Regulation of allergic responses to food by commensal bacteria

Cathryn Nagler, Ph.D.
Department of Pathology
Committee on Immunology
University of Chicago

Sean N. Parker Center for Allergy & Asthma Research
Stanford University
January 27, 2017
Oral tolerance - physiological induction of mucosal and systemic non-responsiveness to dietary antigens
Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7443–7446, October 1986
Immunology

Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen

(Orally induced immunologic unresponsiveness/autoimmunity)

Cathryn Nagler-Anderson*, Loretta A. Bober†, M. Elizabeth Robinson*, Gregory W. Siskind‡, and G. Jeanette Thorbecke*§

*Department of Pathology, NYU School of Medicine, New York, NY 10016; †Schering Research Division, Bloomfield, NJ 07003; and ‡Department of Medicine, Cornell University School of Medicine, New York, NY 10021

Communicated by Michael Heidelberger, June 16, 1986
Table 2 | Oral tolerance in animal models of disease

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Human disease</th>
<th>Induction</th>
<th>Effective oral antigens</th>
<th>Prophylactic or therapeutic</th>
<th>Dose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>Multiple sclerosis</td>
<td>Commonly induced by injection of susceptible animals with myelin proteins plus pertussis toxin as an adjuvant to permeabilize the blood–brain barrier</td>
<td>Whole myelin, myelin basic protein, proteolipoprotein, myelin oligodendrocyte glycoprotein, glatramer acetate (copolymer 1)</td>
<td>Prophylactic and therapeutic</td>
<td>High dose</td>
<td>42–44</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Induced by injection of type II collagen in adjuvant</td>
<td>Collagen types II and IX, HSP65</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>63,94,95,125</td>
</tr>
<tr>
<td>Adjuvant-induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Induced by injection of Freund's adjuvant, bacterial products or HSPs</td>
<td>HSP60, HSP65, type II collagen</td>
<td>Therapeutic</td>
<td>Low dose</td>
<td>114</td>
</tr>
<tr>
<td>Experimental autoimmune uveitis</td>
<td>Autoimmune uveitis</td>
<td>Induced by immunization with sequestered retinal antigens or IRBP</td>
<td>Retinal S-antigen, IRBP, HLA-B27 mimotope (HLA-B27PD)</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>78,79,97</td>
</tr>
<tr>
<td>Experimental autoimmune myasthenia gravis</td>
<td>Myasthenia gravis</td>
<td>Immunization with acetylcholine receptor</td>
<td>Acetylcholine receptor</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>77</td>
</tr>
<tr>
<td>Non-obese diabetic mice</td>
<td>Type 1 diabetes</td>
<td>Spontaneous destruction of pancreatic islet cells</td>
<td>Insulin</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>90,98,99,121</td>
</tr>
<tr>
<td>Rat insulin promoter LCMV diabetes model</td>
<td>Type 1 diabetes</td>
<td>Transgenic expression of LCMV proteins under the rat insulin promoter. Infection with LCMV initiates disease</td>
<td>Insulin</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>101</td>
</tr>
<tr>
<td>Middle cerebral artery occlusion</td>
<td>Stroke</td>
<td>Surgical occlusion of the middle cerebral artery</td>
<td>Myelin basic protein</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>64</td>
</tr>
<tr>
<td>LDL-receptor-deficient mice</td>
<td>Atherosclerosis</td>
<td>Mice lacking the LDL receptor are fed a high-fat diet</td>
<td>HSP65</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>65,66</td>
</tr>
<tr>
<td>Tissue transplant</td>
<td>Tissue transplant</td>
<td>Surgical transplantation of allogeneic tissue</td>
<td>Donor cells, donor MHC proteins</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>80,92</td>
</tr>
</tbody>
</table>

Several common models are used to study oral tolerance. Models are prophylactic if the regimen of oral feeding is begun prior to induction or onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after induction or onset of disease. Low doses correspond to <1 mg per day, whereas >1 mg per day is considered a high dose. This division is based on studies with myelin proteins in experimental autoimmune encephalomyelitis and might not accurately reflect doses with other antigens in other disease models. HSP, heat-shock protein; IRBP, interphotoreceptor retinoid-binding protein; LCMV, lymphocytic choriomeningitis virus; LDL, low-density lipoprotein.
## Oral tolerance in human diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Oral antigen</th>
<th>Dose</th>
<th>Prophylactic or therapeutic</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food allergy</td>
<td>Allergen</td>
<td>Increasing dose over time</td>
<td>Therapeutic</td>
<td>About 80% of patients are successfully desensitized</td>
<td>130</td>
</tr>
<tr>
<td>Autoimmune uveitis</td>
<td>Sequestered retinal antigens, HLA-B27PD</td>
<td>4 mg capsules 3 times a week for 12 weeks</td>
<td>Therapeutic</td>
<td>Marginal clinical benefit. All patients relapsed after cessation of treatment</td>
<td>131,132</td>
</tr>
<tr>
<td>Autoimmune uveitis</td>
<td>Retinal S-antigen, soluble retinal antigens</td>
<td>30 mg S-antigen or 50 mg soluble retinal antigens or both. Decreasing dose, starting from 3 times a week for 8 weeks, ending with once a week</td>
<td>Therapeutic</td>
<td>No benefit, with possible exacerbation of disease in patients receiving a mixture of soluble retinal antigens</td>
<td>133</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Collagen</td>
<td>0.1 mg bovine type II collagen daily for 1 month, followed by 0.5 mg daily for 6 months</td>
<td>Therapeutic</td>
<td>No benefit</td>
<td>135</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td>20, 100, 500 or 2,500 μg chicken type II collagen daily for 24 weeks</td>
<td>Therapeutic</td>
<td>Clinically significant response at 20 μg dose</td>
<td>136</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td>0.05, 0.5 or 5 mg bovine type II collagen daily for 6 months</td>
<td>Therapeutic</td>
<td>Response at 0.5 mg</td>
<td>137</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td>0.5 mg bovine type II collagen daily for 3 months</td>
<td>Therapeutic</td>
<td>Response at 0.5 mg</td>
<td>138</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td>0.1 mg chicken type II collagen daily for 1 month, followed by 0.5 mg for 2 months</td>
<td>Therapeutic</td>
<td>Improvement in most clinical measures, 4 out of 28 patients had complete remission</td>
<td>139</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>Insulin</td>
<td>7.5 mg insulin</td>
<td>Prophylactic</td>
<td>No benefit</td>
<td>*</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td></td>
<td>2.5 mg or 7.5 mg insulin</td>
<td>Therapeutic</td>
<td>No benefit</td>
<td>140</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Myelin</td>
<td>300 mg bovine myelin</td>
<td>Therapeutic</td>
<td>No clinically significant benefit</td>
<td>52,141</td>
</tr>
</tbody>
</table>

*For trial results see National Institutes of Health News website in Further Information. In contrast to experimental animal models, most human clinical trials have attempted to induce oral tolerance after the onset of disease (therapeutically). Treatments are prophylactic if the regimen of oral feeding is begun prior to the onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after the onset of disease. HLA-B27PD, HLA-B27 mimotope.*

FOOD ALLERGIES IN THE U.S.

15 MILLION
Americans have food allergy, a serious medical condition.

People can be allergic to any food, but there are 8 FOODS THAT CAUSE THE MOST REACTIONS.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Eggs</th>
<th>Peanut</th>
<th>Tree Nuts</th>
<th>Soy</th>
<th>Wheat</th>
<th>Fish</th>
<th>Shellfish</th>
</tr>
</thead>
</table>

Reactions can range from a mild response to **anaphylaxis**, a severe and potentially deadly reaction.

**Every 3 minutes** a food allergy reaction sends someone to the ER.

The number of people who have the disease is growing, increasing **50% among children** between 1997 and 2011.

It now affects **1 IN 13 children**
A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses

Xiu-Min Li, MD, Denise Serebrisky, MD, Soo-Young Lee, MD, Chih-Kang Huang, MS, Ludmilla Bardina, MS, Brian H. Schofield, JD, J. Steven Stanley, PhD, A. Wesley Burks, MD, Gary A. Bannon, PhD, and Hugh A. Sampson, MD

New York, NY, Baltimore, Md, and Little Rock, Ark

Intragastric sensitization with peanut+ cholera toxin (CT)

Challenge

Sacrifice

Li et al JACI 2000, 106; 150
TLR4 signaling influences susceptibility to allergic responses to food

Bashir et al. *J. Immunol.* 2004, 172; 6978
Commensal bacteria populate our skin and mucosal surfaces and profoundly influence our health.

There are as many *E. coli* in our gut as there are people on earth!

- 10 trillion human cells
  - 20,000 genes
- 100 trillion bacterial cells
  - 20,000,000 genes

There are as many *E. coli* in our gut as there are people on earth!
We exist in a dynamic interrelationship with our commensal microbiome!

Healthy individuals “tolerate” their commensal bacteria but are also constantly receiving signals from the microbiome that impact both systemic and mucosal immunity.
The commensal microbiota confers many health benefits to the host.
Culture independent methods of analysis have transformed our understanding of the composition of the microbiome

The 16S rRNA gene is highly conserved among bacterial species.

“Universal” primers target conserved regions of this gene and allow for amplification and sequencing of species specific hypervariable regions for bacterial classification.

Structure of 16S ribosomal RNA
The goal of the Human Microbiome Project was to characterize the microbial content of sites in the human body and examine whether changes in the microbiome can be related to disease.

- 300 healthy subjects
- 15-18 body sites
- multiple times

www.hmpdacc.org
Diversity of the human microbiome is determined by microbial habitat

The composition of the microbiota varies by site: Bacteroidetes and Firmicutes dominate in the gut.
Carriage of microbial taxa varies while metabolic pathways remain stable within a healthy population.

Antibiotic use

Western high fat, low fiber diet

Elimination of enteropathogens (H. pylori, helminths)

Vaccination/reduced exposure to infectious disease

Caesarean birth/formula feeding

Alteration of commensal microbiota “dysbiosis”

Inflammatory Bowel Disease

Obesity

Food Allergy

Diabetes

Autism

Asthma

Feehley et al. Seminars in Immunopathology 2012, 34; 671
The gastrointestinal microbiota changes throughout life

- **Newborn**
  - Initial gut bacteria (founder species) depends upon delivery mode
  - Vaginal delivery: *Lactobacillus, Prevotella spp.*
  - Vertical inheritance from mother

- **Early childhood**
  - New strains (less certain in origin) outcompete old ones
  - C-section: *Staphylococcus, Corynebacterium, Propionibacterium spp.*
  - Rapid increase in diversity
  - Higher susceptibility to certain pathogens
  - Early microbiota development = high instability
  - Shifts in response to diet, illness

- **Adult**
  - Highly distinct, differentiated microbiota
  - Microbial community may continue to change, but at a slower rate than in childhood

- **Elderly**
  - Substantially different gut communities than in younger adults

Dominguez-Bello, MG et al, *Gastroenterology* 2011, 140;1713
Does neonatal administration of oral antibiotics alter the composition of the fecal microbiota?

Antibiotic cocktail: kanamycin, gentamicin, colistin, metronidazole, vancomycin

Daily antibiotic gavage

Intragastric sensitization with peanut+cholera toxin (CT)

Weaning

Dilute antibiotics in drinking water

Sacrifice

Challenge
Antibiotic treatment dramatically alters the composition of the microbiota and increases sensitization to food.
How does bacterial colonization change the response to sensitization to a food allergen?
Germ free mice are a powerful tool to examine whether the presence of particular bacterial species protects against sensitization to food.
A novel gnotobiotic model of food allergy

SPF or GF
C57BL/6 mice
Weaning

D0 D2 D7 D14 D21 D28 D35 D36

Intragastric sensitization with peanut + cholera toxin (CT)
Challenge

Sacrifice

Peanut Specific IgE (ng/mL)

Peanut Specific IgG1 (µg/mL)

Total IgE (ng/mL)

CT only
PN + CT

*  **
To identify allergy protective bacterial populations we selectively colonized gnotobiotic mice with representatives of the numerically predominant taxa in the murine colon.

1. Clostridia are resident bacteria associated with the apical mucosa.

2. Bacteroides are enriched among the transient bacteria associated with digesta.
Accumulation of Foxp3^+ regulatory T cells in the gut is dependent on indigenous spore-forming bacteria from the Clostridia class.

Atarashi et al. *Science* 2011, 331; 337
Selective colonization of gnotobiotic mice differentially induces Tregs in the colonic lamina propria

Stefka, Feehley et al PNAS 2014, 111; 13145
A Clostridia containing commensal microbiota protects against allergic sensitization to food

Stefka, Feehley et al *PNAS* 2014, 111; 13145
Clostridia colonization uniquely induces expression of a subset of genes in the colonic epithelium

Stefka, Feehley et al. PNAS 2014, 111; 13145
Expression of IL-22 is significantly increased upon Clostridia colonization

**Stefka, Feehley et al PNAS 2014, 111; 13145**
IL-22 is a barrier protective cytokine

Stefka, Feehley et al. PNAS 2014, 111; 13145
Clostridia-induced IL-22 is necessary to reduce intestinal permeability to food antigen

C57BL/6 mice

Weaning

D-7

Daily Abx gavage

D0

Clost. or IL-22Fc

D6

Challenge

ELISA for Ara h 6

Arachis hypogaea

Stefka, Feehley et al PNAS 2014, 111; 13145
Clostridia-induced IL-22 is necessary to reduce intestinal permeability to food antigen

C57BL/6 mice

Weaning

Daily Abx gavage

Clostridia

Challenge

Isotype or αIL-22

ELISA for Ara h 6

Arachis hypogaea

Ara h 6 in serum (ng/mL)

NT

Abx

AbxClostridia

Abx+IL-22Fc

Ara h 6 in serum (ng/mL)

AbxClostridia+isotype PN

AbxClostridia+αIL-22 PN
A consortium of *Bacteroides*+*Akkermansia* does not induce IL-22 and enhances intestinal permeability to food antigen

* Bacteroides+Akkermansia consortium (Elaine Hsiao, Cal Tech)
  70% *Bacteroides*, 25% *A. muciniphila*, 3% *Parabacteroides*, 1% *Turicibacter*
Depletion of IL-22 primes for an IL-17 response and an increase in peanut-specific IgG

C57BL/6 mice

D-7 D0 D7 D14 D21 D28 D35 D36

Weaning/ Clostridia

D-­‐7

Daily antibiotic gavage

D0

150µg i.p. αIL-22 (8E11, Genentech) or isotype control

D7

Intragastric sensitization with peanut+cholera toxin (CT)

D14

Challenge

D21

Sacrifice

D28

Stefka, Feehley et al PNAS 2014, 111; 13145
Treatment with anti-IL-22 decreases expression of anti-microbial peptides (Reg3b) and increases Clostridia abundance.
Tolerance to dietary allergen requires the induction of a bacteria-induced barrier protective response.
Can we develop novel strategies to modulate the composition of the microbiota to prevent or treat food allergies?
Supplementation of extensively hydrolyzed casein formula with *Lactobacillus GG* accelerates acquisition of tolerance in children with cow’s milk allergy

**Visit 1**
- Full anamnestic and clinical evaluation
- SPT and APT
- Oral food challenge (OFC)

**Visit 2** (6 months)
- Full clinical evaluation

**Visit 3** (12 months)
- Full clinical evaluation
- SPT and APT
- Oral food challenge (OFC)

Supplementation of extensively hydrolyzed casein formula (EHCF) with *Lactobacillus GG* accelerates acquisition of tolerance in children with cow’s milk allergy.

The cow’s milk allergic (CMA) infant microbiome exhibits significantly increased diversity

Berni Canani, Sangwan, Stefka et al *ISMEJ* 2016, 10; 742
The composition of the fecal microbiota is altered in cow’s milk allergic (CMA) infants

Berni Canani, Sangwan, Stefka et al ISMEJ 2016, 10; 742
Demographic variables do not explain differences in bacterial abundance

Berni Canani, Sangwan, Stefka et al  *ISMEJ* 2016, 10; 742
Treatment with LGG supplemented formula changes microbial community structure to enhance production of fecal butyric acid and promote tolerance to CMA

Berni Canani, Sangwan, Stefka et al *ISMEJ* 2016, 10; 742
Genus level differential feature selection revealed a relationship between the abundance of bacterial strains, increased fetal butyrate concentration and acquisition of tolerance.

Berni Canani, Sangwan, Stefka et al, *ISMEJ* 2016, 10; 742
modified from
M. Velasquez-Manoff,
*Nature* 2015, 518: S4
Is sensitization to a food allergen increased in mice colonized with an allergic infant microbiota?

Healthy infant microbiota → Non-allergic

Cow’s milk allergic (CMA) infant microbiota → Allergic

Sensitize with β lactoglobulin (BLG)/CT
Developing microbiome-modulating therapeutics to prevent or treat food allergy

CLOSTRA BIO

- Drug formulations from microbial metabolites
  
  Healthy infant microbiota ➔ Non-allergic
  
  Cow’s milk allergic (CMA) infant microbiota ➔ Allergic

- Pre-biotic dietary supplements

  Sensitize with β lactoglobulin (BLG)/CT

- Therapeutic bacterial species (or mixtures)

www.clostrabio.com