The role of IgG4 in the pathogenesis of eosinophilic esophagitis

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EoE is considered a non-IgE mediated food allergy

• EoE is triggered by food antigens, as indicated by remission induced by elimination diets.
• Elimination diets guided by SPT or serum specific IgE levels do not reliably identify food triggers.
• Omalizumab did not alter symptoms of eosinophilic esophagitis or eosinophil counts in biopsy samples compared with placebo.

5. Clayton et al Gastroenterology 2014; 147:602-9
**EoE in adults is associated with IgG4 and not mediated by IgE**

(A) Tissue IgG4 total IgG

(B) Immunofluorescent staining for IgG4 in an eosinophilic esophagitis subject shows patchy granular intercellular IgG4, in red, with a blue DAPI nuclear counterstain. (C) The control has no IgG4 staining.

Clayton et al Gastroenterology 2014; 147:602-9

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**Esophageal IgG4 deposits in EoE derive from dense plasma cell infiltrates in the lamina propria.**

(A) Trichrome stain shows dense lamina propria fibrosis. (B) Clusters of inflammatory cells were found in the deep lamina propria. (C) The deep lamina propria infiltrates are often perivascular, particularly around small blood vessels, with increased endothelial cellularity. (D) These infiltrates are mainly plasma cells, with sparse lymphocytes, eosinophils, and neutrophils. (E) IgG4 immunostaining shows abundant IgG4 plasma cells mostly in the deep lamina propria, with only sparse IgG4 cells superficially. (F) IgG4 immunoperoxidase staining showed up to 300 IgG4 plasma cells per high-power field. Nearly all the plasma cells are IgG4 positive.

Clayton et al Gastroenterology 2014; 147:602-9
Because this is an hour-long talk, and there have only been 7 papers, you could do ~one slide per paper and summarize the literature to date before going into your question. That is more logical to me than going to your project and then back-tracking.

Emily McGowan, 12/18/2018

Add in the citation to this paper

Emily McGowan, 12/18/2018
Serum IgG4 antibodies to common EoE trigger foods often are present

Serum total IgG4 in eosinophilic esophagitis (top left) is increased 1.9-fold (*P = .016), broadly overlapping the controls. Serum IgG4 reactivity to the 4 most common trigger foods (wheat, milk, egg, and nuts) and to the highest serum IgG4 anti-food content of any tested food are all increased in eosinophilic esophagitis (each **P ≤ 3 x 10^-4). However, for each food studied, 1 or more controls had abundant food-specific IgG4 antibodies.

Clayton et al Gastroenterology 2014; 147:602-9

EoE: IgG4-Associated

- Esophageal Findings:
  - Granular deposits of IgG4
  - Abundant IgG4-containing plasma cells

- IgG4 deposits distinguish EOE from GERD

- IgG4 levels correlate with esophageal eosinophil counts

- IgG4 is specific but not sensitive for EoE diagnosis in both pediatric and adult tissue samples

Clayton et al, 2014
Zuckerberg et al, 2015
Wright et al, 2016
Mohammad et al, 2018
Rosenberg et al, 2018
Pope et al, 2018
Weidlich et al, 2019
Add in citation in bottom
Emily McGowan, 12/18/2018
Overview of IgG4

- Least abundant IgG subclass
- Flexible hinge region allows Fab arm exchange
- Limited ability to form immune complexes
- Can bind Fc portion of IgG
- Increases in response to IL-4 and IL-13, as well as IL-10, and IL-21
- Considered a "non-inflammatory antibody"
- Is thought to be pathologic in certain diseases (pemphigus vulgaris, idiopathic membranous glomerulonephritis, TTP)

Stone et al, NEJM, 2012

Barrier defects in patients with type 2 diseases

Schleimer RP et al. JACI 2017;139:1752-61.
This is a slide from one of my talks. Feel free to adapt it as desired.

Emily McGowan, 12/18/2018
Rationale

• A complex interplay between epithelial barrier dysfunction and the immune response underlies the pathophysiology of EoE.
• This impaired epithelial barrier enables allergens, such as wheat, to infiltrate the esophageal mucosa.
• Foods that are often consumed in large quantities, such as dairy and wheat, could provide a local source of antigen, and IgG4 against food antigens is produced locally in large quantities.
• IgG4 is soluble and should only be present in the tissue if bound to antigen or FcR.

Schleimer RP et al. JACI 2017;139:1752-61.

Hypothesis

• Food-specific IgG4 is forming immune complexes with food antigen in the esophageal mucosa of patients with EoE, and these immune complexes decrease with disease remission
Objectives

• Is food-specific IgG4 forming immune complexes with food antigen in the esophageal mucosa of patients with EoE?

• Is IgG4-food allergen complexes involved in the pathogenesis of EoE through the generation of immune complexes?

Measurements

• **Immunofluorescent Tissue Staining:**
  - Using formalin-fixed, paraffin-embedded esophageal tissue

• **Advanced microscopy**
  - Stimulated Emission Depletion (STED) microscopy
  - Electron microscope
  - Spatially Targeted Optical MicroProteomics (STOMP)

• **Confirmation for “immune complex (protein-protein interaction)”**
  - Co-Immunoprecipitation
What is your question for this hypothesis?
Emily McGowan, 12/18/2018

I would title this slide "Question" and ask, "Is IgG4 involved in the pathogenesis of EoE through the generation of immune complexes?" Then, put your hypothesis: Milk-specific IgG4 is forming immune complexes with milk antigen in the ___ (what level of tissue) of patients with EoE, and these immune complexes decrease with disease remission? (?
Emily McGowan, 12/18/2018

Also, it may not just be milk. Could wheat, egg, soy, etc be doing the same thing?
Emily McGowan, 12/18/2018
IgG4 co-localizes with wheat allergen in the esophageal tissue.

A, Control; B, EoE; The green fluorescence is IgG4, and the red fluorescence is gliadin. The nucleus is stained with DAPI (blue dye).

Co-localization of IgG4 and food allergen

A (Left) FITC green fluorescence with IgG4 primary Antibody; B (Middle) Cy5 red fluorescence with wheat protein; C (Right) Combined Green and red fluorescence plus blue dye (DAPI for nucleus)
This signal of co-localization decreases with disease remission

Swallowed steroids  Food elimination

A, treatment with swallowed steroids; B, treatment with milk and wheat elimination diet; The green fluorescence is IgG4, and the red fluorescence is gliadin. The nucleus is stained with DAPI (blue dye).

Manders’ colocalization coefficient (MCC) analysis

Manders’ M1 and M2 numbers were used to describe the contribution of each one from two selected channels to the pixels of interest. Channel 1 is Cy5 (with a wheat allergen), which is labeled as red, whereas Channel 2 is FITC (with IgG4), which is green fluorescence.
Co-localization of IgG4 and Milk (Bos d5) in the different stage of disease progression

Control  EoE active phase  In remission

Next step:

We see co-staining of IgG4 and wheat and milk in the esophageal mucosa. We also see this decrease with disease remission.

- Can we further confirm the immune complex with other techniques?
  - STED vs FRET
  - Electron microscopy
  - Co-immunoprecipitation
- What are the associated cells and signals surrounding the IgG4-food co-localization?
  - STOMP
Stimulated Emission Depletion (STED) super-resolution microscopy

- Structures smaller than 200nm are lost in a blur (Ernst Abbe) \[ d = \frac{\lambda}{2n \sin \alpha} \]

https://www.youtube.com/watch?v=B4m_Y747gzw

In the top row: A, area of interest overall view by Confocal; A target complex- double staining with both Orange Green 488 and TRITC (B); Single Staining from Orange Green 488 (C)). Signal staining TRITC (D). In the bottom row: corresponding condition performed by tauSTED (E, F, G, H). The green fluorescence is IgG4, and the red fluorescence is wheat protein.
In the top row: A, area of interest overall view by Confocal; B, area of interest by Confocal; C, fluorescence intensity measurement of the targeted complex in A (Green- Orange Green 488; Red- TRITC). In the bottom row: corresponding condition performed by tauSTED (D,E,F). The green fluorescence is IgG4, and the red fluorescence is wheat protein.

Electron microscopy (ultrastructure evaluation)

Electron-dense deposits (arrows) between squamous cells (A) and the lamina propria (B).

Double staining example

Double-label immunoelectron microscopy using the cryosectioning technique showing colocalization of caveolin-1 and Rab5 in the intracellular vesicles of human umbilical vein endothelial cells (HUVEC).


Immunogold Deposits (wheat allergen) in EoE

A, Mast cell in EoE tissue; B, An enlarged view intercellularly demonstrating immunogold labelling with gliadin.
Co-Immunoprecipitation

IP is a method that enables the isolation of a protein or protein complex (with 3 approaches):
- Incubate sample with antibody against protein of interest
- Separate antibody/protein complex from remaining sample
- Analysis

Co-Immunoprecipitation preliminary data

Co-IP of Beta-lactoglobulin (36 kDa, dimer)

Input

(-) tissue  (+) tissue

Serum

(-) tissue  (+) tissue

M 1 2 3 M

Input

β-Lac

IgG4

Co-IP

β-Lac

36 kDa

(+tissue) eos 40 in the esophageal tissue but unclear dx
Spatially Targeted Optical MicroProteomics (STOMP) investigate proteins in the subcellular structure surrounding the complex formation.

Overview of automated Spatially Targeted Optical Micro Proteomics (autoSTOMP). A-B, Structures of interest (SOI) are identified by fluorescence imaging using Zeiss Zen Black software. C, Images are exported to FIJI software to generate a "MAP". D, the MAP is imported into Zen Black using a custom STOMP Macro to guide UV excitation by the 2-photon laser to conjugate the affinity tag to SOI proteins. E-H, autoSTOMP modifications (E-F); G, a custom Python script to obtain tile array; H, the SikuliX icon recognition software is used to automate the basic STOMP protocol (B-D) and loop through the tile array (G).

Part I (STOMP)- IgG4 only
autoSTOMP can identify sub-regions of multiple, small tissue sections across a slide.

A. An esophageal tissue biopsy (~1mm³) was serial sectioned onto slides (up to 8 per slide). A typical section has a size of ~5 tiles (340 μm x 340 μm, each tile, the field view of 25x magnification objective lens). Structures of interest stained for IgG4 (red) are indicated in the schematic as red, counterstain for nuclei (DAPI), scale bar = 500 μm. B-C. The Sikulixscript allows the user to set the boundary points for each section in Zen black. The boundary points then direct a whole-surface scan and tile array for each section (C). D. Instructions are given during the interaction of user and Fiji macro. SOI are manually selected in each section. E. A Python script maps the physical coordinates of each SOI on the microscope stage to generate a tile array of discrete SOI. F-H. A Sikulixscript automates imaging SOI-by-SOI in Zen black (F), MAP file generation from SOI image in Fiji (G), and biotin-BP photo-crosslinking in Zen black (H, magenta) as described in Figure 1.

autoSTOMP proteins associated with IgG4 positive regions of esophageal biopsies

A. An image of a IgG4 (red) stained biopsy section was coordinates defining each SOI are indicated in white dots, nuclei (DAPI) counterstain. Scale bar = 1 mm. B. The SOI locations were be imaged and thresholded to generate MAP files as described in Figure 3. E-H. The SOI from multiple sections are selectively biotinylated. Then sections are dissociated from the slide in lysis buffer. D-H. SOI proteins are isolated by streptavidin (SA) affinity chromatography. E-F, SOI proteins are pelleted with the SA beads (E), washed and isolated by on-bead trypsin digestion (F). G-H, Non-SOI proteins are reserved after SA-binding (G), followed by removal of detergents and salts and trypsin digested (H) for mass spec analysis. The ratio of SOI to non-SOI proteins are used as a measure of enrichment.
Part II (STOMP) - IgG4 with food allergens

(autoSTOMP process images of IgG4 and gliadin staining)

(row 1 & 3) Single color channels, red (IgG4) and green (wheat allergen) and their merged channels are displayed in a row. (row 2 & 4) the map of the single color channel (IgG4), of wheat allergen, and the map of the co-stain. (row 1 & 2) the images of the whole tile where cluster-like region is found. (row 3 & 4) only the cluster-like region is shown while the rest is masked in black.
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Questions?