Microbiota, Epigenetica e Allergia alle Proteine del Latte Vaccino

Prof. Roberto Berni Canani, MD, PhD
Resp.Programma Intradipartimentale di Allergologia Pediatria
Dipartimento di Scienze Mediche Traslazionali
Laboratorio Europeo per lo Studio delle Malattie
Indotte da Alimenti (ELFID)
CEINGE Biotecnologie Avanzate
Università degli Studi di Napoli "Federico II"

LO SCENARIO DELL'AA E' MUTATO

↑ Rischio di persistenza e severità delle manifestazioni cliniche

↑ Necessità di ospedalizzazione

↑ Impatto economico

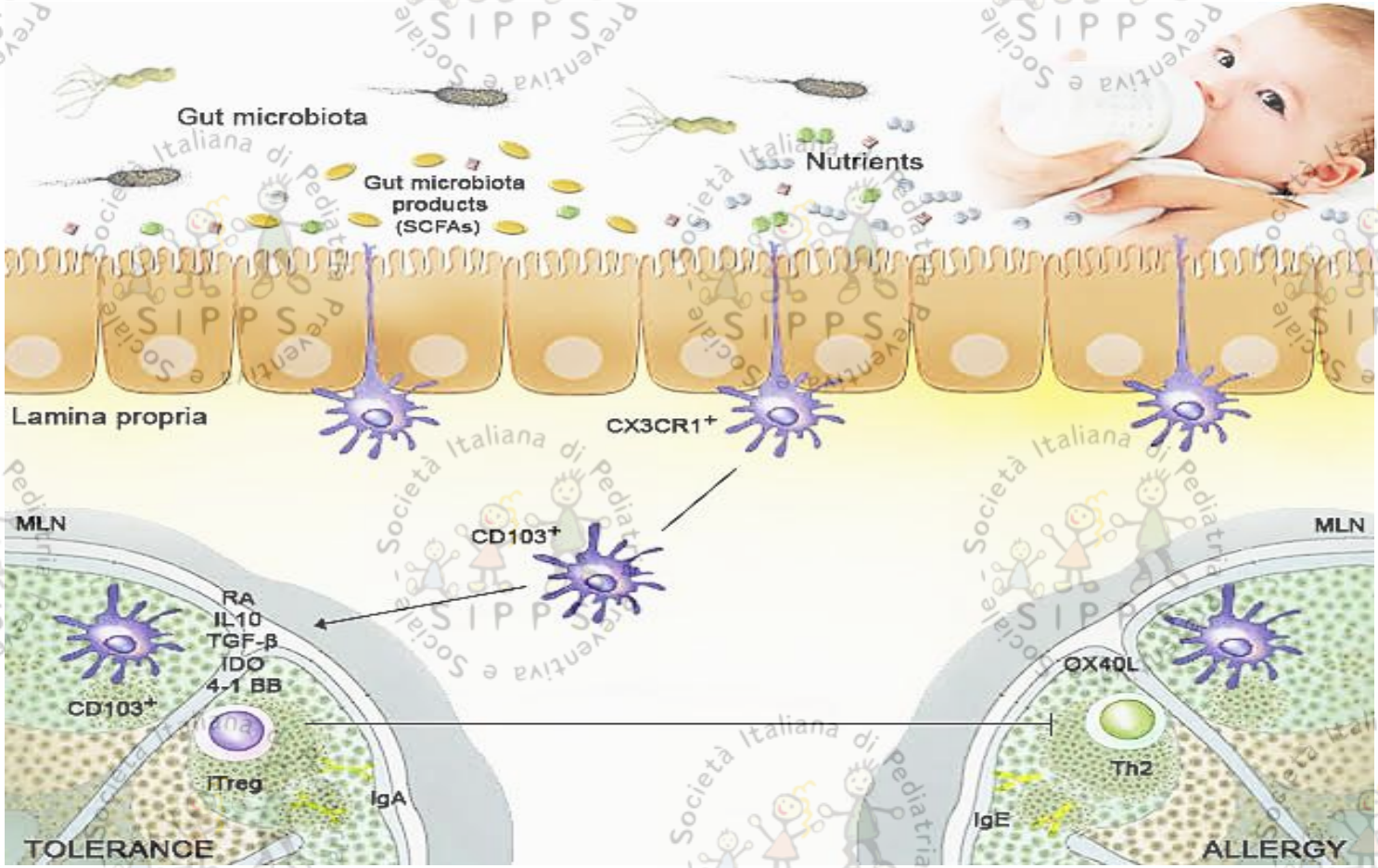
↑ Rischio di sviluppare altre malattie atopiche *(>50% asma, eczema, orticaria, oculorinite)*

↓

Favorire sviluppo e mantenimento della tolleranza orale

Host A, et al. PAI 2002
Skripak JM, et al. JACI 2007
Ross MP, et al. JACI 2008
Wang J, et al. JCI 2011
Chen FM, et al. JMII 2012
Virta LJ et al. JPGN 2013
Gupta R, et al. JAMA Ped 2013
Berni Canani R et al. Clin Exp Allergy 2013
Nocerirno R et al. JACI 2015

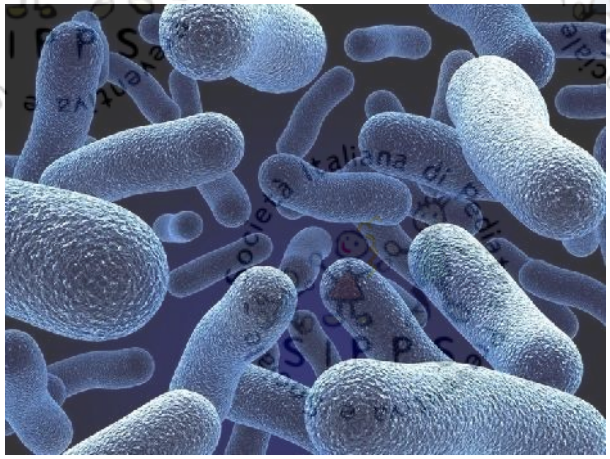
Tolleranza orale: soppressione risposta immunitaria ad Ag della dieta mediata da cellule T regolatorie Ag-specifiche

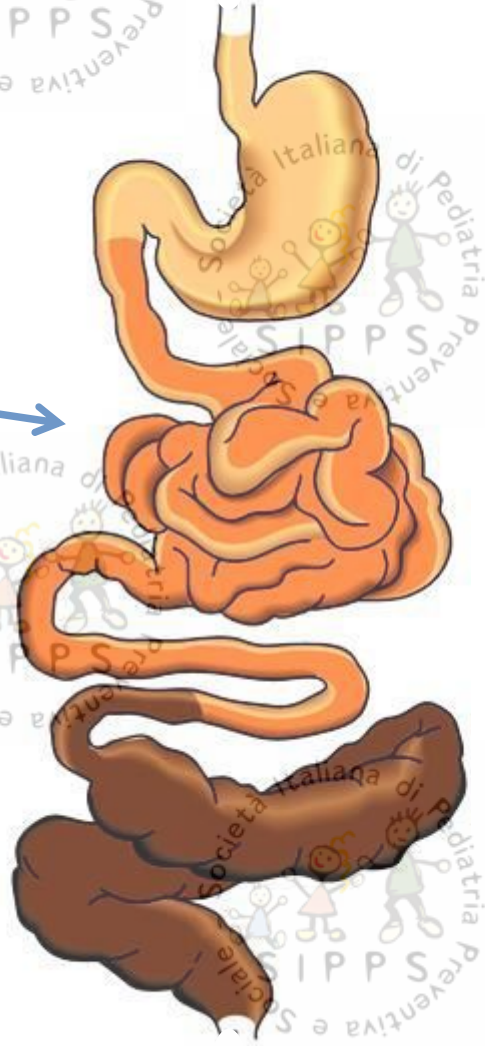


Modulazione dinamica indotta sin dalle prime epoche della vita dalla esposizione a fattori ambientali (dieta) e dallo sviluppo del microbiota intestinale

Di Costanzo M and Berni Canani R JPGN 2016

Principali fattori coinvolti nell'acquisizione della tolleranza orale: fattori dietetici e microbiota



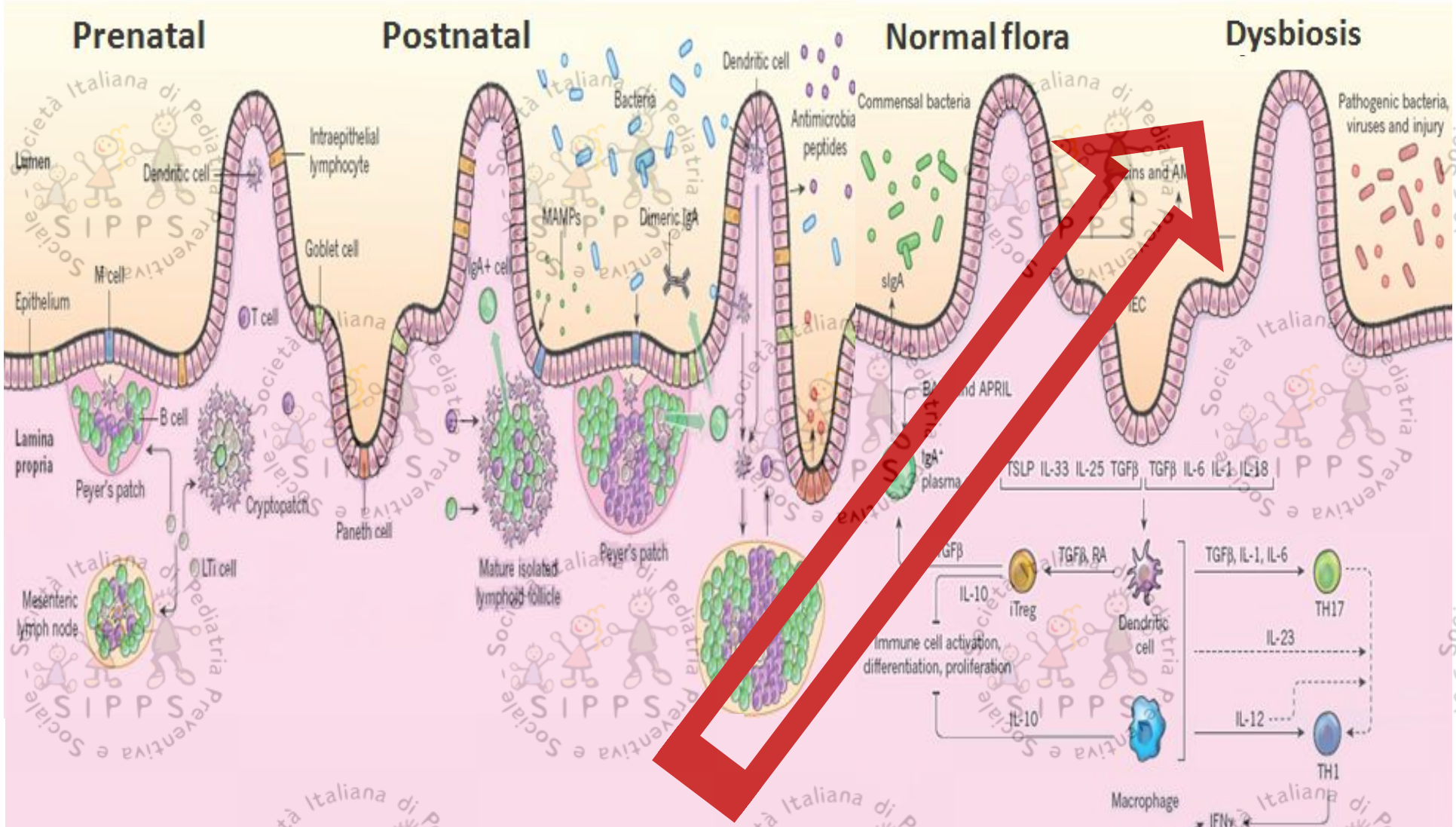


Kim KS et al. Science, 2016

L'assenza di peptidi nella dieta inibisce l'acquisizione di tolleranza: effetti della dieta elementare

- Ridotti livelli di Ig sieriche
- Ridotto numero linfociti nella lamina propria del piccolo intestino
- Ridotte dimensioni delle placche del Payer
- Ridotto numero di FoxP3+ CD4+ Treg
- Aumentata produzione di IgE specifiche
- Aumentare severità della risposta allergica

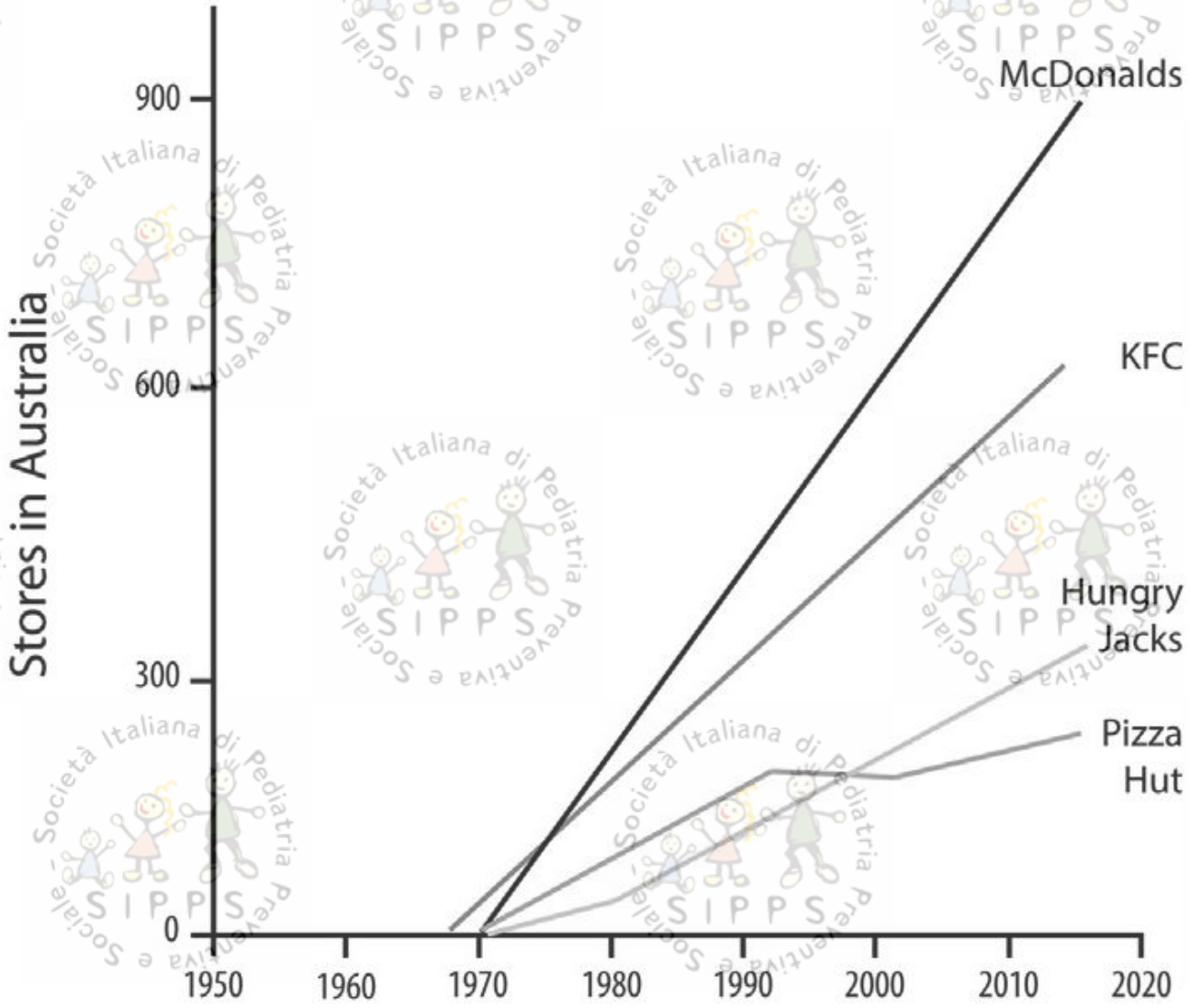
Pereira P, et al. Eur J Immunol 1986
Wastmann BS, et al. Proc Soc Exp Biol Med 1991
Kim KS et al. Science 2016



- Parto cesareo
- Formula
- Antibiotici
- Inibitori dell'acidità gastrica
- Dieta ricca di grassi saturi e povera di fibre

Modified from Maynard CL, et al. Nature 2012

AA IN UN PAESE INDUSTRIALIZZATO

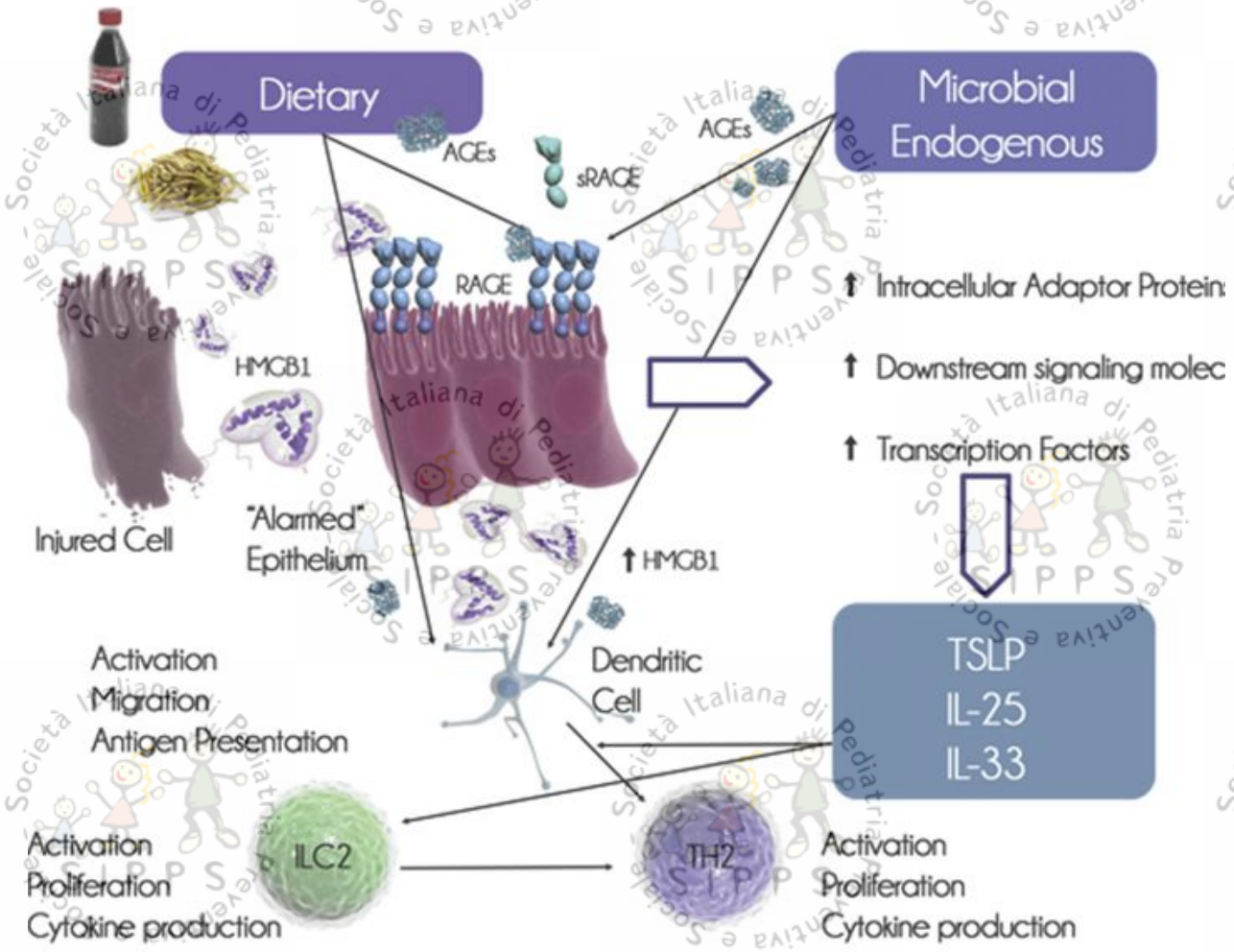


Smith PK, JACI 2016 in press

AGEs (Advanced Glycation End Products)

COMPOSTI CHIMICI PRODOTTI DALLA COMBINAZIONE DI ZUCCHERI CON PROTEINE O GRASSI (GLICAZIONE AVANZATA)

Superfici dorate o abbrustolite di cibi fritti o grigliati, pane tostato, ecc.



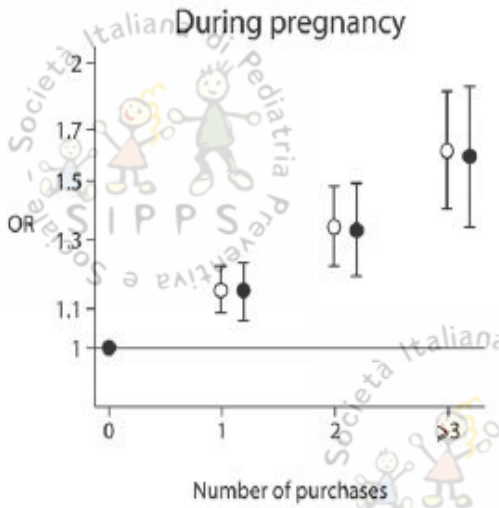
Smith PK, JACI 2016 in press

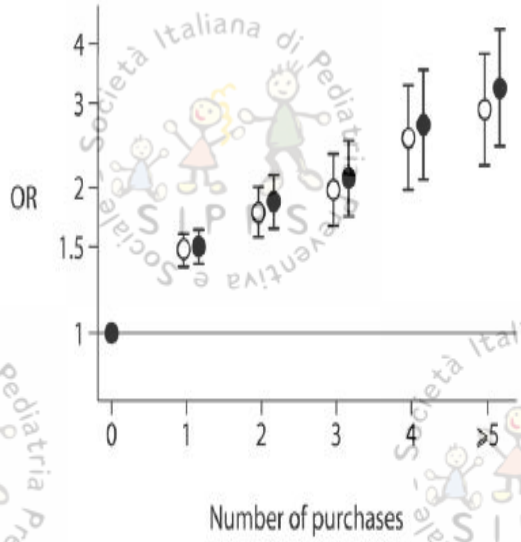
Mother's and Offspring's Use of Antibiotics and Infant Allergy to Cow's Milk

Johanna Metsälä,[a,b] *Annamari Lundqvist,*[c] *Lauri J. Virta,*[d] *Minna Kaila,*[e] *Mika Gissler,*[f,g] *and Suvi M. Virtanen*[a,b,h]

Antibiotic use by the mother

Antibiotic use by the child

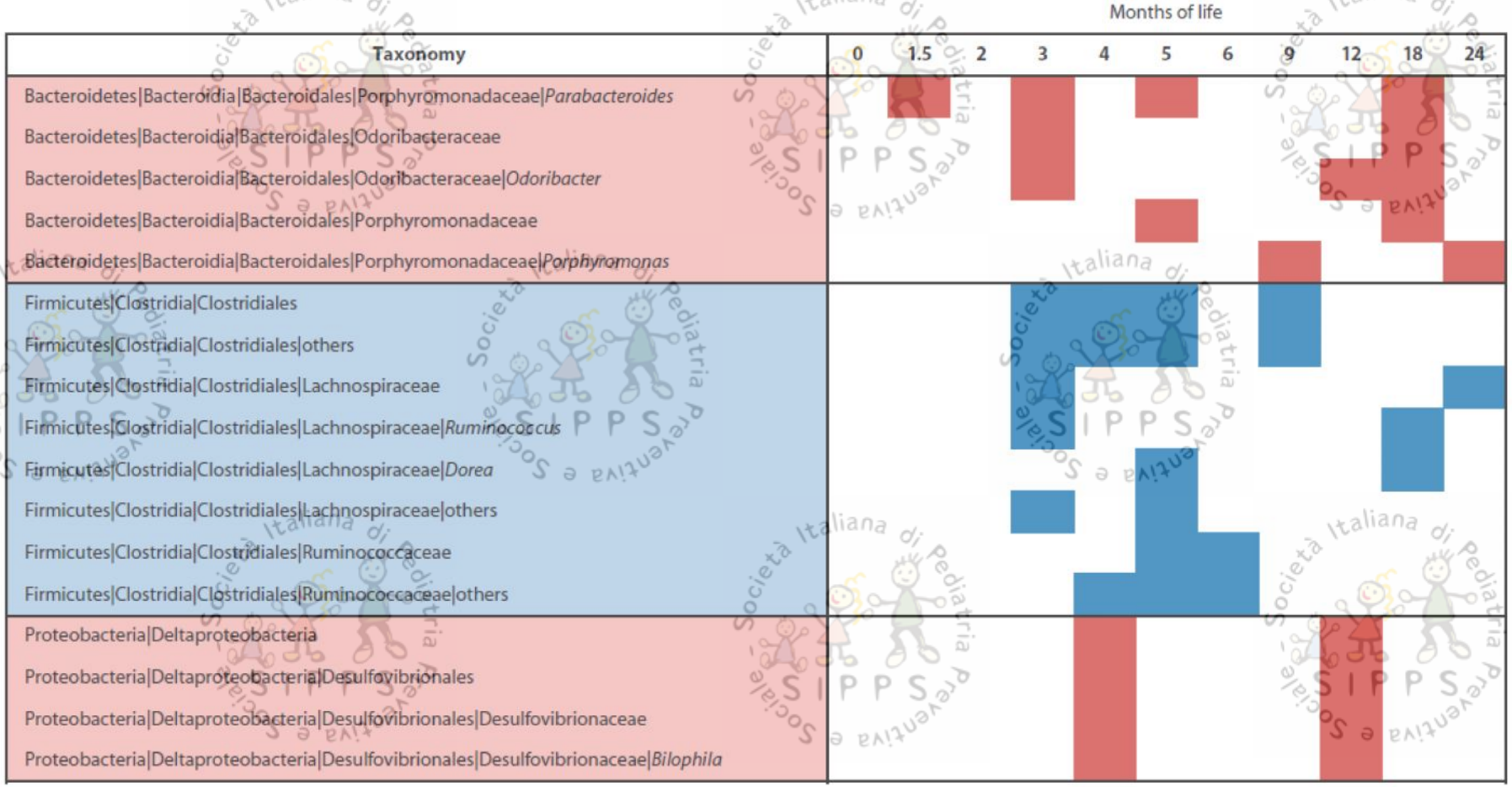




Epidemiology • Volume 24, Number 2, March 2013

Antibiotics, birth mode, and diet shape microbiome maturation during early life

Nicholas A. Bokulich,[1] Jennifer Chung,[1] Thomas Battaglia,[1] Nora Henderson,[1] Melanie Jay,[1,2] Huilin Li,[3] Arnon D. Lieber,[1] Fen Wu,[1,2] Guillermo I. Perez-Perez,[1,4] Yu Chen,[1,2] William Schweizer,[5] Xuhui Zheng,[4] Monica Contreras,[1] Maria Gloria Dominguez-Bello,[1] Martin J. Blaser[1,4,6]*

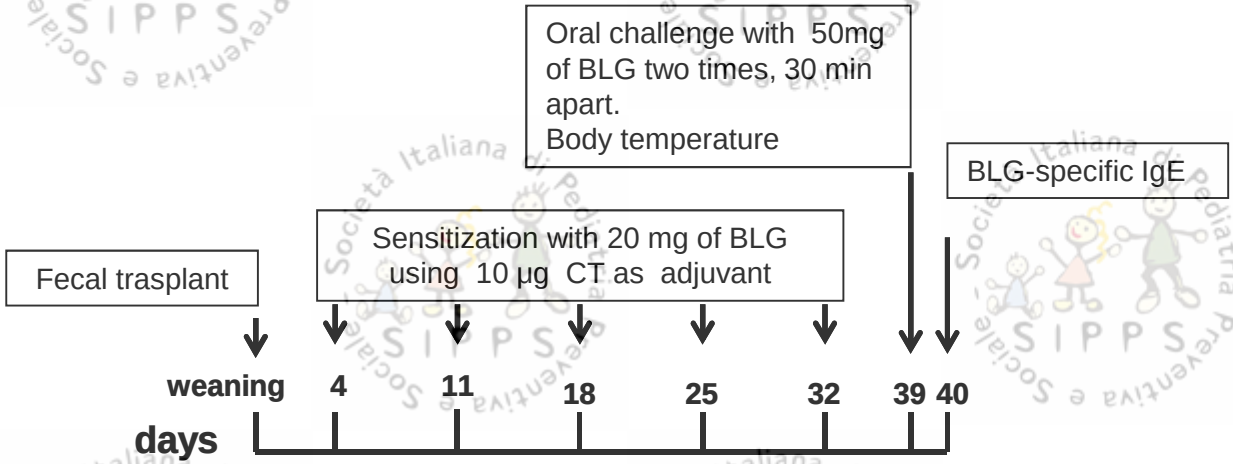


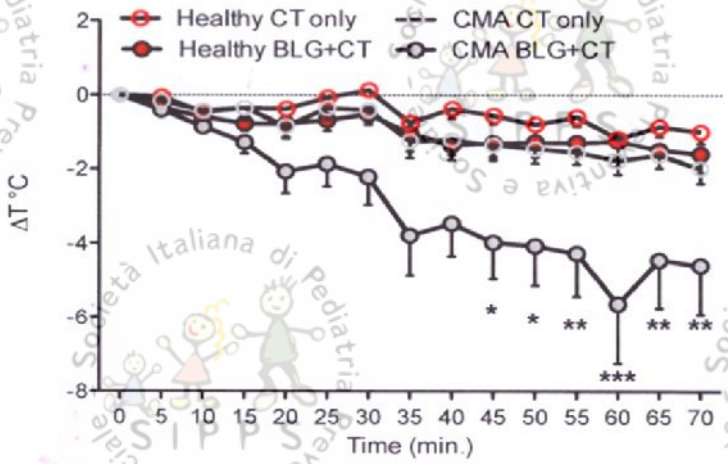
www.ScienceTranslationalMedicine.org 15 June 2016 Vol 8 Issue 343 343ra82

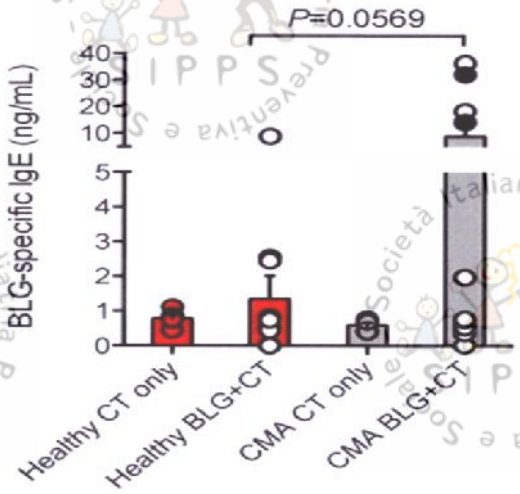
Modello di topo “umanizzato”

La colonizzazione del tratto GI con feci di lattante sano protegge dall'AA

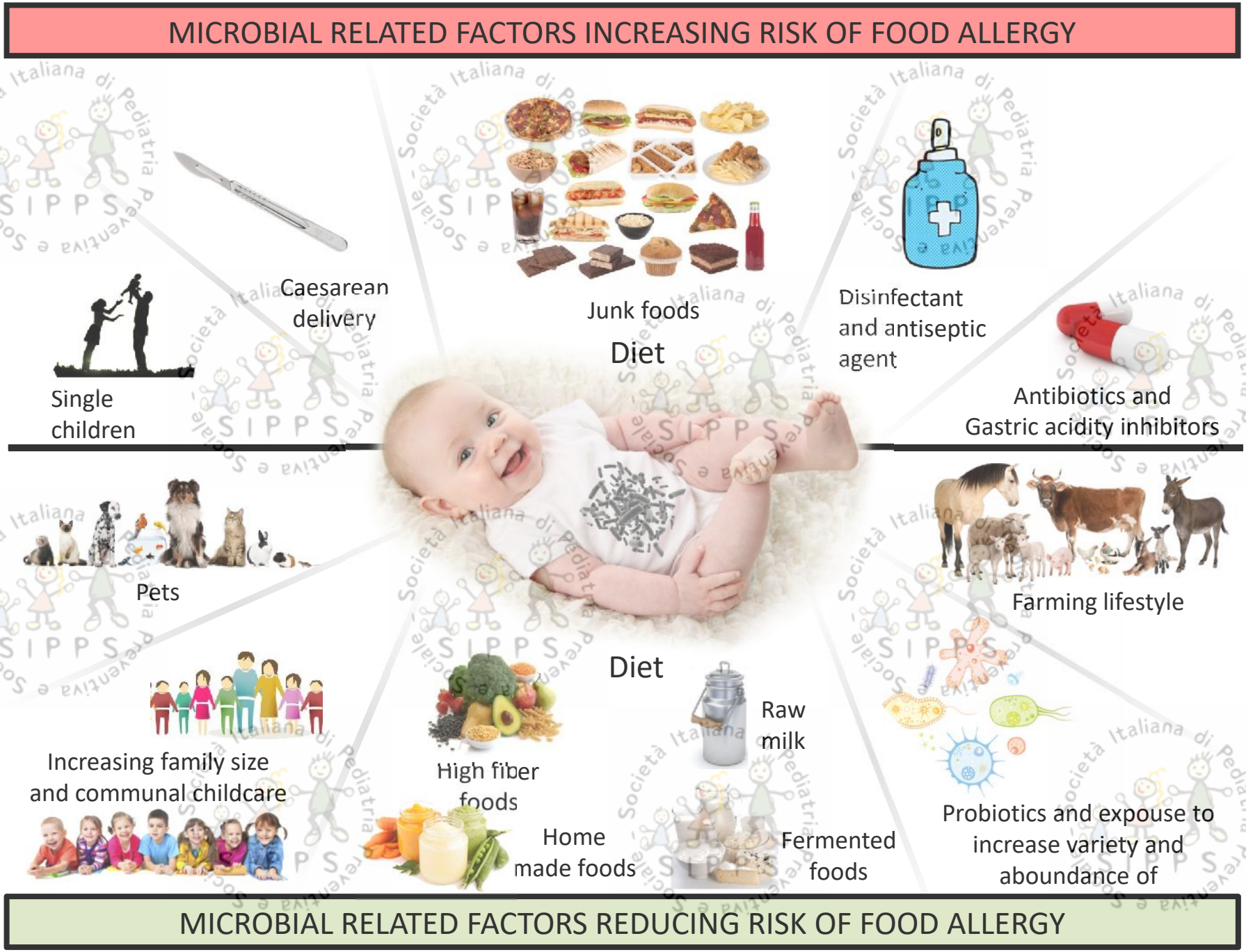
GF C3H/HeN 3-4 weeks old







Berni Canani R and Nagler C, submitted

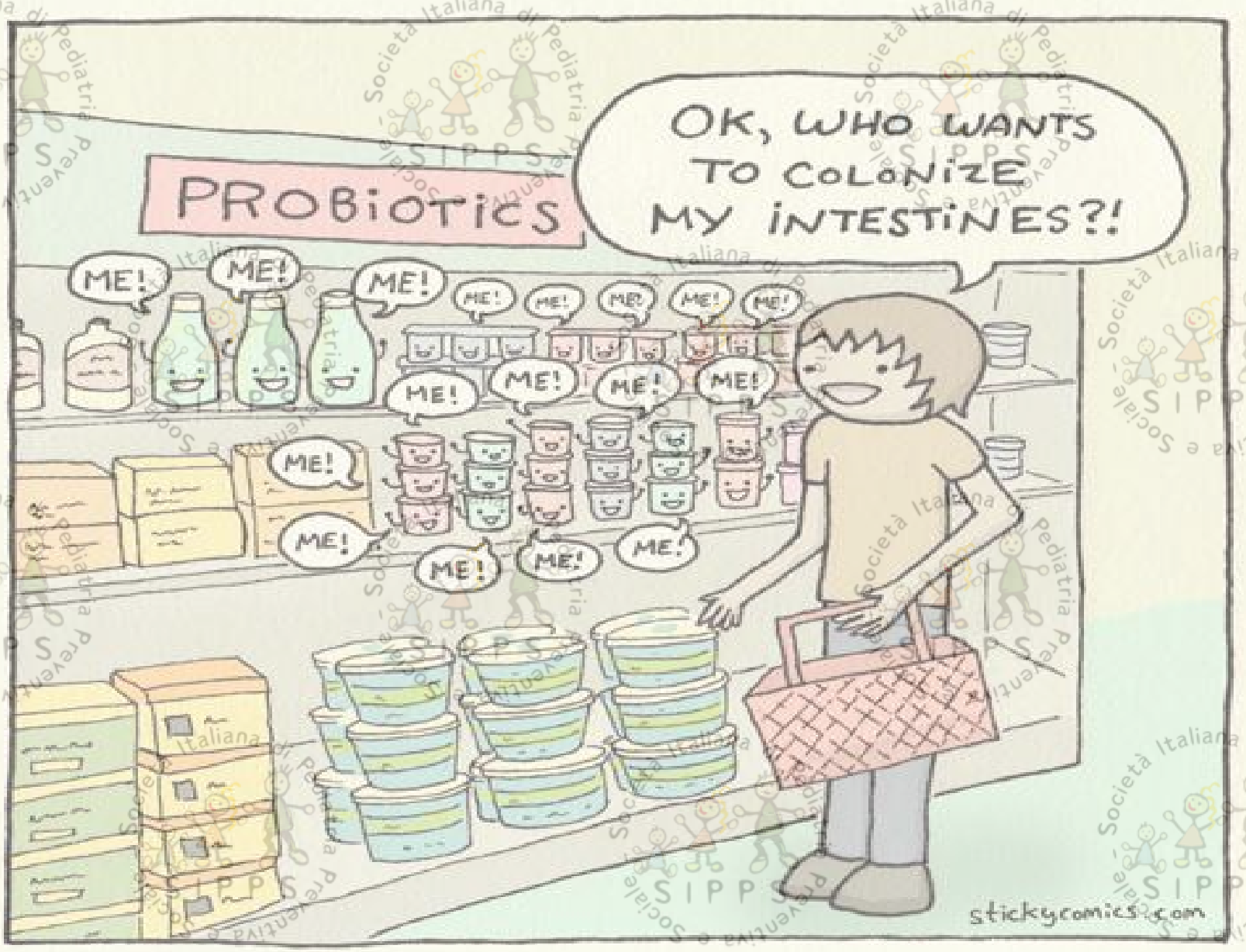


Berni Canani R et al. Biotascope in press


HELP

MICROBIOTA e APLV

- ✓ Disbiosi caratterizzata da ridotta presenza di batteri produttori di butirrato
- ✓ Disbiosi precede la comparsa di APLV
- ✓ Alcuni ceppi di probiotici possono modificare composizione e funzioni del microbiota intestinale del bambino affetto da APLV



Rare

Strain-specific effects

Neurological effects
Immunological effects
Endocrinological effects
Production of specific bioactives

Frequent

Observed at species level

Vitamin synthesis
Direct antagonism
Gut barrier reinforcement
Bile salt metabolism
Enzymatic activity
Neutralization of carcinogens

Widespread

Among studied probiotics

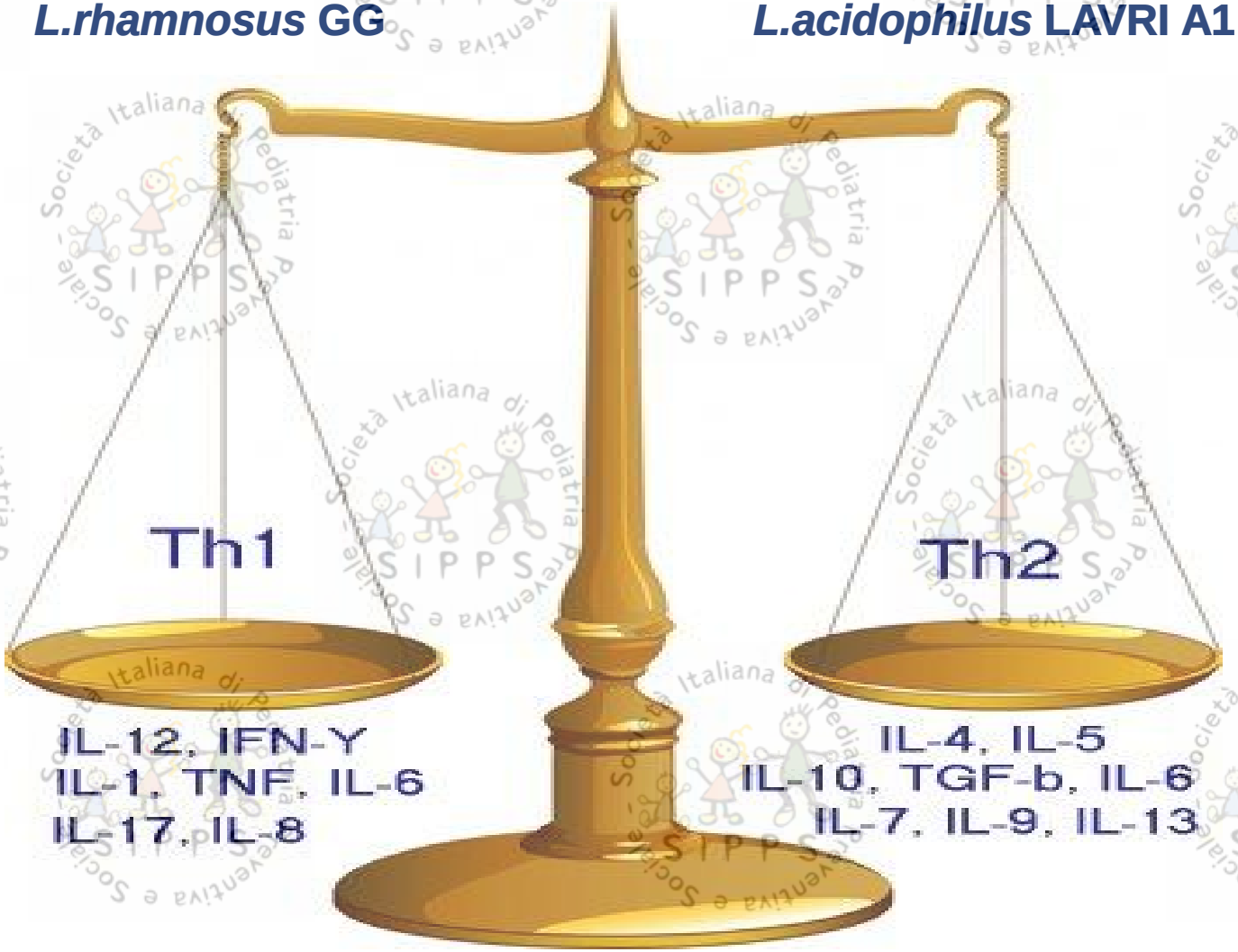
Colonisation resistance
Enterocytes growth
Regulation of intestinal transit
Normalization of perturbed microbiota
Competitive exclusion of pathogens

NATURE REVIEWS | GASTROENTEROLOGY & HEPATOLOGY 2014;11:506–14

“Curare” la disbiosi del bambino con APLV?

***L.casei* CRL431/*B. lactis* Bb12**

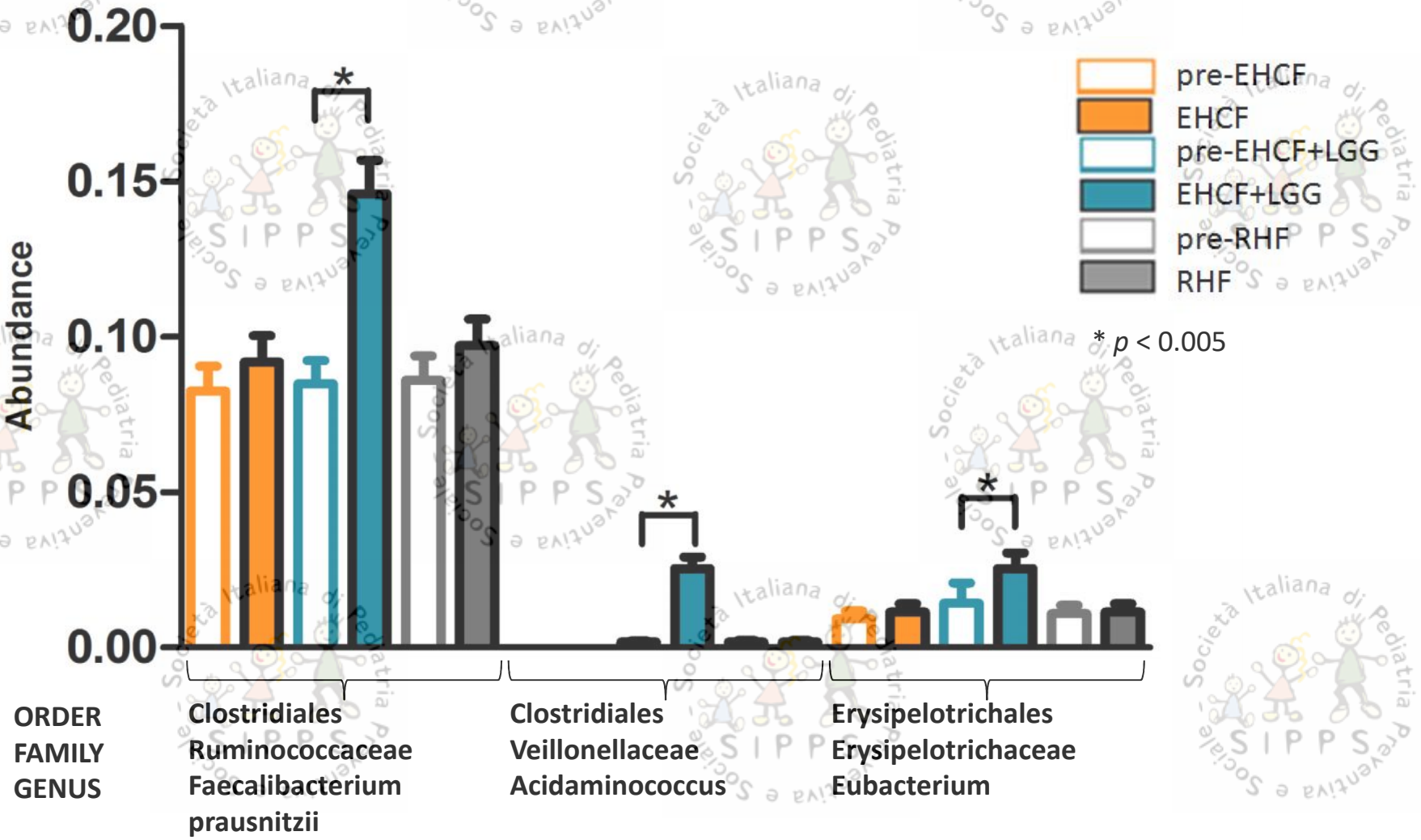
***L.rhamnosus* GG** ***L.acidophilus* LAVRI A1**



Berni Canani R et al. Curr Opin Allergy Clin Immunol 2015

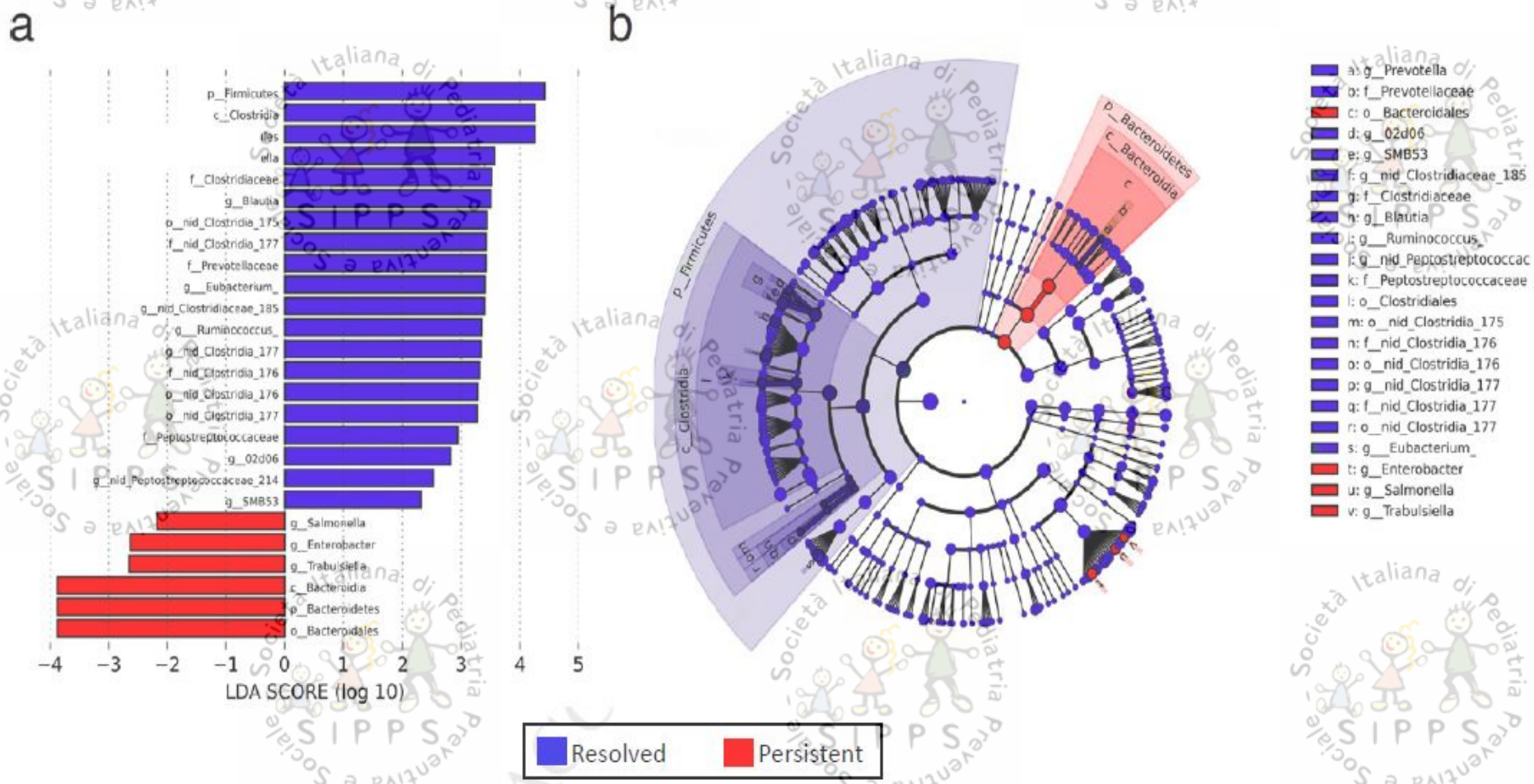
LGG expands protective bacteria in the gut lumen of CMA infants

The ISME Journal
Multidisciplinary Journal of Microbial Ecology
The ISME Journal TOP TEN



Modified from Berni Canani R, et al. ISME Journal 2015

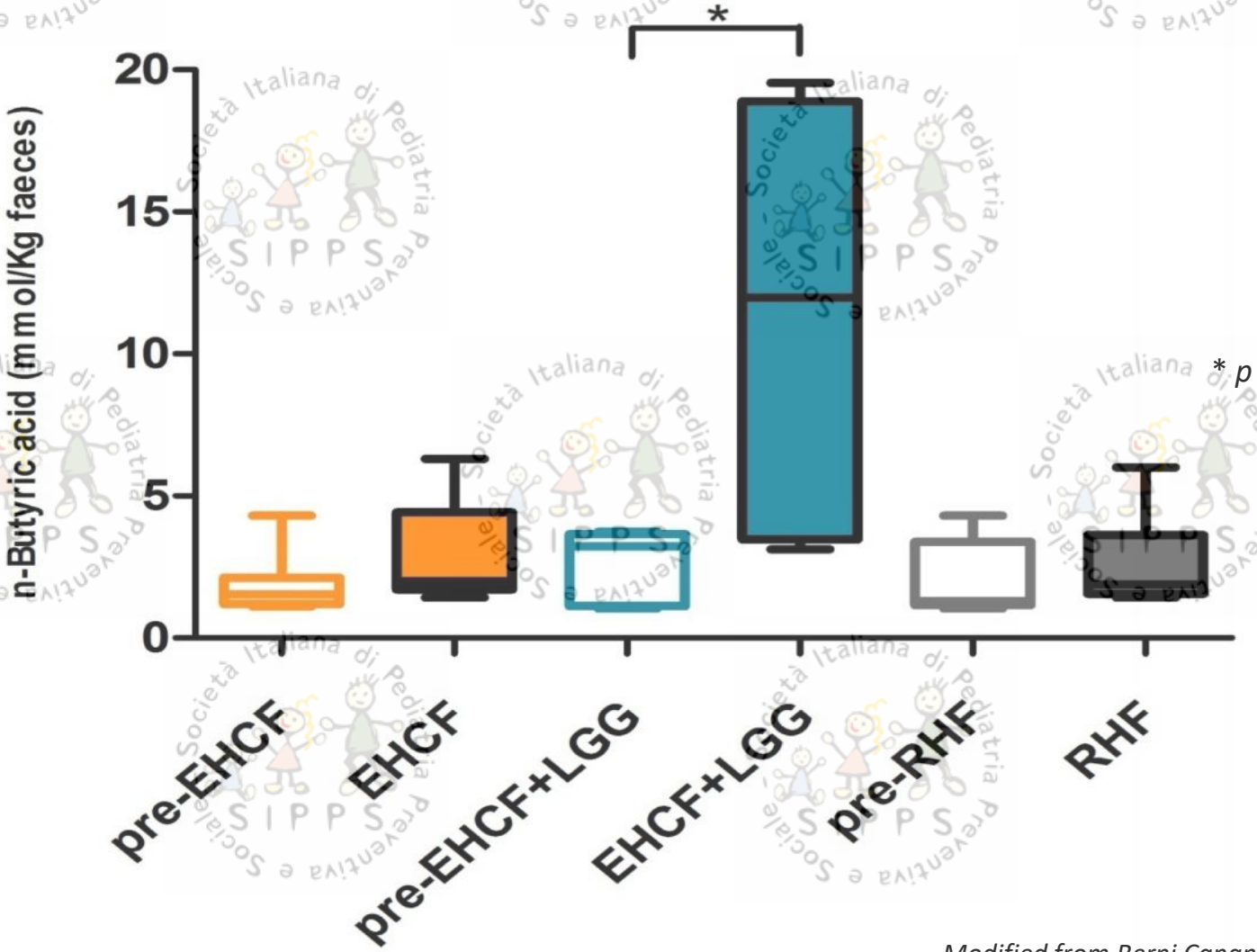
Early-life gut microbiome composition and milk allergy resolution



Bunyavanich S edt al. JACI in press

LGG expands butyrate-producing bacteria in CMA infants

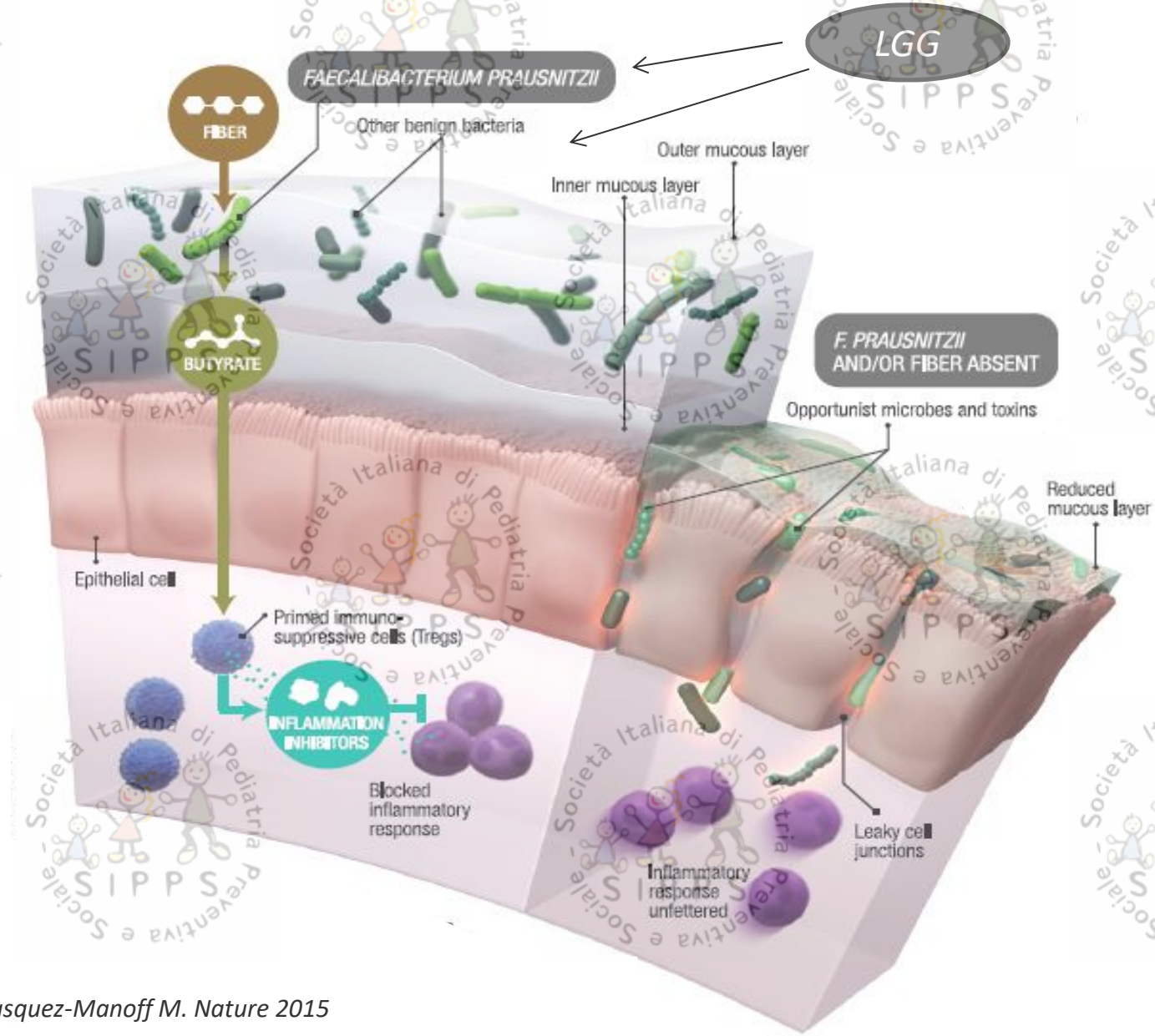
The ISME Journal
Multidisciplinary Journal of Microbial Ecology
The ISME Journal TOP TEN



* $p < 0.005$

Modified from Berni Canani R, et al. ISME Journal 2015

LGG: The Peace-keeper for CMA

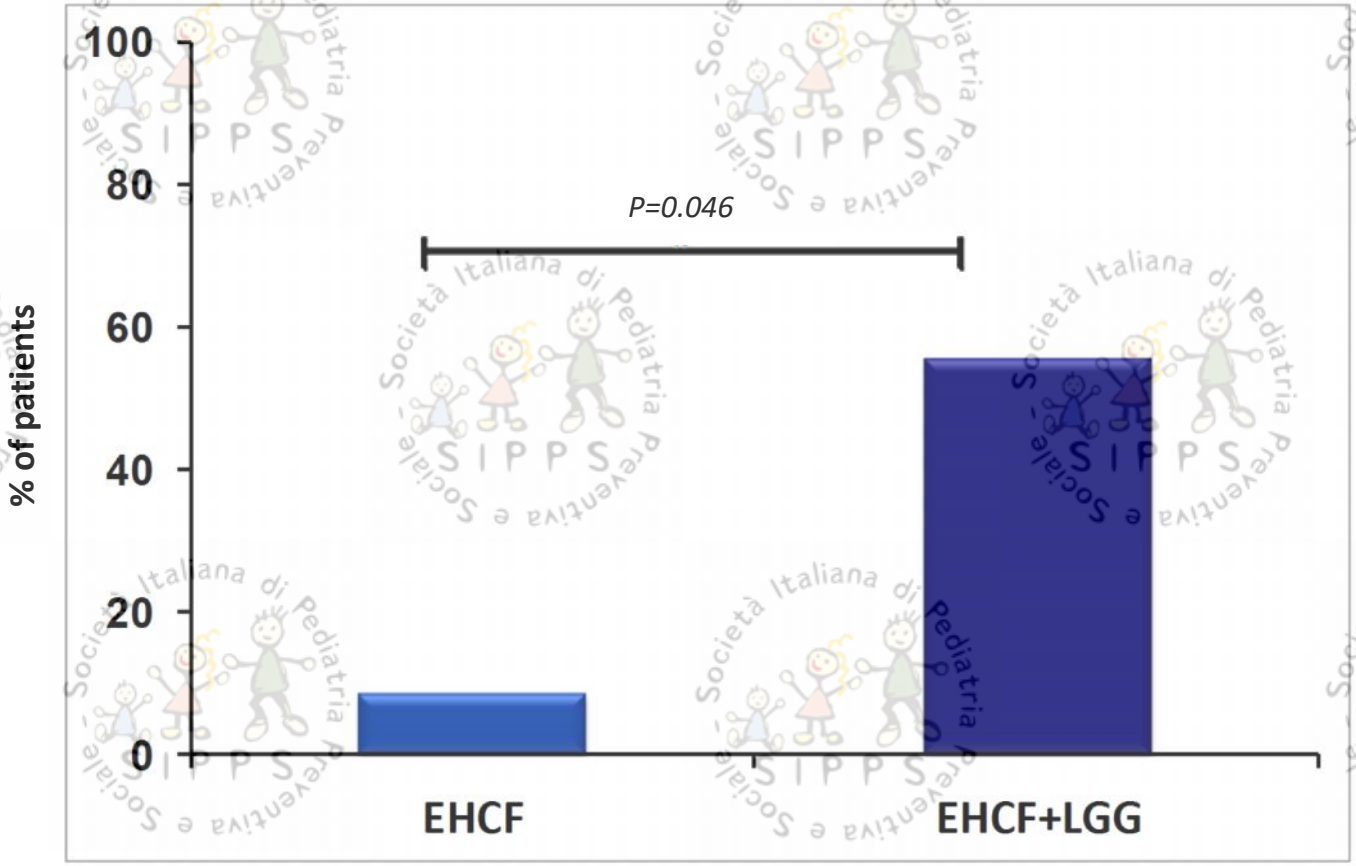


Modified from Velasquez-Manoff M. Nature 2015

LGG accelerates cow's milk protein tolerance acquisition

THE JOURNAL OF Allergy AND Clinical Immunology

Rate of children with IgE-mediated CMA acquiring tolerance at 12 m

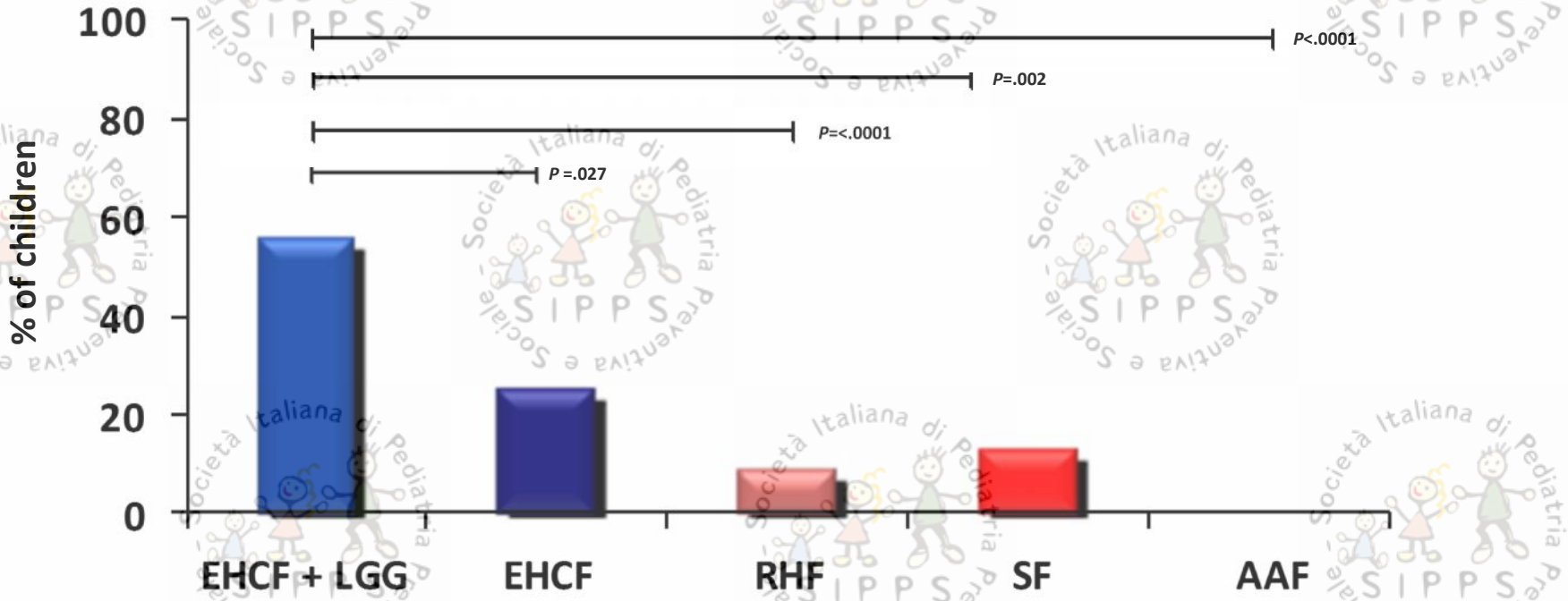


Berni Canani R, et al. 2012

EHCF+LGG leads to tolerance at an earlier age than other formulae

The JOURNAL of PEDIATRICS

Rate of children with IgE-mediated CMA acquiring tolerance at 12 m

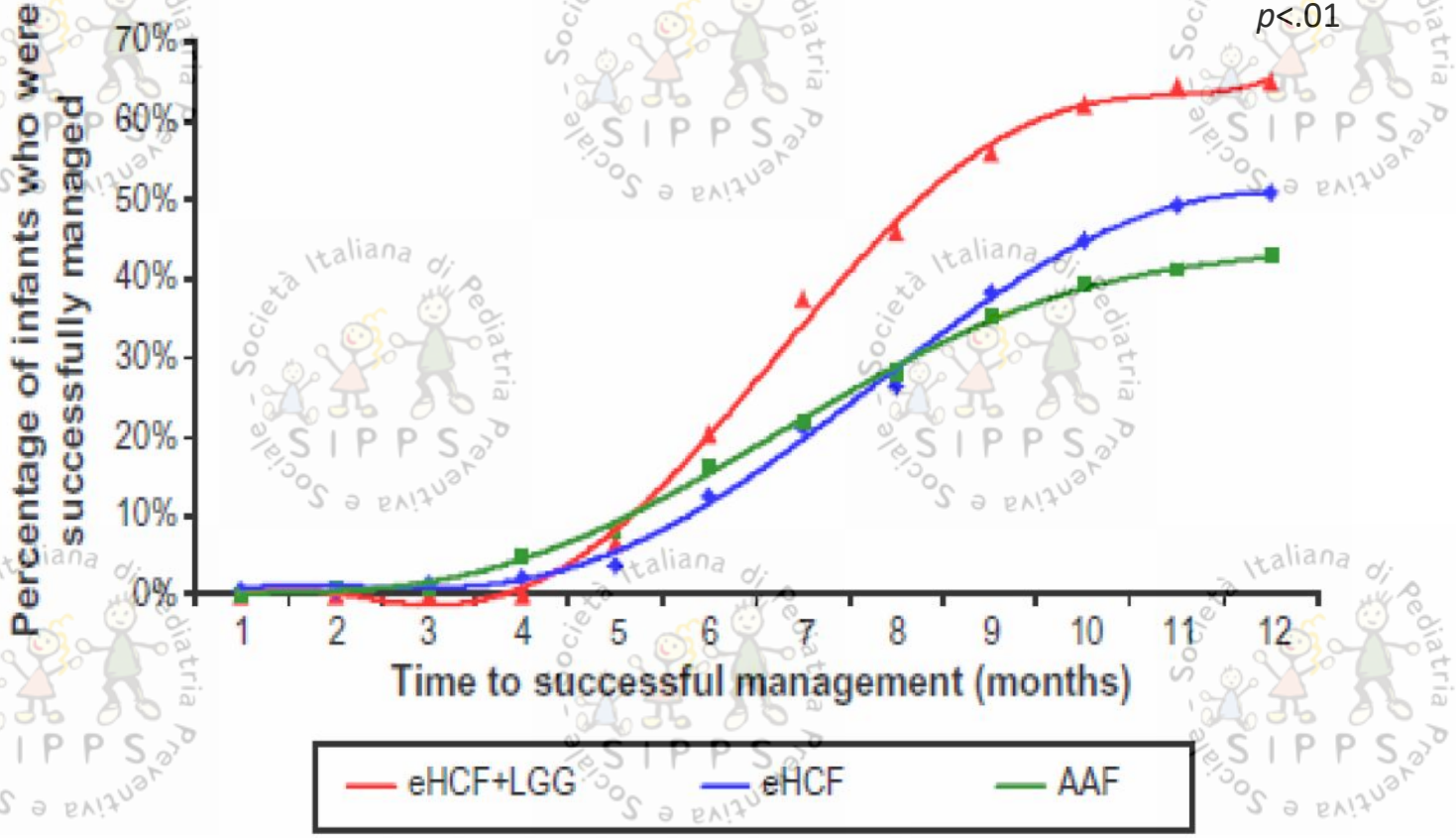


Berni Canani R, et al. 2013

Cost-effectiveness of using an extensively hydrolyzed casein formula plus the probiotic *Lactobacillus rhamnosus* GG compared to an extensively hydrolyzed formula alone or an amino acid formula as first-line dietary management for cow's milk allergy in the US

Olga Ovcinnikova[1]
Monica Panca[1]
Julian F Guest[1,2]

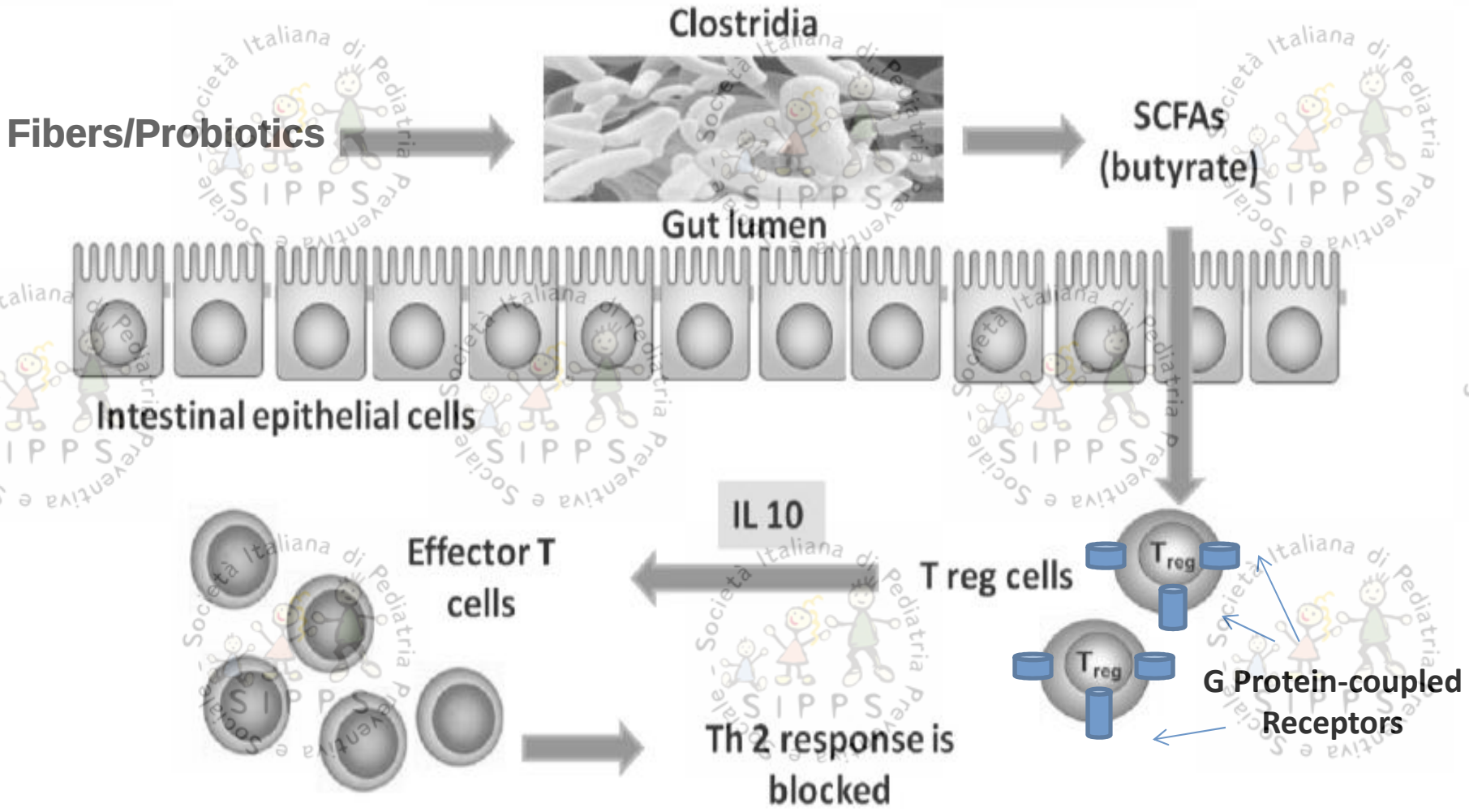
[1]CATALYST Health Economics Consultants, Northwood, London,
[2]Faculty of Life Sciences and Medicine, King's College, London, UK



ClinicoEconomics and Outcomes Research 2015:7 145–152

Potential Beneficial Effects of Butyrate against Food Allergy

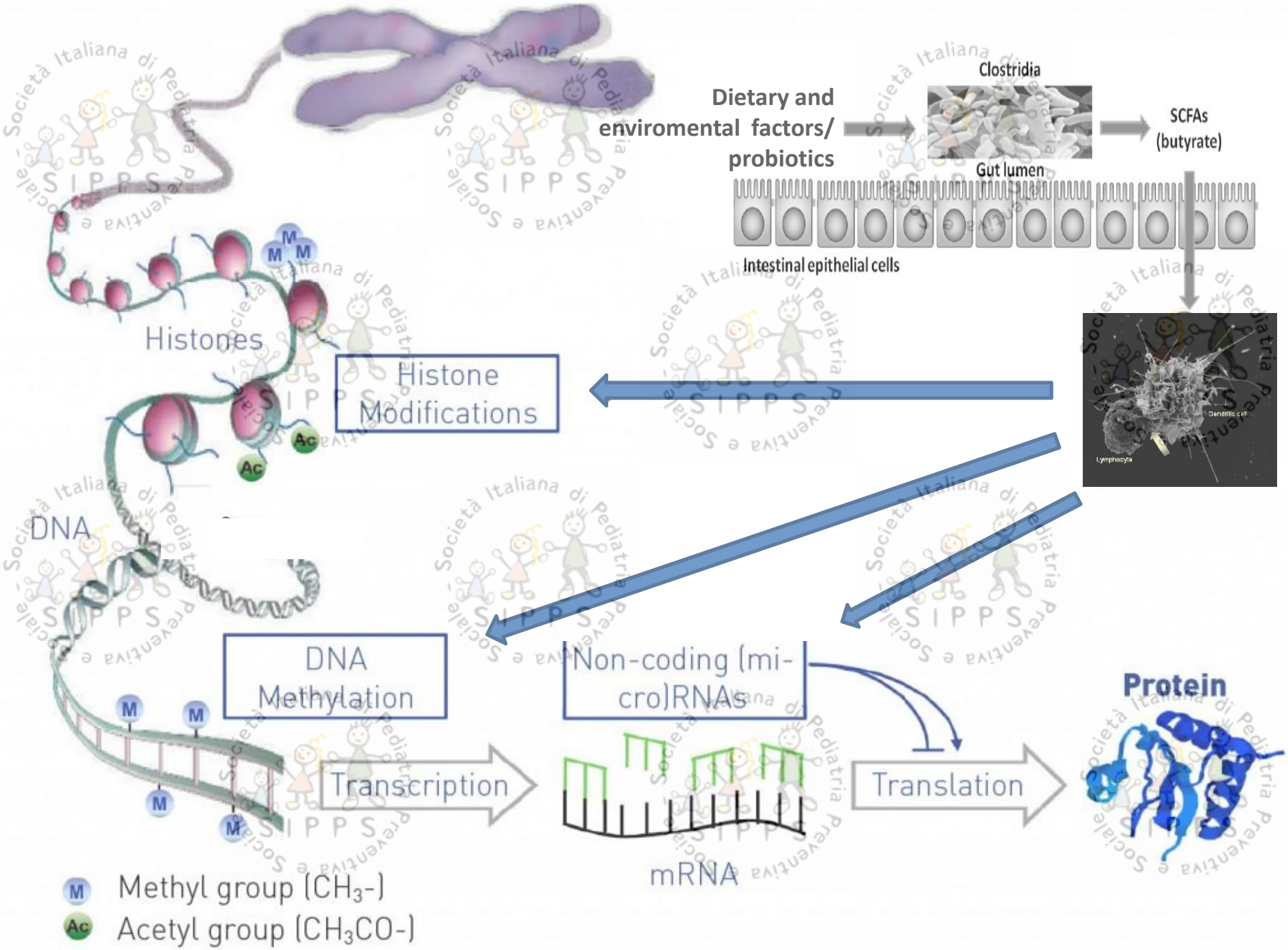
Margherita Di Costanzo[1], Lorella Paparo[1], Rosita Aitoro[1], Linda Cosenza[1], Rita Nocerino[1], Tommaso Cozzolino[1], Vincenza Pezzella[1], Gianfranco Vallone[2] and Roberto Berni Canani[1,3,]*



In: Butyrate: Food Sources, Functions and Health Benefits

Epigenetics mechanisms: heritable changes in gene expression not involving changes in DNA primary sequence

Berni Canani R et al. Nutr Res Rev 2011
Berni Canani R et al. Clin Epigenet 2015


Dietary and
enviromental factors/
probiotics
Clostridia
SCFAs
(butyrate)
Gut lumen
Intestinal epithelial cells
Histones
Histone
Modifications
DNA
DNA
Methylation
Non-coding (mi-
cro)RNAs
Transcription
mRNA
Translation
Protein
Methyl group (CH_3-)
Acetyl group (CH_3CO-)

LGG aumenta il numero di batteri produttori di butirrato: attivazione di meccanismi epigenetici

- Modulation of histone deacetylases 6 and 9
- Th1 cytokine genes demethylation
- Treg activation through DNA demethylation

Up-regulation of Th1 response

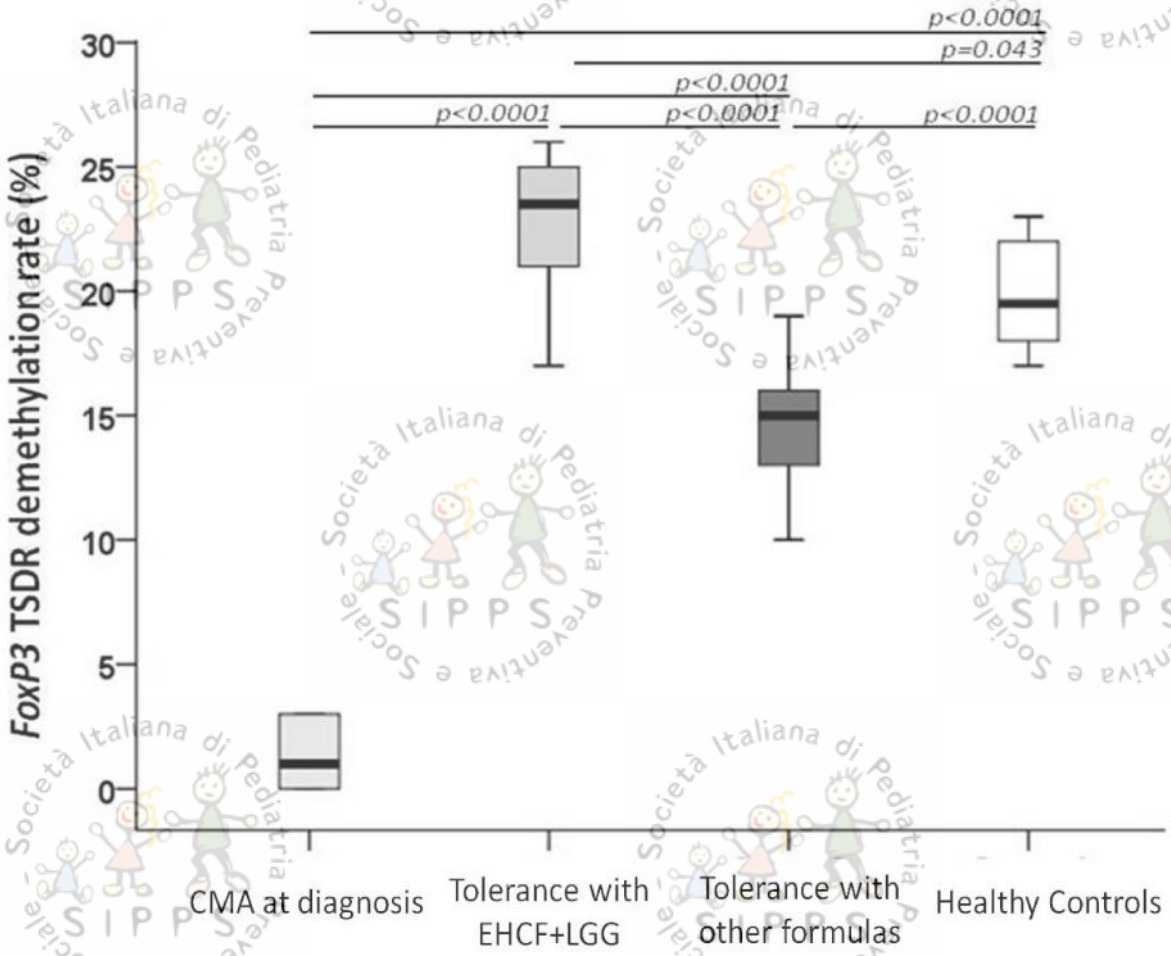
- Selective miRNAs modulation

Down-regulation of Th2 response

Berni Canani R et al. Clin Epigenet 2015
Paparo L et al. Clin Epigenet 2016

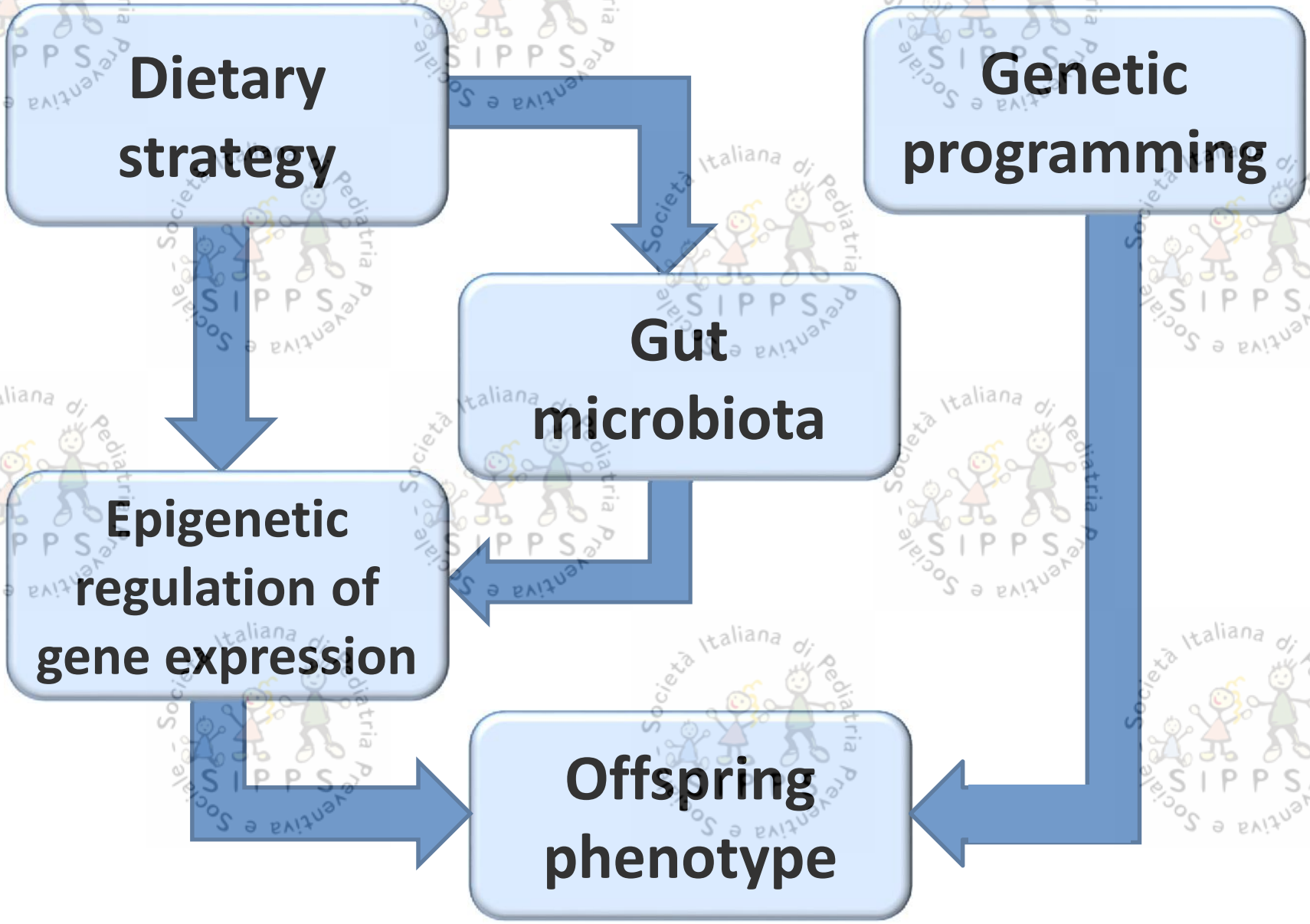
Modulation of TSDR FoxP3 demethylation CMA disease course

CLINICAL EPIGENETICS

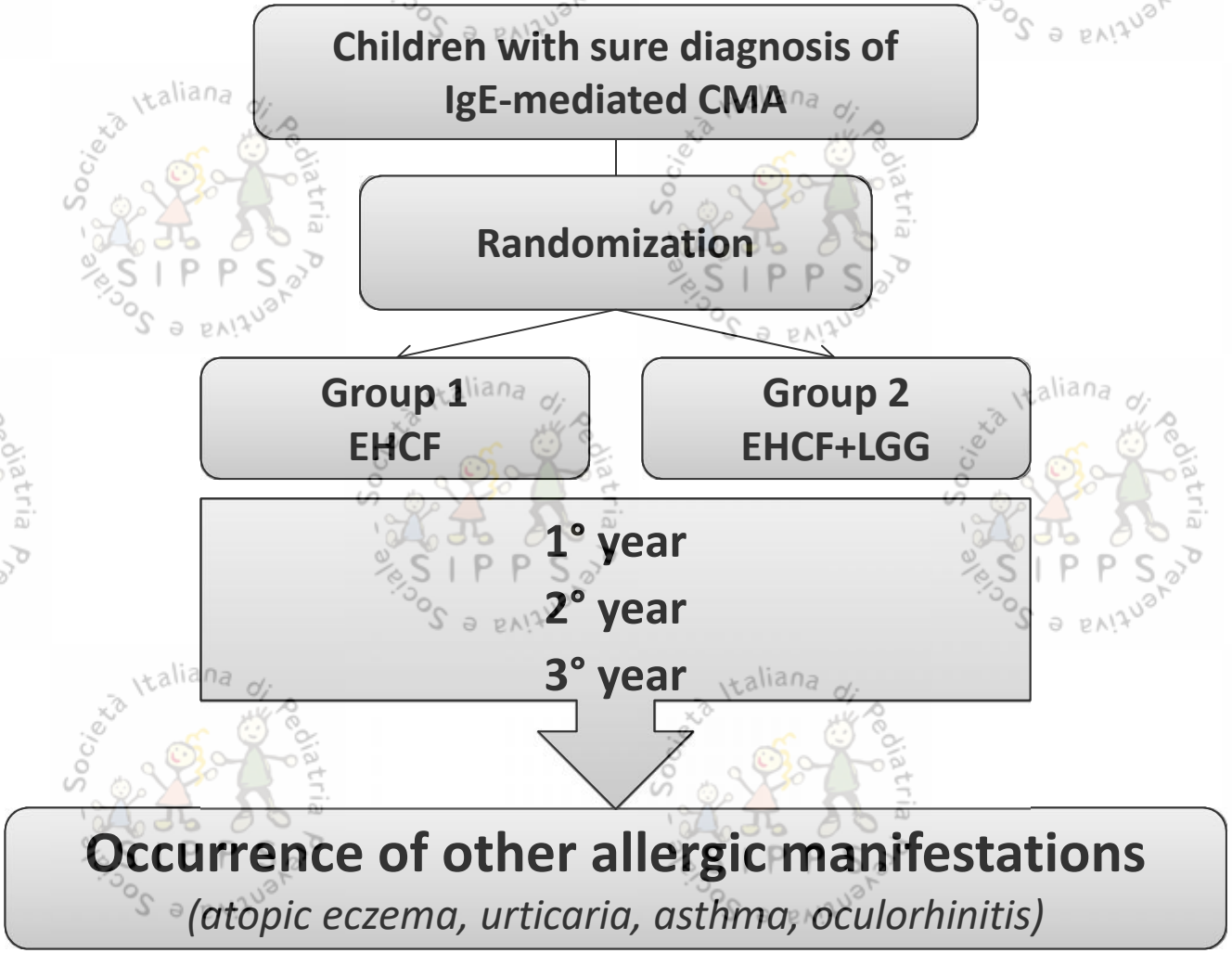


Paparo L et al. Clin Epigenet 2016

New Targets: New Strategies of Intervention

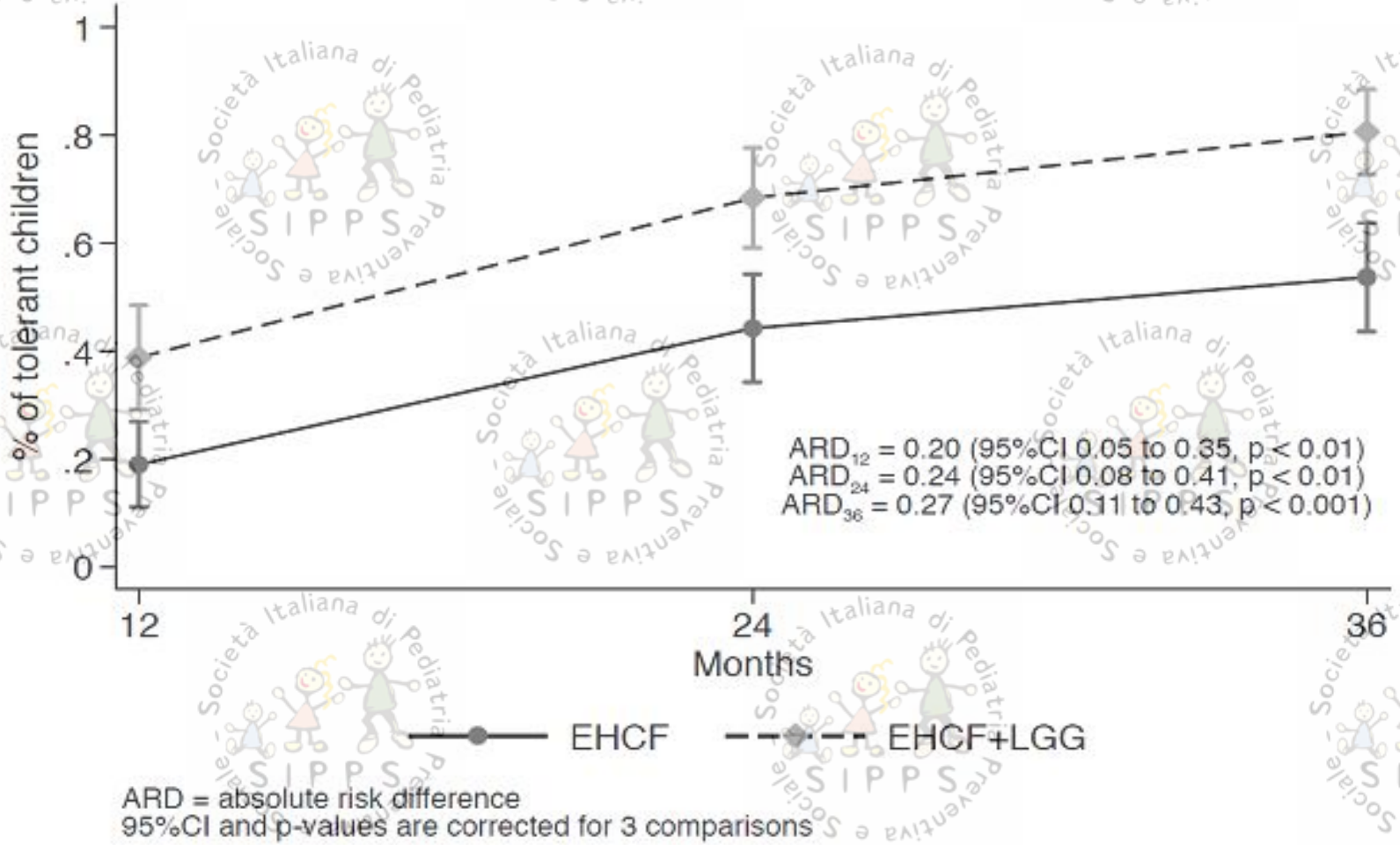


Prospective study: Preventive effect elicited by LGG on atopic manifestations in CMA children



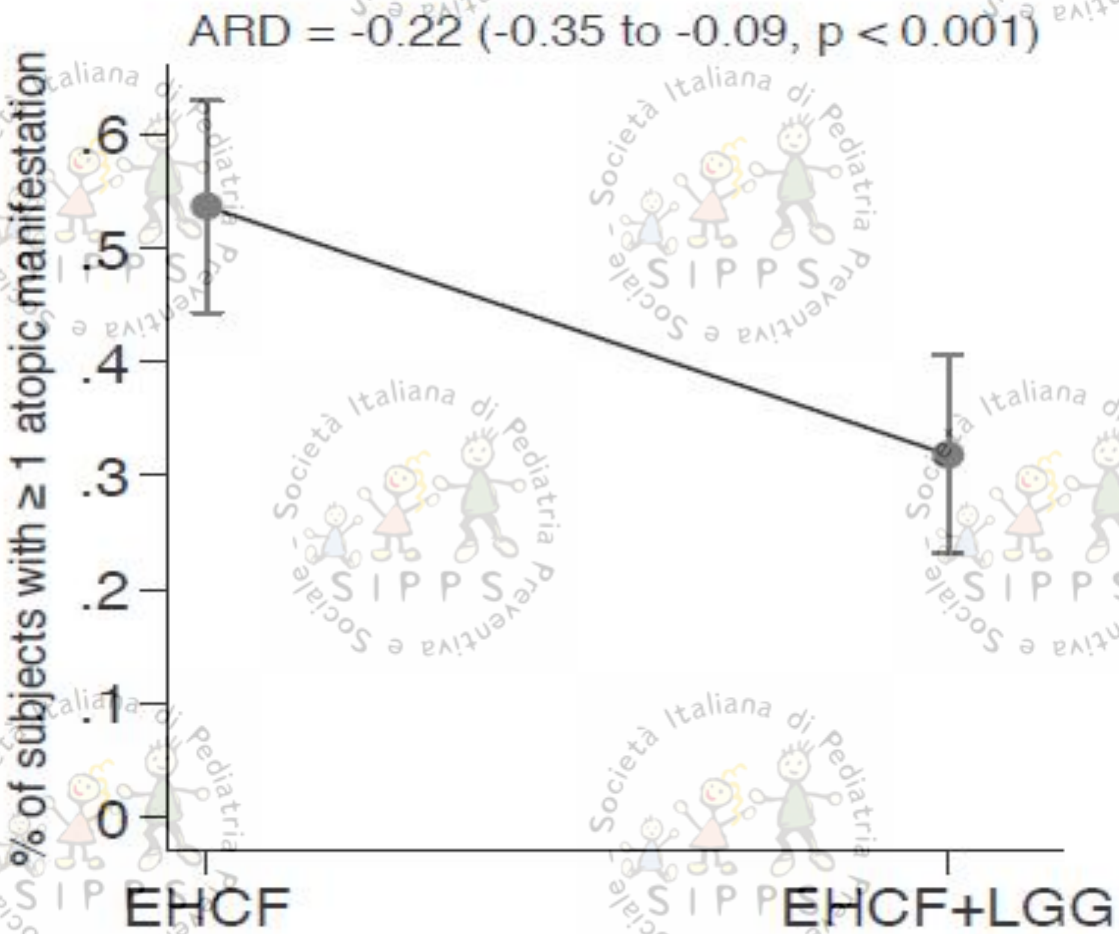
Berni Canani R, et al. 2016 submitted

Oral Tolerance Acquisition



Berni Canani R 2016 submitted

Rate of subjects experienced ≥ 1 atopic manifestation ITT, n=110/group



Berni Canani R 2016 submitted

Conclusioni

- Persistenza e severità dell'AA sono sensibilmente aumentate nelle ultime 2 decadi
- Ruolo rilevante di fattori ambientali che agiscono direttamente o indirettamente ("disbiosi") sul sistema immunitario attraverso meccanismi epigenetici
- La conoscenza di questi meccanismi ha reso possibile l'individuazione di nuove strategie di intervento
- Modulando positivamente questi meccanismi è possibile favorire l'acquisizione della tolleranza e proteggere dalla marcia atopica il bambino con APLV

Team

University of Naples "Federico II"

CEINGE Advanced Biotechnologies
F.Salvatore, M.Capasso, V.D'Argenio, V.Del Monaco

Dept. of Biology
M.P.Mollica, G.Trinchese

Dept. of Experimental Pharmacology
A.Calignano, R.Meli, D.Tronino, C.Pirozzi

Dept. of Translational Medical Science
Pediatric Allergy Unit
R.Nocerino, L.Paparo, R.Aitoro, L.Cosenza, V.Granata, M.diCostanzo, L.Leone, A.Amoroso,T.Cozzolino, C.DiScala, V.Pezzella, Y.Maddalena, B.Buono, G.Gioielli

University of Chicago

Dept. of Pathology
C.Nagler, P.Johnston, T.Feehley

Argonne National Labs
J.Gilbert, D.Antonopoulos,

Comer Children's Hospital – Pediatric GI Unit
S.Guandalini, T. Patton

Dati da modelli animali

C3H/HeJ 3-4 weeks old

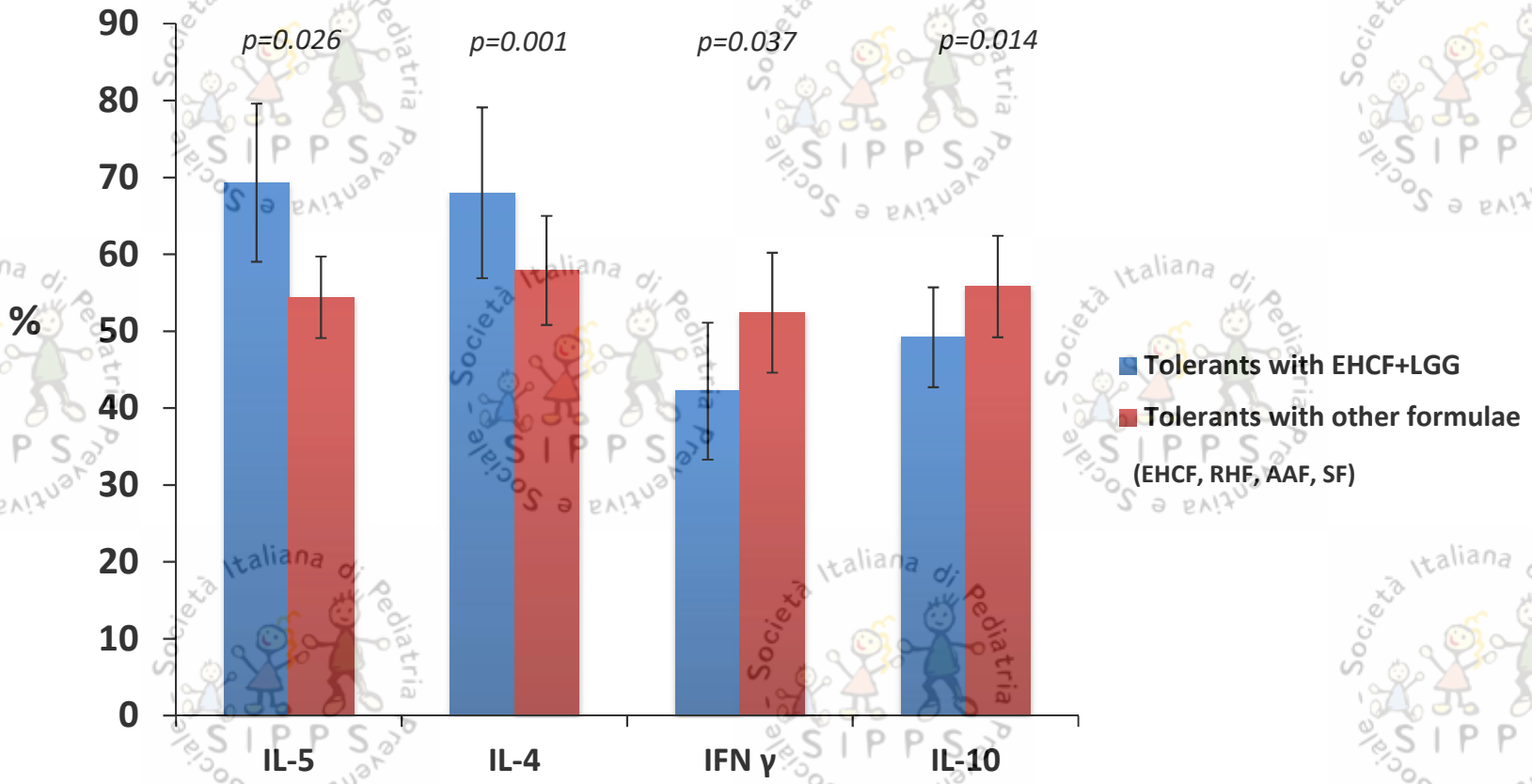
- Animali Germ-free
- Trattamento con antibiotici
- Assenza di TLR4

} Maggiore suscettibilità per lo sviluppo di AA

Bashir MEH et al. J Immunol 2004
Sefik E et al. Science 2015
Ohnmacht C et al. Science 2015

Different DNA methylation rate of IL-4, IL-5, IL-10, INF-γ genes observed in children who outgrew CMA receiving different formulae

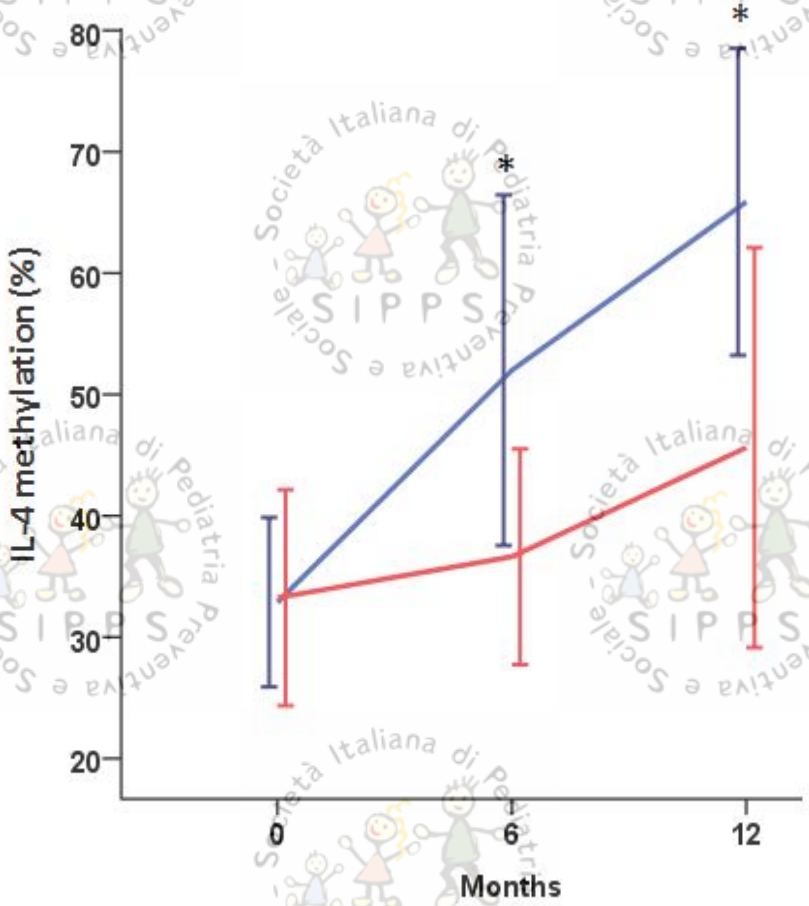
CLINICAL EPIGENETICS



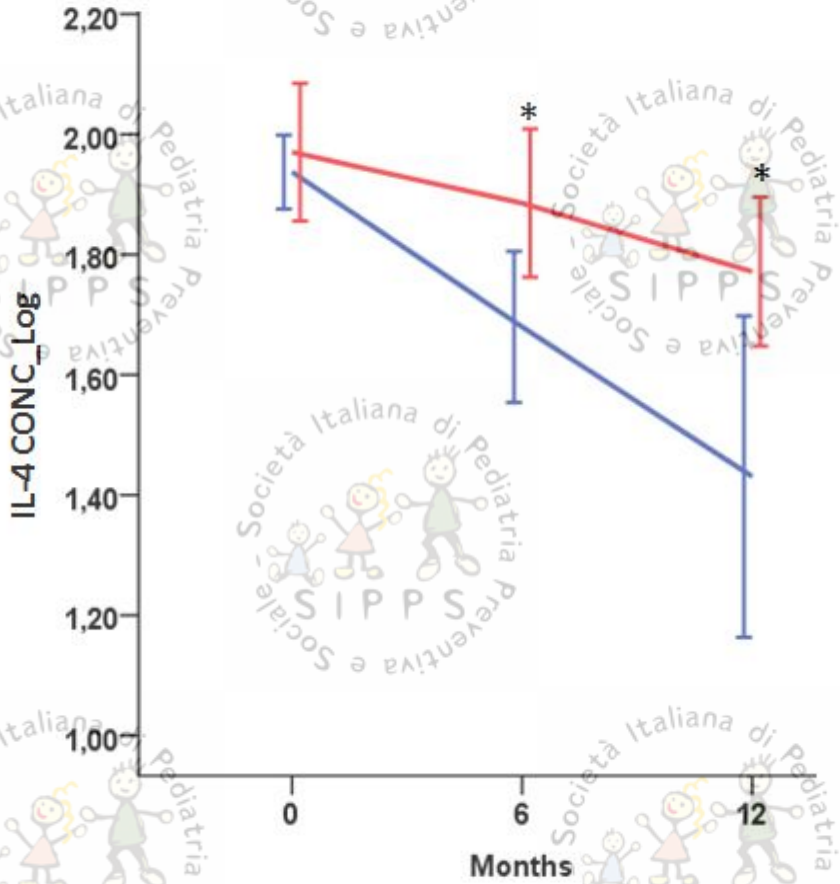
Berni Canani R, et al. 2015

Dietary influence on epigenetic mechanisms in children with IgE-mediated cow's milk allergy

IL-4 gene DNA methylation rate



*p<0.05



*p<0.05

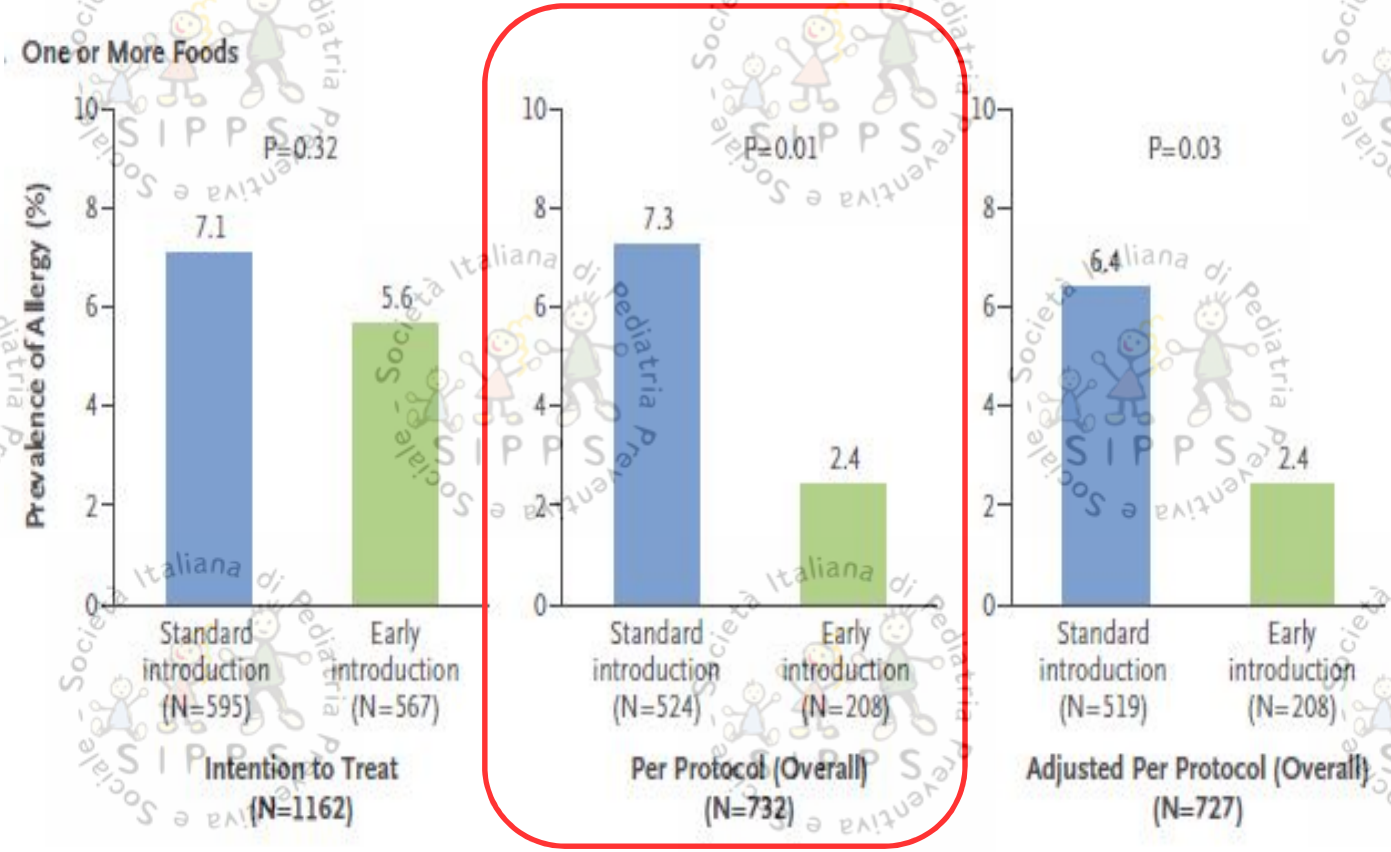
Group 1, EHCF + LGG
Group 2, SF

Nocerino R et al. EAACI MEETING 2016

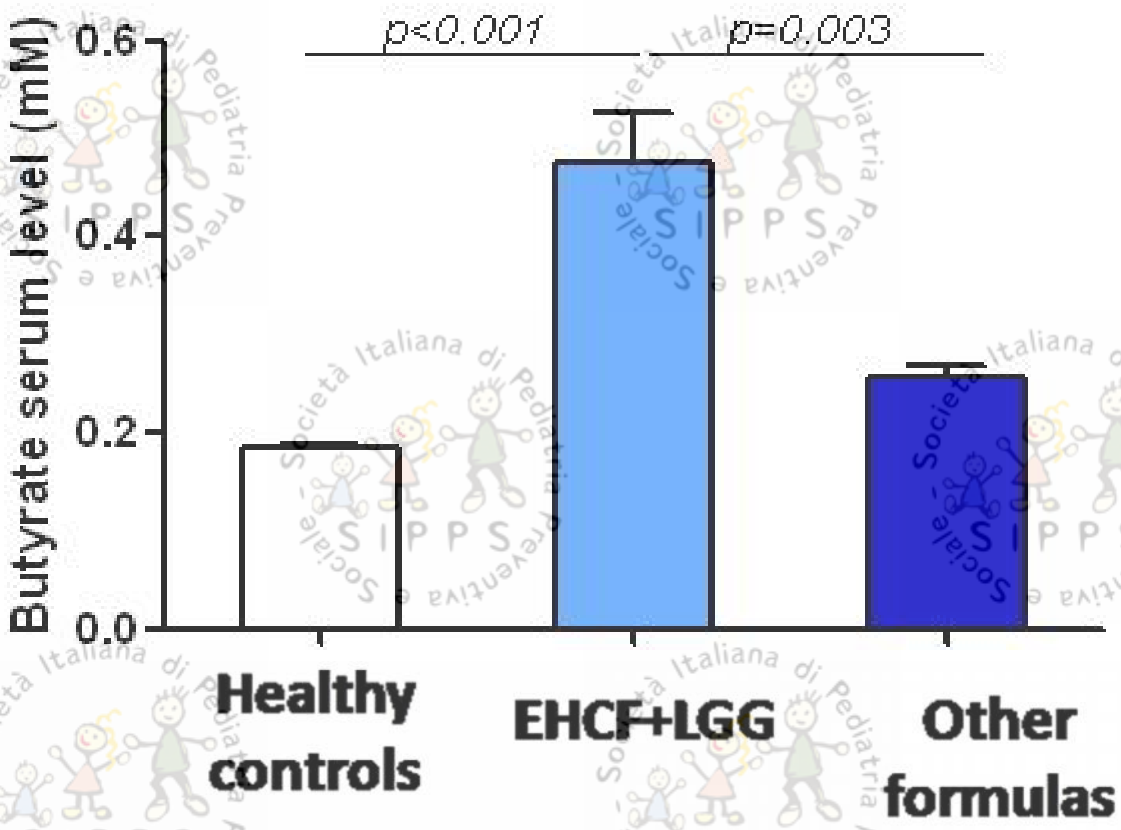
ORIGINAL ARTICLE

Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants

Michael R. Perkin, Ph.D., Kirsty Logan, Ph.D., Anna Tseng, R.D., Bunmi Raji, R.D., Salma Ayis, Ph.D., Janet Peacock, Ph.D., Helen Brough, Ph.D., Tom Marrs, B.M., B.S., Suzana Radulovic, M.D., Joanna Craven, M.P.H., Carsten Flohr, Ph.D., and Gideon Lack, M.B., B.Ch., for the EAT Study Team*



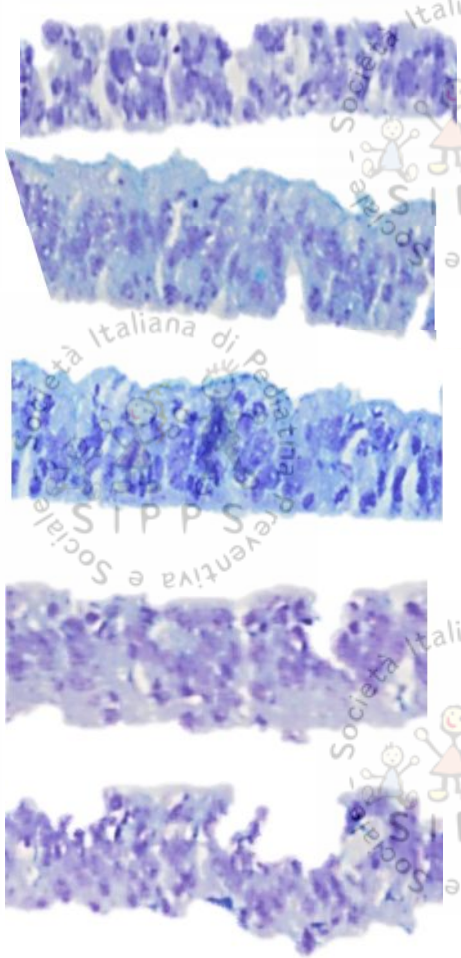
IgE-mediated CMA children treated with EHCF+LGG show higher butyrate serum levels



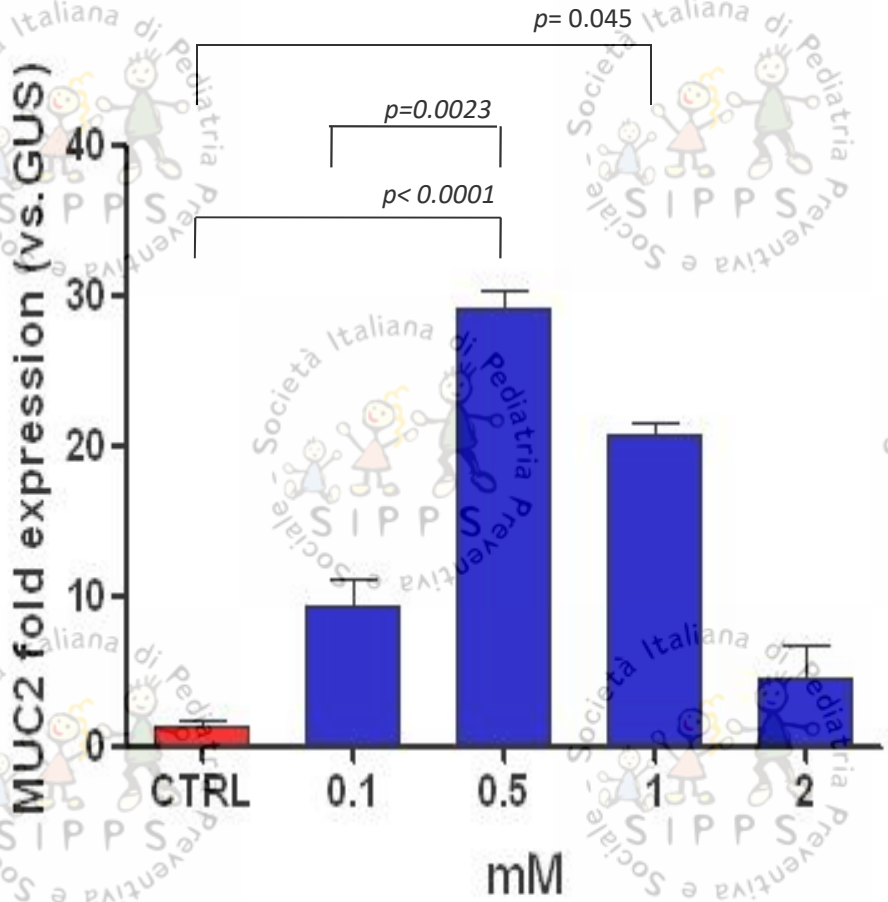
Butyrate concentration in systemic circulation = 0.1 to 1.2 mM
(*Cummings JH et al. Gut 1987)*

Berni Canani R data on file

Butyrate stimulates mucus production and MUC2 expression in human enterocytes



Dose	Thickness (µm)
CTRL	-
0.1mM	3±2
0.5mM	5±2
1mM	-
2mM	-

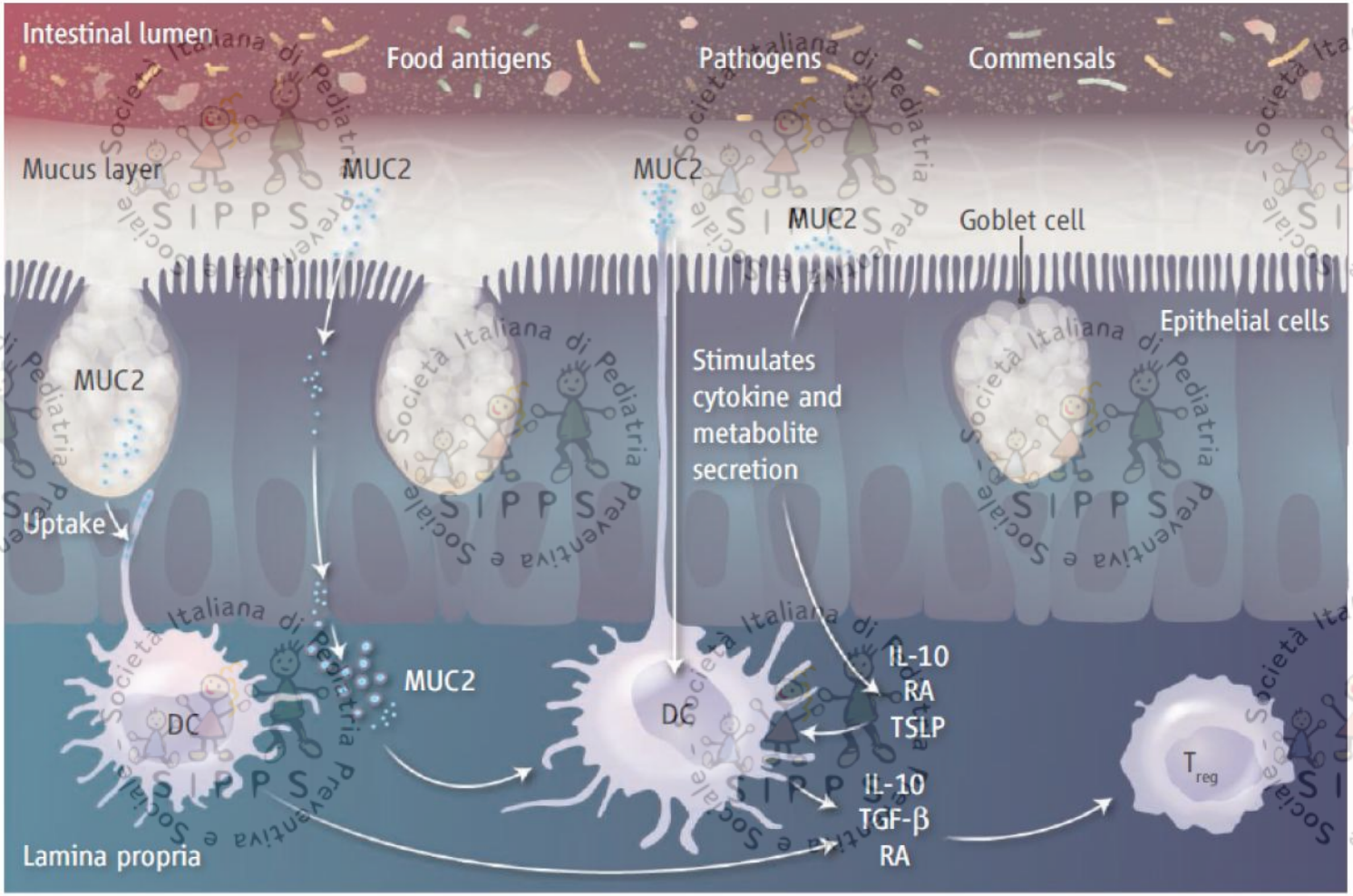


Berni Canani R data on file

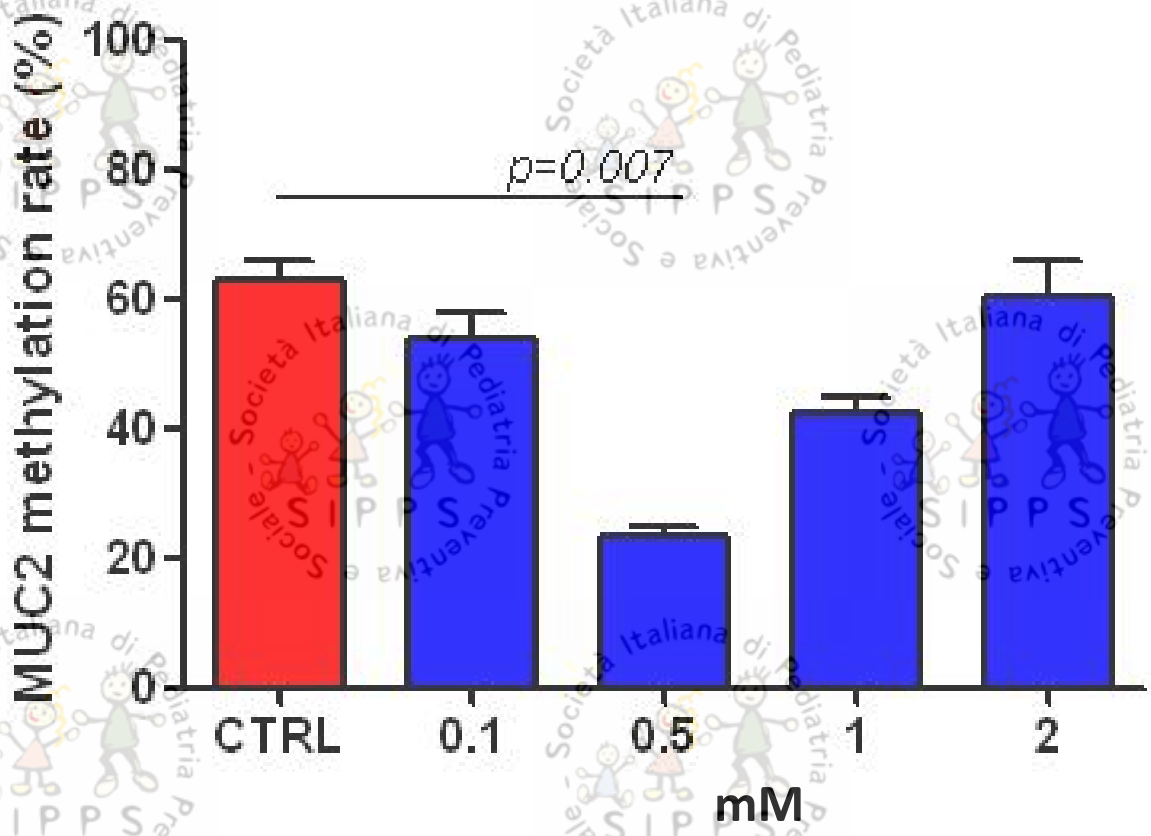
Mucus Coat, a Dress Code for Tolerance

Yasmine Belkaid and John Grainger

Mucus is a determinant of gut immune specification and immune tolerance.



Butyrate induces MUC2-DNA demethylation in human enterocytes



Berni Canani R data on file

The Changing Pattern of CMA

+ Increased risk of persistence until later ages

+ Increased severity of clinical manifestations

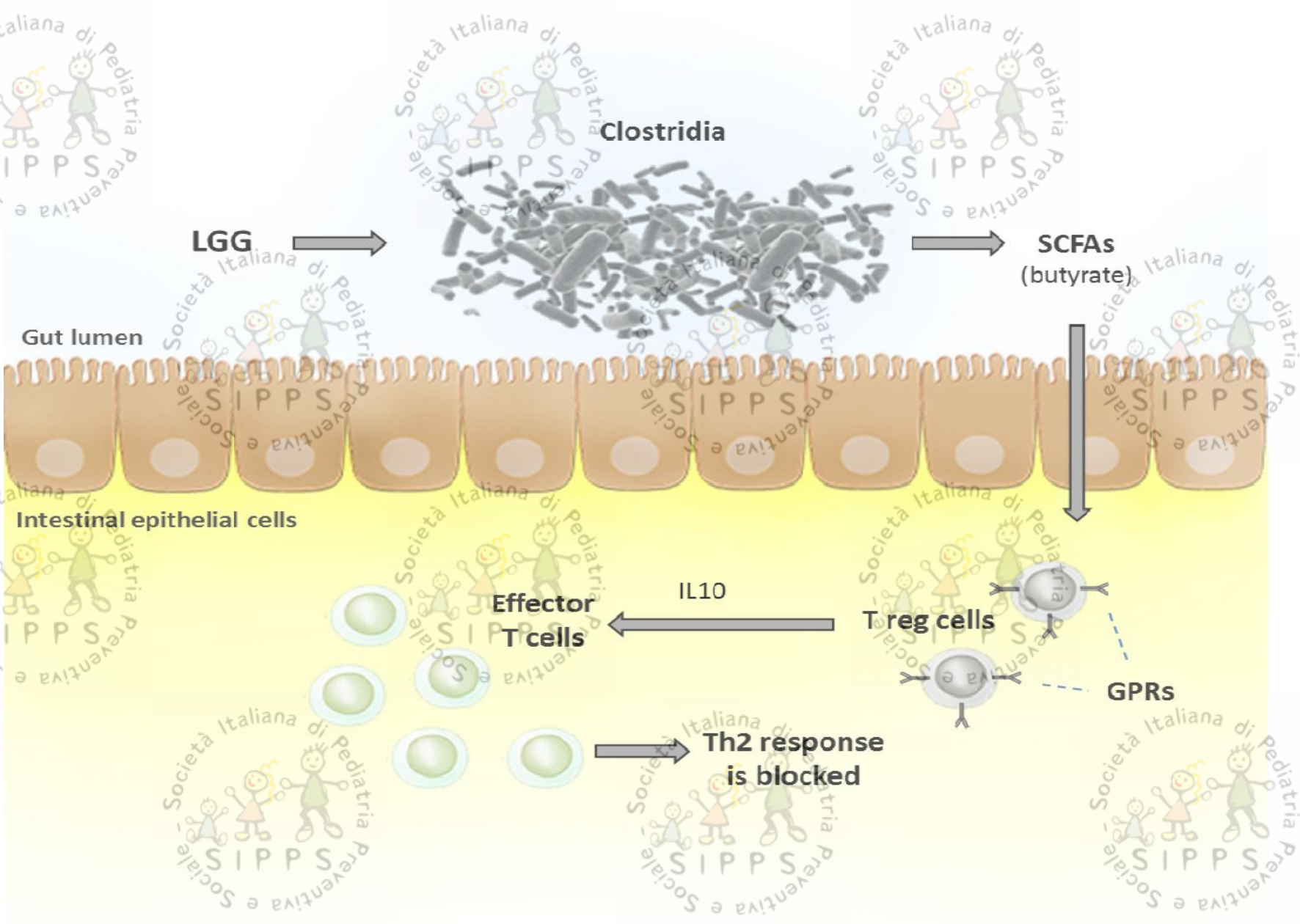
+ Increased need of hospitalization

+ Increased economic impact

+ Increased risk of atopic march *(>50% asthma, atopic eczema, allergic rhinitis)*

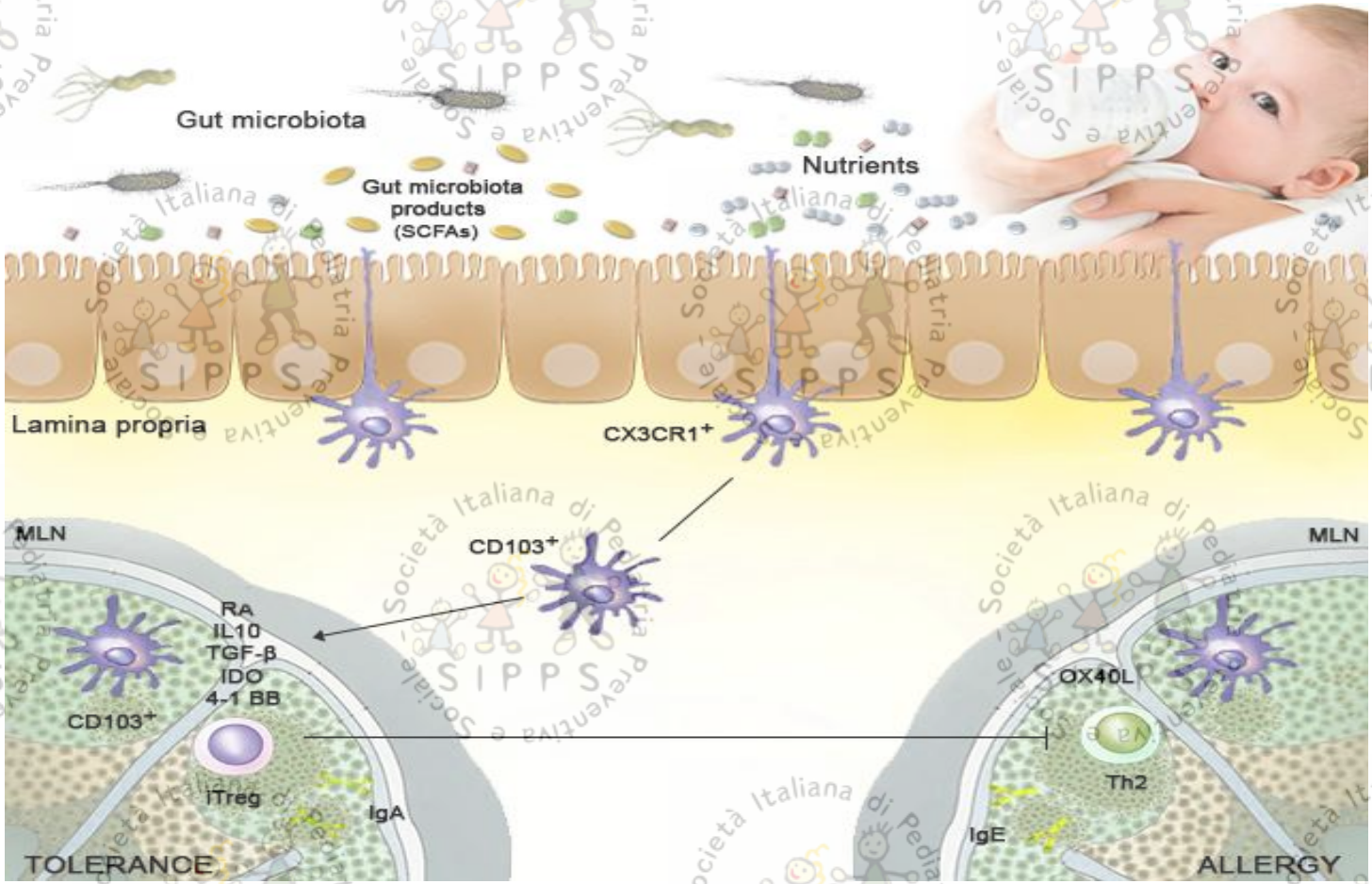
= Strong need to develop effective strategies to stimulate oral tolerance acquisition

Skripak JM, et al. JACI 2007
Ross MP, et al. JACI 2008
Wang J, et al. JCI 2011
Chen FM, et al. JMII 2012
Virta LJ et al. JPGN 2013
Gupta R, et al. JAMA Ped 2013
Prescott SL et al. WAO J 2013
Berni Canani R, et al. Clin Exp All 2013
Nocerino R, et al. JACI 2015



Berni Canani R et al. 2016

Oral tolerance: suppression of immune response to dietary antigens mediated by Ag-specific regulatory T cells

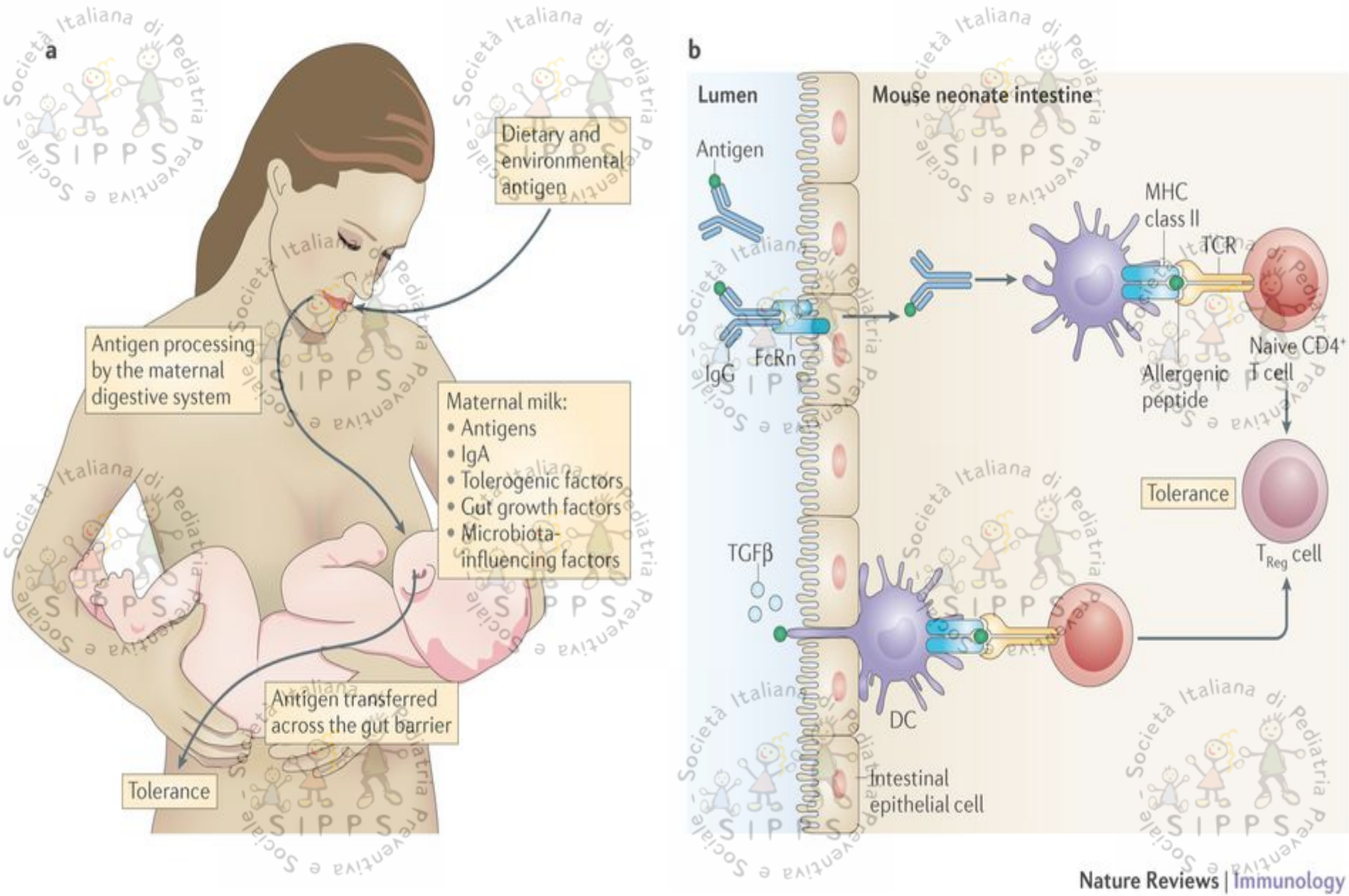


A dynamic modulation of this network is introduced at birth by exposure to enviromental factors and by acquisition of gut microbiota

Di Costanzo M and Berni Canani R JPGN 2016

Conflicts of Interest Disclosure

In the past 12 months, I have had the following relevant financial relationships with the following manufacturers of any commercial product discussed in this lecture: Danone (research grant), Humana (research grant), Kraft-Heinz (research grant, speaker), Mead Johnson Nutriton (research grant, speaker), Novalac (research grant) as part of publically funded research projects with support of the Italian Ministry of Health.


a
Dietary and environmental antigen
Antigen processing by the maternal digestive system
Maternal milk:
• Antigens
• IgA
• Tolerogenic factors
• Gut growth factors
• Microbiota-influencing factors
Antigen transferred across the gut barrier
Tolerance
b
Lumen
Mouse neonate intestine
Antigen
IgG
FcRn
MHC class II
TCR
Naive CD4+ T cell
Allergenic peptide
Tolerance
TGFβ
DC
T_{Reg} cell
Intestinal epithelial cell
Nature Reviews | Immunology

Administration of a probiotic with peanut oral immunotherapy: A randomized trial

Mimi L. K. Tang, PhD,[a,b,c] Anne-Louise Ponsonby, PhD,[d] Francesca Orsini, MSc,[e] Dean Tey, FRACP,[a,c] Marnie Robinson, FRACP,[a,c] Ee Lyn Su, FRACP,[a,c] Paul Licciardi, PhD,[c] Wesley Burks, MD,[f] and Susan Donath, MA[b,e]

Melbourne, Australia, and Chapel Hill, NC

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TABLE III. Clinical outcomes

	PPOIT group	Placebo group	RR,* NNT,† or mean difference‡
2-wk Sustained unresponsiveness			
n (%)	23/28 (82.1)	1/28 (3.6)	23 (3.33-158.84)*§
			1.27 (1.06-1.59)†
2-wk Sustained unresponsiveness, sensitivity 1			
n (%)	23/31 (74.2)	1/31 (3.2)	23 (3.31-159.93)*§
			1.41 (1.14-1.84)†
2-wk Sustained unresponsiveness, sensitivity 2			
n (%)	23/31 (74.2)	4/31 (12.9)	5.75 (2.25-14.69)*§
			1.63 (1.24-2.39)†
Desensitization			
n (%)	26/29 (89.7)	2/28 (7.1)	12.55 (3.28-47.99)*§
			1.21 (1.03-1.47)†
Peanut SPT at T1			
Mean (SD), n	4.83 (3.98), 29	14.54 (5.63), 27	−9.71 (−12.31 to −7.11)‡§
Peanut SPT at T3			
Mean (SD), n	4.46 (4.44), 28	14.75 (6.09), 28	−10.29 (−13.14 to −7.43)‡

T1 refers to the last day of treatment, and T3 refers to 3 months after the end of treatment.
*RR (95% CI).
†NNT (95% CI).
‡Mean difference (95% CI).
§$P < .001$.