

THE PROBIOTICS AGE: FROM E. METCHNIKOFF TO THE HUMAN MICROBIOME PROJECT.

INFLAMMATIONS, INFECTIONS, FUNCTIONAL DISEASES

Interactions between gut microbiota and intestinal epithelium

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"...are we more microbe than man?"

Nature Reviews Gastroenterol Hepatol 9, 555, 2012

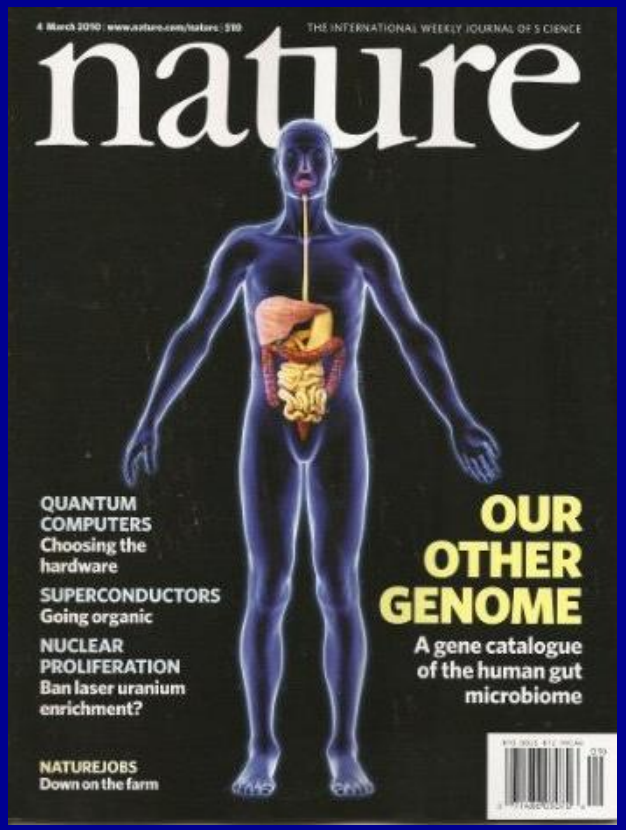
INSIGHT FEATURE

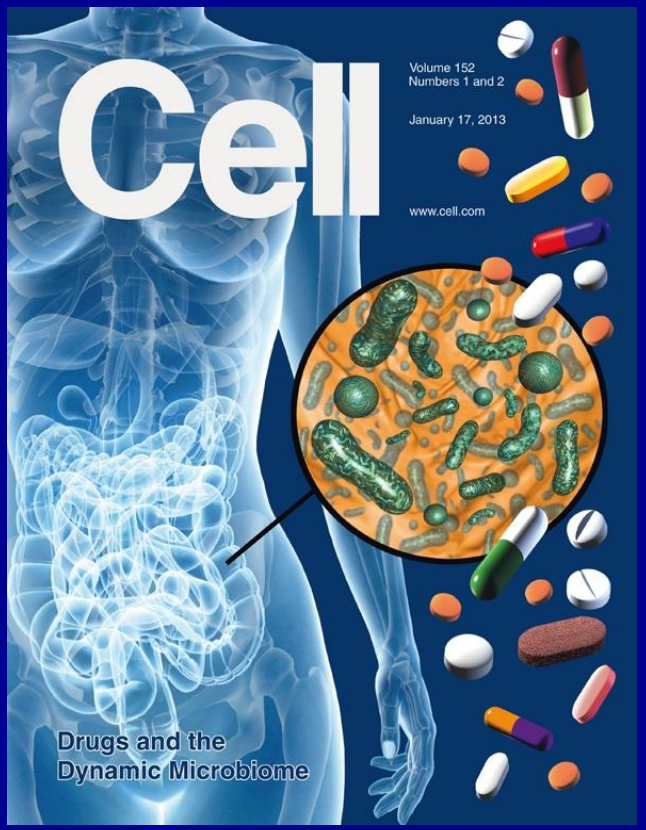
NATURE|Vol 449|18 October 2007|**doi:10.1038/nature06244**

The Human Microbiome Project

Peter J. Turnbaugh, Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight & Jeffrey I. Gordon

A strategy to understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease.


4 March 2010 : www.nature.com/nature : $10
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
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YOU ARE ONLY 10% HUMAN !!!



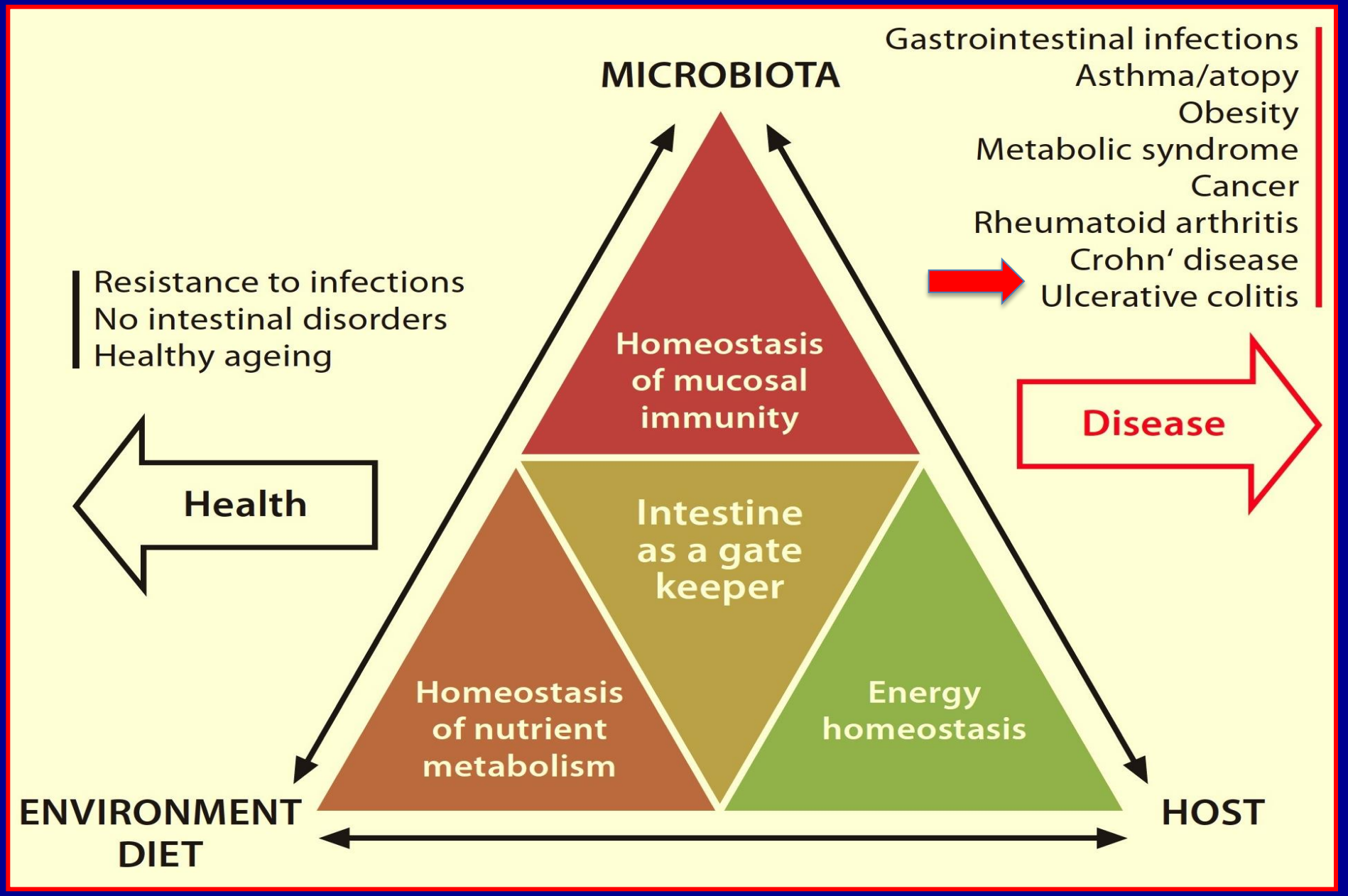
$= 10^{12}$ to 10^{13} cells



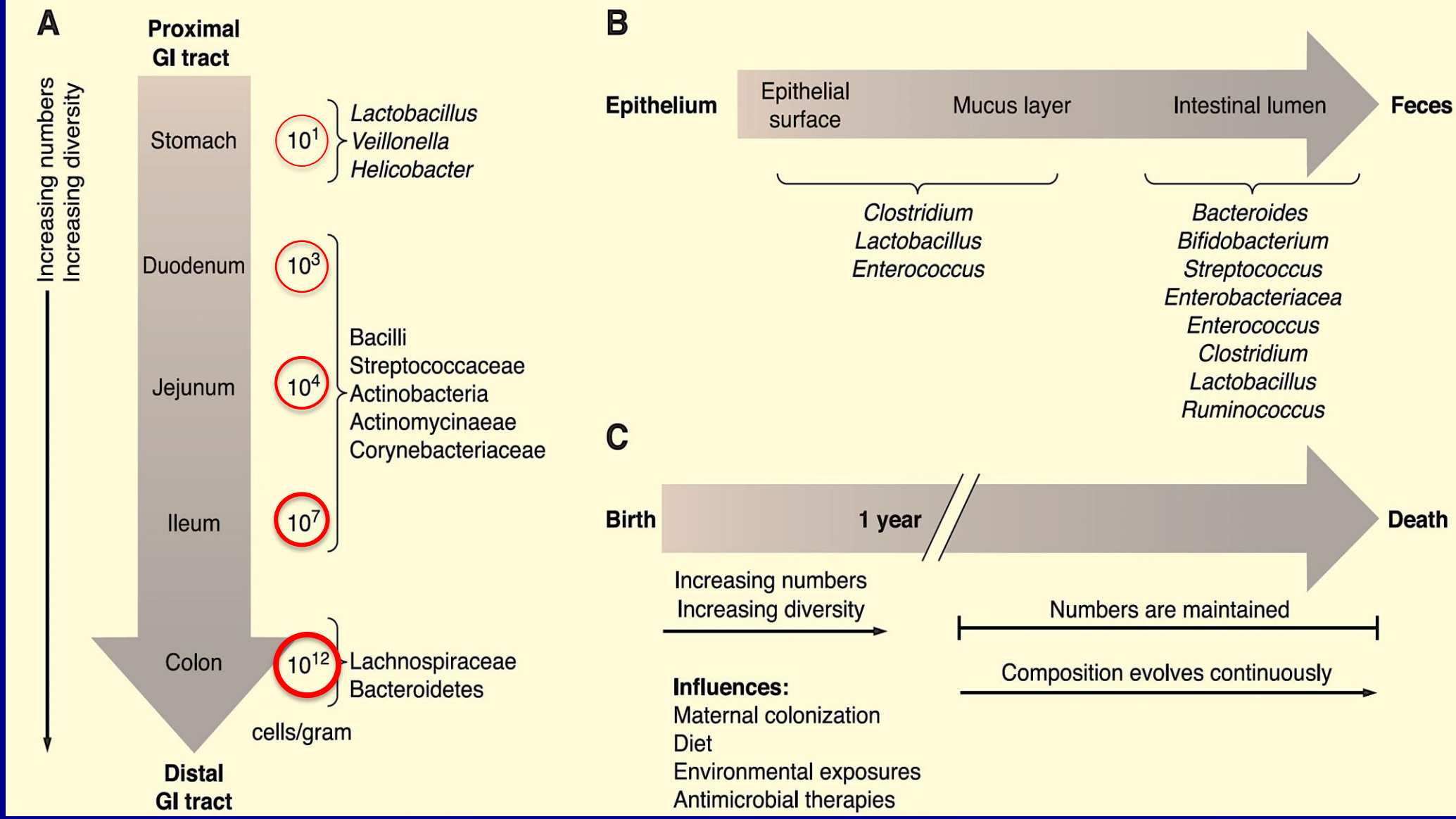


$= 10^{13}$ to 10^{14} intestinal bacteria

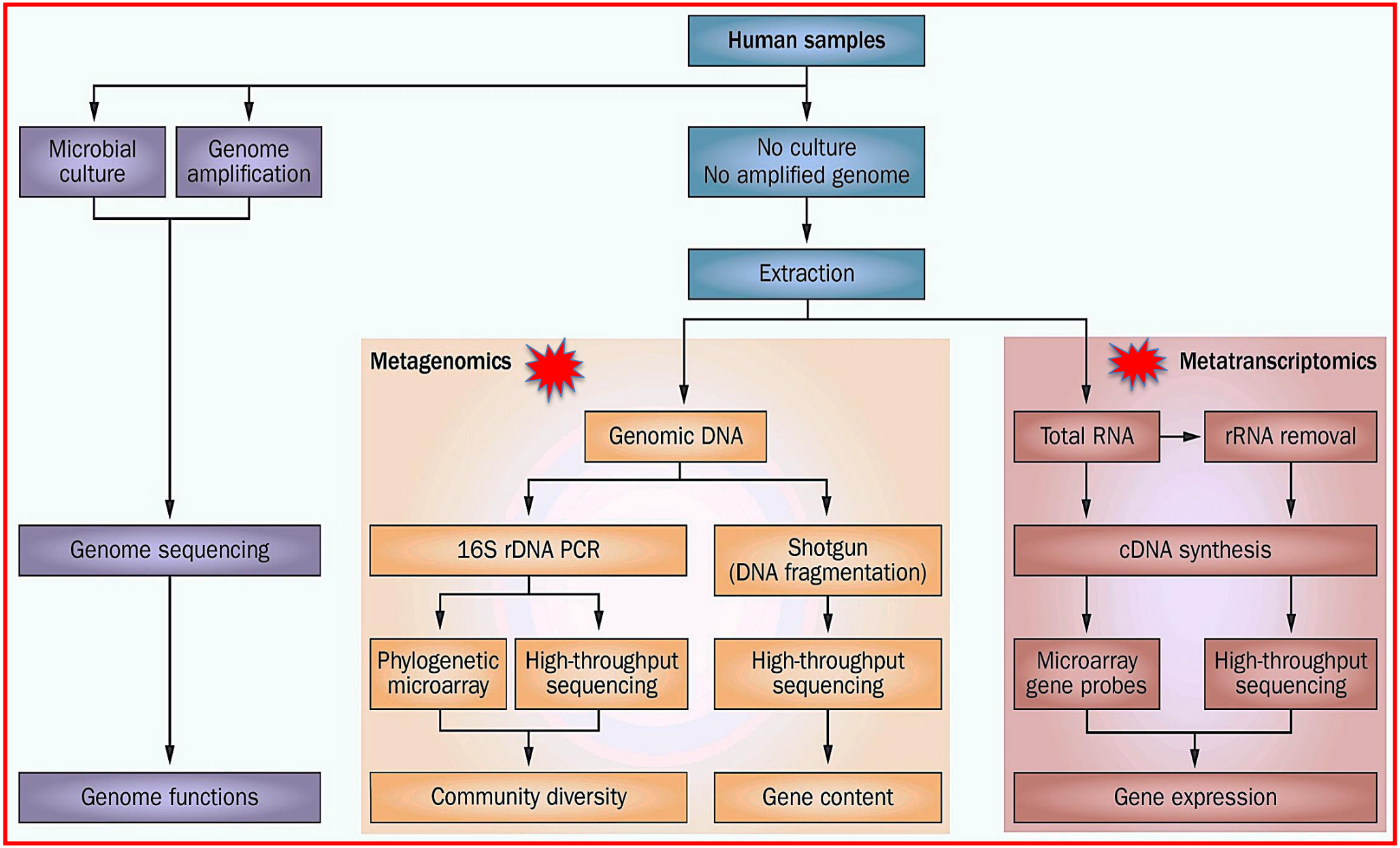
CROSS-TALK BETWEEN HOST CELLS, RESIDENT MICROBIOTA AND ENVIRONMENT



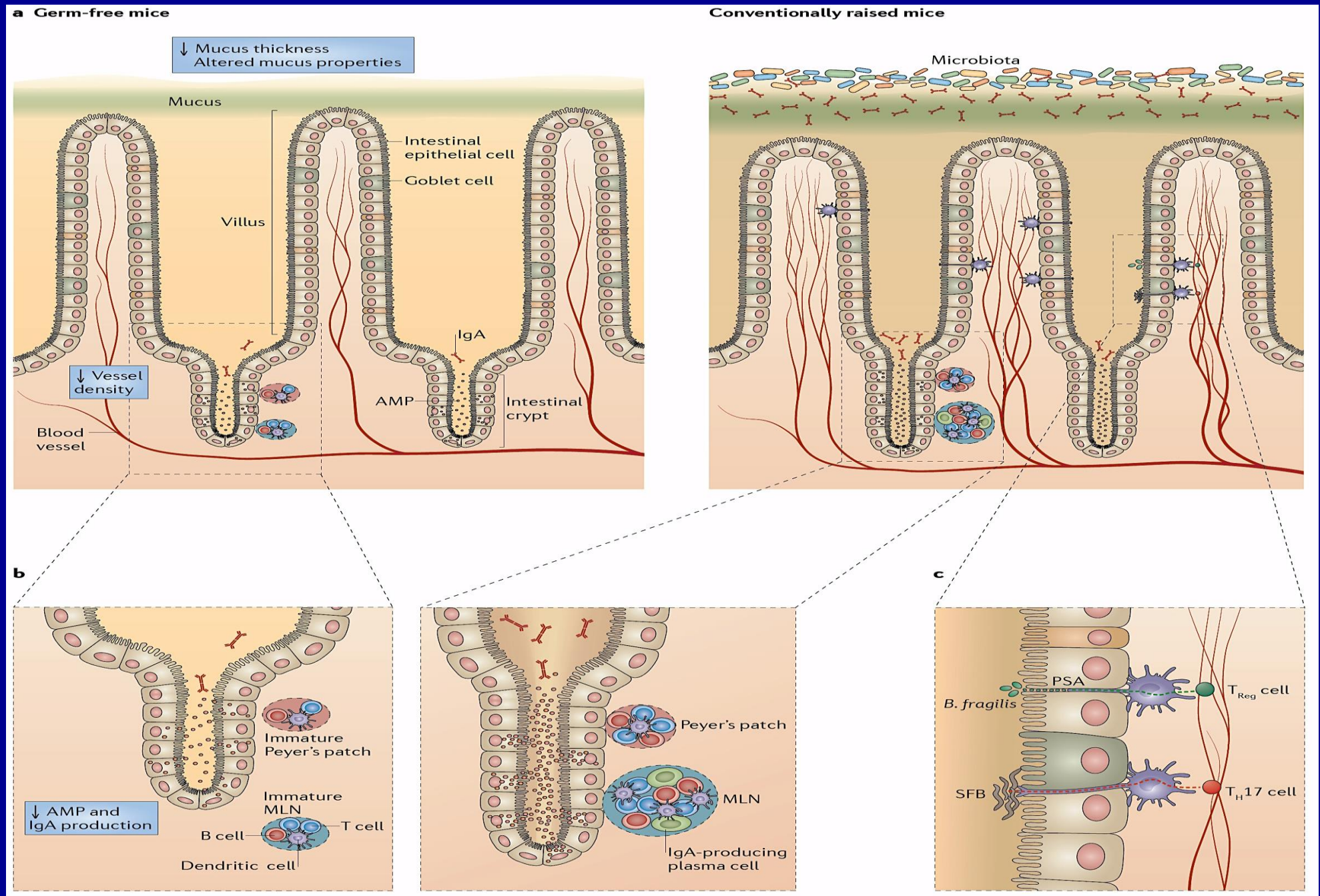
SPATIAL AND TEMPORAL ASPECTS OF INTESTINAL MICROBIOTA COMPOSITION



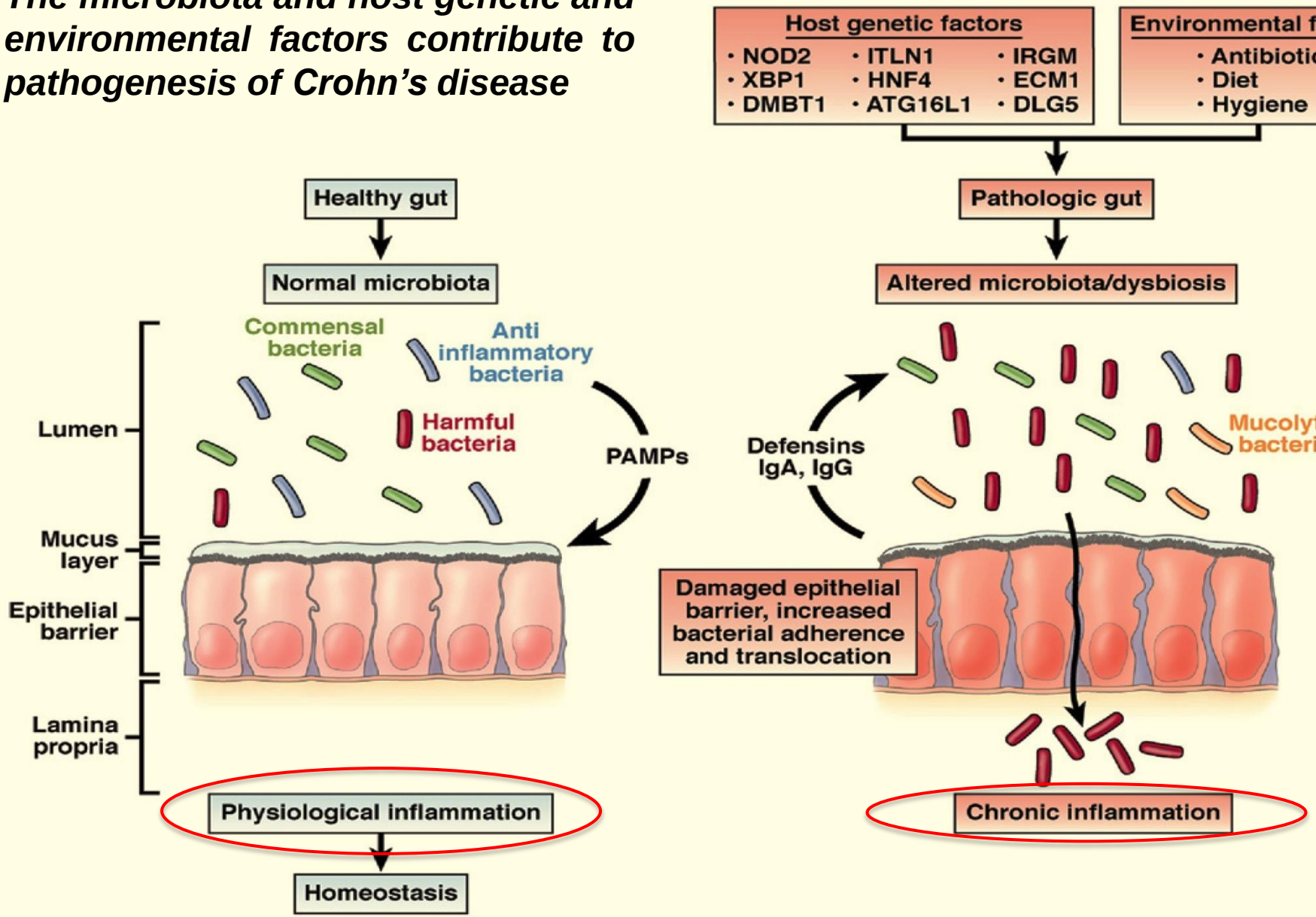
Technologies to investigate the gut microbiota



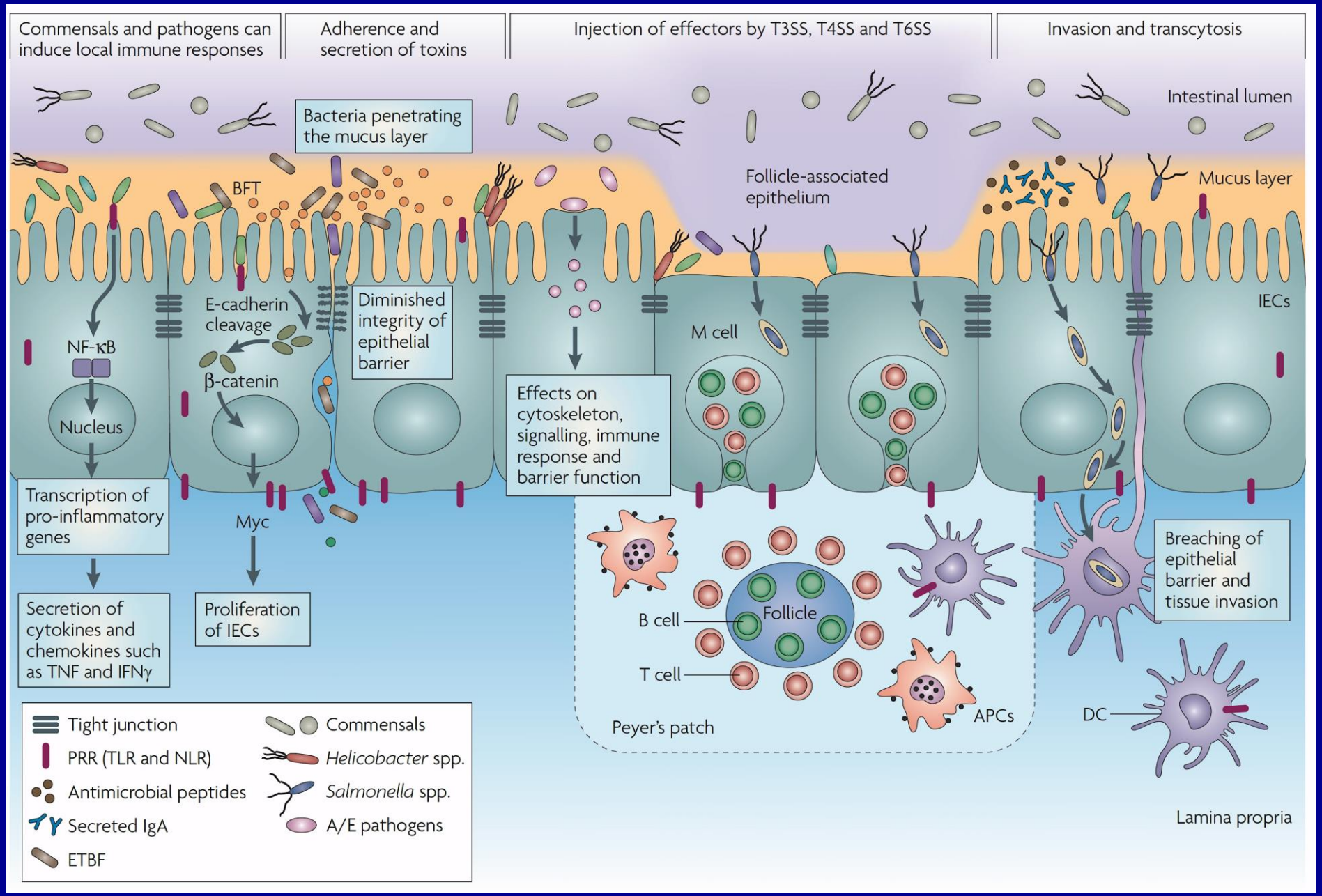
MICROBIOTA-INDUCED MATURATION OF THE GASTROINTESTINAL TRACT



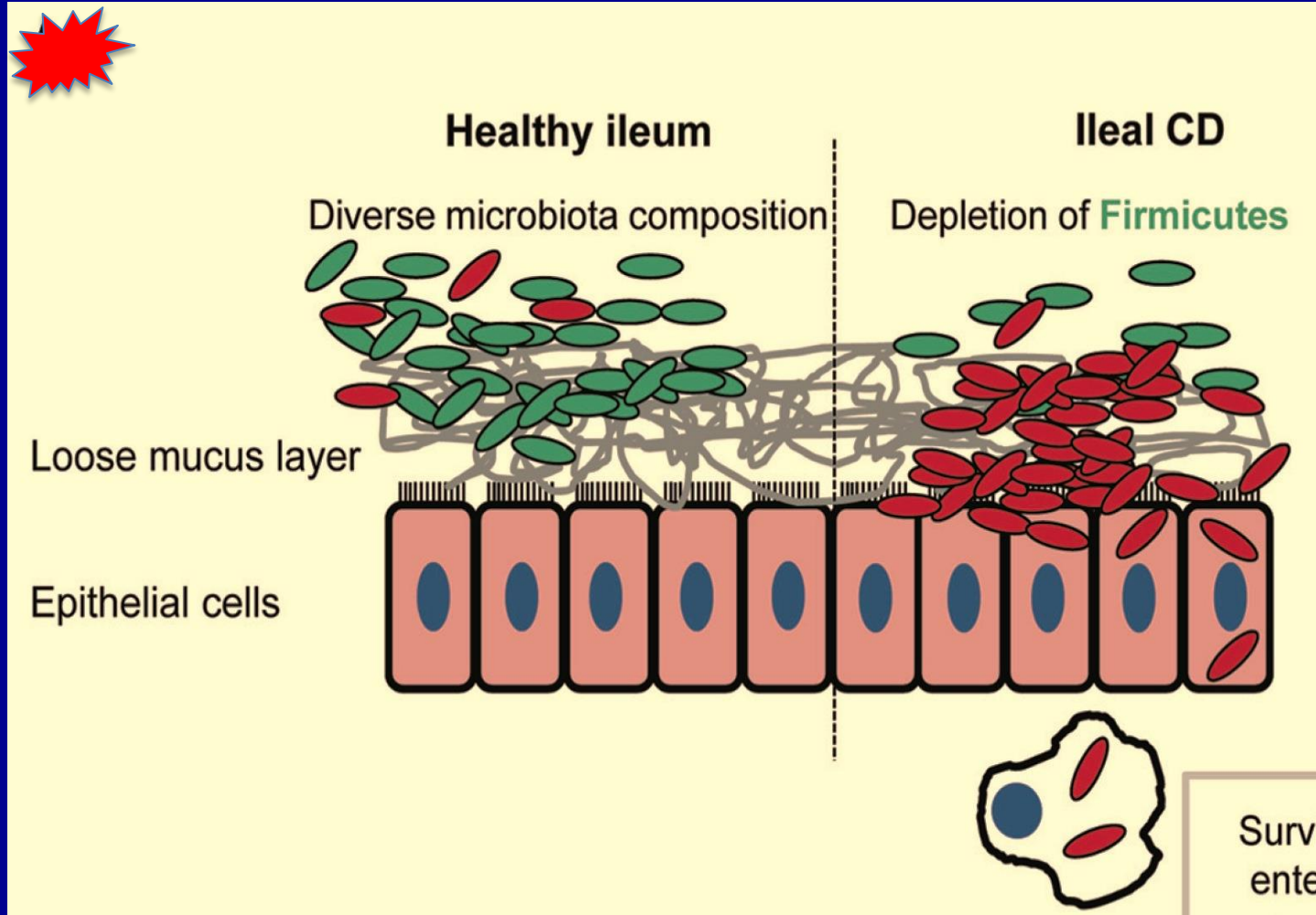
The microbiota and host genetic and environmental factors contribute to pathogenesis of Crohn's disease



INTERACTIONS BETWEEN GUT MUCOSA EPITHELIUM AND MICROBIOTA



PRO-INFLAMMATORY EFFECTS OF GUT BACTERIA IN IBD



Increase in *E. coli*, *Bacteroides/Prevotella* spp.

→ high load of LPS

Adherence of AIEC strains

E. coli type-1 pili expression
Impaired epithelial *CEACAM6* expression

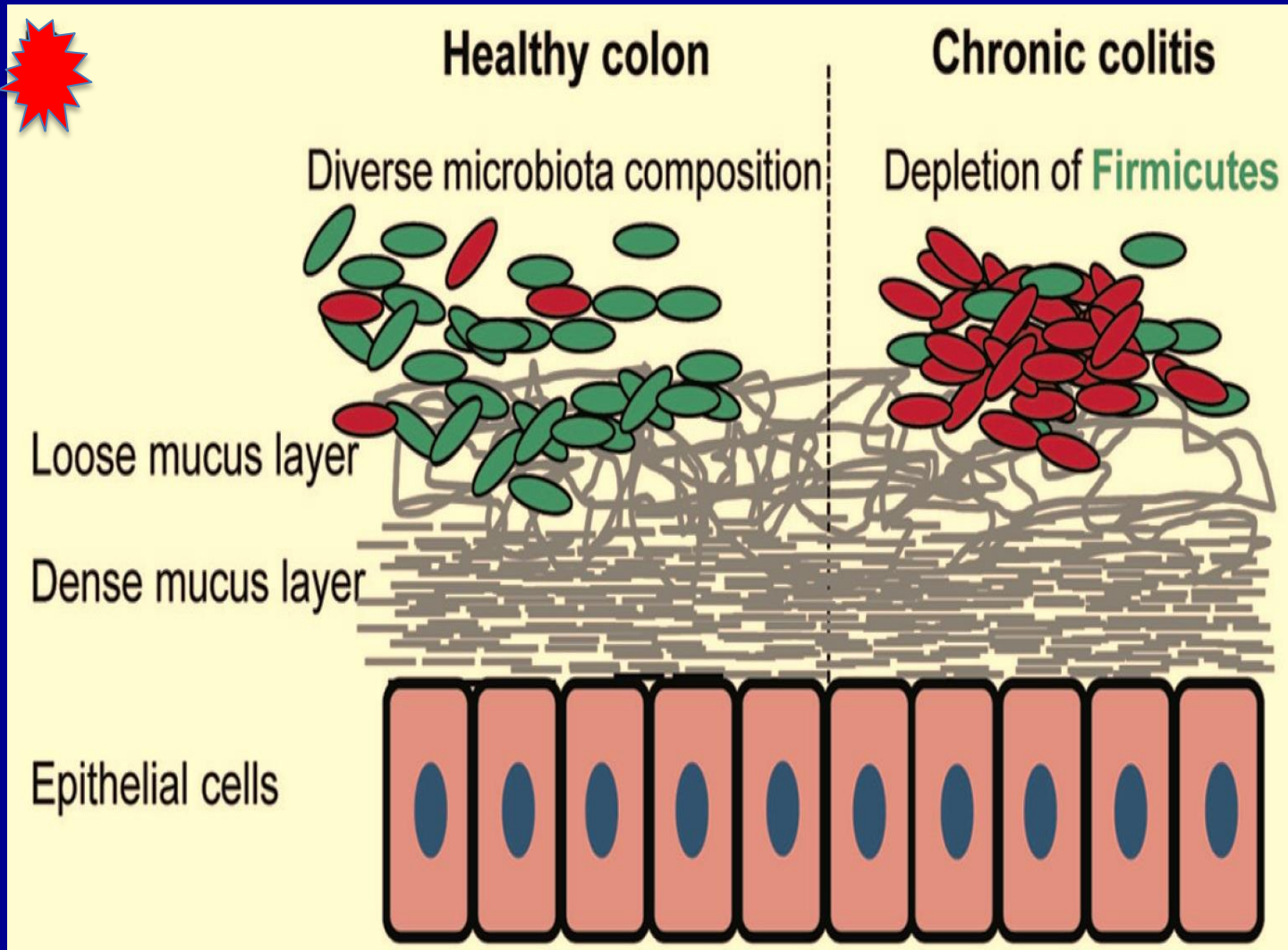
Invasion of AIEC strains

mediated by OmpA-GP96-interactions

Survival and replication AIEC in enterocytes and macrophages

Impaired expression of *NOD2*, *ATG16L* and *IRGM*

PRO-INFLAMMATORY EFFECTS OF GUT BACTERIA IN IBD

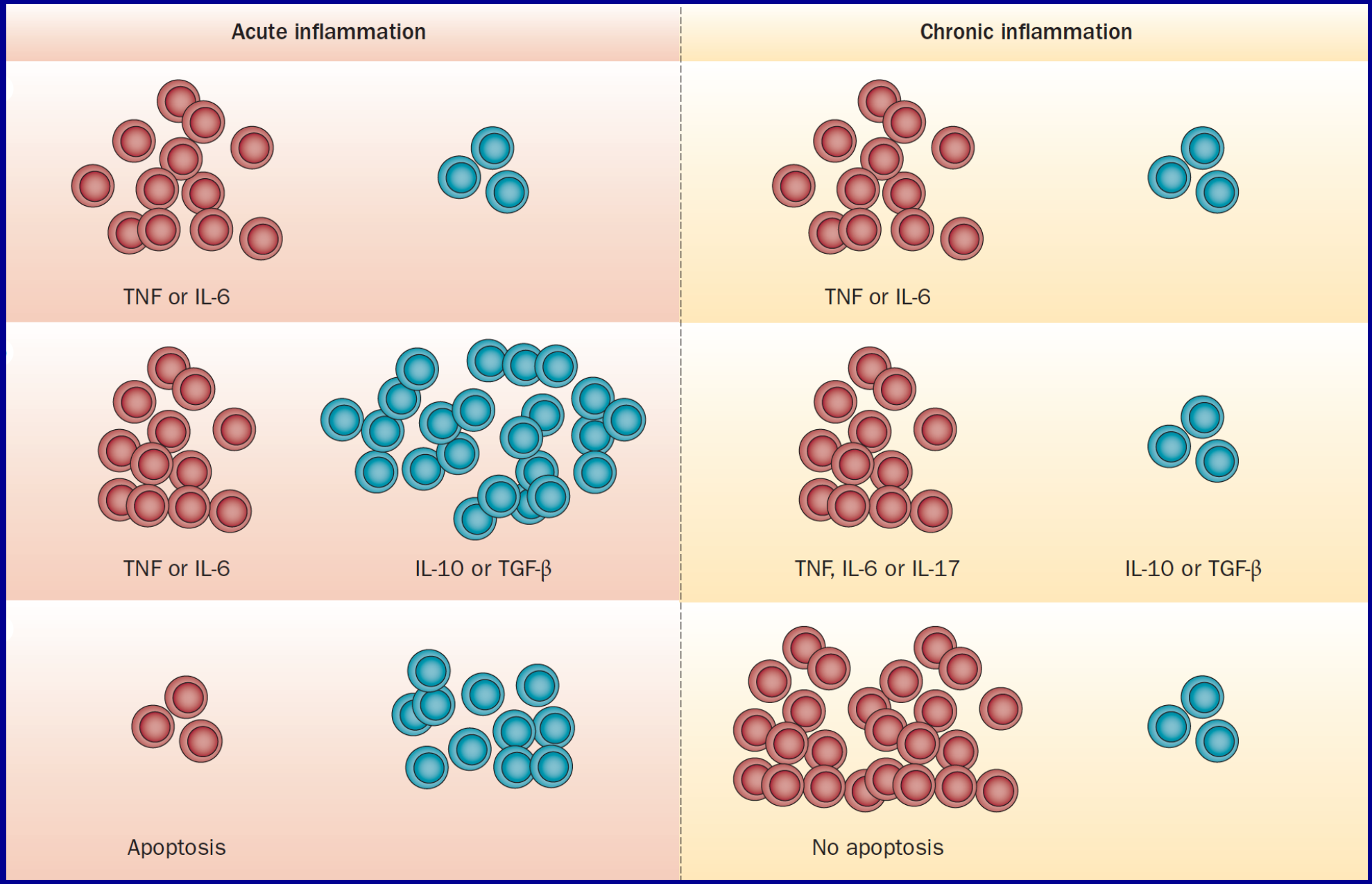


Increase in *E. coli*, *Bacteroides/Prevotella* spp.

→ high load of LPS

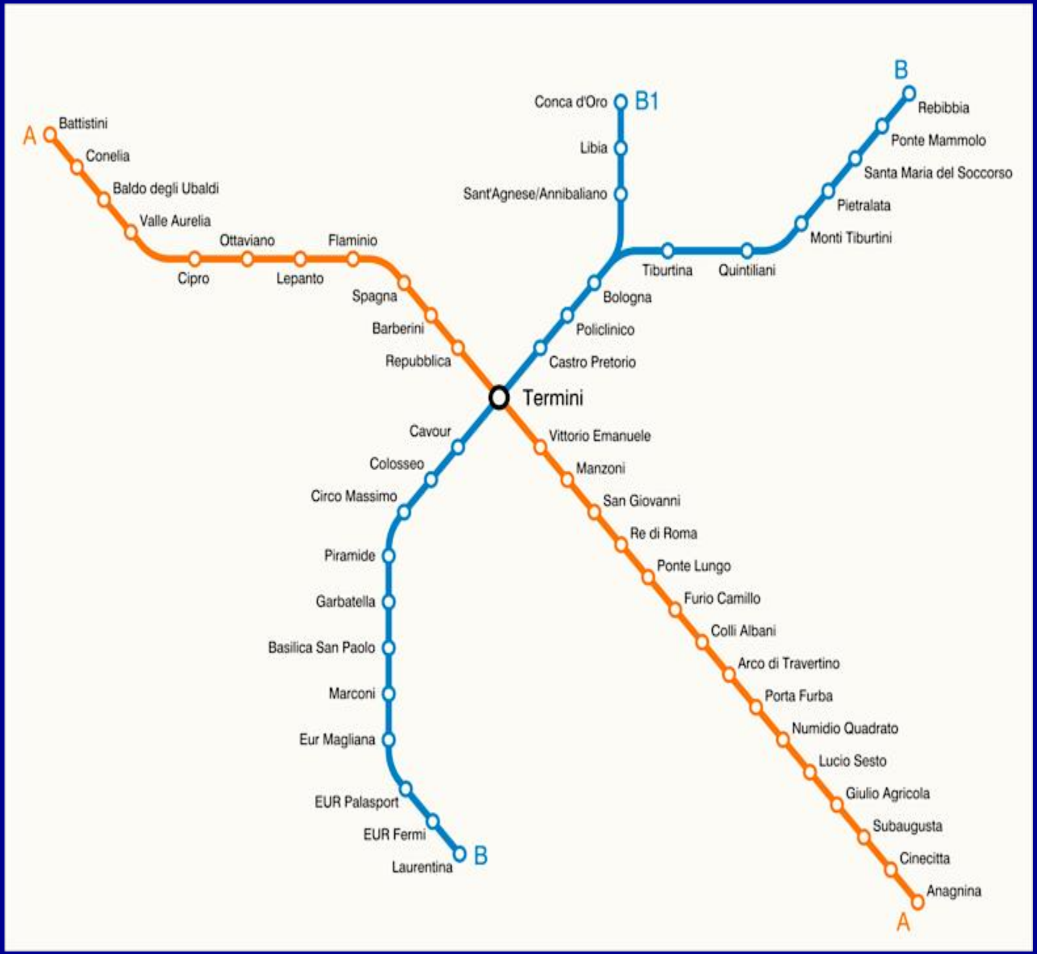
High abundance of *E. coli* with a high number of virulence- and fitness-associated genes

Suggested model of perpetuation of intestinal inflammation in IBD



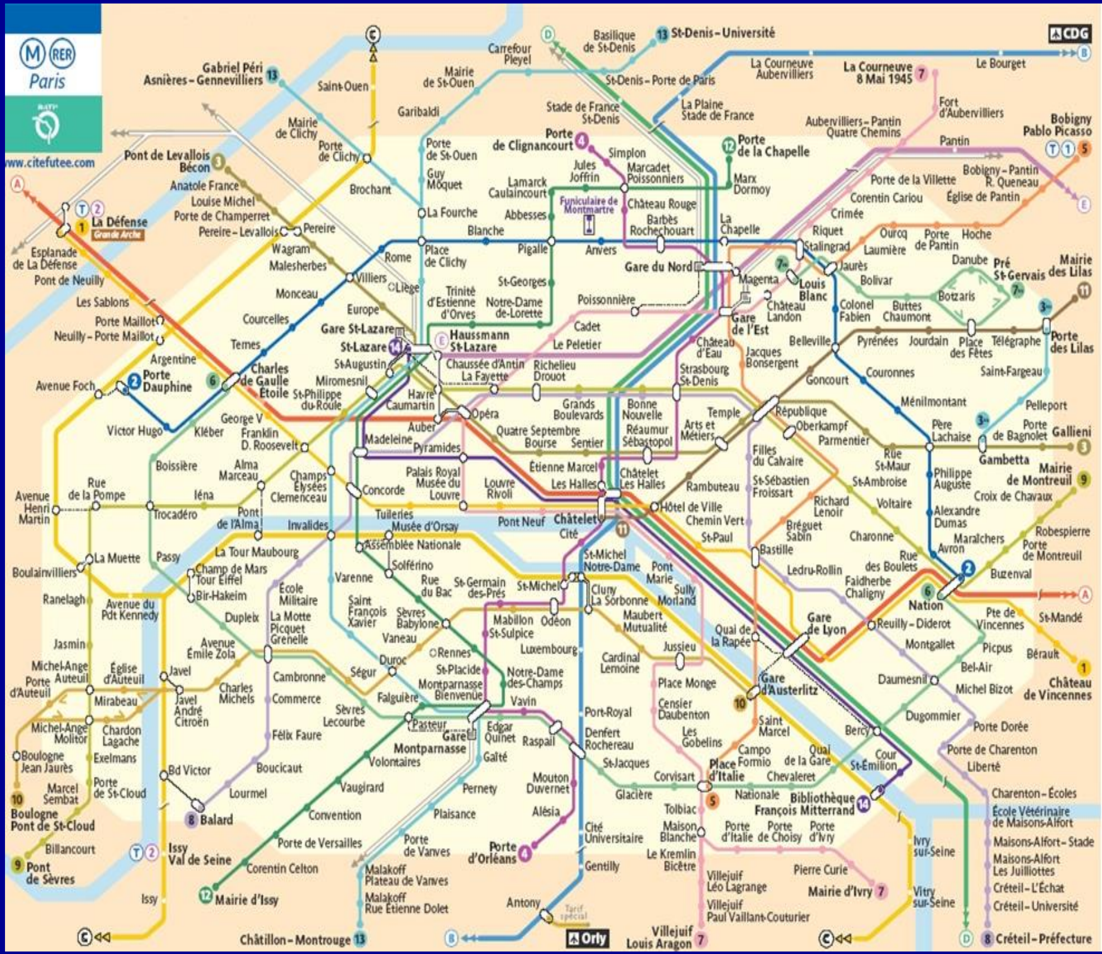
PATHOGENETIC MECHANISMS IN IMMUNE-MEDIATED CHRONIC DISORDERS OF THE GASTROINTESTINAL TRACT

"PHYSIOLOGICAL INFLAMMATION" OF THE GUT MUCOSA



Rome subway

CHRONIC UNCONTROLLED INFLAMMATION OF THE GUT MUCOSA



Paris subway

Differential ability of various bacterial species to induce or prevent experimental colitis

All bacterial species are not equal!



HLA B_{27} transgenic rat

Germ free, no colitis

Cecal bacteria

Bacteroides vulgatus

E. coli

Cecal bacteria + Lactobacillus GG



Aggressive colitis



Moderate colitis



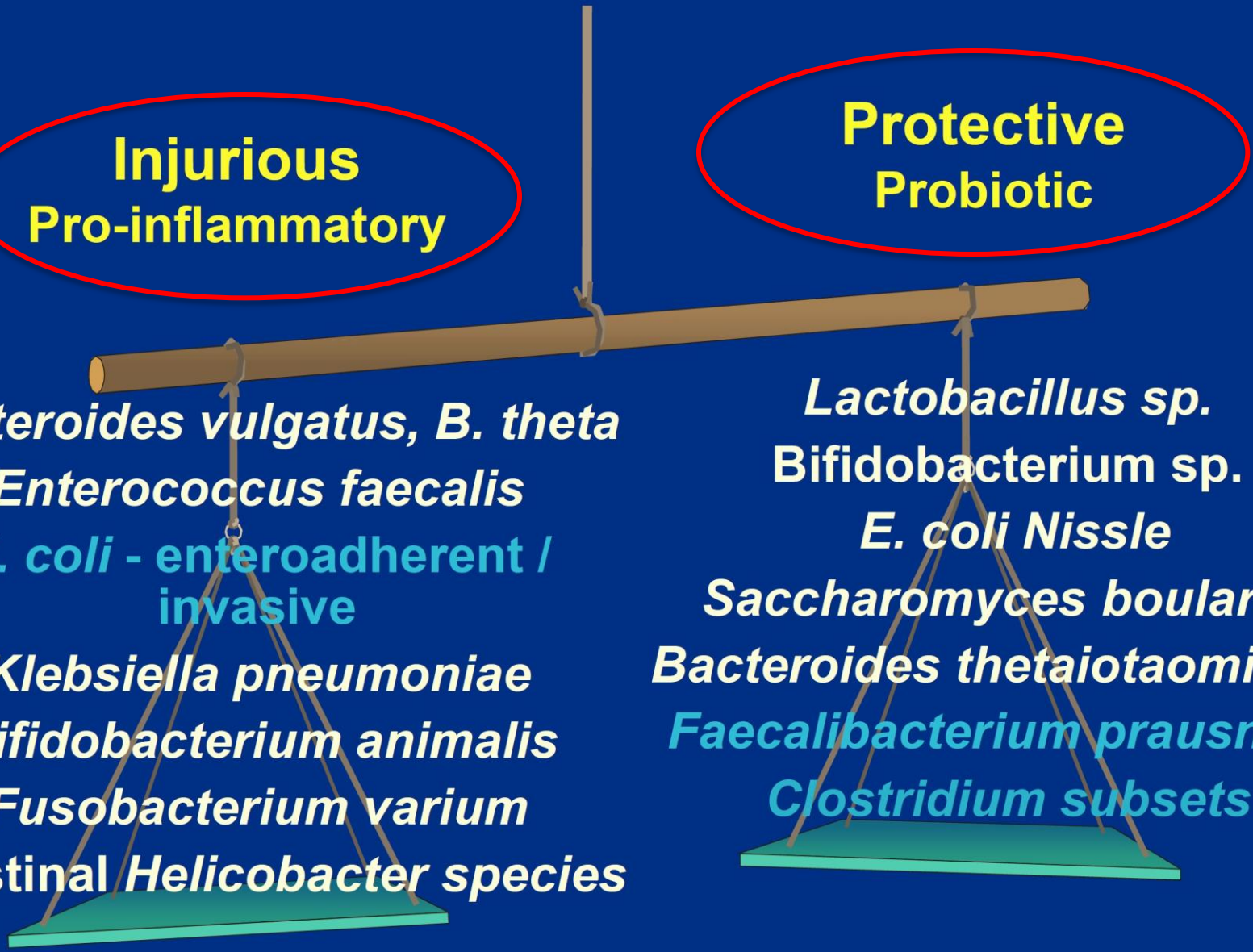
No colitis



Protection

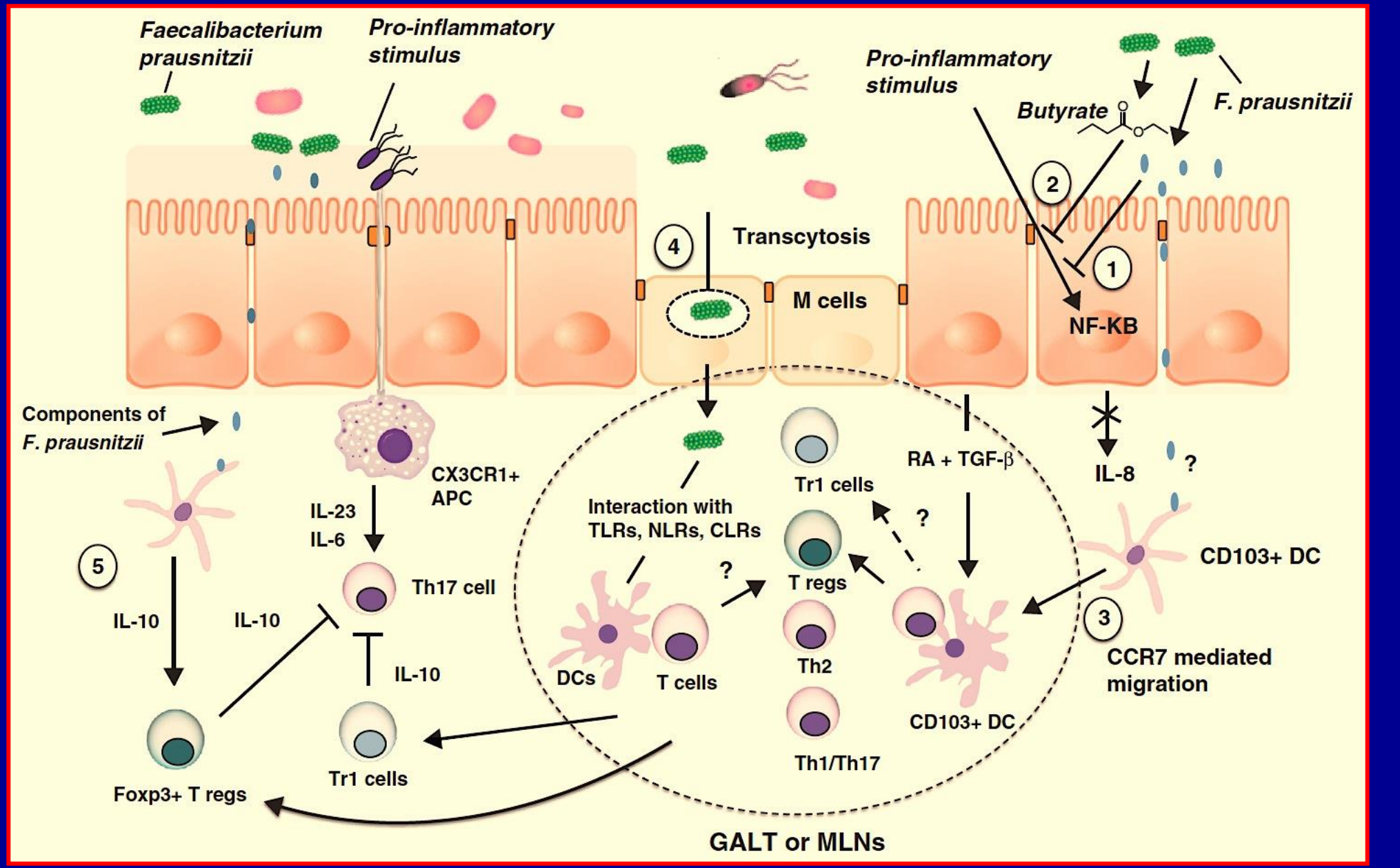
Rath et al., J Clin Invest 1996;98:945
Rath et al., Infect Immunity 1999; 67:2969
Dieleman et.al., Gut 2003; 52:370

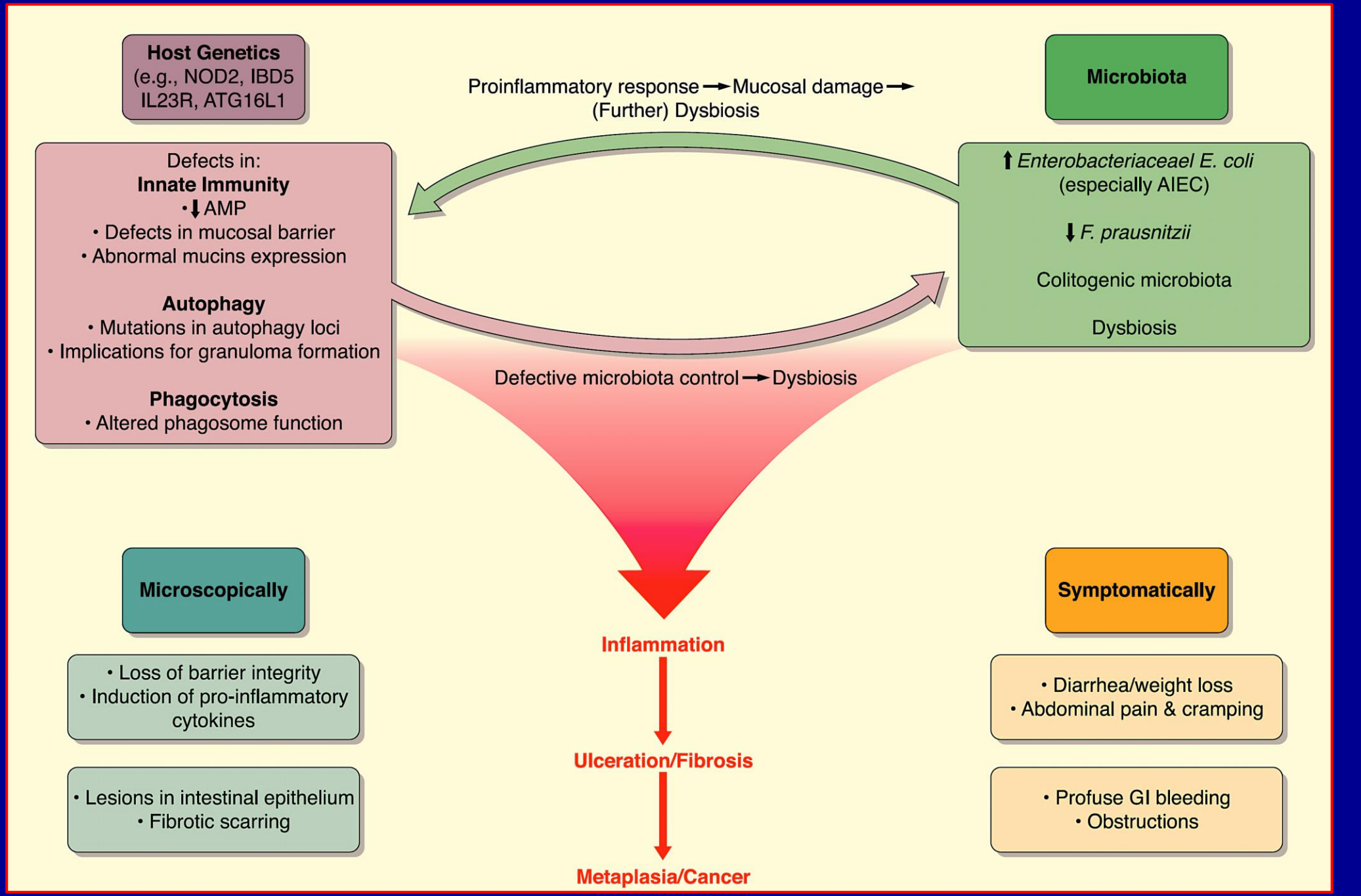
Therapeutic Manipulation of Intestinal Bacteria *(individual profile)*: Selectively Alter Beneficial vs. Detrimental Species



POSSIBLE MECHANISMS OF F. PRAUSNITZII ANTI-INFLAMMATORY EFFECTS

Current Opinion in Microbiology 2013, 16:255-261

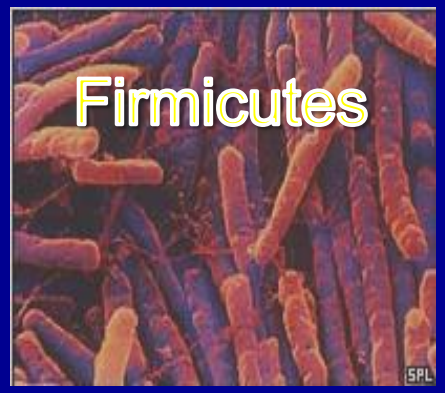


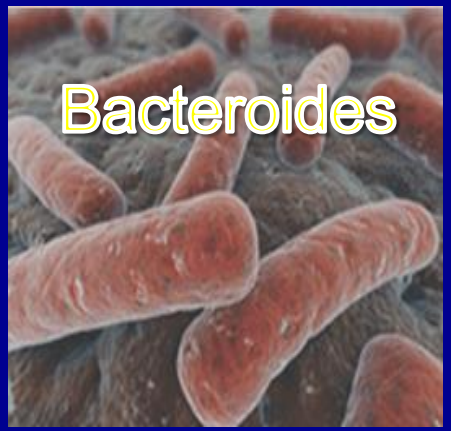

Host Genetics
(e.g., NOD2, IBD5
IL23R, ATG16L1
Proinflammatory response → Mucosal damage →
(Further) Dysbiosis
Microbiota
Defects in:
Innate Immunity
• ↓AMP
• Defects in mucosal barrier
• Abnormal mucins expression
Autophagy
• Mutations in autophagy loci
• Implications for granuloma formation
Phagocytosis
• Altered phagosome function
↑ Enterobacteriaceael E. coli
(especially AIEC)
↓ F. prausnitzii
Colitogenic microbiota
Dysbiosis
Defective microbiota control → Dysbiosis
Microscopically
• Loss of barrier integrity
• Induction of pro-inflammatory
cytokines
• Lesions in intestinal epithelium
• Fibrotic scarring
Inflammation
Ulceration/Fibrosis
Metaplasia/Cancer
Symptomatically
• Diarrhea/weight loss
• Abdominal pain & cramping
• Profuse GI bleeding
• Obstructions

DYSBIOSIS in IBD (Peterson, Cell Host & Microbe, 2008)

Cell count
(log CFU/mL)

IBD







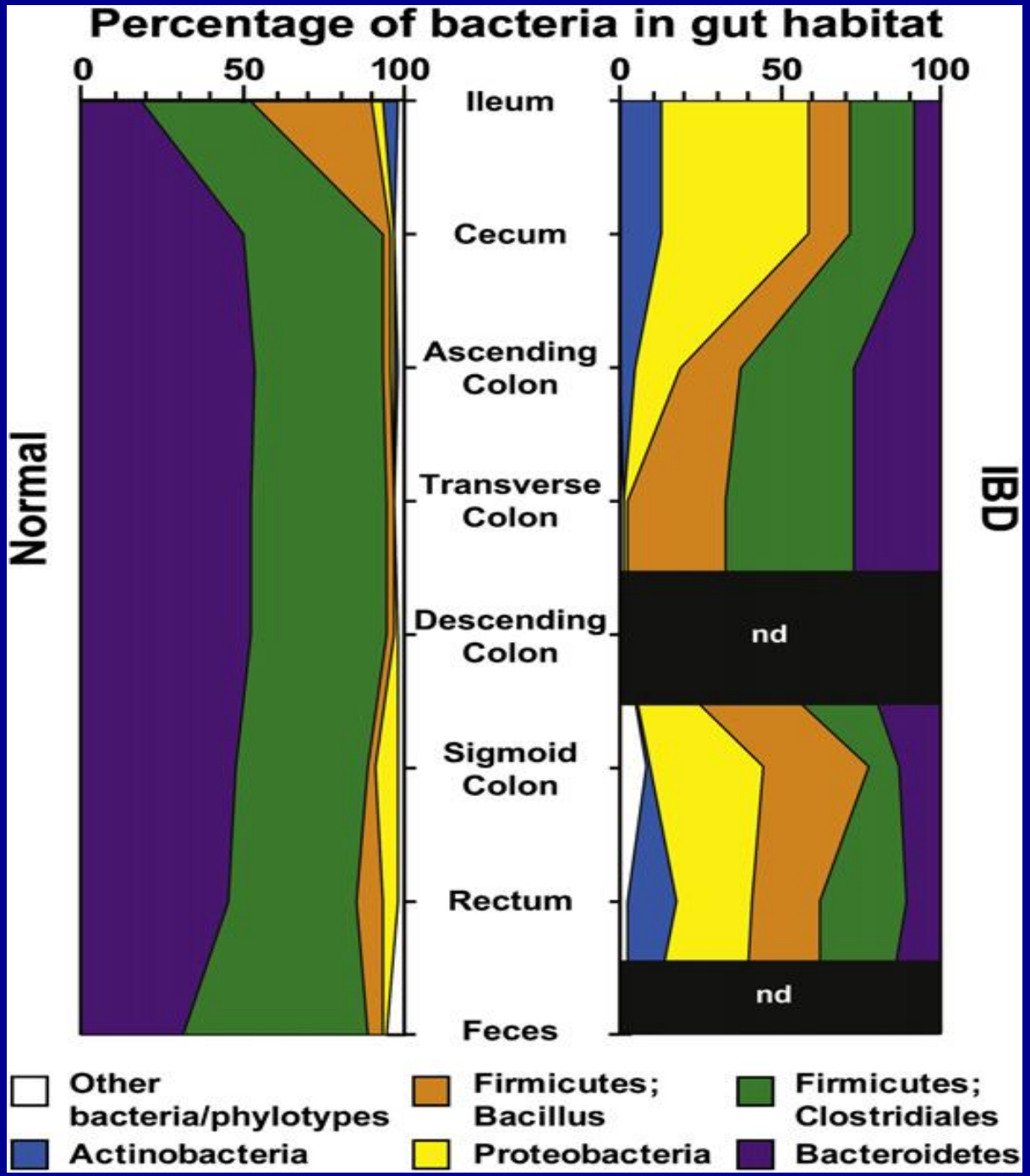


Metagenomic Approaches for defining the pathogenesis of inflammatory bowel disease
Cell Host & Microbe 2008;3:417

IBD groups had marked decreases in two main constituents of gut community

- **Bacteroidetes**
- **Lachnospiraceae**

Alterations in the representation of components of microbiota may be biomarkers that help predict disease predisposition, activity, severity, response to therapy



EVIDENCE THAT INFLAMMATION IN IBD RESULTS FROM THE INTERACTION OF THE GUT MICROBIOTA WITH THE MUCOSAL IMMUNE COMPARTMENTS

1) **The gut microbiota is an essential factor in driving inflammation in IBD.**

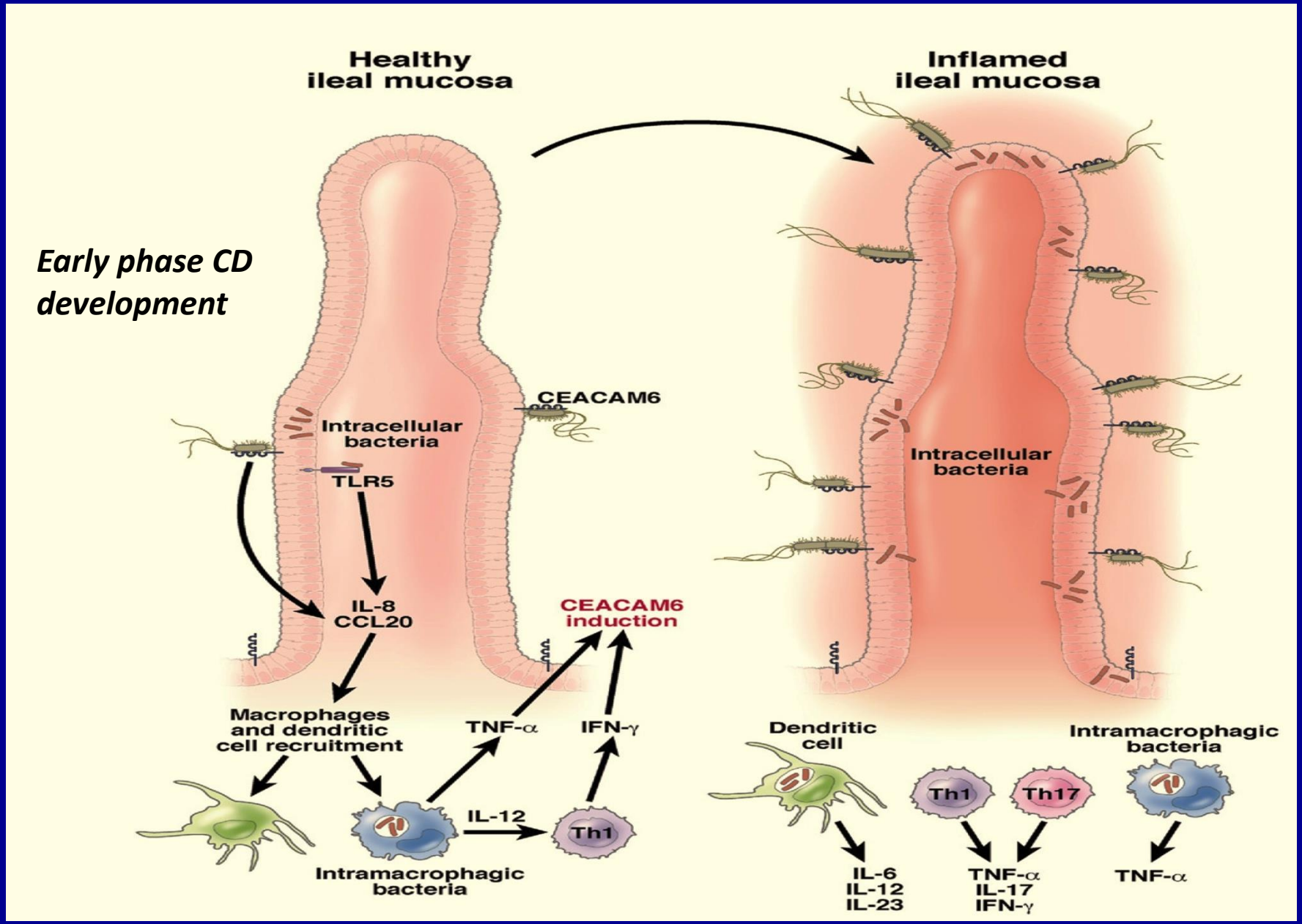
2) ***In Crohn's disease (CD), diversion of the fecal stream induces inflammatory remission and mucosal healing in the excluded intestinal segment whereas infusion of intestinal contents reactivates the disease.***

3) ***In active ulcerative colitis (UC), short-term treatment with an enteric-coated preparation of broad-spectrum antibiotics reduced metabolic activity of the microbiota and mucosal inflammation.***

4) **Patients with UC or CD show abnormal mucosal secretion of IgG antibodies against commensal bacteria and mucosal T cells are hyper-reactive against antigens of the common microbiota.**

AIEC INFECTION AND LOOP OF COLONIZATION AND INFLAMMATION

Early phase CD development



Gastroenterology 2011;140:1720-8

Characterization of Adherent-invasive *Escherichia coli* Isolated from Pediatric Patients with Inflammatory Bowel Disease

Anna Negroni, PhD, Manuela Costanzo, PhD,* Roberta Vitali, PhD,* Fabiana Superti, PhD,† Lucia Bertuccini, PhD,† Antonella Tinari, MSc,† Fabio Minelli,‡ Giovanni Di Nardo, MD,§ Federica Nuti, MD,§ Maria Pierdomenico, PhD,* Salvatore Cucchiara, PhD, MD,§ and Laura Stronati, PhD**

Background: Crohn's disease (CD) and ulcerative colitis (UC), known as inflammatory bowel diseases (IBD), are characterized by an abnormal immunological response to commensal bacteria colonizing intestinal lumen and mucosa. Among the latter, strains of adherent-invasive *Escherichia coli* (AIEC), capable of adhering to and invading epithelium, and to replicate in macrophages, have been described in CD adults. We aimed at identifying and characterizing AIEC strains in pediatric IBD.

Methods: In all, 24 CD children, 10 UC, and 23 controls were investigated. Mucosal biopsies, taken during colonoscopy, were analyzed for the presence of AIEC strains by an adhesive-invasive test. Protein expression of the specific AIEC receptor, the carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), was evaluated by western blot and immunohistochemistry, while tumor necrosis factor alpha (TNF-α) and interleukin (IL)-8 mRNA expression was detected by real-time polymerase chain reaction (PCR), after bacterial infection. Transmission electron microscopy and trans-epithelial electric resistance assays were performed on biopsies to assess bacteria-induced morphological and functional epithelial alterations.

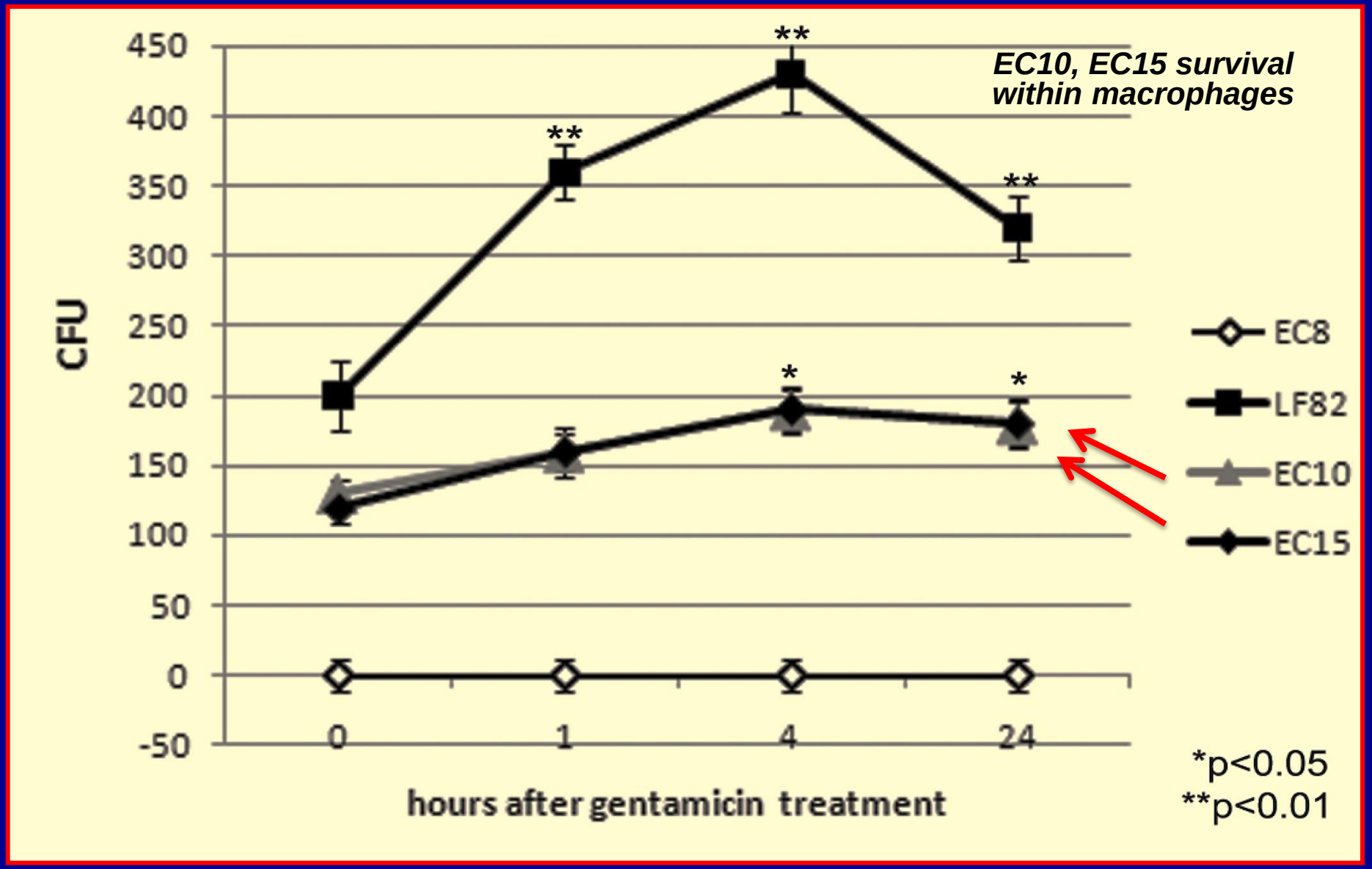
Results: Two bacterial strains, EC15 and EC10, were found to adhere and invade the Caco2 cell line, similar to the well-known AIEC strain LF82 (positive control): they upregulated CEACAM6, TNF-α, and IL-8 gene/protein expression, in vitro and in cultured intestinal mucosa; they could also survive inside macrophages and damage the epithelial barrier integrity. Lesions in the inflamed tissues were associated with bacterial infection.

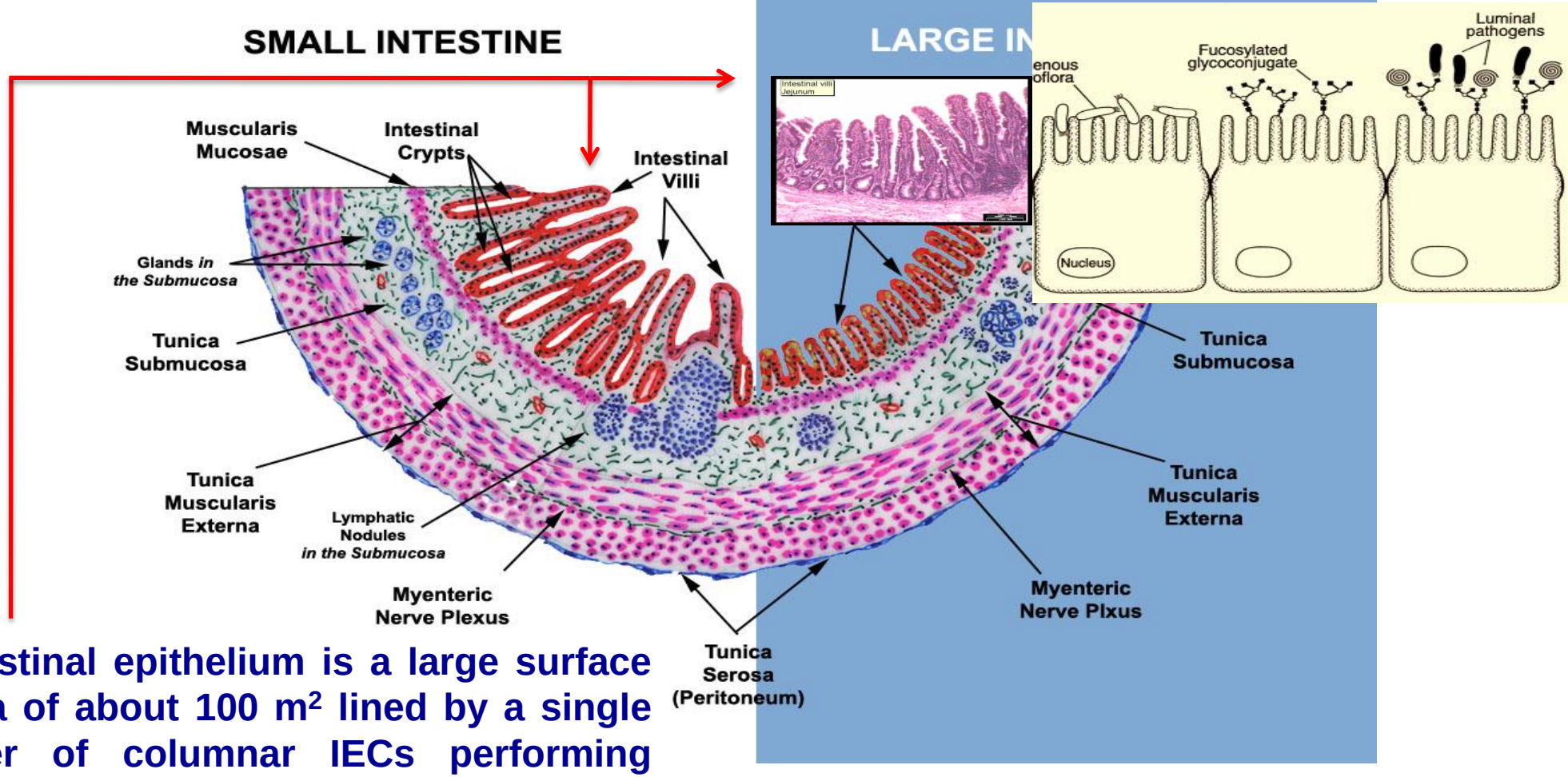
Conclusions: This is the first study showing the presence of adhesive-invasive bacteria strains in the inflamed tissues of children with IBD. Collective features of these strains indicate that they belong to the AIEC spectrum, suggesting their possible role in disease pathogenesis.

(Inflamm Bowel Dis 2012;18:913–924)

Key Words: Crohn's disease, ulcerative colitis, intestinal microbiota, Escherichia coli, adherent-invasive strains

CHARACTERIZATION OF ADHERENT-INVASIVE ESCHERICHIA COLI ISOLATED FROM PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE. Inflammatory Bowel Disease 2012;18:912



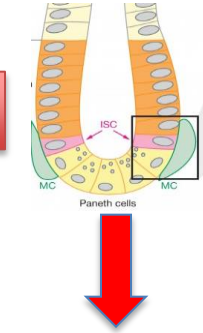


Intestinal epithelium is a large surface area of about 100 m^2 lined by a single layer of columnar IECs performing several functions:

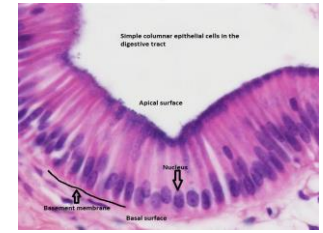
- ***processing and absorbing dietary nutrients***
- ***secreting compounds influencing microbial composition***
- ***sampling of the intestinal microenvironments***
- ***sensing both beneficial and harmful microbes***
- ***inducing and modulating immune responses***

To fulfill these functions, IECs comprise several specialized cell types with distinctive functions

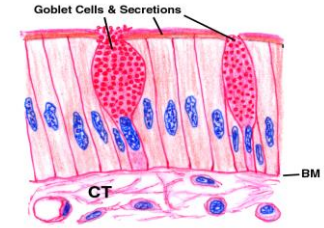
STEM CELL



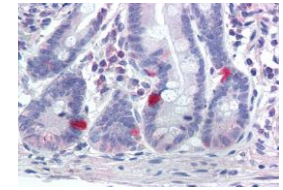
❖ ***Columnar cells***: *(the most abundant) absorbe nutrients by apical villi;*



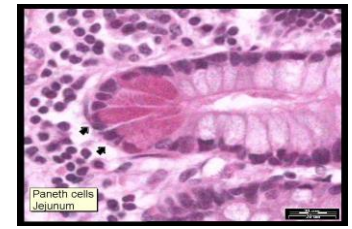
❖ ***Goblet cells***: *produce and secrete mucins in order to form a protective luminal mucus layer;*



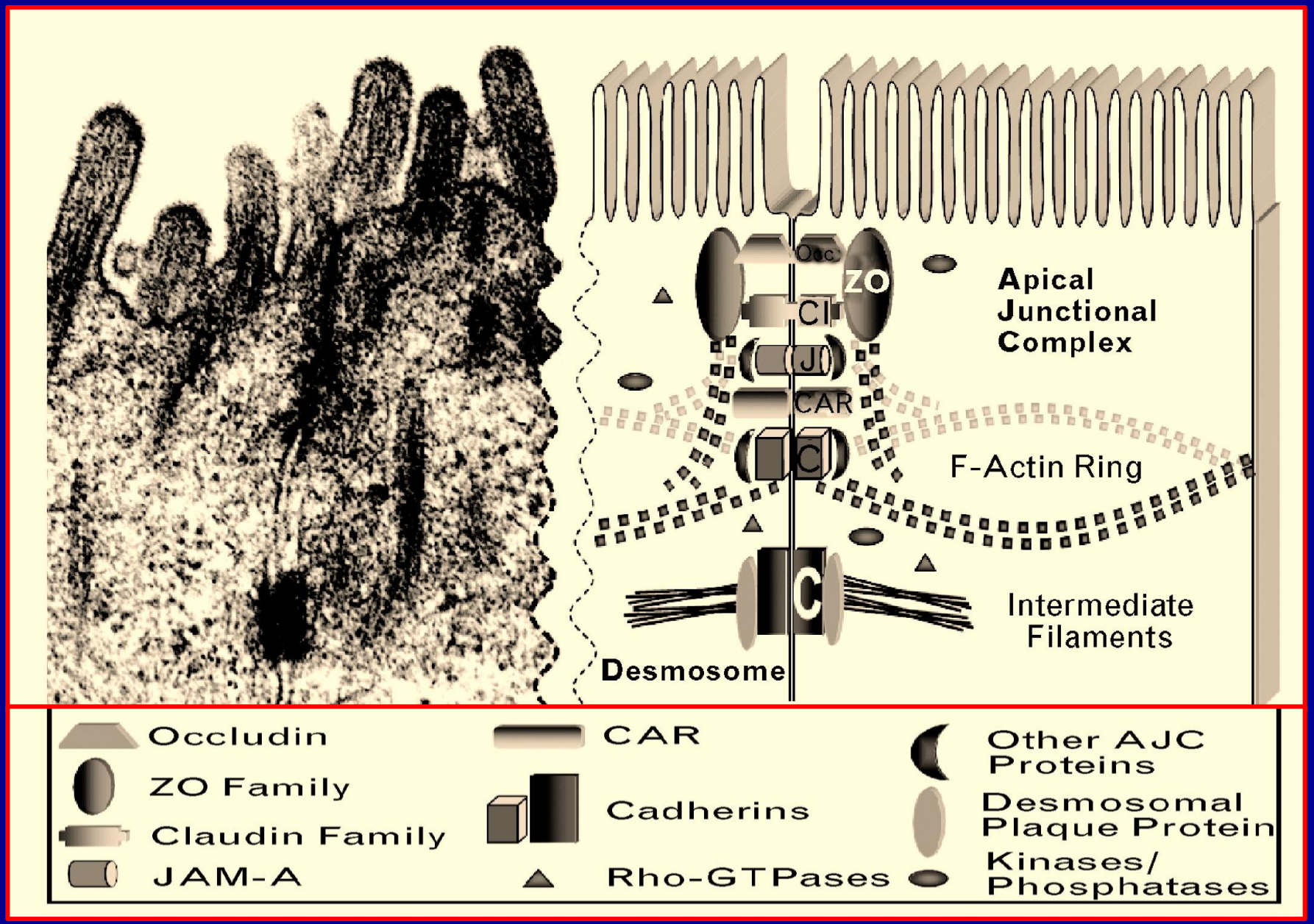
❖ ***Neuroendocrine cells***: *release hormones in an endocrine and paracrine fashion;*



❖ ***Paneth cells***: *secrete antimicrobial peptides*



Transmission electron micrograph of intercellular junctions in human intestinal epithelial cells



American Journal of Pathology, Vol. 166, No. 2, February 2005
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Growth Factors, Cytokines, Cell Cycle Molecules

Interferon-γ and Tumor Necrosis Factor-α Synergize to Induce Intestinal Epithelial Barrier Dysfunction by Up-Regulating Myosin Light Chain Kinase Expression

Fengjun Wang,*† W. Vallen Graham,† Yingmin Wang,† Edwina D. Witkowski,† Brad T. Schwarz,† and Jerrold R. Turner†

From the Institute of Burn Research, Southwest Hospital, Third Military Medical University, Chongqing, China; and the Department of Pathology,† The University of Chicago, Chicago Illinois*

Numerous intestinal diseases are characterized by immune cell activation and compromised epithelial barrier function. We have shown that cytokine treatment of epithelial monolayers increases myosin II regulatory light chain (MLC) phosphorylation and decreases barrier function and that these are both reversed by MLC kinase (MLCK) inhibition. The aim of this study was to determine the mechanisms by which interferon (IFN)-γ and tumor necrosis factor (TNF)-α regulate MLC phosphorylation and disrupt epithelial barrier function. We developed a model in which both cytokines were required for barrier dysfunction. Barrier dysfunction was also induced by TNF-α addition to IFN-γ-primed, but not control, Caco-2 monolayers. TNF-α treatment of IFN-γ-primed monolayers caused increases in both MLCK expression and MLC phosphorylation, suggesting that MLCK is a TNF-α-inducible protein. These effects of TNF-α were not mediated by nuclear factor-κB. However, at doses below those needed for nuclear factor-κB inhibition, sulfasalazine was able to prevent TNF-α-induced barrier dysfunction, MLCK up-regulation, and MLC phosphorylation. Low-dose sulfasalazine also prevented morphologically evident tight junction disruption induced by TNF-α. These data show that IFN-γ can prime intestinal epithelial monolayers to respond to TNF-α by disrupting tight junction morphology and barrier function via MLCK up-regulation and MLC phosphorylation. These TNF-α-induced events can be prevented by the clinically relevant drug sulfasalazine. *(Am J Pathol 2005, 166:409–419)*

A principal function of epithelial surfaces is the maintenance of a barrier to hydrophilic solutes. In intestinal epithelium, this barrier function is compromised in a spectrum of infectious, immune-mediated, and idiopathic diseases.[1] In Crohn's disease these barrier defects, measured as increases in paracellular permeability, may precede clinical evidence of disease,[2] can serve as markers of impending disease reactivation,[3] and correlate with systemic immune activation.[4] Thus, defects in epithelial barrier function are intimately associated with Crohn's disease pathogenesis.

One feature common to Crohn's disease and other intestinal diseases with compromised barrier function is TH1-polarized immune activation, with associated elevations of mucosal interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Several lines of evidence suggest that these cytokine elevations are responsible for the observed barrier defects. First, *in vitro* studies have shown that, at relatively high doses, IFN-γ and TNF-α can induce barrier dysfunction in cultured epithelial monolayers.[5–9] At lower doses, these cytokines can synergize to disrupt barrier function *in vitro*.[8,10,11] *In vivo*, both in human patients and animal models, TNF-α and IFN-γ antagonism can diminish disease severity and restore barrier function.[12–15] Thus, TNF-α and IFN-γ are critical to the barrier disruption that occurs both *in vitro* and *in vivo*.

Although both *in vitro* and *in vivo* data confirm that TNF-α and IFN-γ can cause dysfunction of the epithelial tight junction barrier, the mechanisms of this effect remain unknown. TNF-α can cause epithelial apoptosis, but this does not appear to be the mechanism by which barrier function is compromised.[8,11,16,17] Other mecha-

Supported by the National Institutes of Health (grant DK61931, grant DK42086 to The University of Chicago Digestive Disease Center, and grant CA14599 to The University of Chicago Cancer Center) and the Crohn's & Colitis Foundation of America.

Accepted for publication November 2, 2004.

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Research article

Open Access

Adherent-invasive *Escherichia coli*, strain LF82 disrupts apical junctional complexes in polarized epithelia

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Email: Eytan Wine - wine@ualberta.ca; Juan C Ossa - juan.ossa@sickkids.ca; Scott D Gray-Owen - scott.gray.owen@utoronto.ca; Philip M Sherman* - philip.sherman@sickkids.ca

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Published: 26 August 2009

BMC Microbiology 2009, **9**:180 doi:10.1186/1471-2180-9-180

Received: 14 April 2009
Accepted: 26 August 2009

This article is available from: http://www.biomedcentral.com/1471-2180/9/180

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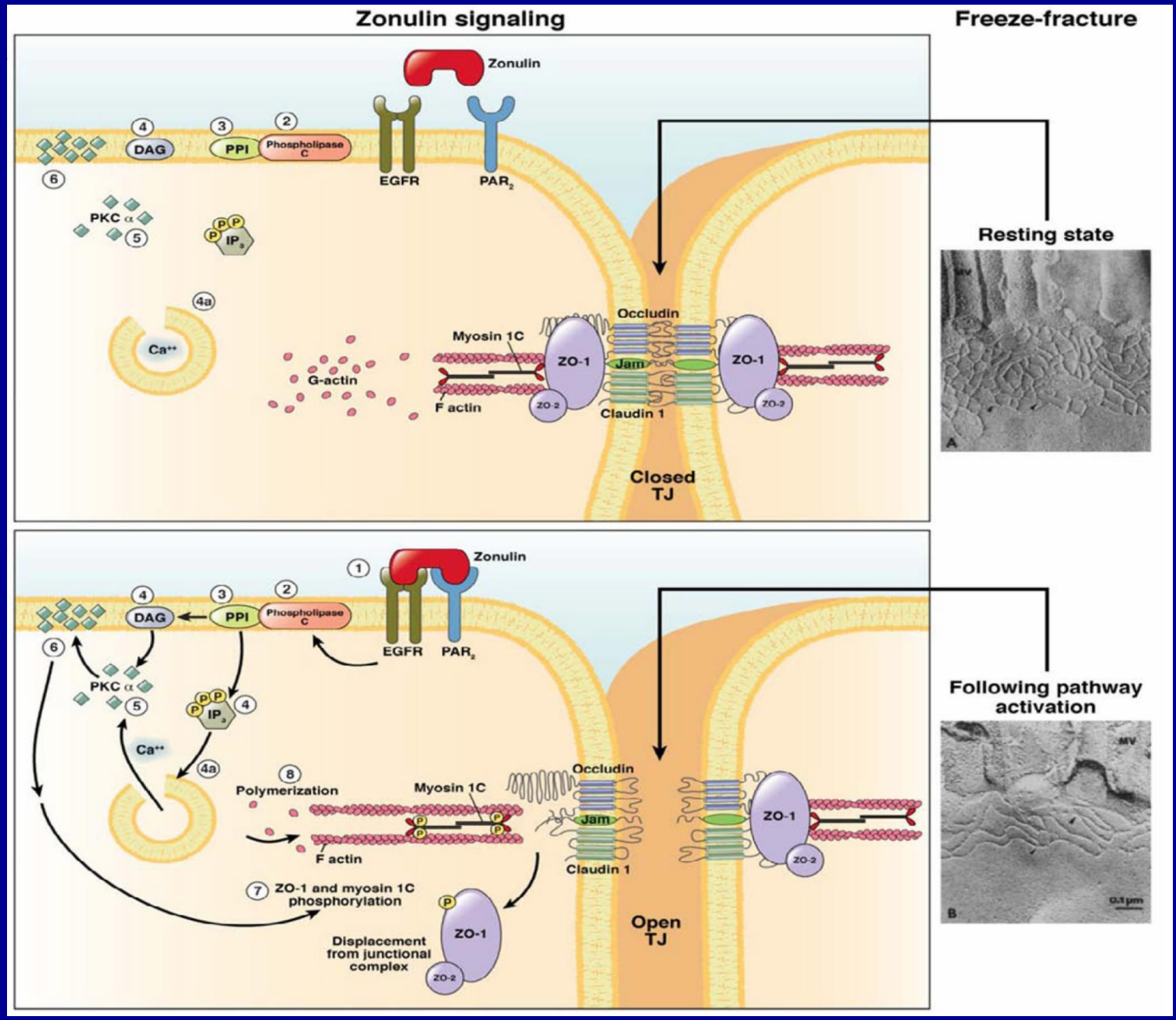
Abstract

Background: Although bacteria are implicated in the pathogenesis of chronic inflammatory bowel diseases (IBD), mechanisms of intestinal injury and immune activation remain unclear. Identification of adherent-invasive *Escherichia coli* (AIEC) strains in IBD patients offers an opportunity to characterize the pathogenesis of microbial-induced intestinal inflammation in IBD. Previous studies have focused on the invasive phenotype of AIEC and the ability to replicate and survive in phagocytes. However, the precise mechanisms by which these newly identified microbes penetrate the epithelial lining remain to be clarified. Therefore, the aim of this study was to delineate the effects of AIEC, strain LF82 (serotype O83:H1) on model polarized epithelial monolayers as a contributor to intestinal injury in IBD.

Results: Infection of T84 and Madin-Darby Canine Kidney-I polarized epithelial cell monolayers with AIEC, strain LF82 led to a reduction in transepithelial electrical resistance and increased macromolecular (10 kilodalton dextran) flux. Basolateral AIEC infection resulted in more severe disruption of the epithelial barrier. Increased permeability was accompanied by a redistribution of the tight junction adaptor protein, zonula occludens-1, demonstrated by confocal microscopy and formation of gaps between cells, as shown by transmission electron microscopy. After 4 h of infection of intestine 407 cells, bacteria replicated in the cell cytoplasm and were enclosed in membrane-bound vesicles positive for the late endosomal marker, LAMP1.

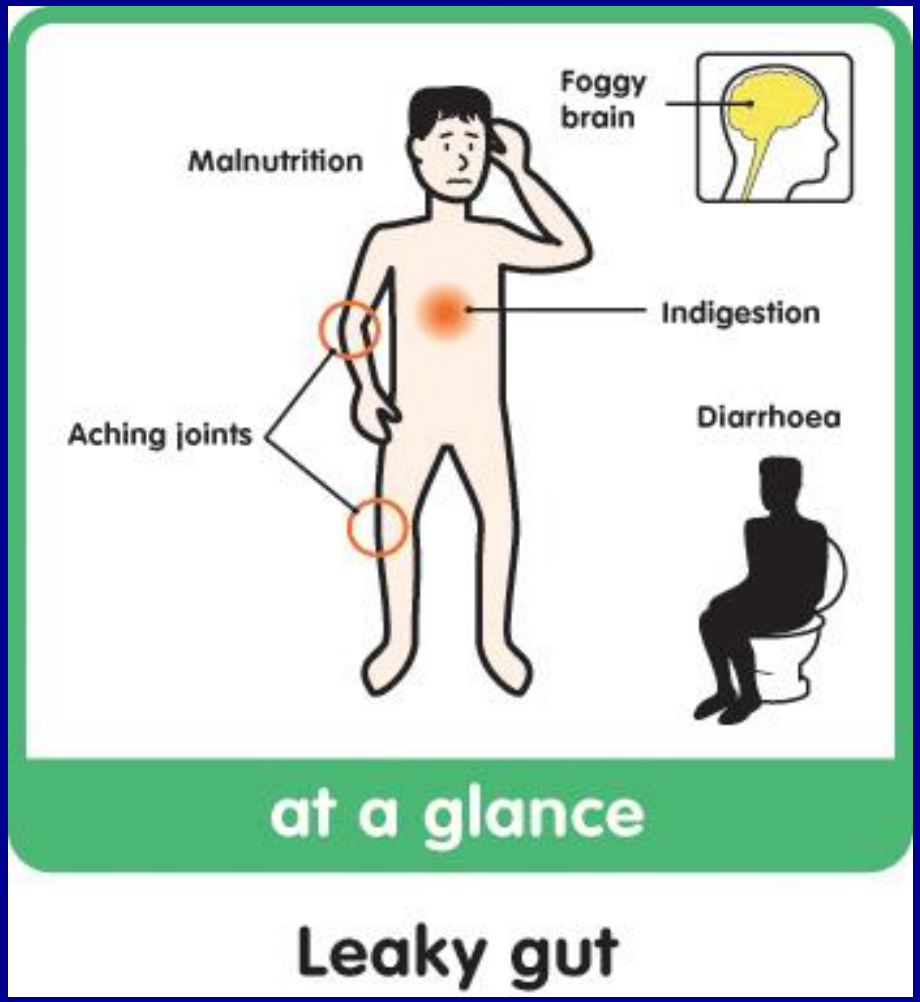
Conclusion: These findings indicate that AIEC, strain LF82 disrupts the integrity of the polarized epithelial cell barrier. This disruption enables bacteria to penetrate into the epithelium and replicate in the host cell cytoplasm. These findings provide important links between microbes related to IBD, the intestinal epithelial cell barrier and disease pathogenesis.

SCHEMATIC REPRESENTATION OF THE ZONULIN MECHANISM OF ACTION



A “LEAKY GUT” has a pivotal role in the *pathogenesis of gastrointestinal and systemic diseases*





What do we know?

Intestinal barrier function is modulated by:

- the immune system, including the TH1-cytokine IFN-γ, the TH2 cytokines IL-4 and IL-13, TNF-α, T cells, mast cells, and eosinophils;
- the gut microflora

Altered intestinal barrier function and increased intestinal permeability is associated with:

- food allergies
- IBD
- celiac disease
- type I diabetes





MECHANISMS UNDERLYING INFLAMMATORY BOWEL DISEASES

GUT MICROBIOTA

↑ aggressive
↓ protective

GENES

barrier function,
immunity,
bacterial killing

IMMUNE RESPONSE

↑ Th1, ↑ Th17
defective innate

ENVIRONMENT

infections,
NSAIDs, diet,
smoking, stress



Diet, microbes and host genetics: the perfect storm in inflammatory bowel disease







MECHANISMS UNDERLYING INFLAMMATORY BOWEL DISEASES

GUT MICROBIOTA

↑ aggressive
↓ protective

GENES

barrier function,
immunity,
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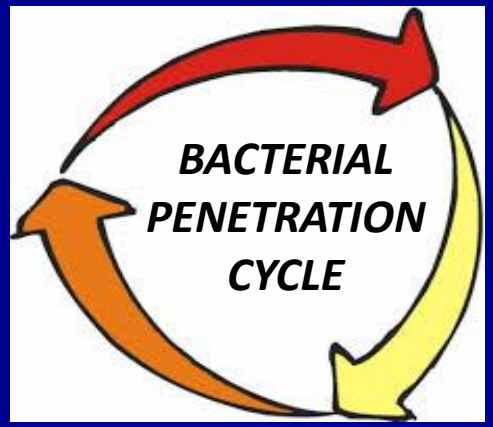
IMMUNE RESPONSE

↑ Th1, ↑ Th17
defective innate

ENVIRONMENT

infections,
NSAIDs, diet,
smoking, stress







CHANGE IN CONSUMPTION OF FOOD OR ADDITIVES

IMPAIRMENT OF BARRIER FUNCTION OF GUT EPITHELIUM

ADHERENCE-INVASION OF NON PATHOGENIC BACTERIA

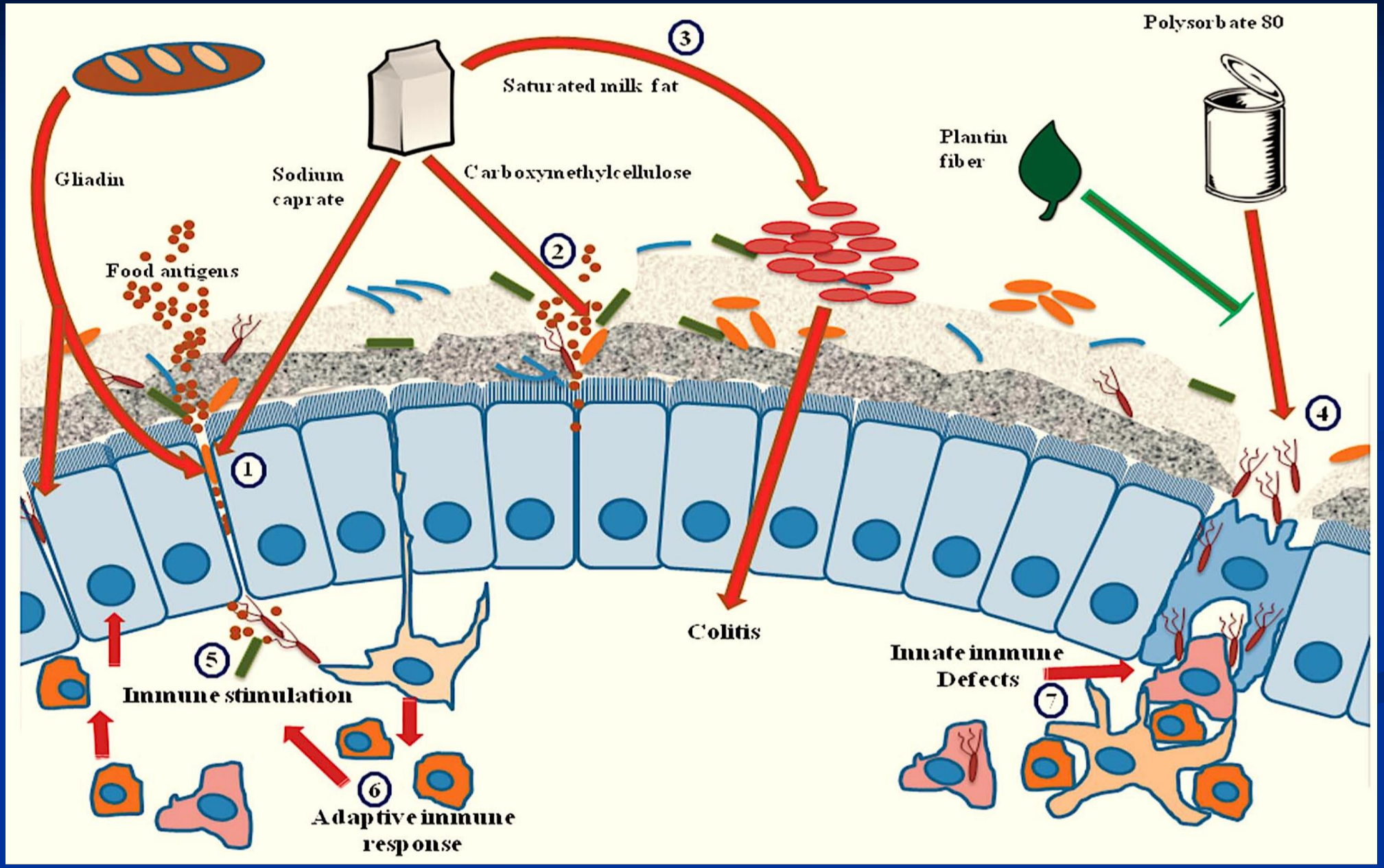
PENETRATION-REPLICATION WITHIN EPITHELIAL CELLS, DCs, MACROPHAGES

If disease susceptibility genes

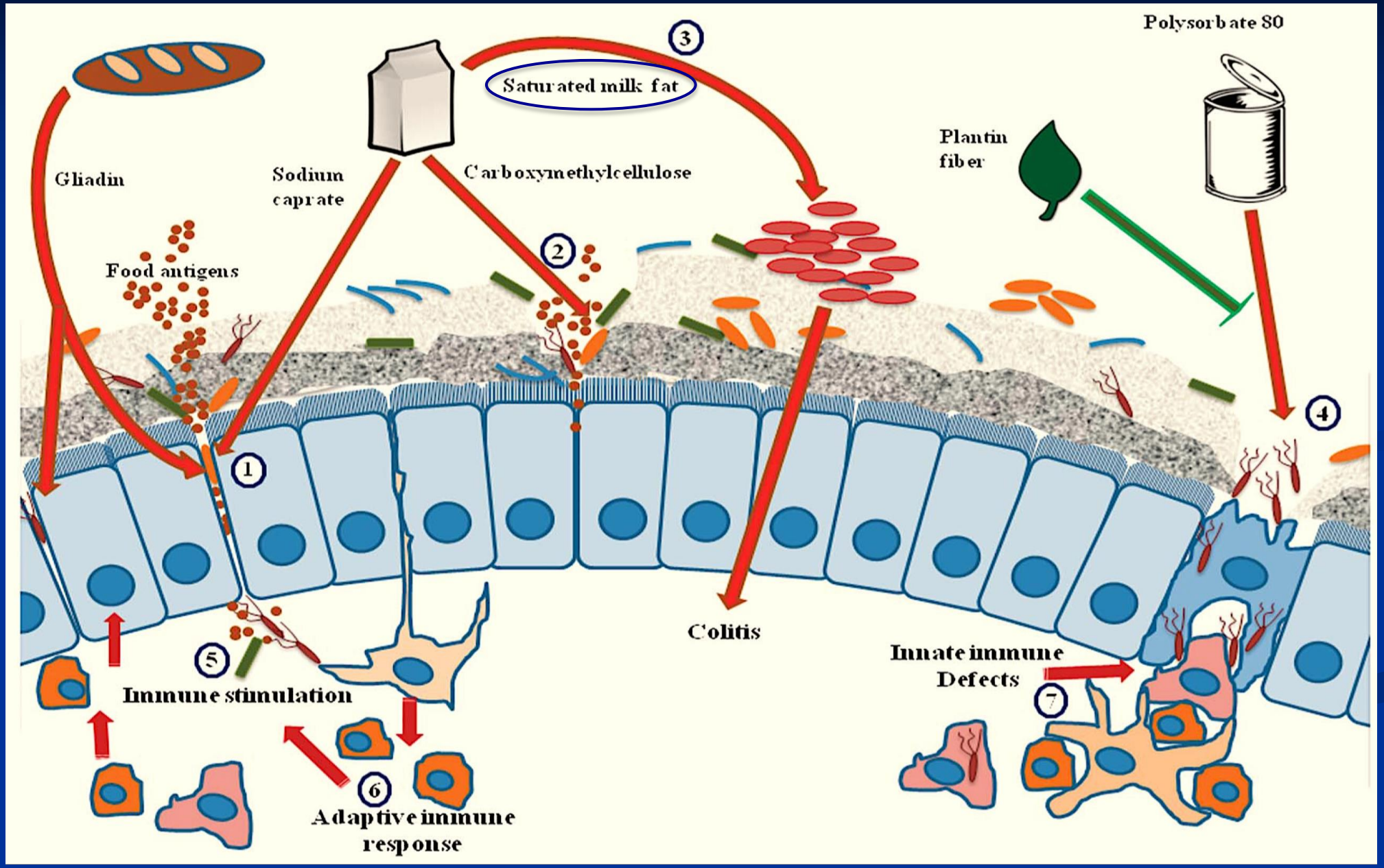
CONTINUOUS TRIGGERING OF THE ADAPTIVE IMMUNE SYSTEM

INFLAMMATION

Examples for effects of dietary components on intestinal permeability and translocation of bacteria

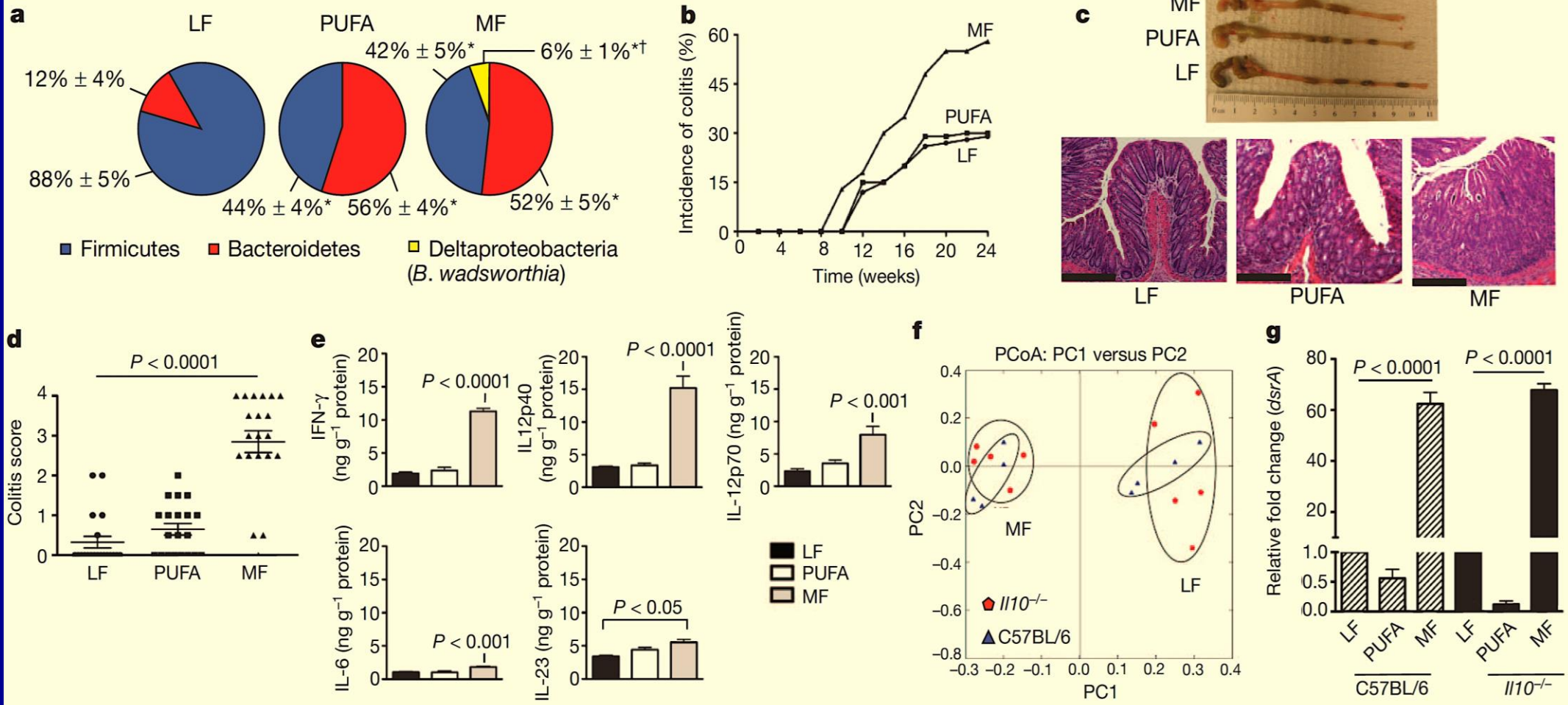


Examples for effects of dietary components on intestinal permeability and translocation of bacteria

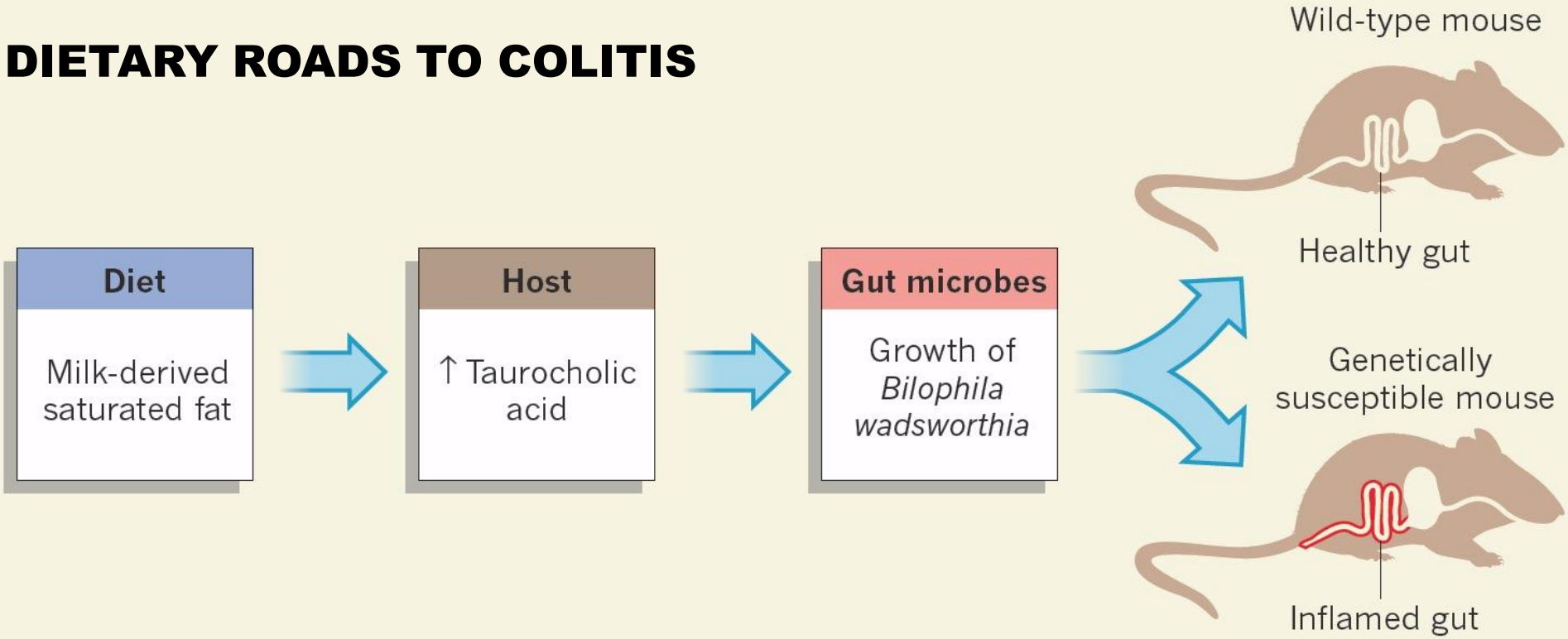


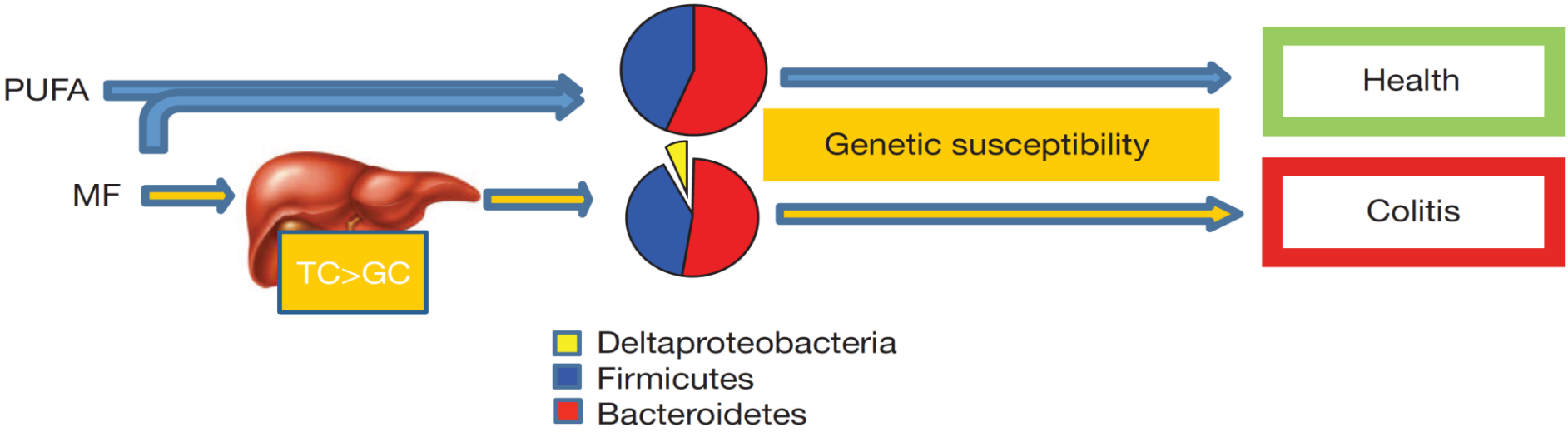
Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10*$^{-/-}$ mice

Suzanne Devkota[1], Yunwei Wang[1], Mark W. Musch[1], Vanessa Leone[1], Hannah Fehlner-Peach[1], Anuradha Nadimpalli[1], Dionysios A. Antonopoulos[2], Bana Jabri[1] & Eugene B. Chang[1]



DIETARY ROADS TO COLITIS





Butyricicoccus pullicaecorum in inflammatory bowel disease

Eeckhaut V, *et al*. *Gut* 2013;**62**:1745–1752.

Venessa Eeckhaut,[1] Kathleen Machiels,[2] Clémentine Perrier,[2] Carlos Romero,[3] Sofie Maes,[1] Bram Flahou,[1] Marjan Steppe,[1] Freddy Haesebrouck,[1] Benedikt Sas,[4] Richard Ducatelle,[1] Severine Vermeire,[2] Filip Van Immerseel[1]

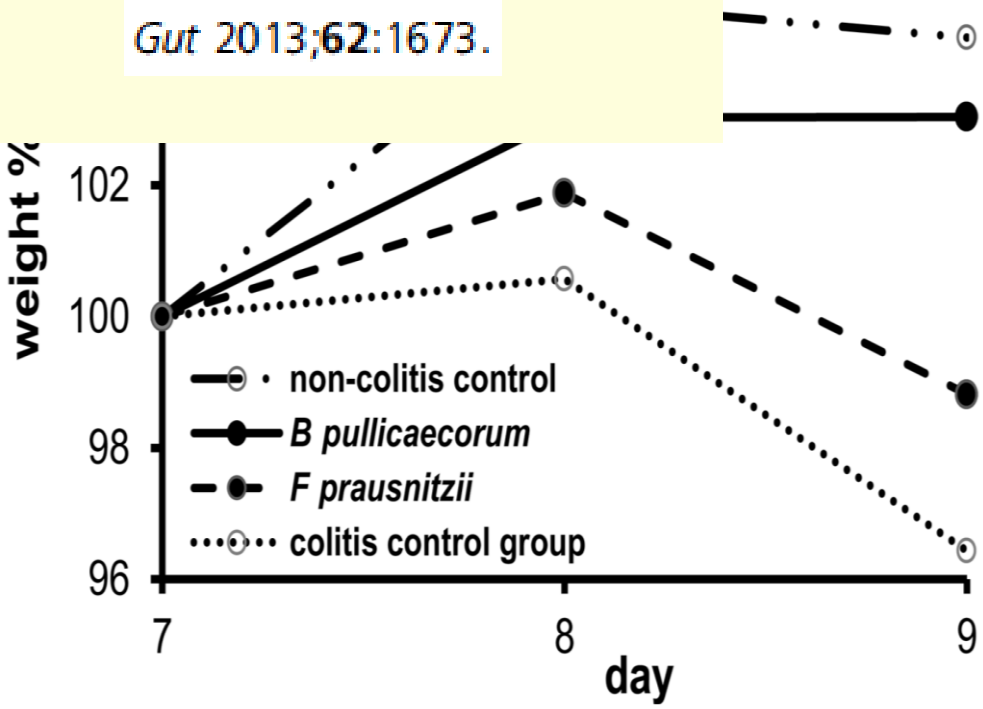
Butyrate-producing bacteria as pharmabiotics for inflammatory bowel disease

Gut 2013;**62**:1673.

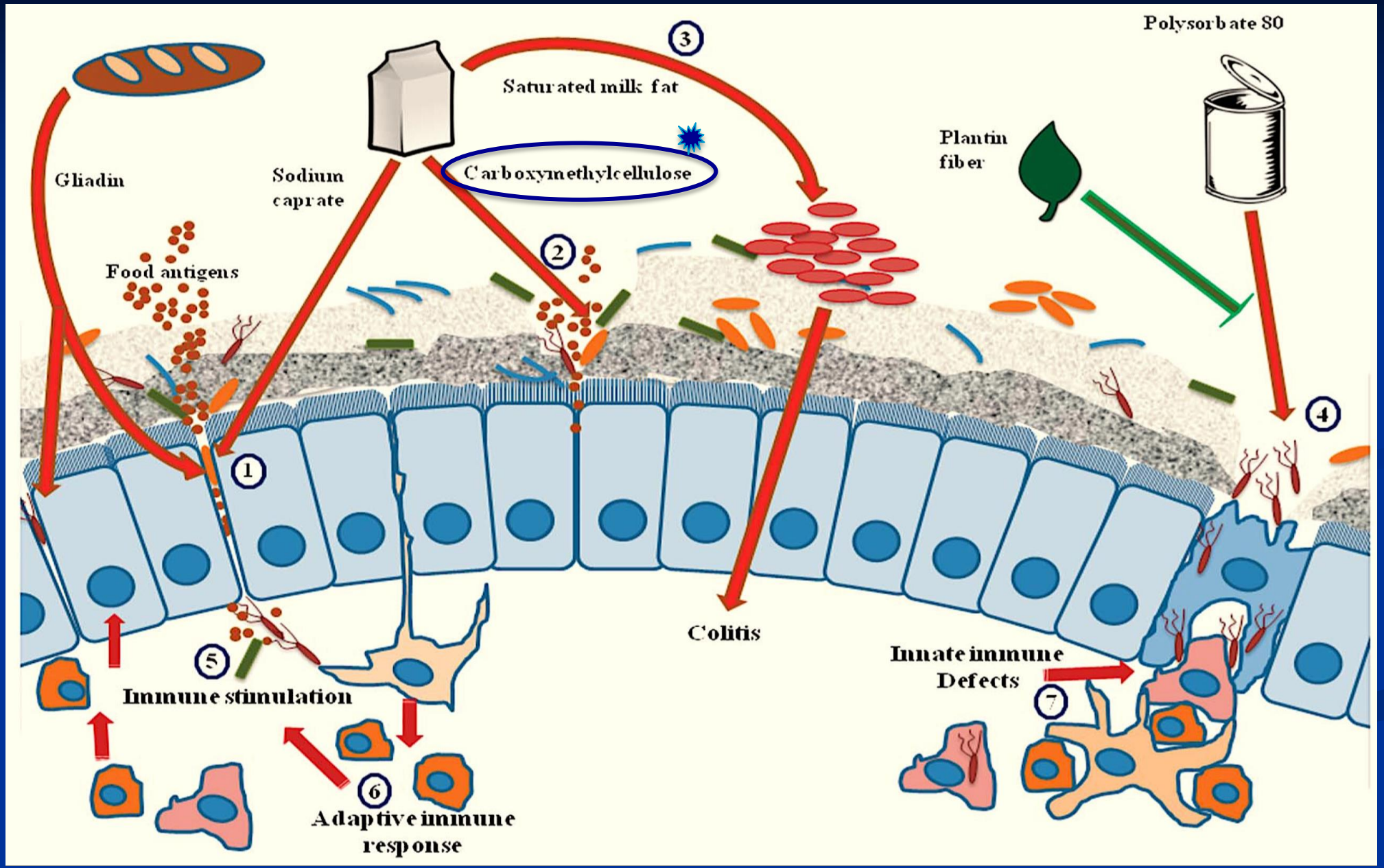
Philippe Marteau

Table 4 The effect o … nduced colitis *prausnitzii* and *Butyric* … microscopic manifestat…

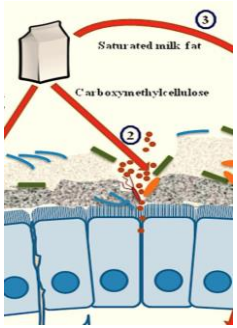
Group	Colon/ body weight (mg/g)	Colon weight/ length (mg/cm)	Macroscopic lesion score	Microscopic lesion score
Colitis control	8.73^{a}±0.68	173^{a}±12.71	6.4^{a}±0.96	15.2^{a}±0.57
F prausnitzii	8.03^{a}±0.70	175^{a}±16.43	4.7^{a}±0.88	12.0^{b}±1.05
B pullicaecorum	6.25^{b}±0.34	129^{b}±8.73	1.8^{b}±0.47	8.2^{c}±1.70
Non-colitis control	5.37^{b}±0.14	104^{b}±2.04	0^{b}	0.8^{d}±0.13



Effects of dietary components on intestinal permeability and translocation of bacteria



Bacterial Overgrowth and Inflammation of Small Intestine After Carboxymethylcellulose Ingestion in Genetically Susceptible Mice



Alexander Swidsinski, MD, Victoria Ung,† Beate C. Sydora, PhD,† Vera Loening-Baucke, MD,* Yvonne Doerffel, MD,‡ Hans Verstraelen, MD,§ and Richard N. Fedorak, MD†*

Background: Detergents and emulsifiers added to food may destroy the mucus barrier, which normally isolates bacteria from the intestinal wall, and lead to chronic bowel inflammation in susceptible persons. We investigated the influence of 2% carboxymethylcellulose (CMC) on the biostructure of the intestinal microbiota in IL-10 gene-deficient mice.

Methods: Twenty to 27-week-old IL-10 gene-deficient mice received either 2% CMC solution ($n = 7$) or water ($n = 6$) orally for 3 weeks. Intestinal bacteria were investigated using fluorescence in situ hybridization in paraffin-fixed sections of the intestine.

Results: CMC-treated IL-10 gene-deficient mice demonstrated a massive bacterial overgrowth, distention of spaces between villi, with bacteria filling these spaces, adherence of bacteria to the mucosa, and migration of bacteria to the bottom of the crypts of Lieberkuehn. Leukocytes migrated into the intestinal lumen in 4 of the 7 CMC mice. The changes were similar to those observed in Crohn's disease in humans and were absent in control animals.

Conclusions: CMC induces bacterial overgrowth and small bowel inflammation in susceptible animals. Because of its ubiquity in products and its unrestricted use in food of the industrial world, CMC is an ideal suspect to account for the rise of IBD in the 20th century.

(Inflamm Bowel Dis 2009;15:359–364)

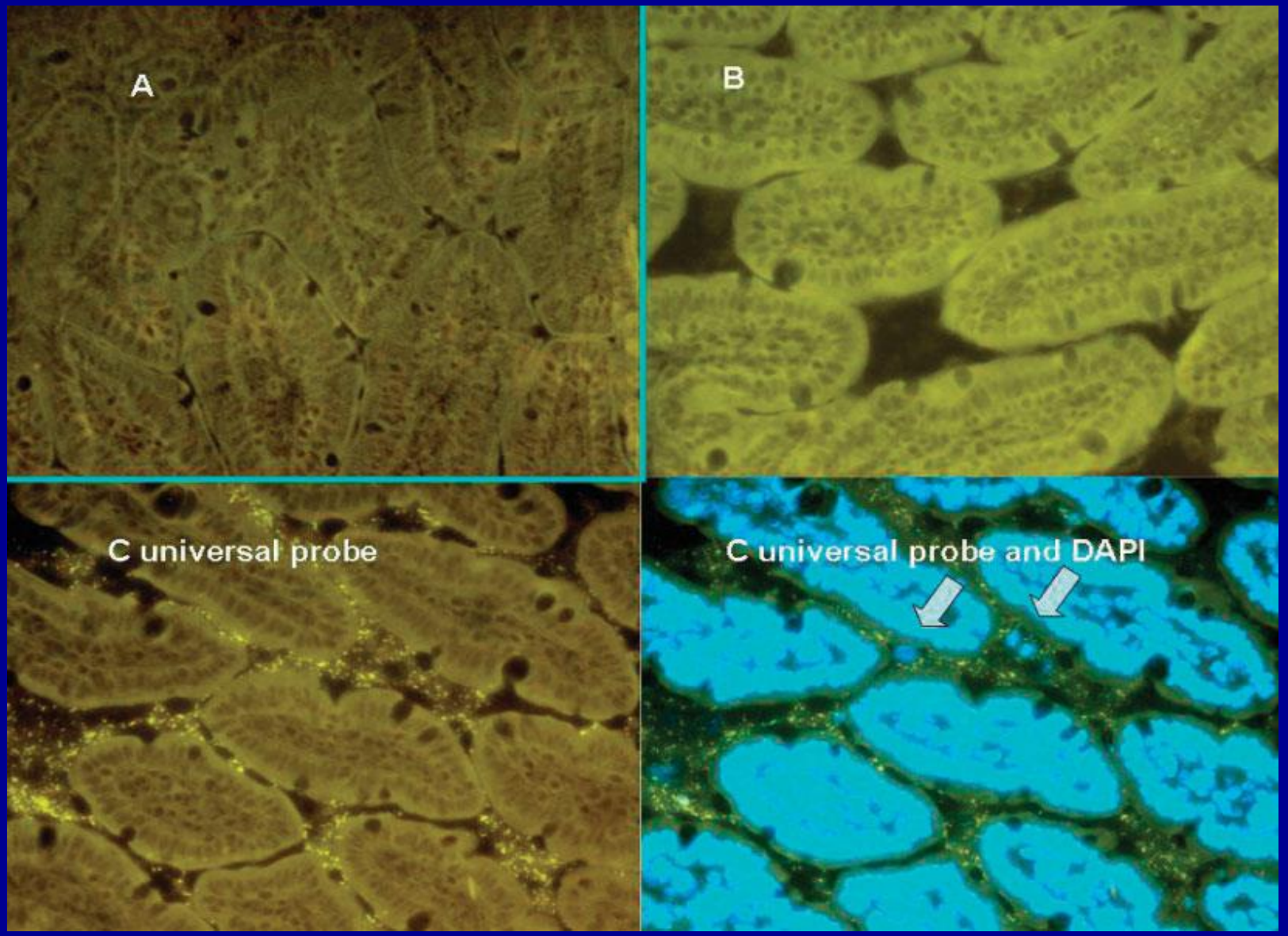
Key Words: carboxymethylcellulose, biostructure, intestinal microbiota

The healthy mucosa is protected from contact with potentially harmful bacteria such as *Bacteroides, Enterobacteriaceae, Enterococci* or *Clostridium perfringens* by an impenetrable, highly viscous mucus barrier. Before bacteria can adhere to and invade the mucosa, they must cross the 40–240-μm thick mucus layer, which is normally free of bacteria.[1] As long as the mucus barrier is intact, the organism ignores the high concentrations of bacteria in the lumen of the large intestine and tolerates the bacteria without an immune response.[2] The mucus barrier is deficient in patients with inflammatory bowel disease (IBD), where bacteria contact the mucosa and build polymicrobial biofilms on the epithelial surface prior to the inflammatory response.[3] The reasons for this deficiency are unclear.

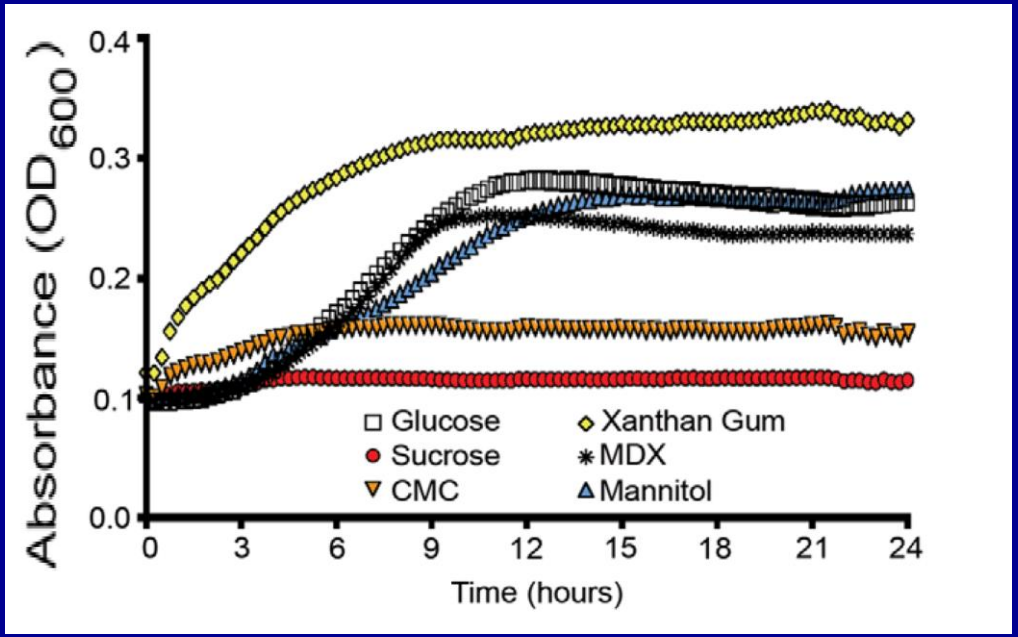
Since the beginning of the 20th century there has been a steady increase in the incidence of both Crohn's disease (CD) and ulcerative colitis (UC).[4] In parallel, detergents are increasingly utilized in households and emulsifiers are increasingly added to food. The "cleaning" effect of emulsifiers and detergents on the colonic mucus has never been investigated. The most prominent emulsifying substance added to the vast majority of food products is carboxymethylcellulose (CMC).

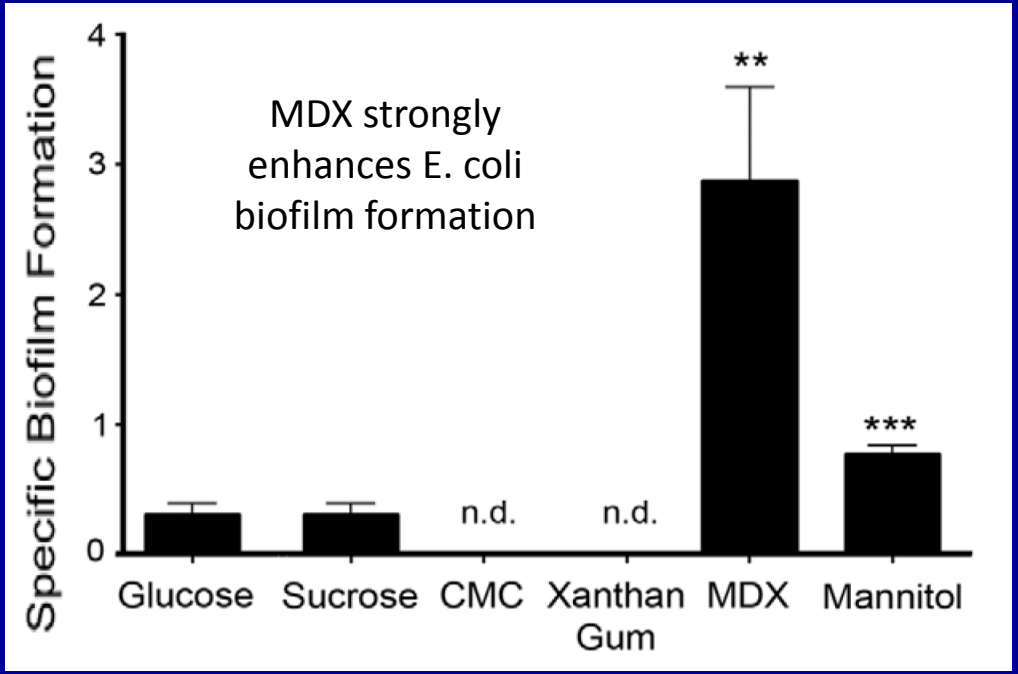
The present study investigates the changes in the biostructure of the intestinal microbiota after 3 weeks of daily exposure to CMC in IBD-susceptible IL-10 gene-deficient mice.

Bacterial Overgrowth and Inflammation of Small Intestine After Carboxymethylcellulose Ingestion in Genetically Susceptible Mice. Inflamm Bowel Dis 2009;15:359-364



Jejunum of IL-10 gene-deficient mice (A: water-treated; B,C: CMC-treated mouse) hybridized with the universal Eub338-Cy3 probe (orange fluorescence) 400.





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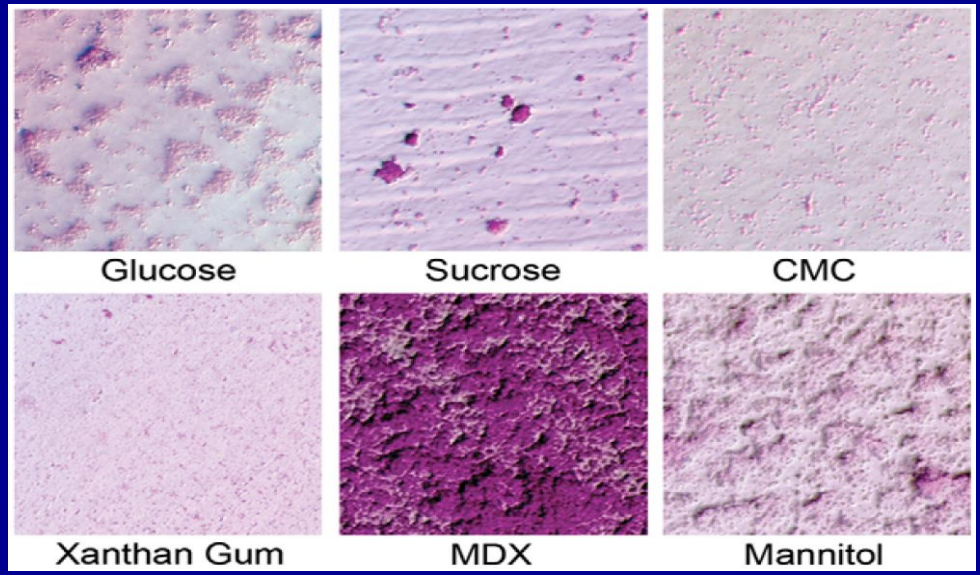
Crohn's Disease-Associated Adherent-Invasive *Escherichia coli* Adhesion Is Enhanced by Exposure to the Ubiquitous Dietary Polysaccharide Maltodextrin

Kourtney P. Nickerson[1,2], Christine McDonald[1,2]*

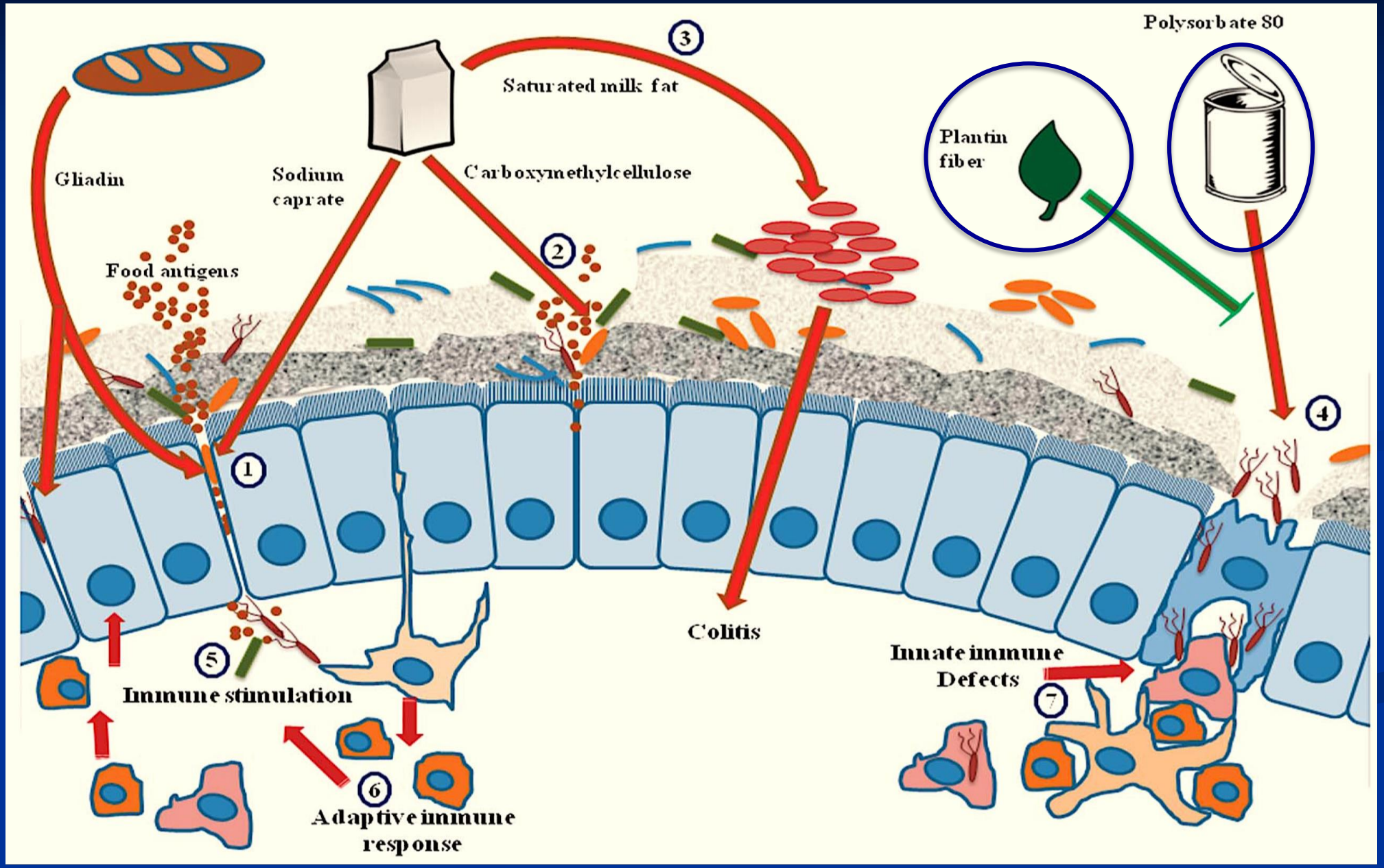
1 Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, United States of America, **2** Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, Ohio, United States of America

Abstract

Crohn's disease (CD) is associated with intestinal dysbiosis evidenced by an altered microbiome forming thick biofilms on the epithelium. Additionally, adherent-invasive *E. coli* (AIEC) strains are frequently isolated from ileal lesions of CD patients indicating a potential role for these strains in disease pathogenesis. The composition and characteristics of the host microbiome are influenced by environmental factors, particularly diet. Polysaccharides added to food as emulsifiers, stabilizers or bulking agents have been linked to bacteria-associated intestinal disorders. The escalating consumption of polysaccharides in Western diets parallels an increased incidence of CD during the latter 20th century. In this study, the effect of a polysaccharide panel on adhesiveness of the CD-associated AIEC strain LF82 was analyzed to determine if these food additives promote disease-associated bacterial phenotypes. Maltodextrin (MDX), a polysaccharide derived from starch hydrolysis, markedly enhanced LF82 specific biofilm formation. Biofilm formation of multiple other *E. coli* strains was also promoted by MDX. MDX-induced *E. coli* biofilm formation was independent of polysaccharide chain length indicating a requirement for MDX metabolism. MDX exposure induced type I pili expression, which was required for MDX-enhanced biofilm formation. MDX also increased bacterial adhesion to human intestinal epithelial cell monolayers in a mechanism dependent on type 1 pili and independent of the cellular receptor CEACAM6, suggesting a novel mechanism of epithelial cell adhesion. Analysis of mucosa-associated bacteria from individuals with and without CD showed increased prevalence of *malX*, a gene essential for MDX metabolism, uniquely in the ileum of CD patients. These findings demonstrate that the ubiquitous dietary component MDX enhances *E. coli* adhesion and suggests a mechanism by which Western diets rich in specific polysaccharides may promote dysbiosis of gut microbes and contribute to disease susceptibility.

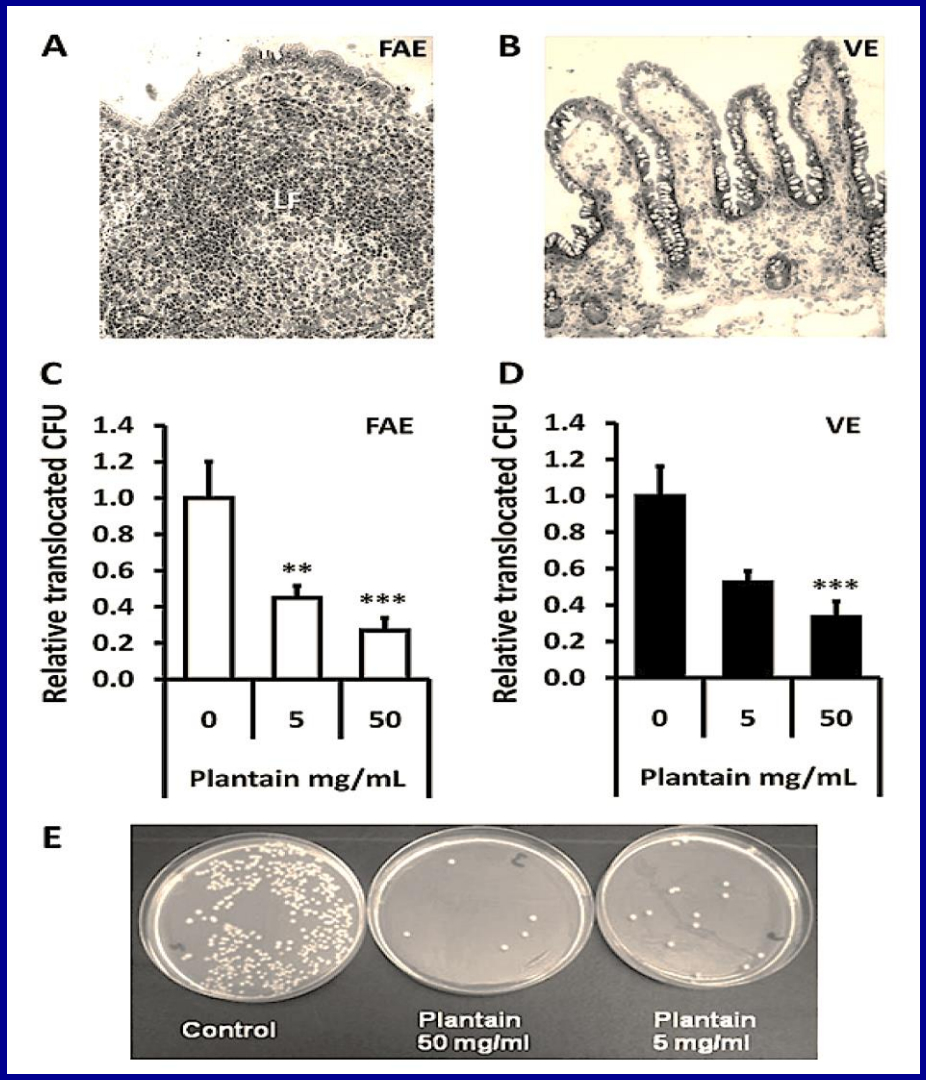


Effects of dietary components on intestinal permeability and translocation of bacteria

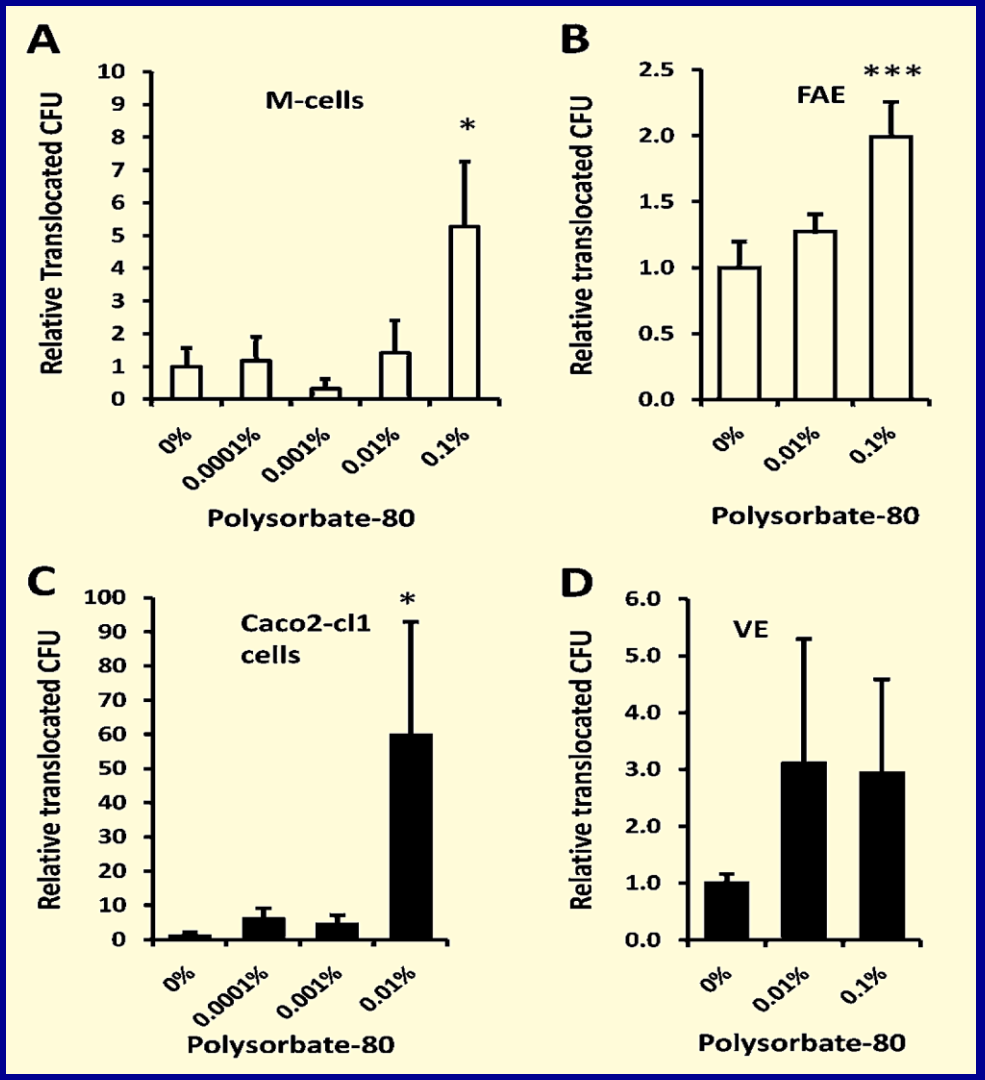


Translocation of Crohn's disease *Escherichia coli* across M-cells: contrasting effects of soluble plant fibres and emulsifiers

Gut 2010;**59**:1331—1339. doi:10.1136/gut.2009.195370



Plantain NSP blocks translocation of E coli across the human intestinal epithelium in Ussing chambers.



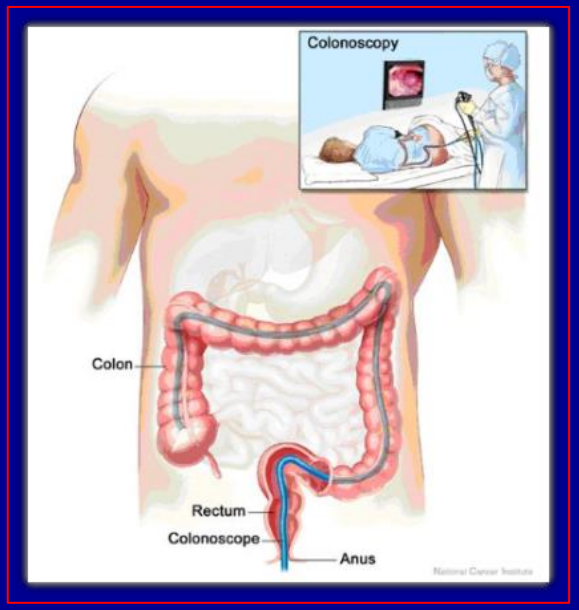
Dietary emulsifier polysorbate-80 increases translocation of E coli across the intestinal epithelium

GENETICA

(GWAS)







SIEROLOGIA

Markers:
p-ANCA, ASCA, anti-OMPC, anti-cBir1…..etc……



1. ISTOLOGIA (routine)

2. IMMUNOLOGIA MUCOSALE
TH1, TH2, TH17…..
Signaling pathways
Molecole di adesione…..

3. MICROBIOMA
(Gut mucosa associated bacteria (specie, taxa….)

Feci, Urine:

METABOLOMICA

Foreseeable (?) clinical scenario:……2018..……2020…..

2 patients with Crohn's disease: similar presentation, different outcomes

Onset: two 12y/o boys with failure to grow for one year, anemia, weight loss, abdominal pain, diarrhea sometimes bloody

Evaluation: ileal Crohn's disease for 35 cm, inflammatory, ulcers, no fistulae, no stenosis

Treatment (classic): methyl-prednisolone + azathioprine (as mainenance)

Patient A

Initial benign course
Felt well – after 6 months: flare up, re-evaluation, again steroids + AZA, response less satisfactory than earlier, need for frequent evaluations

Patient B

Course with complications: Intra-abdominal abscess, drained, ileal resection.
Fever, low quality of life
Antibiotic courses
Post-surgery Infliximab

2 patients with Crohn's disease, 2 different treatments (2018……2020…?)

Patient A

- Tissue shows low *F. prausnitzii*, high adherent/invasive *E.coli (AIEC)*
- Genotype-NOD2 polymorphism, elevated OMP-C
- Treatment: Ciprofloxacin x 1 month, *F. prausnitzii* probiotic weekly, daily plantain extract (prevent AIEC binding) (backup-early resection and prevent recurrence with *F. prausnitzii* probiotic)
- No clinical symptoms or Rx complications for 15 years

Patient B

- Genotype IRGM polymorphism, low Clostridium group XIVa (Firmicutes) (associated with activation of Treg cells), high titers ASCA and cBir1, high IL-17 levels on biopsies
- Treatment: induction with anti-IL-23, prolonged administration of GM-CSF and novel Clostridium cocktail
- No further Crohn's disease symptoms for 15 years

DEFINING IBD SUBTYPES

Genetic susceptibility	Luminal antigenic drive	Immune responses	Clinical phenotypes
Gene I	Bacteria I	Immune responses I	IBD I
Gene II	Bacteria II	Immune responses II	IBD II
Gene III	Bacteria III	Immune responses III	IBD III



IBD1, IBD2, IBD3, IBD4……IBDn