

Regulation of allergic responses to food by commensal bacteria



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Oral tolerance - physiological induction of mucosal and systemic non-responsiveness to dietary antigens

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

(orally induced immunologic unresponsiveness/autoimmunity)

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Communicated by Michael Heidelberger, June 16, 1986

Table 2 | **Oral tolerance in animal models of disease**

Animal model	Human disease	Induction	Effective oral antigens	Prophylactic or therapeutic	Dose	References
Experimental autoimmune encephalomyelitis	Multiple sclerosis	Commonly induced by injection of susceptible animals with myelin proteins plus pertussis toxin as an adjuvant to permeabilize the blood–brain barrier	Whole myelin, myelin basic protein, proteolipoprotein, myelin oligodendrocyte glycoprotein, glatiramer acetate (copolymer 1)	Prophylactic Prophylactic and therapeutic	High dose Low dose	42–44 63,94,95,125
Collagen-induced arthritis	Rheumatoid arthritis	Induced by injection of type II collagen in adjuvant	Collagen types II and IX, HSP65	Prophylactic	Low dose	74
Adjuvant-induced arthritis	Rheumatoid arthritis	Induced by injection of Freund's adjuvant, bacterial products or HSPs	HSP60, HSP65, type II collagen	Therapeutic	Low dose	114
Experimental autoimmune uveitis	Autoimmune uveitis	Induced by immunization with sequestered retinal antigens or IRBP	Retinal S-antigen, IRBP, HLA-B27 mimotope (HLA-B27PD)	Prophylactic	Low dose	78,79,97
Experimental autoimmune myasthenia gravis	Myasthenia gravis	Immunization with acetylcholine receptor	Acetylcholine receptor	Prophylactic	Low dose	77
Non-obese diabetic mice	Type 1 diabetes	Spontaneous destruction of pancreatic islet cells	Insulin	Prophylactic	Low dose	90,98,99,121
Rat insulin promoter LCMV diabetes model	Type 1 diabetes	Transgenic expression of LCMV proteins under the rat insulin promoter. Infection with LCMV initiates disease	Insulin	Prophylactic	Low dose	101
Middle cerebral artery occlusion	Stroke	Surgical occlusion of the middle cerebral artery	Myelin basic protein	Prophylactic	Low dose	64
LDL-receptor-deficient mice	Atherosclerosis	Mice lacking the LDL receptor are fed a high-fat diet	HSP65	Prophylactic	Low dose	65,66
Tissue transplant	Tissue transplant	Surgical transplantation of allogeneic tissue	Donor cells, donor MHC proteins	Prophylactic	Low dose	80,92

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.

Table 3 | **Oral tolerance in human diseases**

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

*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.



Milk



Eggs



Peanut



Tree Nuts



Soy



Wheat



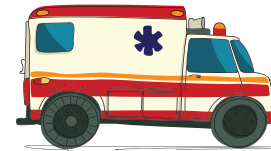
Fish



Shellfish

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**.



It now affects
1 IN 13
children

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

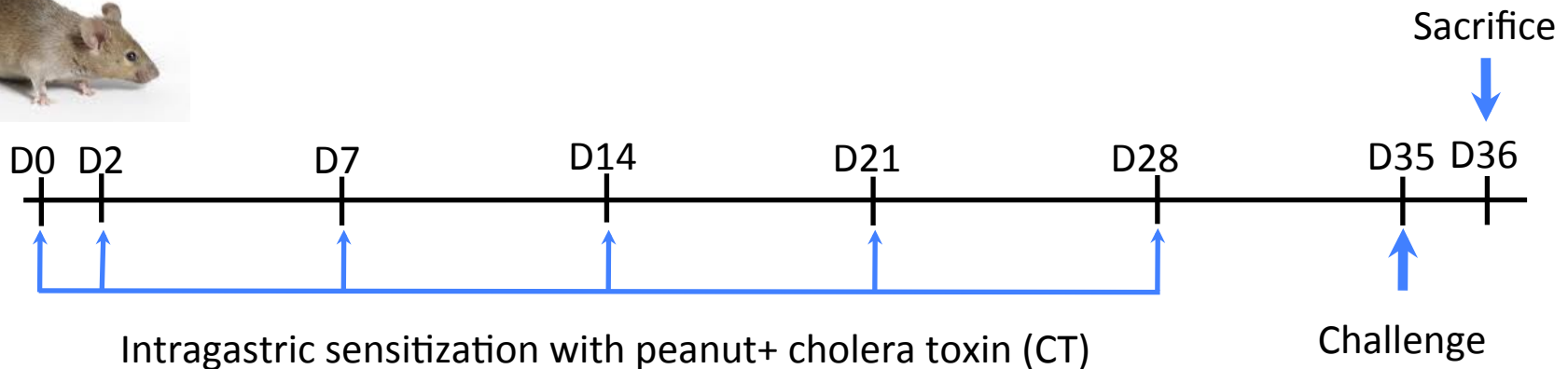


A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses

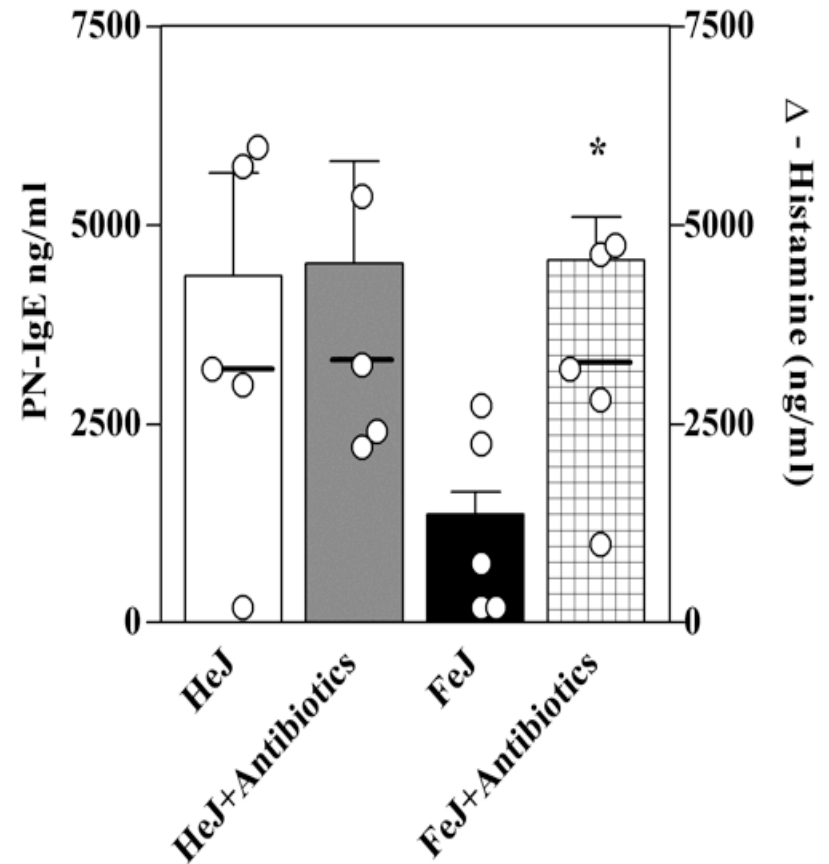
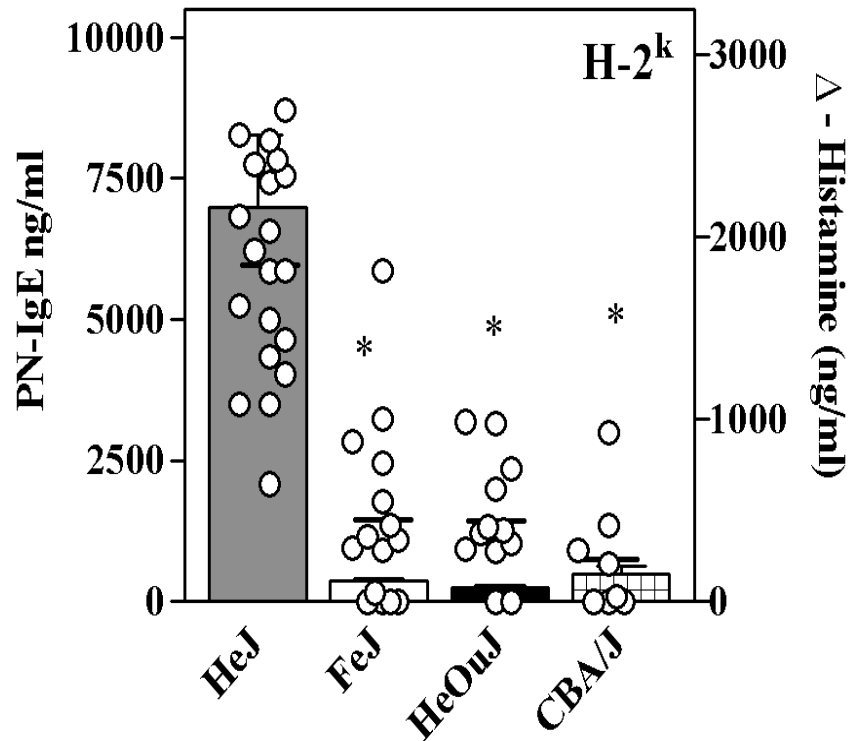
Xiu-Min Li, MD,^a Denise Serebrisky, MD,^a Soo-Young Lee, MD,^a Chih-Kang Huang, MS,^a Ludmilla Bardina, MS,^a Brian H. Schofield, JD,^b J. Steven Stanley, PhD,^c A. Wesley Burks, MD,^c Gary A. Bannon, PhD,^c and Hugh A. Sampson, MD^a
New York, NY, Baltimore, Md, and Little Rock, Ark



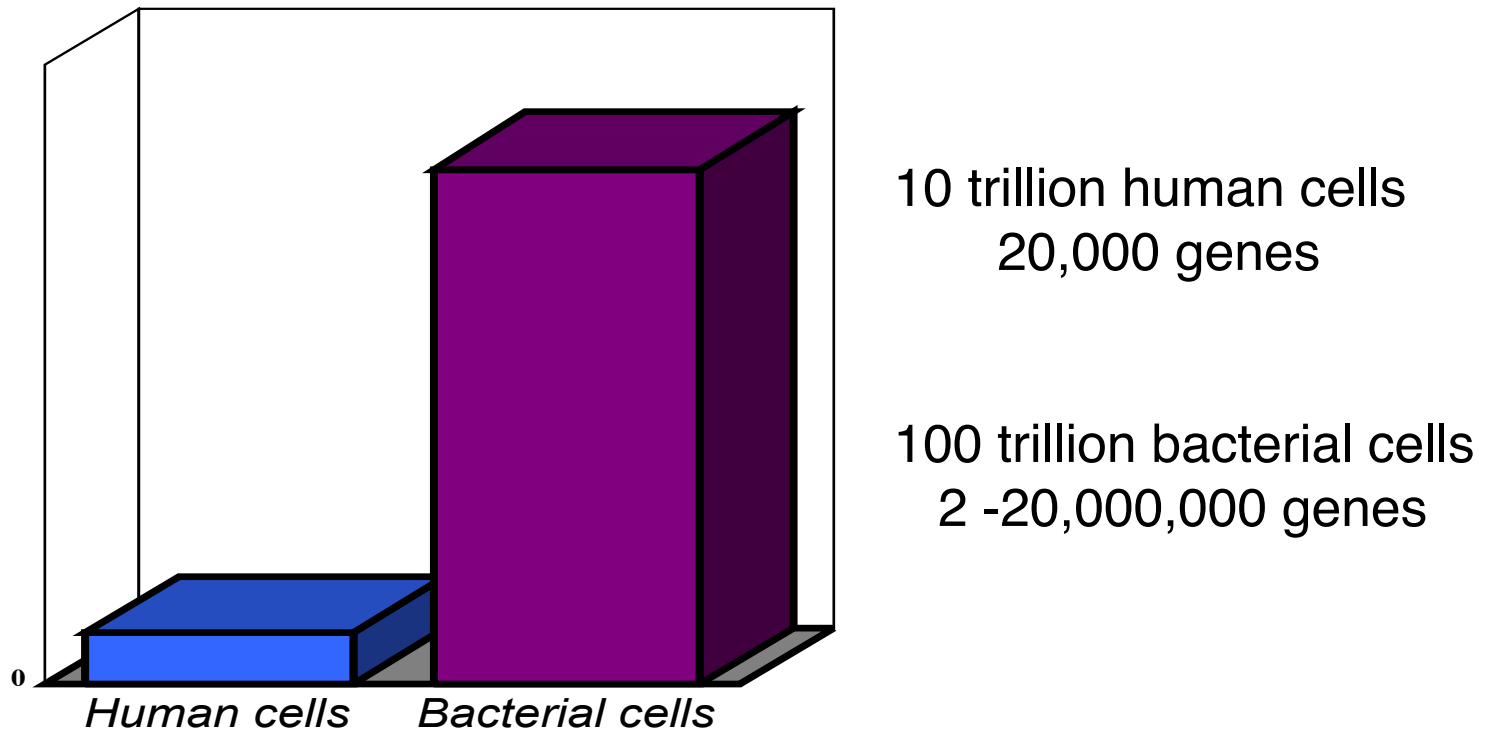
C3H/HeJ



TLR4 signaling influences susceptibility to allergic responses to food



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!

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

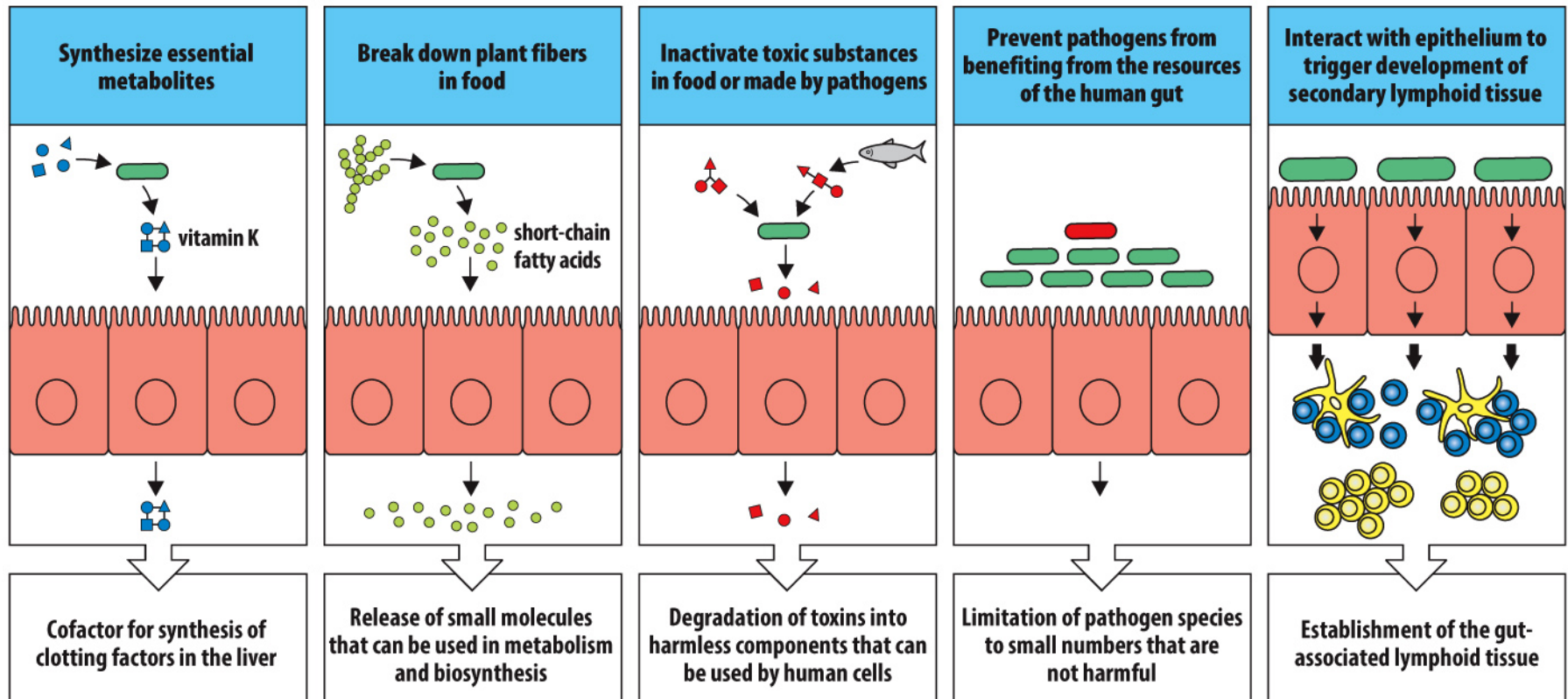
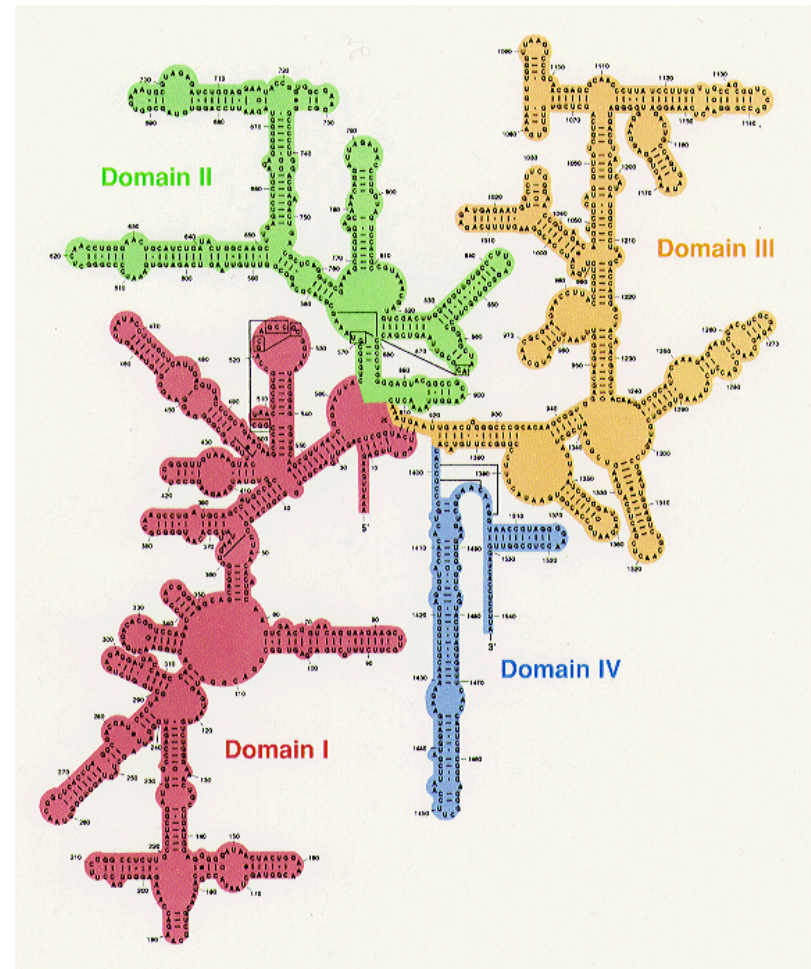


Figure 10.5 The Immune System, 4th ed. (© Garland Science 2015)

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

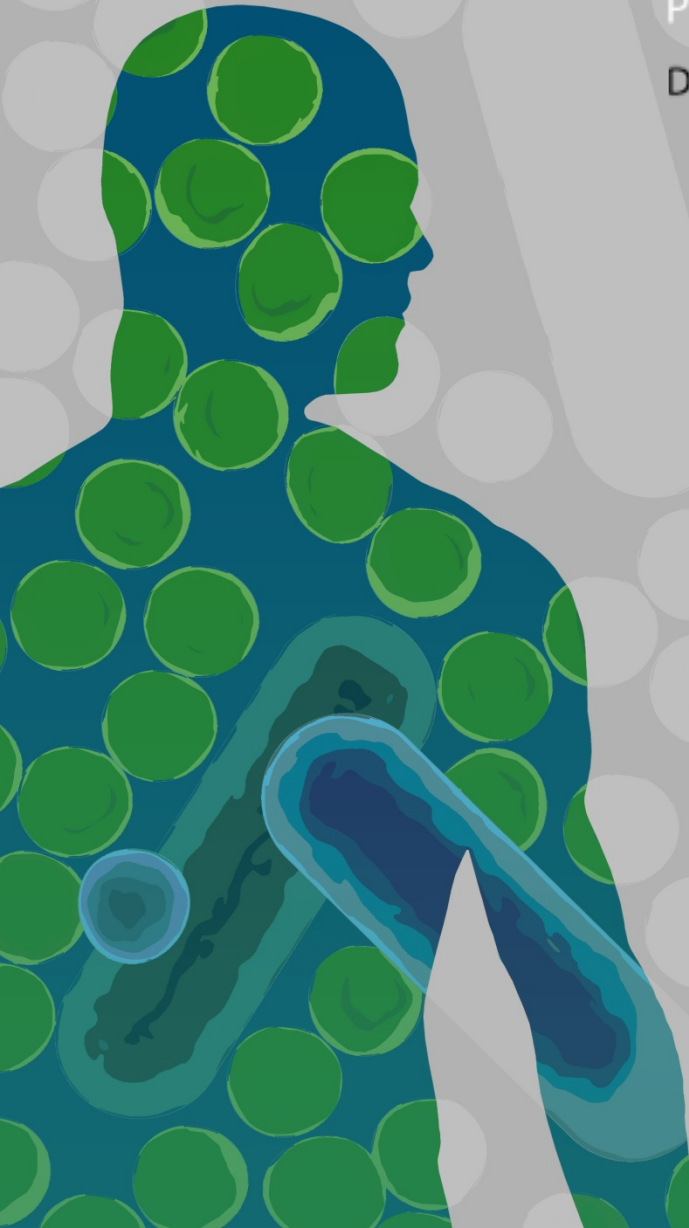
- 300 healthy subjects
- 15-18 body sites
- multiple times

HUMAN
MICROBIOME
PROJECT
DACC

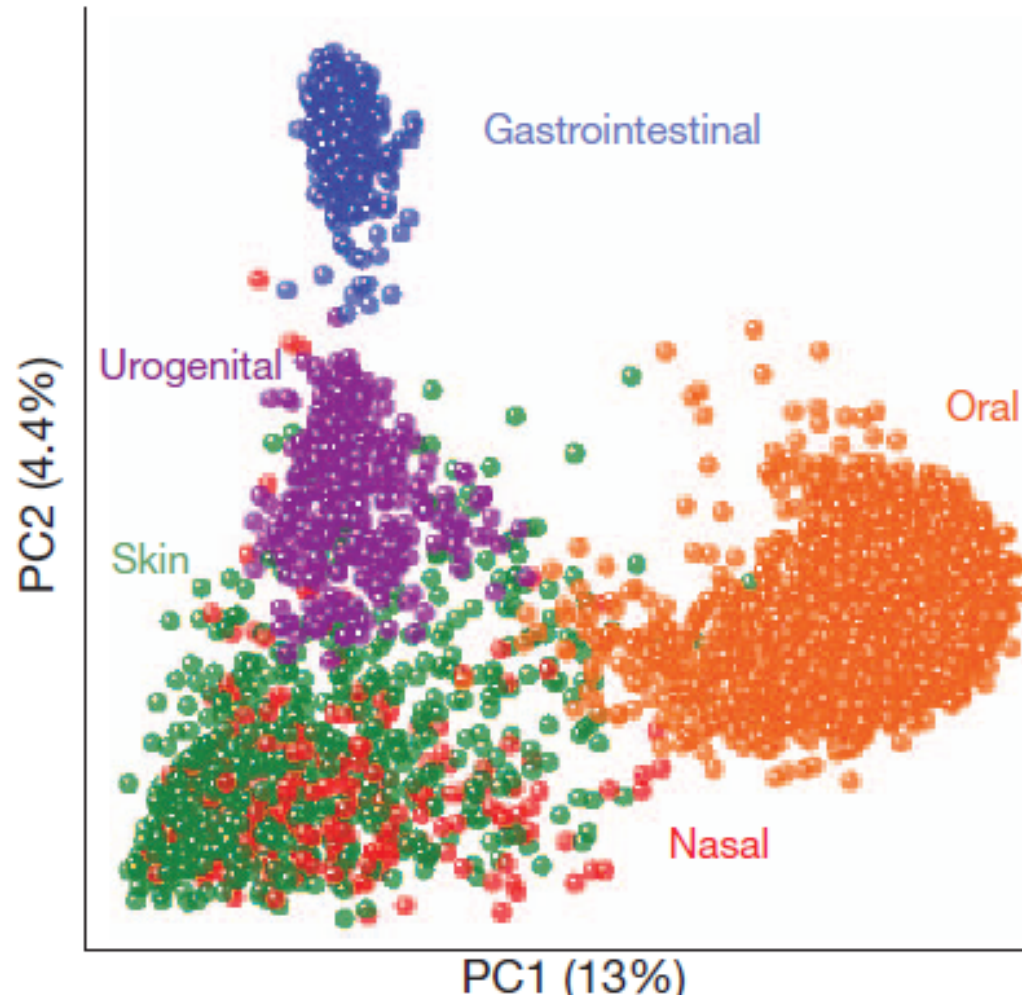


The goal of the Human Microbiome Project was to characterize the microbial content of sites in the human body and examine whether **changes** in the microbiome can be related to disease.

www.hmpdacc.org

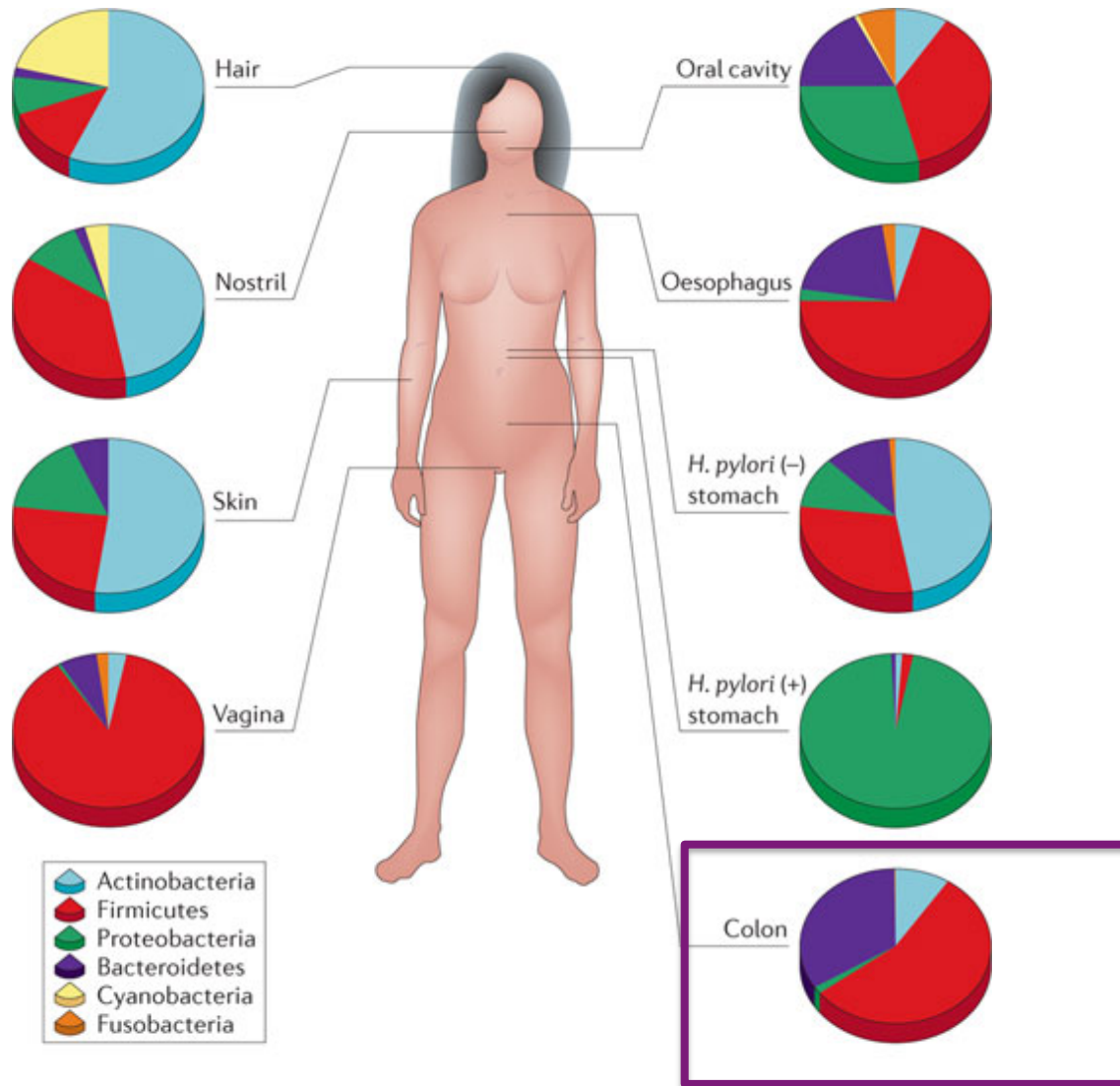


Diversity of the human microbiome is determined by microbial habitat

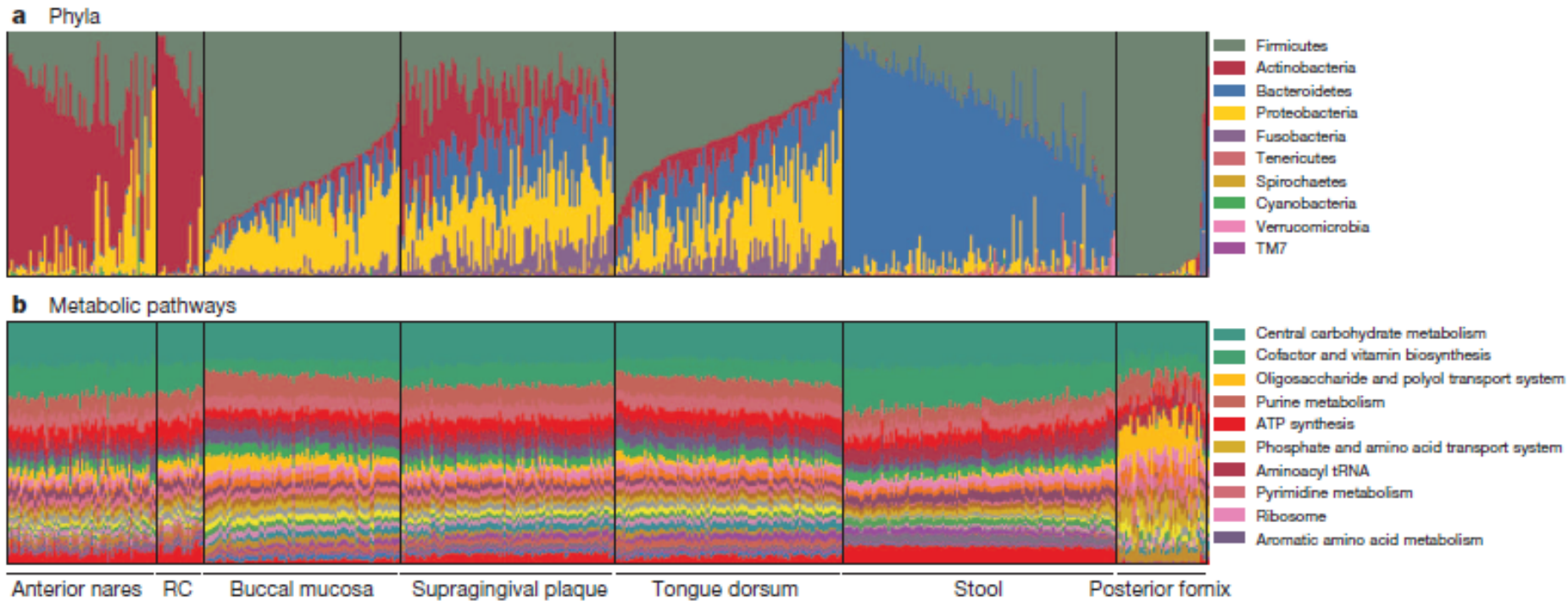


The Human Microbiome Project Consortium, *Nature* 2012, 486; 207

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 entero-
pathogens
(*H. pylori*,
helminths)



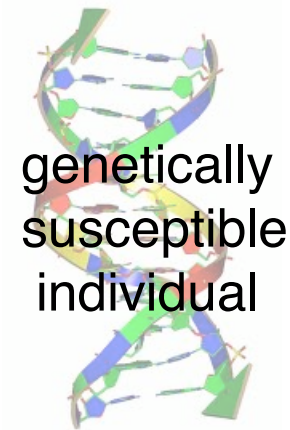
Vaccination/reduced
exposure to
infectious
disease



Caesarean
birth/formula
feeding



Alteration of
commensal
microbiota
“dysbiosis”



genetically
susceptible
individual

Inflammatory
Bowel
Disease

Obesity

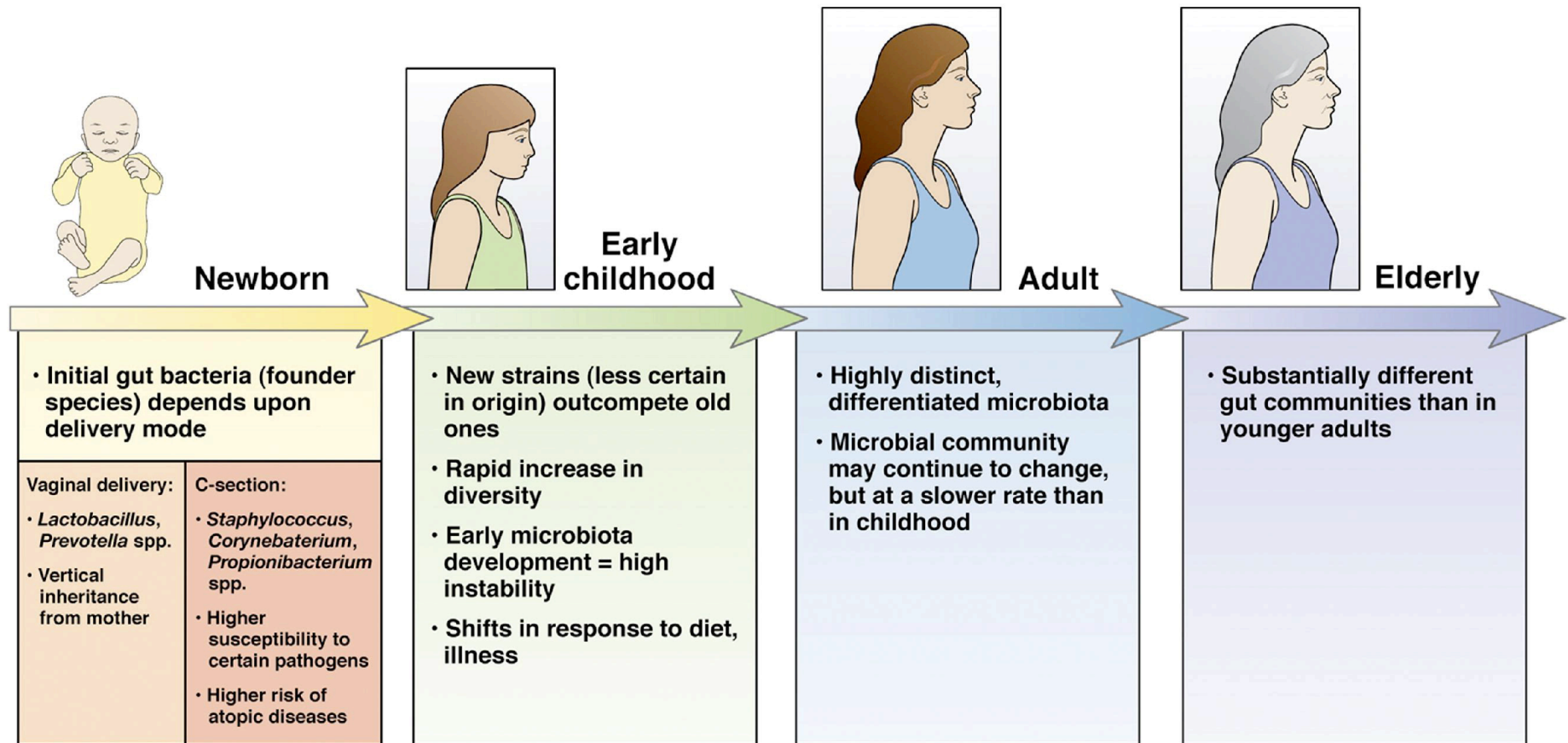
Food Allergy

Diabetes

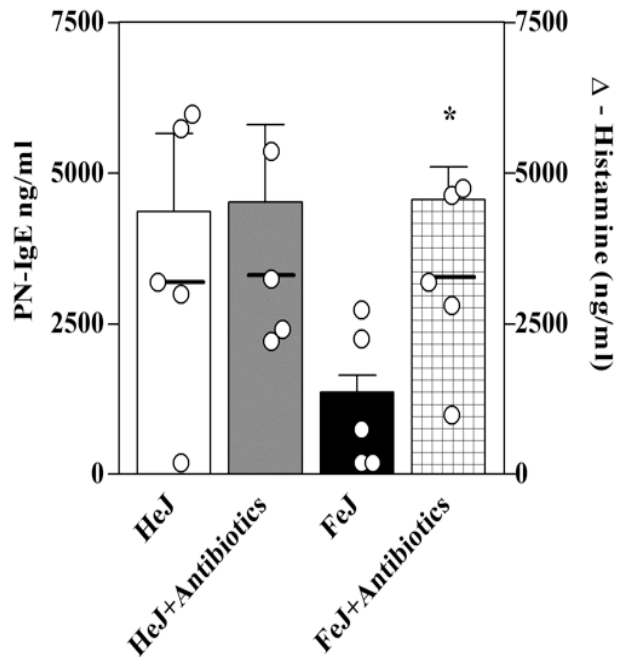
Autism

Asthma

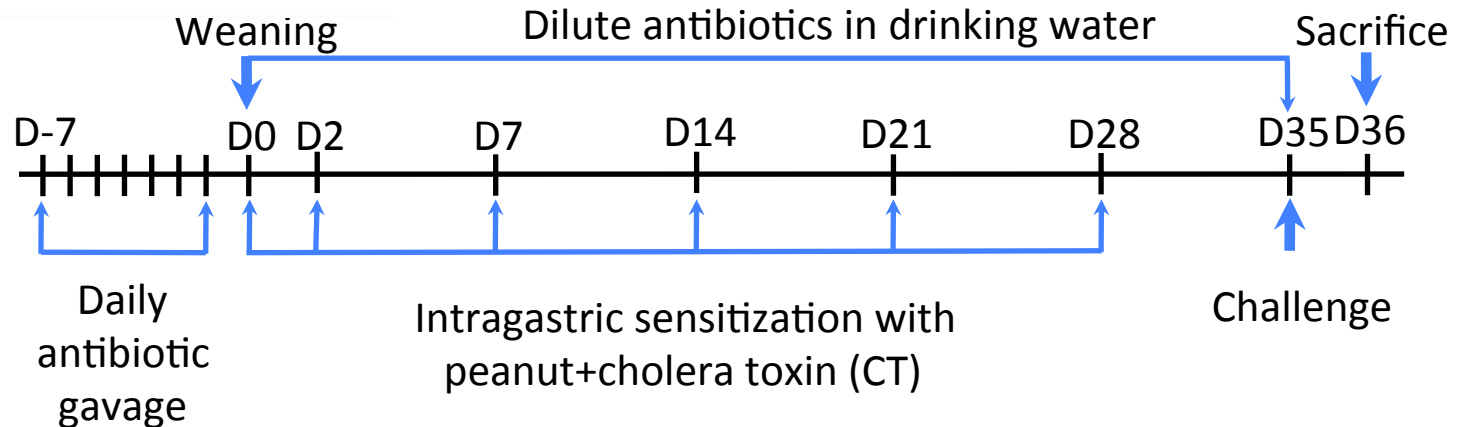
The gastrointestinal microbiota changes throughout life



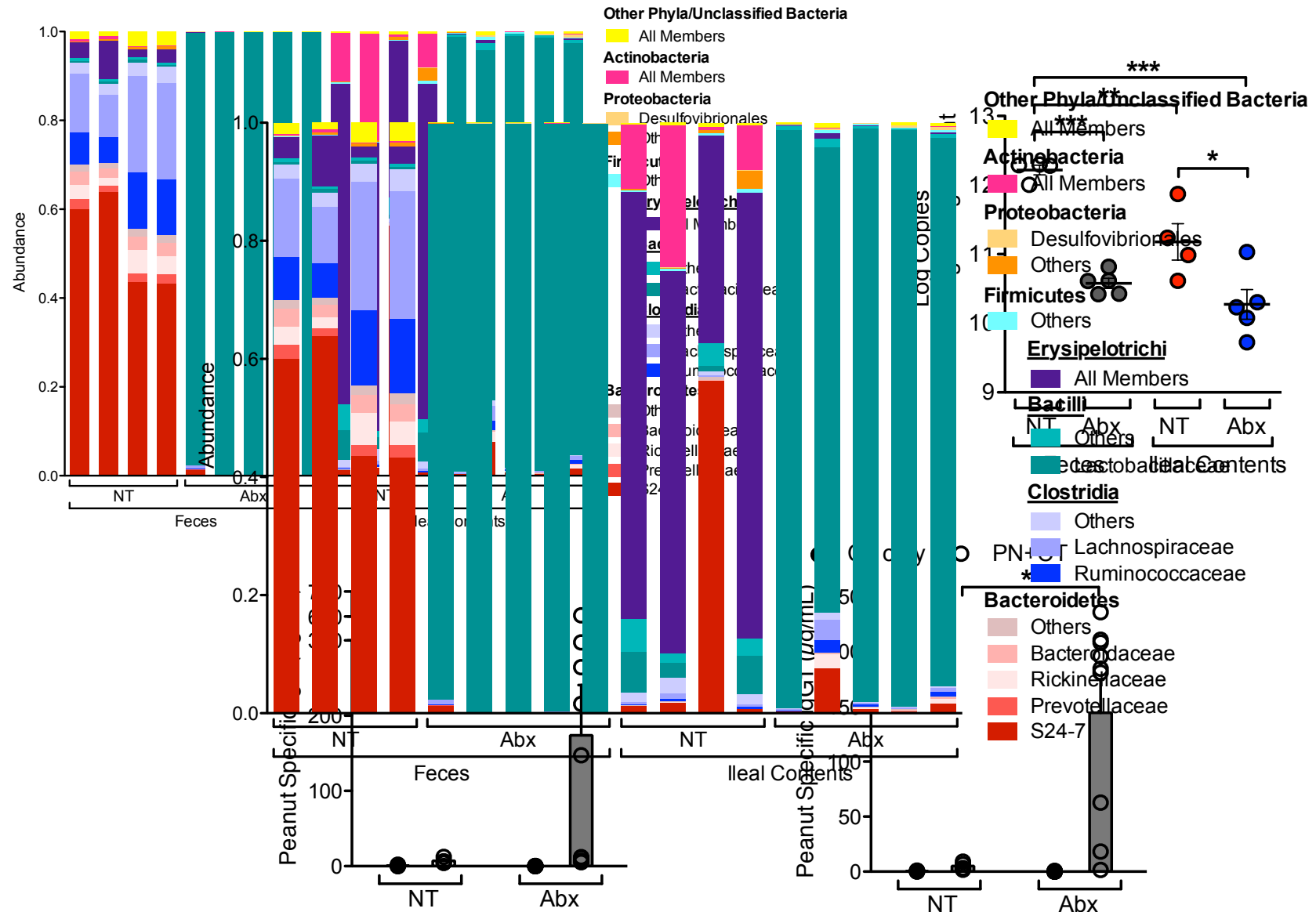
Does neonatal administration of oral antibiotics alter the composition of the fecal microbiota?



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



Antibiotic treatment dramatically alters the composition of the microbiota and increases sensitization to food

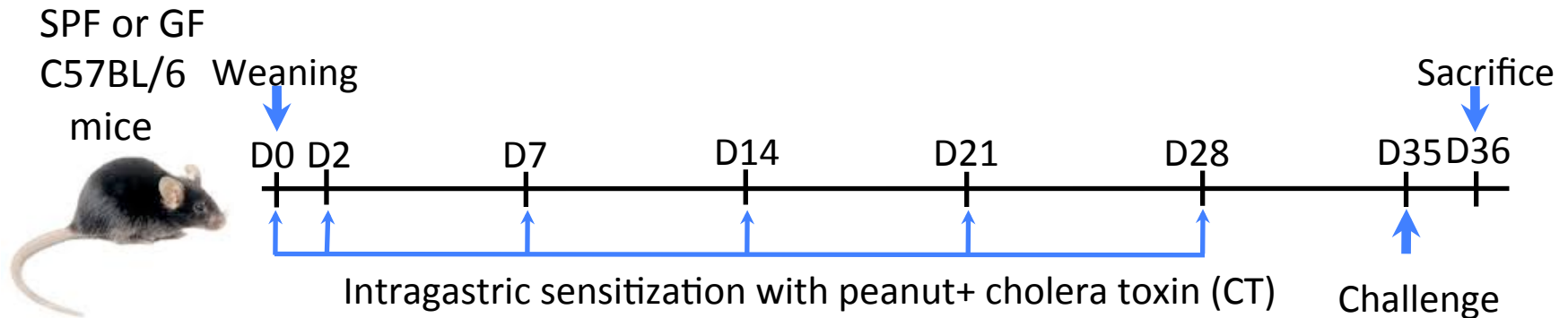
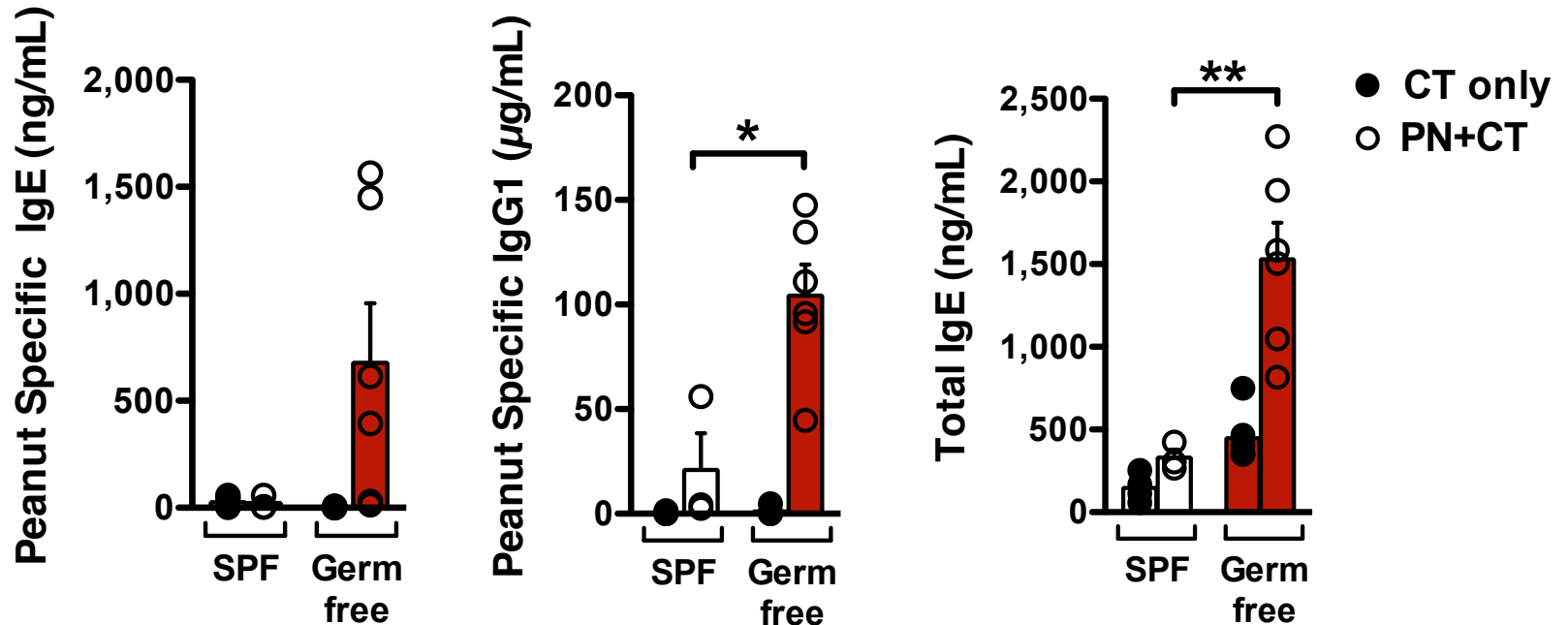


How does bacterial colonization change the response to sensitization to a food allergen?

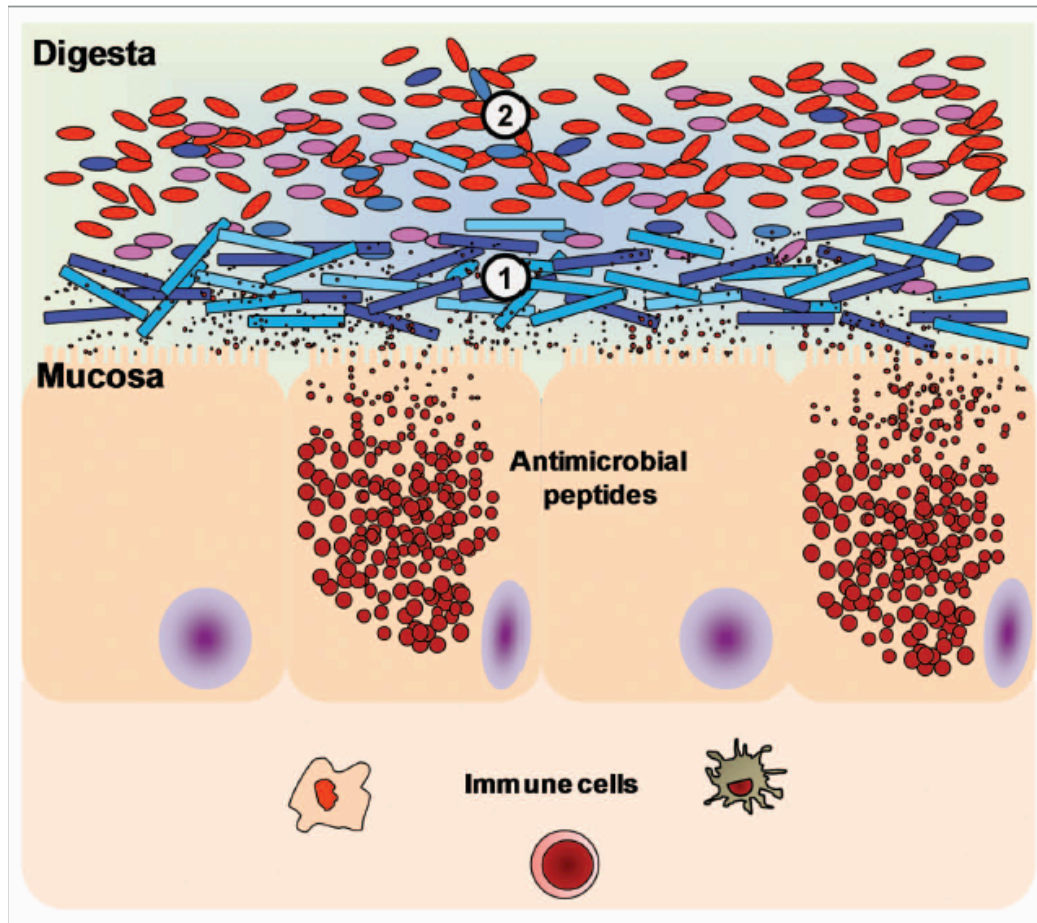
Germ free mice are a powerful tool to examine whether the presence of particular bacterial species protects against sensitization to food



A novel gnotobiotic model of food allergy



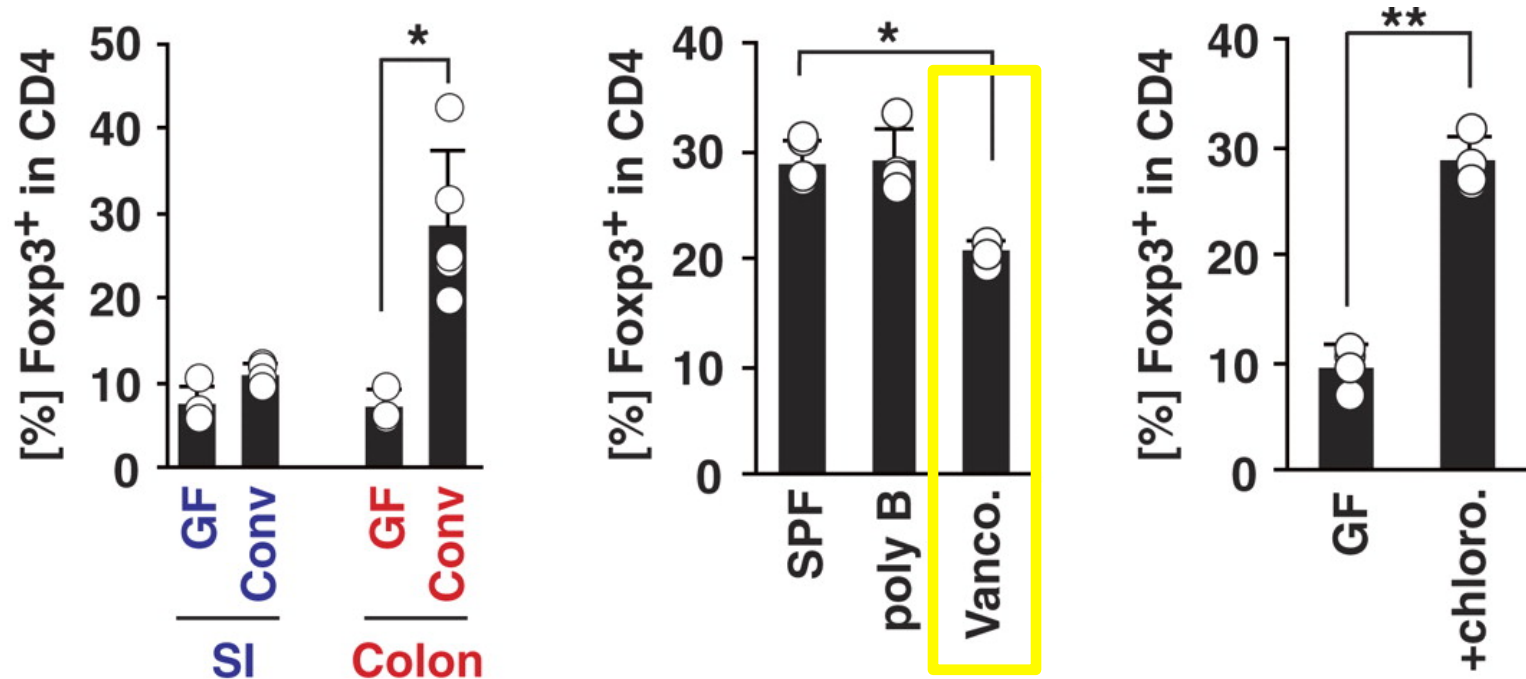
To identify allergy protective bacterial populations we selectively colonized gnotobiotic mice with representatives of the numerically predominant taxa in the murine colon



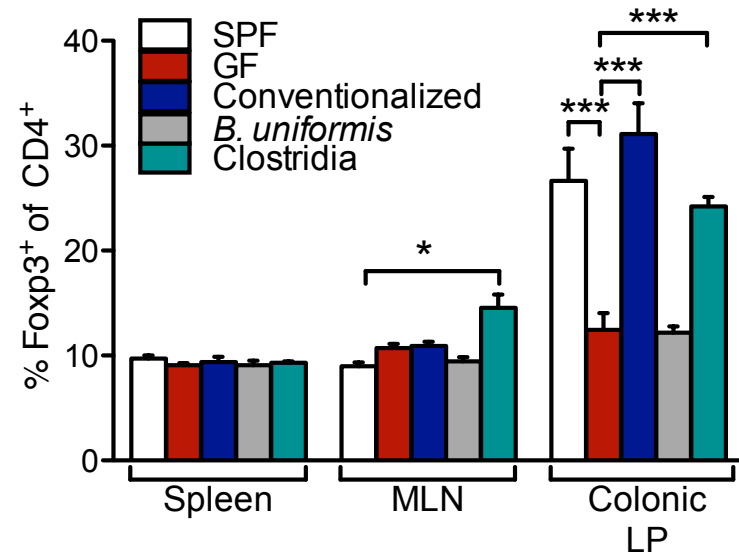
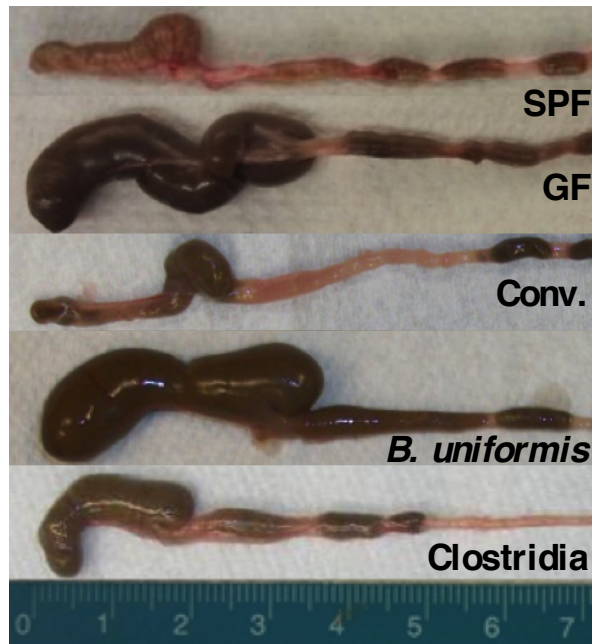
② **Bacteroides**
are enriched among the
transient bacteria
associated with digesta

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

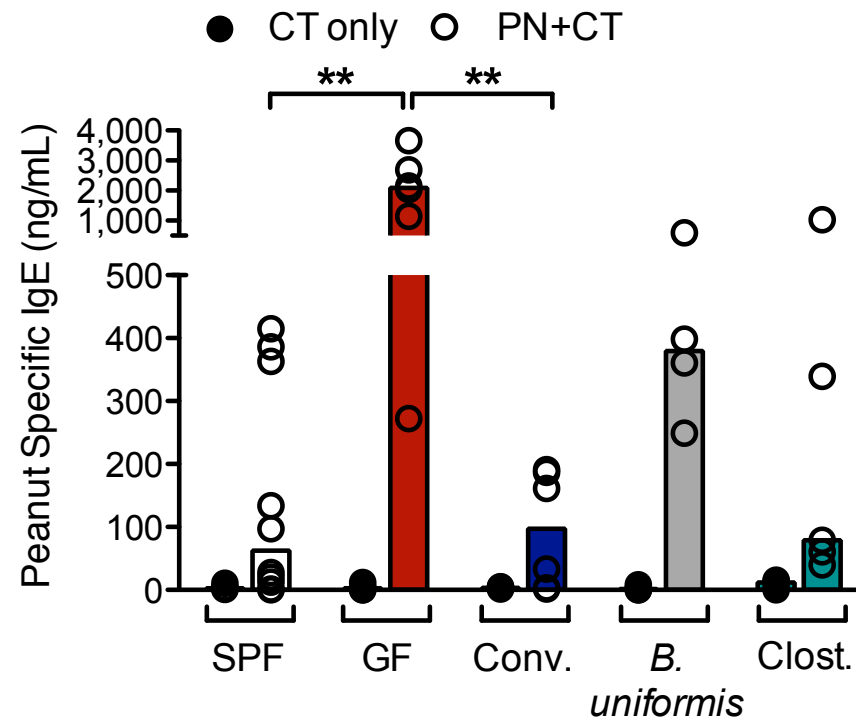
Accumulation of Foxp3⁺ regulatory T cells in the gut is dependent on indigenous spore-forming bacteria from the Clostridia class



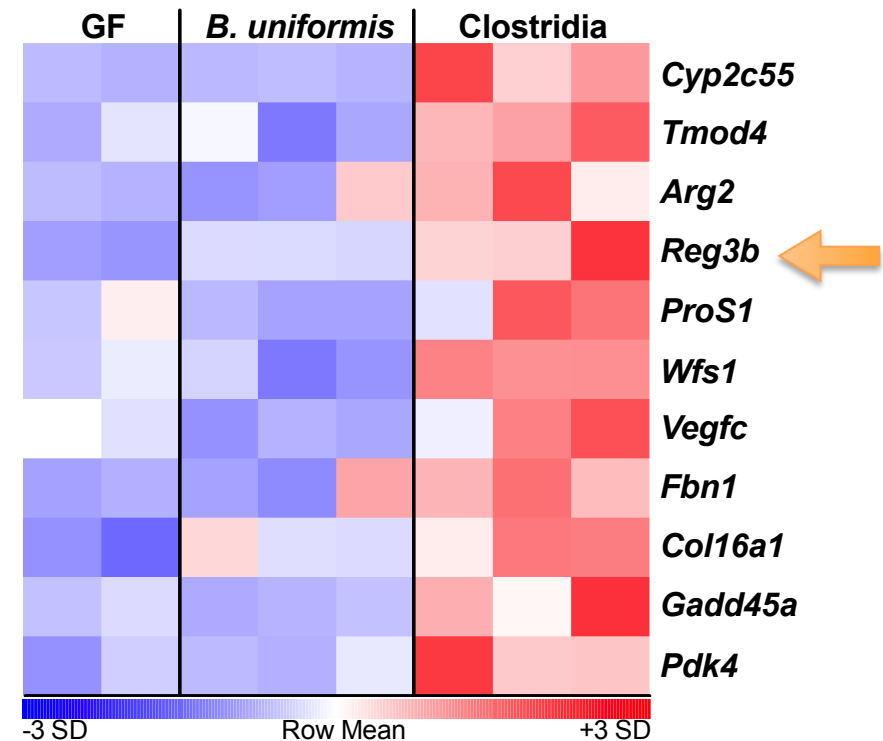
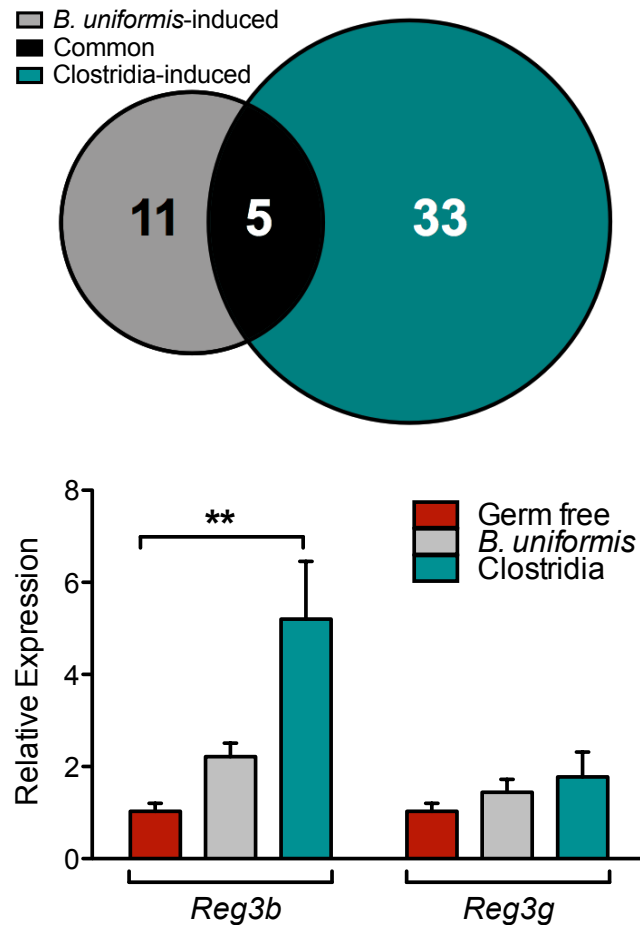
Selective colonization of gnotobiotic mice differentially induces Tregs in the colonic lamina propria



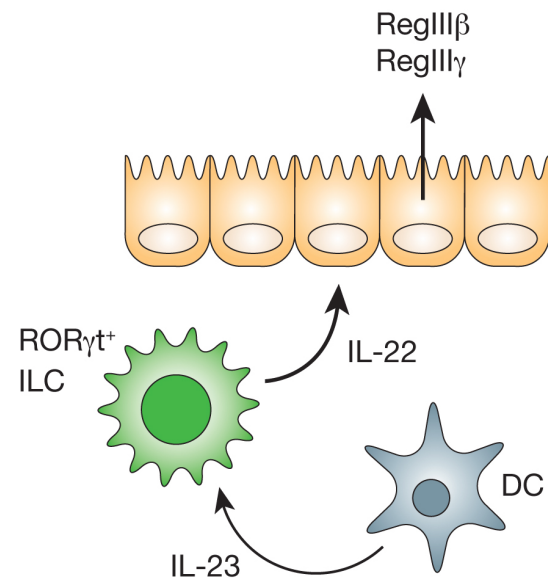
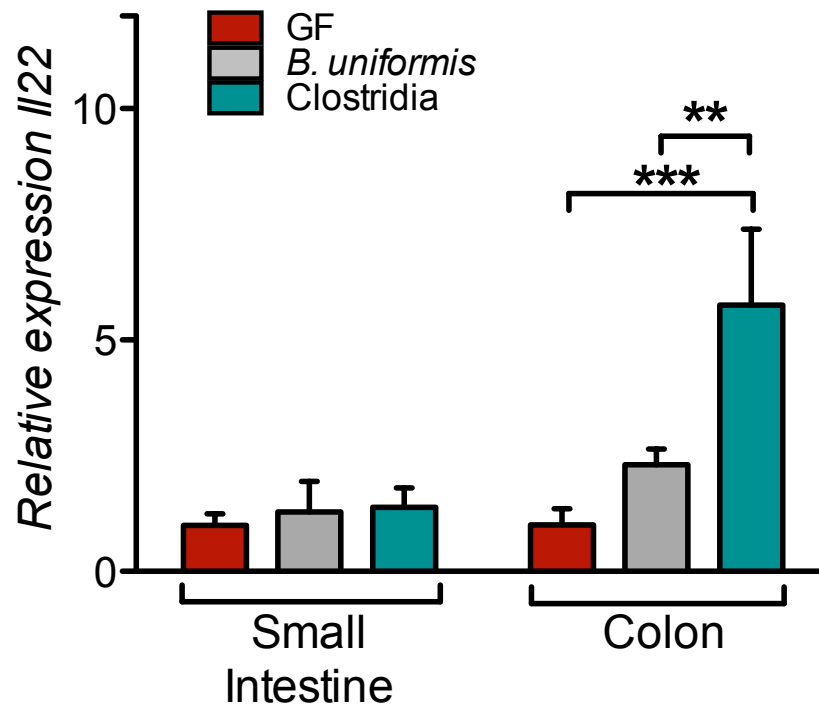
A Clostridia containing commensal microbiota protects against allergic sensitization to food



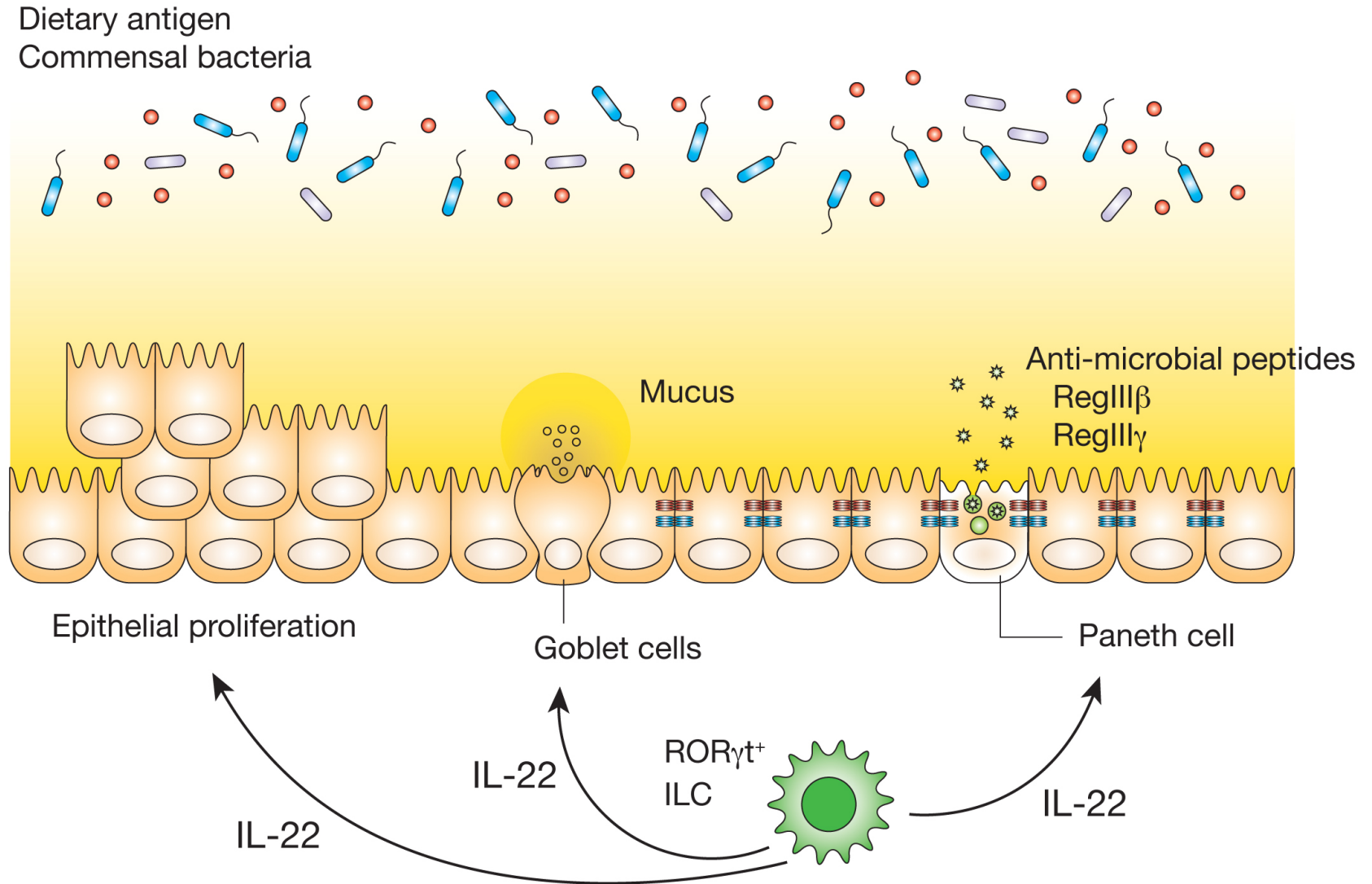
Clostridia colonization uniquely induces expression of a subset of genes in the colonic epithelium



Expression of IL-22 is significantly increased upon Clostridia colonization

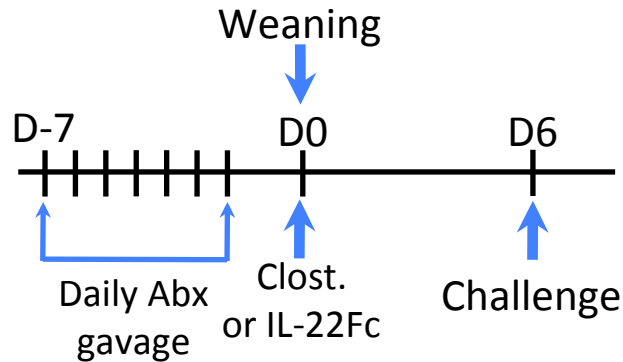


IL-22 is a barrier protective cytokine



Clostridia-induced IL-22 is necessary to reduce intestinal permeability to food antigen

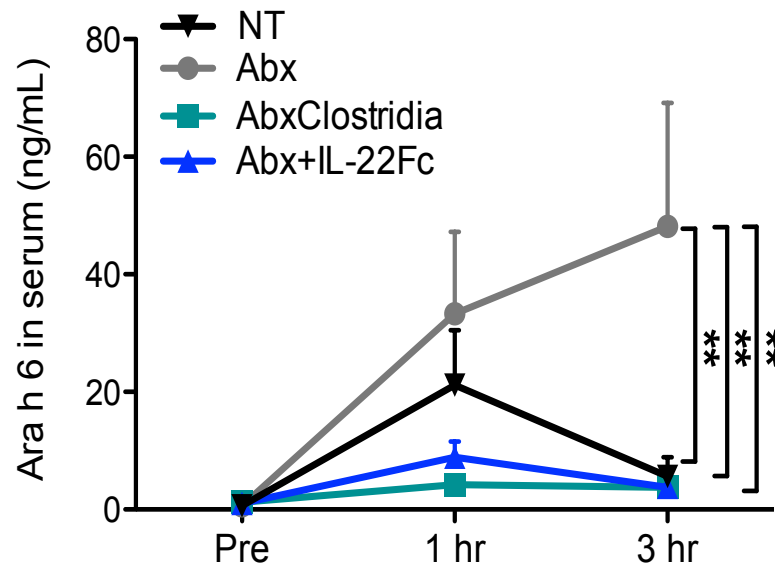
C57BL/6
mice



ELISA for Ara h 6

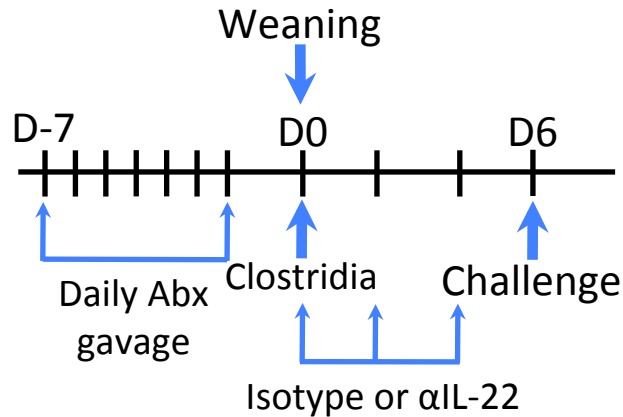


Arachis hypogaea



Clostridia-induced IL-22 is necessary to reduce intestinal permeability to food antigen

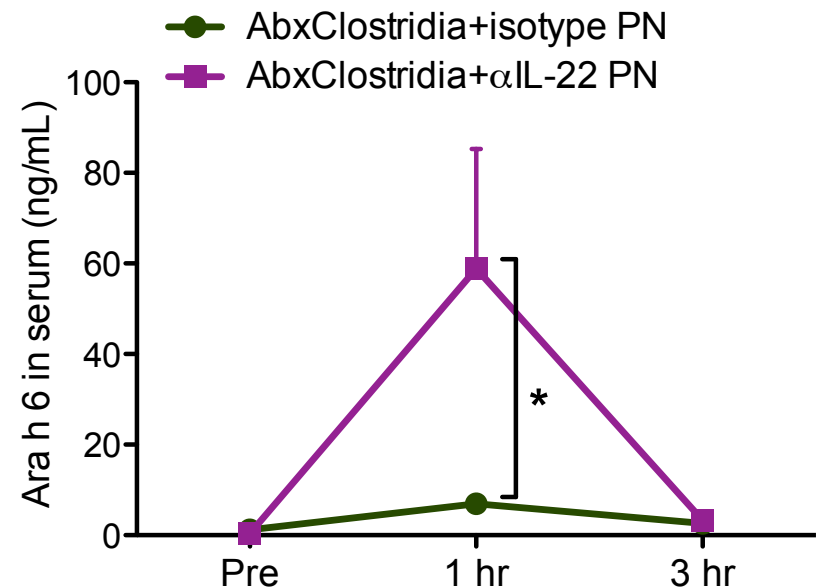
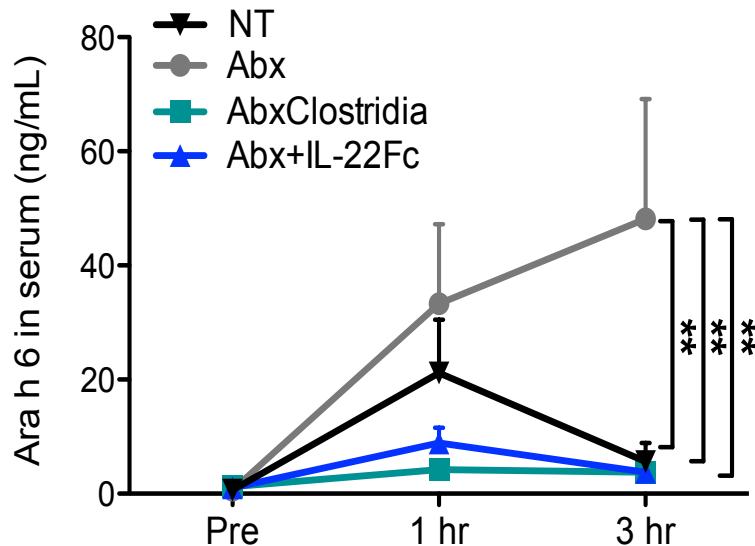
C57BL/6
mice



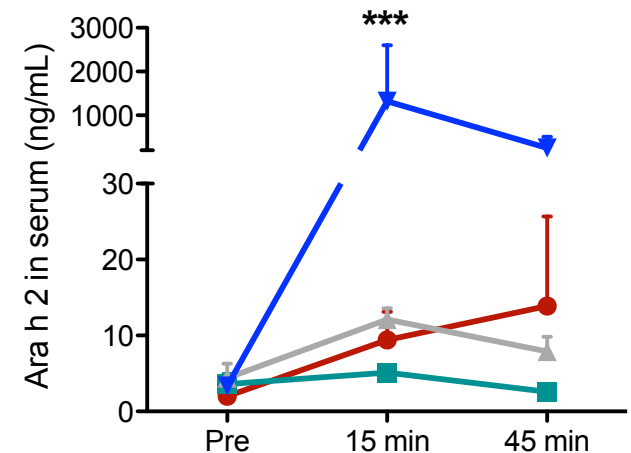
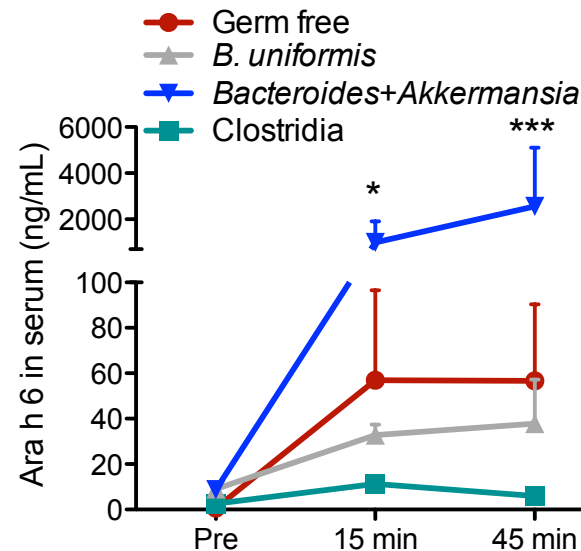
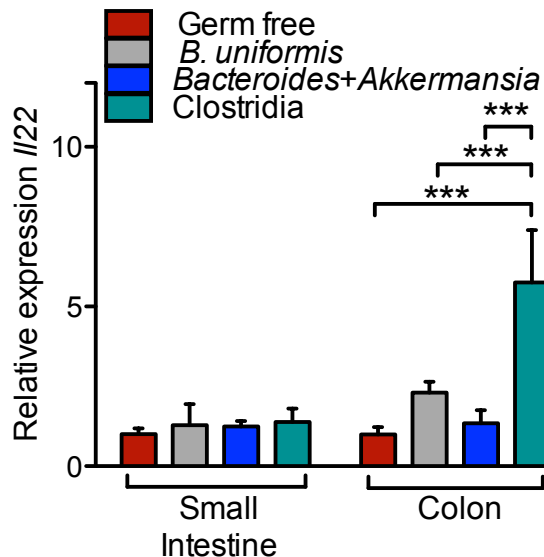
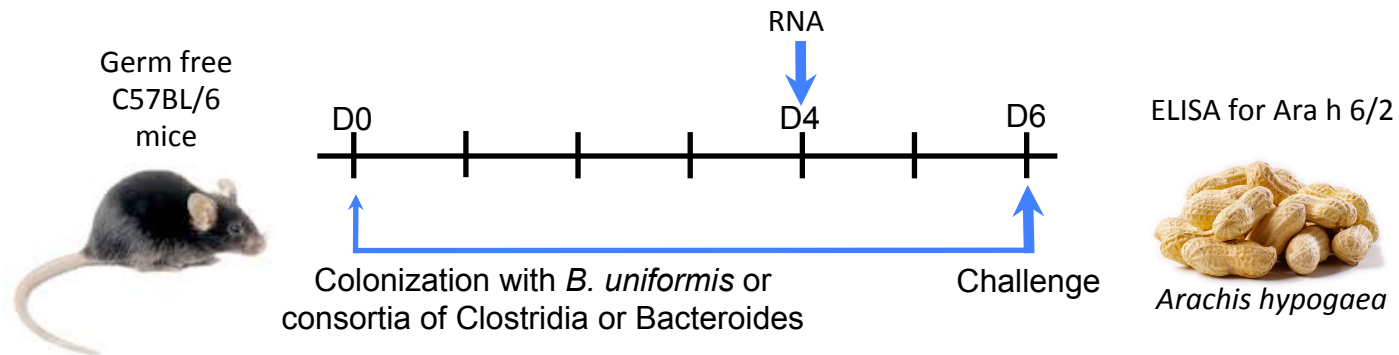
ELISA for Ara h 6



Arachis hypogaea

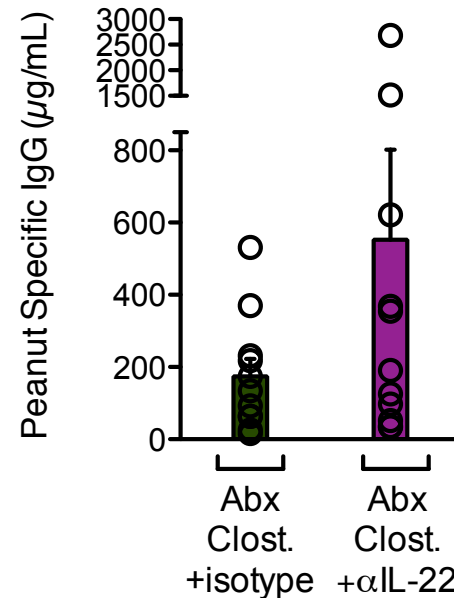
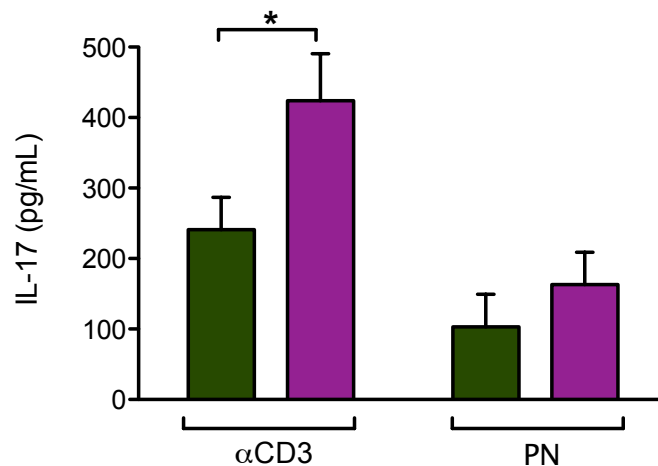
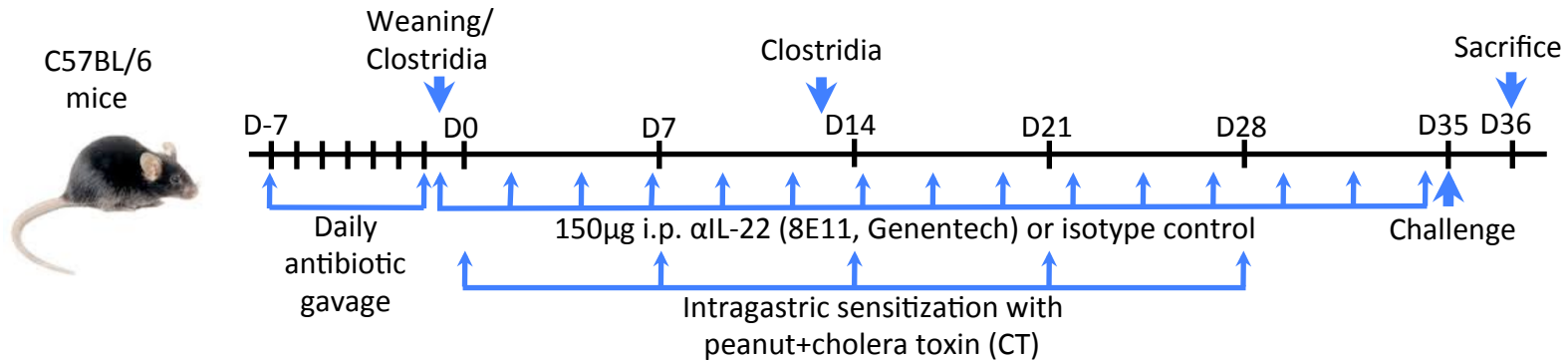


A consortium of *Bacteroides*+*Akkermansia* does not induce IL-22 and enhances intestinal permeability to food antigen

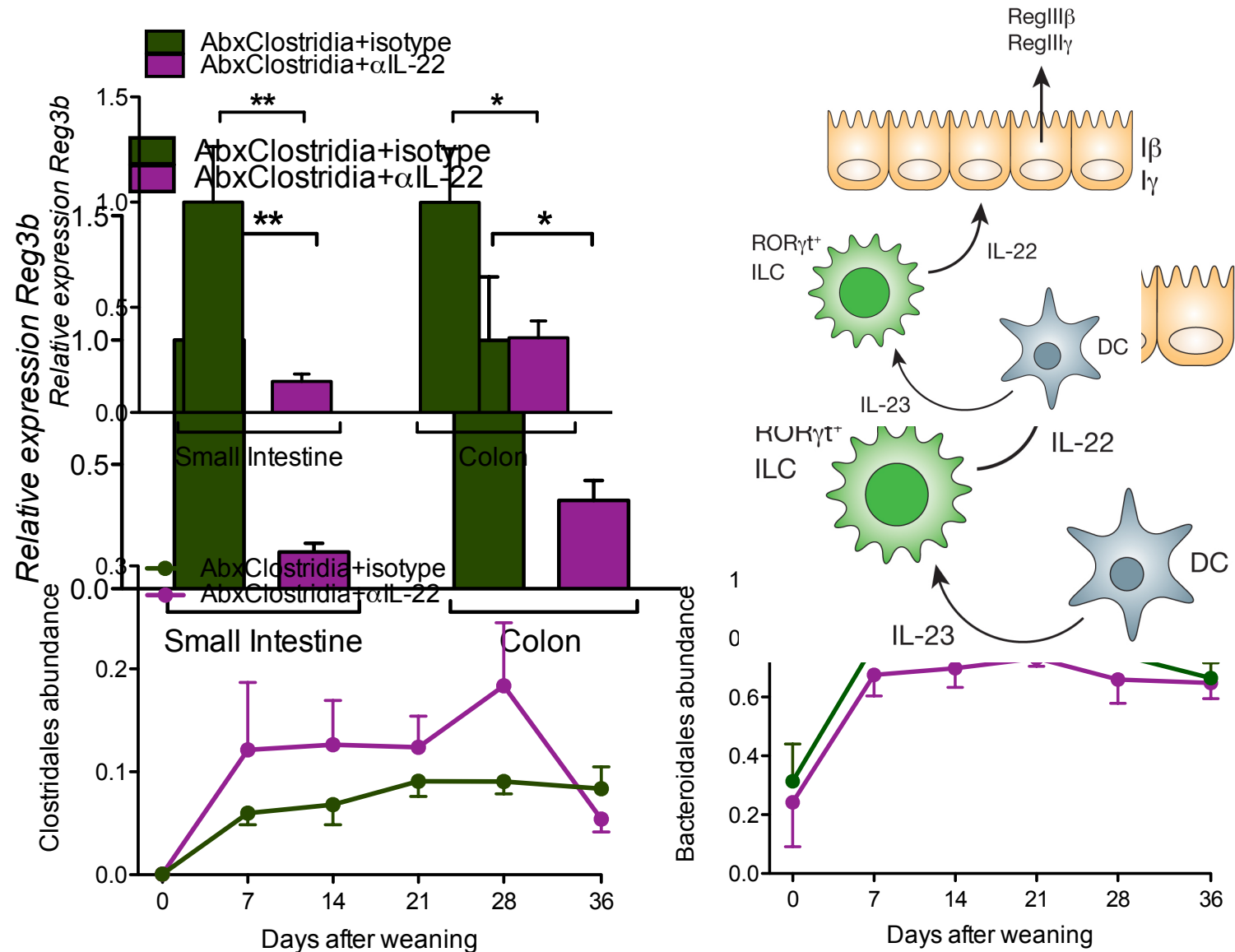


Bacteroides+*Akkermansia* consortium (Elaine Hsiao, Cal Tech)
70% *Bacteroides*, 25% *A. muciniphila*, 3% *Parabacteroides*, 1% *Turicibacter*

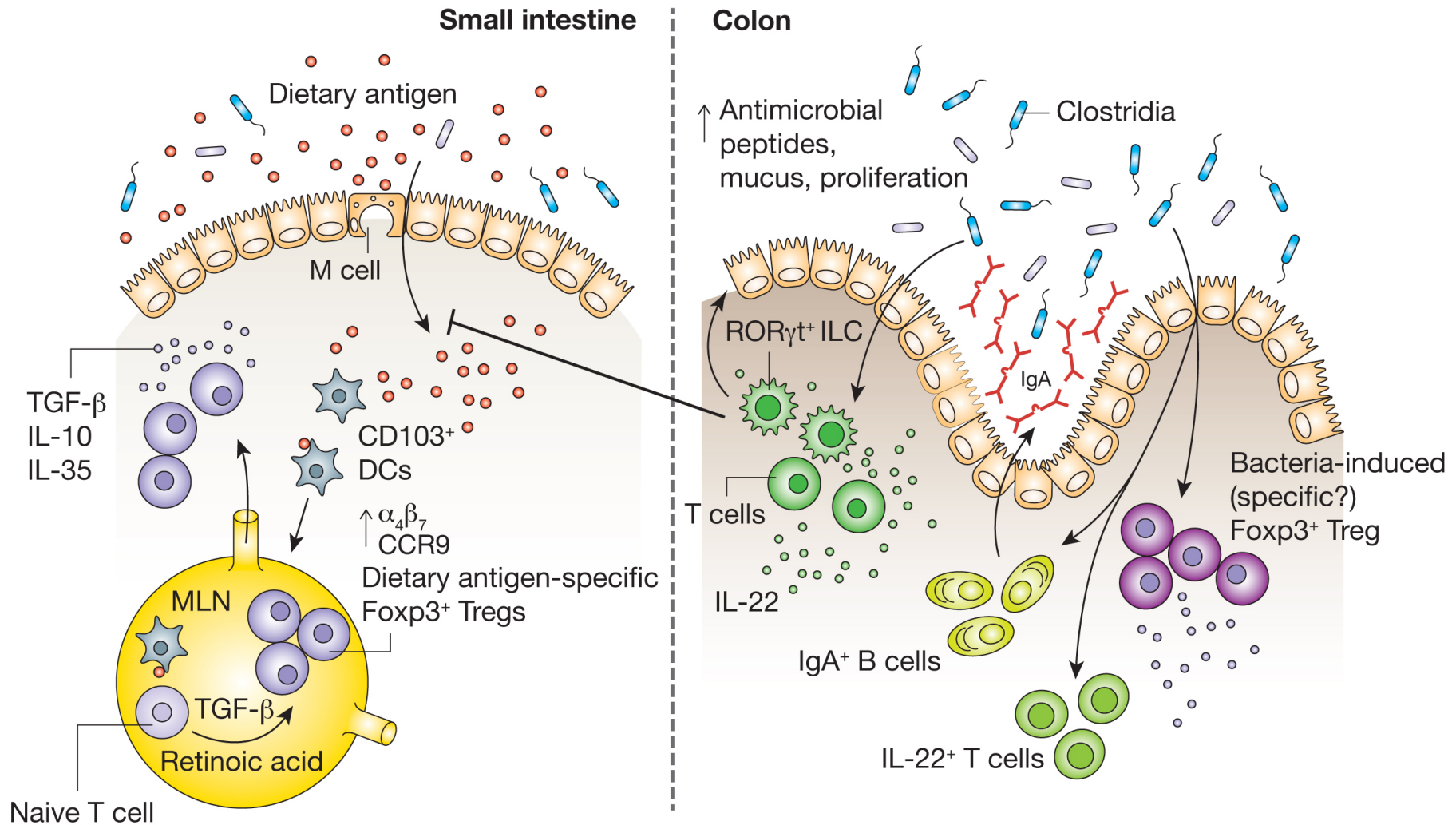
Depletion of IL-22 primes for an IL-17 response and an increase in peanut-specific IgG



Treatment with anti-IL-22 decreases expression of anti-microbial peptides (Reg3b) and increases Clostridia abundance

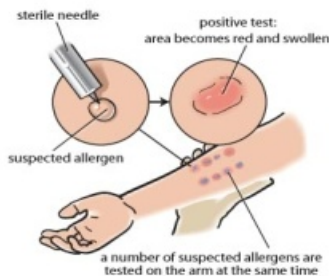


Tolerance to dietary allergen requires the induction of a bacteria-induced barrier protective response

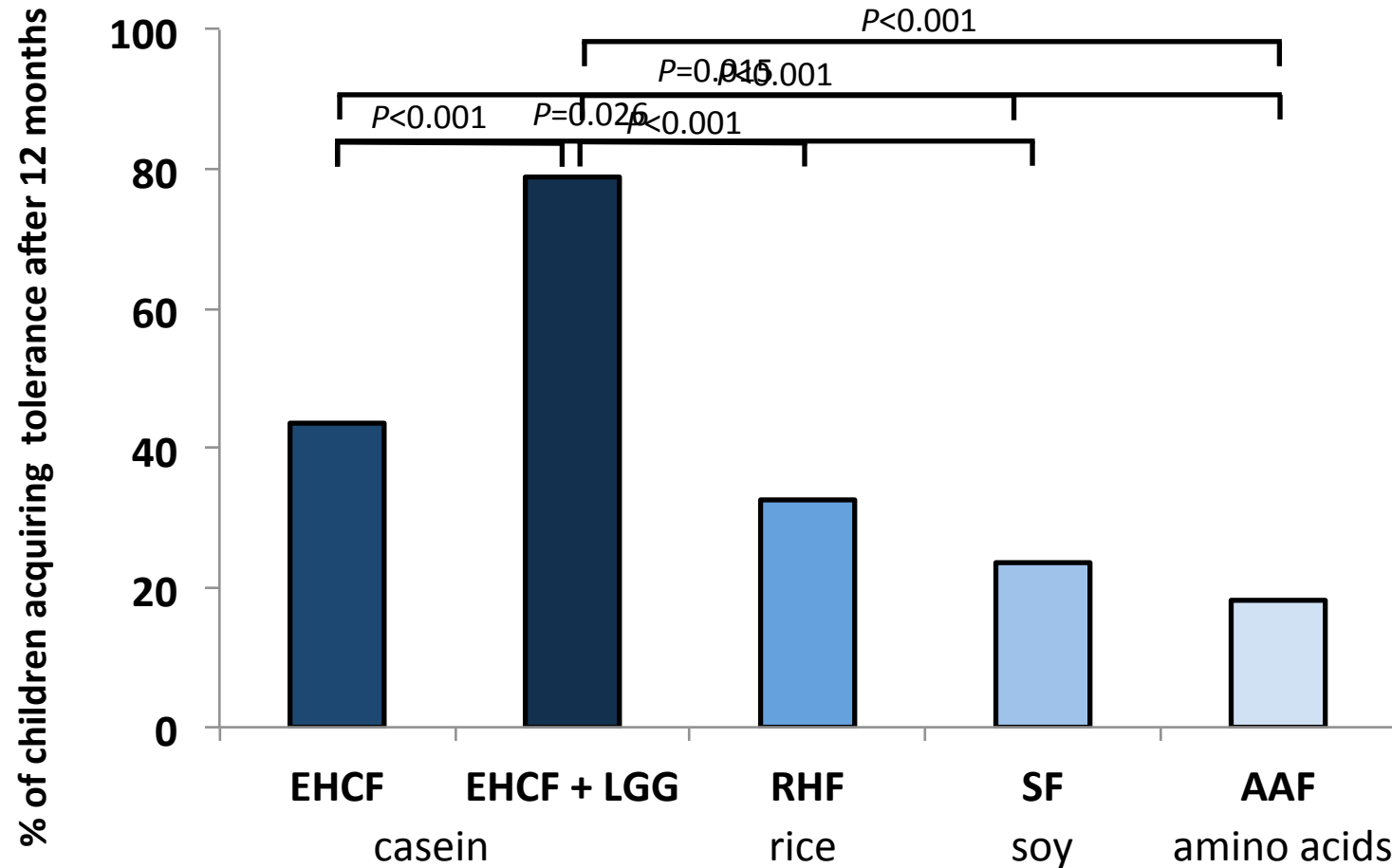


Can we develop novel strategies to modulate the composition of the microbiota to prevent or treat food allergies?

Supplementation of extensively hydrolyzed casein formula with *Lactobacillus GG* accelerates acquisition of tolerance in children with cow's milk allergy

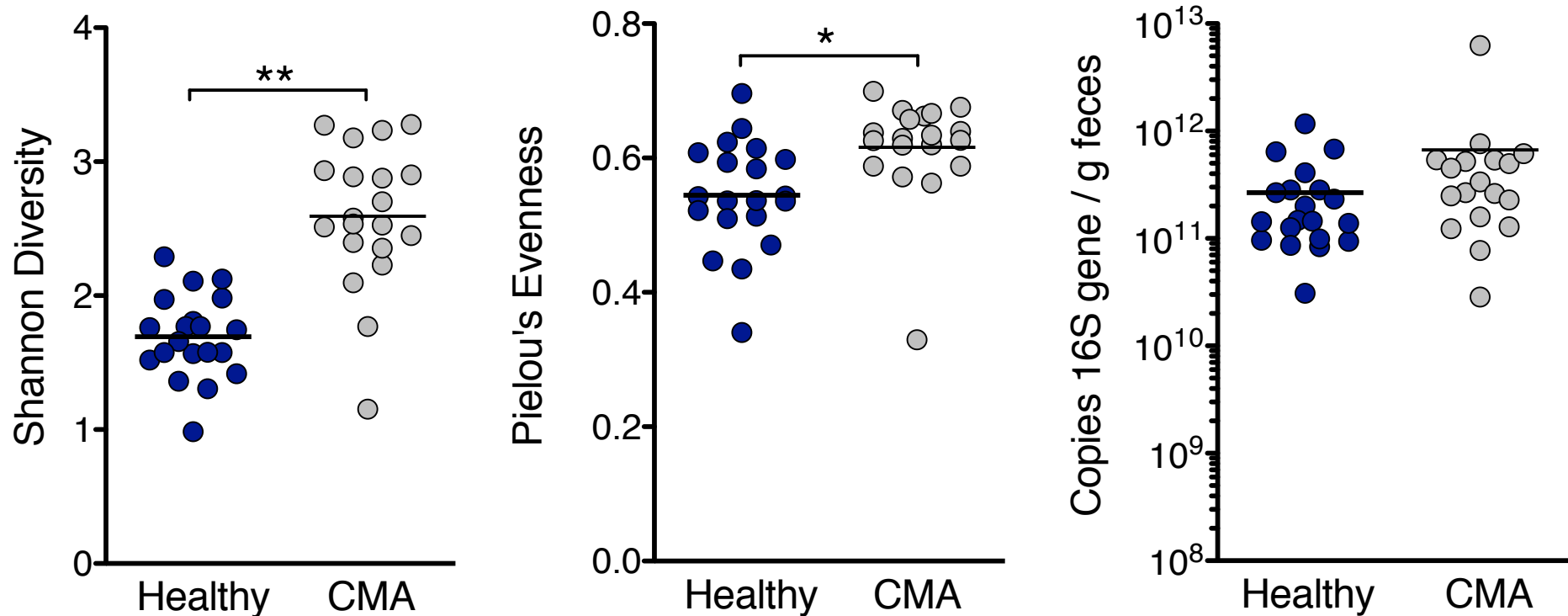


Supplementation of extensively hydrolyzed casein formula (EHCF) with *Lactobacillus GG* accelerates acquisition of tolerance in children with cow's milk allergy

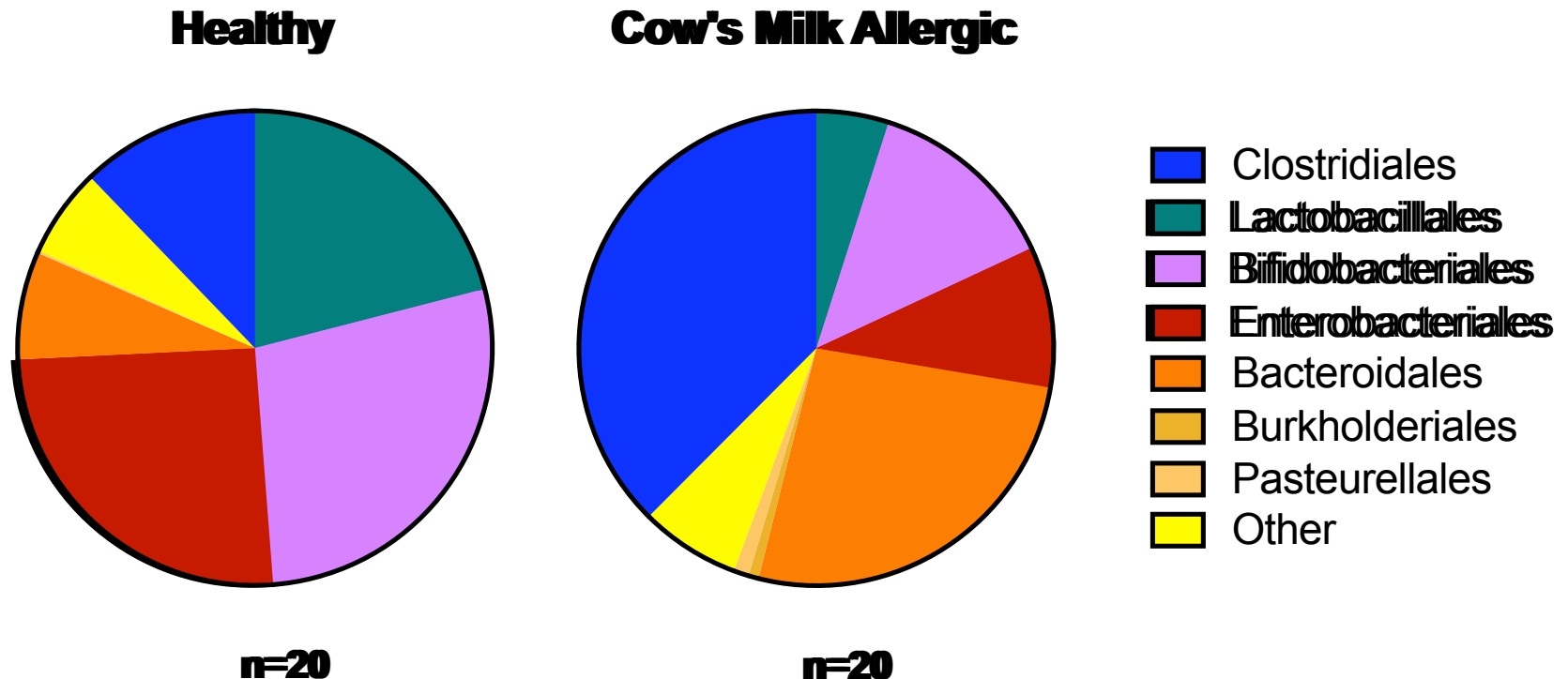


Berni Canani R, et al. *The Journal of Pediatrics* 2013, 163; 771

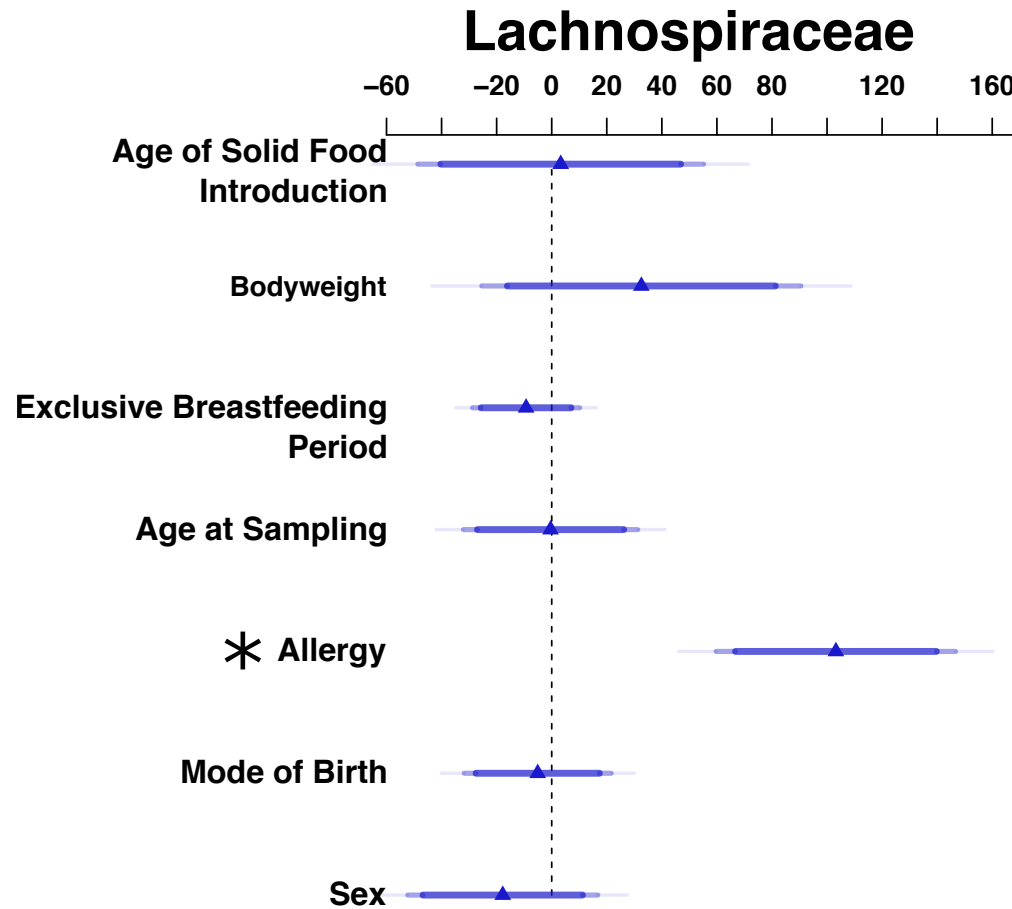
The cow's milk allergic (CMA) infant microbiome exhibits significantly increased diversity



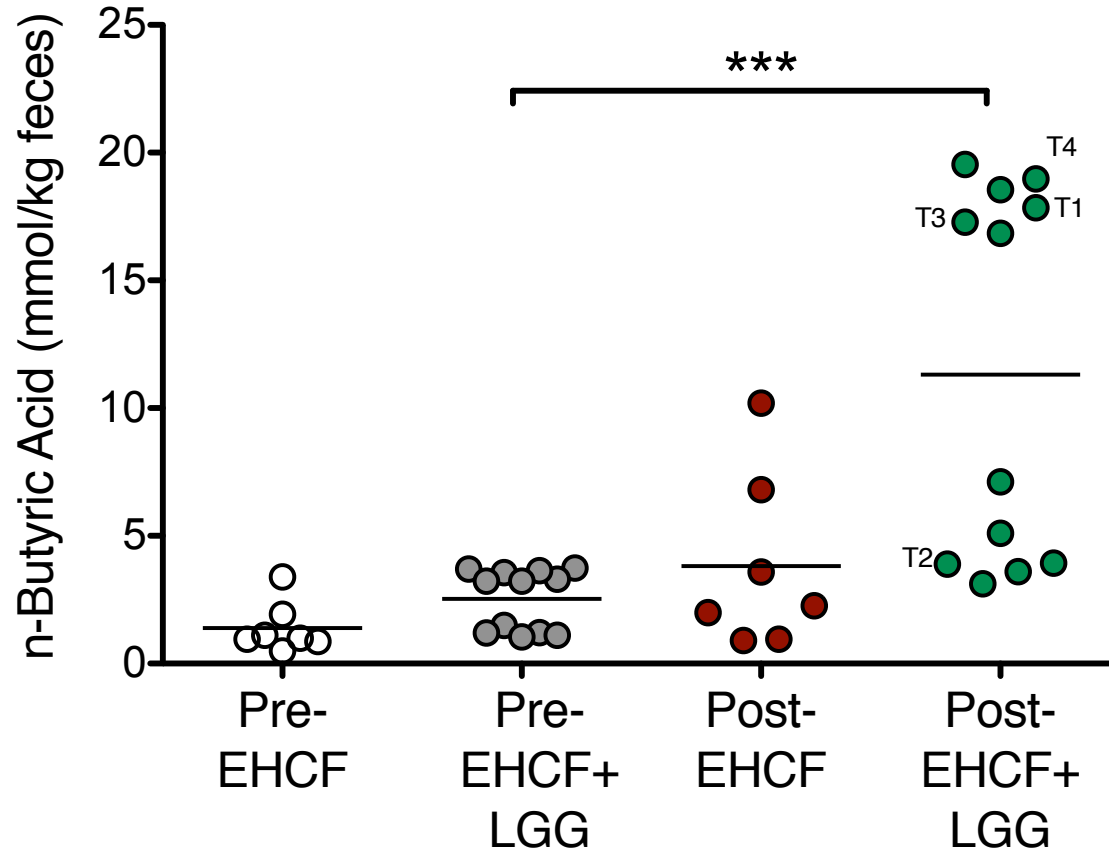
The composition of the fecal microbiota is altered in cow's milk allergic (CMA) infants



Demographic variables do not explain differences in bacterial abundance



Treatment with LGG supplemented formula changes microbial community structure to enhance production of fecal butyric acid and promote tolerance to CMA

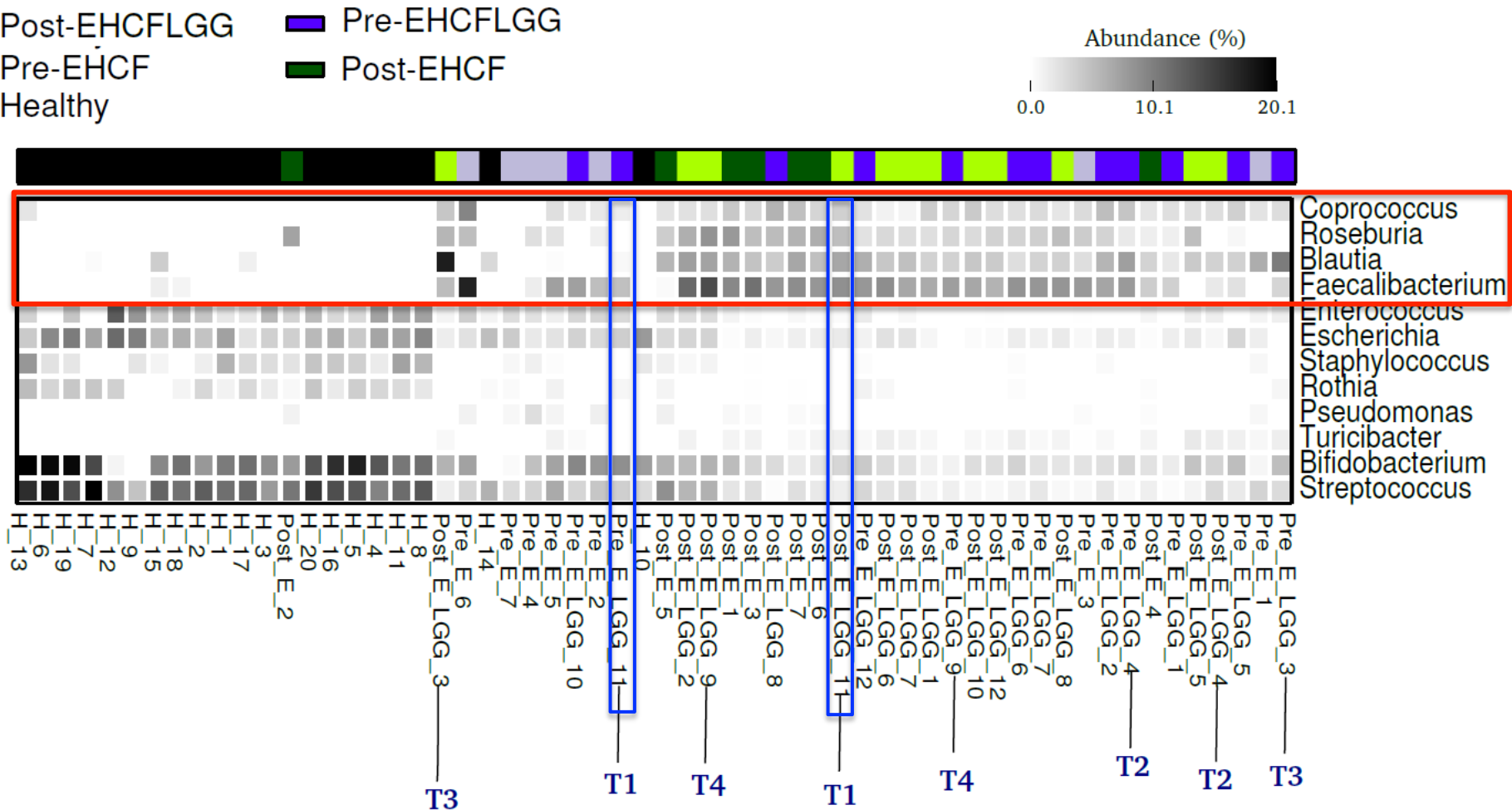


**Tolerance to CMA
at 12 months**

EHCF 0/7

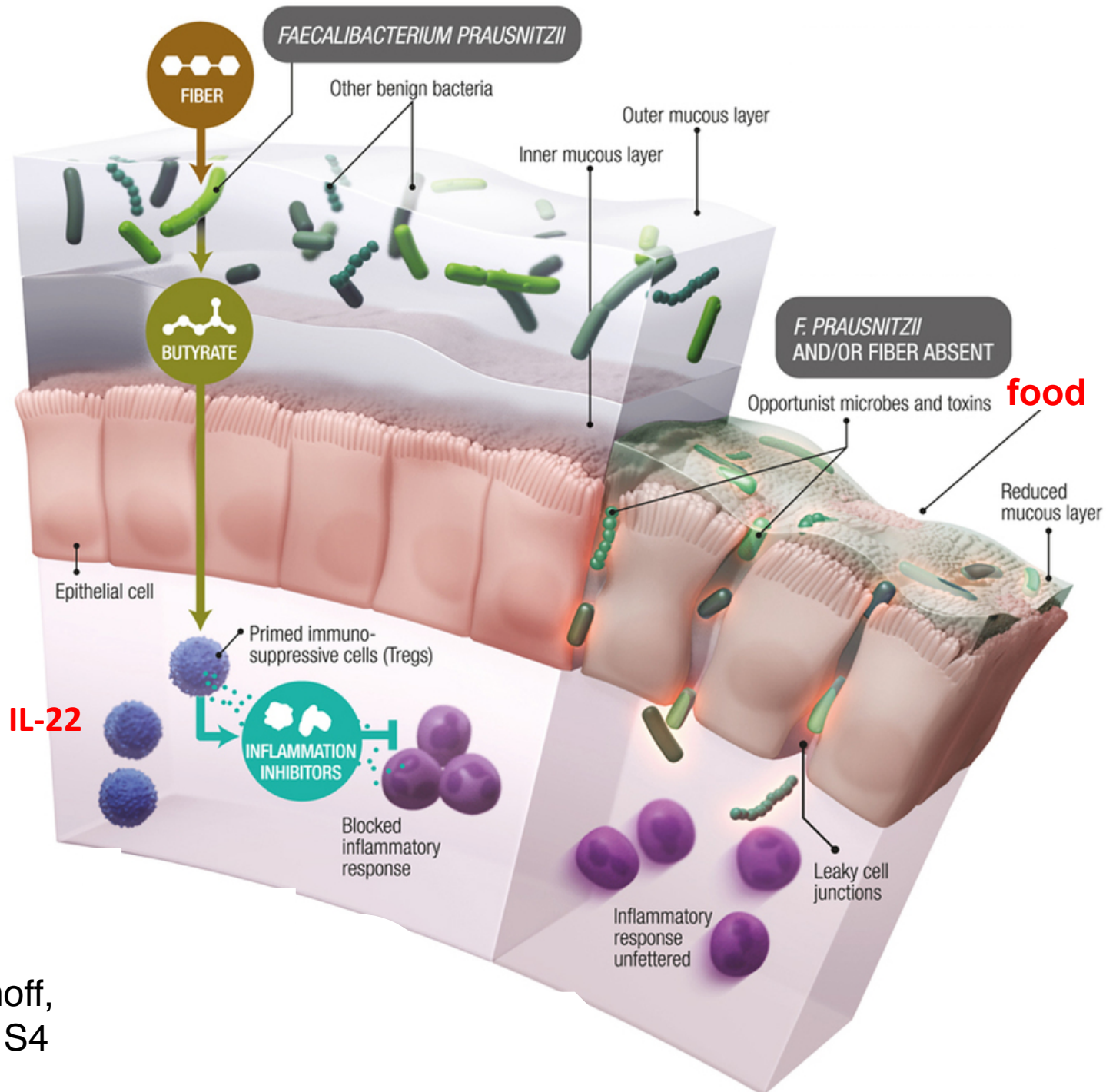
EHCF + LGG 5/12*

Genus level differential feature selection revealed a relationship between the abundance of bacterial strains, increased fetal butyrate concentration and acquisition of tolerance



GUT MICROBIOME

The Peace-keepers



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

Sensitize with β lactoglobulin (BLG)/CT

Cow's milk
allergic (CMA)
infant microbiota



Allergic



Developing microbiome-modulating therapeutics to prevent or treat food allergy

CLOSTRABIO

- Drug formulations from microbial metabolites

Healthy infant
microbiota



Non-allergic

- Pre-biotic dietary supplements

Sensitize with β lactoglobulin (BLG)/CT

- Therapeutic bacterial species (or mixtures)

Cow's milk
allergic (CMA)
infant microbiota



Allergic