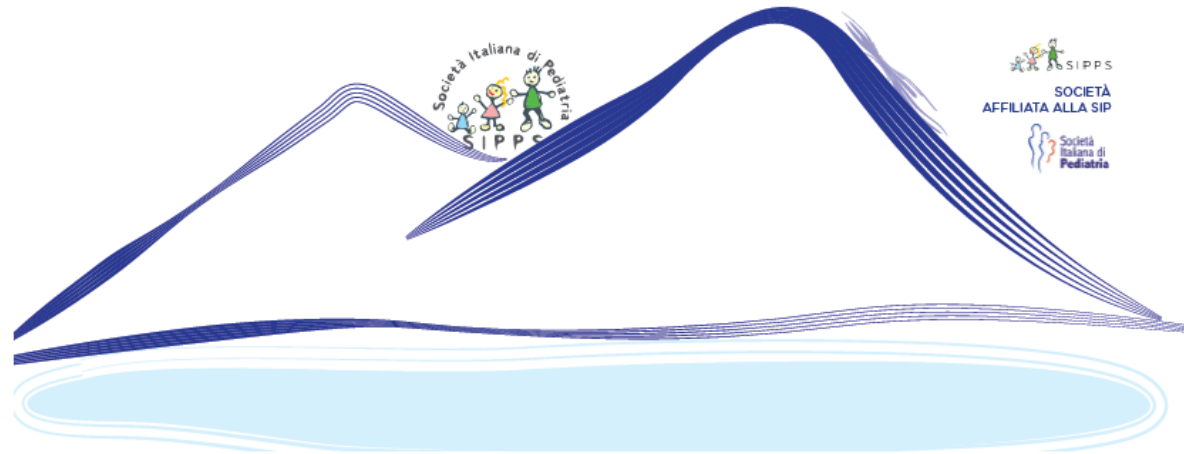


# Napule è...

PEDIATRIA PREVENTIVA E SOCIALE



Microbiota  
intestinale ed  
immunità  
mucosale

Alessandro  
Fiocchi  
Napoli  
1 maggio 2015

Editor-in-chief,  
the WAO Journal

LUCI OMBRE ABBAGLI

Prevenzione

Nutrizione

Allergologia

Dermatologia

Gastroenterologia



- The human microbioma: influences on natural immunity
- The human microbioma: influences on adaptive immunity
- Illustrate the GLAD-p recommendations for the use of probiotics in allergy prevention



**SKEPTICISM**



- 1. The CUPPA document**
2. The human microbiome
3. Microbiome & innate immunity
4. Microbiome & adaptive immunity
5. Microbiome & disease
6. The GLAD-p recommendations
7. Conclusions

# Why probiotics in pediatric allergy?

- In Italy, trust in probiotics' ability to promote immune health is driving the market
- This opens new opportunities to companies who want to expand their probiotic range
- The already massive market of probiotic supplements grew from €143m to €219m in just five years, between 2008 & 2012
- By contrast, North America recorded €206m sales
- In Italy, the market is expected to grow to €279m by 2015 (a rise of 28 per cent)

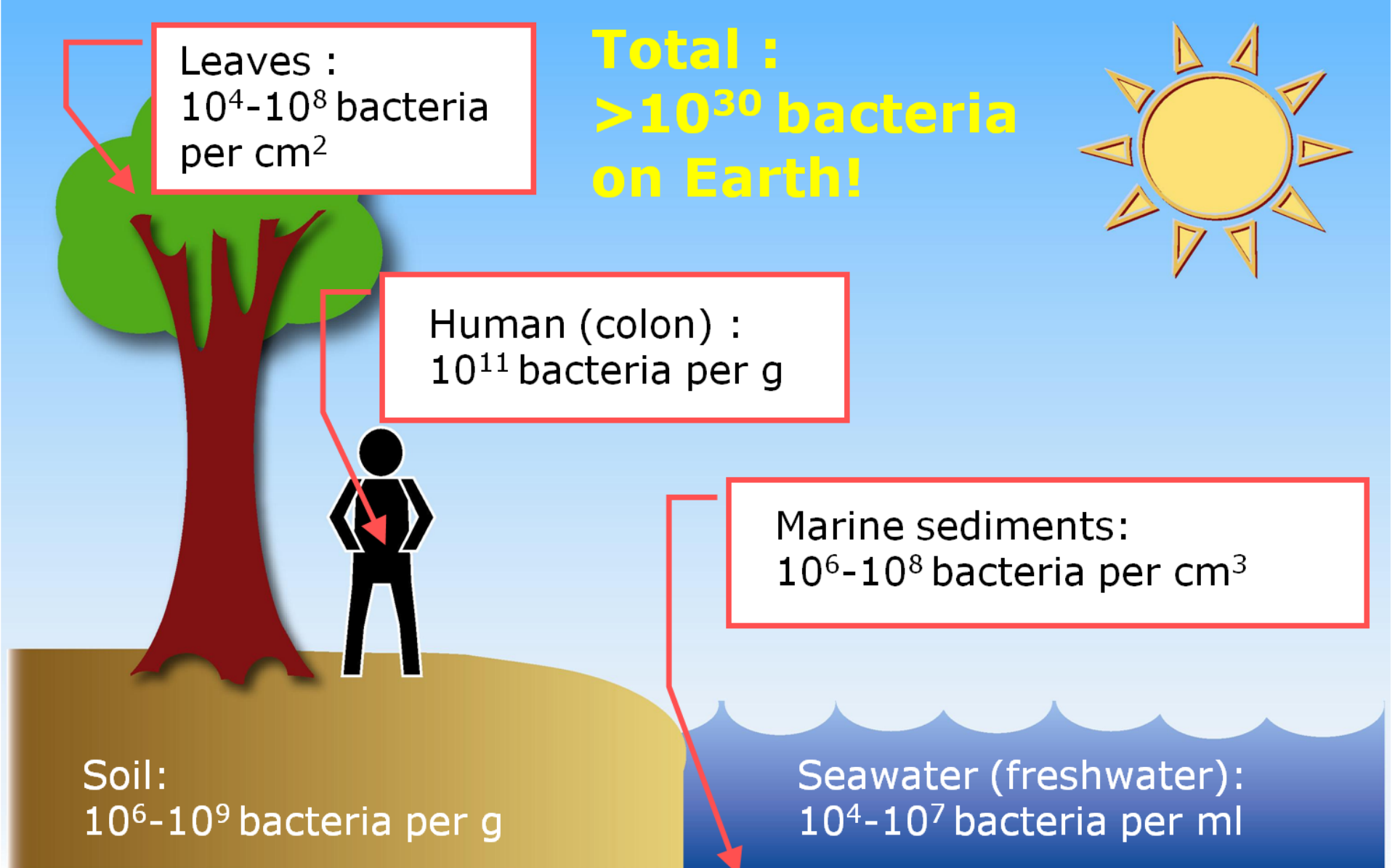
# Why probiotics in pediatric allergy?



- Probiotics, originally conceived for treatment of gastrointestinal diseases and later applied to allergy prevention, are increasingly proposed for the treatment of allergic disease.
- A matter of concern for allergists because the translation from possible prevention effects to therapeutic efficacy remains to be demonstrated.



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Leaves :  
 $10^4$ - $10^8$  bacteria  
per  $\text{cm}^2$

**Total :**  
 **$>10^{30}$  bacteria**  
**on Earth!**



Human (colon) :  
 $10^{11}$  bacteria per g

Marine sediments:  
 $10^6$ - $10^8$  bacteria per  $\text{cm}^3$

Soil:  
 $10^6$ - $10^9$  bacteria per g

Seawater (freshwater):  
 $10^4$ - $10^7$  bacteria per ml

Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R, Dreborg S, Goodman R, Kuitunen M, Haahtela T, Heine RG, Lack G, Osborn DA, Sampson H, Tannock GW, Lee BW; on behalf of the WAO Special Committee on Food Allergy and Nutrition. Clinical Use of Probiotics in Pediatric Allergy (CUPPA): A World Allergy Organization Position Paper. World Allergy Organ J. 2012; 5:148-67



Microbes are ubiquitous in the biosphere

Microbes have existed and evolved over 3.8 billion years

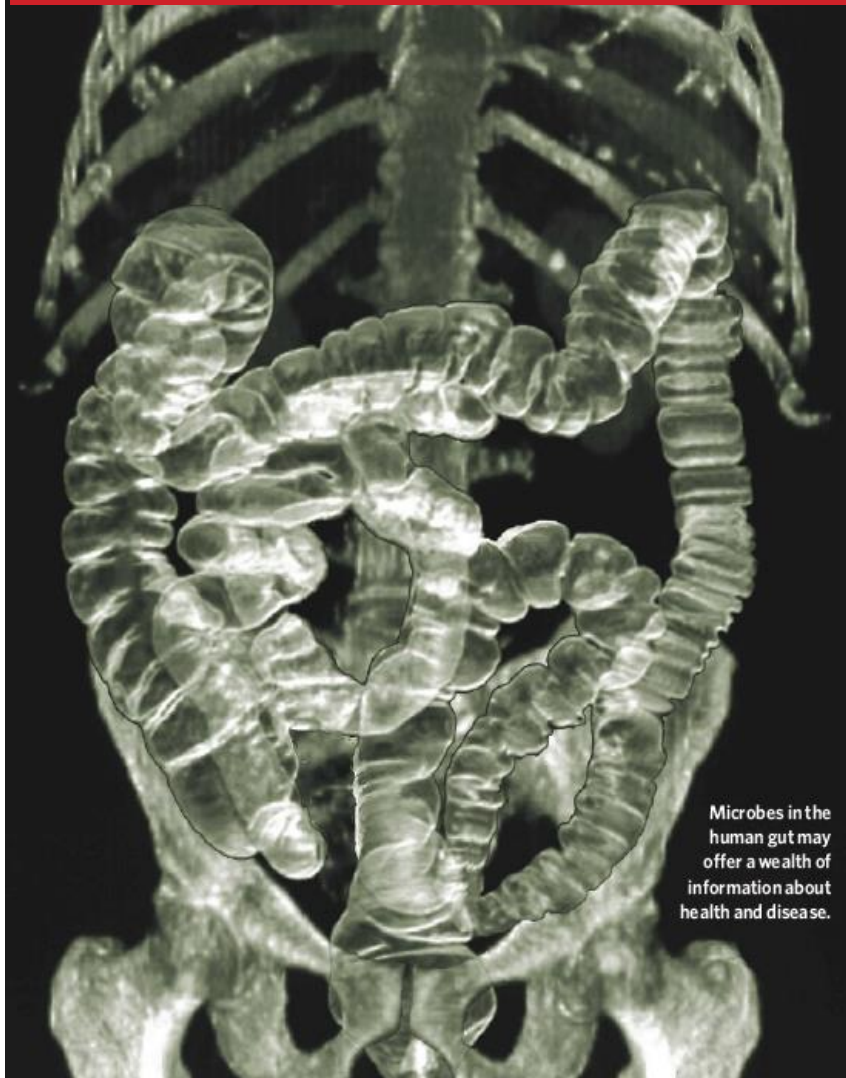
The intestinal tract of humans harbors 100 trillion cells

The number of human cells x 10

GI microbiota: bacteria, archaea, eukarya, and viruses

NATURE | Vol 453 | 29 May 2008

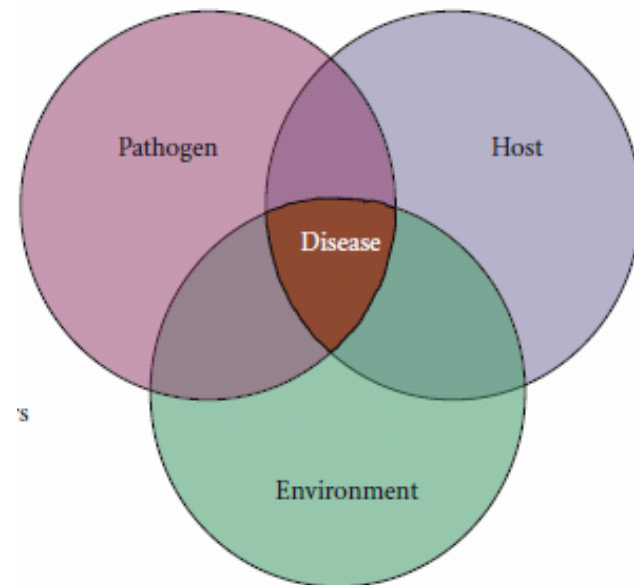
HUMAN MICROBIOME NEWS FEATURE



Microbes in the human gut may offer a wealth of information about health and disease.

The human body teems with microbes. In the first of two features, **Asher Mullard** looks at the global efforts to catalogue this vast 'microbiome'. In the second, **Apoorva Mandavilli** meets the surgeons who have a rare opportunity to watch an ecosystem being established as they transplant guts from one person to another.

## THE INSIDE STORY





## How does the body recruit a microbiome? .

- Humans are born mostly microbially sterile
- Founding microbiota acquired during delivery from vaginal canal
- Babies born via Caesarean section have an initial microbiota dominated by bacteria associated with the skin
- The limited neonatal microbiota increases in diversity as environmental exposure accumulates with age.



## How does the body recruit a microbiome? .

Infancy is a time of great plasticity for the developing microbiota

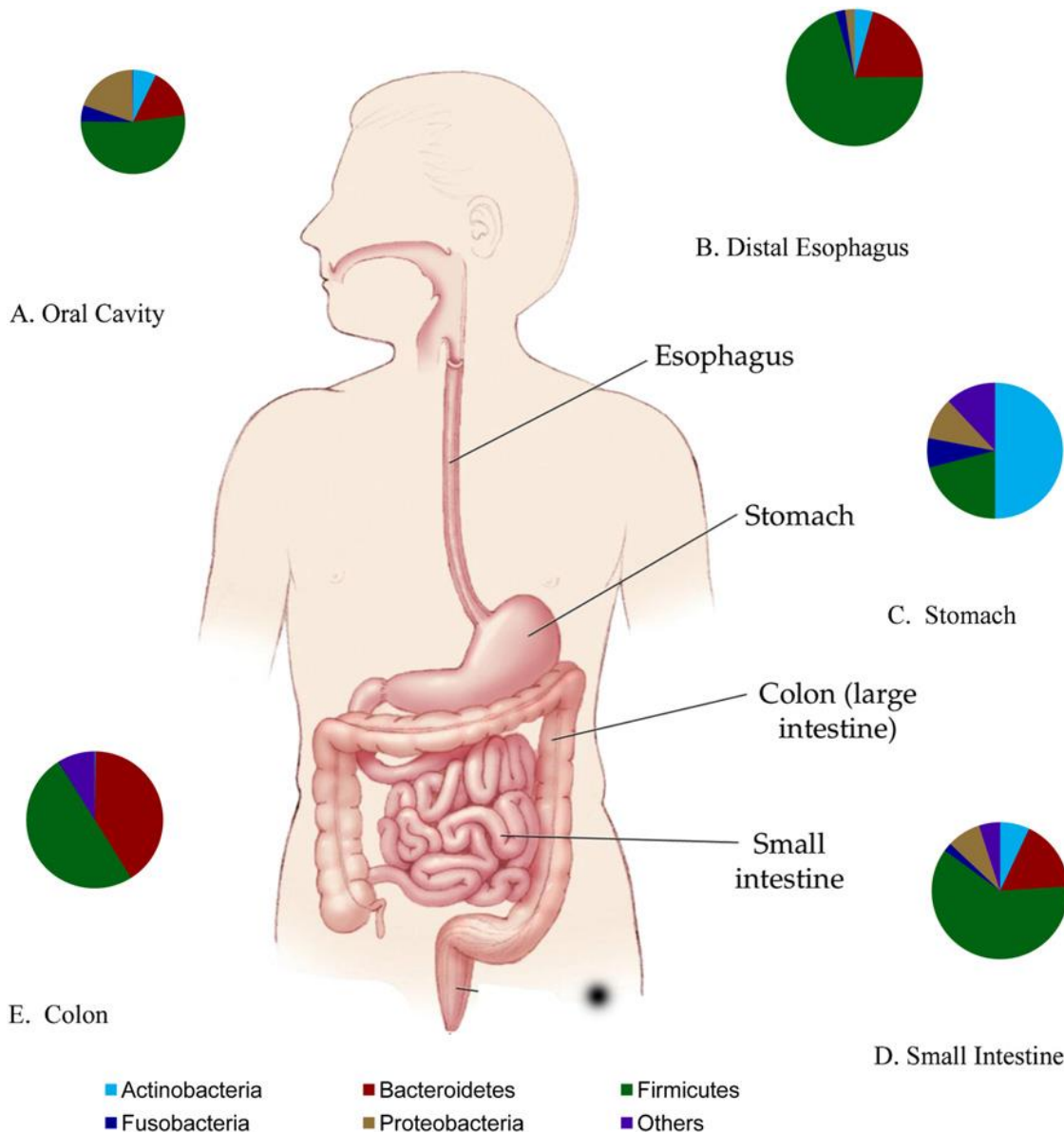
Neonatal antibiotic treatment induces changes in the microbial metabolome

→ promotes obesity

→ and depletes mucosa-associated bacteria crucial for fortifying the epithelial barrier to prevent allergic sensitization to food



# Men & microbes



Dave M. The human gut microbiome: current knowledge, challenges, and future directions. *Transl Res.* 2012;160:246-57



# How does the body recruit a microbiome? .

The microbiota of an individual stabilizes with adulthood

Continues to be shaped by environmental stimuli

The intestinal microbiota of modern urban settings differs from that of rural pre-industrialized societies

→ Diabetes

→ Inflammatory bowel disease

→ Obesity

→ Allergies

→ asthma.

Therefore, → hard to understand

1. how each compartment problem should be addressed
2. by which microorganism
3. at what time in the disease process
4. at what dose
5. for how long.



The idea that supplemental probiotic bacteria can be used to manipulate allergy homeostatic mechanisms remains a hypothesis

Pr

Th

ho

- prev

- treatment.

"Todo es  
e para los  
enen fe".  
go, ayúdame  
que me falte.  
r, aumenta mi fe.  
amén.



**Act of Faith**

O my God, I firmly believe that you are one God in three divine Persons, Father, Son, and Holy Spirit. I believe that your divine Son became man and died for our sins and that he will come to judge the living and the dead. I believe these and all the truths which the Holy Catholic Church teaches because you have revealed them who are eternal truth and wisdom, who can neither deceive nor be deceived. In this faith I intend to live and die. Amen.

Sadler



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→ pathogen-associated molecular patterns (PAMPs)

→ metabolic by-products

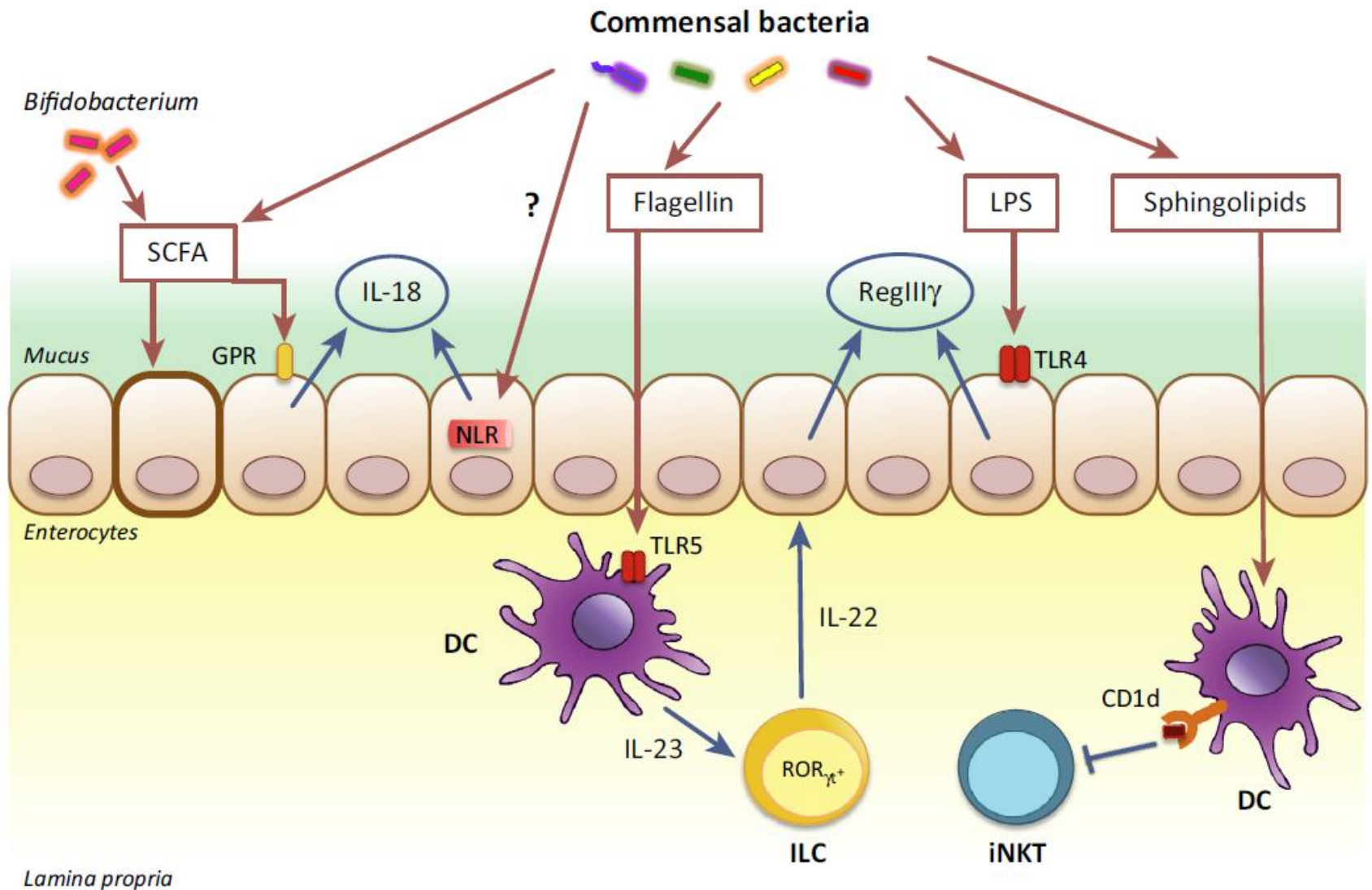
→ PAMPs (LPS) induce antimicrobial peptides (RegIIIg) from IEC

→ flagellin is recognized by CD103+ lamina propria DCs

→ innate lymphoid cells (ILC) secrete IL-22,

→ IL-22 induces antimicrobial peptides (AMPs)

# Microbiota influence on the innate immune responses

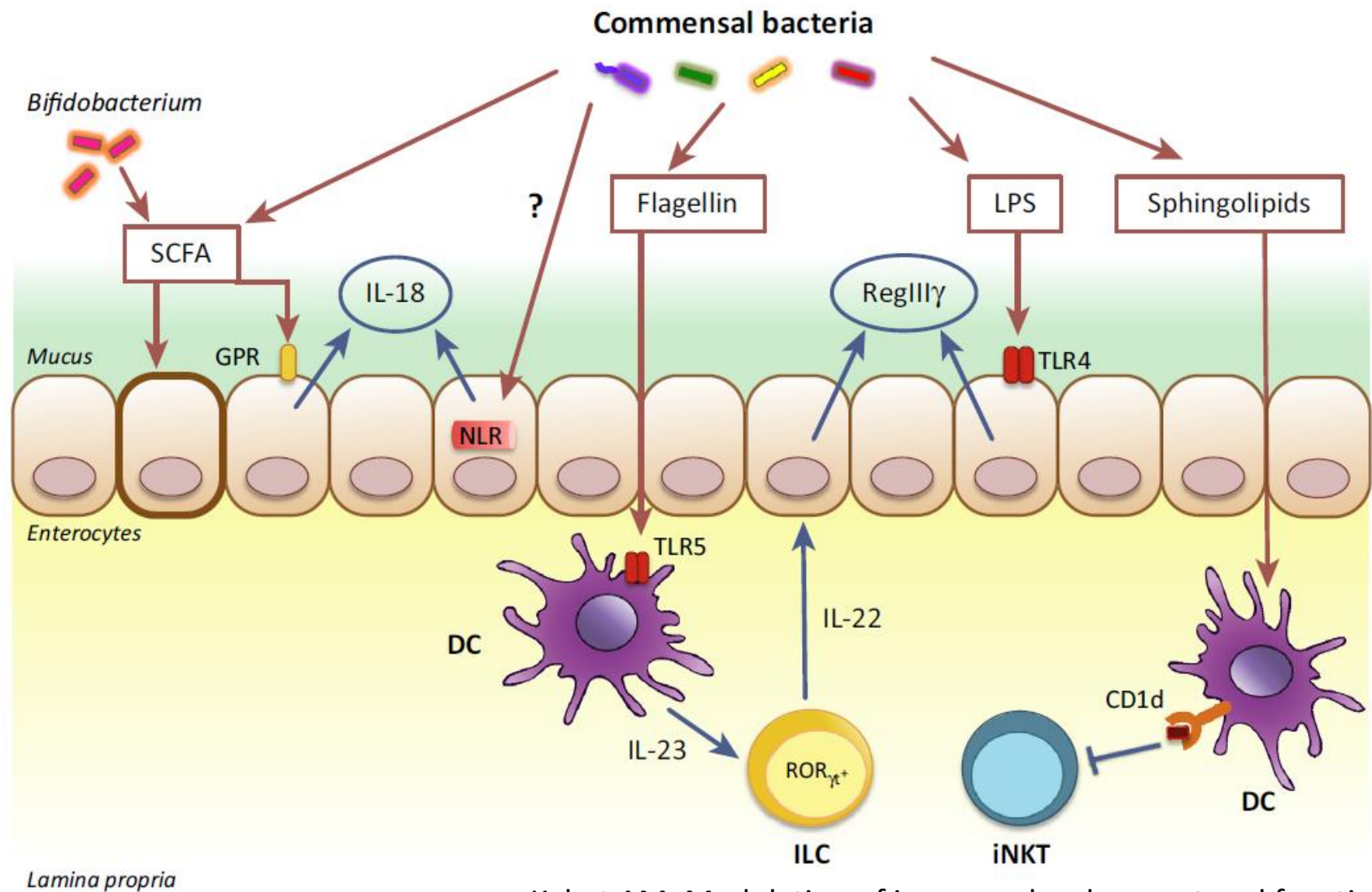


Kabat AM. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014;35:507-17

# Microbiota induce antimicrobial peptides from IEC

- pathogen-associated molecular patterns (PAMPs)
- metabolic by-products
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# Microbiota influence on the innate immune responses

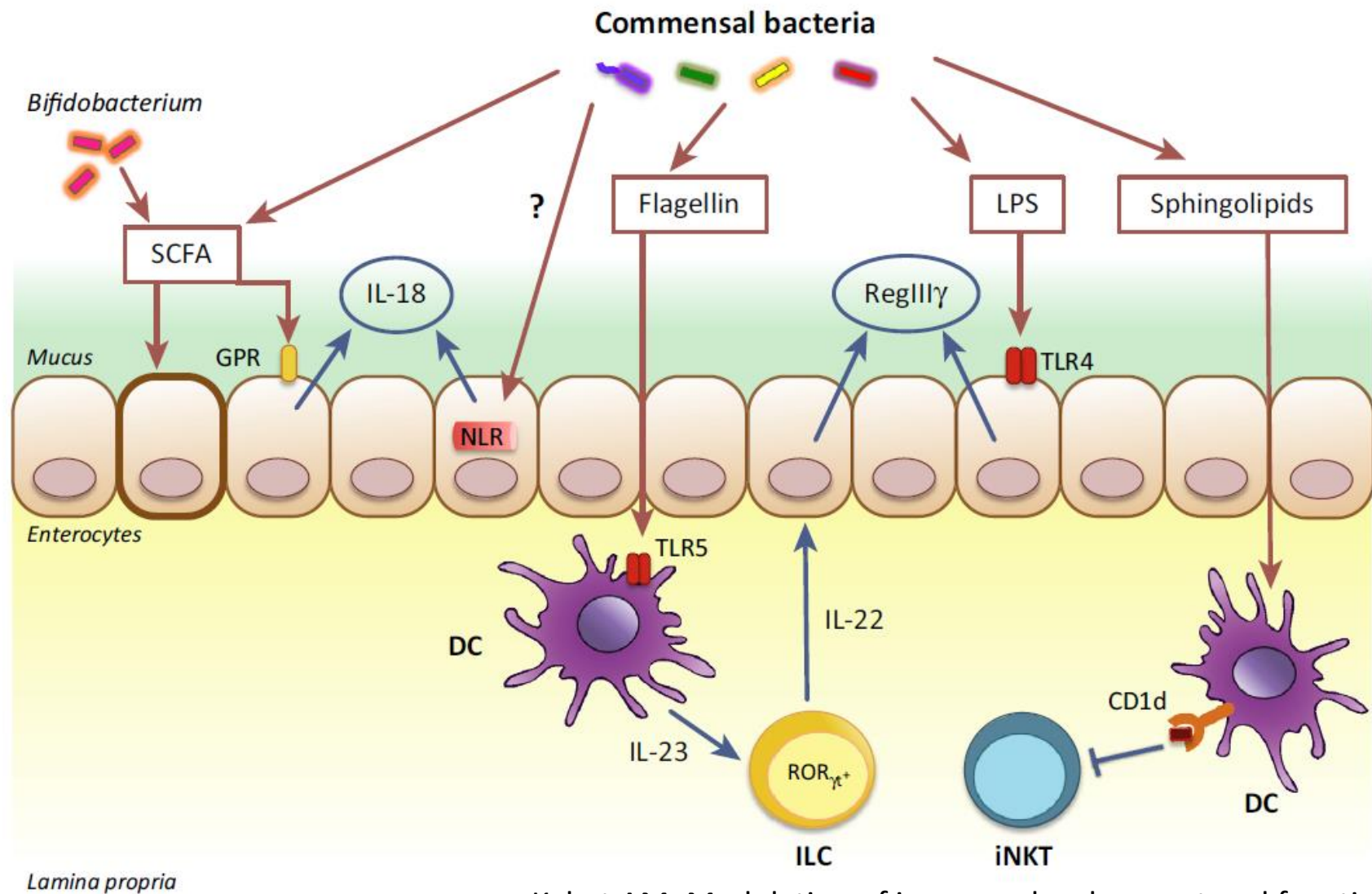


Kabat AM. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014;35:507-17

# Microbiota induces IL-18 from IEC

- Microbiota activates the NOD-like family receptors (NLRs)
- IL-18 production
- SCFA activates the G protein-coupled receptor (GPR)
- IL-18 production
- SCFAs (e.g. acetate by *Bifidobacterium*) induce antiapoptotic response in the IEC
- promote epithelial cell barrier function
- Microbiota-derived sphingolipids presented on CD1d by DC ↓ colonic invariant natural killer T cells (iNKT) development.

# Microbiota influence on the innate immune responses



Kabat AM. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014;35:507-17

# Microbiota influence on the innate immune responses

Microbiota activates the NOD-like family receptors (NLRs)

→ IL-18 production

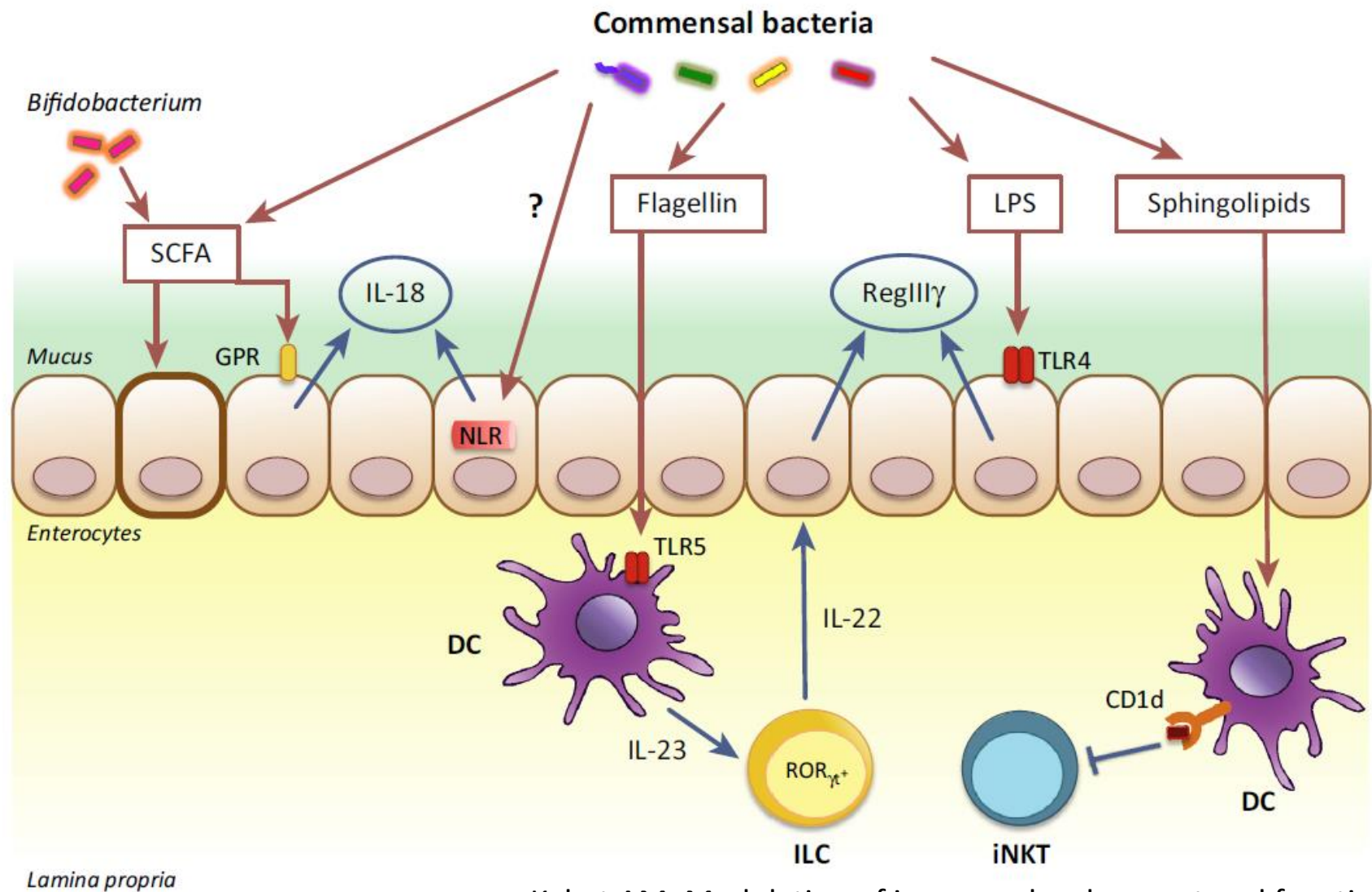
SCFA activates the G protein-coupled receptor (GPR) 109<sup>°</sup>

→ IL-18 production

SCFAs (e.g. acetate by Bifidobacterium) induce antiapoptotic response in the IEC → promote epithelial cell barrier function

Microbiota-derived sphingolipids presented on CD1d by DC → colonic invariant natural killer T cells (iNKT) development.

# Microbiota influence on the innate immune responses



Kabat AM. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014;35:507-17



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# Microbiota induces IgA plasma cells

## Bacterial antigens through M cells

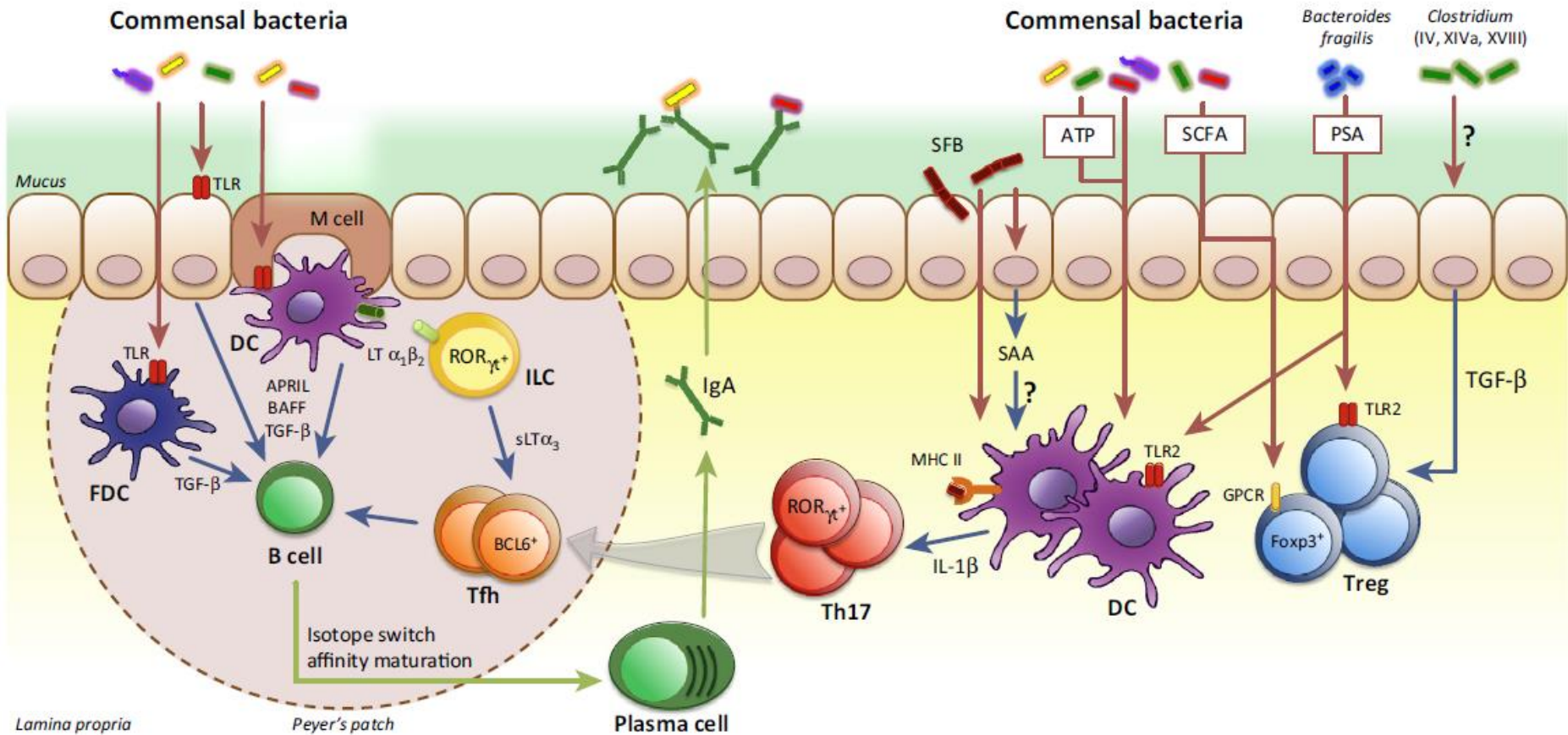
→ B cell activating factor of the TNF family (BAFF) →

→ proliferation-inducing ligand (APRIL) →

→ transforming growth factor (TGF)- $\beta$  →

→ B cells → (Ig) A+ plasma cells

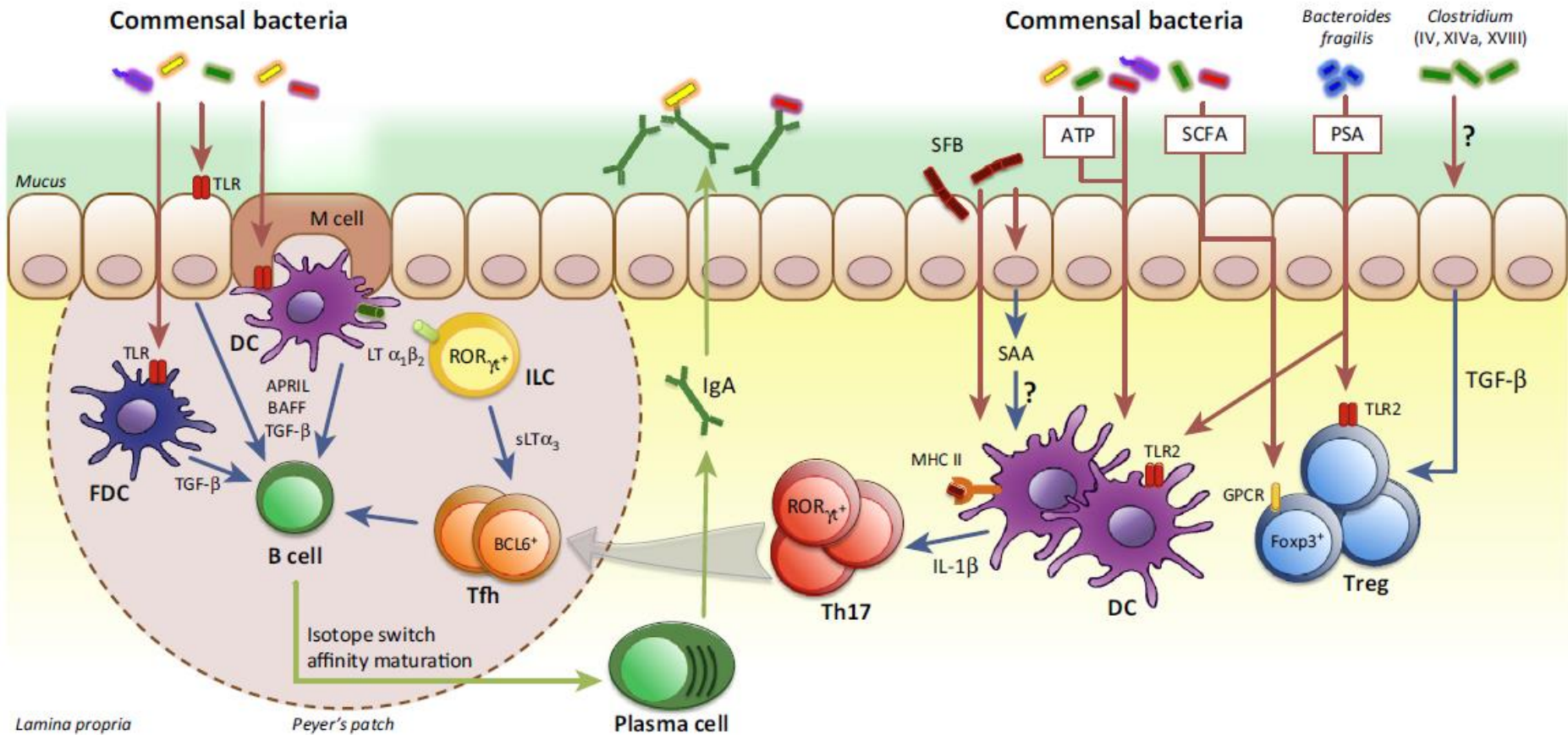
# Microbiota influence on the adaptive responses



# Microbiota drives IgA induction

- follicular dendritic cells (FDC) in the Payer's patches → TGF- $\beta$  → differentiation of B cells into IgA+ plasma cells
- innate lymphoid cells (ILC), → membrane bound lymphotoxin (LTa1b2) → DC → T cell-independent IgA induction.
- soluble form of ILC-derived lymphotoxin (sLTa3) → T follicular helper (Tfh) population → T cell homing to the lamina propria → T cell-dependent IgA induction

# Microbiota influence on the adaptive responses

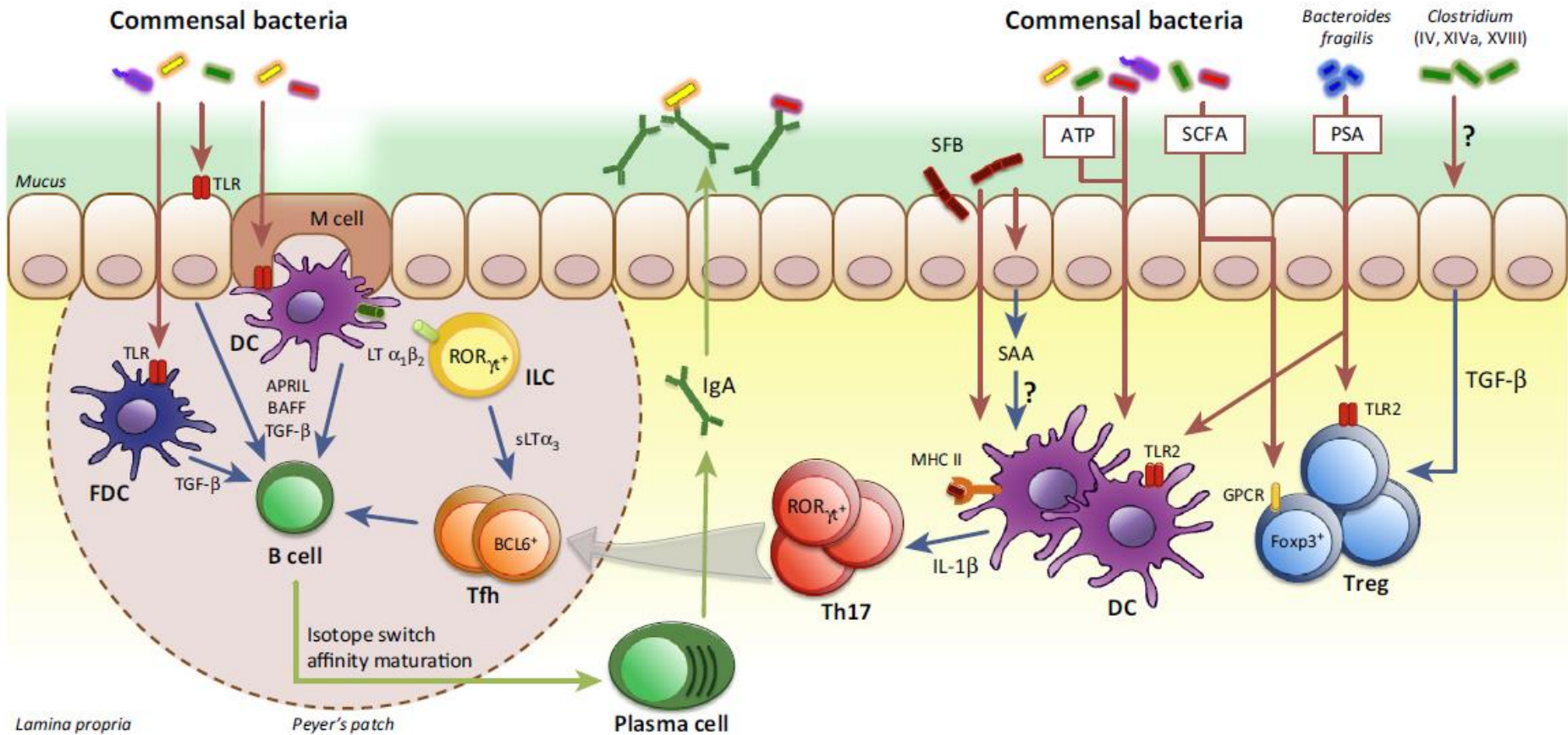


# Microbiota promotes Th17 differentiation

- Segmented filamentous bacteria (SFB), in close contact with IEC,
  - serum amyloid A (SAA) → DC → T helper (Th) 17.
- Presentation of SFB antigens by DC on major histocompatibility complex (MHC) → Th17 induction
- interleukin (IL)-1 $\beta$  production by mononuclear phagocytes → promotes Th17 differentiation
- ATP produced by certain commensals → DCs and → induction of Th17 cells.

Th17 cells can differentiate into Tfh cells → contribute to IgA production.

# Microbiota influence on the adaptive responses



Kabat AM. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014;35:507-17

Polysaccharide A (PSA) produced by *Bacteroides fragilis*

→ toll-like receptor (TLR) 2 → Treg differentiation

→ DCs → Treg differentiation

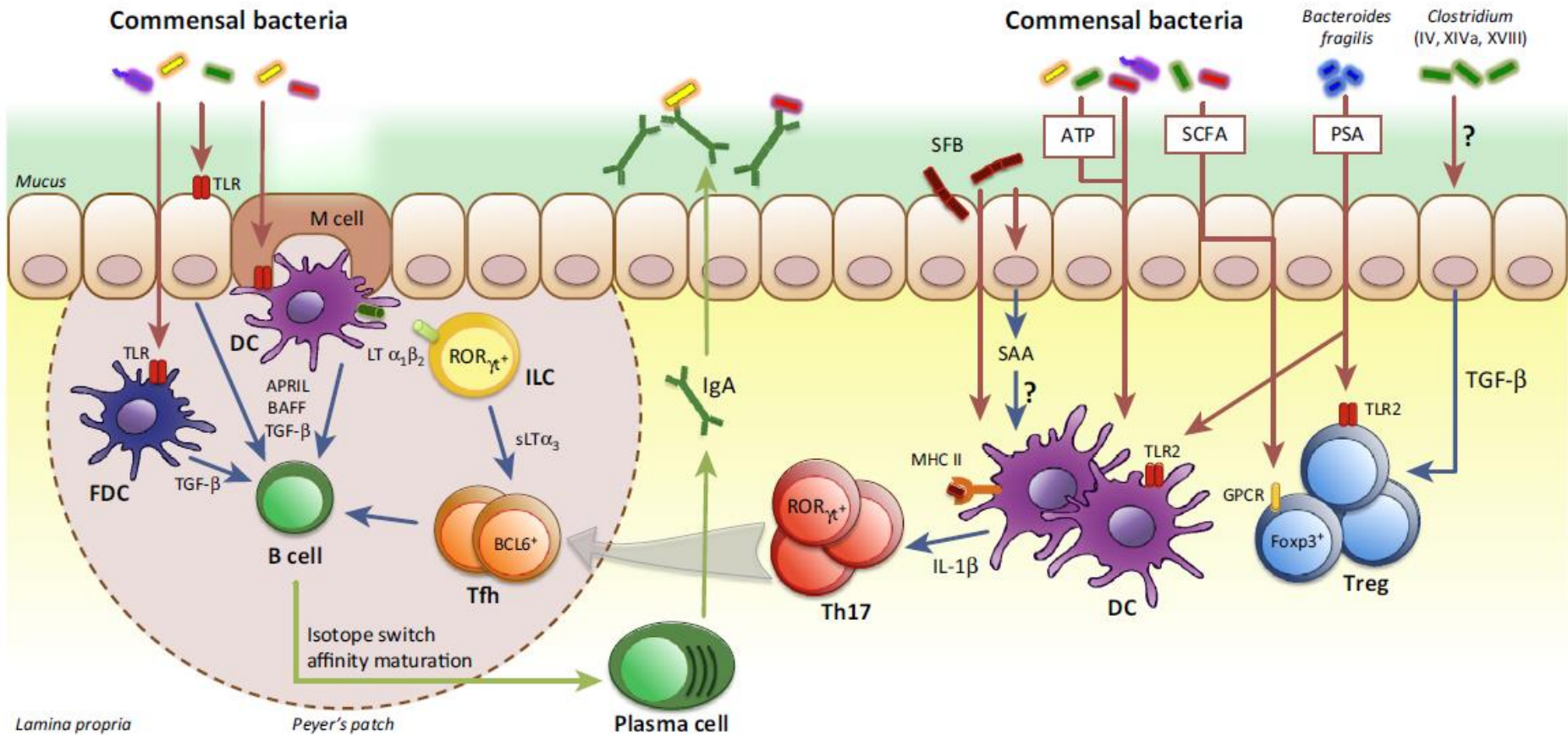
SCFA

→ via G protein-coupled receptor (GPCR) → Treg cell generation

→ via IEC → Treg cell generation

Clostridium species (clusters IV, XIVa, and XVIII) → TGF- $\beta$  production  
in IEC, → Treg differentiation in the colon.

# Microbiota influence on the adaptive responses





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**CARTE DE LA NAVIGATION  
DES ARGONAUTES  
DU MONDE PRIMITIF**

*suivant les Periples  
de TIMÉE, d'HÉCATÉE,  
d'APOLLONIS et d'ÉRYTHRE  
Pour servir à l'histoire de la  
Grèce.*

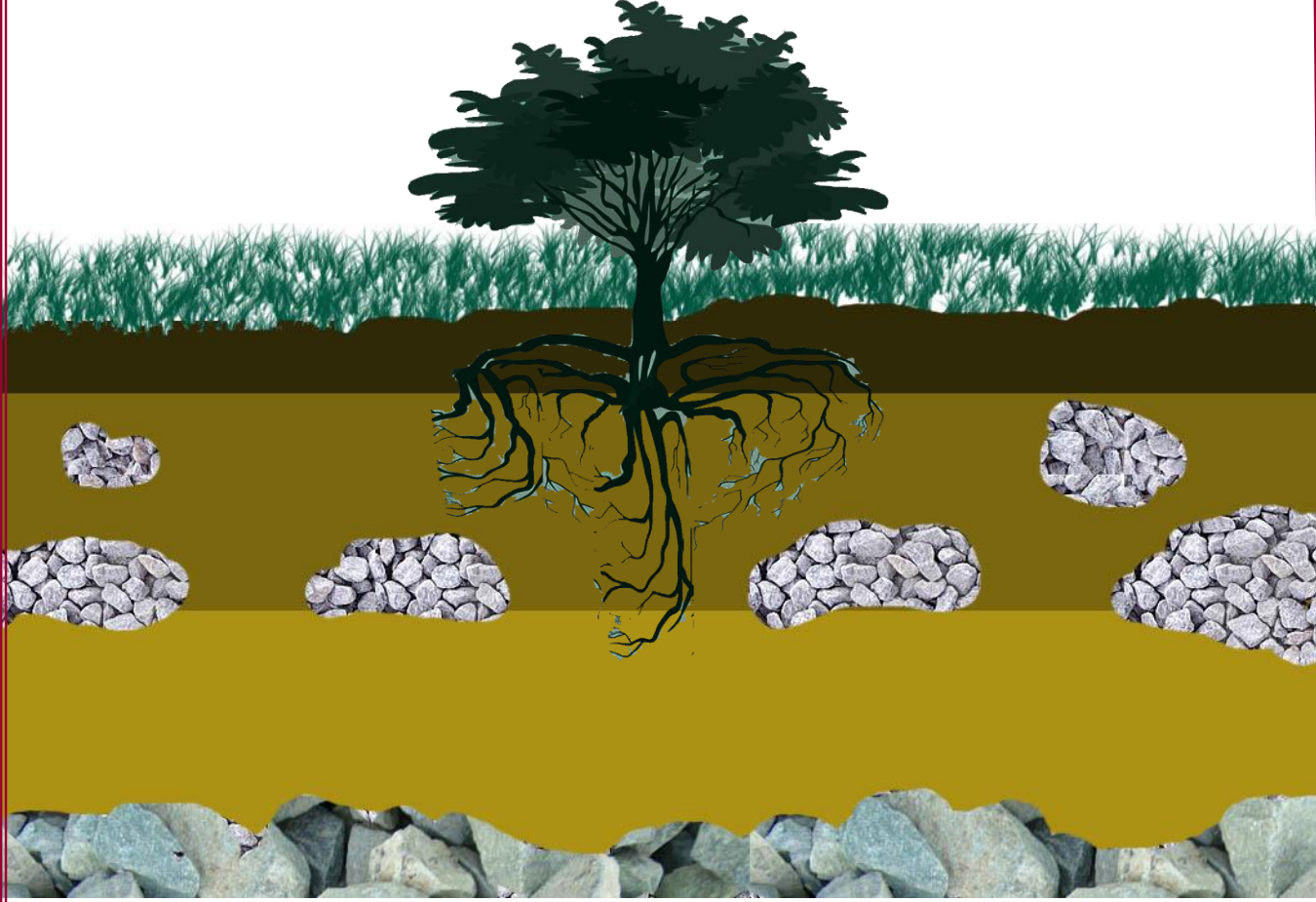




*Man's White Elephant*



Trends in hospital admission rates  
of food allergy by age (1990-2003)

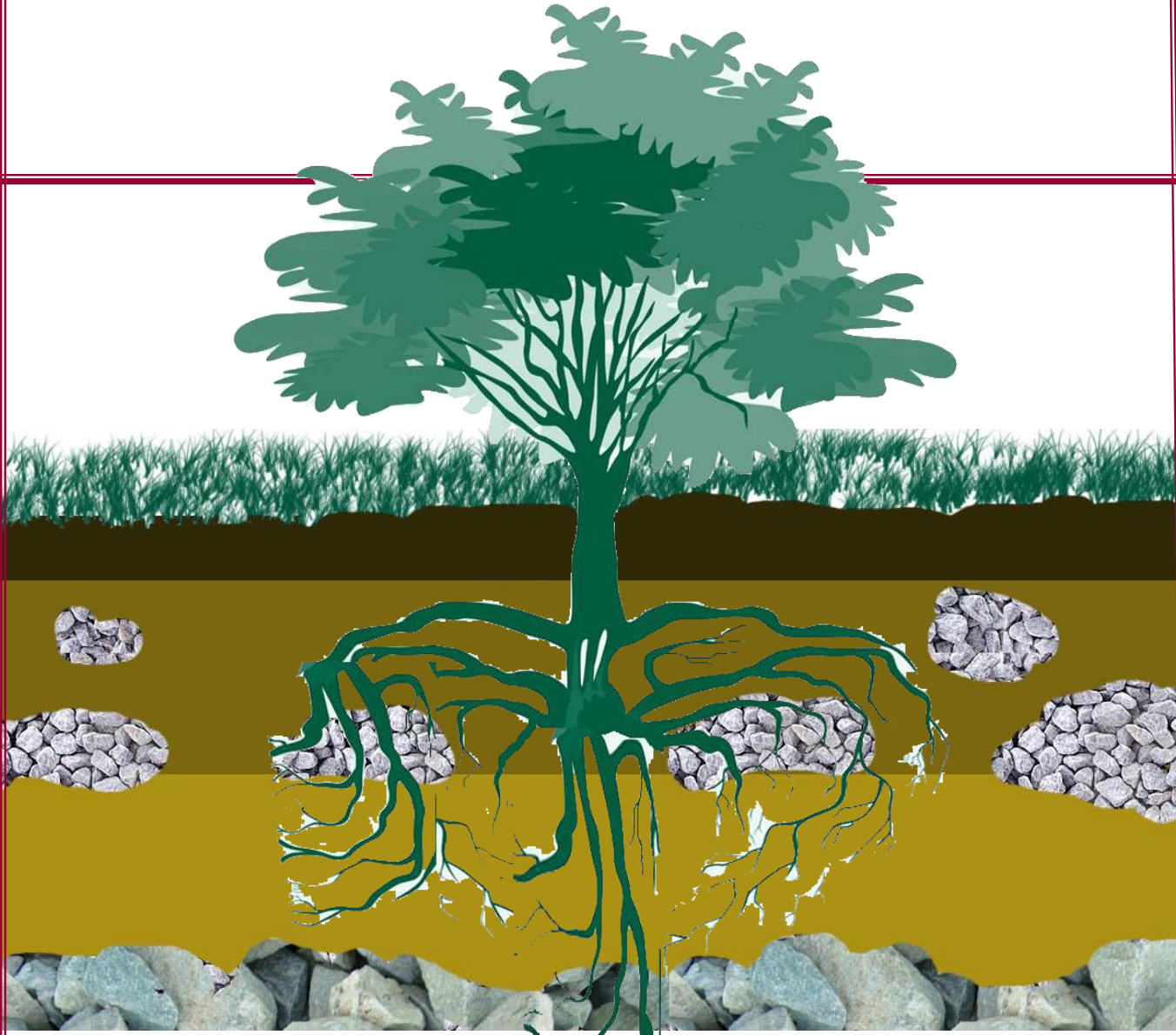


1990 – 20:1.000.000

Gupta R. Time trends in allergic disorders in the UK.  
Thorax 2007; 62:91-6



Trends in hospital admission rates  
of food allergy by age (1990-2003)



2003 – 120:1.000.000

Gupta R. Time trends in allergic disorders in the UK.  
Thorax 2007; 62:91-6

# NUTRIRE LA VITA

Il pasto che educa e cura

Bambino Gesù  
OSPEDALE PEDIATRICO



Official Partner

SAVE THE DATE  
31 MAGGIO 2015 - ORE 9:30  
Conference Center di EXPO (zona sud centrale)  
EXPO 2015 - Milano



*L'Ospedale Pediatrico Bambino Gesù è lieta di invitarla a*

## **ALLERGEAT**

L'ALLERGIA TRA LE MALATTIE NON TRASMISSIBILI:  
IL PARADOSSO DELL'ABBONDANZA.

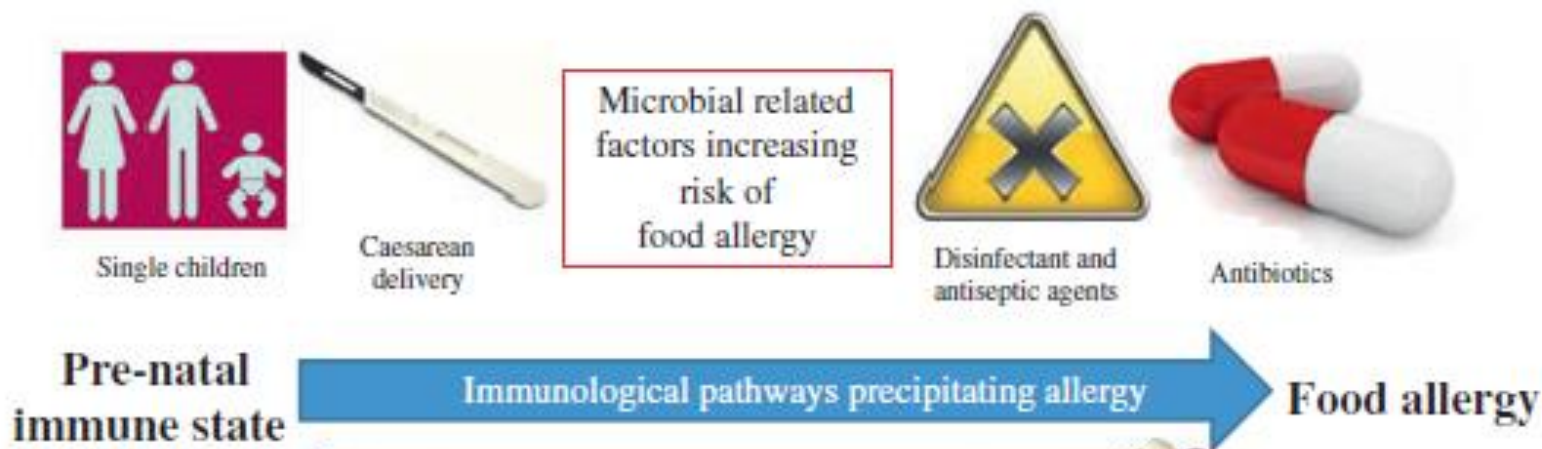
Allergeat vuole lanciare una riflessione sul tema della disegualianza come generatrice di malattie. Le malattie allergiche colpiscono le popolazioni più ricche. Cosa c'è nel loro stile di vita che genera malattia? L'alimentazione? L'igiene? Lo svezzamento?

Rifletteremo su questi aspetti, alla luce delle recenti ricerche che indicano uno stile di svezzamento naturale come possibile strumento di prevenzione.

INFO  
[convegni@biomedia.net](mailto:convegni@biomedia.net)



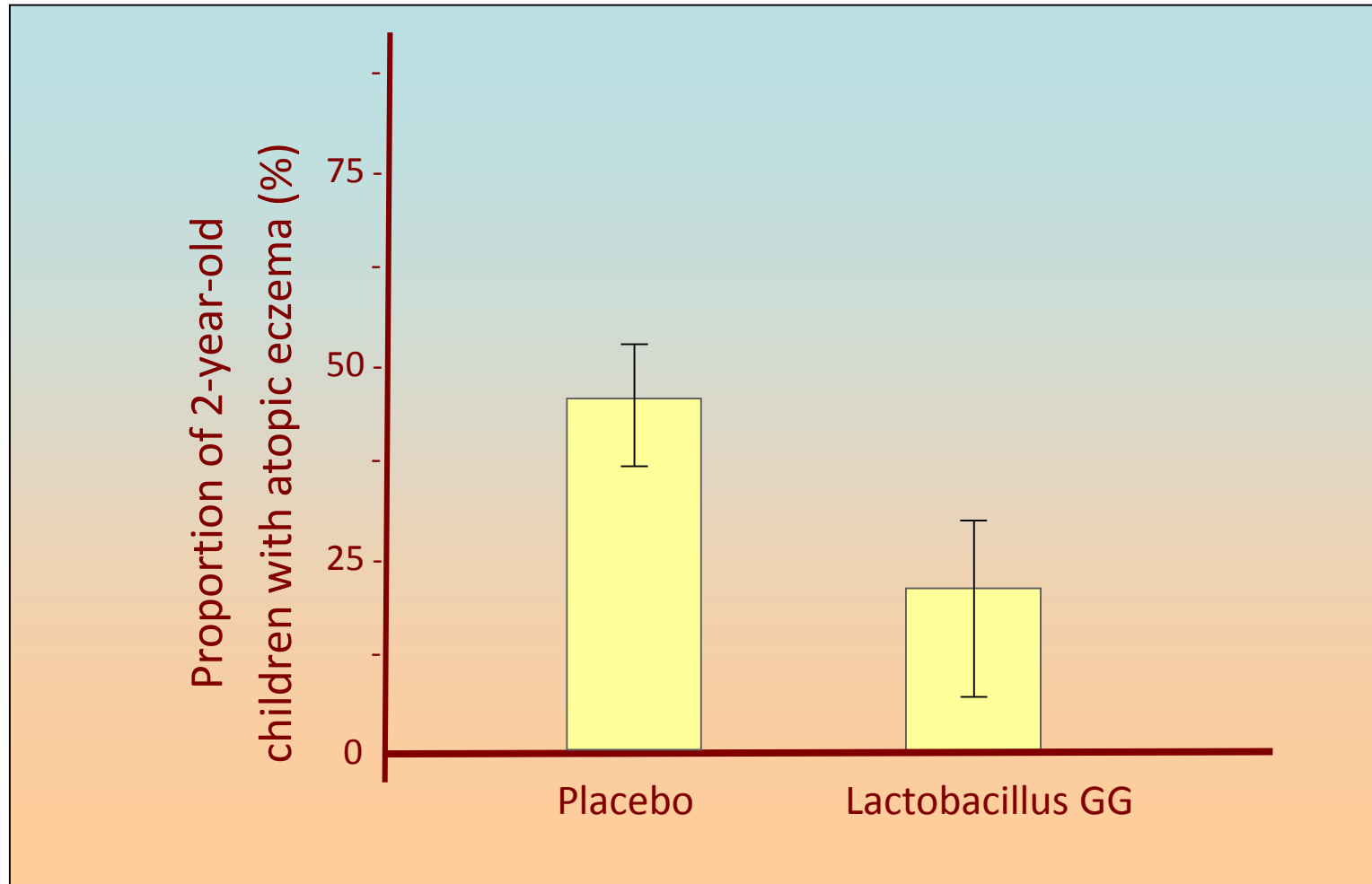
# Proposals for reducing the risk of food allergy



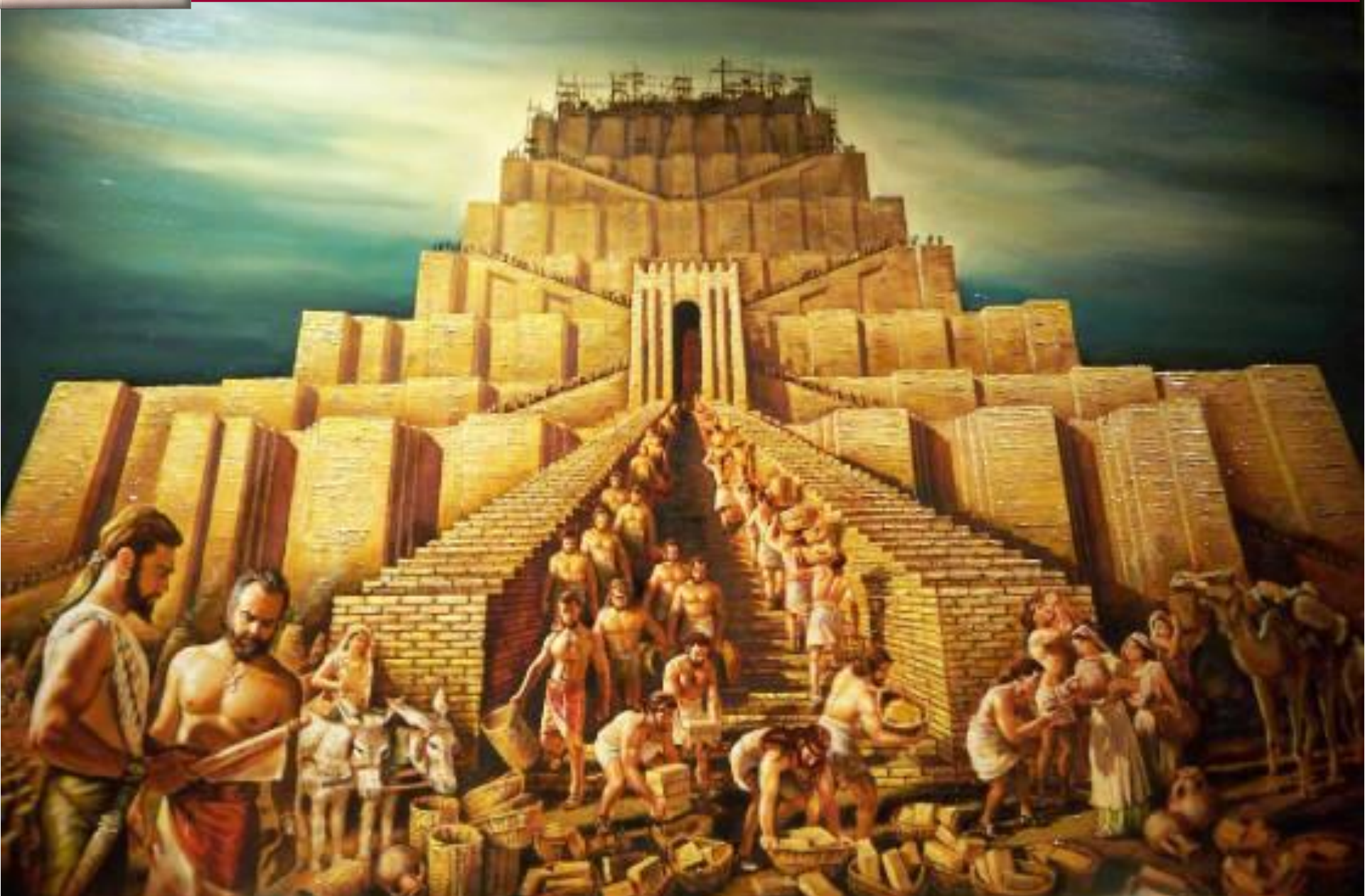
Marrs T. Is there an association between microbial exposure and food allergy? A systematic review. *Pediatr Allergy Immunol.* 2013;24:311-320



## Reduction of AD by 50%



Kalliomaki M. Probiotics and prevention of atopic disease: a randomised placebo controlled trial. *Lancet* 2001; 357:1076-79





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POSITION ARTICLE AND GUIDELINES

Open Access

## World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics

Alessandro Fiocchi<sup>1†</sup>, Ruby Pawankar<sup>2†</sup>, Carlos Cuello-Garcia<sup>3,4</sup>, Kangmo Ahn<sup>5</sup>, Suleiman Al-Hammadi<sup>6</sup>, Arnav Agarwal<sup>3,7</sup>, Kirsten Beyer<sup>8</sup>, Wesley Burks<sup>9</sup>, Giorgio W Canonica<sup>10</sup>, Motohiro Ebisawa<sup>11</sup>, Shreyas Gandhi<sup>3,7</sup>, Rose Kamenwa<sup>12</sup>, Bee Wah Lee<sup>13</sup>, Haiqi Li<sup>14</sup>, Susan Prescott<sup>15</sup>, John J Riva<sup>16</sup>, Lanny Rosenwasser<sup>17</sup>, Hugh Sampson<sup>18</sup>, Michael Spigler<sup>19</sup>, Luigi Terracciano<sup>20</sup>, Andrea Vereda-Ortiz<sup>22</sup>, Susan Waserman<sup>21</sup>, Juan José Yepes-Nuñez<sup>3</sup>, Jan L Brożek<sup>3,21\*</sup> and Holger J Schünemann<sup>3,21</sup>

### Abstract

**Background:** Prevalence of allergic diseases in infants, whose parents and siblings do not have allergy, is approximately 10% and reaches 20–30% in those with an allergic first-degree relative. Intestinal microbiota may modulate immunologic and inflammatory systemic responses and, thus, influence development of sensitization and allergy. Probiotics have been reported to modulate immune responses and their supplementation has been proposed as a preventive intervention.

**Objective:** The World Allergy Organization (WAO) convened a guideline panel to develop evidence-based recommendations about the use of probiotics in the prevention of allergy.

**Methods:** We identified the most relevant clinical questions and performed a systematic review of randomized controlled trials of probiotics for the prevention of allergy. We followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to develop recommendations. We searched for and reviewed the evidence about health effects, patient values and preferences, and resource use (up to November 2014). We followed the GRADE evidence-to-decision framework to develop recommendations.

**Results:** Currently available evidence does not indicate that probiotic supplementation reduces the risk of developing allergy in children. However, considering all critical outcomes in this context, the WAO guideline panel determined that there is a likely net benefit from using probiotics resulting primarily from prevention of eczema. The WAO guideline panel suggests: a) using probiotics in pregnant women at high risk for having an allergic child; b) using probiotics in women who breastfeed infants at high risk of developing allergy; and c) using probiotics in infants at high risk of developing allergy. All recommendations are conditional and supported by very low quality evidence.

**Conclusions:** WAO recommendations about probiotic supplementation for prevention of allergy are intended to support parents, clinicians and other health care professionals in their decisions whether to use probiotics in pregnancy and during breastfeeding, and whether to give them to infants.

**Keywords:** Allergy, Prevention, Probiotics, Practice guidelines, GRADE

\* Correspondence: brozekj@mcmaster.ca  
 †Equal contributors



High risk for allergy in a child =

- a biological parent or sibling with existing or history of
  - allergic rhinitis,
  - asthma,
  - eczema,
  - food allergy.





The WAO guideline panel suggests **using** probiotics in pregnant women at **high risk for allergy in their children**

Considering all critical outcomes → net benefit resulting primarily from prevention of eczema



WAO / McMaster working group.  
GuideLines for Allergic Disease Prevention.  
WAO Journal, 2015; 8:3, 28 January 2015



The WAO guideline panel suggests **using** probiotics in women who breastfeed infants at **high risk of developing allergy**

Considering all critical outcomes → net benefit resulting primarily from prevention of eczema





The WAO guideline panel suggests **using** probiotics in infants at **high risk of developing allergies**

Considering all critical outcomes → net benefit resulting primarily from prevention of eczema





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The deep changes in lifestyle deeply influences the human microbiota

These changes impact on immune system

... diabetes

.... IBD

... celiac disease

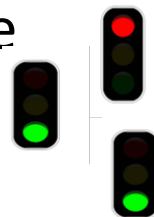
... autoimmune diseases

... obesity



Current recommendations for allergy prevention:

1. avoidance of cigarette smoke
2. promotion of breastfeeding
3. use of hydrolysed formula



GLAD-p recommendations on probiotics:

1. in pregnant women
2. in breastfeeding women
3. in breastfed infants



**NUTRIRE LA VITA**  
Il pasto che educa e cura

Bambino Gesù  
OSPEDALE PEDIATRICO



EXPO  
MILANO  
2015

**ALLERGEAT**  
L'ALLERGIA TRA LE MALATTIE NON TRASMISSIBILI  
IL PARADOSSO DELL'ABBONDANZA

**31 MAGGIO 2015**  
ORE 9:30

CONFERENCE CENTRE DI EXPO  
(ZONA SUD CENTRALE)  
EXPO 2015 - MILANO



Allergeat vuole lanciare una riflessione sul tema della disuguaglianza come generatrice di malattie. Le malattie allergiche colpiscono le popolazioni più ricche. Cosa c'è nel loro stile di vita che genera malattia? L'alimentazione? L'igiene? Lo svezzamento?

Rifletteremo su questi aspetti, alla luce delle recenti ricerche che indicano uno stile di svezzamento naturale come possibile strumento di prevenzione.

**COMITATO D'ONORE**

**Mariella Enoc**  
Presidente Ospedale Pediatrico Bambino Gesù

**Bruno Dallapiccola**  
Direttore Scientifico Ospedale Pediatrico Bambino Gesù

**Massimiliano Raponi**  
Direttore Sanitario Ospedale Pediatrico Bambino Gesù

**Alberto G. Ugazio**  
Direttore Dipartimento Medicina Pediatrica  
Ospedale Pediatrico Bambino Gesù

**PROGRAMMA**

**SALUTO BENVENUTO**

**Mariella Enoc**  
Presidente Ospedale Pediatrico Bambino Gesù

**9.30 SESSIONE 1  
NON URGENZE, MA PRIORITÀ**

Presidente: Ruby Pawankar, Tokyo - Giappone  
Moderatori: Alberto Martelli, Gorbagnate - Italia  
Elvira Verduci, Milano - Italia

**L'epidemia di allergia nel mondo sviluppato**  
Giovanni Passalacqua, Genova - Italia

**Trends della malattia allergica  
nei paesi in via di sviluppo**  
Rose Kamenwa, Nairobi - Kenia

**Sviluppo delle malattie allergiche  
nelle popolazioni migranti**  
Carlo Lombardi, Brescia - Italia

**11.00 LETTURA SPECIALE**  
Presidente: Bruno Dallapiccola, Roma - Italia

**Le malattie non comunicabili.  
La sfida del 21° secolo**  
Francesco Biasi, Milano - Italia

**11.30 SESSIONE 2  
LA PREVENZIONE DELLE MALATTIE  
ALLERGICHE: NUOVE PROSPETTIVE**

Presidente: Giovanni Corsello, Palermo - Italia  
Moderatori: Susanna Esposito, Milano - Italia  
Marzia Duse, Roma - Italia

**Prevenzione nutrizionale delle allergie  
alimentari prima delle linee guida GLAD - P**  
Hania Szajewska, Varsavia - Polonia

**Il progetto GLAD - P**  
Ruby Pawankar, Tokyo - Giappone

**Prevenire l'allergia con lo svezzamento?**  
Alessandro Fiochi, Roma - Italia

**13.30 LETTURA SPECIALE**  
Presidente: Marcello Giovannini, Milano - Italia

**Gli interventi nutrizionali  
per la prevenzione delle allergie sono  
esportabili per la prevenzione di altre  
malattie non comunicabili?**  
Valerio Nobili, Roma, Italia

Allergy Prevention  
can be achieved  
without giving up  
the benefits of civilization