IL MICROBIOMA

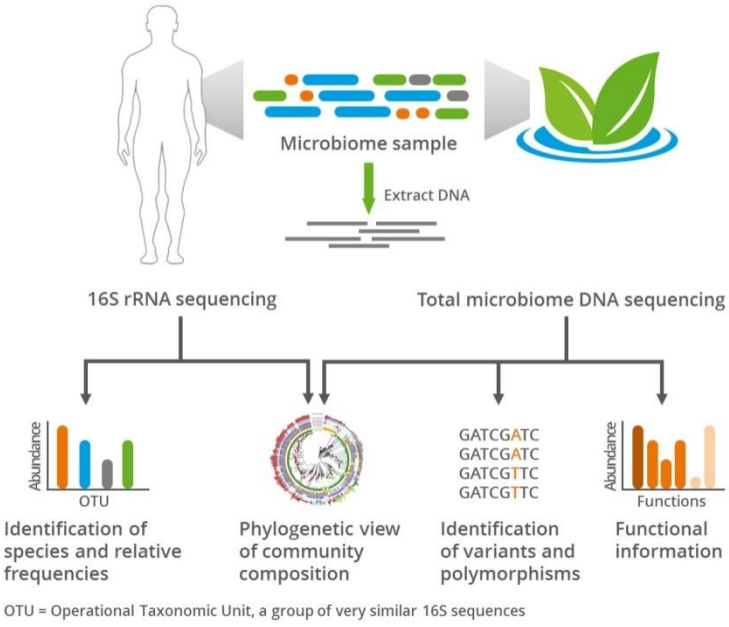
Enrico S. Corazziari

UNIVERSITÀ "SAPIENZA" *Alumni*, ROMA
enrico.corazziari@uniroma1.it

Senior Consultant
U.O. Gastroenterologia
ISTITUTO CLINICO HUMANITAS, ROZZANO
enrico.corazziari@humanitas.it

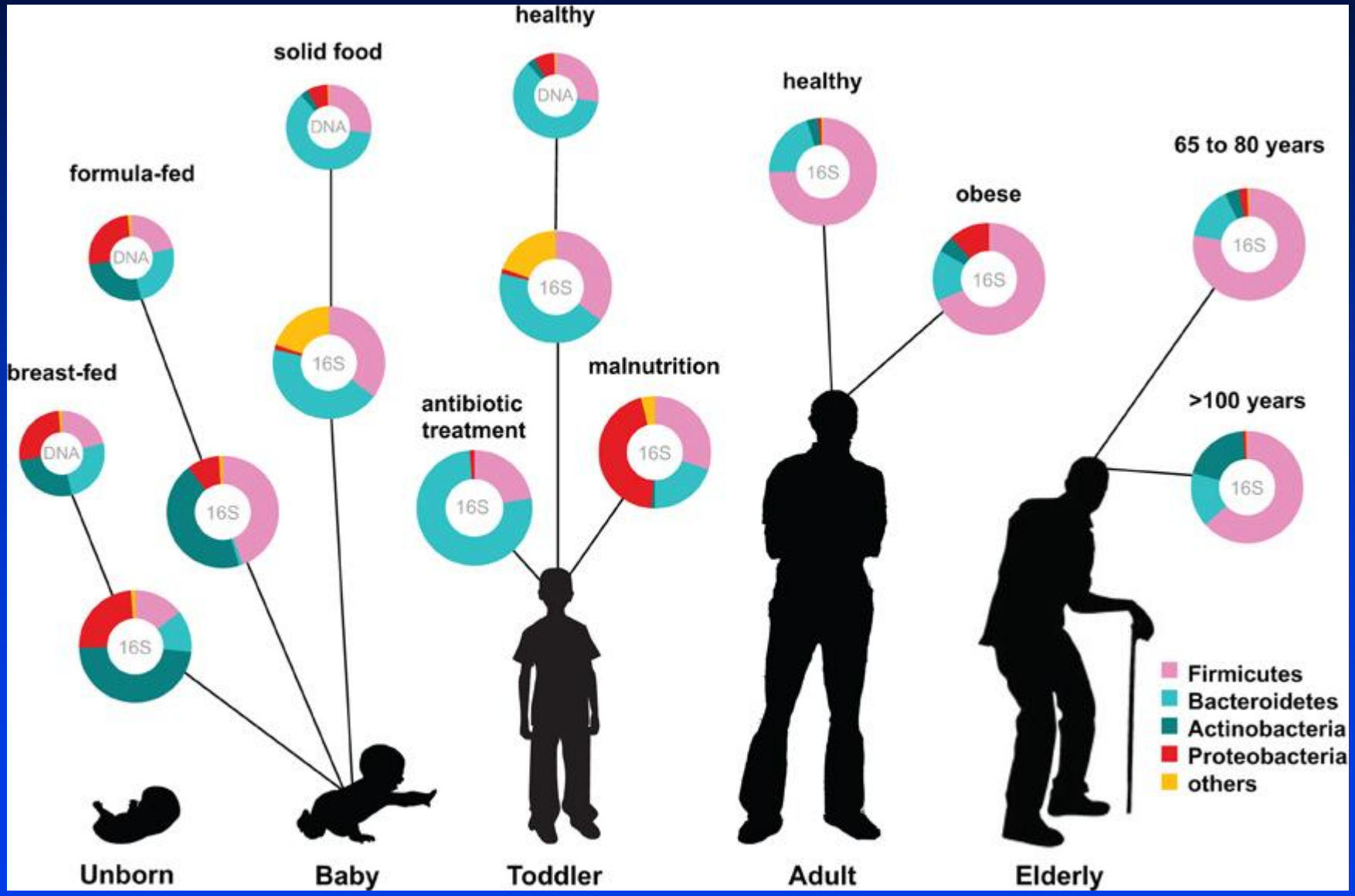
IL NUOVO MONDO

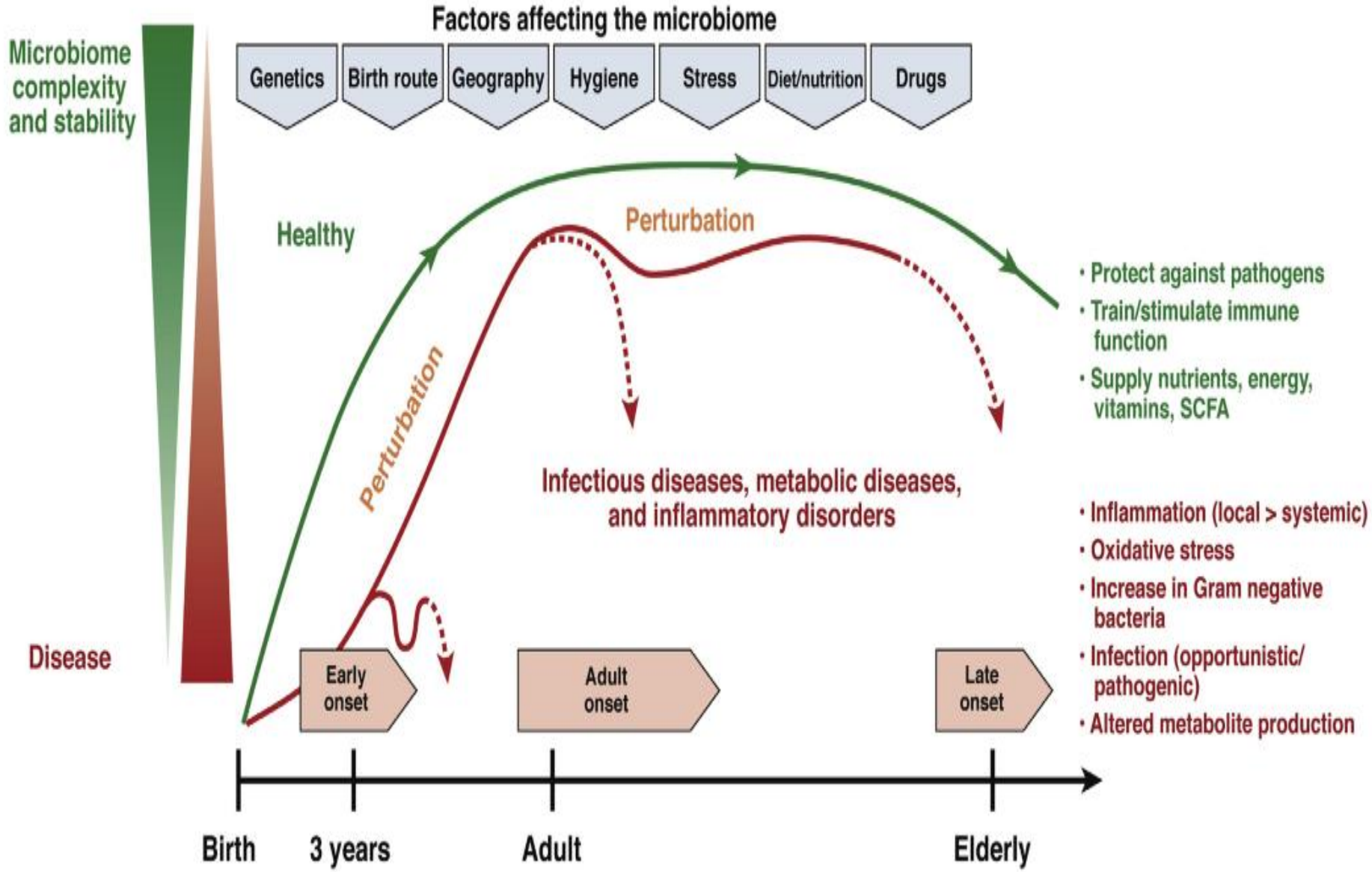
TUTTO DA SCOPRIRE



Human **microbiota is made of symbiotic microorganisms living in the human body**

MICROBIOTA IN THE DIFFERENT AGES

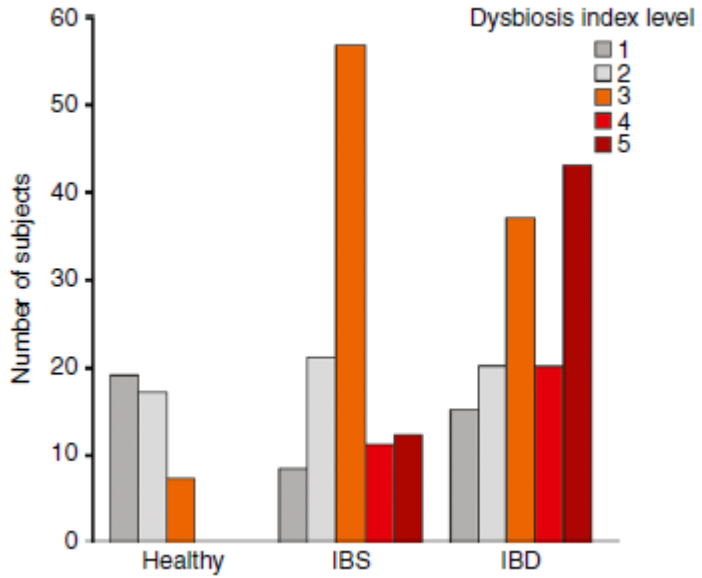




Kostic A D et al. Gastroenterology 2014;146:1489-1499

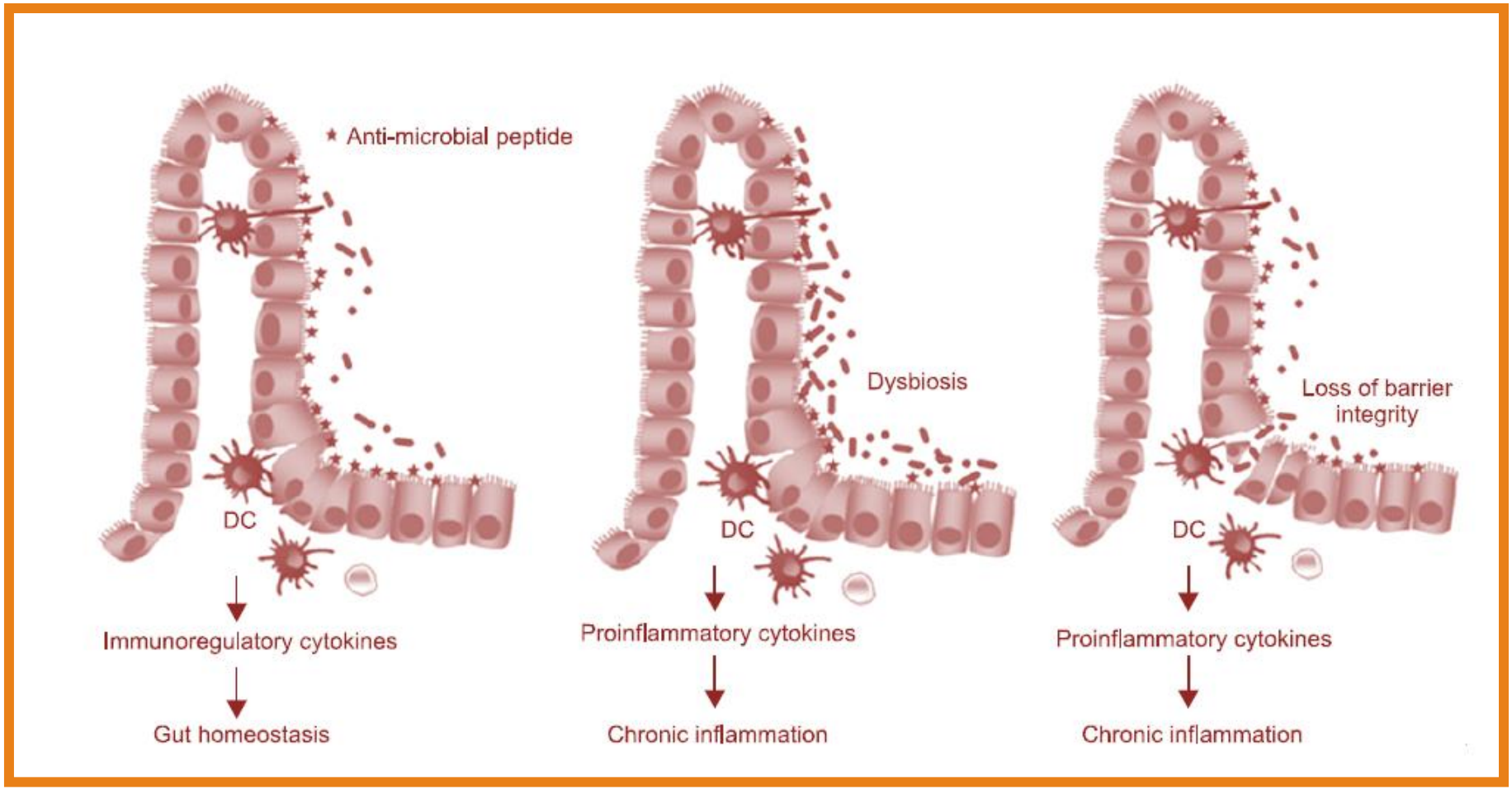
EUBIOSIS Average microbiota representation of healthy individuals

DYSBIOSIS A gradual deviation from Eubiosis

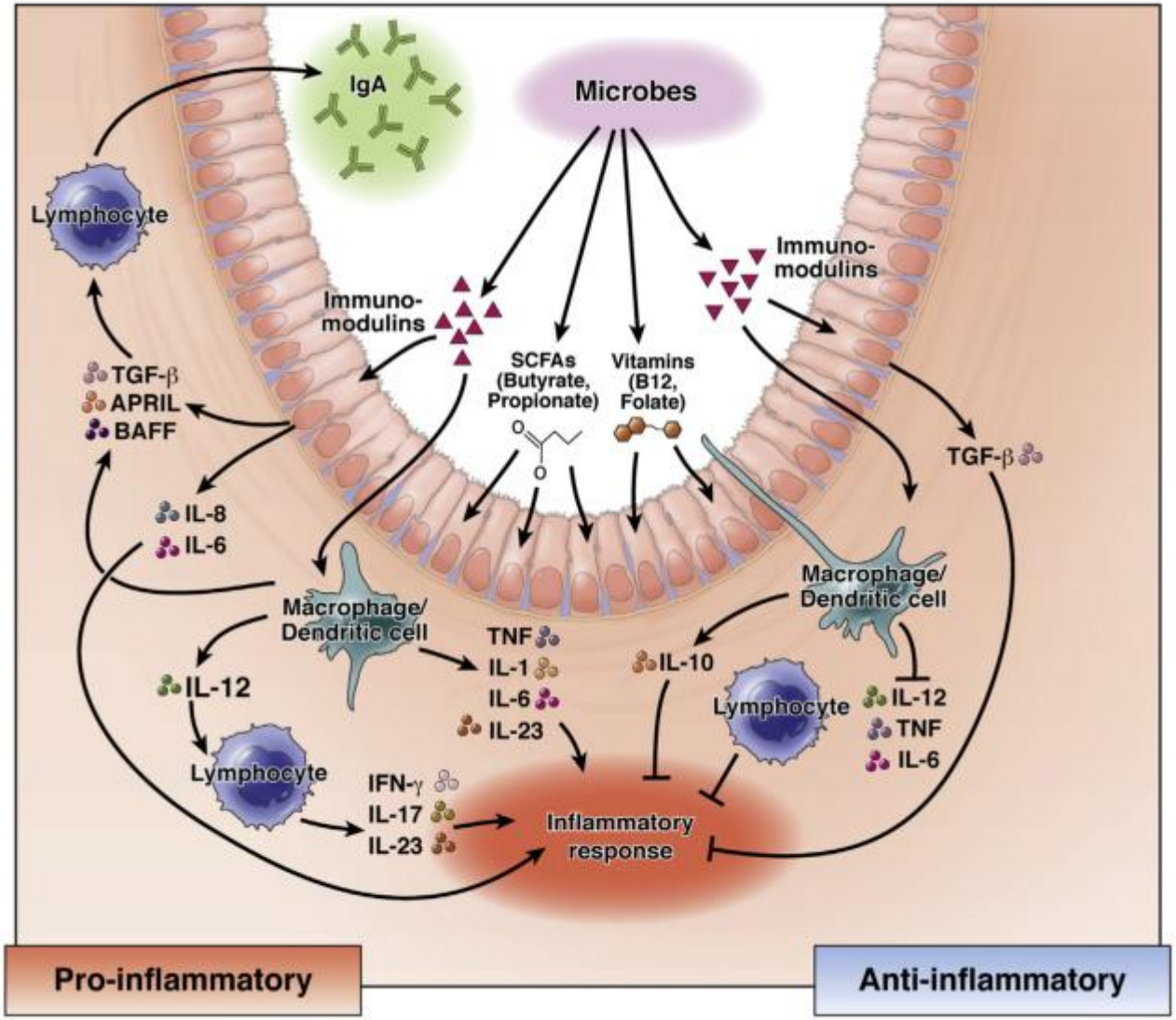


Casen et al APT 2015

MICROBIOTA EUBIOSIS, DYSBIOSIS AND EPITHELIAL IMMUNE BARRIER

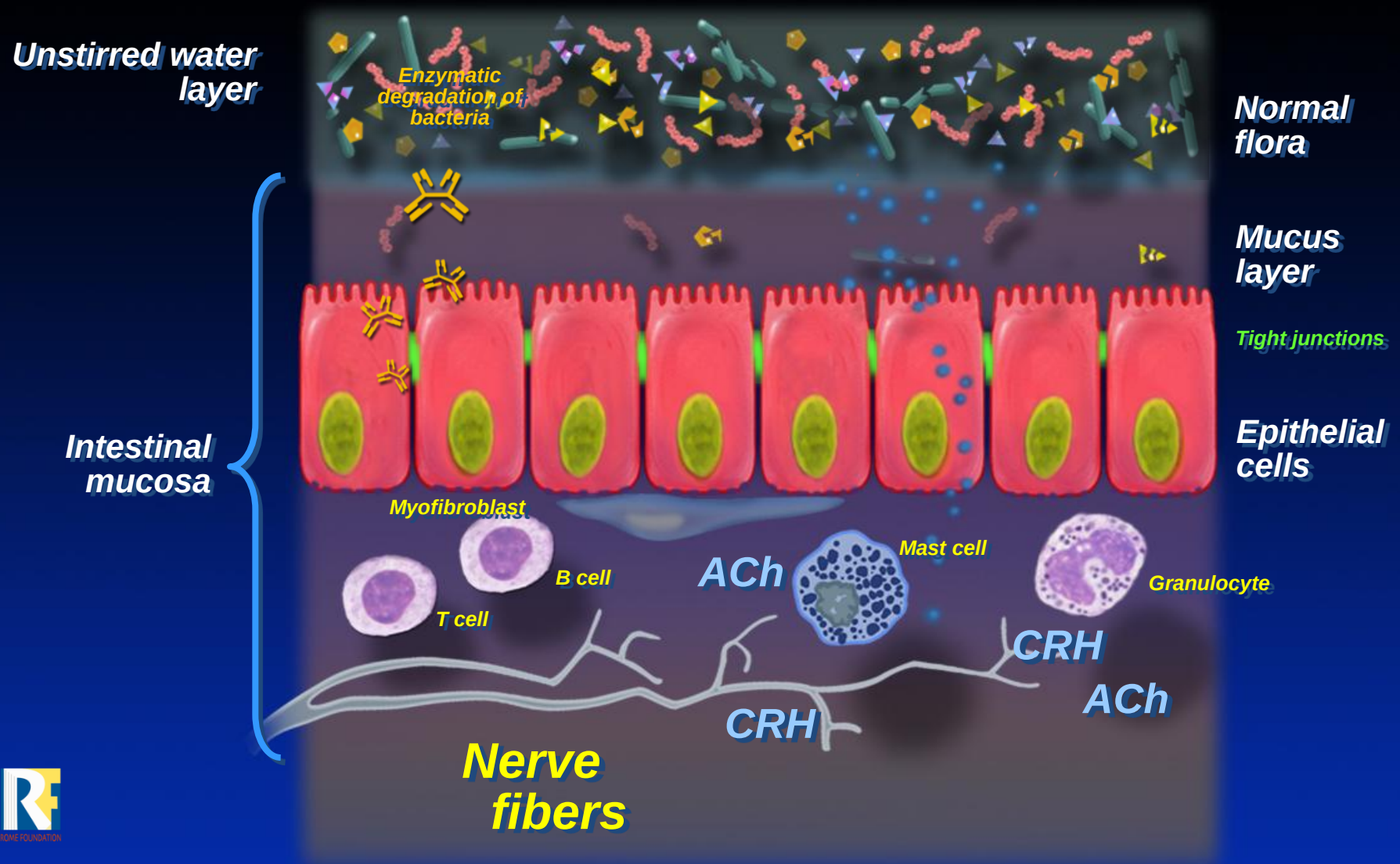


IMMUNOMODULATION OF THE INTESTINAL MICROBIOTA



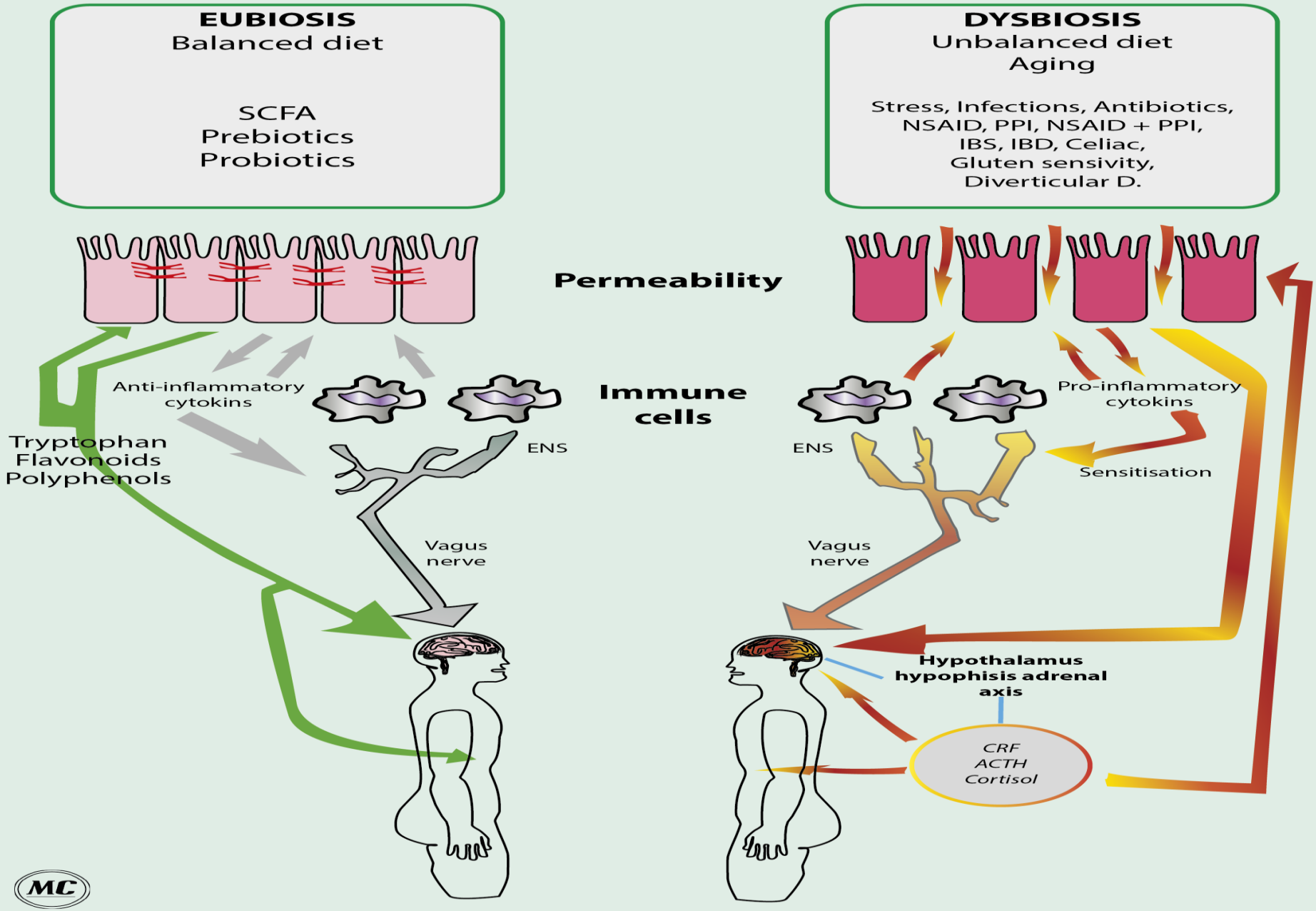
Hollister EB et al. Gastroenterology 2014;146:14491458

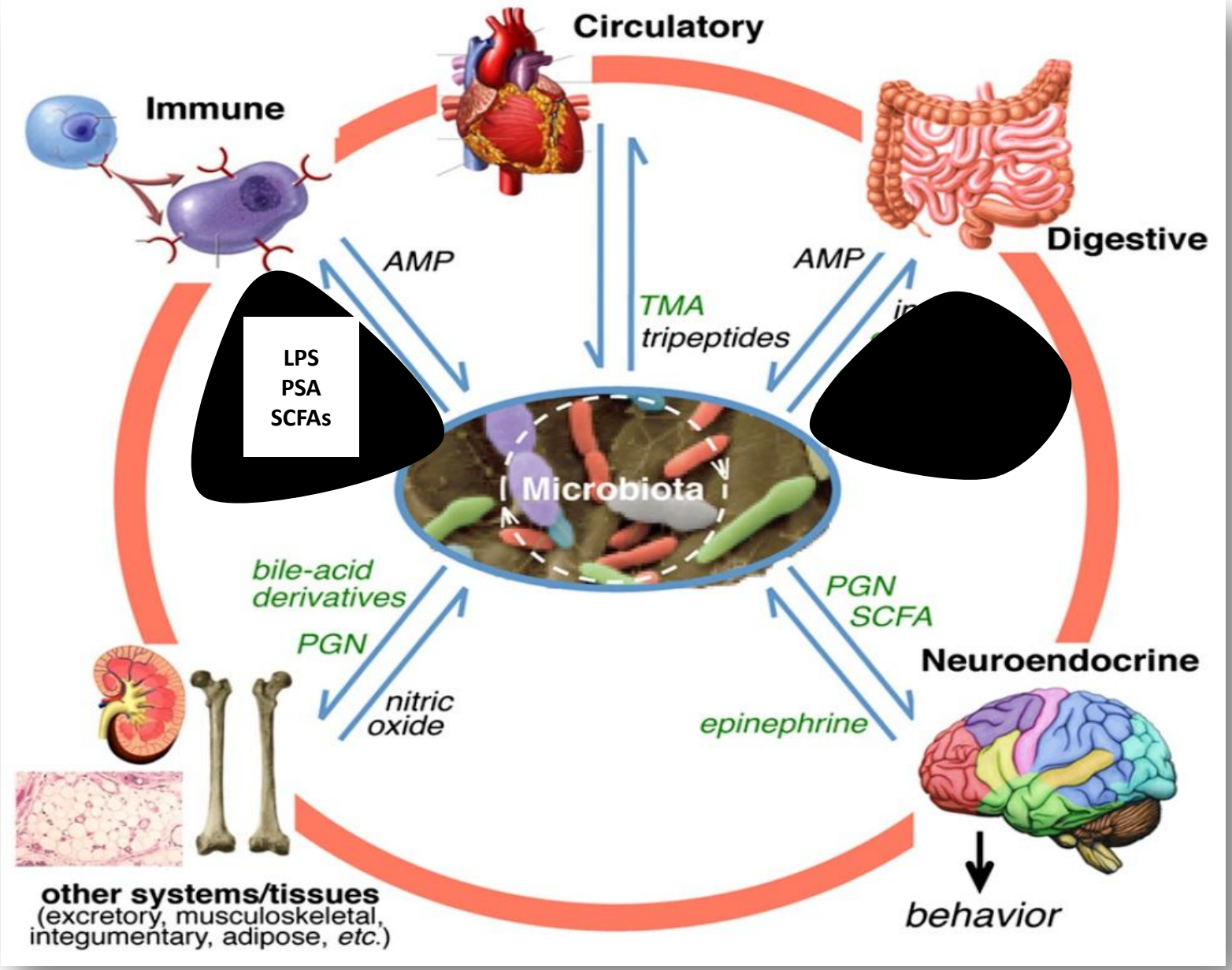
The Mucosal Epithelium Is a Barrier to the Entry of Antigenic Threats From the Intestinal Lumen



Soderholm JD and Perdue M Am J Physiol 2001; 280:G7

INTERACTIONS BETWEEN INTESTINAL ECOSYSTEM, SNC AND ENTIRE ORGANISM




Circulatory
Immune
Digestive
AMP
AMP
TMA
tripeptides
LPS
PSA
SCFAs
Microbiota
bile-acid
derivatives
PGN
nitric
oxide
PGN
SCFA
epinephrine
Neuroendocrine
behavior
other systems/tissues
(excretory, musculoskeletal,
integumentary, adipose, etc.)

DYSBIOSIS & DISEASE

Perturbation of the GI microbiota has been demonstrated in

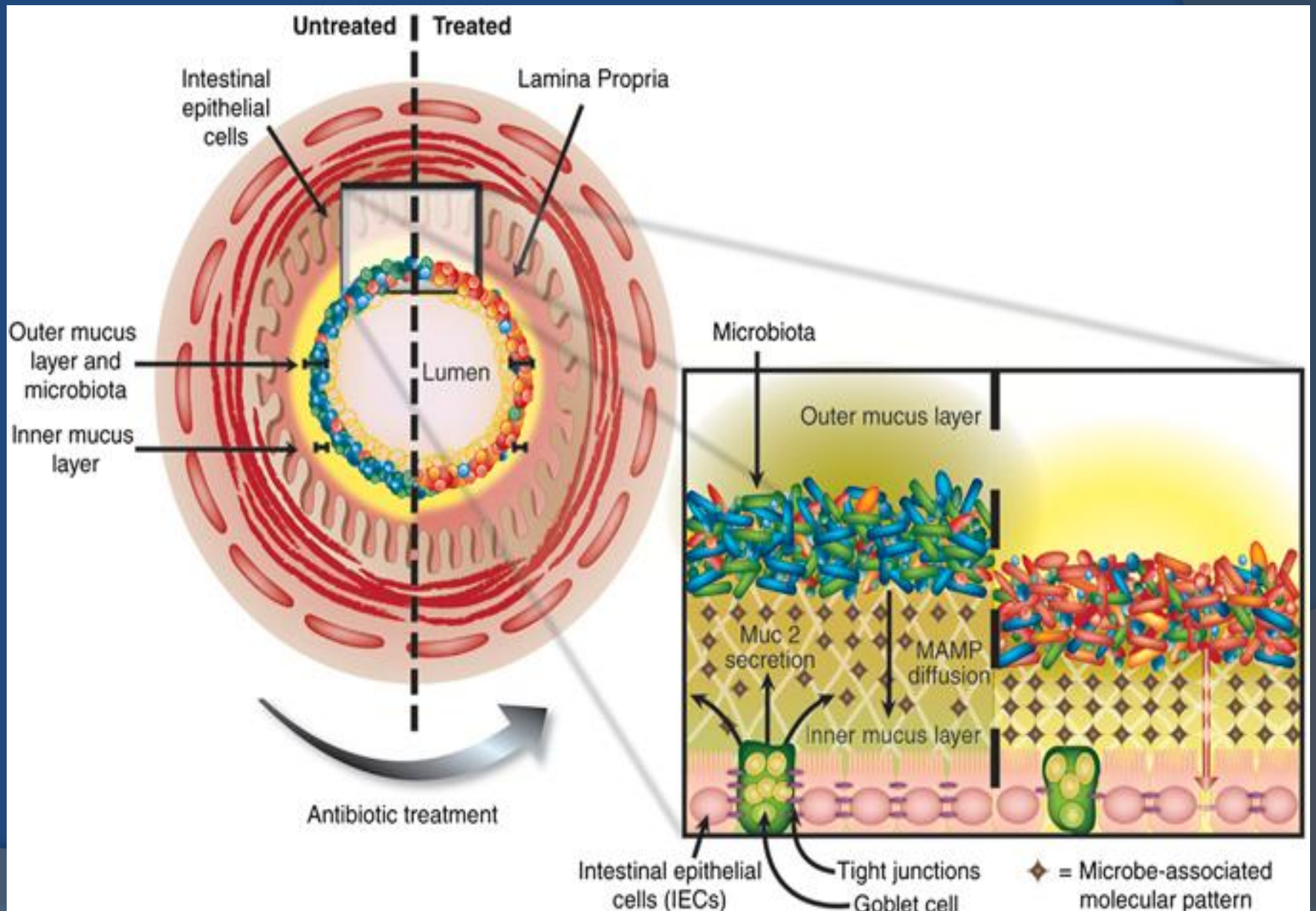
Pathophysiology of

- Antibiotic-Associated diarrhea
- Clostridium Difficile Colitis
- Necrotizing entero-colitis

Association with

- Ulcerative Colitis
- Crohn's Disease
- IBS
- Celiac disease
- Non Alcoholic Steatohepatitis
- Primary Biliary Cirrhosis
- Primary Sclerosing Cholangitis
- Colorectal cancer
- Obesity
- Type 1 & 2 Diabetes
- Atopic dermatitis
- Upper Respiratory Infections
- Bacterial vaginosis
- Urinary Tract Infections
- Behavioral alterations (Autism)
- Psychological alterations
- Degenerative neurological diseases

EFFECT OF ANTIBIOTICS ON INTESTINAL ECOSYSTEM



EFFECTS OF ANTIBIOTICS ON GUT MICROBIOTA

DECREASE

- Taxonomic Richness, Diversity & Eveness

AFFECT

- Gene Expression
- Protein Activity
- Metabolism

IT MAY AFFECT ALL HUMAN PHYSIOLOGY PROCESSES THAT RELY ON MICROBIOTA ACTIVITIES

EFFECT OF ANTIBIOTICS ON INTESTINAL MICROBIOTA

- have short and long term effect on intestinal microbiota
- **May affect all physiology processes that rely on microbiota activities**

An altered microbiota composition may

- Promote pathogen activation
- Increase mucosal permeability
- Induce mucosal low grade inflammation
- Disactivate amtimutagenic and anticarcinogenic effects

SHORT TERM EFFECTS OF ANTIBIOTICS

- Antibiotic Associated Diarrhea(AAD)
- Overgrowth of Nosocomial Pathogens
  - *Klebsiella Pneumoniae*
  - *Staphilococcus Aureus*
  - *Clostridium Difficile*
- Colonization of Opportunistic Pathogen
  - *Candida Albicans*

AFFECT ALL HUMAN PHYSIOLOGY PROCESSES THAT RELY ON MICROBIOTA ACTIVITIES

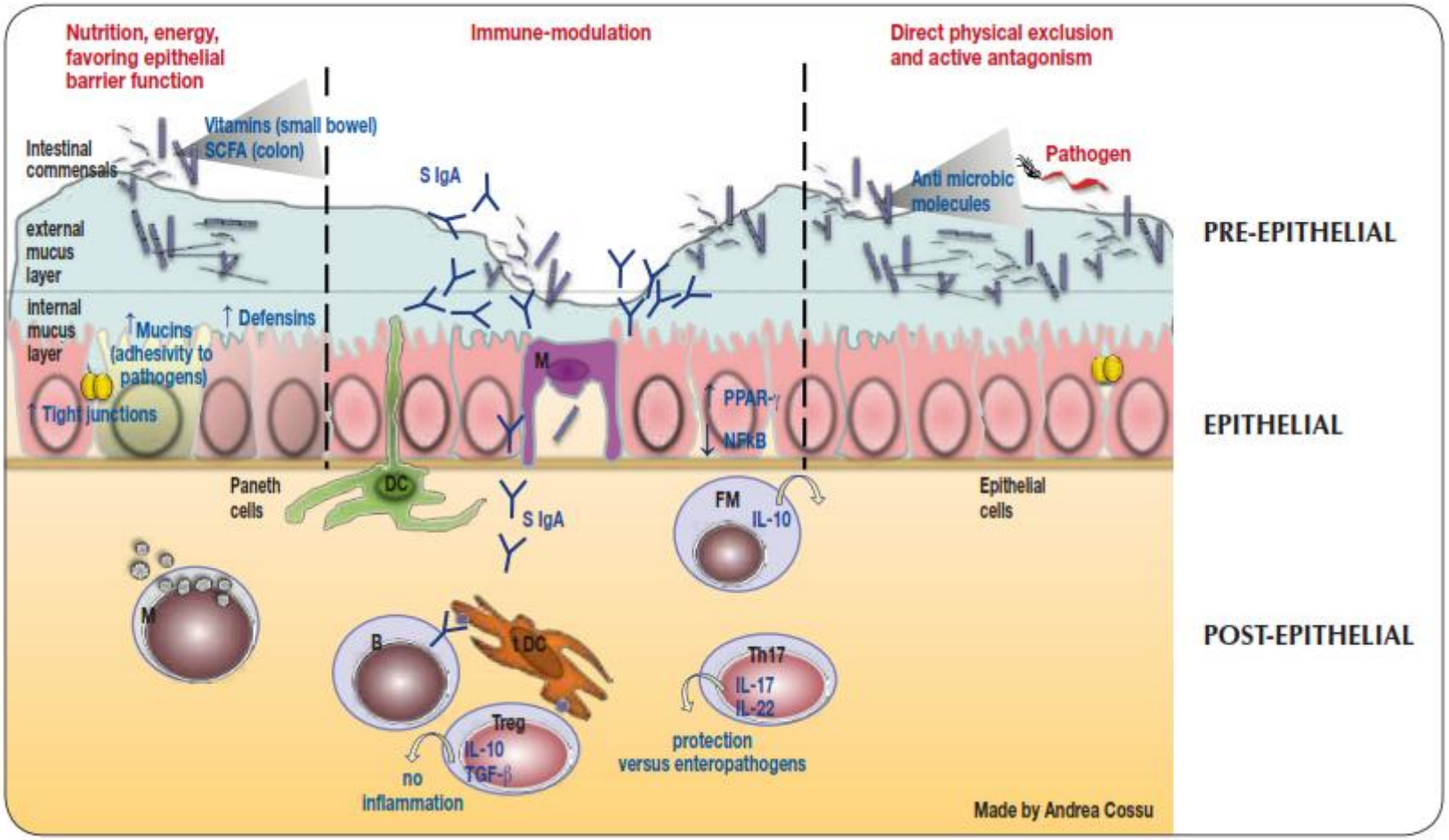
ANTIBIOTIC ASSOCIATED DIARRHEA

LOW RISK: *metronidazole and parenteral aminoglycosides (gentamicin);*

MEDIUM RISK: *tetracyclines (oxytetracycline), sulphonamides (trimethoprim), macrolides (azithromycin, clarithromycin, erytrhomycin), quinolones (ciprofloxacin);*

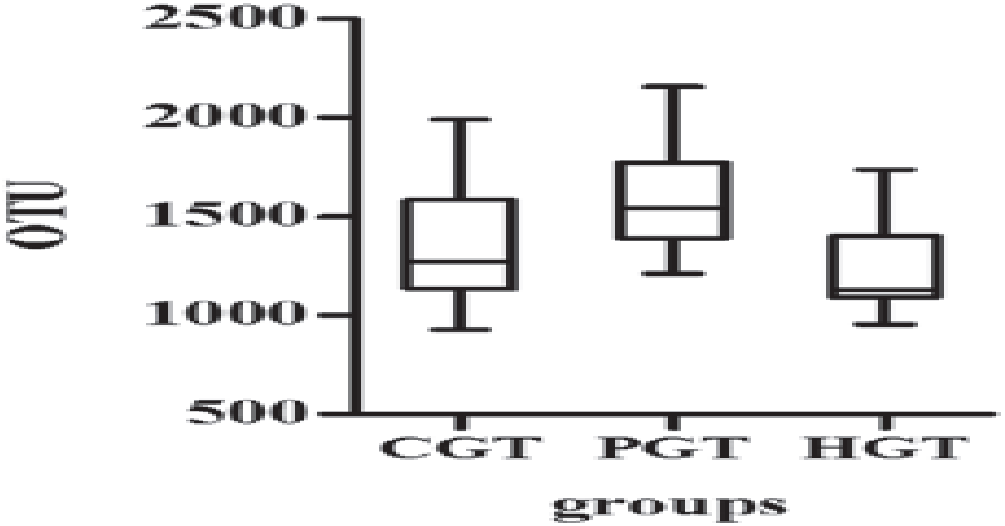
HIGH RISK: *aminopenicillin (amoxicillin, benzylpenicillin, co-amoxiclav, flucloxacillin), cephalosporins (cefalexin, ceftazidime, cefuroxime)*

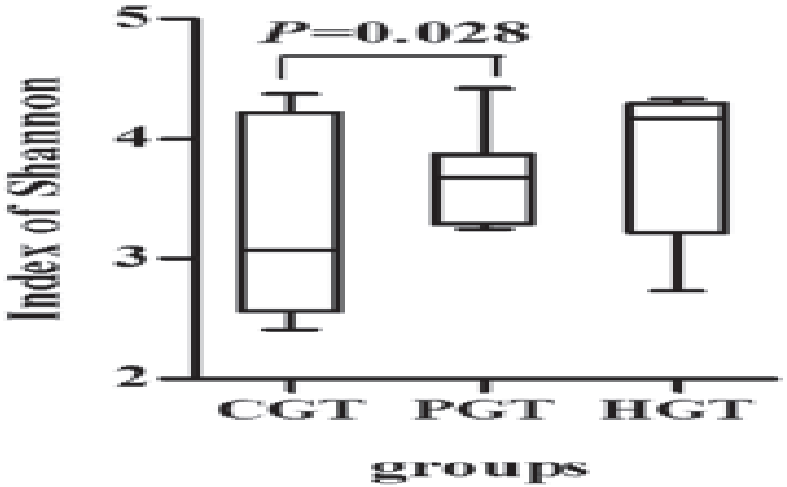
ACTION OF PROBIOTICS ON THE INTESTINAL ECOSYSTEM



MICROBIOTA DIVERSITY WITH PROBIOTICS

CGT=PLACEBO;PGT=PROBIOTICS; HGT =HEALTHY





EFFECT OF PROBIOTICS ON ANTIBIOTIC ACTION ON MICROBIOTA

	Probiotic	*P* value (within group)	Placebo	*P* value	
				Within group	Between group
Total bacteria					
Baseline	10.89 ± 0.22	-	10.81 ± 0.23	-	0.067
End of antibiotic + probiotic/placebo	10.58 ± 0.43	< 0.001	10.49 ± 0.38	< 0.001	0.177
End of probiotic/placebo	10.75 ± 0.30	0.003	10.77 ± 0.26	0.399	0.735
End of follow-up	10.87 ± 0.24	0.527	10.79 ± 0.31	0.568	0.221
Lactobacillus					
Baseline	7.40 ± 0.79	-	7.42 ± 1.56	-	0.391
End of antibiotic + probiotic/placebo	7.13 ± 0.88	0.104	6.91 ± 1.45	0.032	0.642
End of probiotic/placebo	7.42 ± 0.77	0.944	7.07 ± 1.38	0.030	0.331
End of follow-up	7.16 ± 1.50	0.375	6.96 ± 1.87	0.149	0.851
Lactobacillus acidophilus ATCC 700396					

Bacterial counts (log10 counts/g wet weight) at baseline (day 1), after 1 wk treatment period with antibiotic + probiotic or placebo (day 8), after 1 wk of supplementation with probiotic or placebo only (day 15) and after 1 wk follow-up period (day 22). Data are expressed as mean ± SD.

Forssten et al World J Gastroenterol 2014

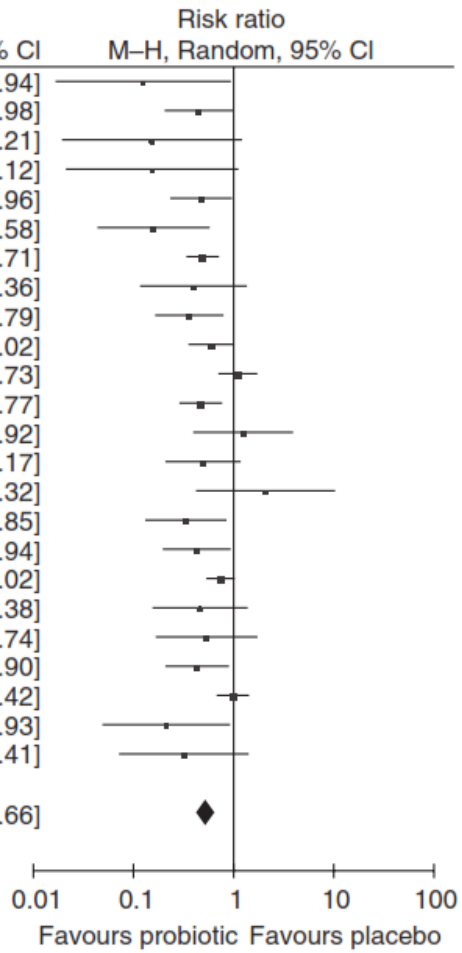
METANALYSIS OF PROBIOTIC TREATMENT FOR ANTIBIOTIC ASSOCIATED DIARRHEA IN ADULTS

(a)

Study or subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk ratio M–H, Random, 95% CI
Armuzzi 2001	1	30	8	30	1.0%	0.13 [0.02, 0.94]
Beausoliel 2007	7	44	16	45	4.5%	0.45 [0.20, 0.98]
Can 2006	1	73	7	78	1.0%	0.15 [0.02, 1.21]
Cimperman 2011	1	13	5	10	1.0%	0.15 [0.02, 1.12]
Cindoruk 2007	9	62	19	62	5.1%	0.47 [0.23, 0.96]
Cremonini 2002	3	63	6	20	2.2%	0.16 [0.04, 0.58]
Gao 2010	37	171	37	84	8.6%	0.49 [0.34, 0.71]
Gotz 1979	3	36	9	43	2.4%	0.40 [0.12, 1.36]
Hickson 2007	7	57	19	56	4.5%	0.36 [0.17, 0.79]
Koning 2008	9	19	15	19	6.8%	0.60 [0.35, 1.02]
Koning 2010	13	17	9	13	7.7%	1.10 [0.71, 1.73]
LaRosa 2003	14	48	31	50	7.2%	0.47 [0.29, 0.77]
Lonnermark 2010	6	80	5	83	2.6%	1.25 [0.40, 3.92]
McFarland 1995	7	97	14	96	4.0%	0.49 [0.21, 1.17]
Myllyluoma 2005	4	23	2	24	1.5%	2.09 [0.42, 10.32]
Nista 2004	5	50	15	50	3.6%	0.33 [0.13, 0.85]
Orrhage 1994	6	20	7	10	4.5%	0.43 [0.20, 0.94]
Psaradellis 2010	47	216	65	221	9.2%	0.74 [0.53, 1.02]
Safdar 2008	4	23	6	16	2.8%	0.46 [0.16, 1.38]
Song 2010	4	103	8	111	2.6%	0.54 [0.17, 1.74]
Surawicz 1989	11	116	14	64	4.9%	0.43 [0.21, 0.90]
Thomas 2001	39	133	40	134	8.7%	0.98 [0.68, 1.42]
Wenus 2008	2	34	8	29	1.8%	0.21 [0.05, 0.93]
Wunderlich 1989	2	23	6	22	1.7%	0.32 [0.07, 1.41]
Total (95% CI)		1551		1370	100.0%	0.53 [0.43, 0.66]
Total events	242		371			

Heterogeneity: $Tau^2 = 0.10$; $Chi^2 = 41.34$, df = 23 ($P = 0.01$); $I^2 = 44\%$

Test for overall effect: $Z = 5.81$ ($P < 0.00001$)



Videlock EJ & Cremonini F. Aliment Pharmacol Ther 2012;35:1355-1369

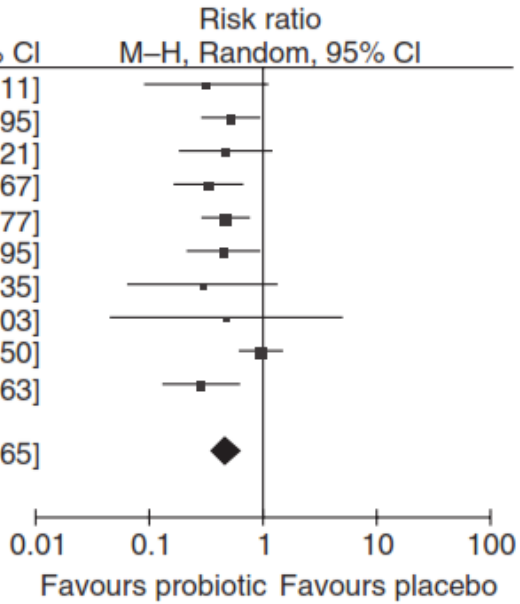
METANALYSIS OF PROBIOTIC TREATMENT FOR ANTIBIOTIC ASSOCIATED DIARRHEA IN CHILDREN

(b)

Study or subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk ratio M–H, Random, 95% CI
Arvola 1999	3	61	9	58	4.9%	0.32 [0.09, 1.11]
Correa 2005	13	80	24	77	14.1%	0.52 [0.29, 0.95]
Jirapinyo 2002	3	8	8	10	7.7%	0.47 [0.18, 1.21]
Kotowska 2005	9	119	29	127	11.6%	0.33 [0.16, 0.67]
LaRosa 2003	14	48	31	50	17.1%	0.47 [0.29, 0.77]
Rusczczynski 2008	9	120	20	120	10.8%	0.45 [0.21, 0.95]
Szajewska 2009	2	34	6	30	3.5%	0.29 [0.06, 1.35]
Szymanski 2008	1	40	2	38	1.6%	0.47 [0.04, 5.03]
Tankanow 1990	10	15	16	23	18.5%	0.96 [0.61, 1.50]
Vanderhoof 1999	7	93	25	95	10.1%	0.29 [0.13, 0.63]
Total (95% CI)		618		628	100.0%	0.48 [0.35, 0.65]
Total events	71		170			

Heterogeneity: $Tau^2 = 0.08$; $Chi^2 = 13.97$, df = 9 ($P = 0.12$); $I^2 = 36\%$

Test for overall effect: $Z = 4.77$ ($P < 0.00001$)



Videlock EJ & Cremonini F. Aliment Pharmacol Ther 2012;35:1355-1369

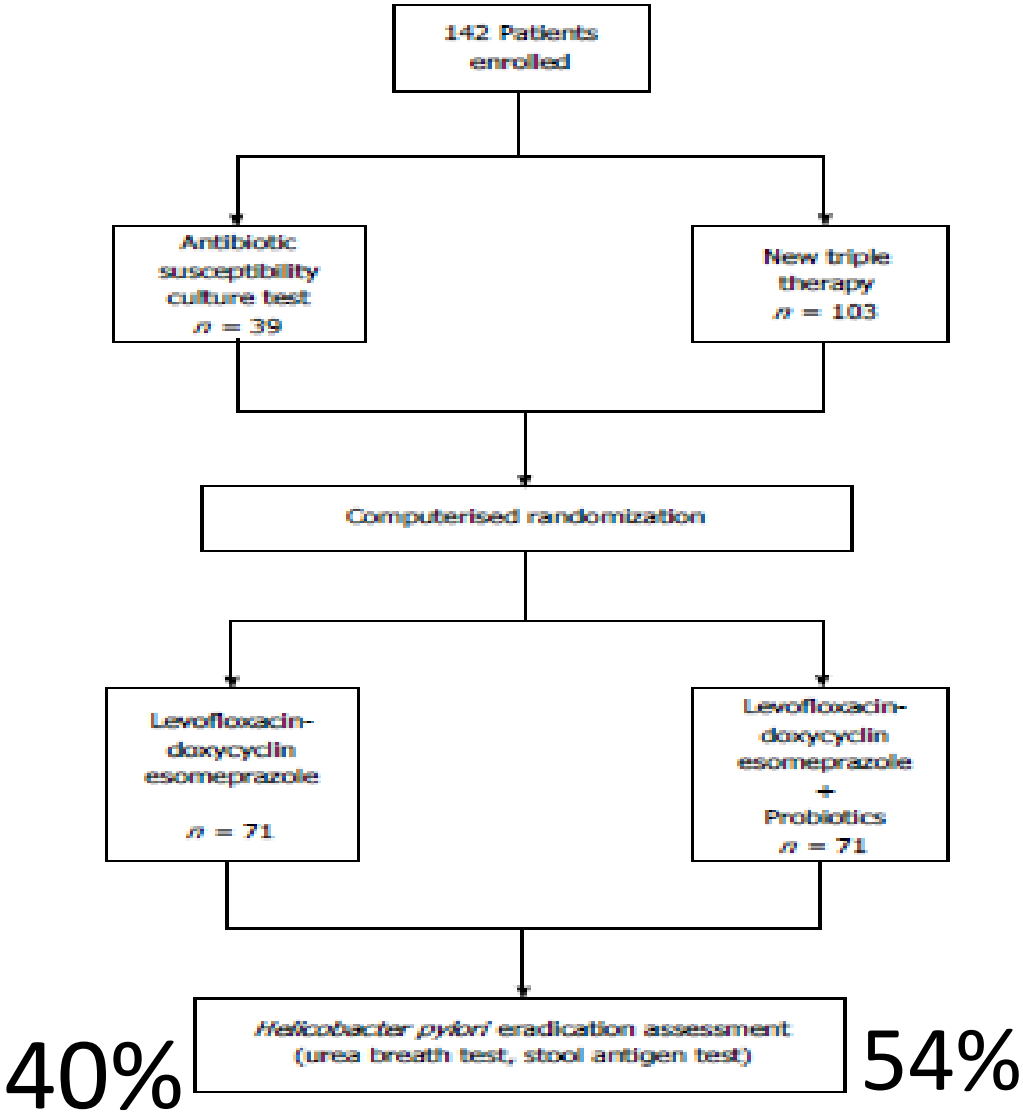
QUALITA' → CEPPO

Famiglia	*Lactobacillaceae*
Genere	*Lactobacillus*
Specie	*rhamnosus*, *acidophilus*, *paracasei*
Ceppo	XY, LPC-S01, CNCMI1572 (L. casei DG®)

È il tipo di ceppo che determina la qualità del probiotico


SCIENCE PHOTO LIBRARY

PROBIOTIC LACTOBACILLUS CASEI DG + ANTIBIOTICS FOR HP ERADICATION



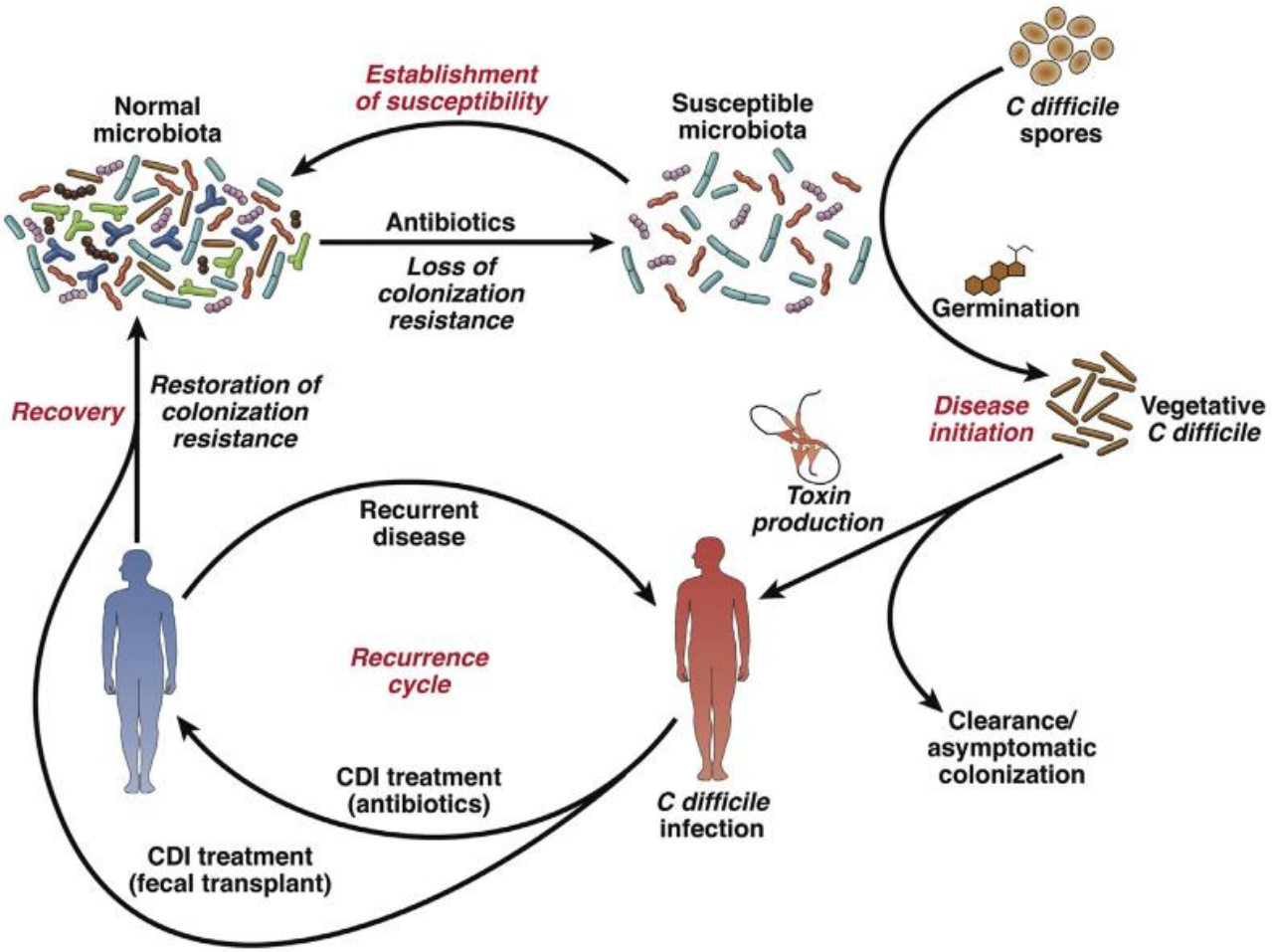
Paoluzi et al World J Gastroenterol 2015

EFFICACY OF PROBIOTIC TREATMEN FOR AAD and HP ERADICATION

	NNT	(95% CI)
All AAD Studies	8	7-11
H. Pylori treatment	5	4-10

Videlock EJ; Cremonini F. APT 2012;35:1355-1369

CYCLE OF C. *difficile* INFECTION

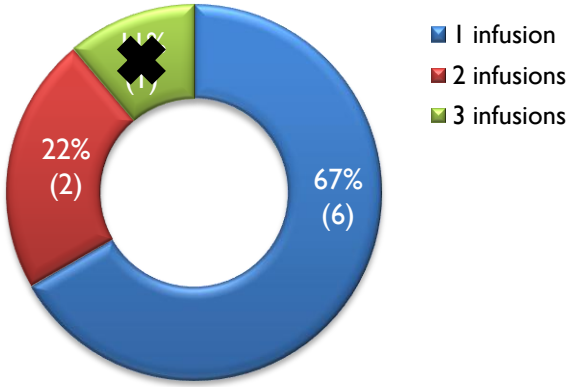


Britton RA et al. Gastroenterology 2014;146:1547-1553

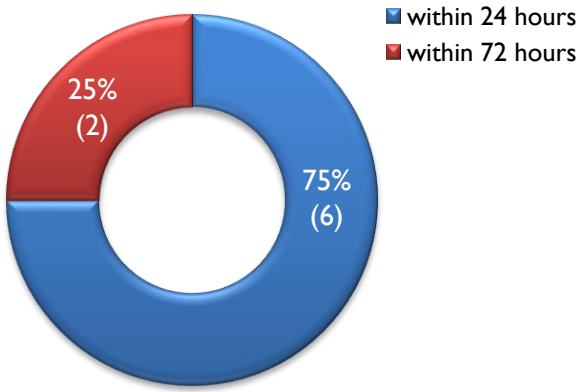
Results

Population sample (mean±sd)	
Number of donors:	5
M/F	3/2
Age (years):	25± 1.3
BMI (kg/m^2):	22.4 ± 1.2
Number of patients:	9
M/F	3/6
Age (years;):	72±10.7
Total procedures:	13
Time of infusion (minutes)	45 ± 12
Resolution:	8/9
Major adverse events (up to 1 year):	None

Total procedures = 13



Resolution of diarrhea



LONG TERM EFFECTS OF ANTIBIOTICS

- **Resistome**
- IBS
- IBD
- Obesity
- Metabolic syndrome
- Atopic disease
- Autoimmune diseases
- Rectocolonic Cancer

Except for resistome there is insufficient evidence to claim that antibiotics may confer clinically significant long term effects

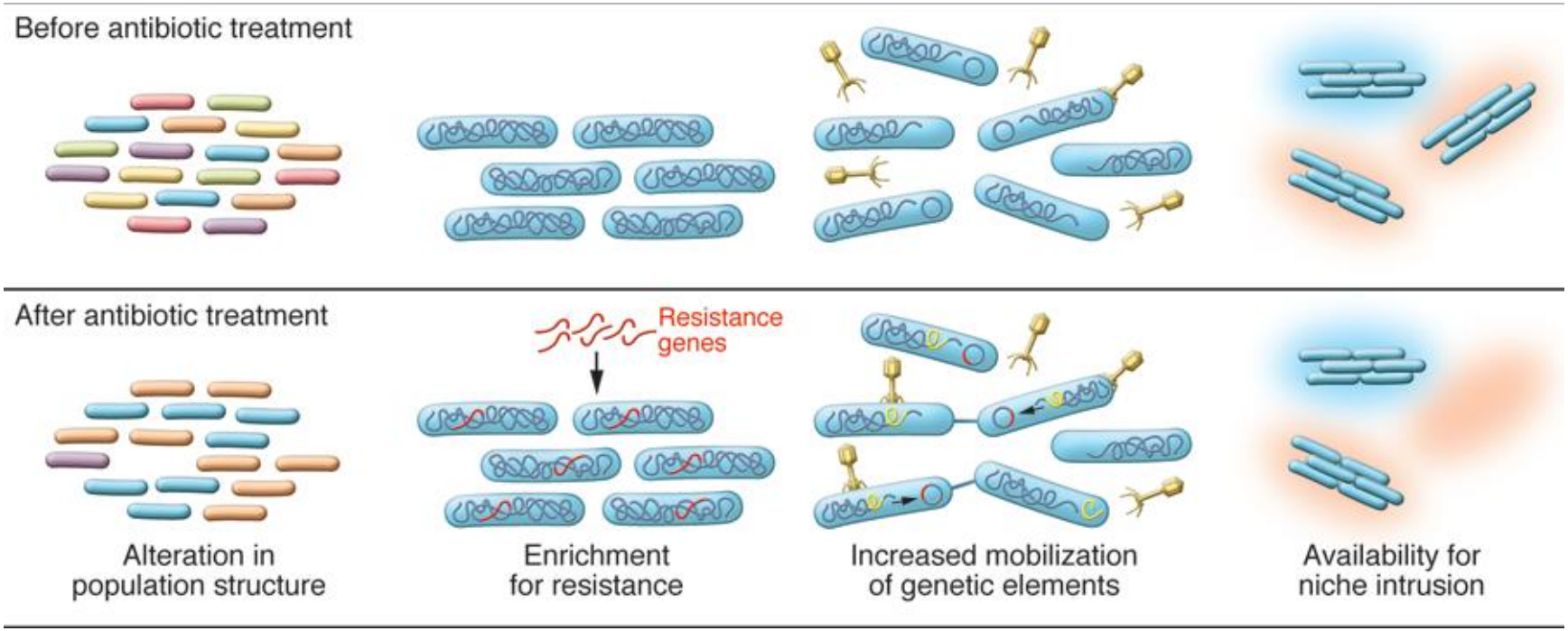
Int J Obes (Lond). 2014 Gut. 2006;55:205-11; Infect Dis Clin North Am. 2010;24:977-93,ix; Gut. 2008;57:1315-21; Eur J Gastroenterol Hepatol. 1998;(1):59-62; Scand J Gastroenterol. 2008;43(8):961-6; Modi et al JCI 2014 Modi et al JCI 2014 Mikkelson et al 2016

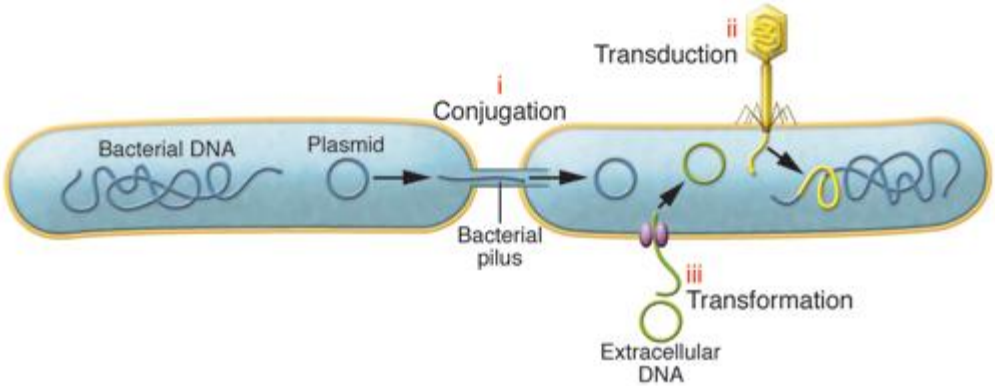
RESISTOME

Antibiotic resistance genes of the gut microbiota

- Resistance gene transfer from commensals to gut-dwelling opportunistic pathogens appears to be a relatively rare event but may contribute to the emergence of multi-drug resistant strains

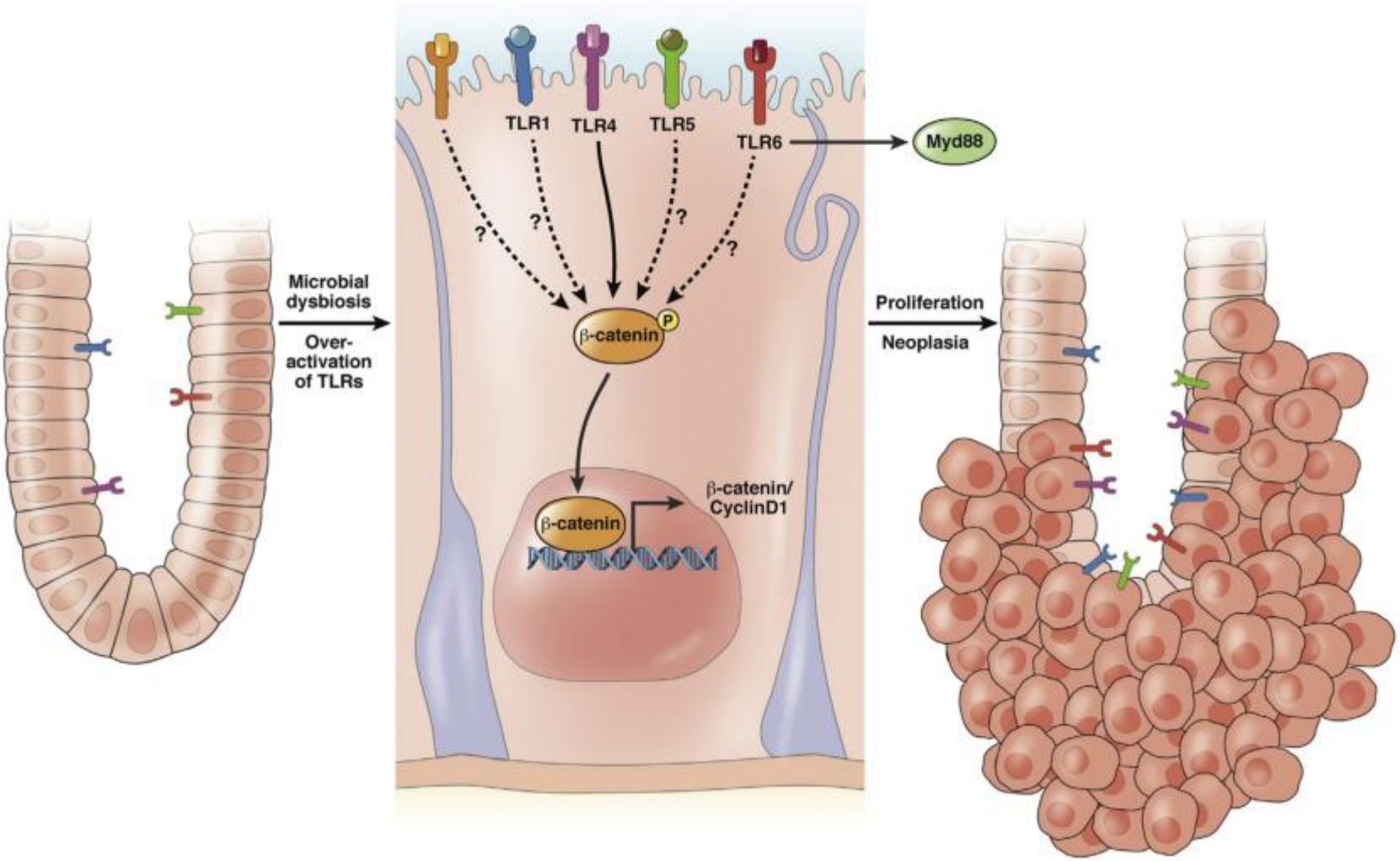
EFFECTS OF ANTIBIOTICS ON MICROBIOTA





Modi et al JCI 2014

TLR ACTIVATION BY MICROBIOTA AND CARCINOGENESIS



Abreu MT et al. Gastroenterology 2014;146:1534-1546

Frequent Use of Antibiotics Is Associated with Colorectal Cancer Risk: Results of a Nested Case–Control Study

Vincent K. Dik[1] · Martijn G. H. van Oijen[1,2] · Hugo M. Smeets[3,4] · Peter D. Siersema[1]

Dig Dis Sci (2016) 61:255–264

	Cases *n*, %	Controls *n*, %	Univariable[c] OR (95 % CI)	Multivariable[d] OR (95 % CI)
Prescriptions[a]				
None	1399 (34.7)	5754 (36.0)	Ref.	Ref.
Very low	1328 (33.0)	5245 (32.8)	1.04 (0.96–1.13)	1.05 (0.96–1.14)
Low	549 (13.6)	2250 (14.1)	1.01 (0.90–1.13)	1.02 (0.91–1.14)
Intermediate	358 (8.9)	1413 (8.8)	1.05 (0.92–1.19)	1.06 (0.93–1.22)
High	395 (9.8)	1326 (8.3)	1.23 (1.08–1.40)	1.26 (1.11–1.44)
p-trend			<0.01	<0.01
Per 5 prescriptions			1.04 (1.01–1.07)	1.05 (1.01–1.09)
Days[b]				
None	1399 (34.7)	5754 (36.0)	Ref.	Ref.
Very low	1243 (30.9)	4971 (31.1)	1.03 (0.95–1.12)	1.03 (0.95–1.13)
Low	711 (17.6)	2722 (17.0)	1.08 (0.97–1.19)	1.09 (0.98–1.21)
Intermediate	377 (9.4)	1550 (9.7)	1.01 (0.89–1.14)	1.02 (0.89–1.16)
High	299 (7.4)	991 (6.2)	1.24 (1.08–1.44)	1.28 (1.10–1.48)
p-trend			<0.01	<0.01
Per 25 days			1.00 (0.99–1.01)	1.00 (0.99–1.01)

ORs odds ratios, *95 % CI* 95 % confidence interval

[a] Cutoff points are based on the 50th, 75th, and 90th percentile of prescriptions within users: very low (1–2), low (3–4), intermediate (5–7), and high (≥8)

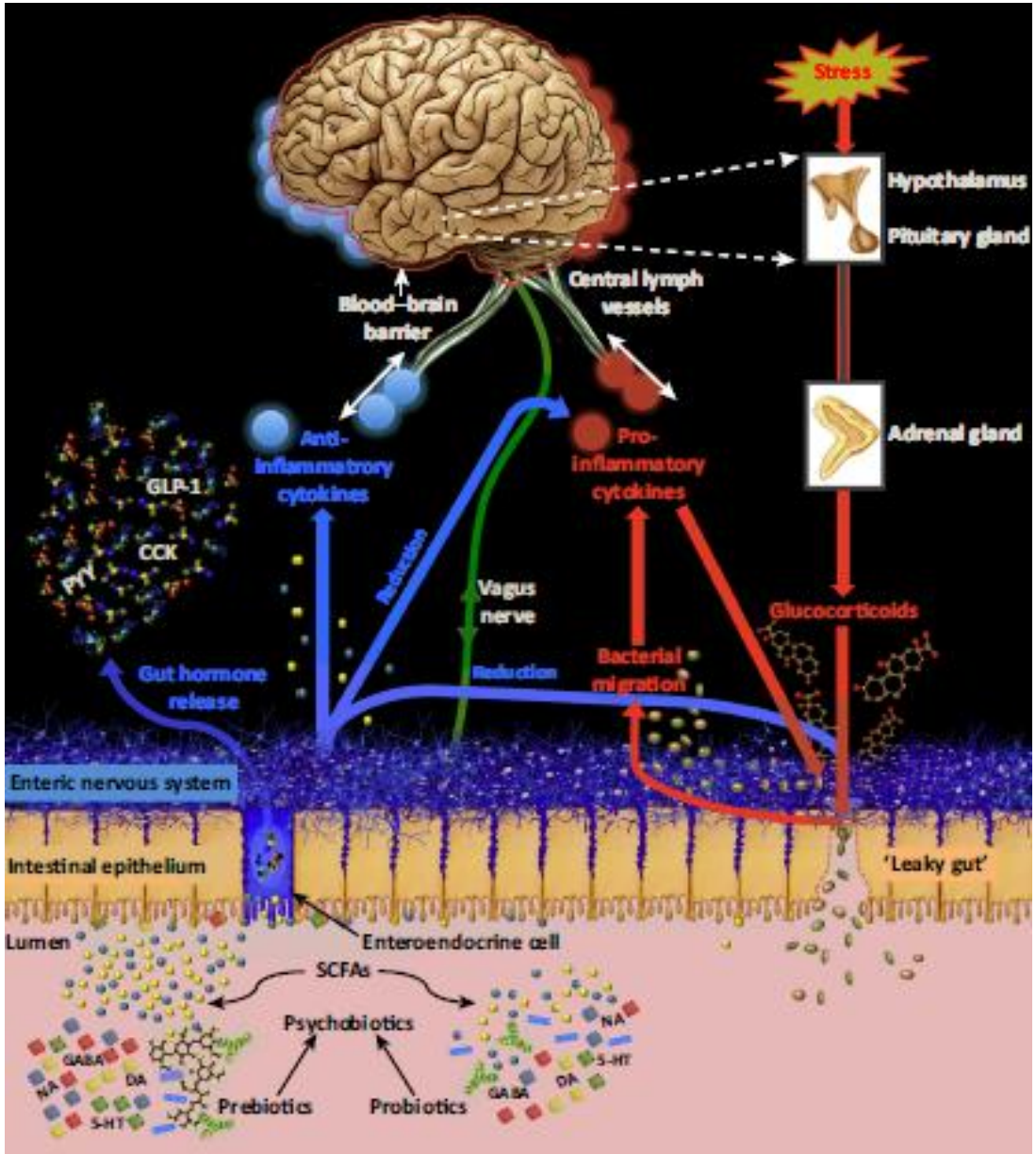
[b] Cutoff points are based on the 50th, 75th, and 90th percentile of prescribed number of days within users: very low (1–15), low (16–34), intermediate (35–70), and high (≥70)

Anticancerous and antimutagenic activity of probiotics

- Mutagen binding, degradation and mutagenesis inhibition
- Prevention of nontoxic procarcinogen conversion to harmful toxic and highly reactive carcinogens
- increasing short chain fatty acids (SCFA) and hence lowering intestinal pH
- Modulation and enhancement of the host's innate immunity

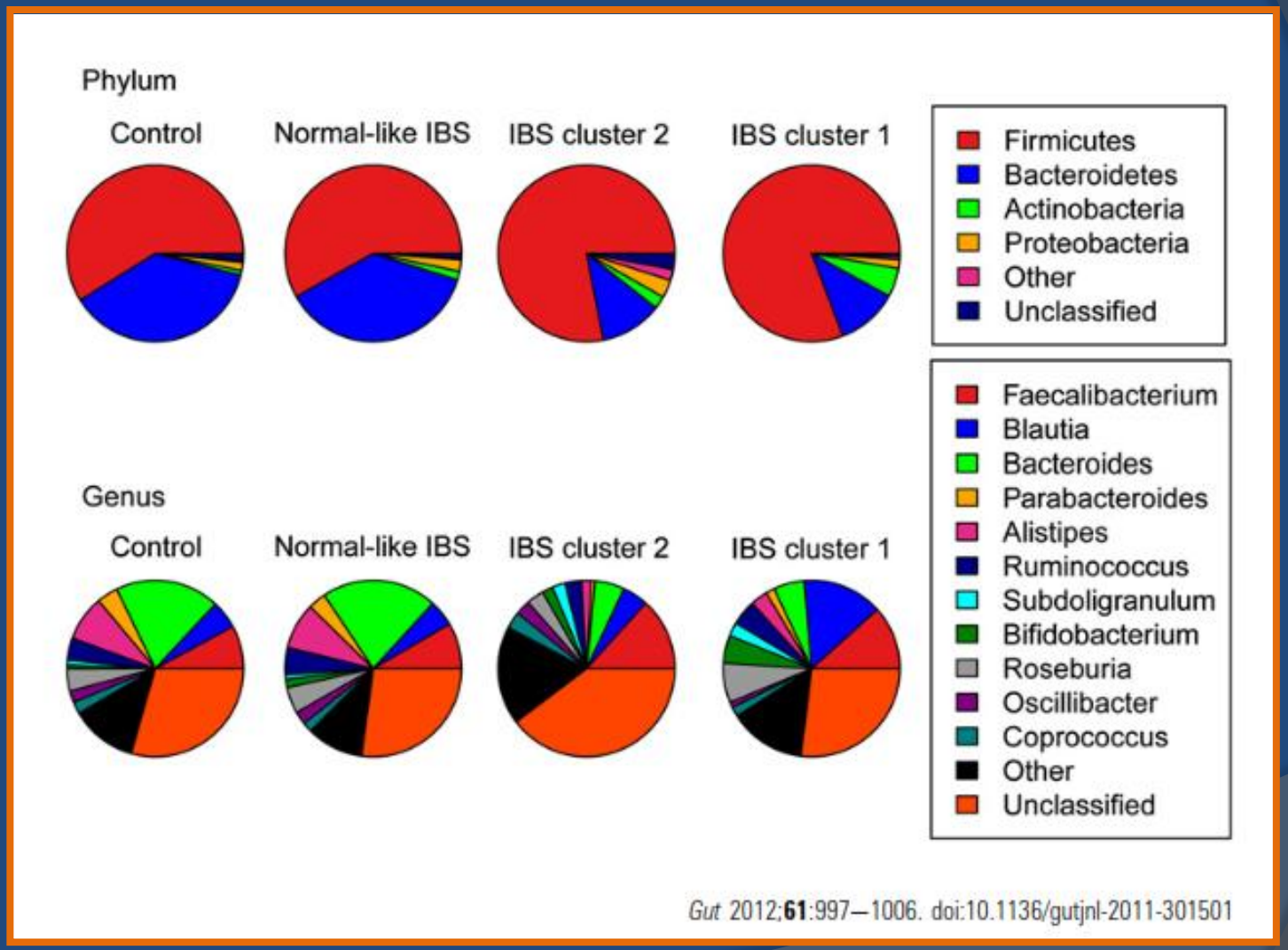
BACTERIA-GUT-BRAIN –AXIS

Psychobiotics

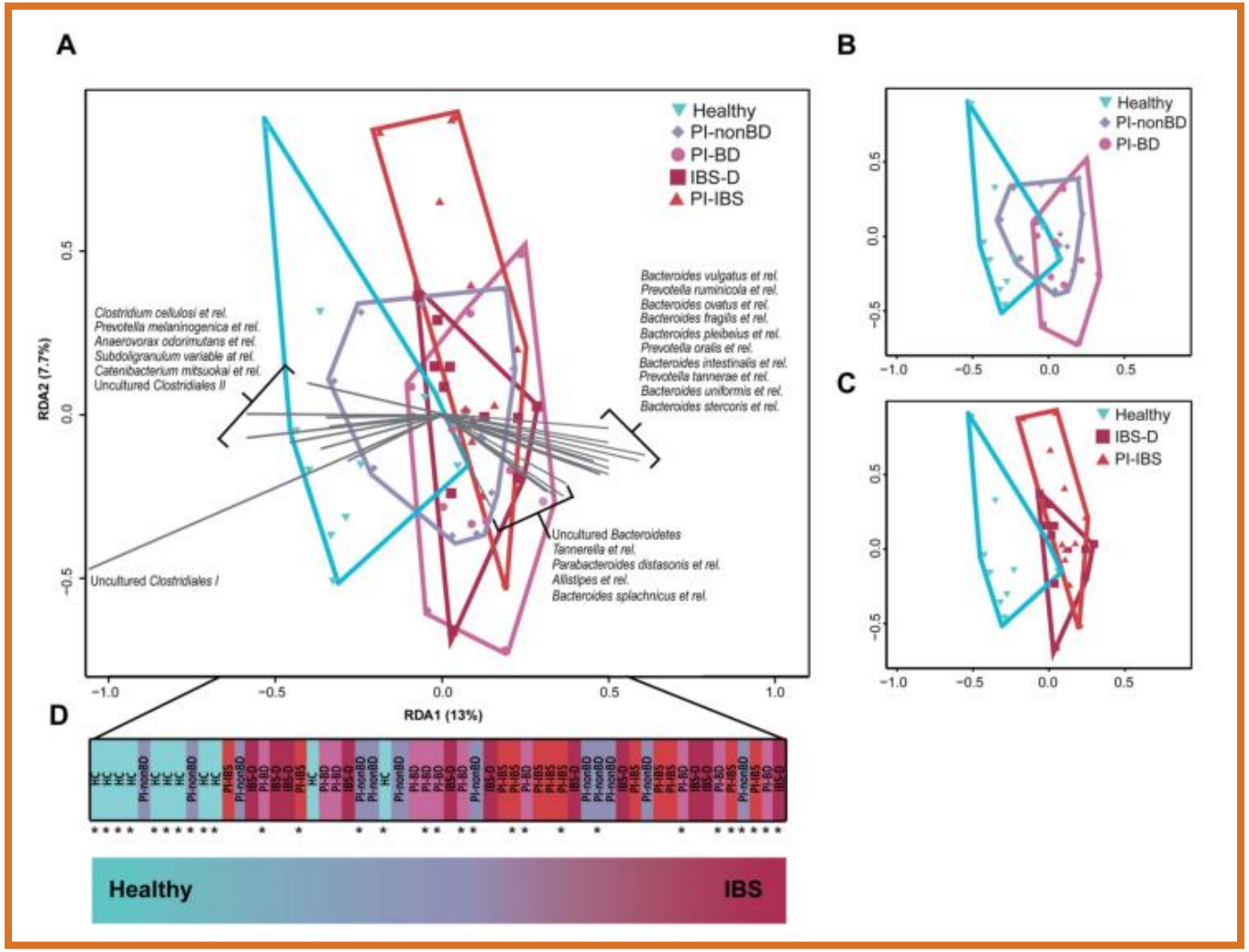


Sarkar et al Trends in Neurosciences 2016

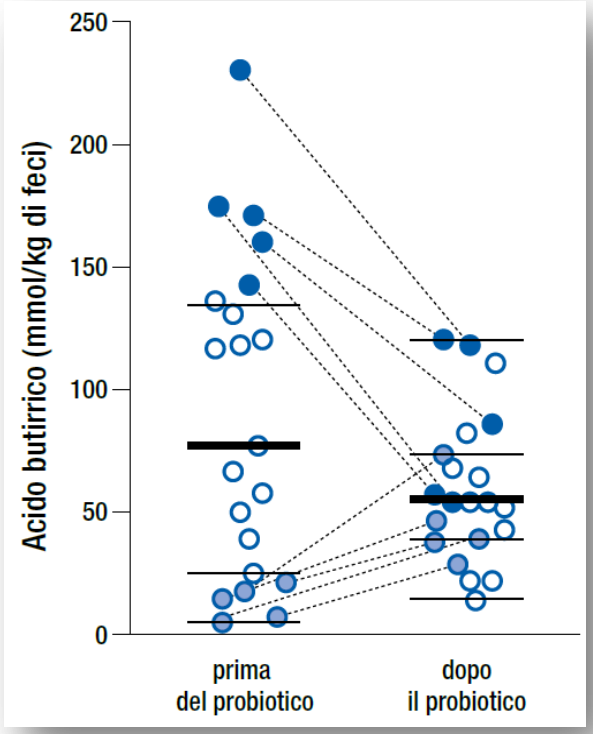
MICROBIOL COMMUNITIES IN HEALTHY AND IBS SUBJECTS

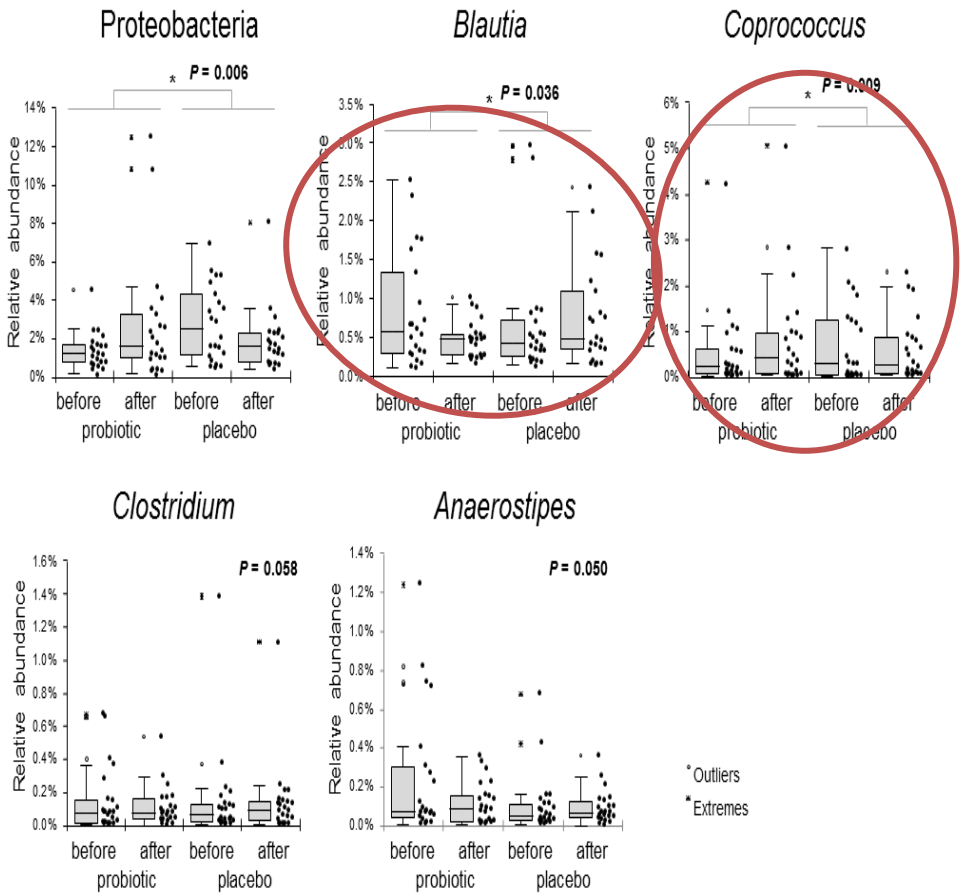


Ian B Jeffery, et al. Gut 2012;61:997-1006 doi 10,1136/gutinl-2011-301501


A
Healthy
PI-nonBD
PI-BD
IBS-D
PI-IBS
Clostridium cellulosi et rel.
Prevotella melaninogenica et rel.
Anaerovorax odorimutans et rel.
Subdoligranulum variable at rel.
Catenibacterium mitsuokai et rel.
Uncultured Clostridiales II
Uncultured Clostridiales I
Bacteroides vulgatus et rel.
Prevotella ruminicola et rel.
Bacteroides ovatus et rel.
Bacteroides fragilis et rel.
Bacteroides plebeius et rel.
Prevotella oralis et rel.
Bacteroides intestinalis et rel.
Prevotella tannerae et rel.
Bacteroides uniformis et rel.
Bacteroides stercoris et rel.
Uncultured Bacteroidetes
Tannerella et rel.
Parabacteroides distasonis et rel.
Alistipes et rel.
Bacteroides splachnicus et rel.
RDA2 (7.7%)
RDA1 (13%)
B
C
D
Healthy
IBS

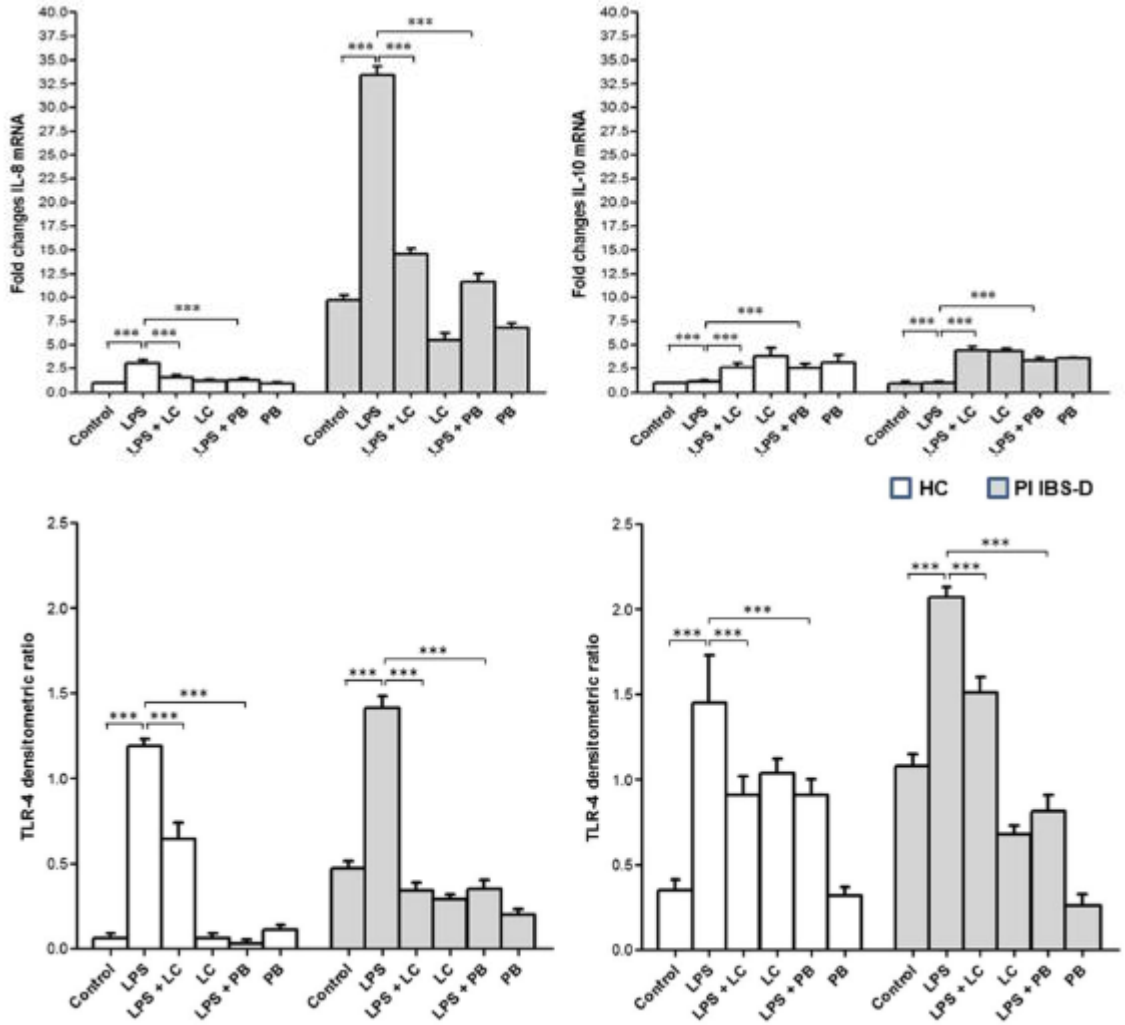
Eubiotic effect of L. paracasei CNCMI1572 (L. casei DG®), modulating Microbiota , by balancing butyrate concentrations





Ferrario C et al. J Nutr. 2014 Nov;144(11):1787-96

EFFECTS OF *L. casei DG*® ON INTERLEUKINS AND TLR4



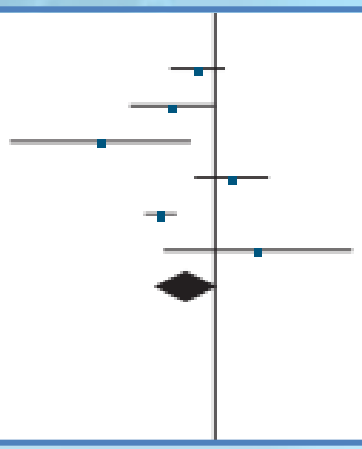
Compare et al BMC Gastroenterol 2017

FOREST PLOT OF RCTS OF LACTOBACILLI PROBIOTICS IN IBS
EFFECT ON GLOBAL SYMPTOM/ABDOMINAL PAIN

1.1.2 *Lactobacillus*							
Nobaek, 2000	21	30	25	30	5.1%	0.84 [0.63, 1.12]	2000
Niedzielin, 2001	11	20	17	20	3.6%	0.65 [0.42, 1.00]	2001
Sinn, 2008	4	20	13	20	1.3%	0.31 [0.12, 0.78]	2008
Dapoigny, 2012	19	26	16	26	4.1%	1.19 [0.81, 1.74]	2012
Ducrotte, 2012	61	108	105	106	6.4%	0.57 [0.48, 0.67]	2012
Farup, 2012	6	9	3	7	1.2%	1.56 [0.59, 4.11]	2012
Subtotal, (95% CI)		213		209	21.7%	0.75 [0.54, 1.04]	
Total events	122		179				

Heterogeneity: $\tau^2 = 0.10$; $\chi^2 = 20.20$, df = 5 ($P = 0.001$); $I^2 = 75\%$

Test for overall effect: $Z = 1.72$ ($P = 0.08$)



AC Ford et al. Am J Gastroenterol 29 July 2014;doi:10,1038/ajg.2014,202

Effect of *Lactobacillus paracasei* CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: A pilot randomized clinical trial

L. paracasei CNCM I-1572 treatment	*p* value	Median relative abundance (mmol/kg)	
		Before	After
Acetate	**0.021**	**36.63** (±22.62)	**47.83** (±26.14)
Propionate	0.289	15.18 (±10.35)	16.37 (±11.97)
Butyrate	**0.047**	**5.99** (±8.30)	**10.52** (±8.51)

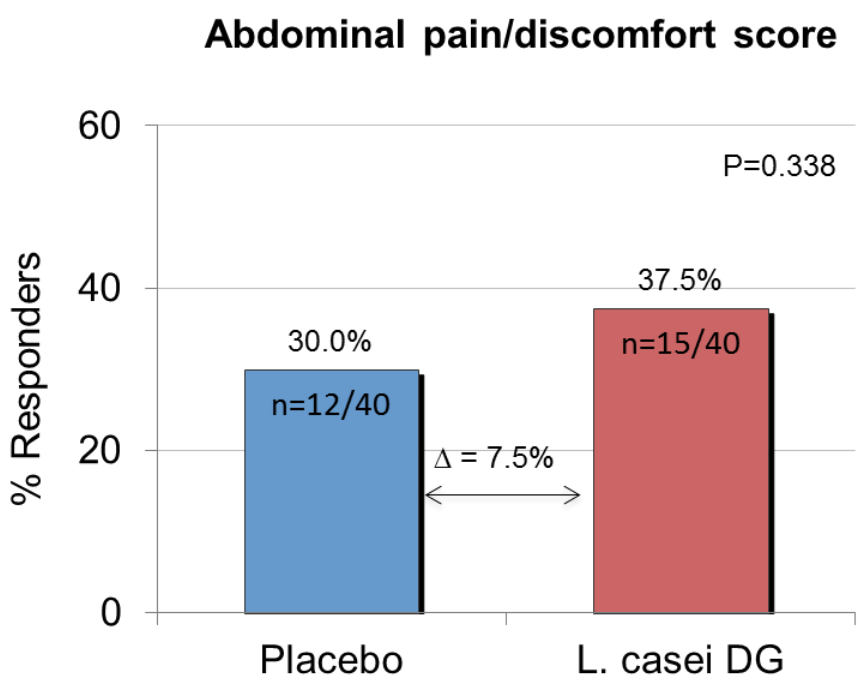
IL-6 and IL-15 decreased significantly after treatment

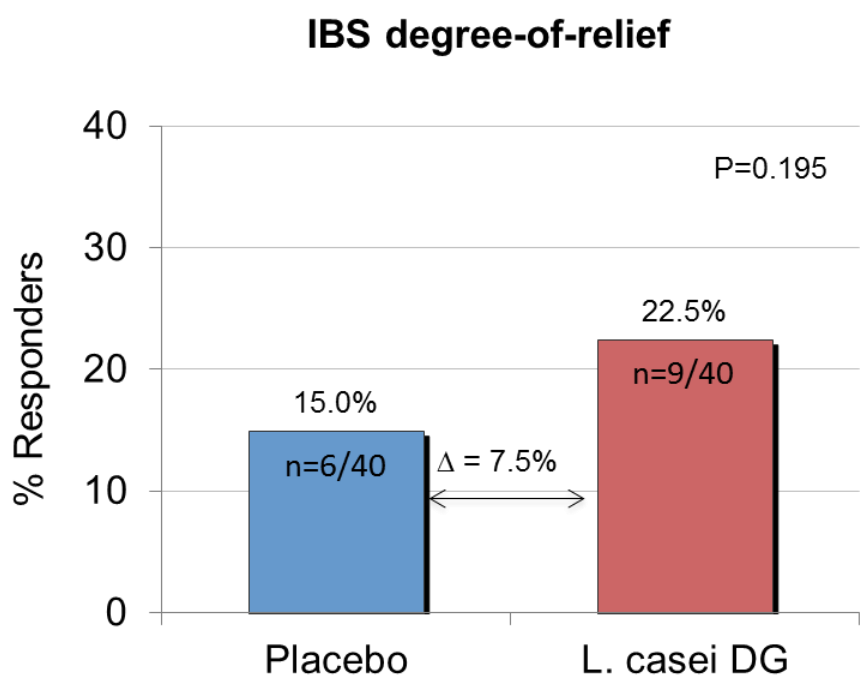
Cremon et al UEG J 2017

Azione antinfiammatoria e immunostimolante

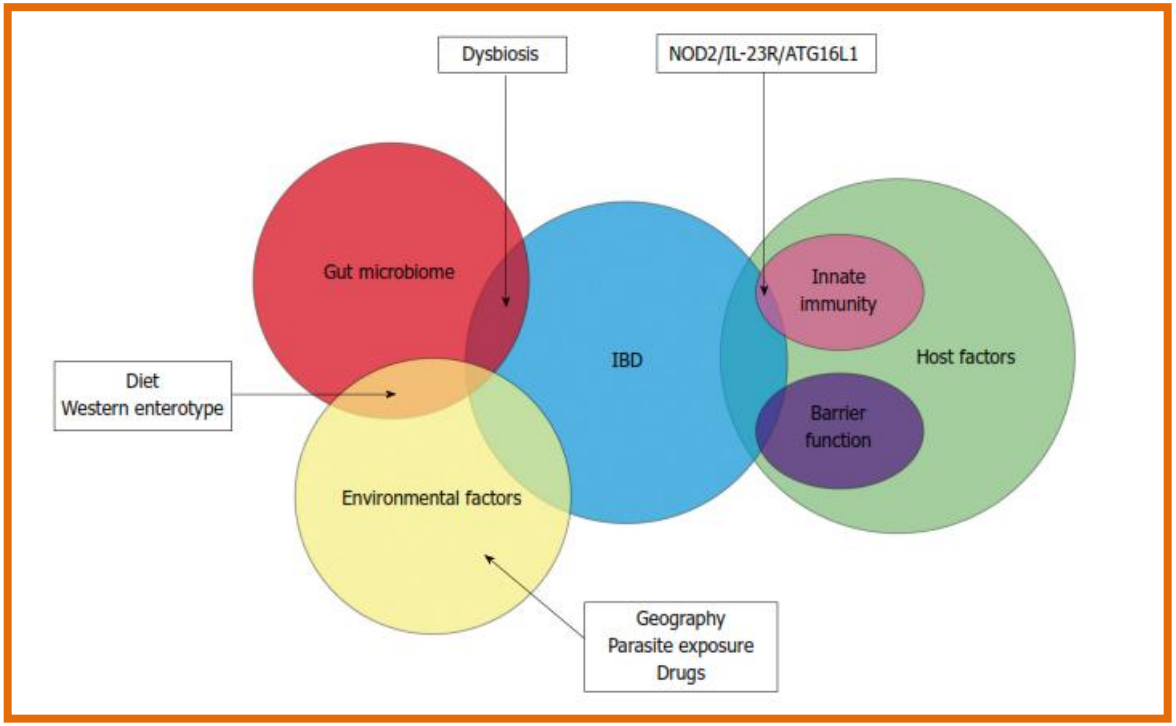
Dolore /fastidio addominale e grado di miglioramento per IBS

Proporzione di rispondenti





PATHOGENESIS OF IBD

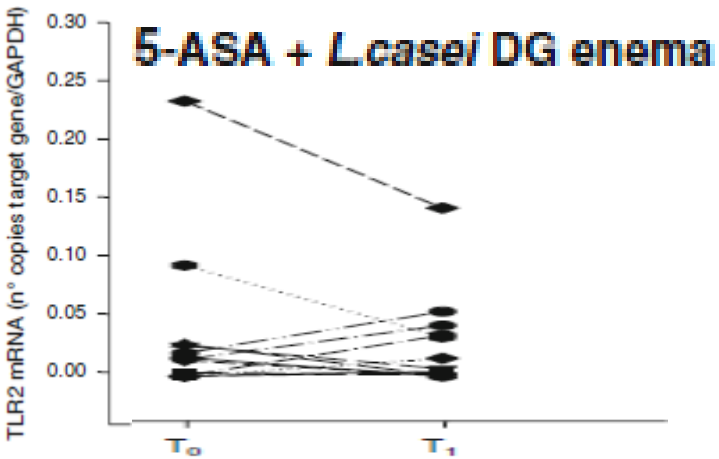


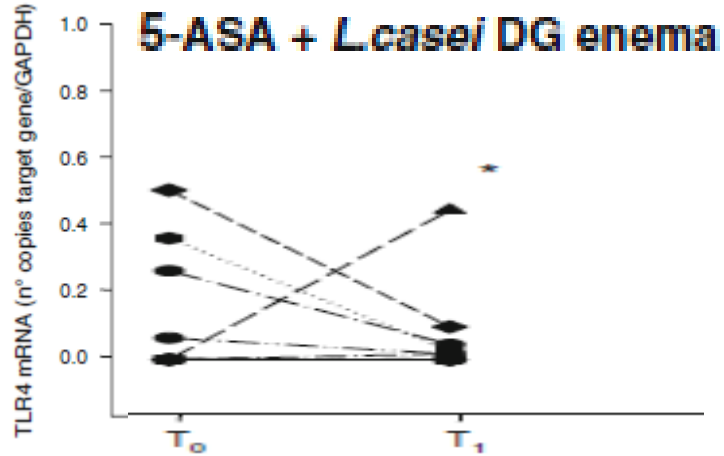
G L Hold, et al. World J Gastroenterol 2014;20:1192-1210

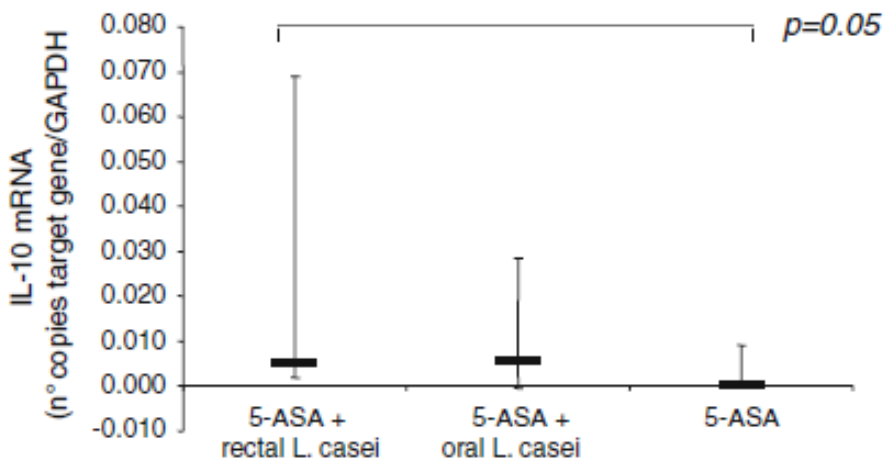
Rectal Administration of *Lactobacillus casei* DG Modifies Flora Composition and Toll-Like Receptor Expression in Colonic Mucosa of Patients with Mild Ulcerative Colitis

	Oral 5-ASA ($n = 7$)	Oral 5-ASA plus oral *L. casei* DG (8×10^8 cfu) ($n = 8$)	Oral 5-ASA plus rectal *L. casei* DG (8×10^8 cfu) ($n = 11$)
Enterobacteriaceae spp.	9.5 (range 2.1–65.4) $\times 10^3$ cfu/10 mg tissue	7.9 (range 4.5–53.4) $\times 10^3$ cfu/10 mg tissue	2.4 (range 0–11) $\times 10^3$ cfu/10 mg tissue*
Lactobacillus spp.	2.1 (range 0–21.8) $\times 10^3$ cfu/10 mg tissue	2.9 (range 0–25.2) $\times 10^3$ cfu/10 mg tissue	10.5 (range 0–75.4) $\times 10^3$ cfu/10 mg tissue*

* $P < 0.01$ compared with UC patients receiving oral 5-ASA alone or 5-ASA plus oral *L. casei* DG





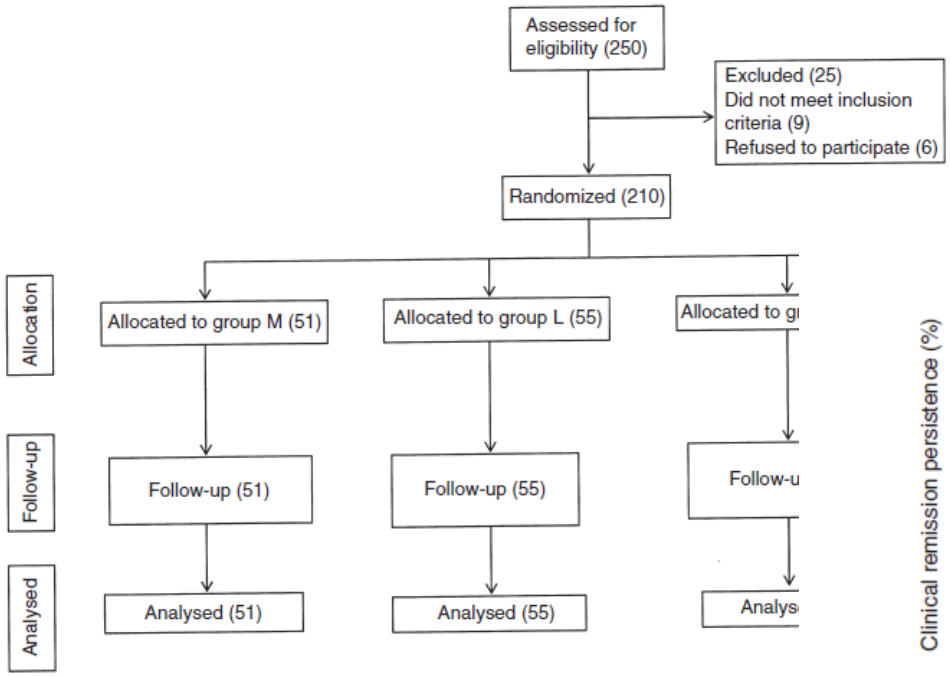


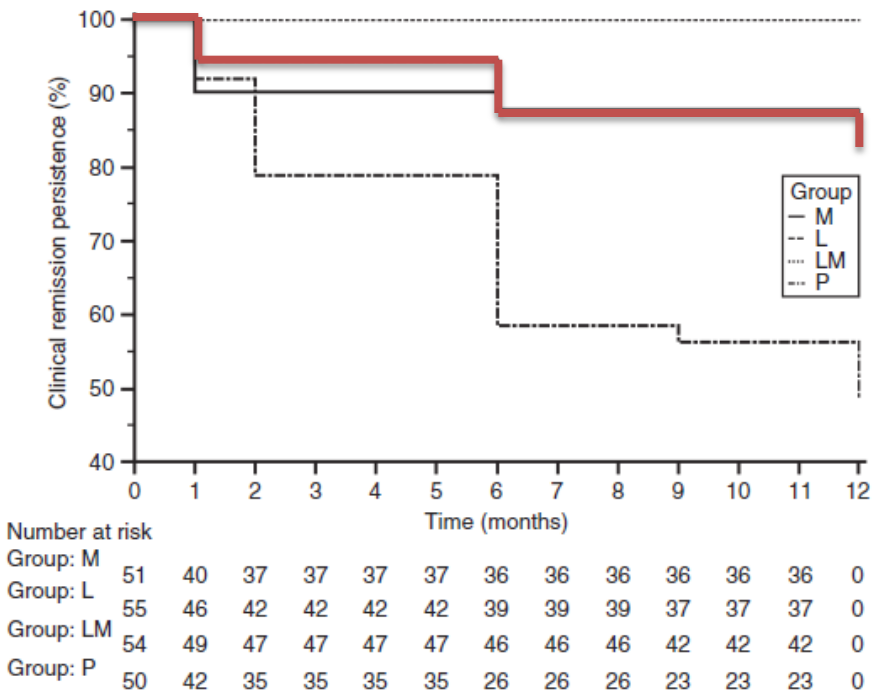
D'Incà et al Dig Dis Sci 2010

AP&T Alimentary Pharmacology and Therapeutics

Randomised clinical trial: mesalazine and/or probiotics in maintaining remission of symptomatic uncomplicated diverticular disease – a double-blind, randomised, placebo-controlled study

A. Tursi*, G. Brandimarte†, W. Elisei‡, M. Picchio§, G. Forti¶, G. Pianese‖, S. Rodino**, T. D'Amico**, N. Sacca**, P. Portincasa††, E. Capezzuto‡‡, R. Lattanzio§§, A. Spadaccini¶¶, S. Fiorella¶¶, F. Polimeni***, N. Polimeni***, V. Stoppino†††, G. Stoppino†††, G. M. Giorgetti‡‡‡, F. Aiello§§§ & S. Danese¶¶¶





Both cyclic mesalazine and ***L. casei DG®*** treatments, particularly when given in combination, appear to be better than placebo for maintaining remission of symptomatic uncomplicated diverticular disease

CONCLUSIONI

Molte condizioni possono alterare lo stato di eubiosi del microbiota intestinale

Probiotici e Trapianto fecale di microbiota esercitano un effetto eubiotico

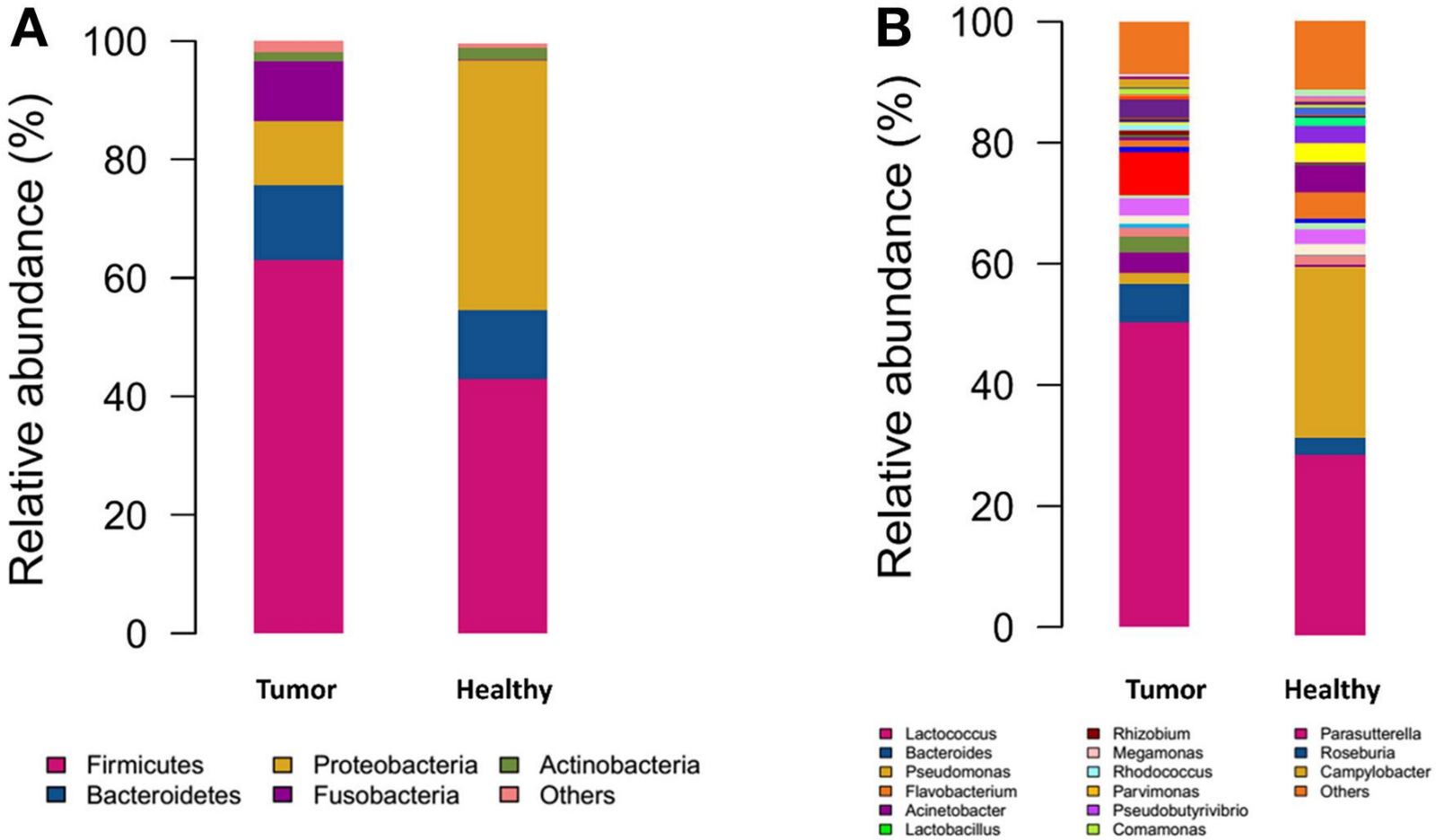
- Il microbiota alterato dagli antibiotici
- Causa effetti a breve termine e il rischio di resistome
- E' associato con effetti a lungo termine , ma senza sufficiente evidenza di un effetto causale
- L'uso degli antibiotici dovrebbe essere limitato a indicazioni cliniche ben delimitate e per ilminore tempo possibile
- I Probiotici dovrebbero essere impiegati in associazione con le terapie antibiotiche per contrastare gli effetti a breve termine e prevenire quelli a lungo termine

CONCLUSIONI

Nella nostra pratica clinica i probiotici, se usati alla dose giornaliera di 9 miliardi CFU per almeno 4 settimane, si sono dimostrati efficaci nella

- Prevenzione e terapia della Diarrea causata da antibiotici
- Sindrome dell'Intestino Irritabile
- Malattia diverticolare sintomatica non complicata
- Prevenzione delle ricadute durante le fasi di remissione della rettocolite ulcerosa
- Prevenzione della proctite attinica
- Prevenzione della pauchite

LUMINAL MICROBIOTA DIVERSITY IN CRC



Gao et al Front Microbiol 2015

QUALITA' → QUANTITA'

Dopo la terapia antibiotica è fondamentale ripristinare il corretto microbiota. Pertanto, dovrebbero essere assunti diversi MILIARDI DI CELLULE VIVE

Deve essere assunto «**un miliardo di cellule vive** per almeno uno dei ceppi presenti».

Tale quantitativo deve essere garantito fino al termine della shelf-life

QUALITA' → QUANTITA'

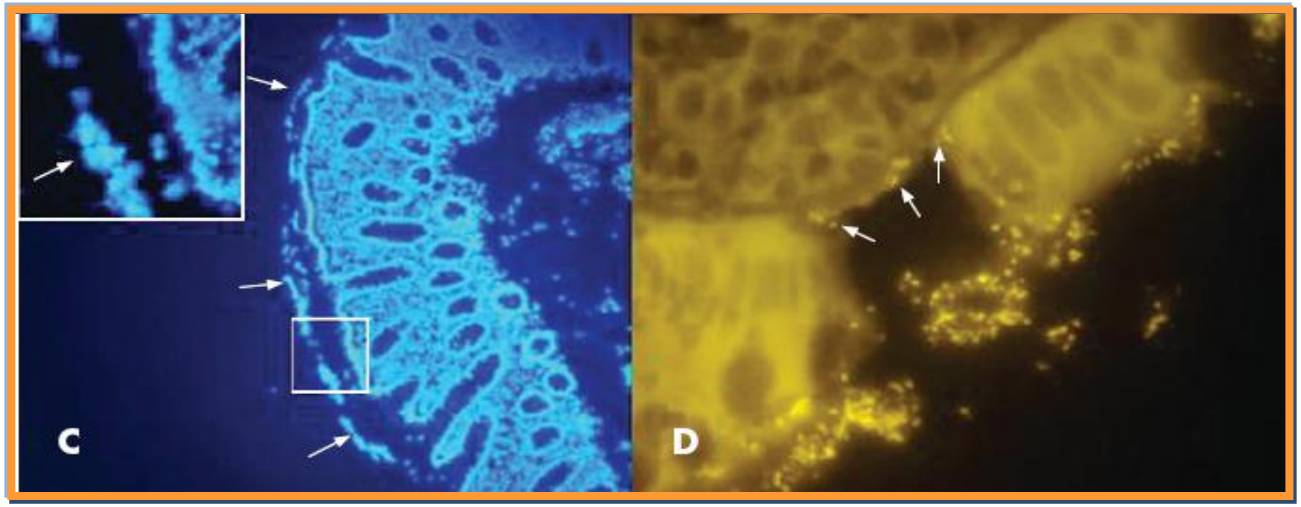
La scelta della dose deve essere basata sulle evidenze disponibili

Considerando tutte le diverse malattie gastrointestinali e le specie probiotiche, una significativa efficacia è stata osservata per dosi di almeno un miliardo, ma per alcune condizioni (IBS) almeno 9 miliardi:

$1–9x10^9$ CFU/day

Colorectal cancer prevention by mesalazine in UC

- **Chemopreventive mechanism** interfering with intracellular signals involved in CRC cell growth
  Stolfi et al J Biomed Biotech 2012



Targets bacterial polyphosphate accumulation reducing bacteria resistance towards oxidative stress, colonization, and biofilm formation, diminishing the capacity of bacteria to persist within chronically inflamed environments- Dahl et al Nat Microbiol 2016

CONCLUSIONS

- Several factors may alter the normal eubiotic microbiota
- Dysbiosis is a microbiota that can be differentiated from that of the average healthy population
- Dysbiosis per se does not imply the presence of a pathological condition
- Dysbiosis is associated with many disease conditions including CRC
- Probiotics exert an eubiotic effect

In the prevention of CRC

- Probiotics exert several favorable antitumorigenic effects, either directly on the epithelial cells, the immune system modulation ,and preventing dysbiosis