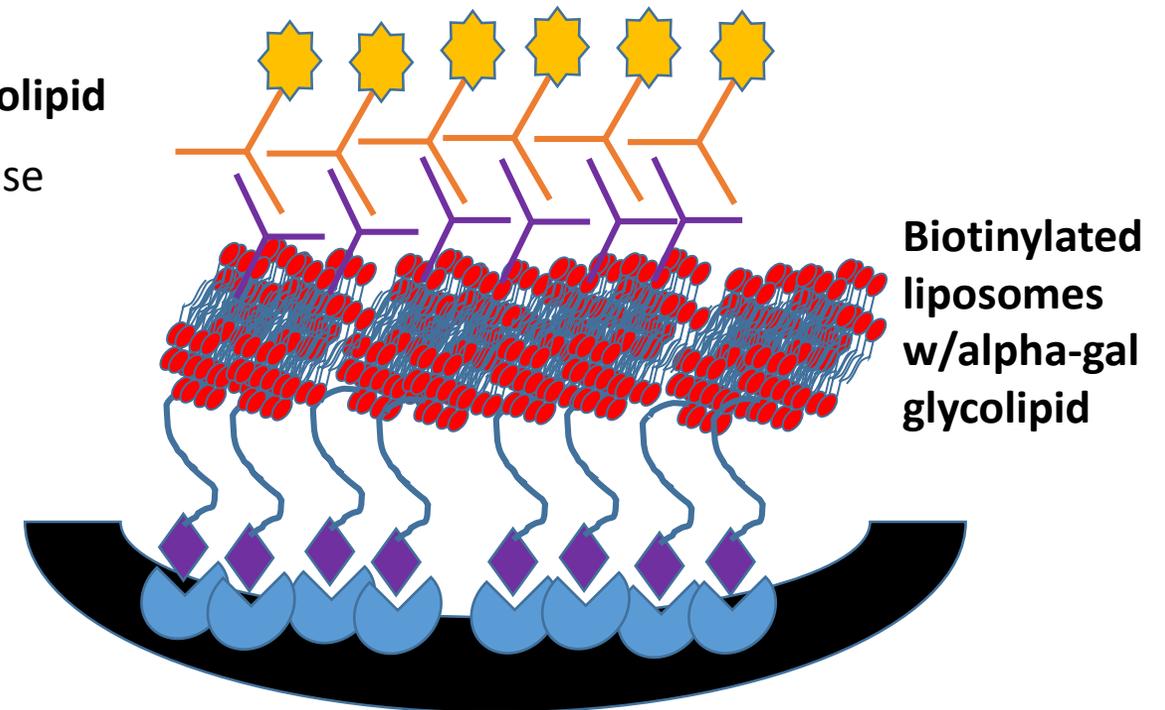
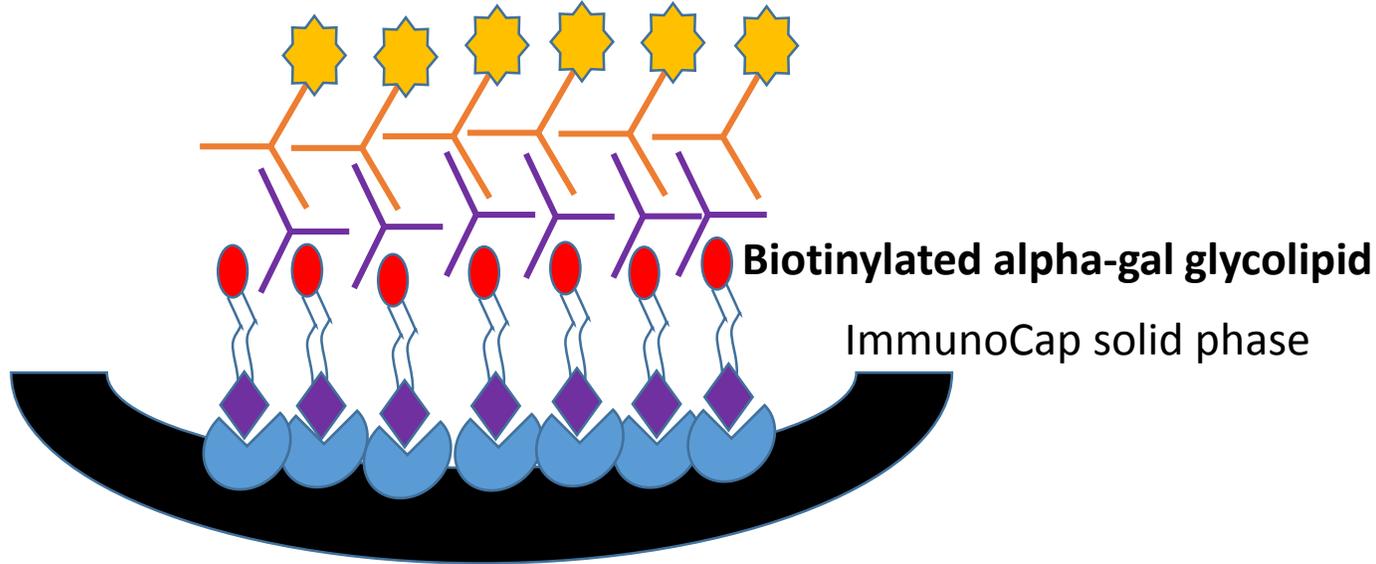
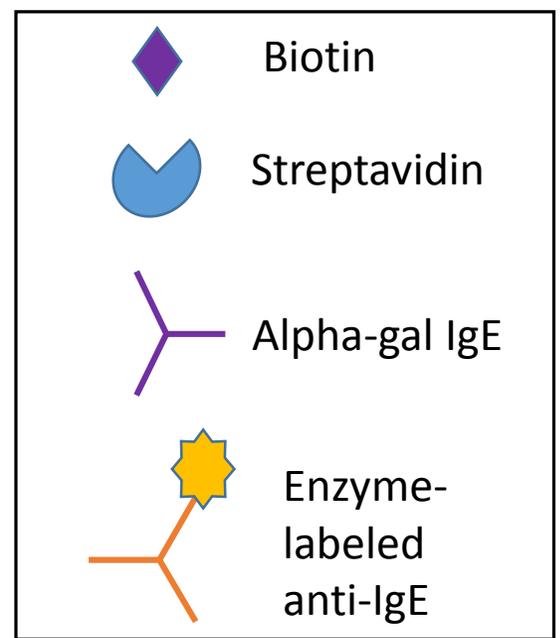
Our current method of detecting IgE-binding to alpha-gal glycolipid may not be optimized



IgE Binding (IU/ml)

Subject	cetuximab	iGB3
1	21.20	2.61
2	7.45	2.46
3	9.76	2.37
4	10.80	2.37
5	37.70	3.20

Subaim 1a: Create new ImmunoCAPs with biotinylated alpha-gal glycolipids and/or biotinylated liposomes that contain alpha-gal glycolipids attached directly to streptavidin CAP

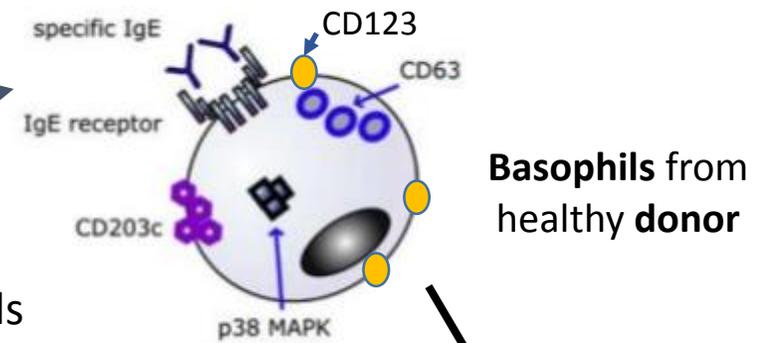
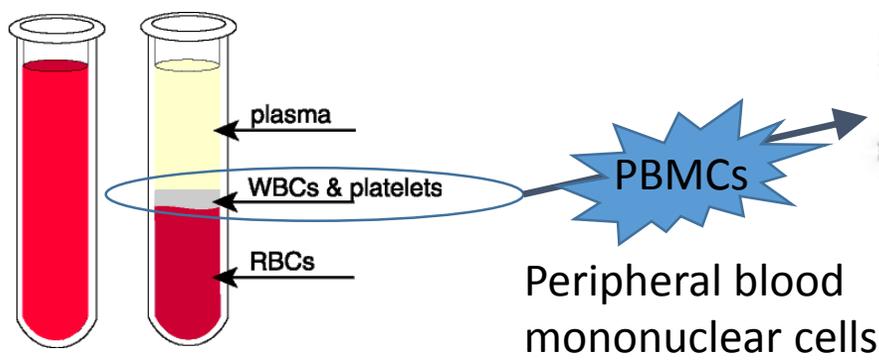


Preliminary Data

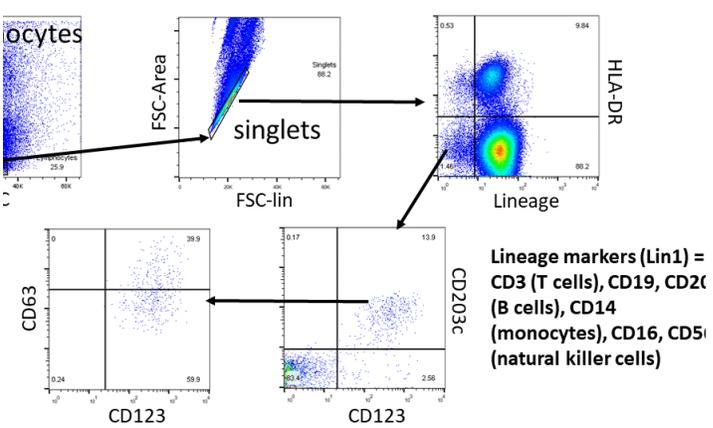
Can alpha-gal specific IgE bind to alpha-gal-containing glycolipids?

Can alpha-gal-containing glycolipids activate allergy effector cells? If so, how?

Whole Human Blood



Basophils from healthy donor



Lineage markers (Lin1) =
CD3 (T cells), CD19, CD20
(B cells), CD14
(monocytes), CD16, CD56
(natural killer cells)

Can alpha-gal containing glycolipid activate basophils?
Approach : Indirect Basophil Activation Test

2
Strip off native IgE

3
Sensitize donor basophils overnight using plasma from patient with alpha-gal allergy

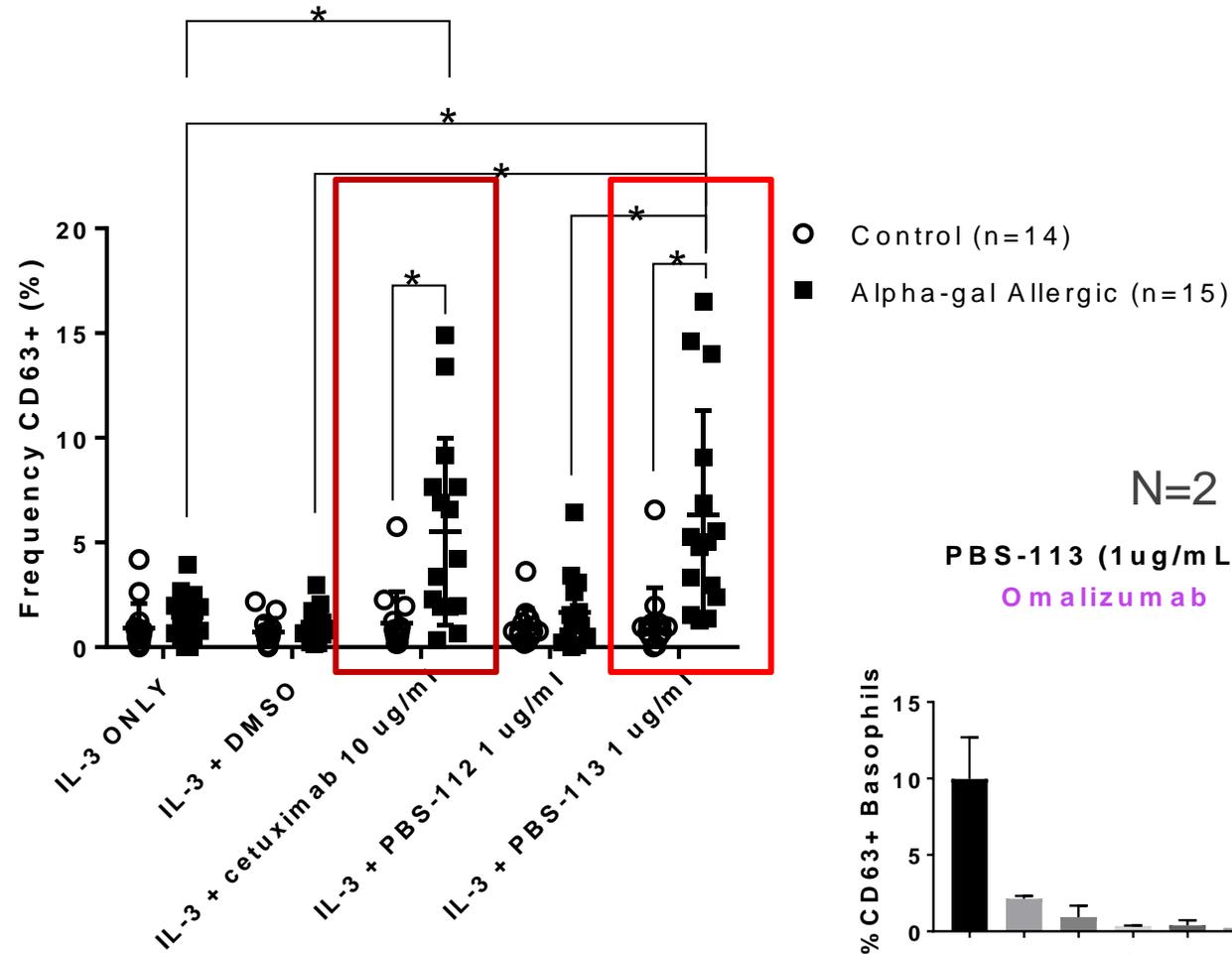
- 4
30 minute incubation with:
- IL-3 + DMSO
 - IL-3 + **Cetuximab*** (glycoprotein)
 - IL-3 + PBS-112 (glycolipid)
 - IL-3 + **PBS-113*** (glycolipid)
- *contains alpha-gal moieties

5
6
Harvest and stain for **basophils** and the **activation marker CD63** and evaluate using **flow cytometry**

7
Flow Cytometer

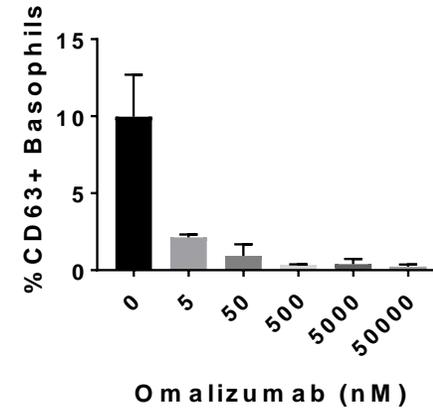


Alpha-gal-containing glycolipids activate basophils sensitized with plasma from alpha-gal allergic subjects in an IgE-dependent fashion

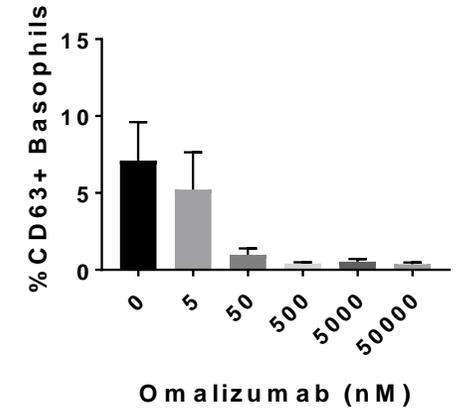


N=2 alpha-gal allergic

PBS-113 (1ug/mL)+
Omalizumab



Cetuximab (10 ug/mL) +
Omalizumab

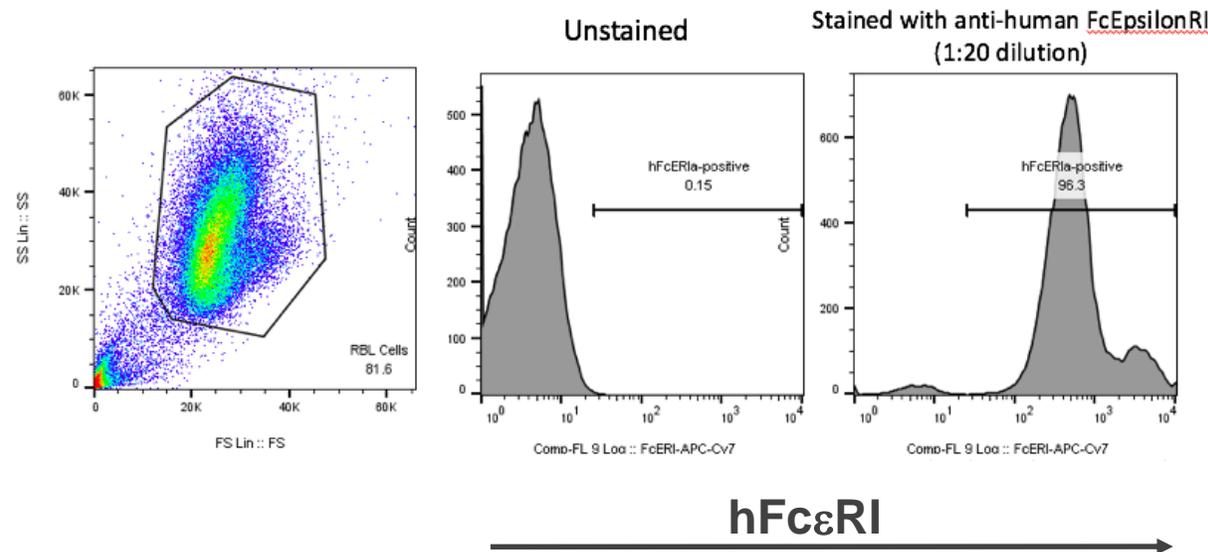


<https://en.wikipedia.org/wiki/Omalizumab>

Omalizumab = Monoclonal antibody against IgE

Subaim 1b: Alternative readouts for allergic effector cell activation

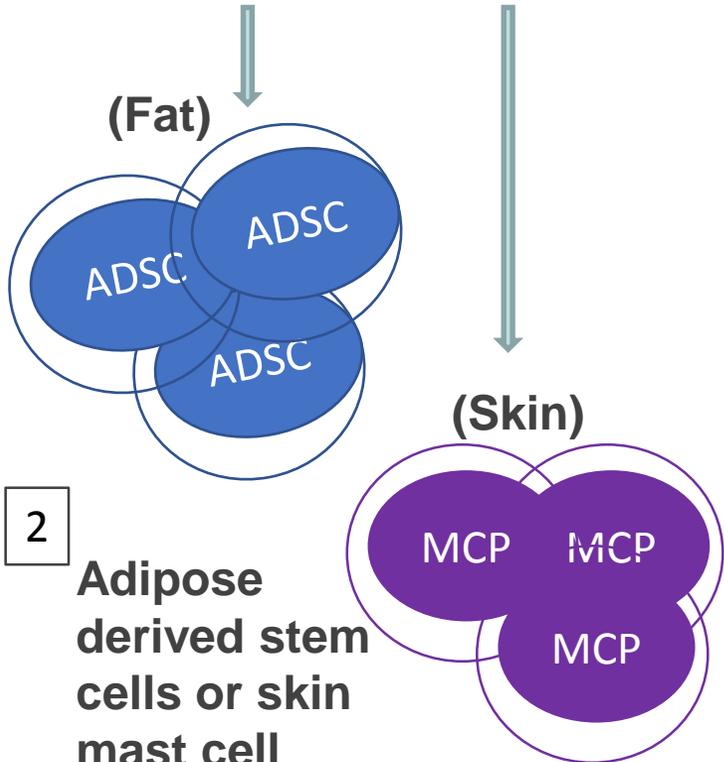
- Increased CD63 expression on primary basophils – an indirect marker of activation w/no measurement of mediator release
- Use transfected humanized rat basophil leukemia cell line RBL-SX38 which expresses hFc ϵ RI
- Sensitize with alpha-gal allergic plasma
- Assay beta hexosaminidase release following stimulation with alpha-gal



Subaim 1c: Alternative readouts for allergic effector cell activation

Surgical Specimens

1



2 Adipose derived stem cells or skin mast cell progenitors

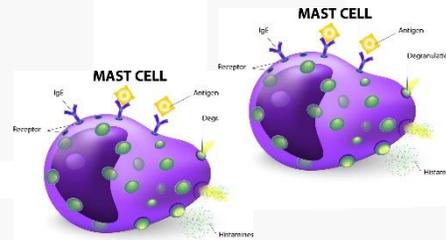
3

Mast Cell Differentiation Media (includes rhSCF)



shutterstock.com • 556378924

4



Cultured primary human mast cells

7

Beta hexosaminidase assay

6

45 minute incubation with:

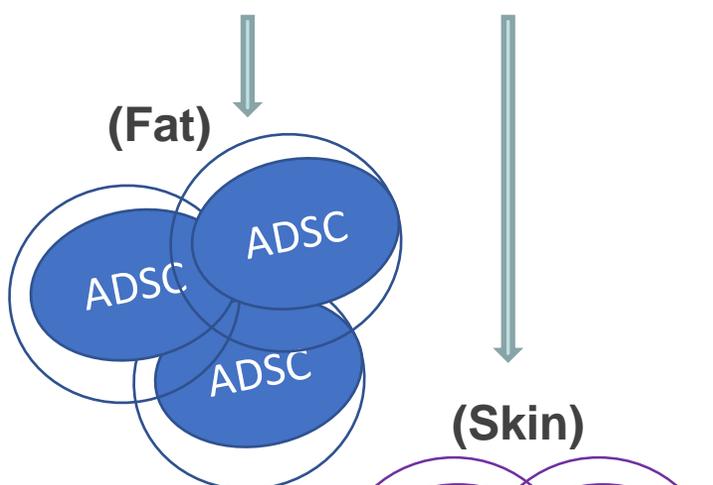
- Beef thyroglobulin* (glycoprotein)
- Cetuximab* (glycoprotein)
- PBS-112 (glycolipid)
- PBS-113* (glycolipid)

5

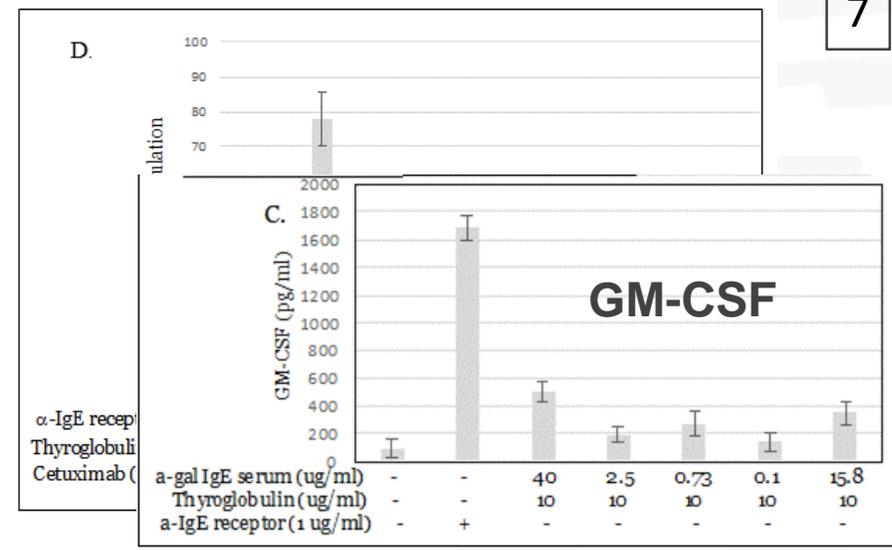
Sensitize with plasma from subject with alpha-gal allergy

Kepley Lab (UNC-Greensboro)

Subaim 1c: Alternative readouts for allergic effector cell activation



2 Adipose derived stem cells or mast cell progenitors



3 Mast Cell Differentiation Media (includes rhSCF)

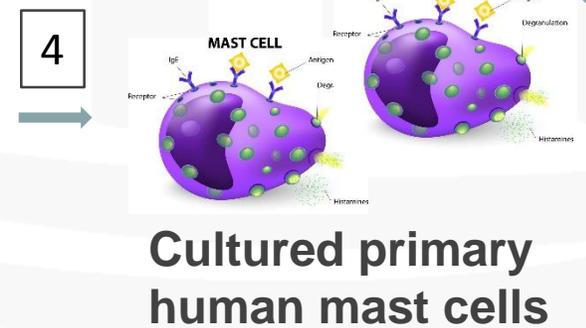


7 Cytokine production

6 24 hour incubation with:

- Beef thyroglobulin* (glycoprotein)
- Cetuximab* (glycoprotein)
- PBS-112 (glycolipid)
- PBS-113* (glycolipid)

5 Sensitize with plasma from subject with alpha-gal allergy

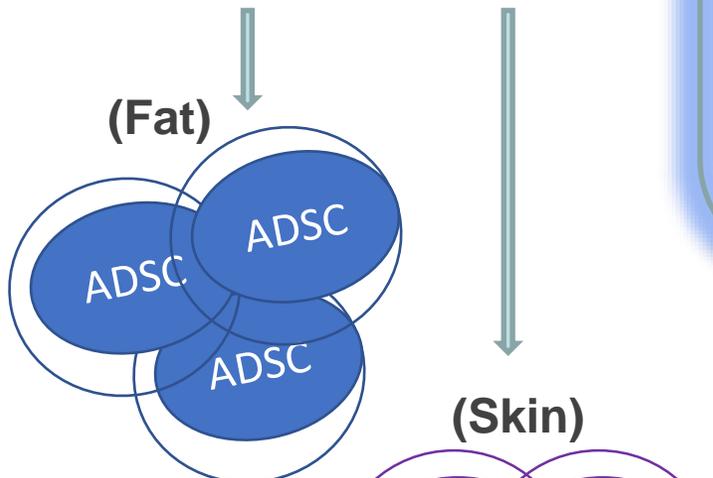


shutterstock.com • 556378924

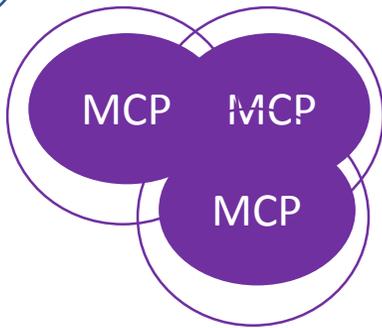


Subaim 1c: Alternative readouts for allergic effector cell activation

Cell culture system increases ability to dissect mechanisms of alpha-gal glycolipid mediated mast cell activation



2 Adipose derived stem cells or mast cell progenitors

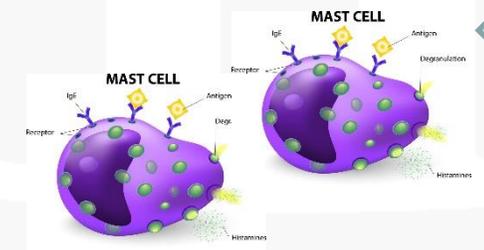


3 Mast Cell Differentiation Media (includes rhSCF)



shutterstock.com • 556378924

4



Cultured primary human mast cells

5 Sensitize with plasma from subject with alpha-gal allergy

6

- 24 hour incubation with:**
- Beef thyroglobulin* (glycoprotein)
 - Cetuximab* (glycoprotein)
 - PBS-112 (glycolipid)
 - PBS-113* (glycolipid)

7

Cytokine production

Acknowledgments



UNC
SCHOOL OF MEDICINE

Iweala Lab (UNC)

Camille Kapita

Julie John

Commins Lab (UNC)

Dr. Scott Commins

Dr. Shailesh Choudhary

Claire Addison

Claire Amelio

Brigham Young University

Dr. Paul B. Savage

Platts-Mills Lab (UVA)

Alex Schuyler

UNC Food Allergy Initiative

Jada Suber

Dr. Kelly Orgel

Dr. Mike Kulis

Dr. Wesley Burks

Oklahoma State University

Dr. Susan Little

Kansas State University

Dr. Brian Herrin

University of Southern Mississippi

Dr. Shahid Karim

Gary Crispell

Brigham and Women's Hospital

Dr. Patrick J. Brennan

Gerald Watts

UNC Division of Rheumatology, Allergy & Immunology

Dr. Edwin Kim

Dr. Beth Jonas

Dr. Richard Loeser

Diane Bresch, RN

UNC Department of Medicine

Dr. Ron Falk

Wash. U. St. Louis Allergy/Immunology

Dr. Maya Jerath

UNC Flow Cytometry Core

- supported in part by NIH P30 CA016086 Cancer Center Core support Grant to the UNC Lineberger Comprehensive Cancer Center

Kepley Lab (UNC-Greensboro)

Dr. Chris Kepley

Mohammad Fereydouni

UNC Alpha-Gal Study Subjects

AAAAI Faculty Development Program

Dr. Corinne Keet

Deborah Levinson

Steve Folstein

Funding sources:

NIH-NIAID T32 AI 007062-39

UM1 AI 30936-01 -- UNC FAI

Summary

- A 30-minute incubation with alpha-gal-containing glycolipids activated basophils sensitized with plasma from alpha-gal allergic subjects in an IgE-dependent fashion
- These results suggest a unique role for glycolipid rarely described in IgE-mediated food allergy
- **Next steps include:**
 - » **developing a cell culture system with mast cells to dissect mechanisms of alpha-gal glycolipid-mediated mast cell activation**
 - » **Using mouse models of alpha-gal syndrome to establish the relevance of alpha-gal-glycolipid allergic effector cell activation *in vivo***



SUPPLEMENTARY SLIDES

1. Brennan PJ et al., Nat Rev Imm 2013
2. Hong GU et al., Cell Signal 2014
3. Blumberg RS et al., J Immunol 1991
4. Rossjohn J et al., Nat Rev Imm 2012

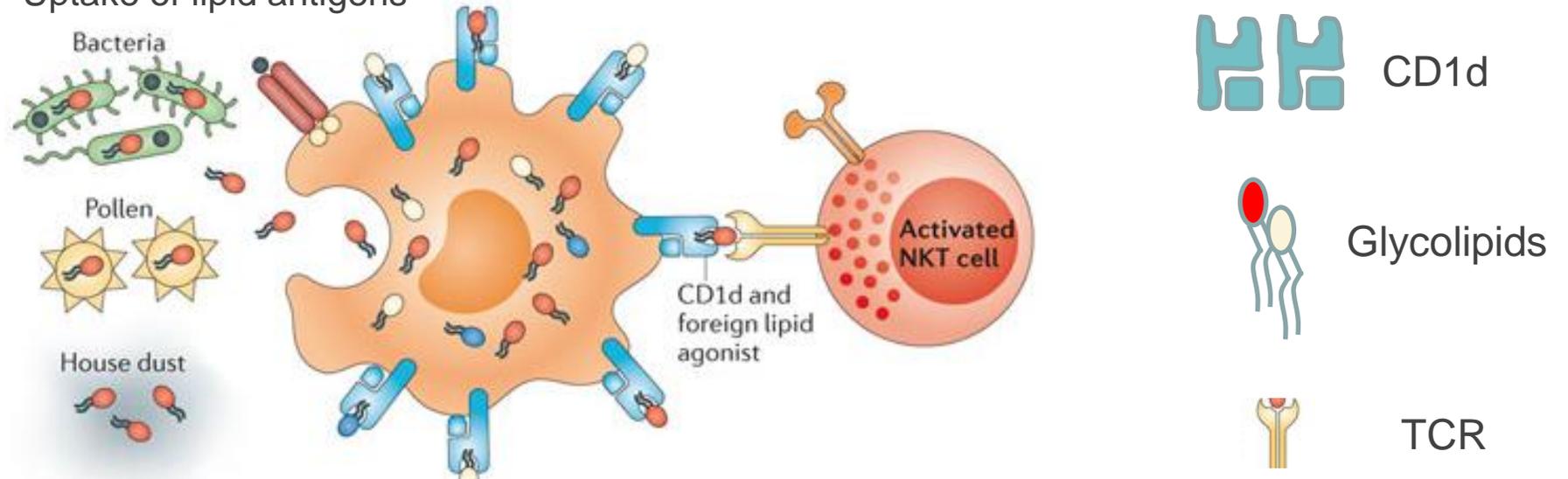
How does the immune system process glycolipid antigens?

- CD1 : MHC I-like proteins that survey intracellular endosomal compartments for glycolipid antigen to bind and present to immune effector cells
 - » 5 isoforms in human, CD1a, b, c, d, and e,

1. Brennan PJ et al., Nat Rev Imm 2013
2. Hong GU et al., Cell Signal 2014
3. Blumberg RS et al., J Immunol 1991
4. Rossjohn J et al., Nat Rev Imm 2012

How does the immune system process glycolipid antigens?

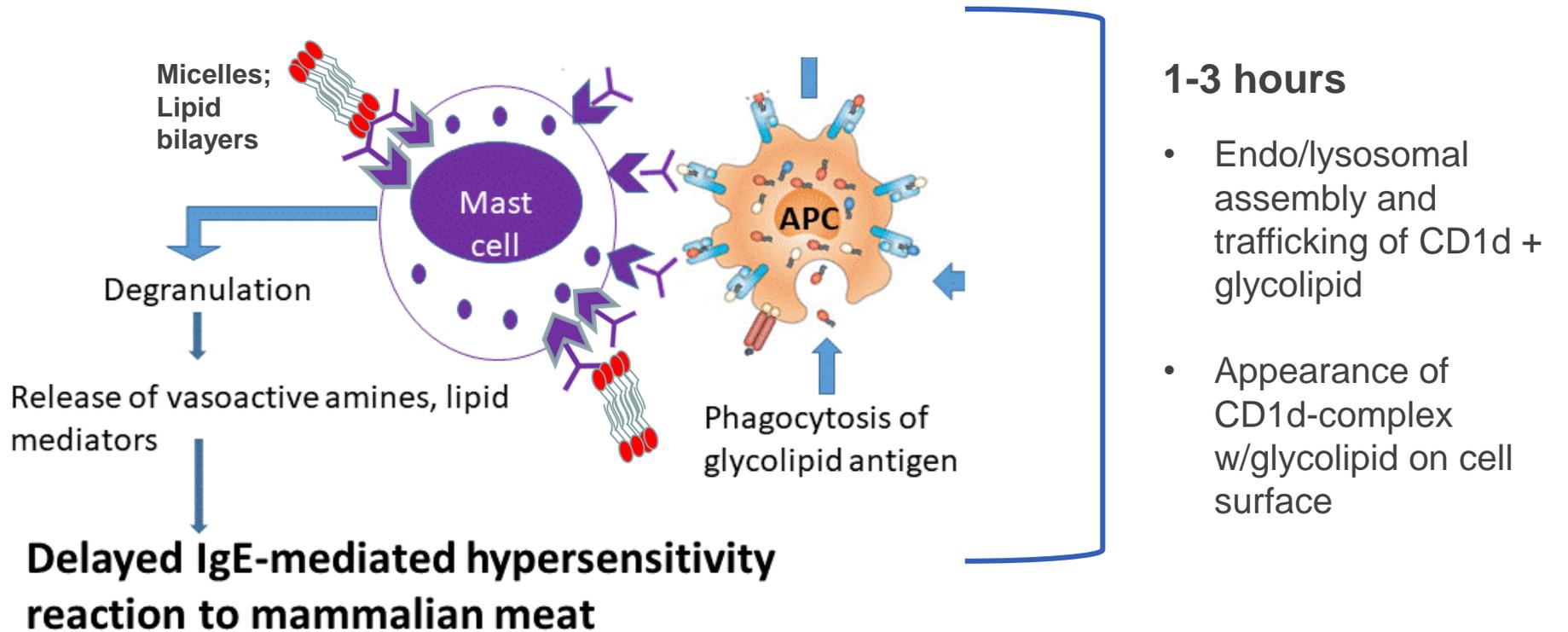
Uptake of lipid antigens



- **CD1d isoform**

- » expressed on both professional and non-professional APCs^{1,2,3}
- » Presents self & foreign lipid antigens to **invariant natural killer T (iNKT) cells**

Hypothesis 2b: Time required to process and present glycolipid to immune effector cells may explain the delayed allergic reactions in alpha-gal allergy



Specific Aims:

Aim 1: Evaluate candidate alpha-gal-containing components of mammalian tissue that can mediate delayed allergic responses in AGS

Working hypothesis: Alpha-gal-containing mammalian glycolipids bind alpha-gal-specific IgE displayed on allergic effector cell (i.e. mast cell and basophil) surfaces, activating these cells

Aim 2: Identify cellular sources of type 2 cytokines critical for the generation of alpha-gal specific IgE in AGS

Working hypothesis: Unconventional T cells that recognize and respond to glycolipid antigen, specifically CD1d-restricted NKT cells, drive the type 2 cytokine environment that supports alpha-gal-specific antibody class switching to IgE, following lone star tick exposure



Specific Aims:

Aim 1: Evaluate candidate alpha-gal-containing components of mammalian tissue that can activate allergy effector cells in AGS

Working hypothesis: Alpha-gal-containing mammalian glycolipids bind alpha-gal-specific IgE displayed on allergic effector cell (i.e. mast cell and basophil) surfaces, activating these cells

Aim 2: Identify alpha-gal-containing components of mammalian tissue that can mediate delayed allergic responses in AGS in vivo

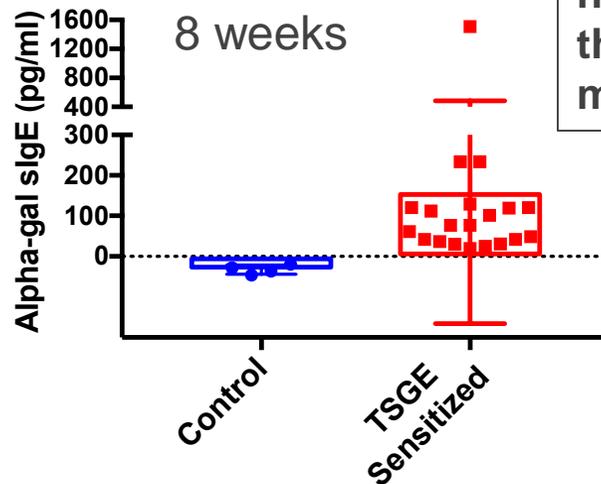
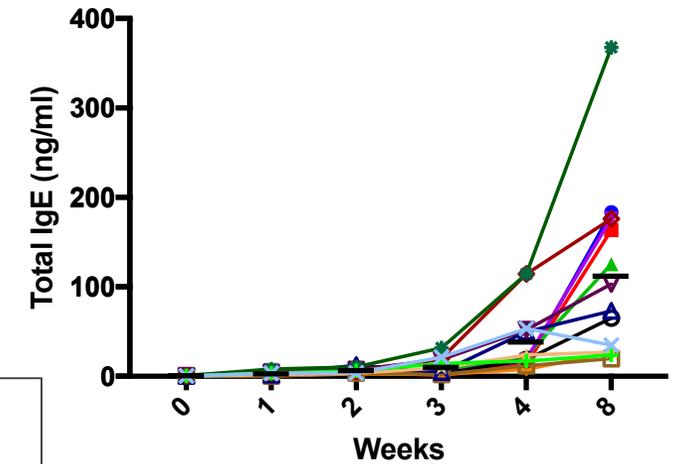
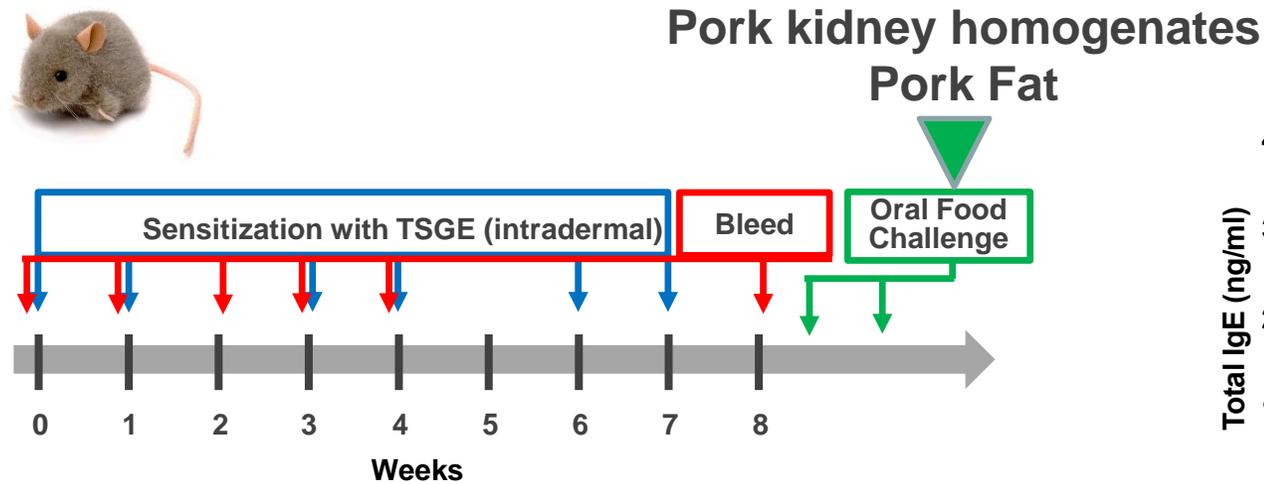
Working hypothesis: Allergic effector cells sensitized with alpha-gal-specific IgE and activated with alpha-gal-containing glycolipids trigger delayed anaphylaxis *in vivo*.

Aim 3: Identify cellular sources of type 2 cytokines critical for the generation of alpha-gal specific IgE in AGS

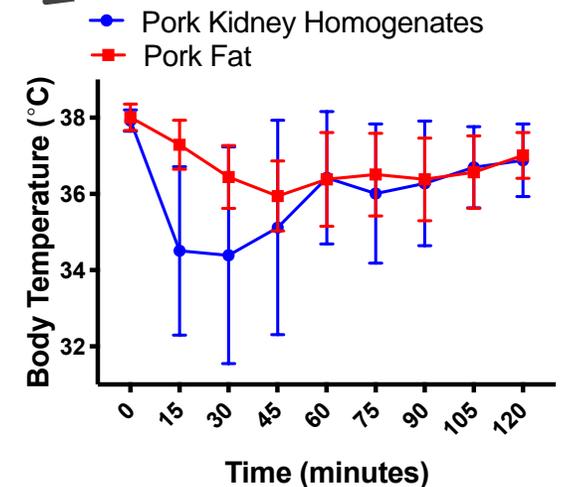
Working hypothesis: Unconventional T cells that recognize and respond to glycolipid antigen, specifically CD1d-restricted NKT cells, drive the type 2 cytokine environment that supports alpha-gal-specific antibody class switching to IgE, following lone star tick exposure

Preliminary Data

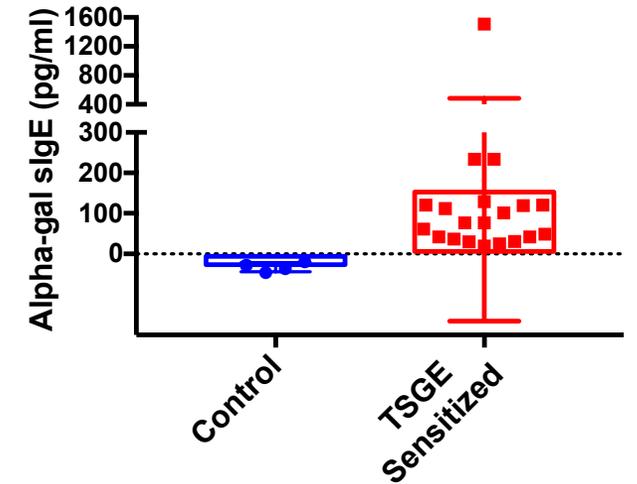
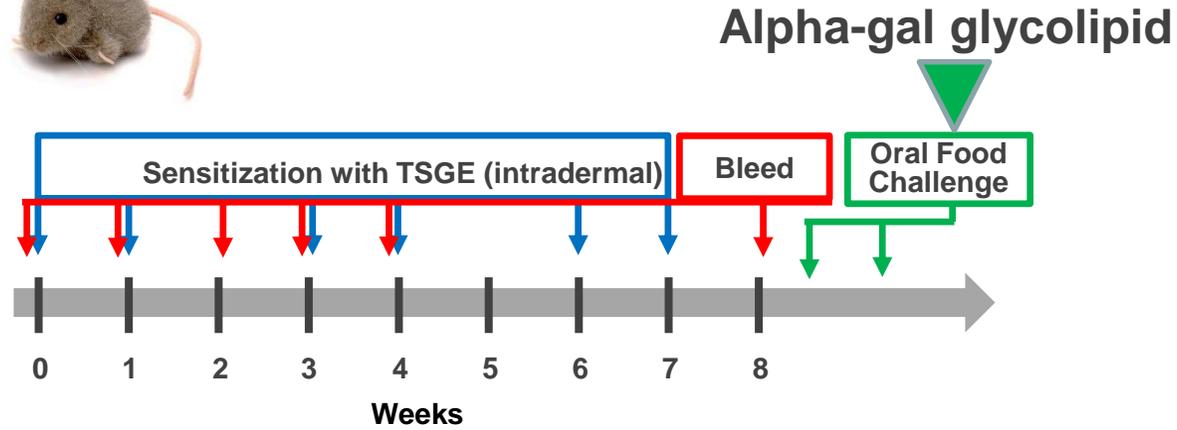
Intradermal injection with TSGE induces a rise in total and alpha-gal specific IgE in alpha-gal KO mice



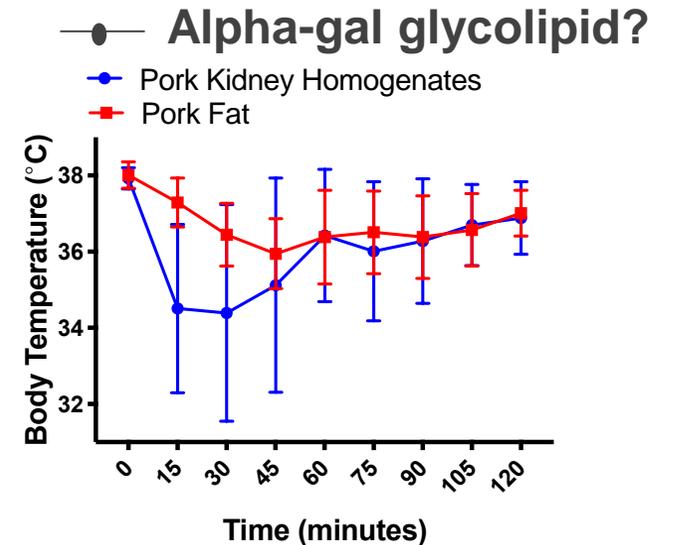
Mice sensitized with TSGE and challenged with pork kidney homogenates or pork fat drop their body temperature 15 to 45 minutes post challenge



Aim 2a: Oral challenge of alpha-gal KO mice with alpha-gal-containing glycolipid following sensitization with tick salivary gland extract (TSGE)

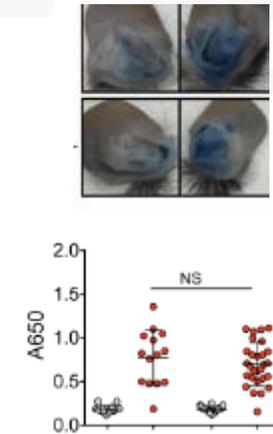
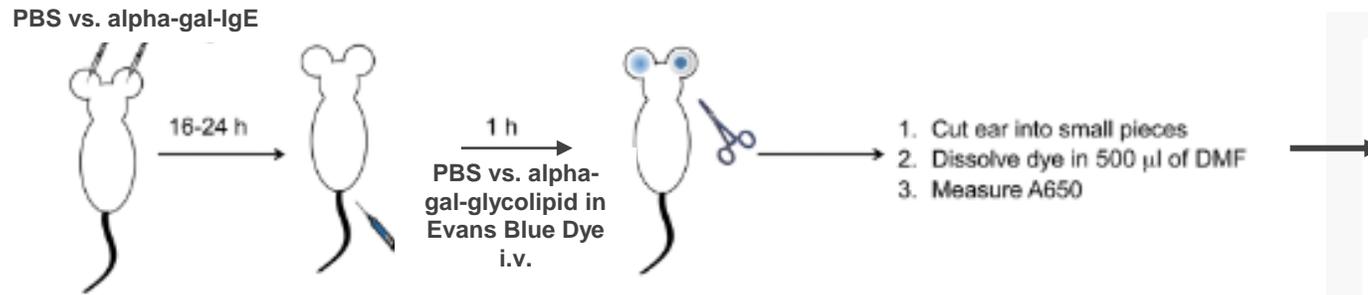


Thermometer with rectal probe to measure body temperature

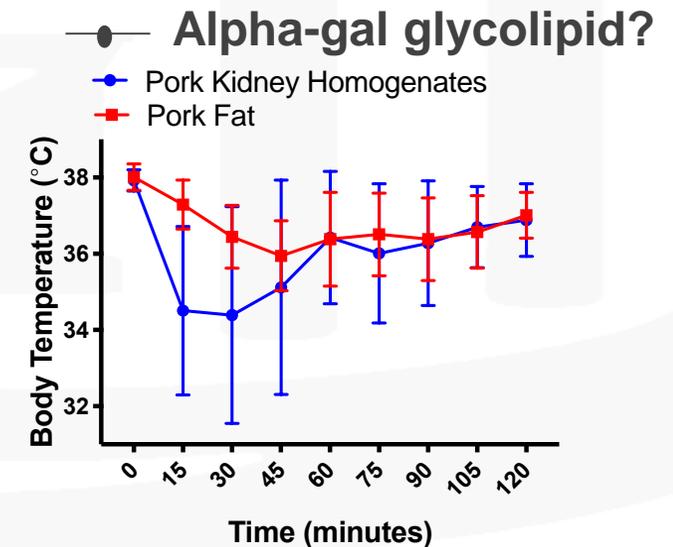
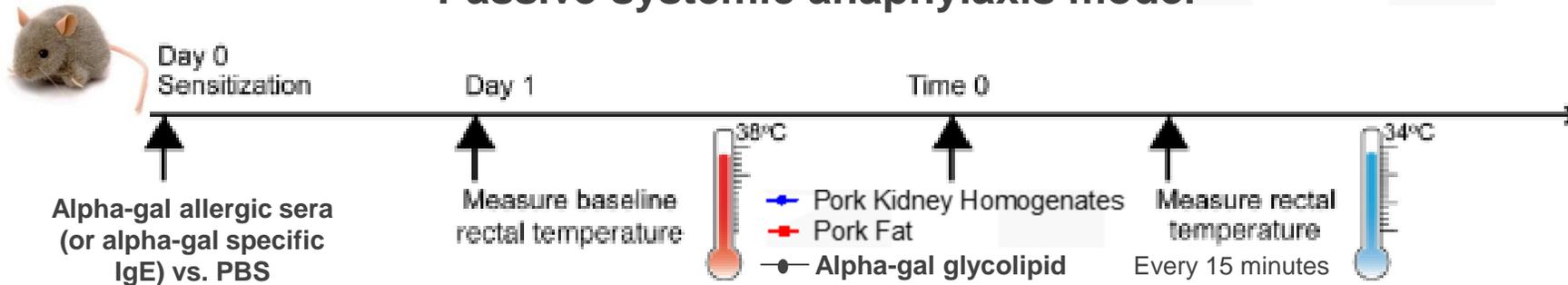


Aim 2b: Passively sensitize humanized mice that express hFcεRI (commercially available) and assess ability of alpha-gal-containing glycolipid to induce anaphylaxis *in vivo*

Passive cutaneous anaphylaxis



Passive systemic anaphylaxis model



Summary

- A 30-minute incubation with alpha-gal-containing glycolipids activated basophils sensitized with plasma from alpha-gal allergic subjects in an IgE-dependent fashion
- These results suggest a unique role for glycolipid rarely described in IgE-mediated food allergy
- **Next steps include:**
 - » **developing a cell culture system with mast cells to dissect mechanisms of alpha-gal glycolipid-mediated mast cell activation**
 - » **Using mouse models of alpha-gal syndrome to establish the relevance of alpha-gal-glycolipid allergic effector cell activation *in vivo***

Acknowledgments



UNC
SCHOOL OF MEDICINE

Iweala Lab (UNC)

Camille Kapita

Julie John

Commins Lab (UNC)

Dr. Scott Commins

Dr. Shailesh Choudhary

Claire Addison

Claire Amelio

Brigham Young University

Dr. Paul B. Savage

Platts-Mills Lab (UVA)

Alex Schuyler

UNC Food Allergy Initiative

Jada Suber

Dr. Kelly Orgel

Dr. Mike Kulis

Dr. Wesley Burks

Oklahoma State University

Dr. Susan Little

Kansas State University

Dr. Brian Herrin

University of Southern Mississippi

Dr. Shahid Karim

Gary Crispell

Brigham and Women's Hospital

Dr. Patrick J. Brennan

Gerald Watts

UNC Division of Rheumatology, Allergy & Immunology

Dr. Edwin Kim

Dr. Beth Jonas

Dr. Richard Loeser

Diane Bresch, RN

UNC Department of Medicine

Dr. Ron Falk

Wash. U. St. Louis Allergy/Immunology

Dr. Maya Jerath

UNC Flow Cytometry Core

- supported in part by NIH P30 CA016086 Cancer Center Core support Grant to the UNC Lineberger Comprehensive Cancer Center

Kepley Lab (UNC-Greensboro)

Dr. Chris Kepley

Mohammad Fereydouni

UNC Alpha-Gal Study Subjects

AAAAI Faculty Development Program

Dr. Corinne Keet

Deborah Levinson

Funding sources:

NIH-NIAID T32 AI 007062-39

UM1 AI 30936-01 -- UNC FAI