

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



Photo credit: Sam Kittner '85

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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 ( $\leq 2$  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 ( $\geq 2$  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:

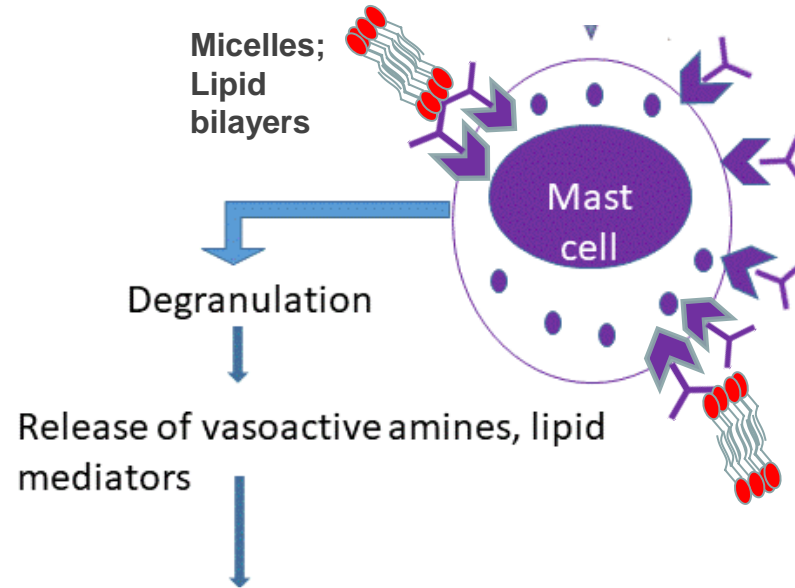
***Aim 1: Evaluate candidate alpha-gal-containing components of mammalian tissue that can mediate delayed allergic responses in AGS***

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***

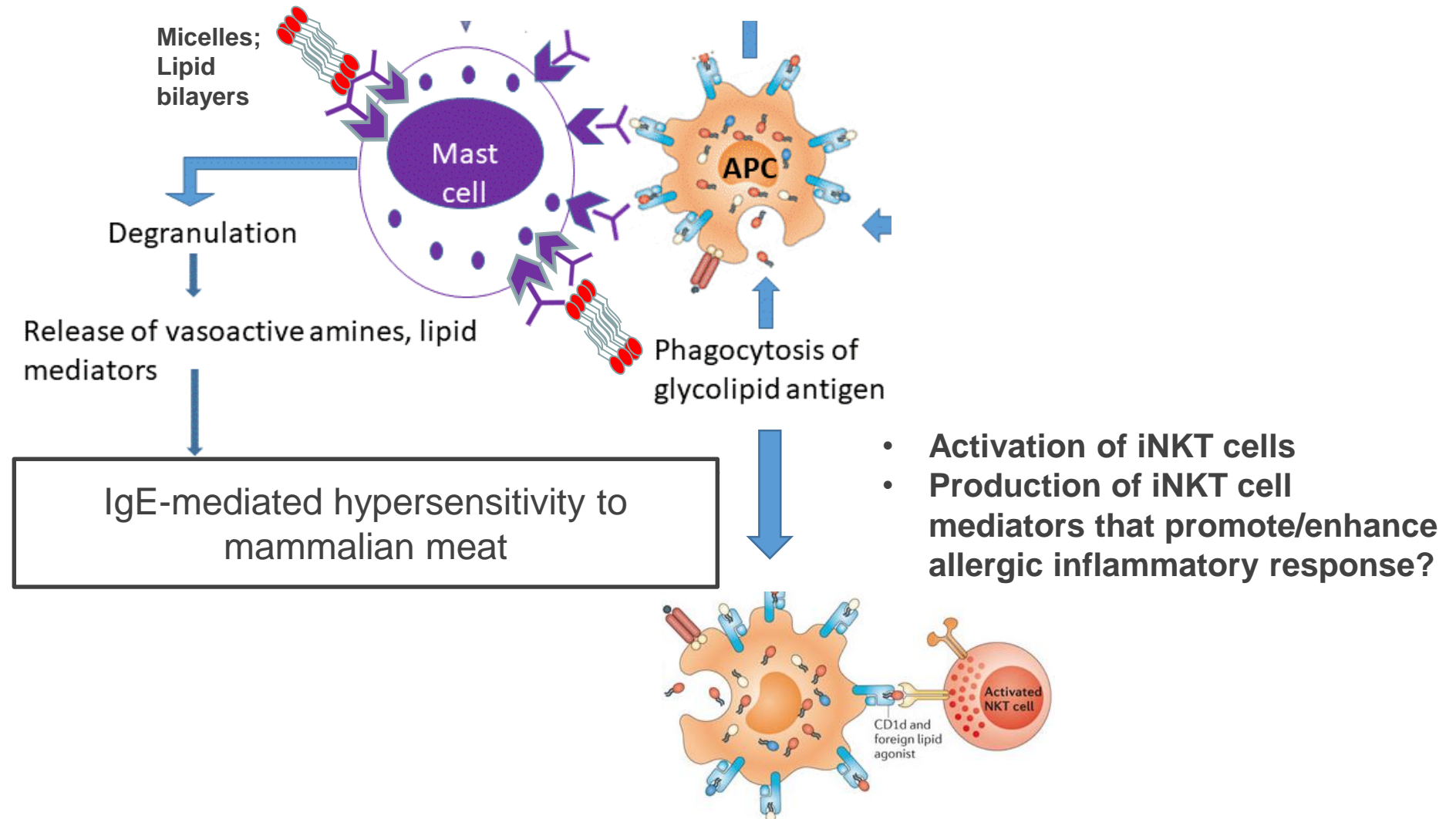
Working hypothesis: Unconventional T cells that recognize and respond to glycolipid antigen, specifically CD1d-restricted NKT cells, drive the type 2 cytokine environment that supports alpha-gal-specific antibody class switching to IgE, following lone star tick exposure

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

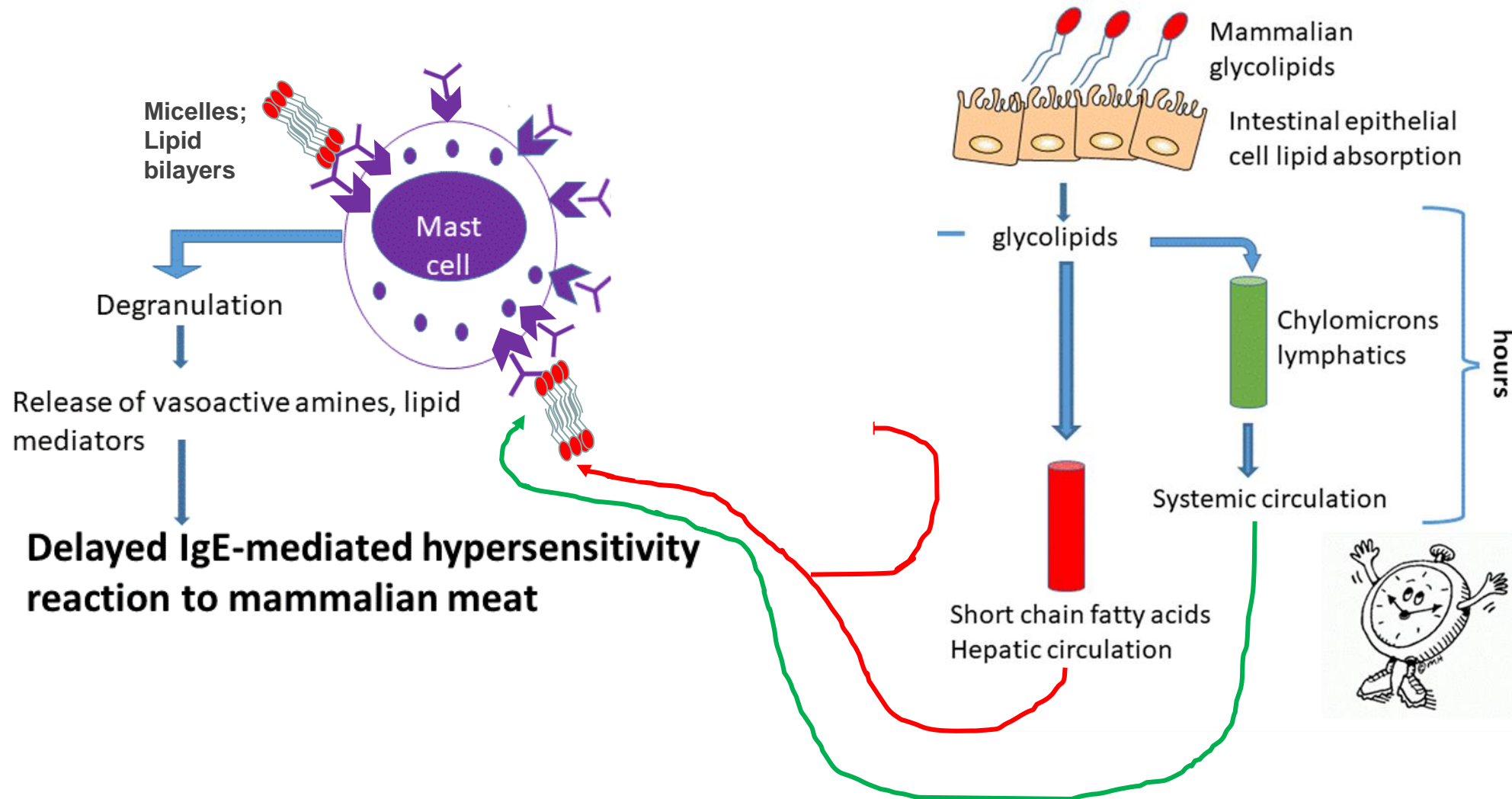




# Central Hypothesis 1: Glycolipids play a role in the effector phase of alpha-gal allergy



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



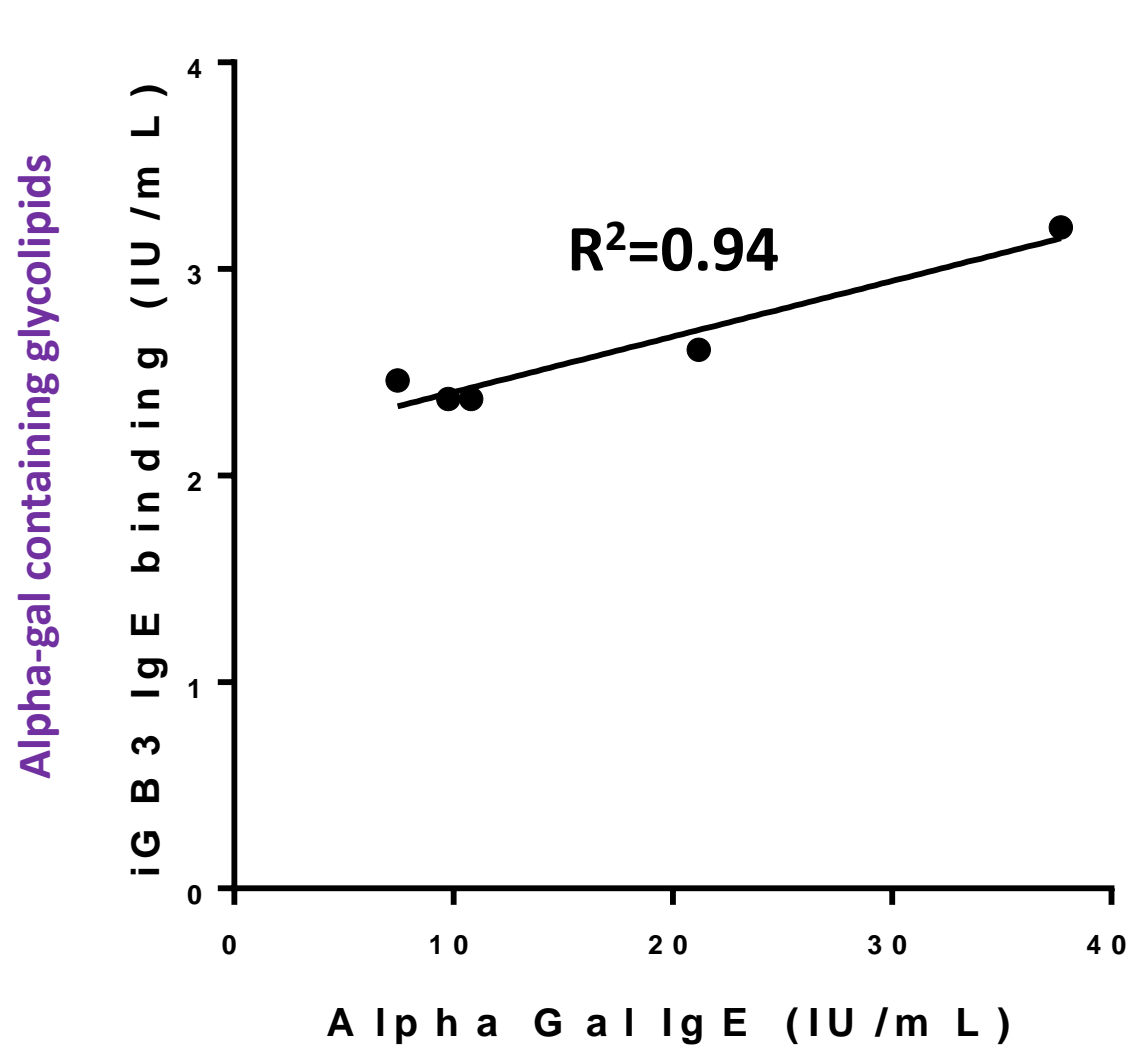


## Preliminary Data

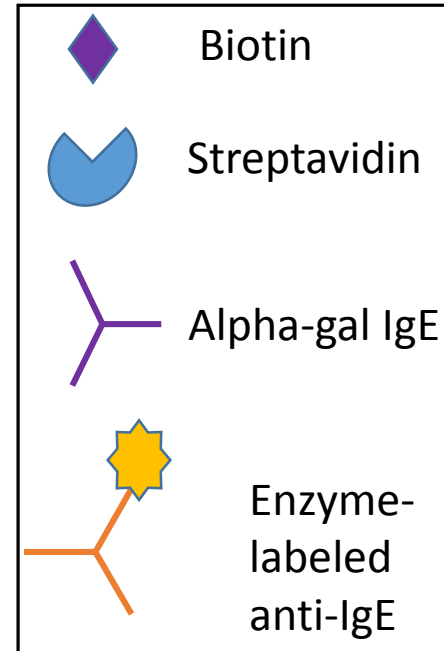
**Can alpha-gal specific IgE bind to alpha-gal-containing glycolipids?**

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

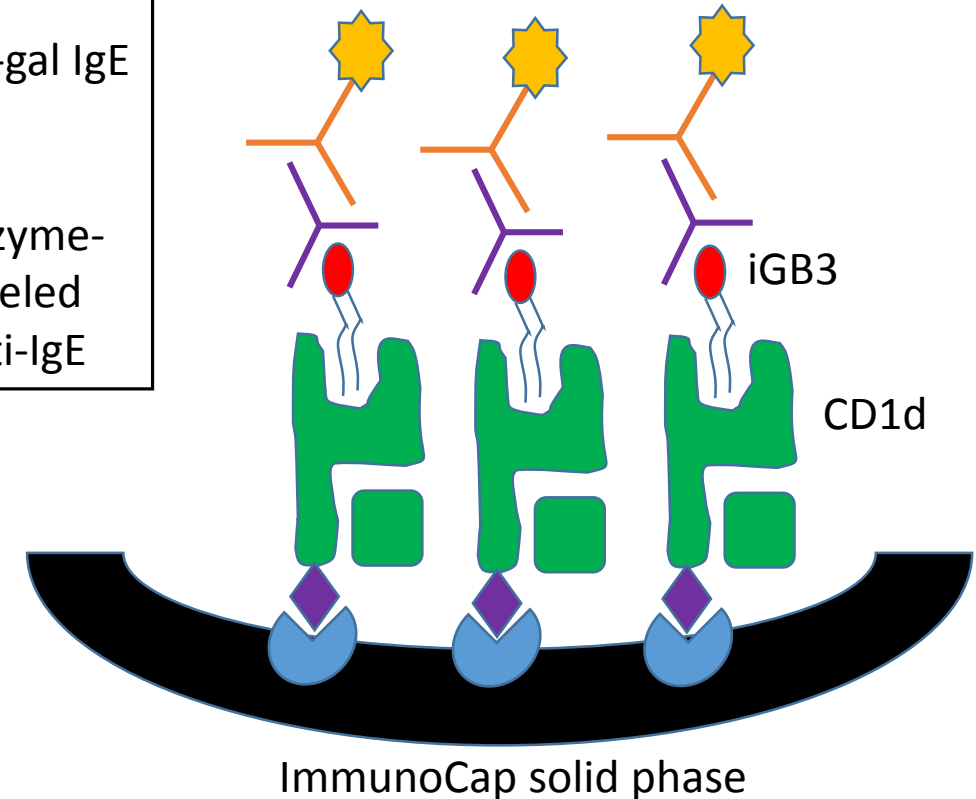
# Serum IgE from alpha-gal allergic patients binds alpha-gal in both glycoproteins and glycolipids

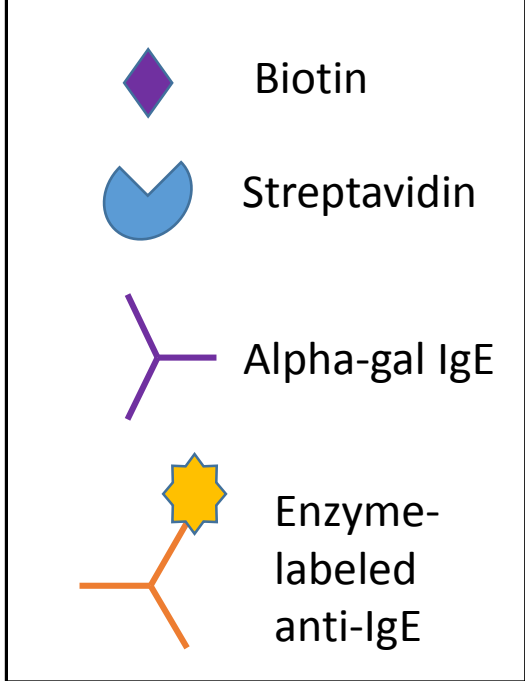


**cetuximab binding – alpha-gal containing glycoprotein**

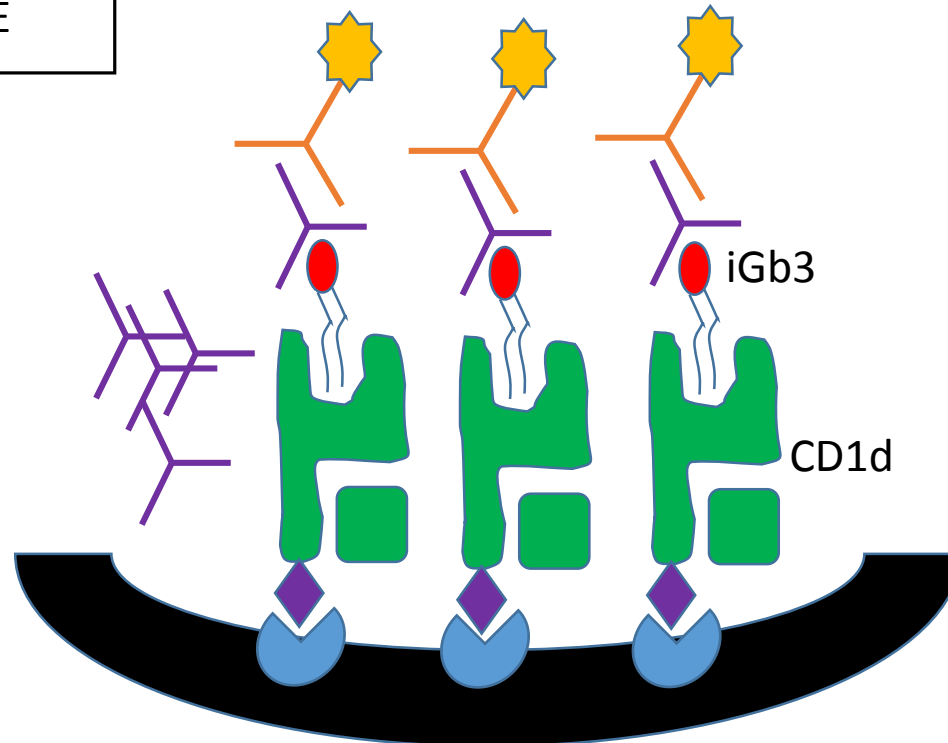


- Incubate w/developing agent
- Measure fluorescence
- Convert to IU/ml





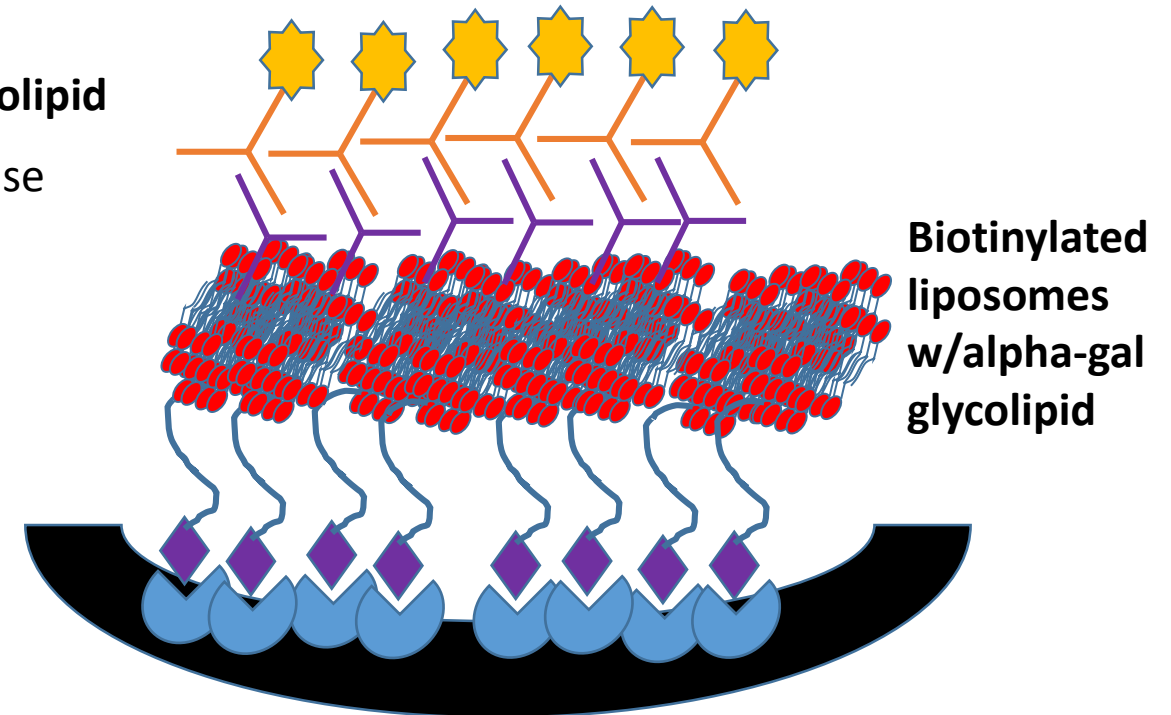
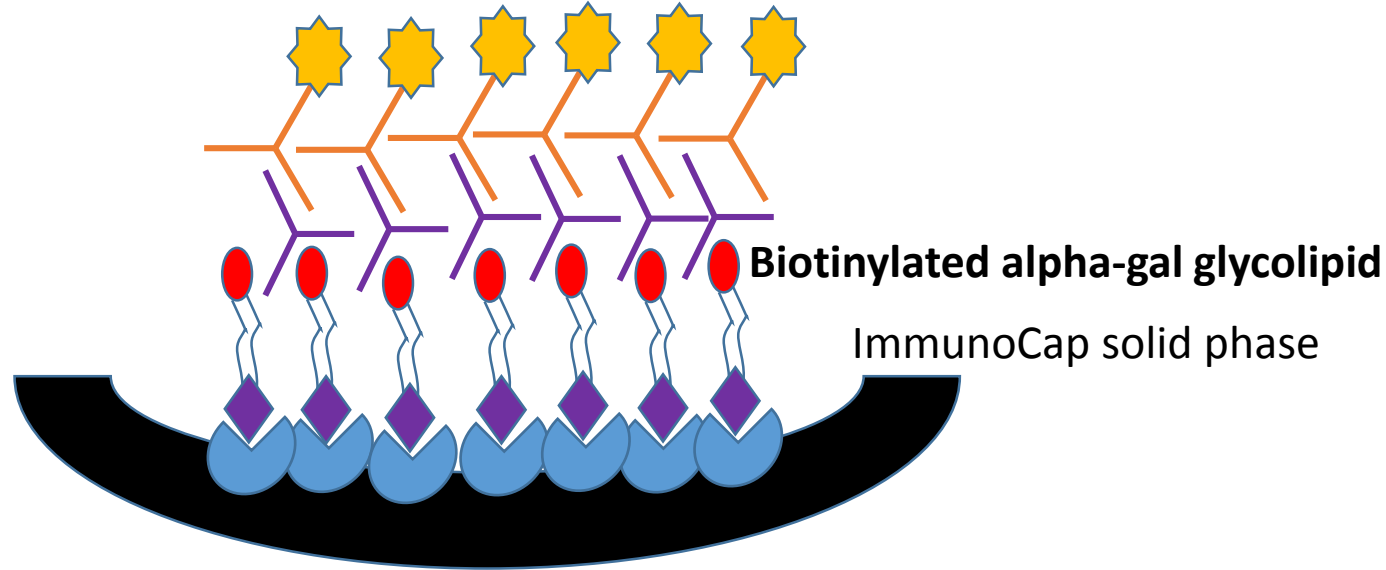
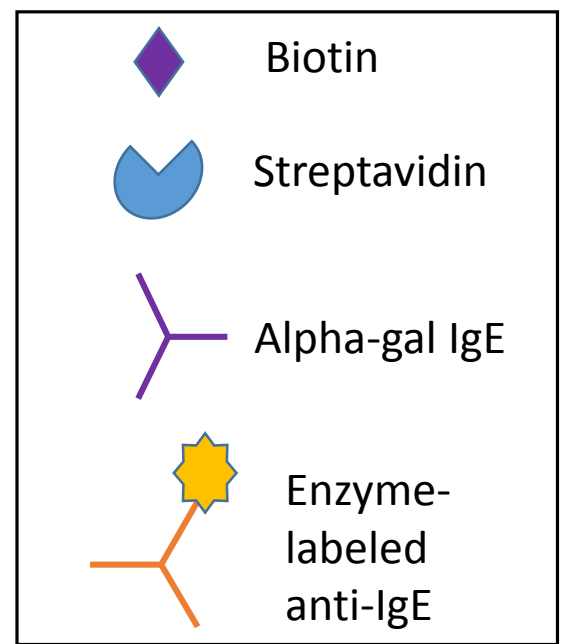
Our current method of detecting IgE-binding to alpha-gal glycolipid may not be optimized



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

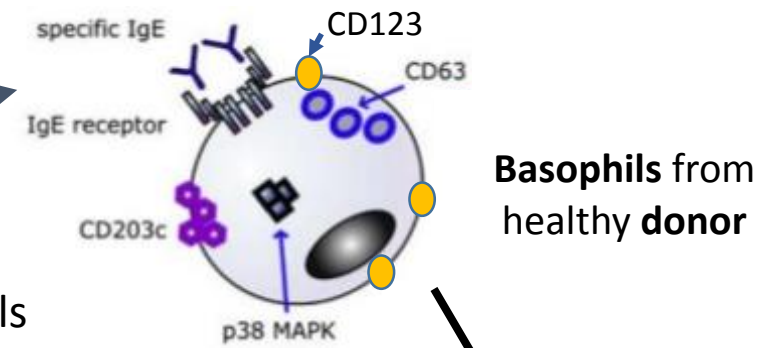
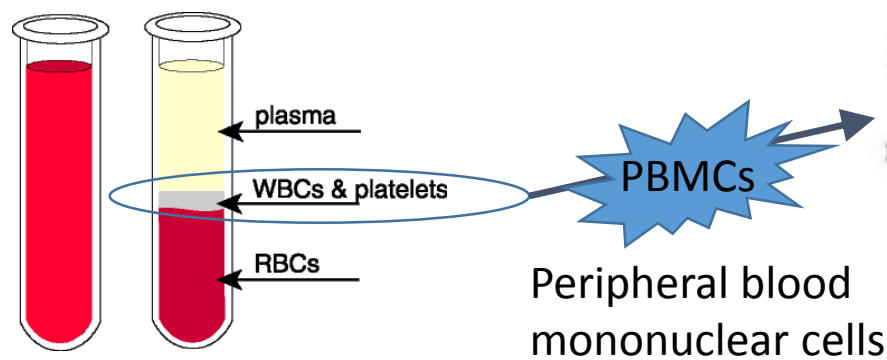


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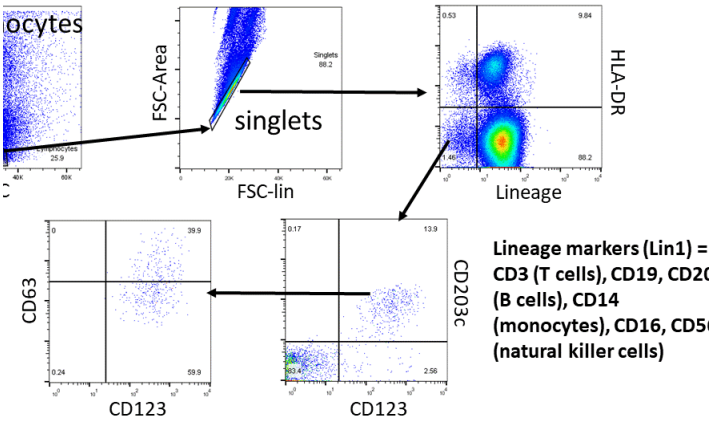
Can alpha-gal specific IgE bind to alpha-gal-containing glycolipids?

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

Whole Human Blood



Basophils from healthy donor



7

Flow Cytometer



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

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

5

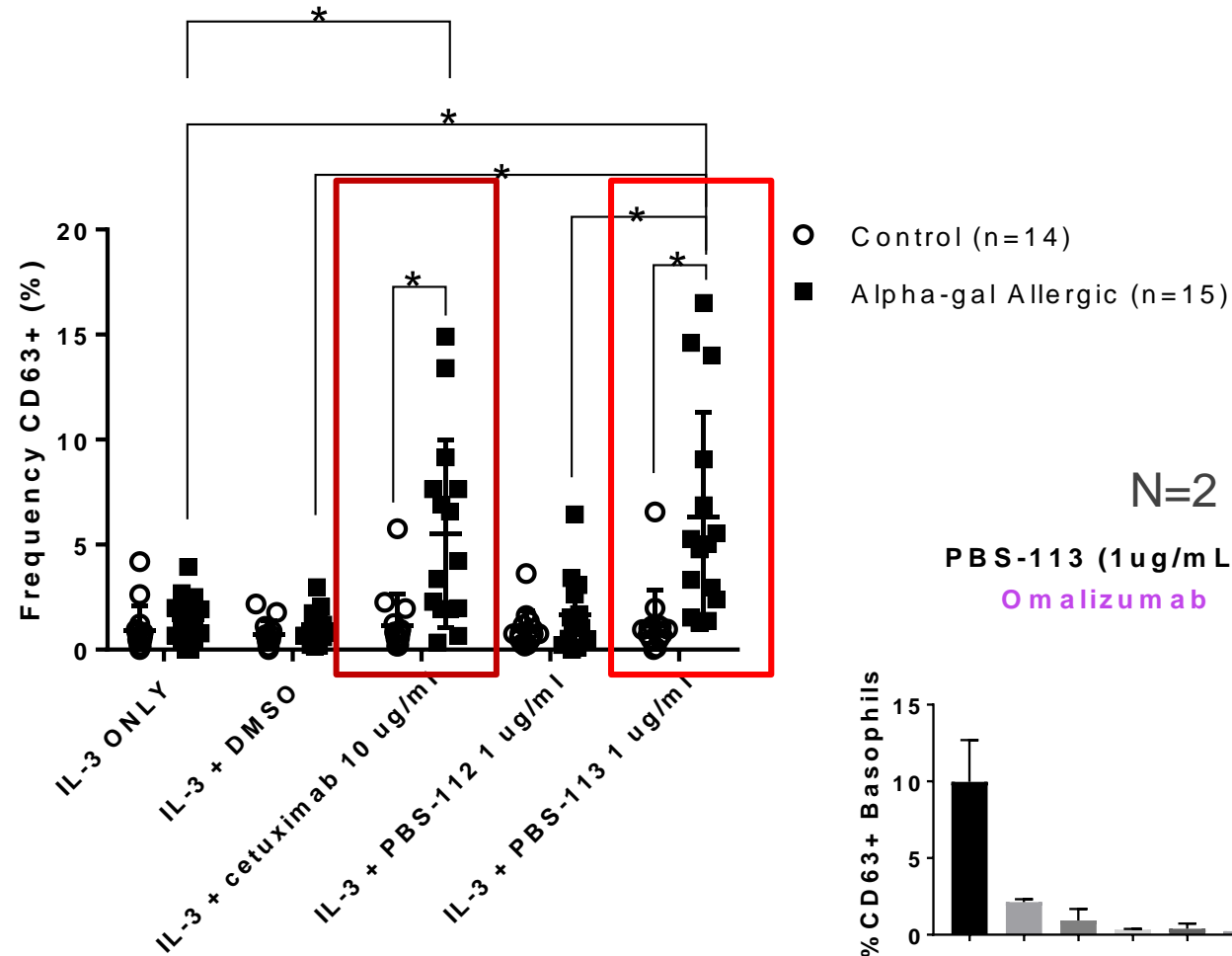
2 Strip off native IgE  
3 Sensitize donor basophils overnight using plasma from patient with alpha-gal allergy

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



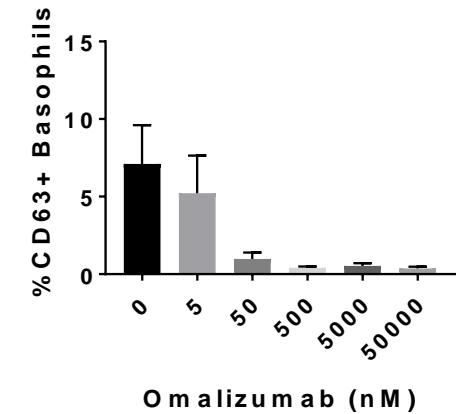
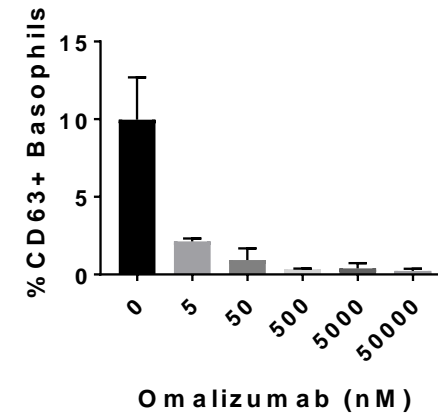
# Alpha-gal-containing glycolipids activate basophils sensitized with plasma from alpha-gal allergic subjects in an IgE-dependent fashion



N=2 alpha-gal allergic

PBS-113 (1ug/mL)+  
Omalizumab

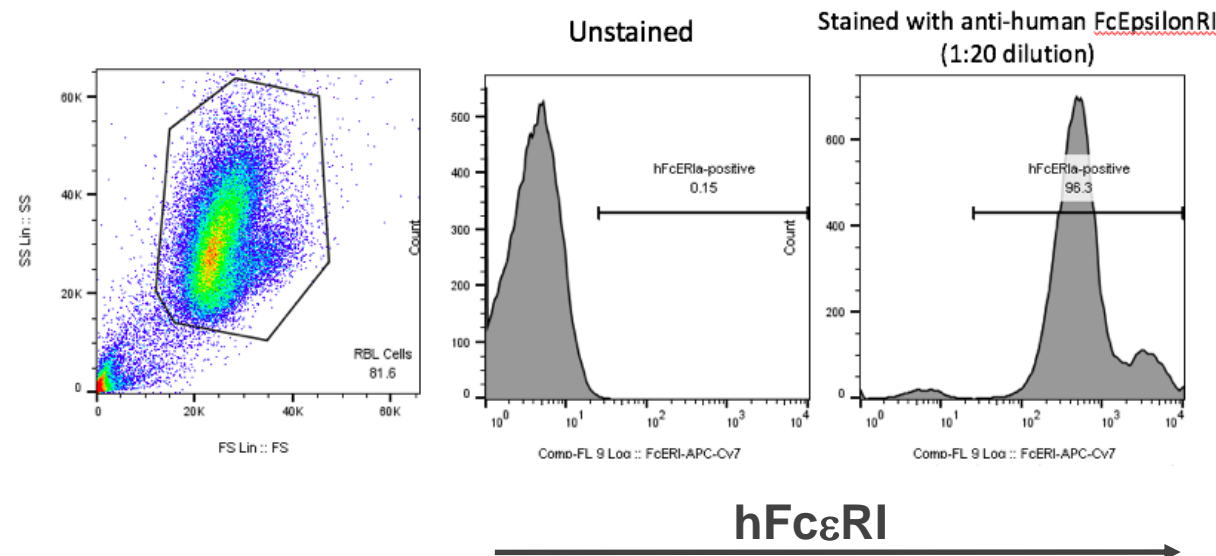
Cetuximab (10 ug/mL) +  
Omalizumab



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 $\epsilon$ RI**
- **Sensitize with alpha-gal allergic plasma**
- **Assay beta hexosaminidase release following stimulation with alpha-gal**

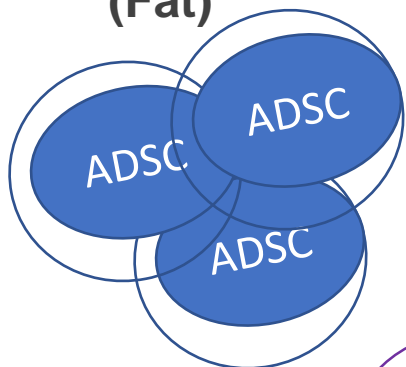


# Subaim 1c: Alternative readouts for allergic effector cell activation

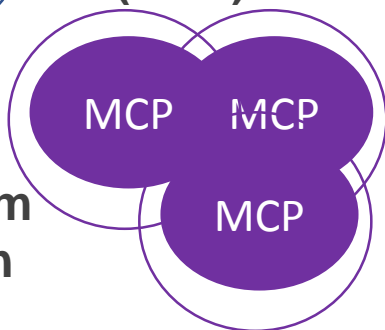
## Surgical Specimens



(Fat)



(Skin)

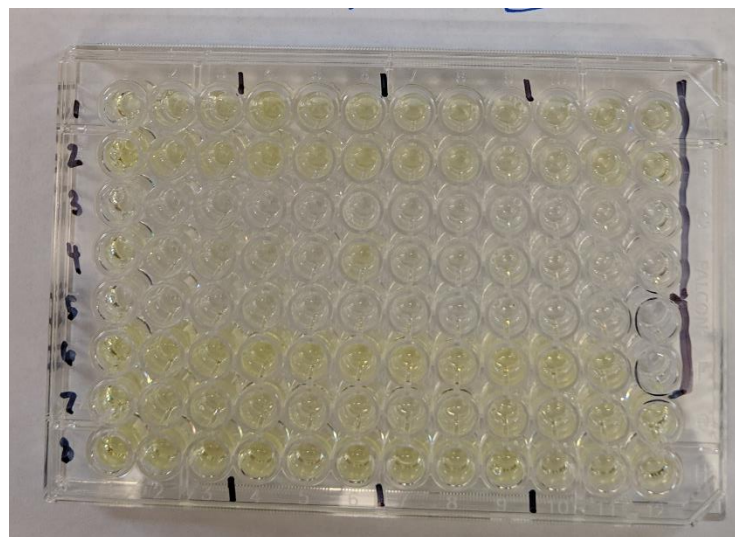


Adipose  
derived stem  
cells or skin  
mast cell  
progenitors

Mast Cell Differentiation  
Media (includes rhSCF)



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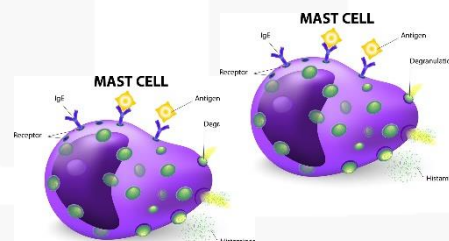


7 Beta hexosaminidase assay

- 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

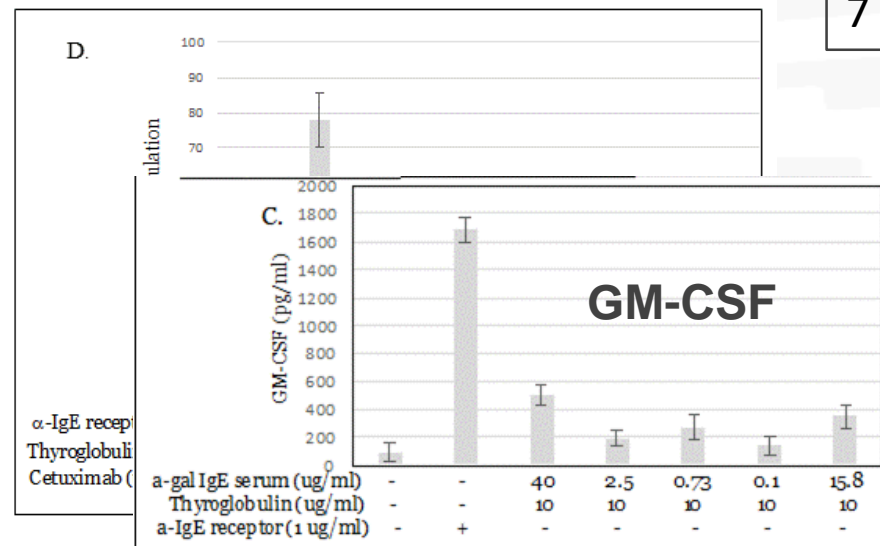
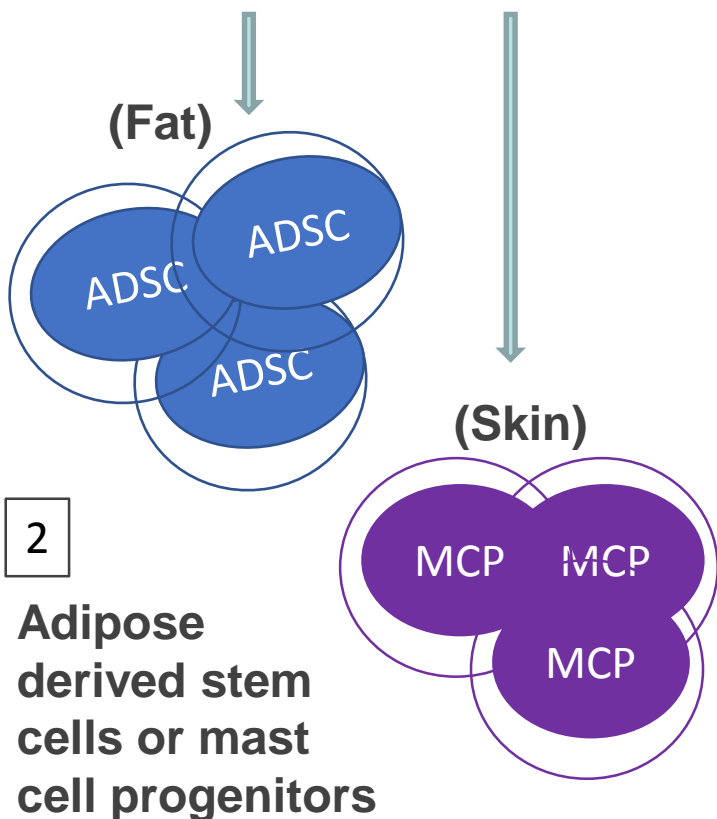
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Cultured primary  
human mast cells

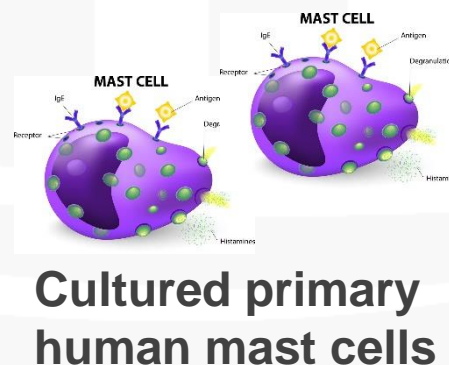
Kepley Lab (UNC-  
Greensboro)

# Subaim 1c: Alternative readouts for allergic effector cell activation



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4



7

## Cytokine production

6

### 24 hour incubation with:

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

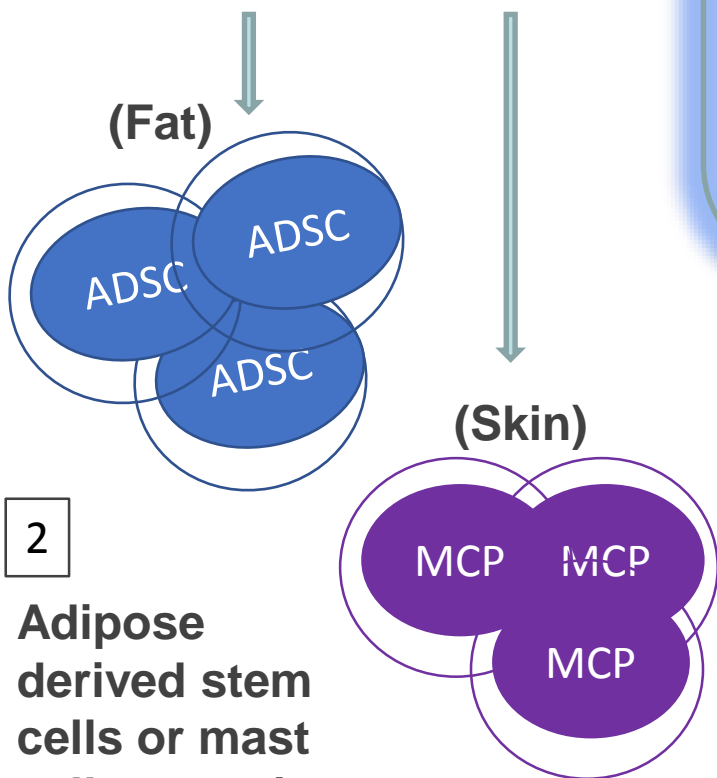
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Sensitize with plasma from subject with alpha-gal allergy

Kepley Lab (UNC-Greensboro)



# Subaim 1c: Alternative readouts for allergic effector cell activation



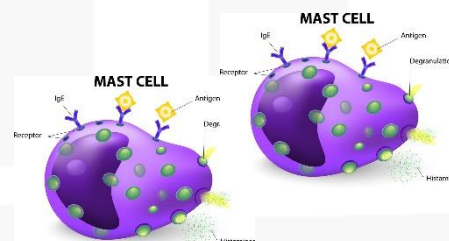
Cell culture system increases ability to dissect mechanisms of alpha-gal glycolipid mediated mast cell activation

3 Mast Cell Differentiation Media (includes rhSCF)



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# Acknowledgments

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**Dr. Chris Kepley**

**Mohammad Fereydouni**

UNC Alpha-Gal Study Subjects

AAAAI Faculty Development Program

**Dr. Corinne Keet**

**Deborah Levinson**

**Steve Folstein**

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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

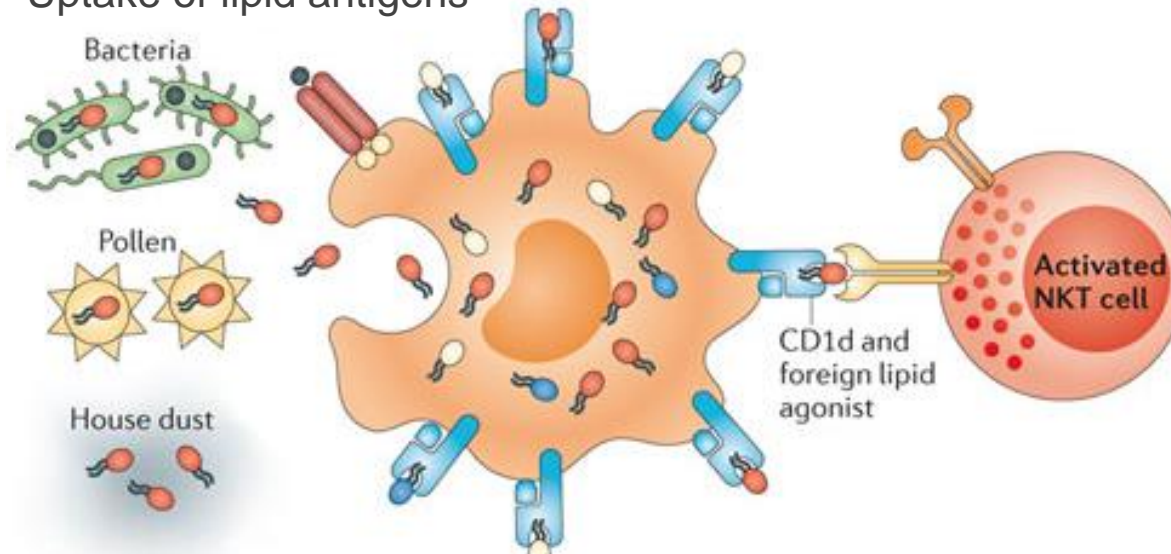
# How does the immune system process glycolipid antigens?

- 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
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# How does the immune system process glycolipid antigens?

Uptake of lipid antigens



CD1d



Glycolipids

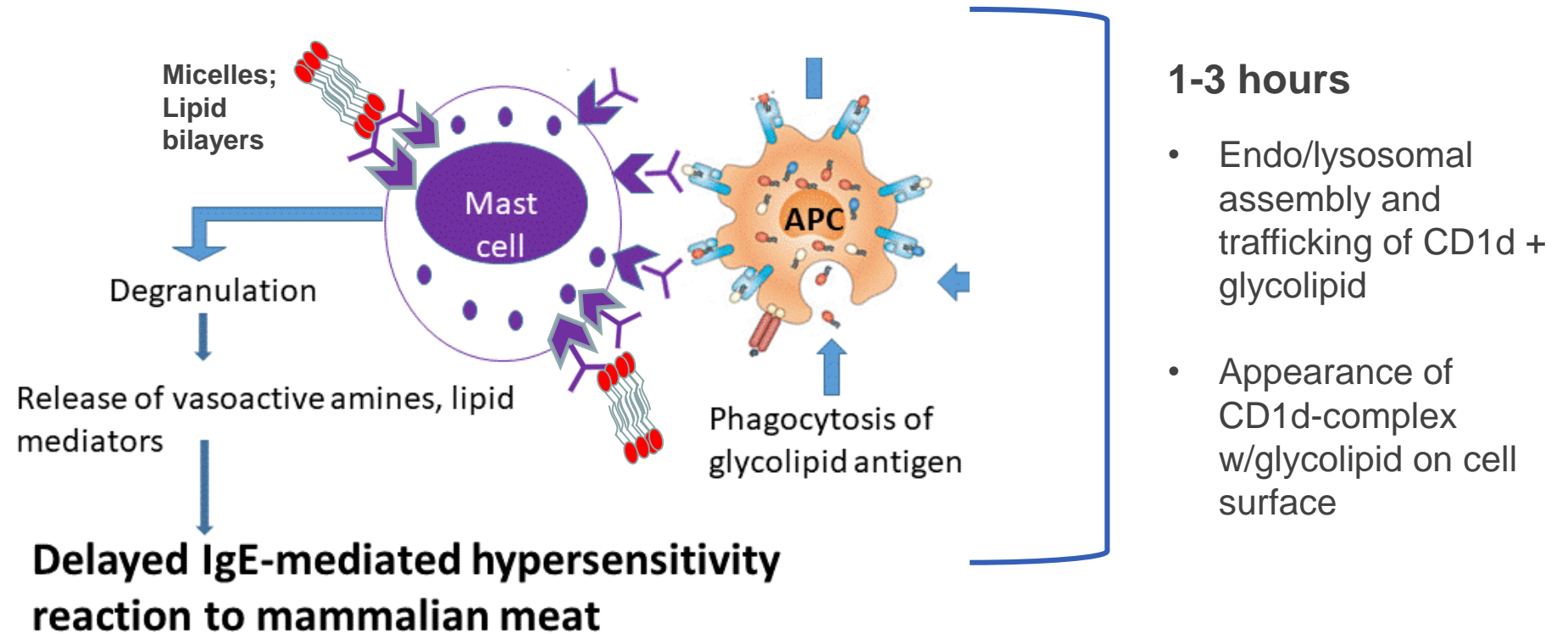


TCR

- **CD1d isoform**

- » expressed on both professional and non-professional APCs<sup>1,2,3</sup>
- » 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***

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

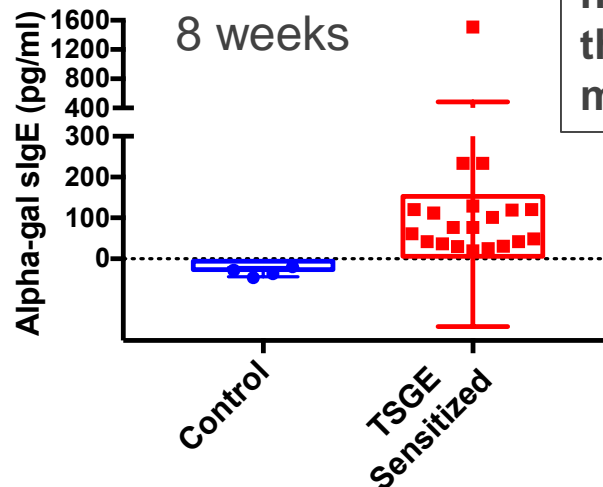
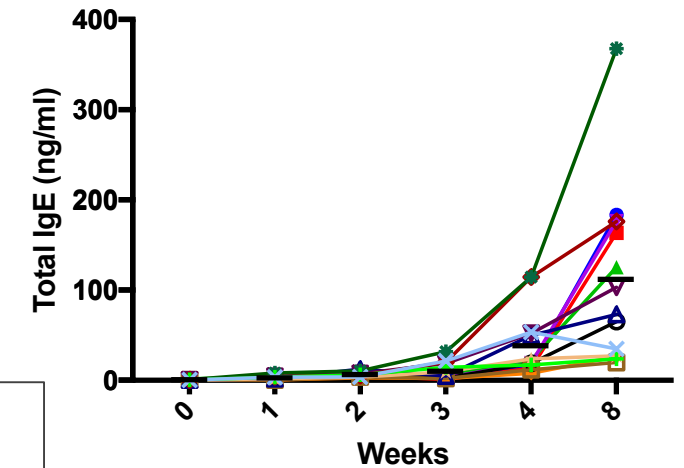
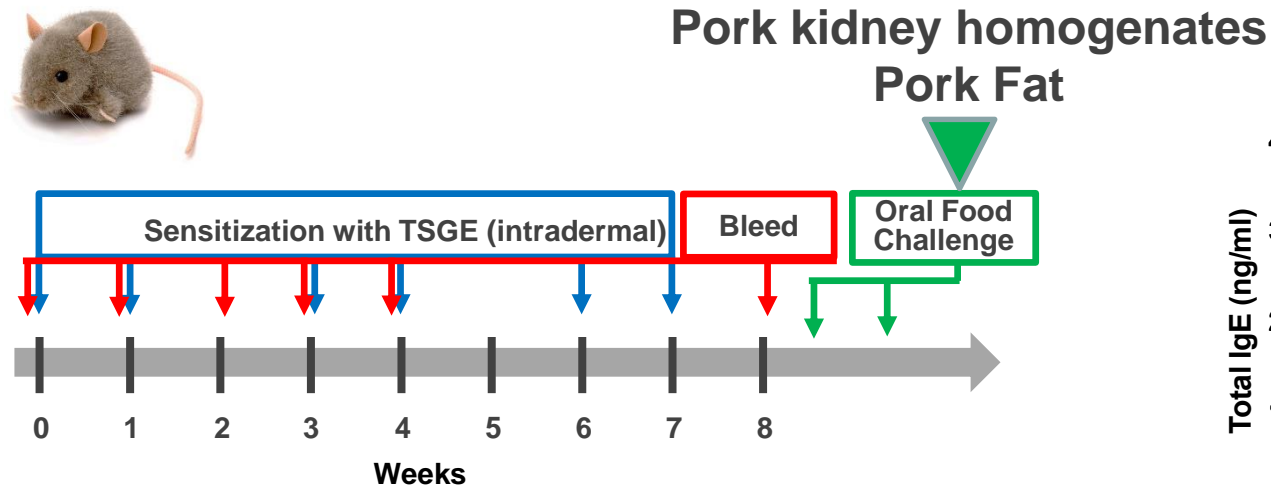
Working hypothesis: Allergic effector cells sensitized with alpha-gal-specific IgE and activated with alpha-gal-containing glycolipids trigger delayed anaphylaxis *in vivo*.

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

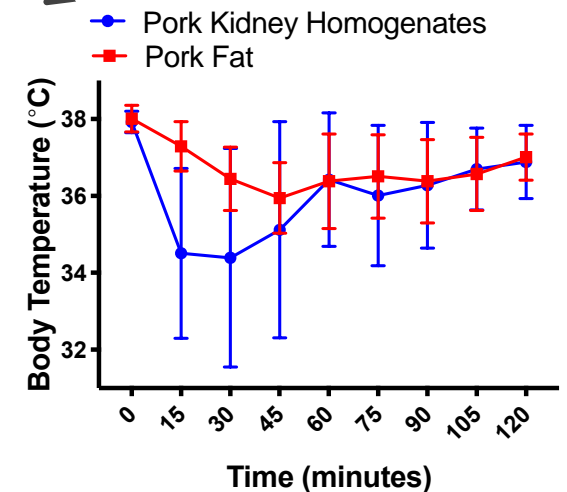
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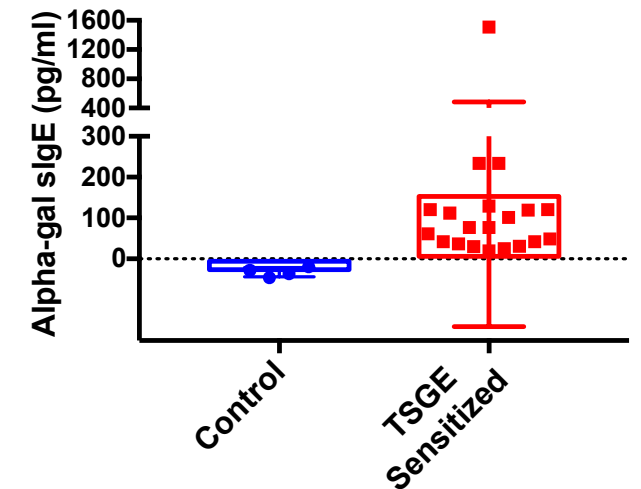
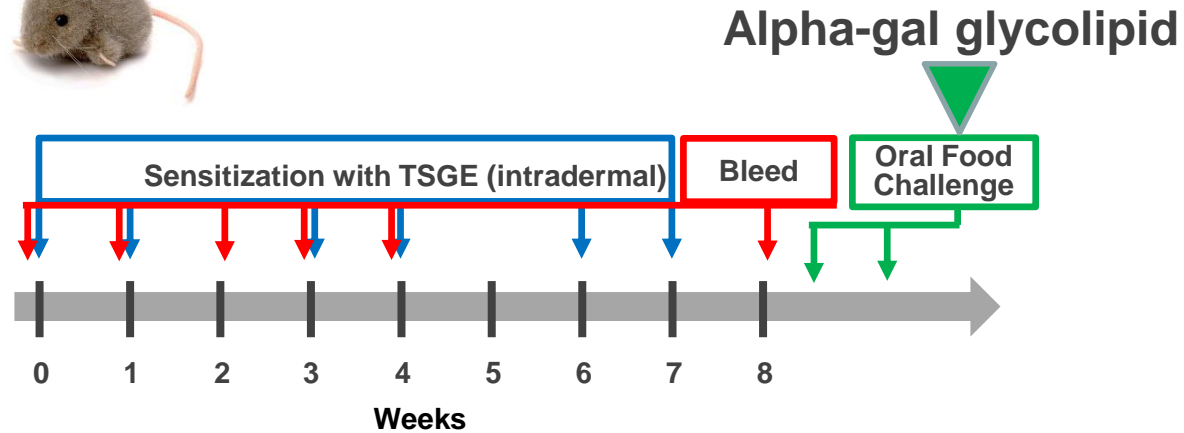
## Intradermal injection with TSGE induces a rise in total and alpha-gal specific IgE in alpha-gal KO mice



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

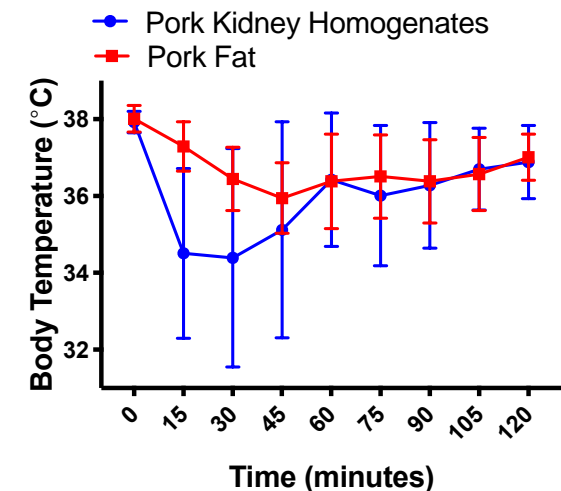


## Aim 2a: Oral challenge of alpha-gal KO mice with alpha-gal-containing glycolipid following sensitization with tick salivary gland extract (TSGE)



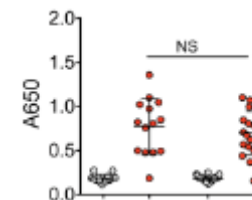
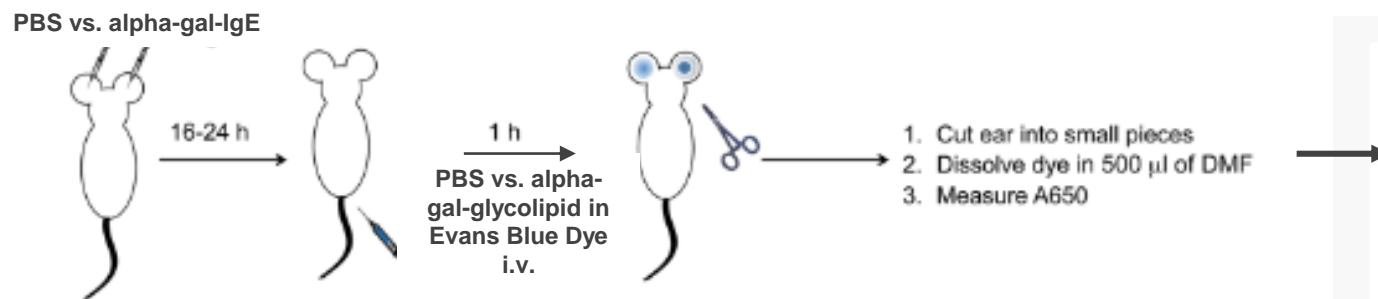
Thermometer with rectal probe to measure body temperature

### Alpha-gal glycolipid?

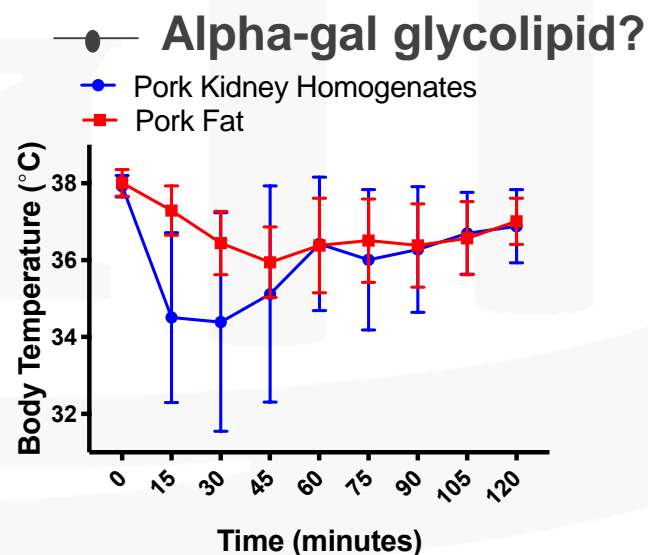
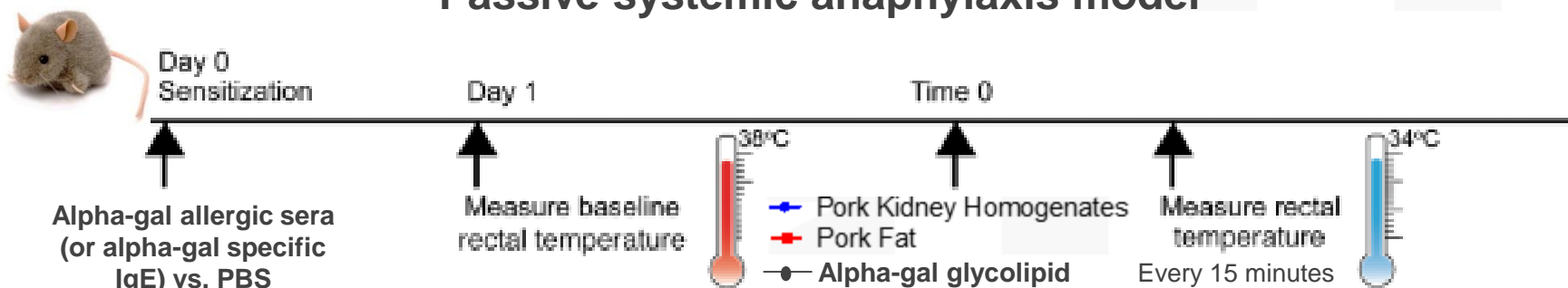


## Aim 2b: Passively sensitize humanized mice that express hFcεRI (commercially available) and assess ability of alpha-gal-containing glycolipid to induce anaphylaxis *in vivo*

### Passive cutaneous anaphylaxis



### Passive systemic anaphylaxis model



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