CONSENSUS HELP
Human Early Life Prevention
MICROBIOTA E IMMUNITÀ

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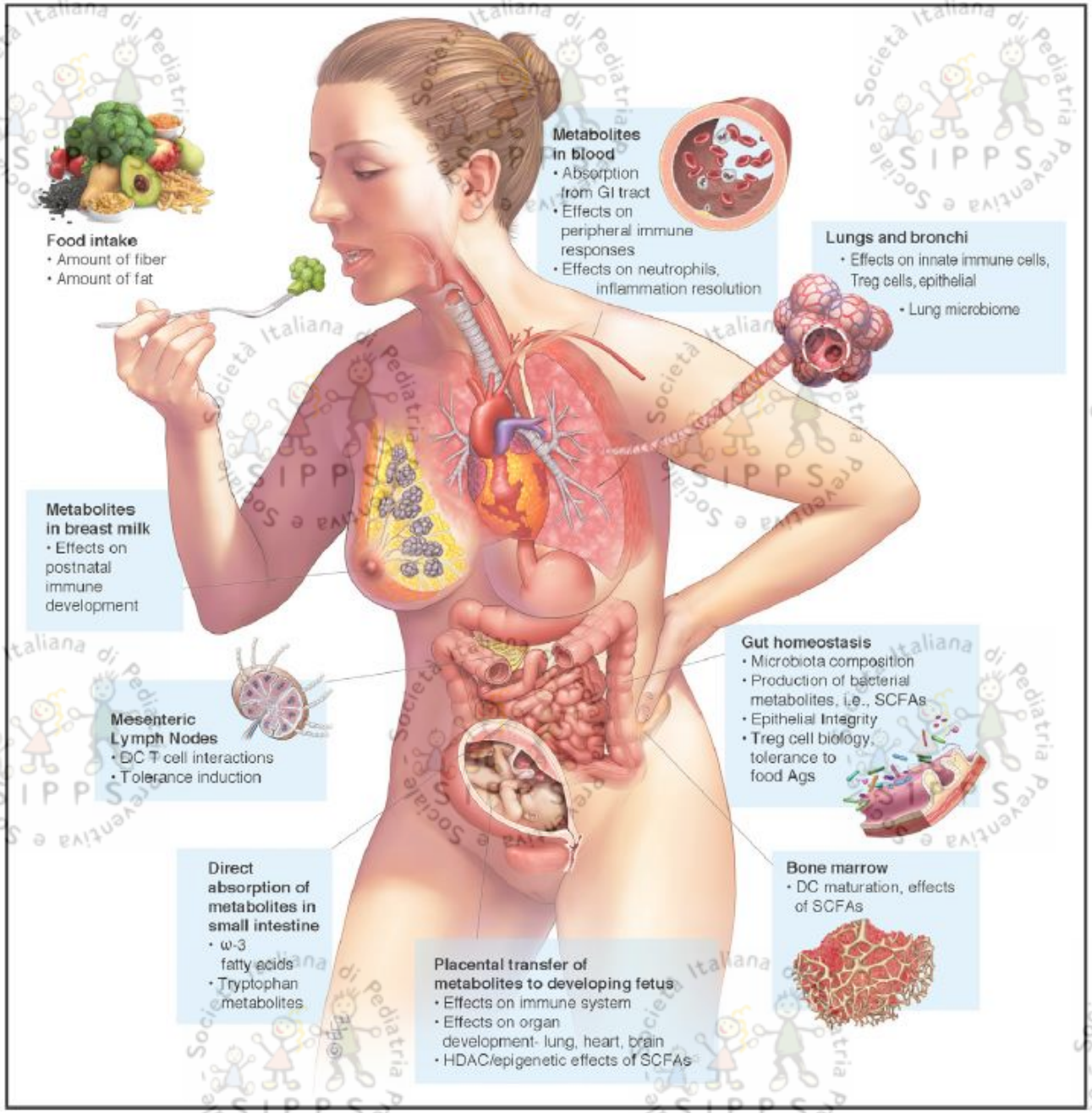
Dipartimento di Scienze Mediche Traslazionali
Sezione di Pediatria
Laboratorio Europeo per lo Studio delle Malattie Indotte da Alimenti (ELFID)
CEINGE Biotecnologie Avanzate
Università degli Studi Napoli "Federico II"

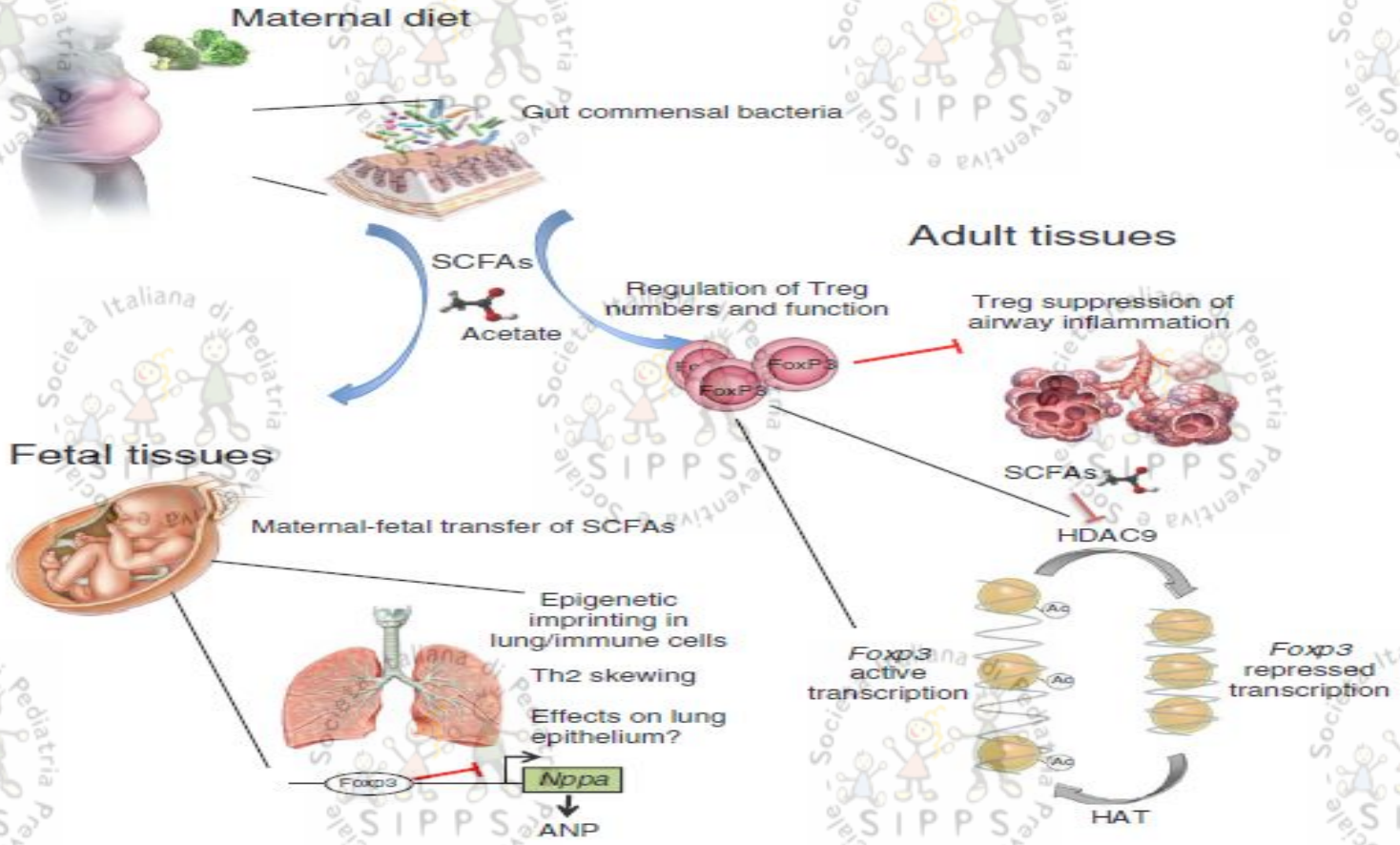

450 mL/die di liquido amniotico
Carboidrati, proteine, lipidi (20Kcal/Kg/die)
Fattori di Crescita (EGF, IGF-1)
DNA batterico
miRNA


Food intake
• Amount of fiber
• Amount of fat
Metabolites in blood
• Absorption from GI tract
• Effects on peripheral immune responses
• Effects on neutrophils, inflammation resolution
Lungs and bronchi
• Effects on innate immune cells, Treg cells, epithelial
• Lung microbiome
Metabolites in breast milk
• Effects on postnatal immune development
Mesenteric Lymph Nodes
• DC T cell interactions
• Tolerance induction
Gut homeostasis
• Microbiota composition
• Production of bacterial metabolites, i.e., SCFAs
• Epithelial Integrity
• Treg cell biology, tolerance to food Ags
Direct absorption of metabolites in small intestine
• ω-3 fatty acids
• Tryptophan metabolites
Bone marrow
• DC maturation, effects of SCFAs
Placental transfer of metabolites to developing fetus
• Effects on immune system
• Effects on organ development- lung, heart, brain
• HDAC/epigenetic effects of SCFAs

nature COMMUNICATIONS 2015

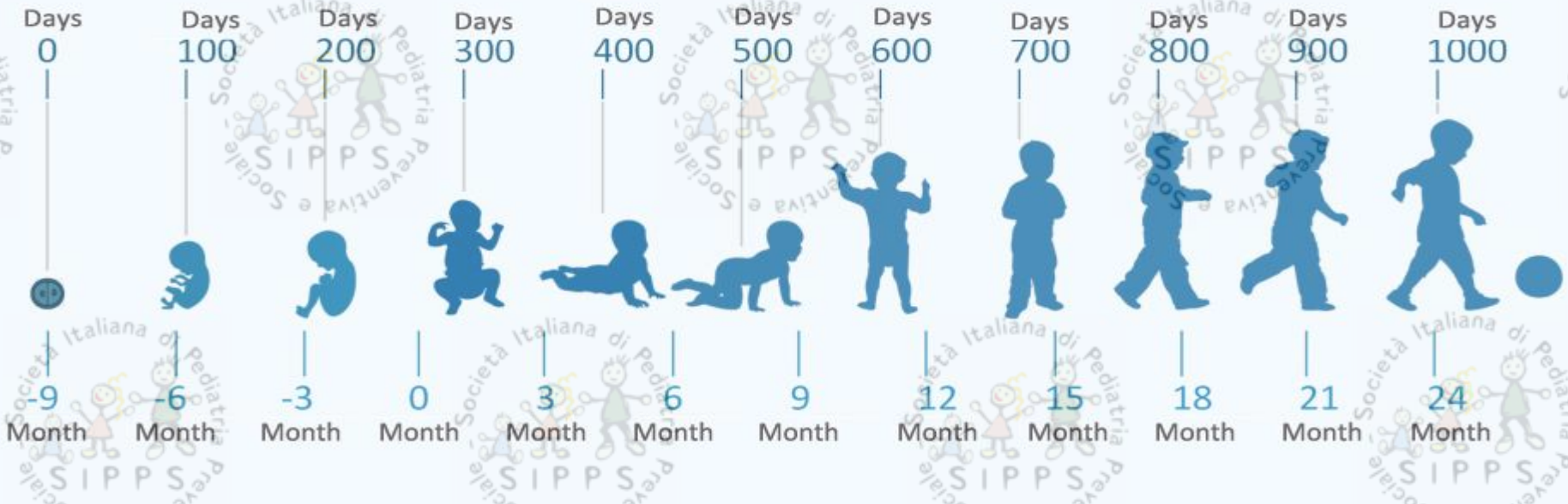
Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites

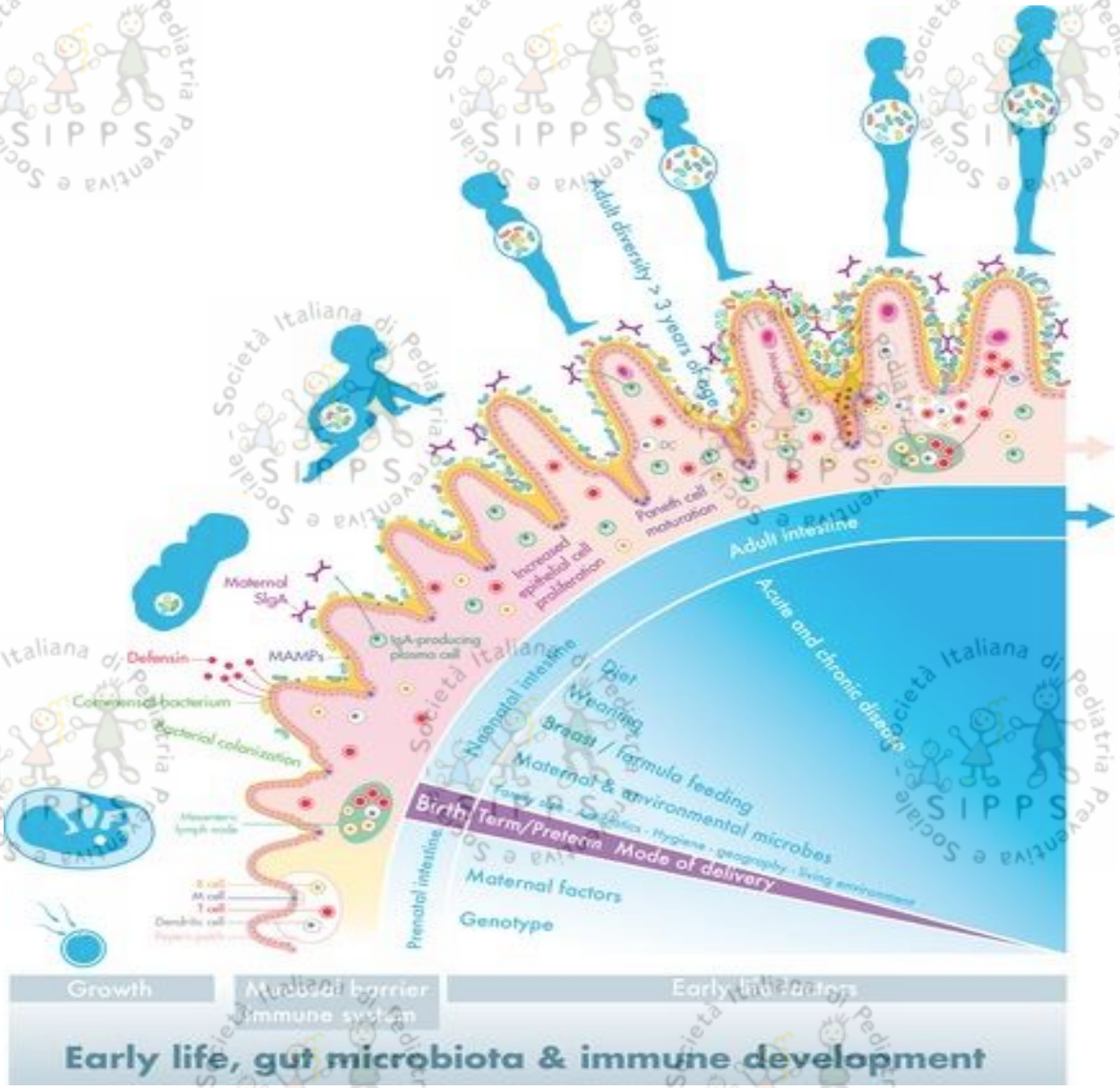
Alison N. Thorburn[1], Craig I. McKenzie[1], Sj Shen[1], Dragana Stanley[2], Laurence Macia[1], Linda J. Mason[1], Laura K. Roberts[1], Connie H.Y. Wong[1], Raymond Shim[1], Remy Robert[1], Nina Chevalier[1,3], Jian K. Tan[1], Eliana Mariño[1], Rob J. Moore[4,5], Lee Wong[6], Malcolm J. McConville[7,8], Dedreia L. Tull[8], Lisa G. Wood[9], Vanessa E. Murphy[9], Joerg Mattes[9], Peter G. Gibson[9] & Charles R. Mackay[1,10]

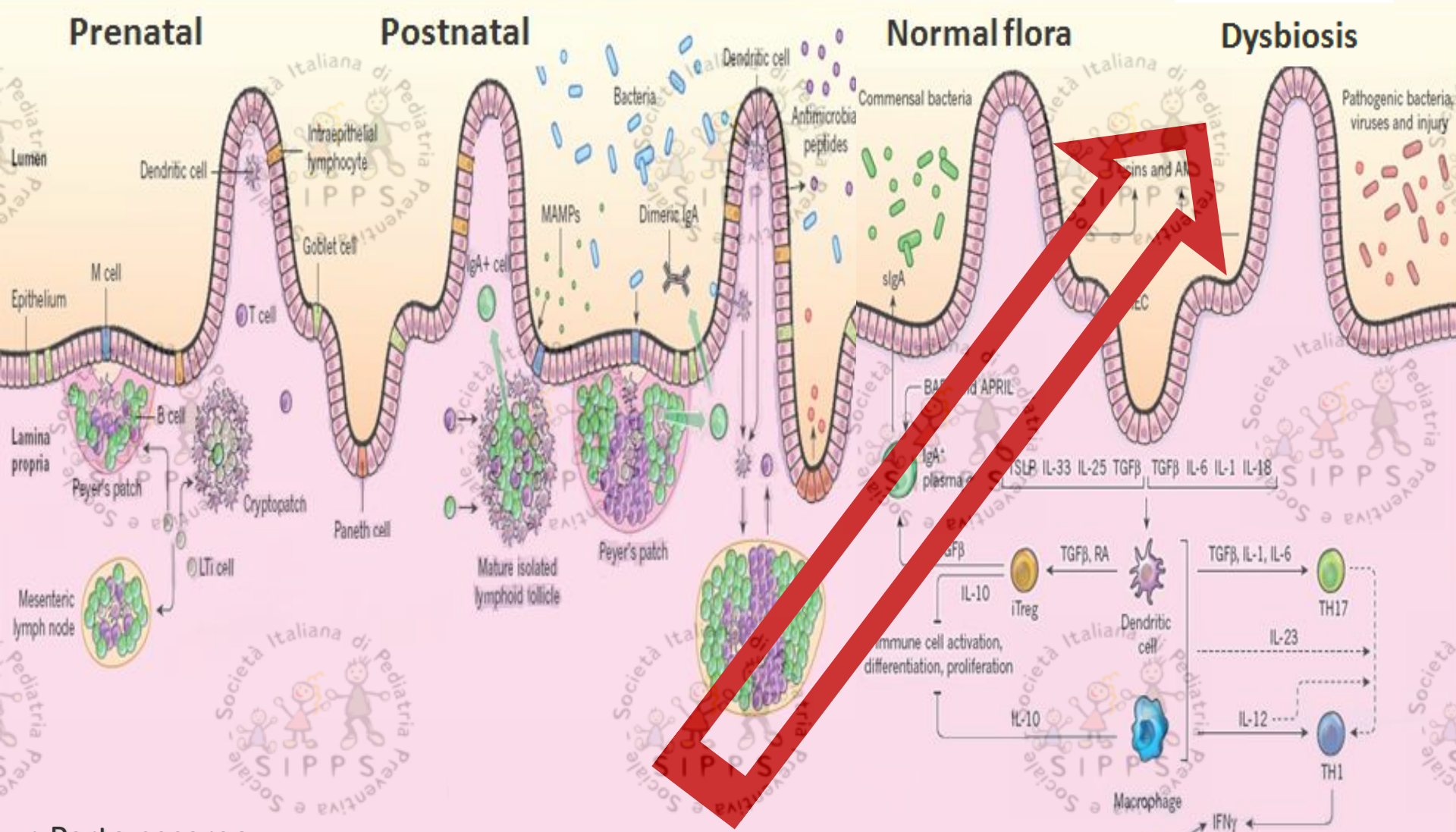


1000

WHAT YOU DO AND EAT IN THE FIRST 1000 DAYS, MAKES A DIFFERENCE FOR THE REST OF YOUR LIFE




Adult diversity > 3 years of age
Maternal SIgA
Defensin
MAMPs
Commensal bacterium
Bacterial colonization
IgA-producing plasma cell
Increased epithelial cell proliferation
Paneth cell maturation
Adult intestine
Acute and chronic disease
Neonatal intestine
Diet
Weaning
Breast / formula feeding
Maternal & environmental microbes
Birth
Term/Preterm
Mode of delivery
Prenatal intestine
Maternal factors
Genotype
Growth
Mucosal barrier
Immune system
Early life factors
Early life, gut microbiota & immune development



- Parto cesareo
- No latte materno
- Antibiotici
- Inibitori acidità gastrica
- Dieta povera di fibre e ricca di grassi saturi

Modified from Maynard CL et al. Nature 2012

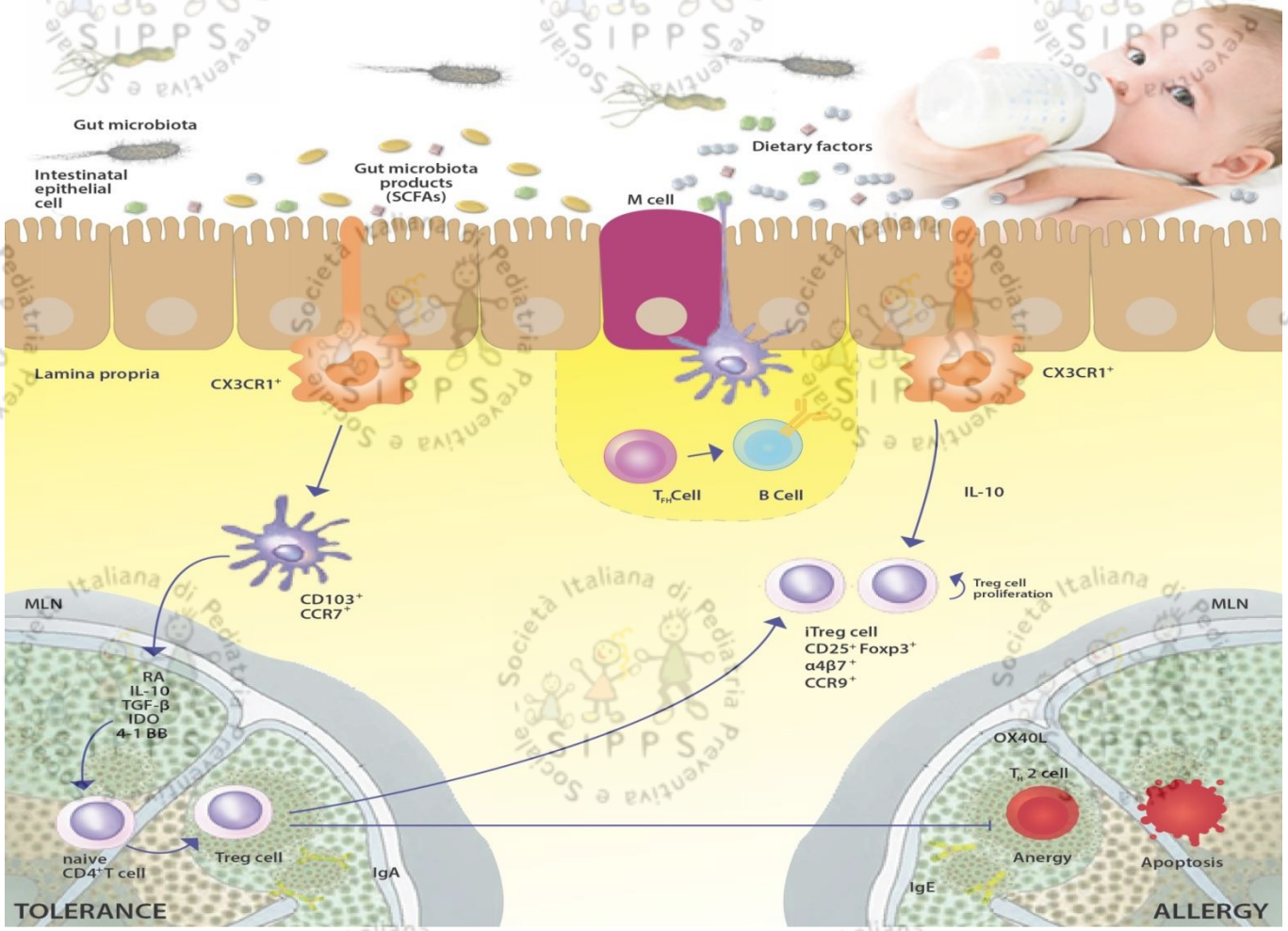
American Academy of Allergy Asthma & Immunology

ALLERGY STATISTICS

Worldwide, the rise in prevalence of allergic diseases has continued in the industrialized world for more than 50 years.

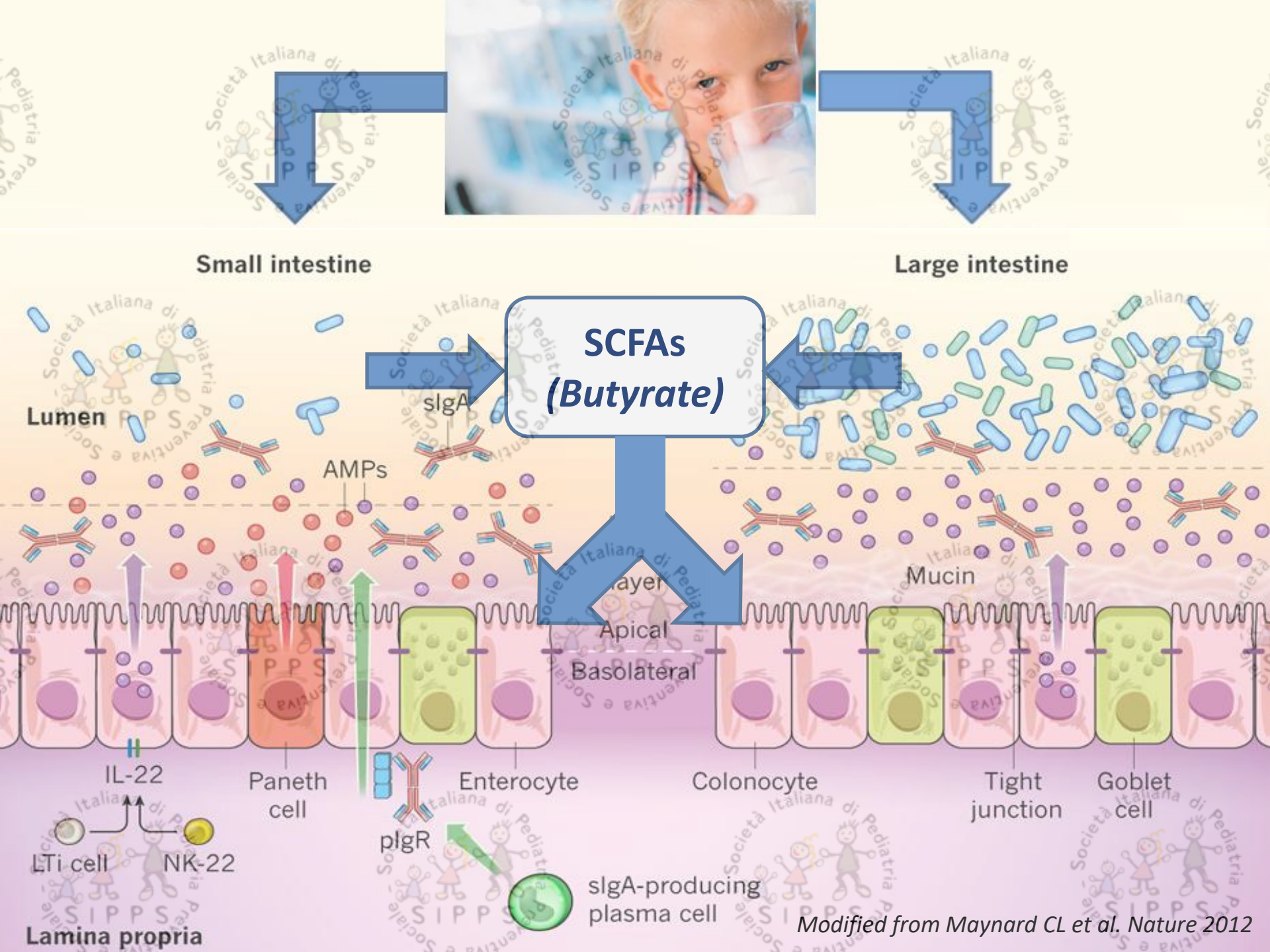
Worldwide, allergy rates to one or more common allergens among school children are currently approaching 40%-50%.

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



Dynamic network modulated from birth by exposure to dietary factors and acquisition of gut microbiota

Modified from Di Costanzo M and Berni Canani R JPGN 2016



Modified from Maynard CL et al. Nature 2012

Diet and food allergy development during infancy: Birth cohort study findings using prospective food diary data

Kate E. C. Grimshaw, PhD, RD,[a] Joe Maskell, MSc,[b] Erin M. Oliver, MSc,[a] Ruth C. G. Morris, Dip HE, RN(Child),[c] Keith D. Foote, MBBS, FRCPCH,[d] E. N. Clare Mills, PhD,[e] Barrie M. Margetts, PhD, FFPH,[f]* and Graham Roberts, DM, MRCPCH[a]* *Southampton, Winchester, and Manchester, United Kingdom*

BY THE AGE OF 2 YEARS

MORE FOOD ALLERGY

LESS FOOD ALLERGY

J Allergy Clin Immunol 2014;133:511-9.

Association Between Adherence to the Mediterranean Diet and Asthma in Peruvian Children

Jessica L. Rice[1] · Karina M. Romero[2] · Rocio M. Galvez Davila[2] · Carla Tarazona Meza[2] · Andrew Bilderback[3] · D'Ann L. Williams[4] · Patrick N. Breysse[4] · Sonali Bose[3] · William Checkley[3,4,5] · Nadia N. Hansel[3,4] · GASP Study Investigators

BY THE AGE OF 9-19 YEARS

MORE ASTHMA

LESS ASTHMA

Lung (2015) 193:893–899

The New York Times

FOOD

The Hippies Have Won

By CHRISTINE MUHLKE APRIL 4, 2017

Dopo 10 giorni:

✓Perdita di >1300 specie batteriche

✓Aumento Bacteroidetes / Riduzione Firmicutes

Tre giorni consecutivi sono sufficienti per indurre effetti simili

Spector TD et al. Cell 2015
Vanamala JKP Cell 2015
Nadeem O et al. Mol Nutr Food Res 2016

Argonne National Laboratory campus

Prof. Jack Gilbert - ANL and U.Chicago, IL
Prof. Danilo Ercolini – U.Chicago, IL
and Univ. Federico II, Naples, Italy

Bacteroidetes

Firmicutes

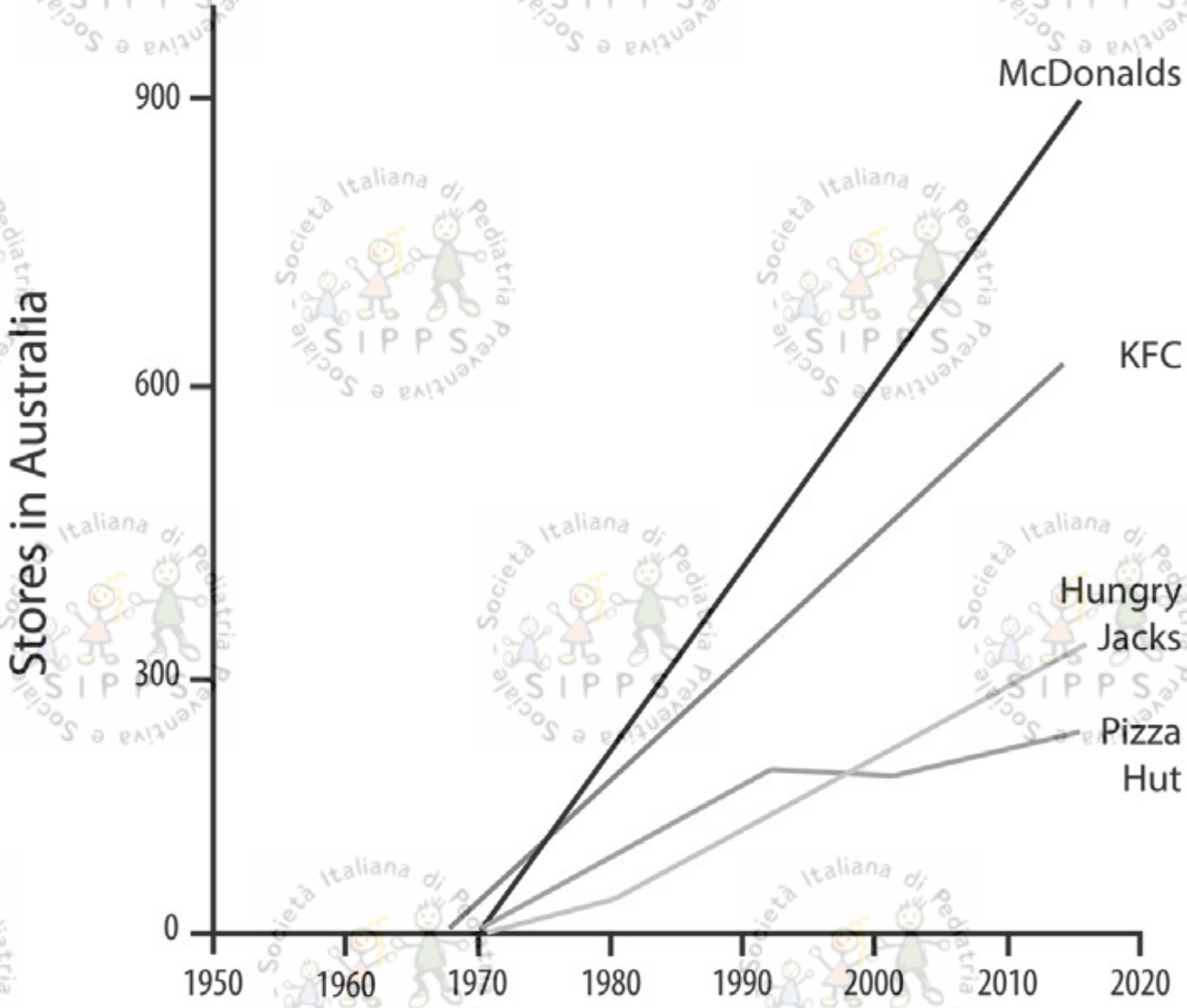
Healthy babies

Non-IgE-mediated

IgE-mediated

Modified from Berni Canani R et al ISME J 2015 and 2017 submitted

AA IN UN PAESE INDUSTRIALIZZATO

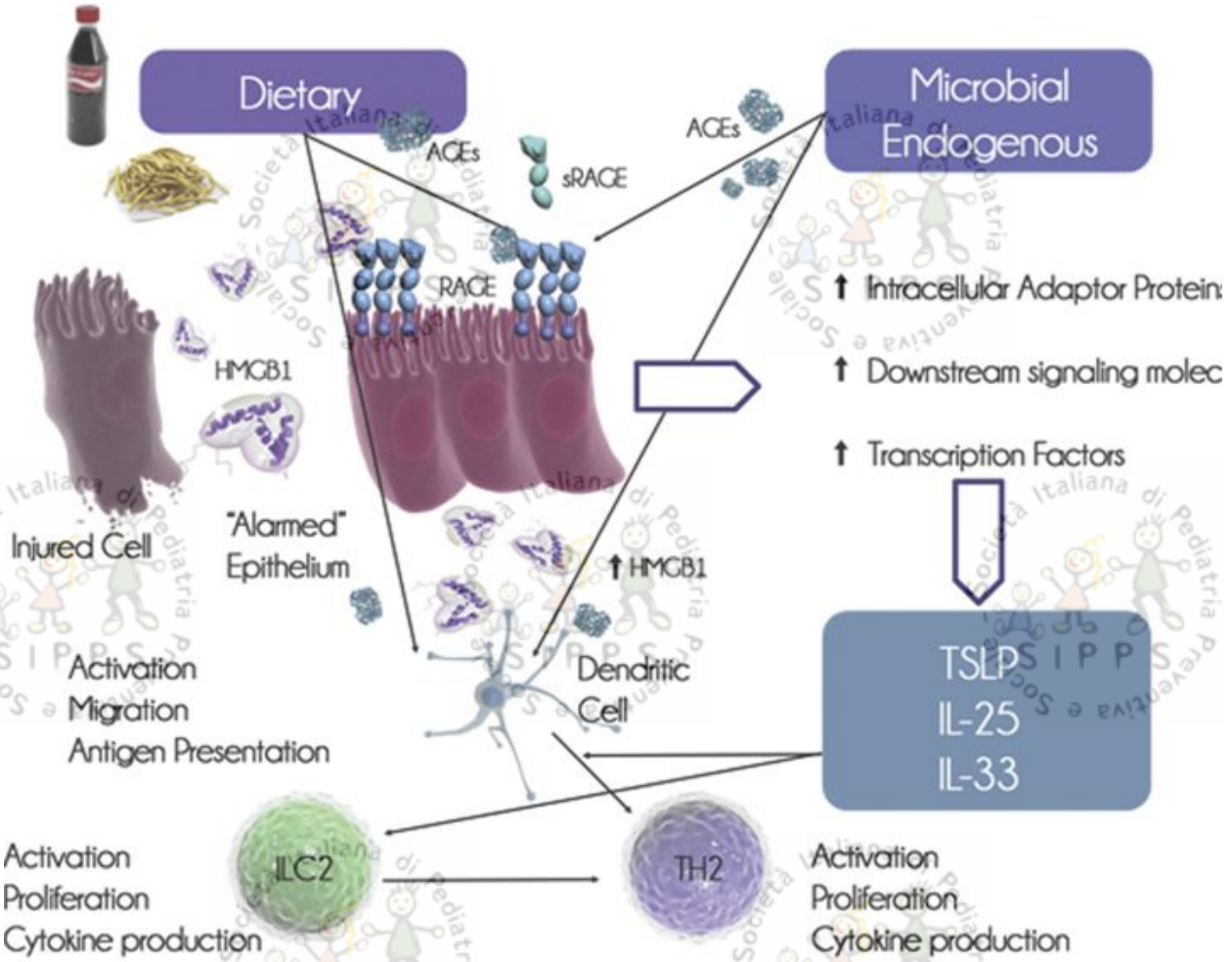


Smith PK, JACI 2016

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

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

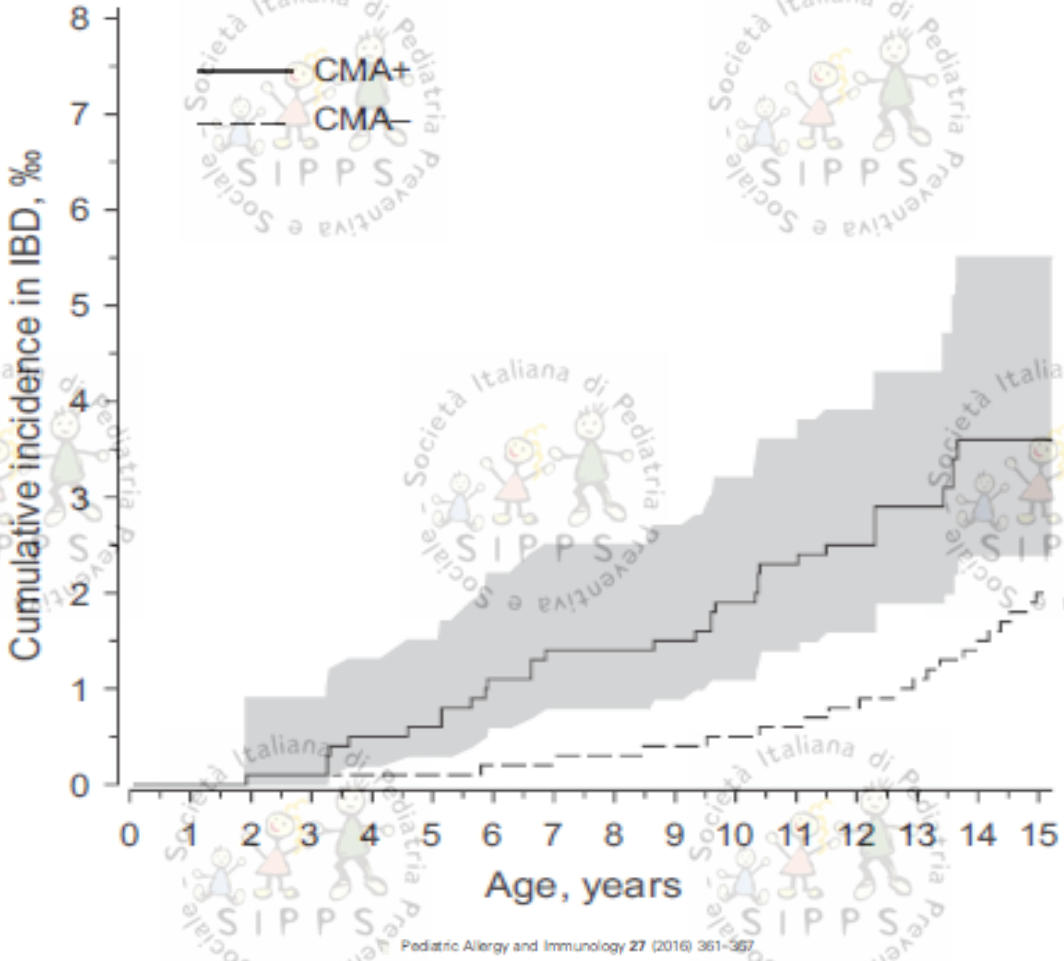
Epidemiology • Volume 24, Number 2, March 2013

Symptoms suggestive of cow's milk allergy in infancy and pediatric inflammatory bowel disease

Lauri J. Virta[1], Hannu Kautiainen[2,3,4] & Kaija-Leena Kolho[5]

[1]Research Department, Social Insurance Institution of Finland, Turku, Finland; [2]Unit of Primary Health Care, Helsinki University Hospital, Helsinki, Finland; [3]Department of General Practice, University of Helsinki, Helsinki, Finland; [4]Unit of Primary Health Care, Kuopio University Hospital, Kuopio, Finland; [5]Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

225.041 children born from Jan 1999 and Dec 2002: 7910 developed CMA



Pediatric Allergy and Immunology 27 (2016) 361–367

Therapy With Gastric Acidity Inhibitors Increases the Risk of Acute Gastroenteritis and Community-Acquired Pneumonia in Children

Roberto Berni Canani, MD, PhD[a], Pia Cirillo, MD[a], Paola Roggero, MD[b], Claudio Romano, MD[c], Basilio Malamisura, MD[d], Gianluca Terrin, MD[a], Annalisa Passariello, MD[a], Francesco Manguso, MD, PhD[e], Lorenzo Morelli, MD[f], Alfredo Guarino, MD[a], for the Working Group on Intestinal Infections of the Italian Society of Pediatric Gastroenterology, Hepatology and Nutrition (SIGENP)

Characteristics	Controls ($n = 95$)	GA Inhibitors ($n = 91$)
Age, median, mo (IQR)	10 (8–15)	10 (8–16)
Male, *n* (%)	50 (53)	48 (53)
Weight, median, kg (IQR)	9.3 (8–10)	9.1 (8–15)
Length, median, cm (IQR)	74 (70–78)	74 (70–80)
Patients presenting with		
Acute gastroenteritis in the previous 4 mo, *n* (%)	17 (18)	18 (20)
Acute gastroenteritis in the follow-up period, *n* (%)	19 (20)	43 (47)[a,b]
Pneumonia in the previous 4 mo, *n* (%)	1 (1)	3 (3)
Pneumonia in the follow-up period, *n* (%)	2 (2)	11 (12)[a,b]

[a] $P < .05$, GA inhibitor users versus control children.

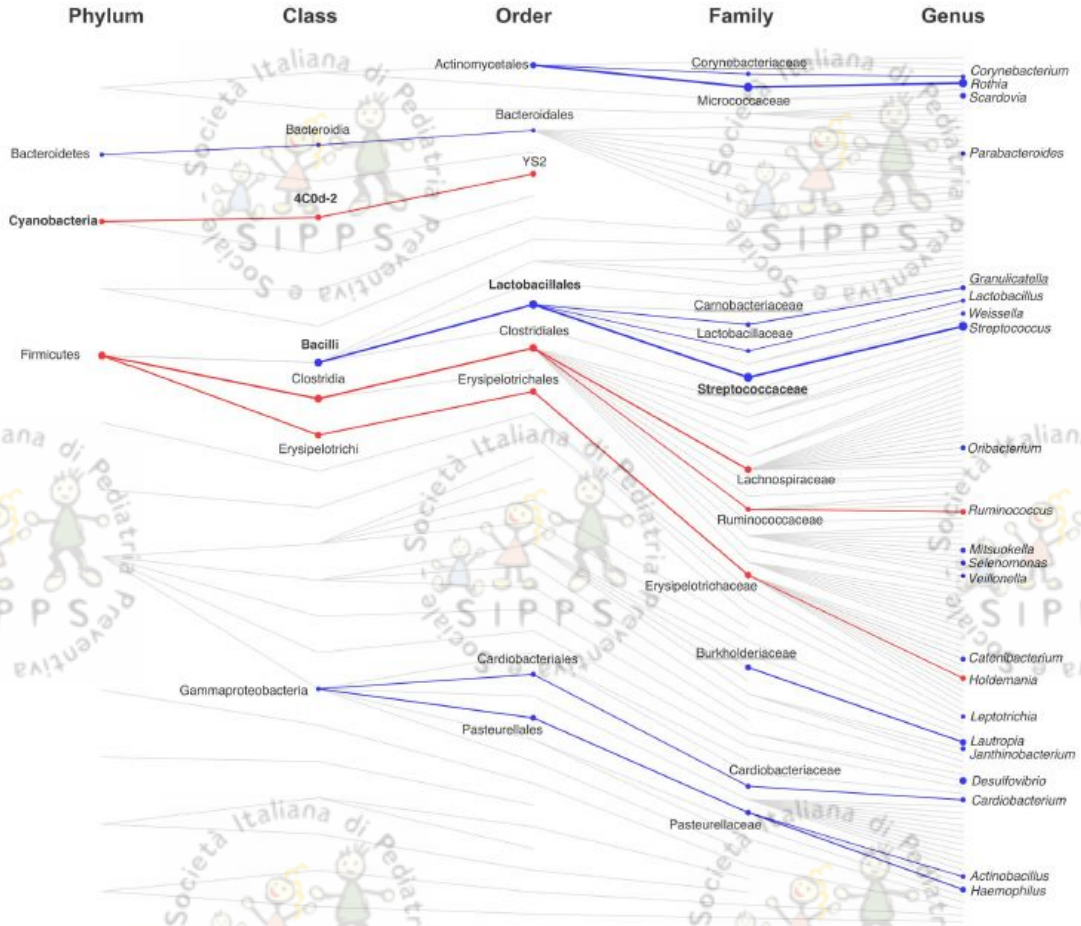
[b] $P < .05$, 4 months before versus 4 months after the enrollment.

IQR indicates interquartile range.

Pediatrics 2006;117;817-820

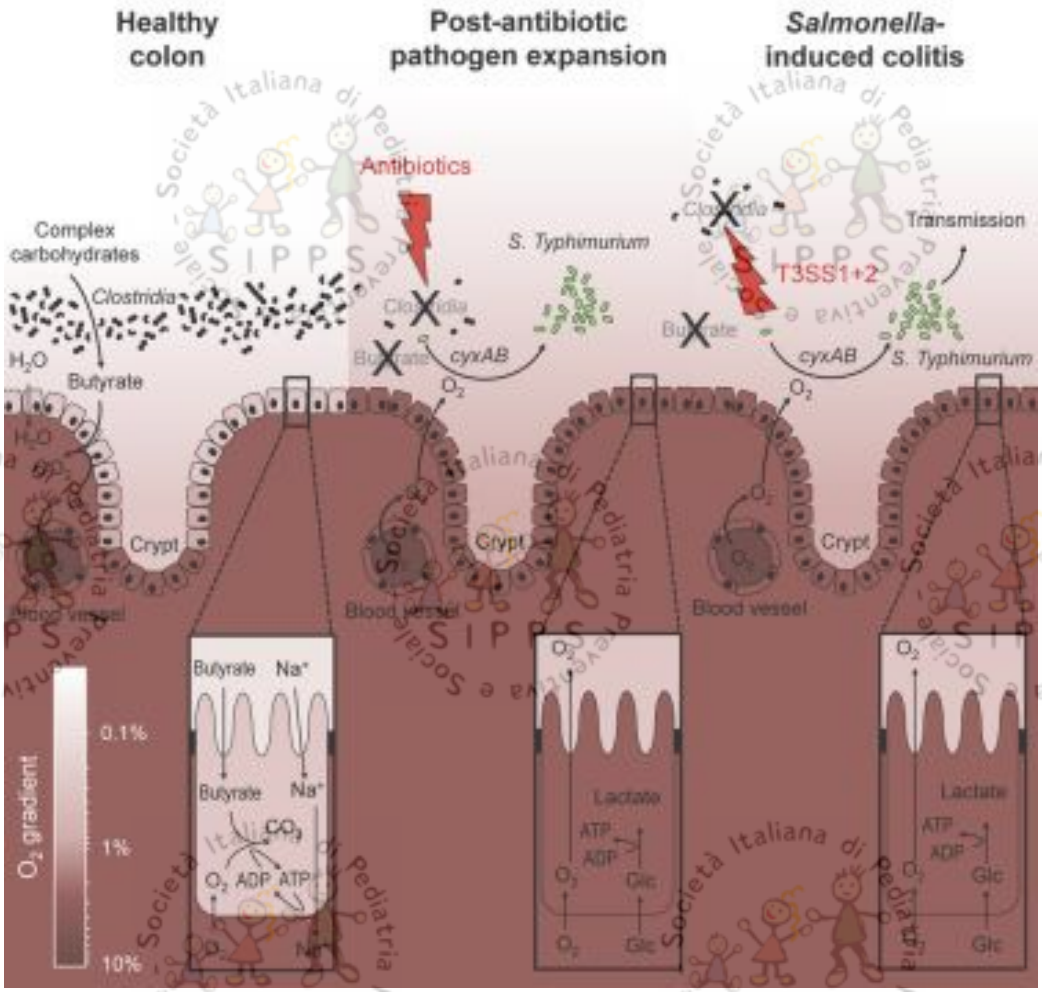
Proton pump inhibitors alter the composition of the gut microbiota

Matthew A Jackson,[1] Julia K Goodrich,[2,3] Maria-Emanuela Maxan,[4] Daniel E Freedberg,[5] Julian A Abrams,[5] Angela C Poole,[2,3] Jessica L Sutter,[2,3] Daphne Welter,[2,3] Ruth E Ley,[2,3] Jordana T Bell,[1] Tim D Spector,[1] Claire J Steves[1]



Gut 2016;**65**:749–756.

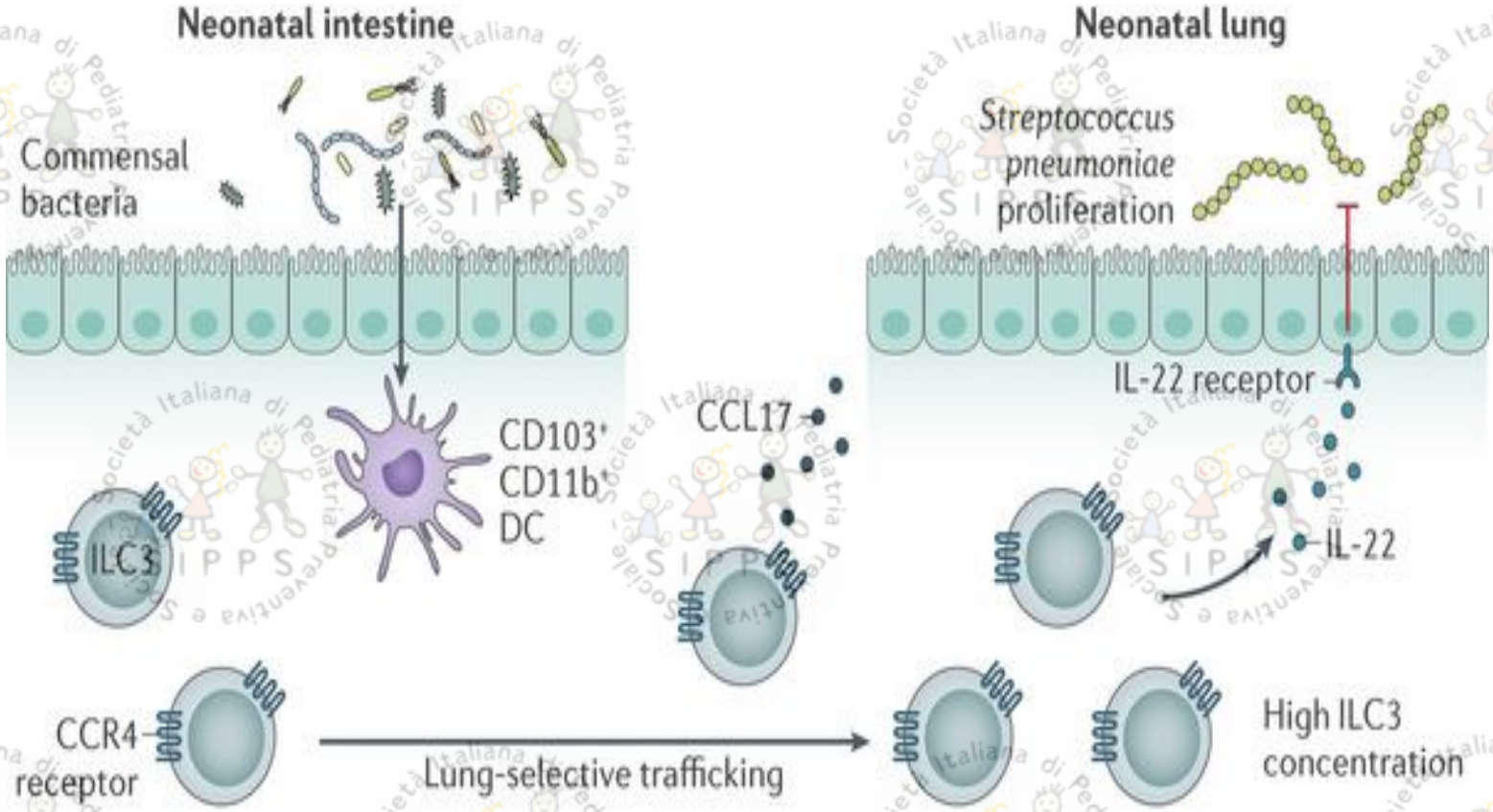
Depletion of butyrate-producing *Clostridia* from the Gut Microbiota Drives an Aerobic Luminal Expansion of *Salmonella*



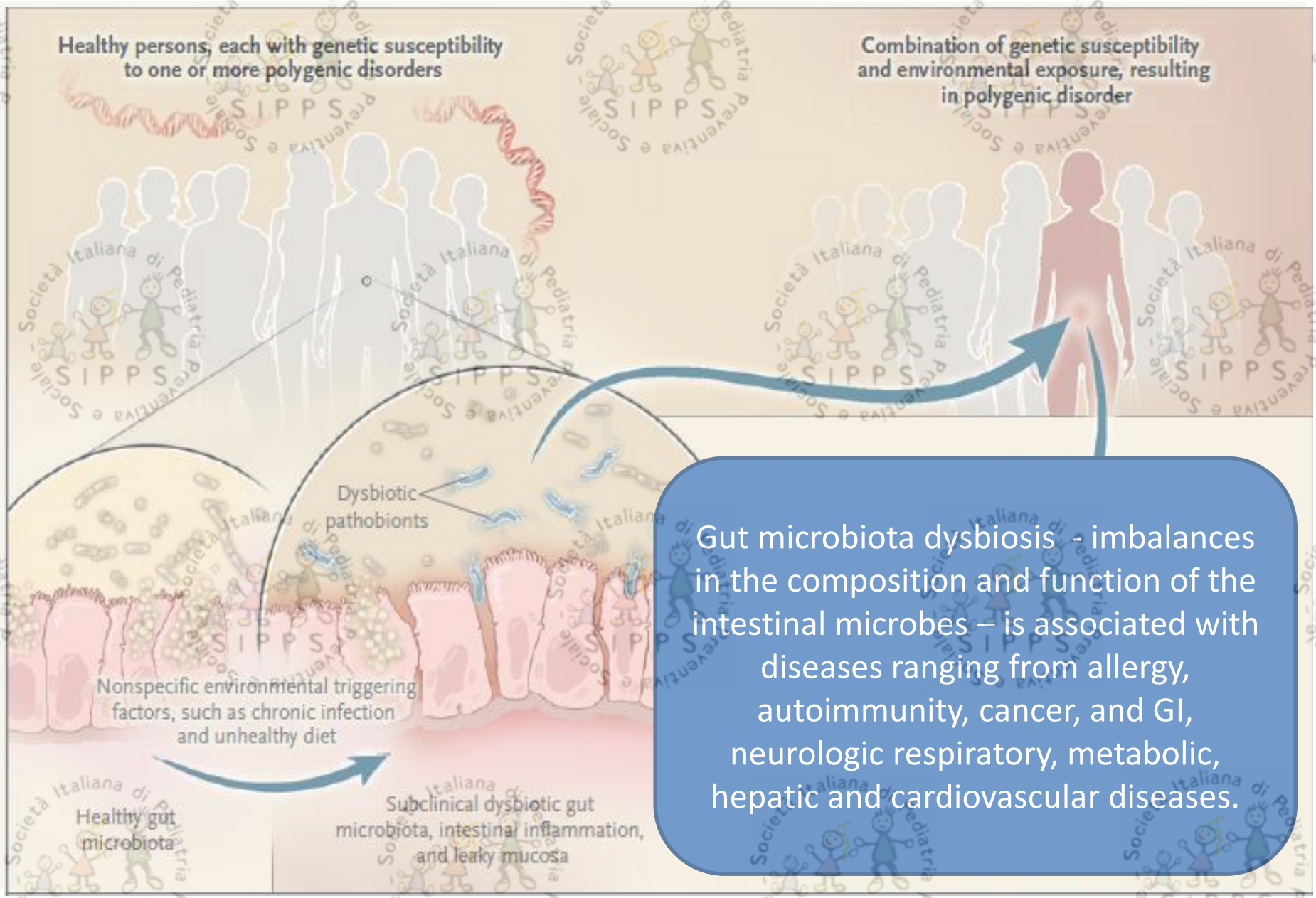
Rivera-Chavez F et al- Cell Host & Microbe 2016

Gut microbiota: Neonatal gut microbiota induces lung immunity against pneumonia

SabrinaTamburini & Jose C. Clemente Nat Rev Gastro Feb 2017



ILC3=Innate lymphoid cells 3



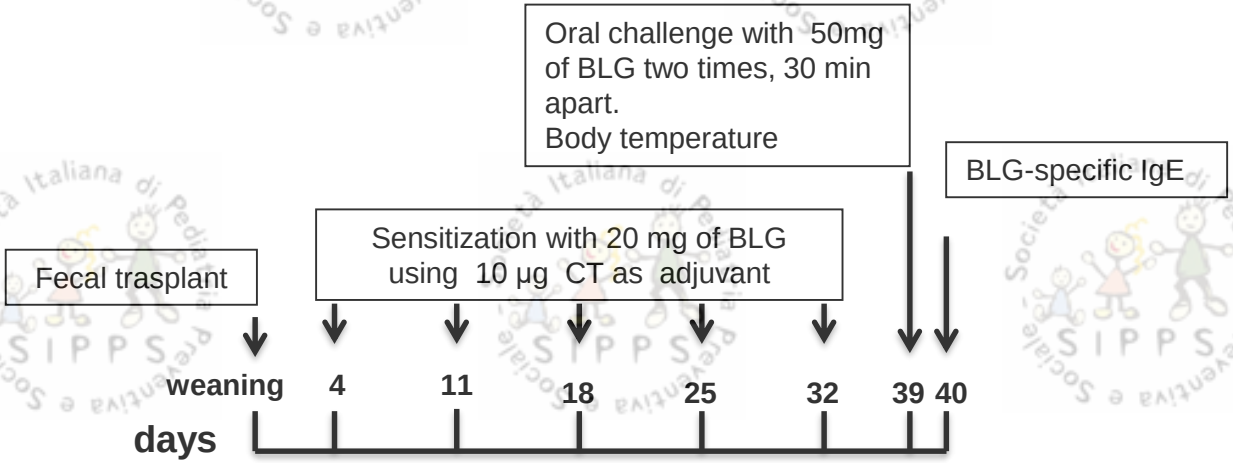
Gut microbiota dysbiosis - imbalances in the composition and function of the intestinal microbes – is associated with diseases ranging from allergy, autoimmunity, cancer, and GI, neurologic respiratory, metabolic, hepatic and cardiovascular diseases.

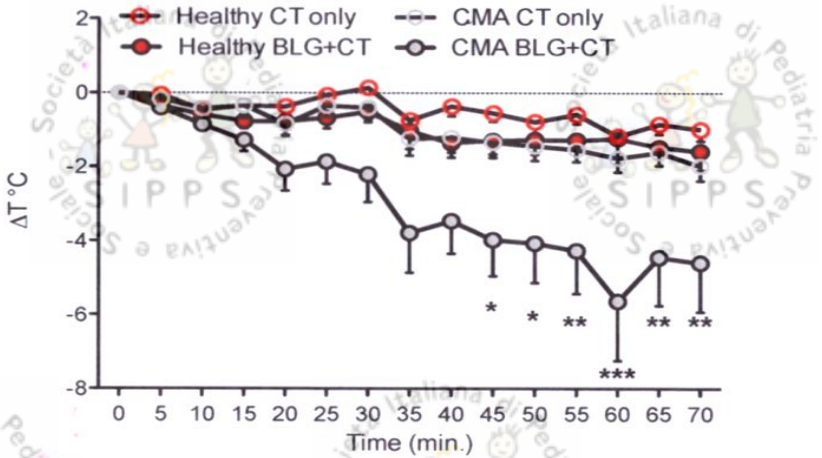
Lynch VS and Pedersen O. N Eng J Med 2016

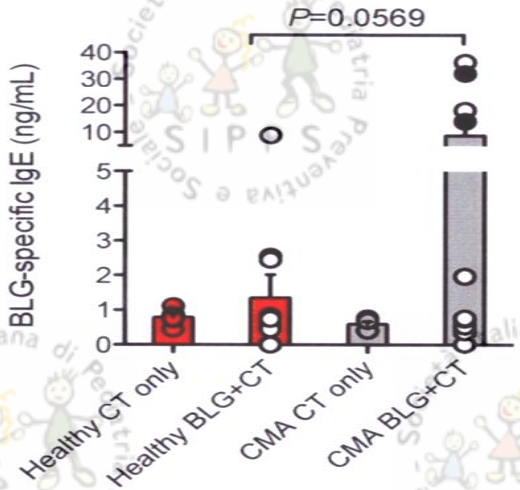
Humanized mice model of FA

Gut microbiota from healthy infant but not from CMA infant protects against FA

GF C3H/HeN 3-4 weeks old







Nagler C and Berni Canani R, submitted

MICROBIAL RELATED FACTORS INCREASING RISK OF FOOD ALLERGY

Caesarean delivery

Junk foods/baby foods

Diet

Disinfectant and antiseptic agent

Single child

Antibiotics and Gastric acidity inhibitors

Pets

Farming lifestyle

Increasing family size and communal childcare

High fiber foods

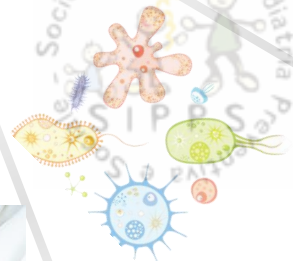
Diet

Raw milk

Fermented foods

Home made foods

Breast milk

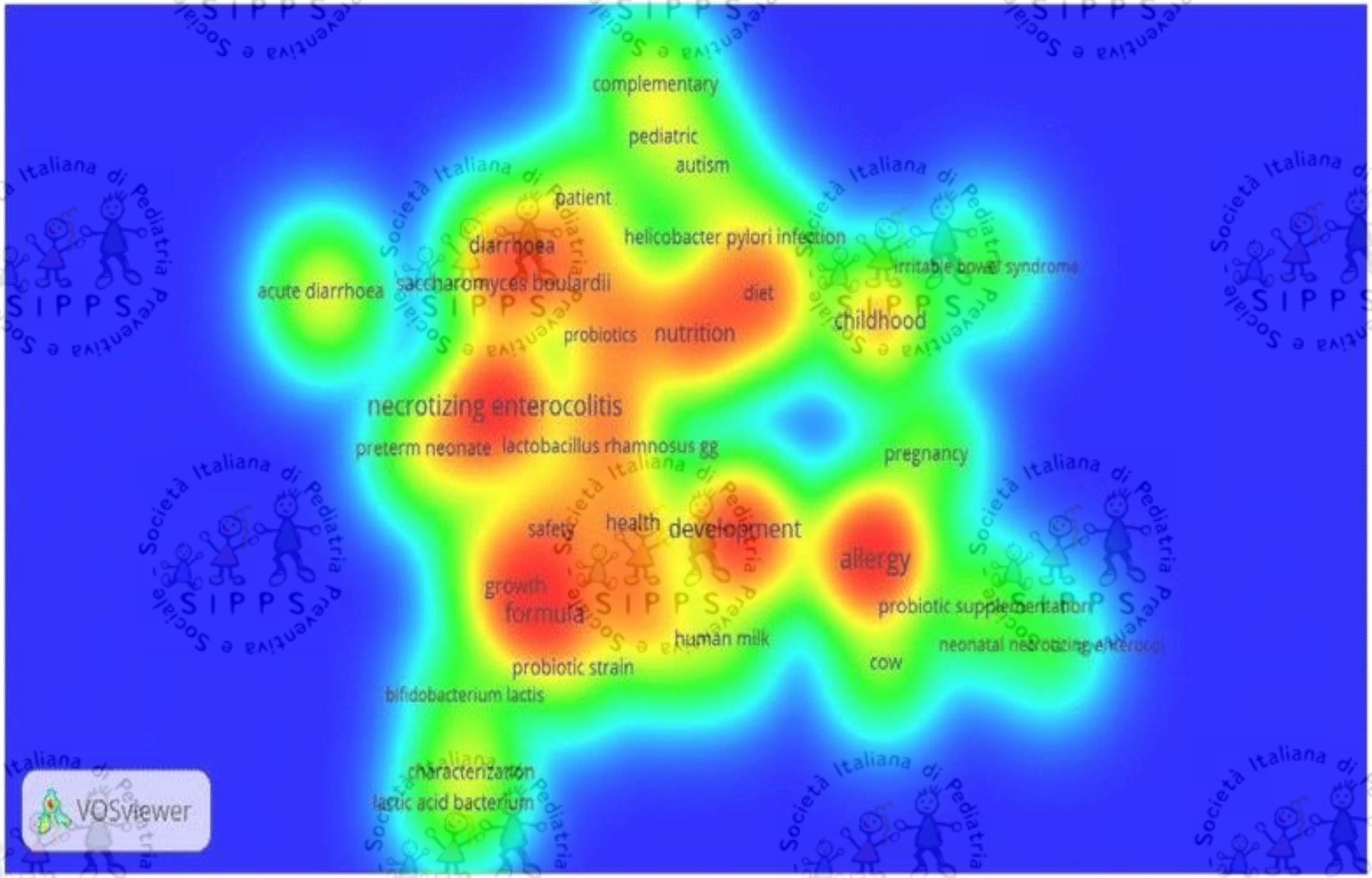


Probiotics and exposure to increased variety and abundance of microorganisms

MICROBIAL RELATED FACTORS REDUCING RISK OF FOOD ALLERGY

Aitoro R et al. Nutrients 2017 in press

Map of the most frequent terms in the title of papers on Probiotics in Pediatrics



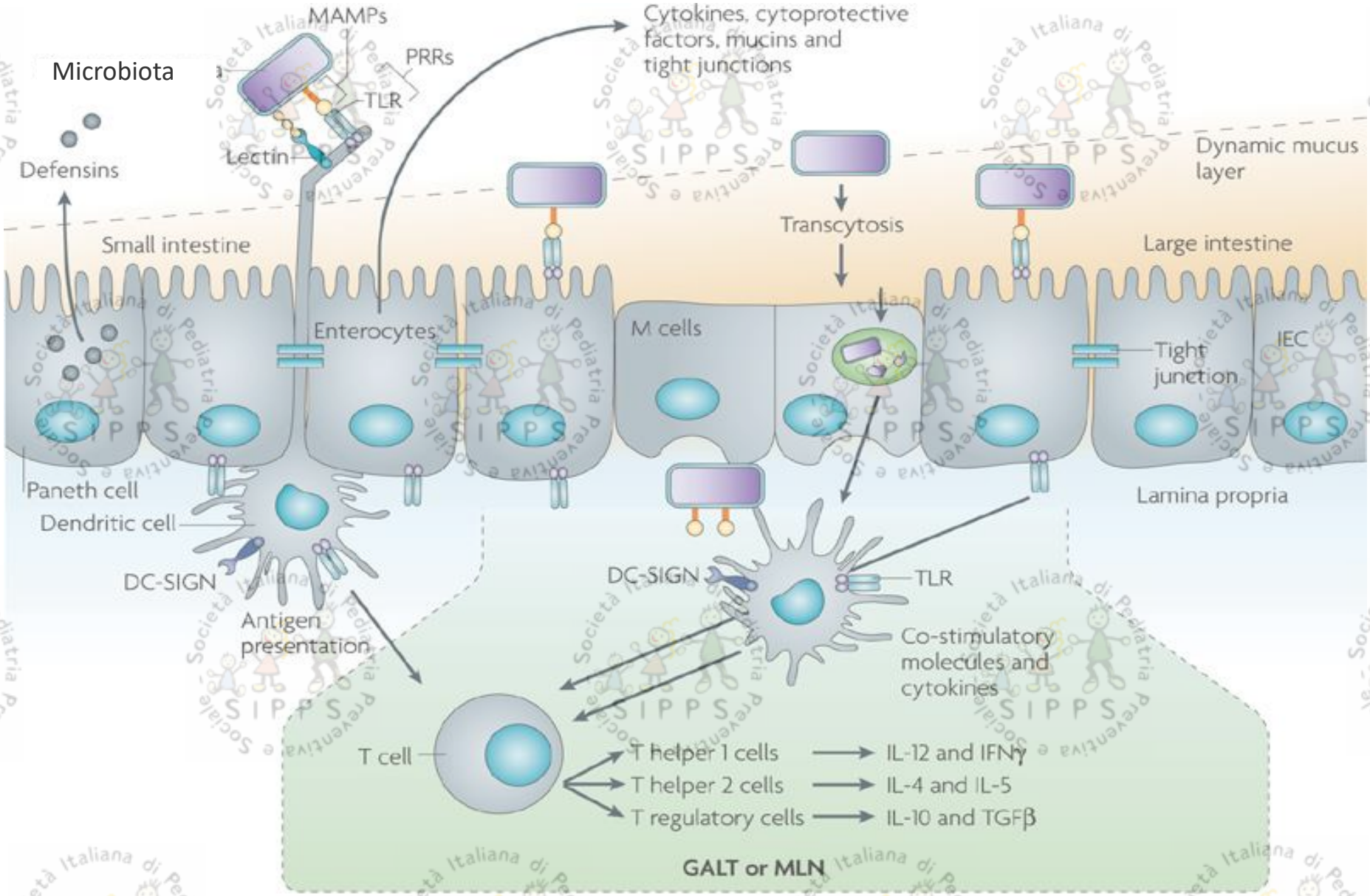
Sweileh WM et al. WAO J 2016

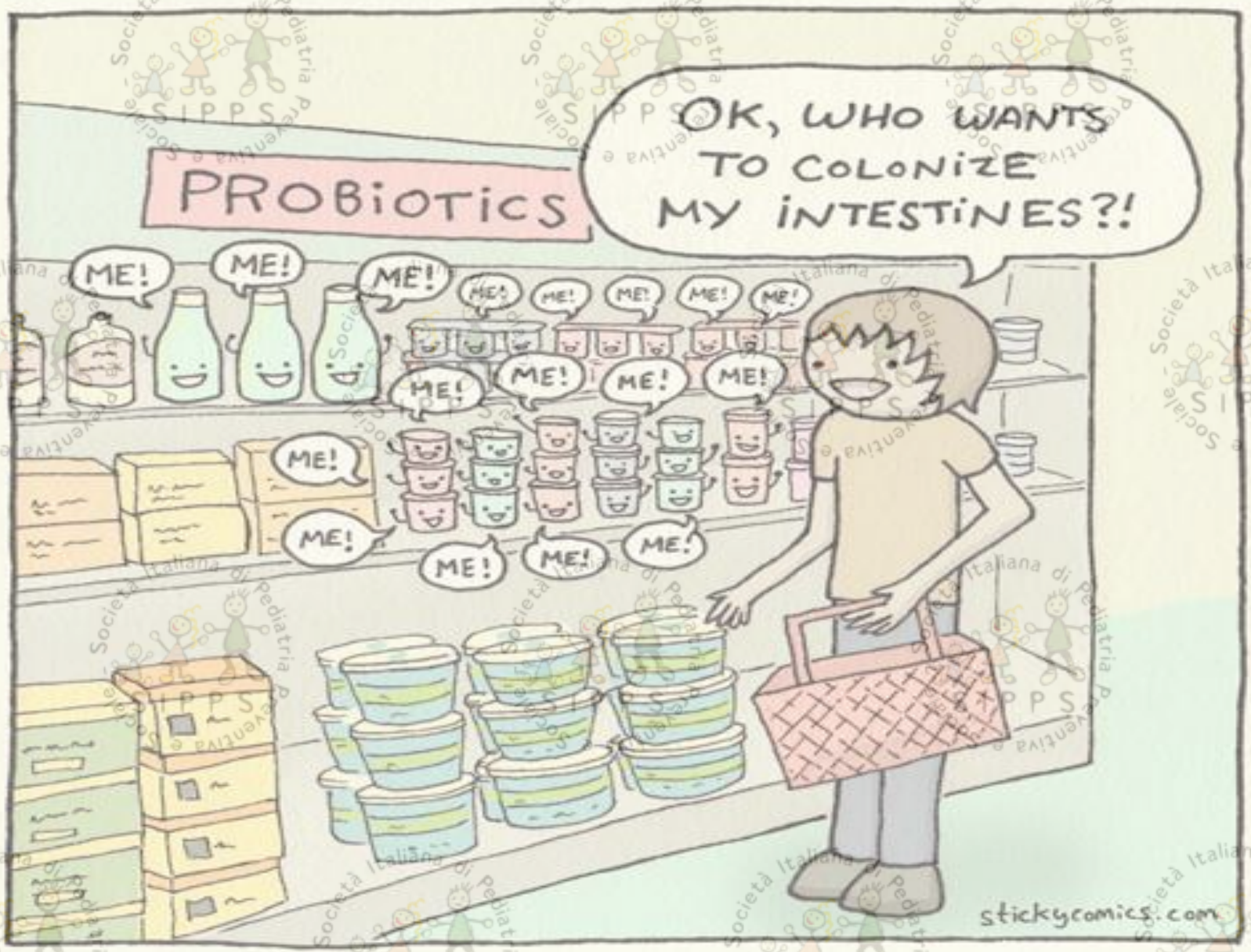
Top 10 list of institutions that published research articles on probiotics in pediatrics (1994-2015)

SCR[a]	Institution	Number of documents N = 2817 (%)	Total citation (Rank)	Citations/article (Rank)	H-index (Rank)	Affiliation country
1st	*Turun yliopisto*	82 (2.91)	8095 (1)	98.72 (2)	34 (1)	Finland
2nd	*Medical University Warsaw*	72 (2.56)	3234 (3)	44.92 (5)	27 (2)	Poland
3rd	*Universita degli Studi di Napoli Federico II*	45 (1.60)	1805 (6)	40.11 (8)	23 (3)	Italy
4th	*Universita degli Studi di Milano*	44 (1.56)	1864 (5)	42.36 (7)	19 (6)	Italy
5th	*Massachusetts General Hospital*	37 (1.31)	2105 (4)	56.89 (3)	22 (4)	USA
6th	*Turun Yliopistollinen Keskussairaala*	36 (1.28)	5103 (2)	141.75 (1)	21 (5)	Finland
7th	*Universite Paris Descartes*	35 (1.24)	1553 (7)	44.37 (6)	18 (7)	France
8th	*Nestle*	32 (1.14)	1072 (9)	33.50 (9)	17 (8)	Switzerland
9th	*University of Florida*	29 (1.03)	1410 (8)	48.62 (4)	16 (9)	USA
9th	*Universitair Ziekenhuis Brussel*	29 (1.03)	567 (10)	19.55 (10)	14 (10)	Belgium

Sweileh WM et al. WAO J 2016

Microbiota


MAMPs
PRRs
TLR
Lectin
Defensins
Cytokines, cytoprotective factors, mucins and tight junctions
Dynamic mucus layer
Transcytosis
Small intestine
Large intestine
Enterocytes
M cells
Tight junction
IEC
Paneth cell
Dendritic cell
DC-SIGN
Antigen presentation
Lamina propria
DC-SIGN
TLR
Co-stimulatory molecules and cytokines
T cell
T helper 1 cells → IL-12 and IFNγ
T helper 2 cells → IL-4 and IL-5
T regulatory cells → IL-10 and TGFβ
GALT or MLN


PROBIOTICS
OK, WHO WANTS TO COLONIZE MY INTESTINES?!
ME!
stickycomics.com

Quality of probiotic products. A Comment by the ESPGHAN Working Group on Prebiotics and Probiotics

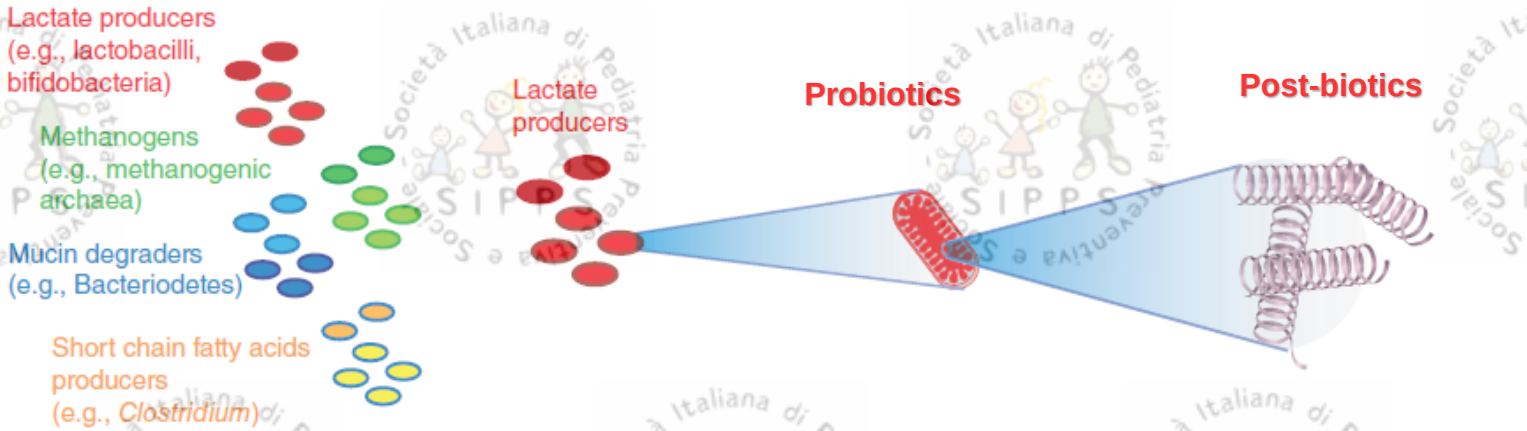
Kolaček S, Hojsak I, Berni Canani R, Guarino A, Indrio F, Orel R, Pot B, Shamir R, Szajewska H, Vandenplas Y, van Goudouver H, Weizmann Z

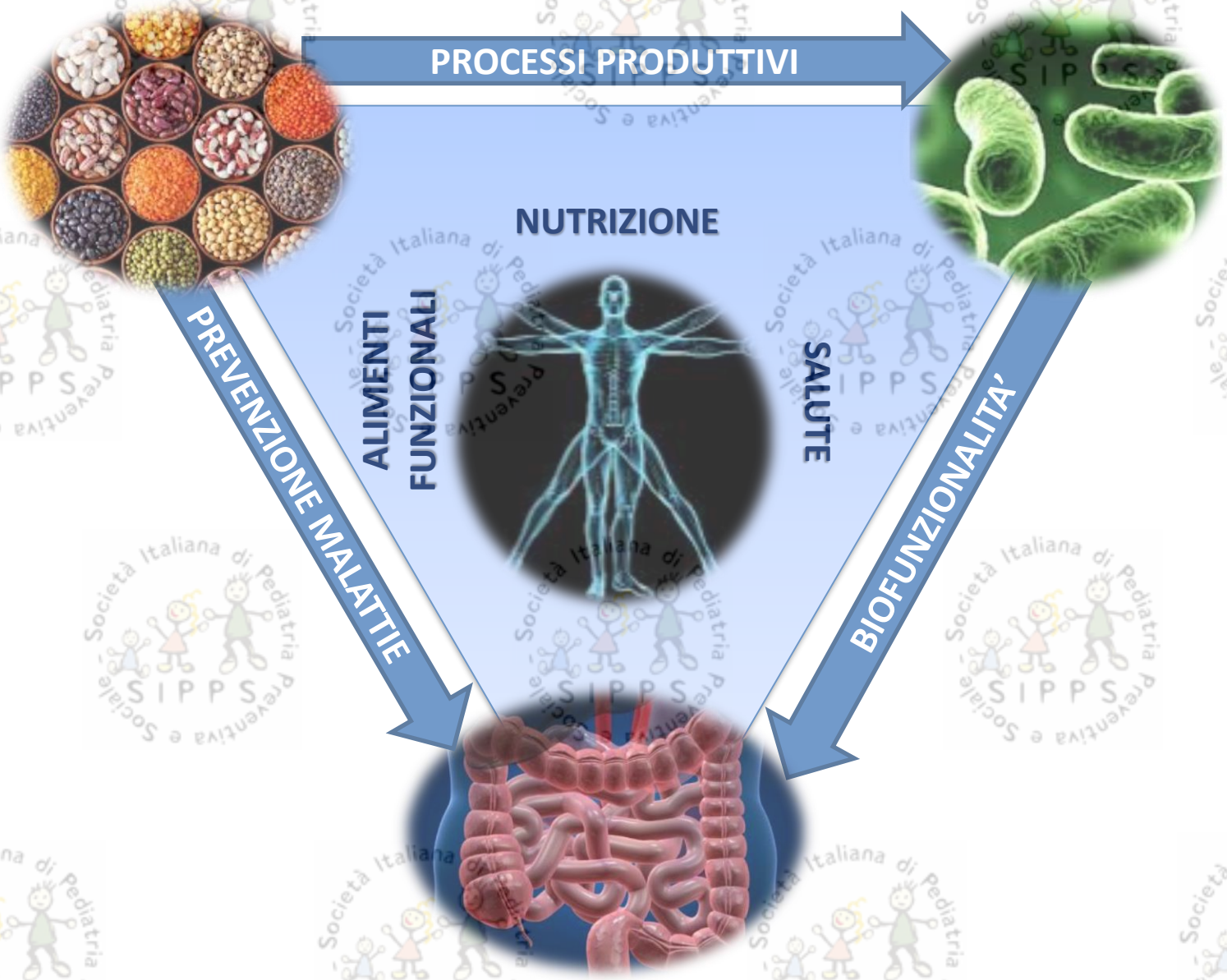
- Precisa identificazione di ceppo e dosi
- Controlli di qualità effettuati in Laboratori Certificati con metodiche standardizzate. Risultati resi pubblici.
- Utilizzo clinico legato a particolari ceppi e dosi
- Eventuali avversi riportati in apposito registro internazionale tenuto da Autorità Competenti

JPGN 2107 in press

Medicines from microbiota

Bernat Olle



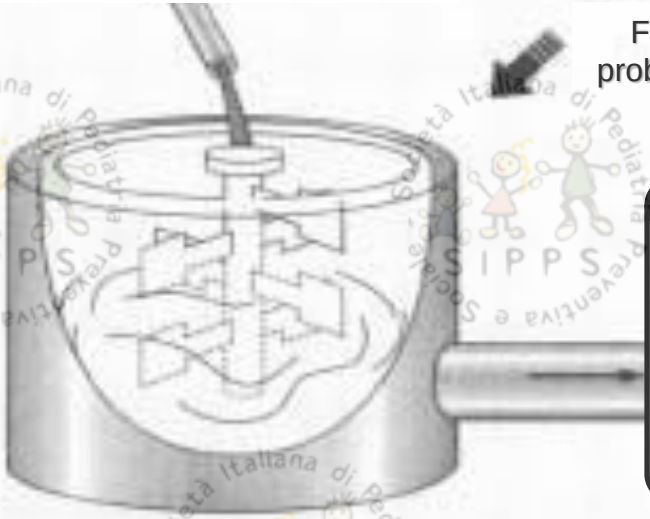

PROCESSI PRODUTTIVI
NUTRIZIONE
ALIMENTI FUNZIONALI
SALUTE
PREVENZIONE MALATTIE
BIOFUNZIONALITA'

Approccio con “postbiotici”

1

Trattamento termico per inattivare il batterio dopo la fermentazione a 85°C per 20 sec

Fermentazione di latte vaccino o riso con il probiotico *L.paracasei* CBA L74 5.9x10^9 CFU/g (BCCM/LMG LMG S-24480) 15 h a 37°C



2

3

Prodotto del processo di fermentazione altamente riproducibile

Cow's milk and rice fermented with *Lactobacillus paracasei* CBA L74 prevent infectious diseases in children: A randomized controlled trial

Rita Nocerino [a], Lorella Paparo [a], Gianluca Terrin [b], Vincenza Pezzella [a], Antonio Amoroso [a], Linda Cosenza [a], Gaetano Cecere [a], Giulio De Marco [a], Maria Micillo [a], Fabio Albano [a], Rosa Nugnes [a], Pasqualina Ferri [a], Giuseppe Ciccarelli [a], Giuliana Giaccio [a], Raffaella Spadaro [a], Ylenia Maddalena [a], Francesco Berni Canani [c], Roberto Berni Canani [a, d, e, *]

[a] Department of Translational Medical Science, University of Naples "Federico II", Naples, Italy
[b] Department of Gynecology-Obstetrics and Perinatal Medicine, University of Rome "La Sapienza", Rome, Italy
[c] ENT Unit, Ospedale Spirito Santo di Pescara, Pescara, Italy
[d] European Laboratory for the Investigation of Food Induced Diseases (ELFID), University of Naples "Federico II", Naples, Italy
[e] CEINGE – Advanced Biotechnologies, University of Naples "Federico II", Naples, Italy

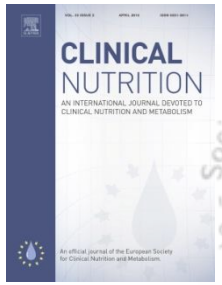
Numero totale di infezioni: riduzione del **60%**

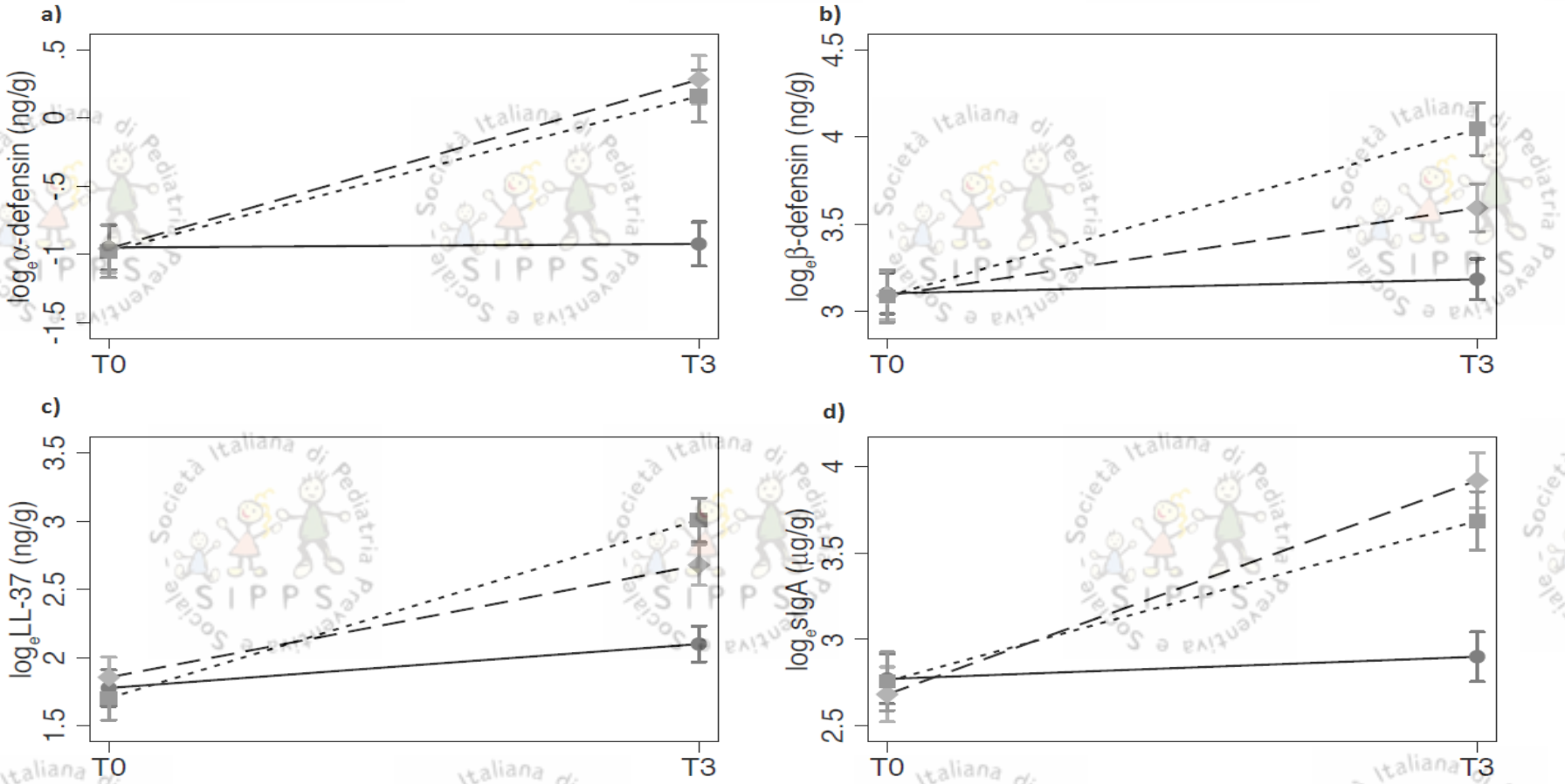
Numero di episodi di diarrea acuta: riduzione del **55%**

Numero di episodi di infezioni a carico delle alte vie del respiro: riduzione del **60%**

Utilizzo di antibiotici: riduzione del **75%**

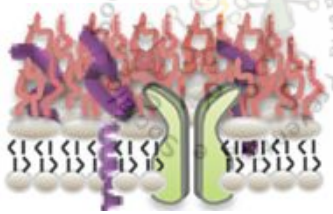
Latte fermentato e riso fermentato con *L.paracasei* CBA L74 esercitano una significativa immunomodulazione





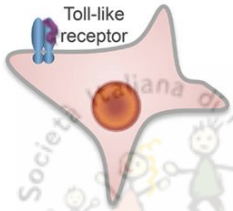
Nocerino R, et al. Clin Nutr 2015

Peptidi dell'immunità innata

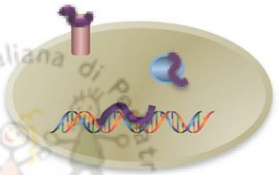


Inibizione legame patogeno cellule bersaglio

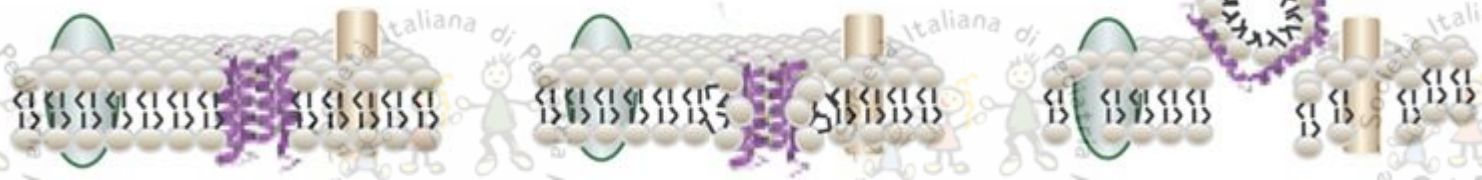
Attivazione sistema immunitario



Interazione diretta con espressione dei geni del patogeno



Alterazione membrana cellulare



Modified from Duplantier AJ Front Immunol 2013

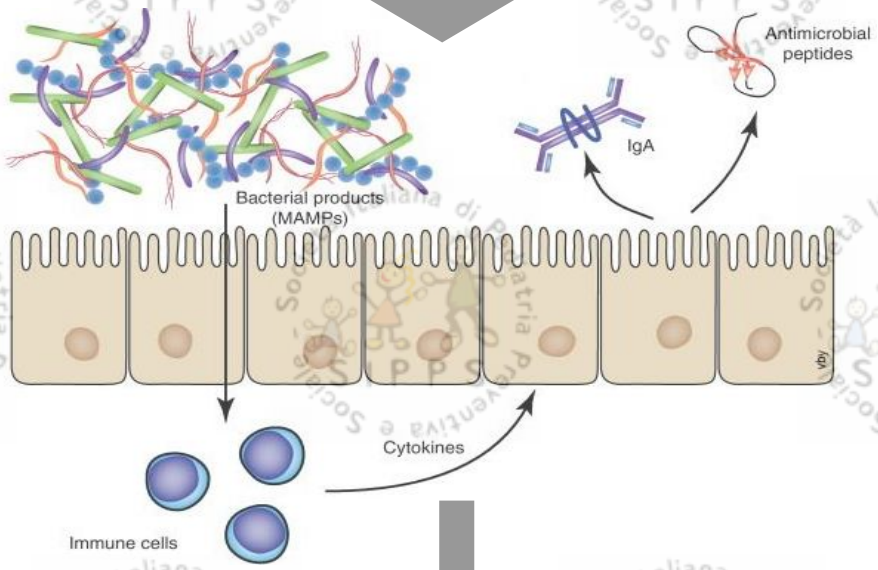
Latte fermentato e riso fermentato con CBA-L74

Peptidi

Microbiota intestinale

Prodotti batterici

Butirrato



Stimolazione dei meccanismi di difesa immunologici e non-immunologici

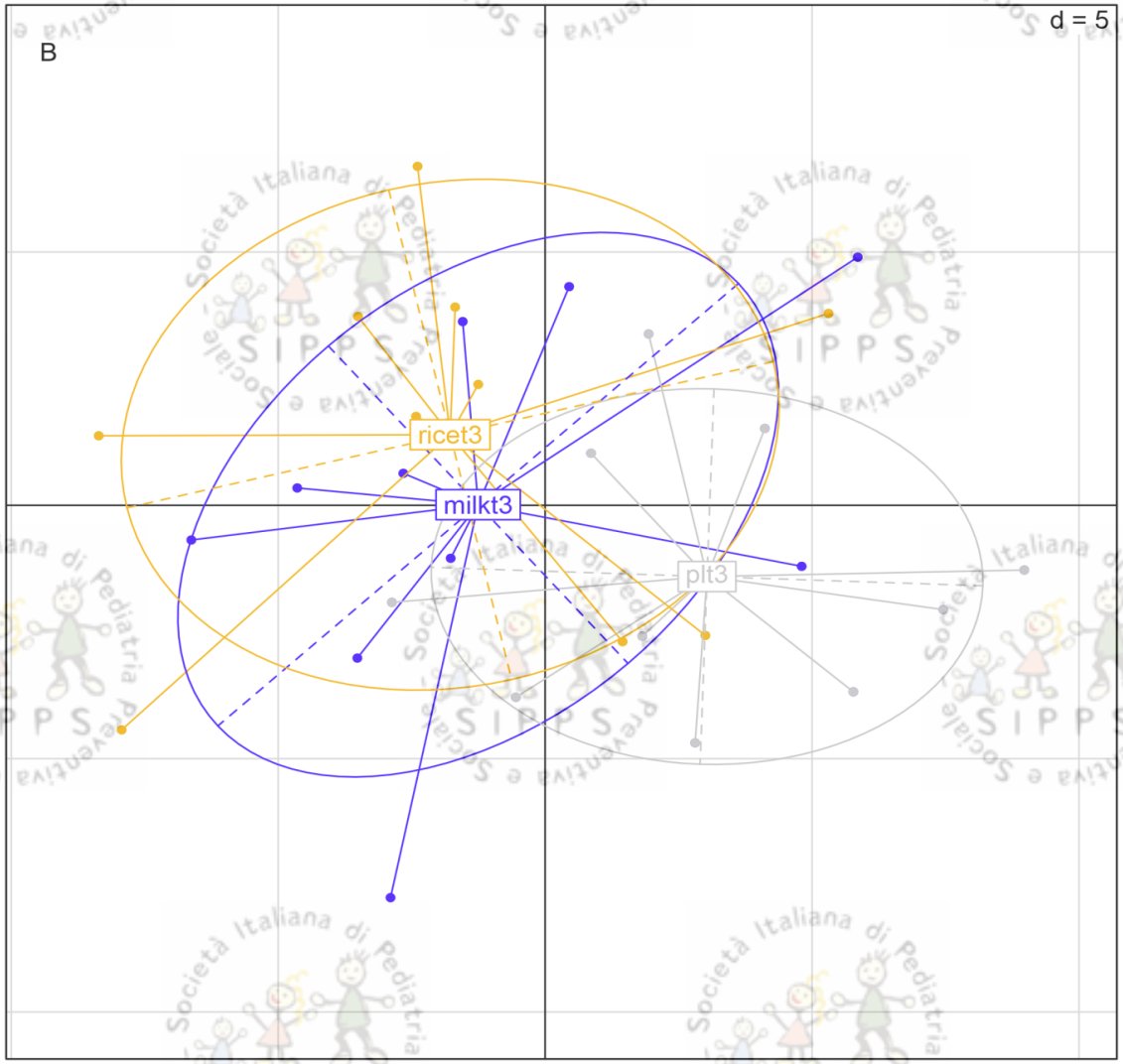

Argonne National Laboratory campus

Prof. Jack Gilbert – ANL and U.Chicago, IL
Prof. Danilo Ercolini – ANL and U.Chicago, IL and Univ. Federico II, Naples, Italy

- No significant effect on OTUs count, Shannon diversity Index, Bacterial load (16s RNA copies/gr of stool)
- Significant increase in *Ruminococcaceae* in FM-treated subjects
- Significant increase in *Lachnospiraceae* in FM-treated subjects

Berni Canani R, et al. manuscript in preparation

Cow's milk fermented with *L.paracasei* CBA L74 shapes gut microbiota composition



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Famiglie batteriche con abbondanza significativamente diversa

CLOSTRIDIACEAEE

CIDs

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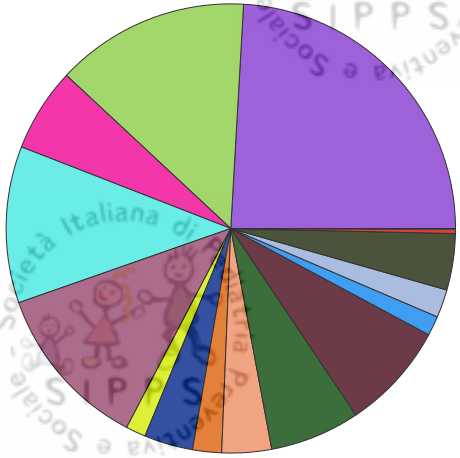
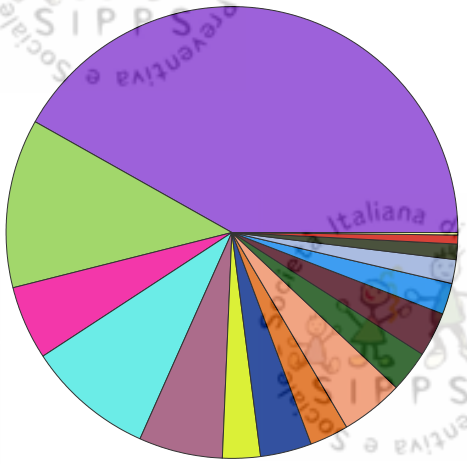
Roseburia Oligotypes

16sRNA Next-generation sequencing



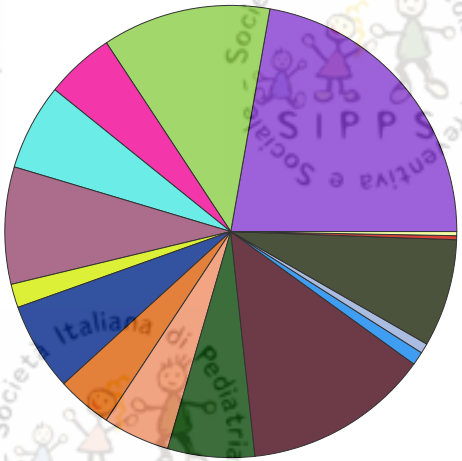
FM CBA L74 T0



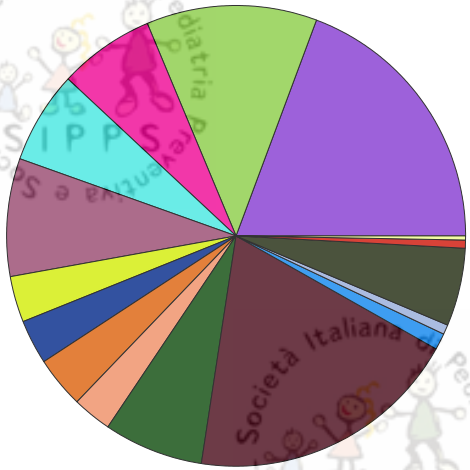
*

FM CBA L74 T3

Specific *Roseburia* oligotype is promoted by FM CBA L74 (*Roseburia* oligotype 1) and shows positive correlation with sIgA (ρ=0.63, *P*=0.038)



Placebo T0

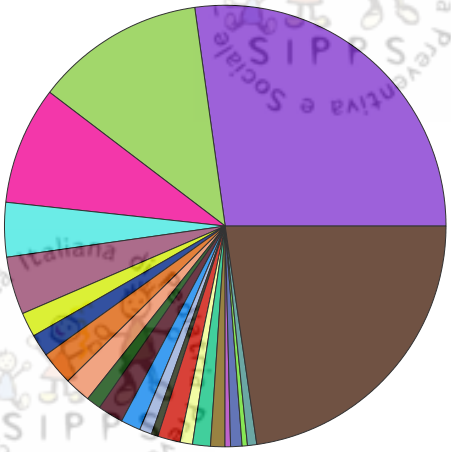


Placebo T3

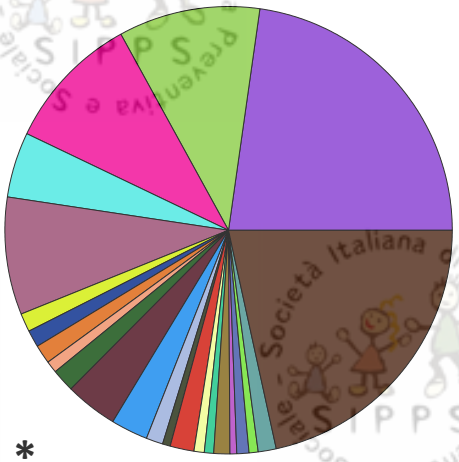
Berni Canani R, et al. manuscript in preparation

Blautia Oligotypes

16sRNA Next-generation sequencing

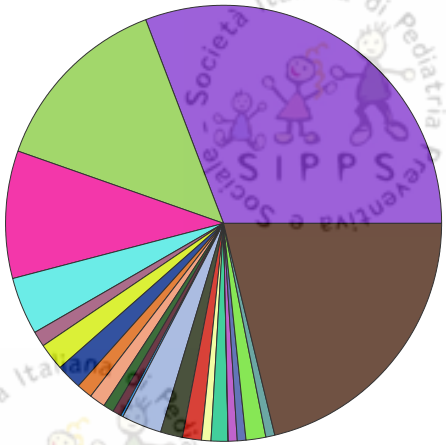


FM CBA L74 T0

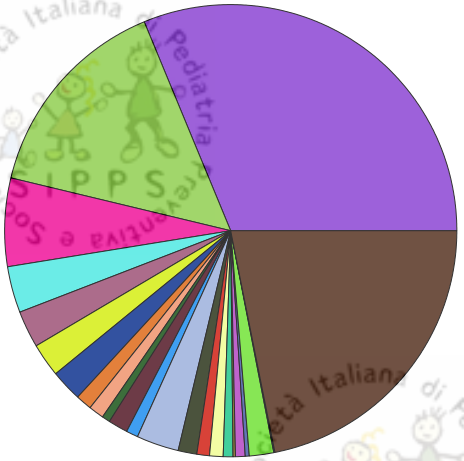


FMCBA L74 T3

FM CBAL74 promotes an increase in oligotypes 5 and 13, that were positively correlated with alpha-defensin (ρ=0.84, P=0.007; ρ=0.58, P=0.040)



Placebo T0



Placebo T3

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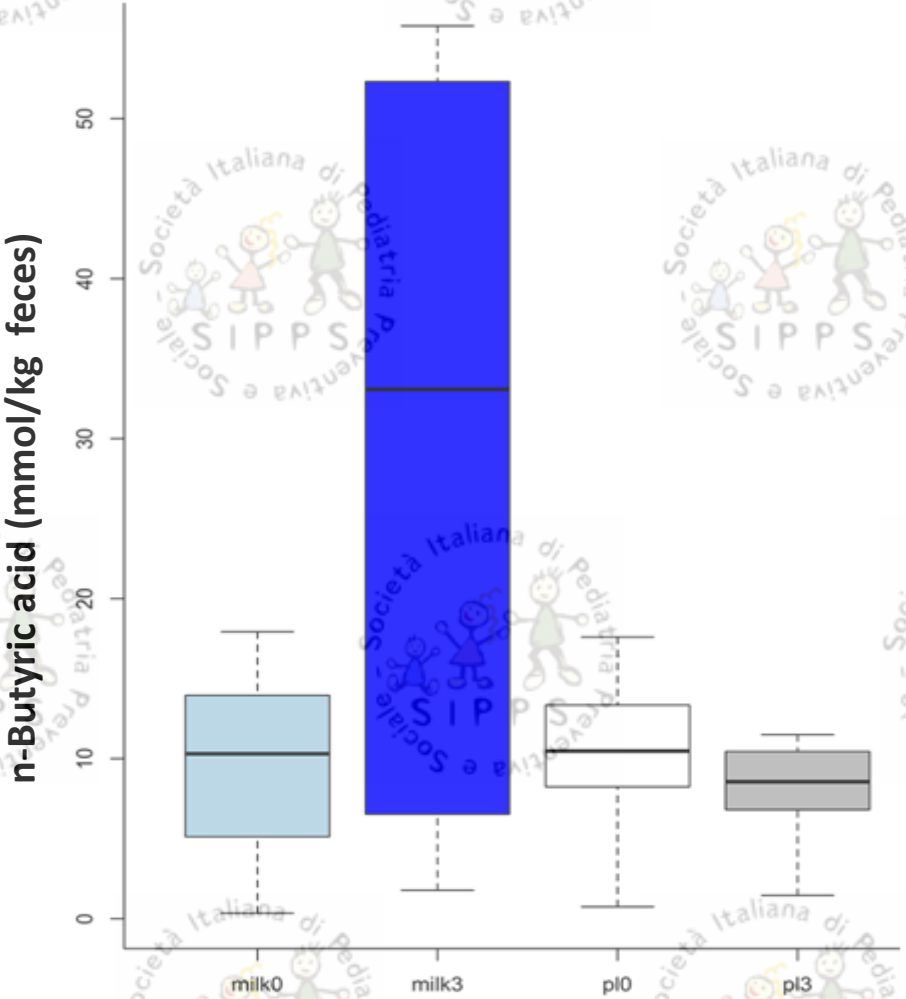
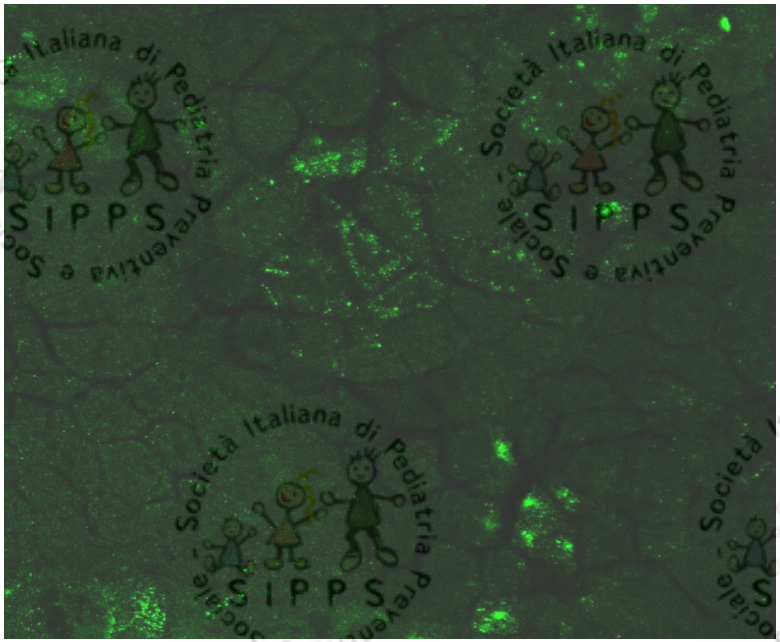
Stimolazione della produzione di butirrato a livello intestinale

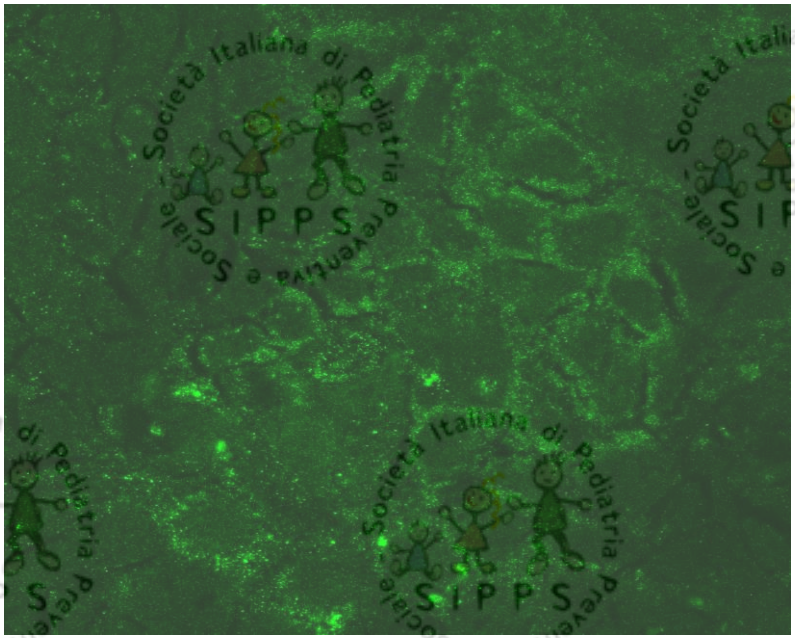


Berni Canani R, et al. manuscript in preparation

Effect of butyrate on LL-37 production by human enterocytes



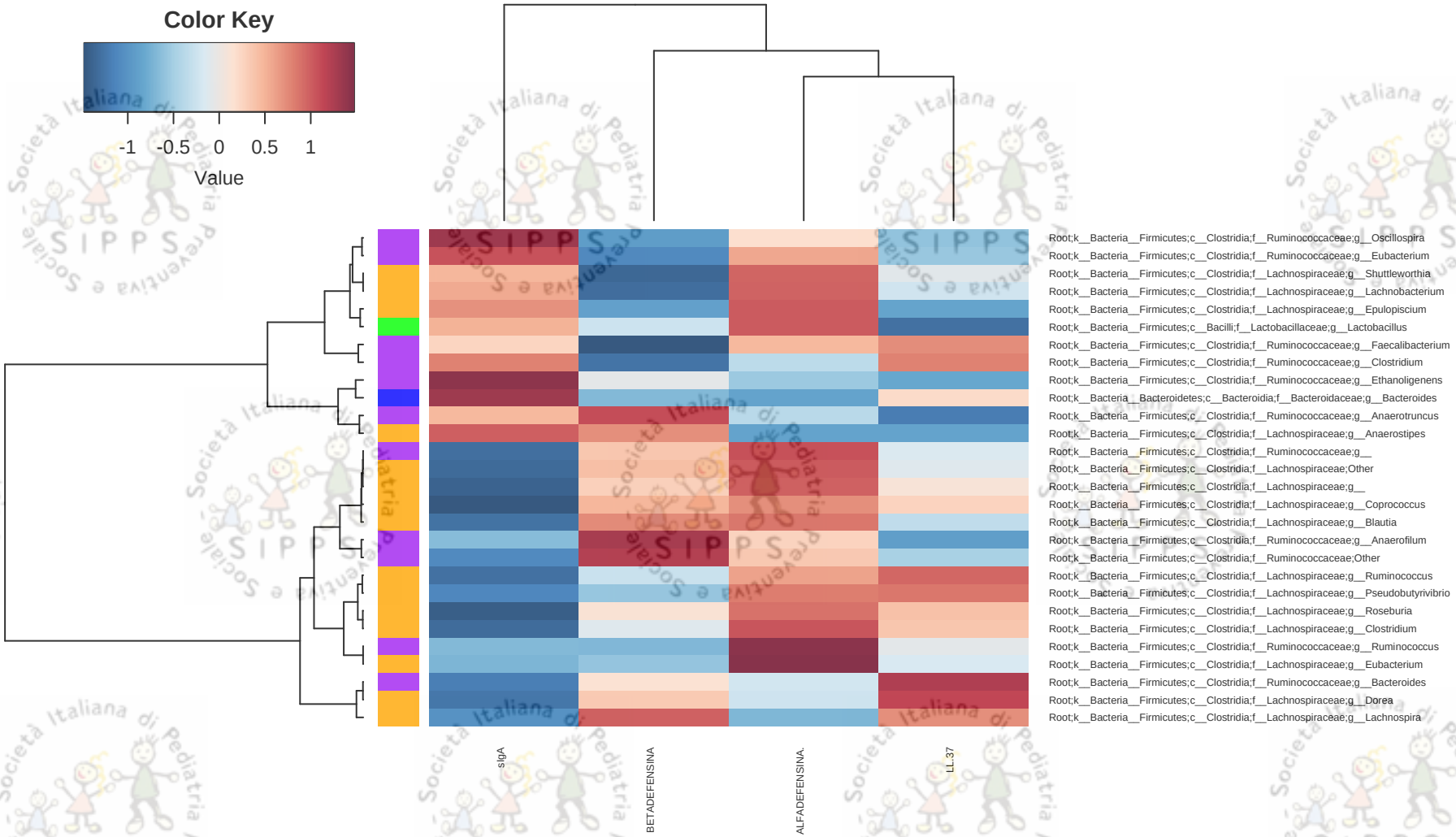
Control



Butyrate

Berni Canani R, et al. manuscript in preparation

Correlazione tra modifiche della composizione del microbiota intestinale e attivazione dell'immunità innata ed acquisita



Berni Canani R, et al. manuscript in preparation

Conclusioni

+ Il microbiota svolge un ruolo fondamentale nel regolare sviluppo e funzioni del sistema immunitario
+ Alterazioni di composizione e funzioni del microbiota sono associati a diverse patologie croniche non trasmissibili
+ Fattori ambientali (*in primis dieta e farmaci*) svolgono un ruolo cruciale nel regolare sviluppo e funzioni del microbiota intestinale
+ La conoscenza di questi meccanismi sta offrendo nuove prospettive di intervento nella prevenzione e trattamento di molte patologie del bambino

Comparative relative abundance (% of total reads) for the genera identified as significantly different

Phyla	Family	Species	Strain
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Blautia/Roseburia*
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Faecalibacterium*
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Eubacterium*
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Oscillospira*

Berni Canani R, et al. manuscript in preparation

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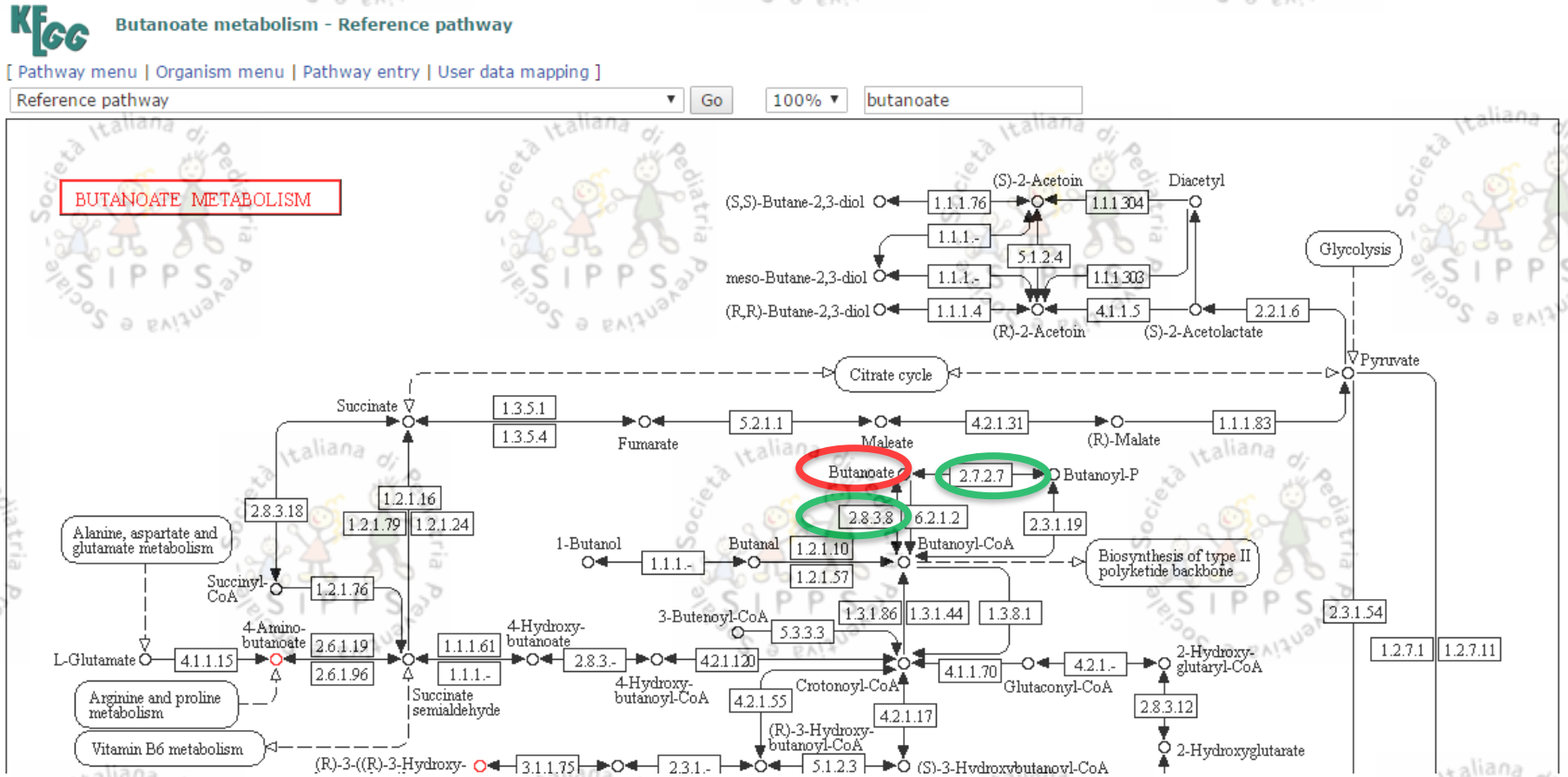
Comer Children's Hospital – Pediatric GI Unit
S.Guandalini

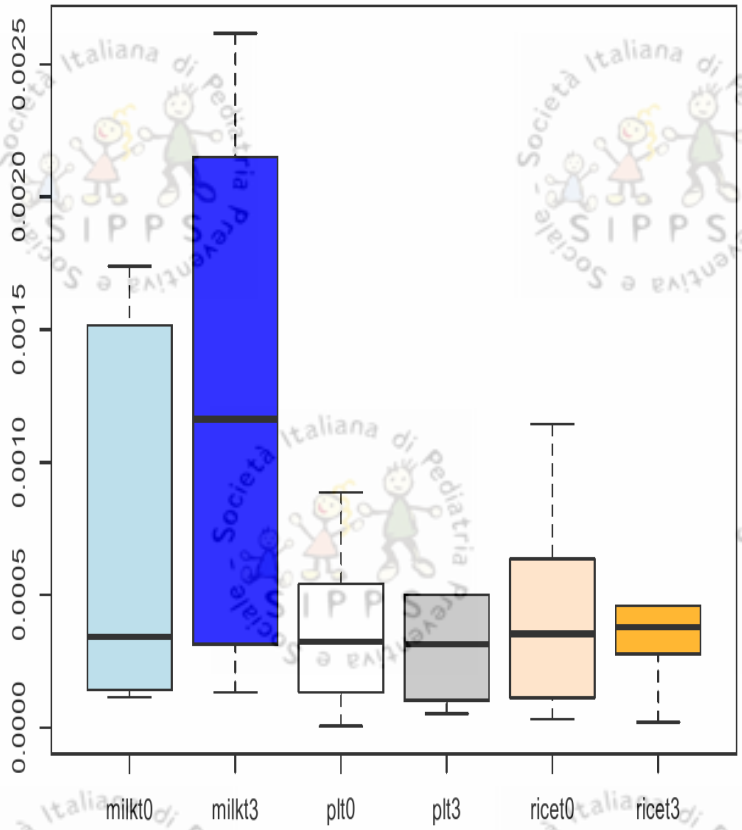
Metagenome prediction using PICRUSt : focus on butyrate

KEGG pathways → Metabolism → Butanoate metabolism (Butyrate)

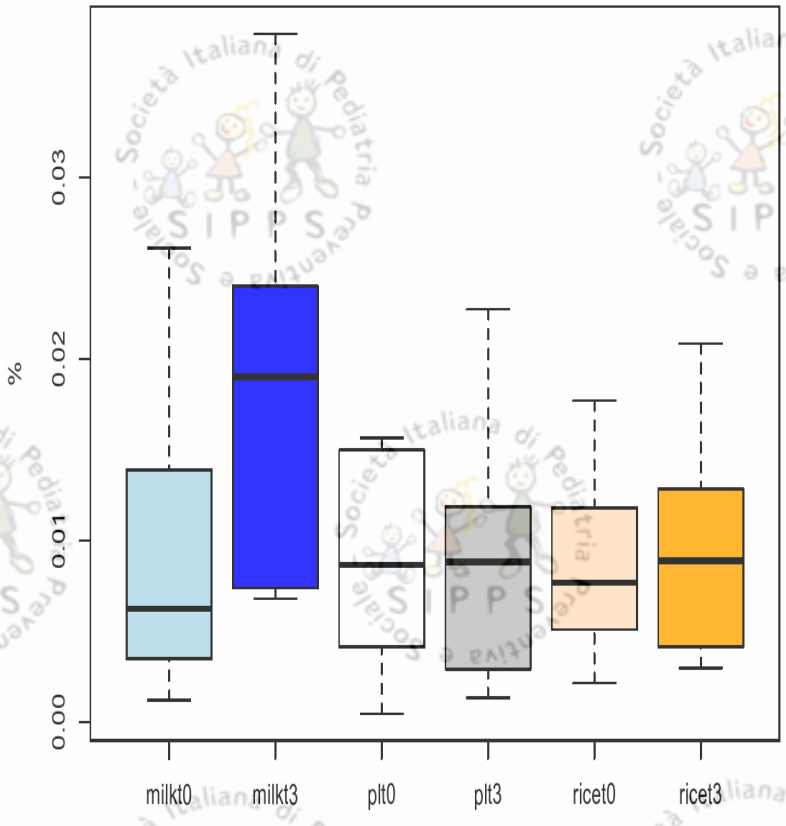


http://www.genome.jp/kegg-bin/show_pathway?map=map00650&show_description=show

The predicted abundance of key enzymes in butyrate production pathways such as butyryl coA transferase (E.C 2.8.3.8) and butyrate kinase (E.C. 2.7.2.7) increased upon FM CBAL74 treatment



butyryl coA transferase (E.C. 2.8.3.8)



butyrate kinase (E.C. 2.7.2.7)

Berni Canani R, et al. manuscript in preparation

Effect of cow's milk fermented with Lactobacillus paracasei CBA L74 on common infectious diseases in children: a multicenter randomized controlled trial

Giovanni Corsello, Maurizio Carta, Roberto Marinello, Marina Picca, Giulio De Marco, Maria Micillo, Dante Ferrara, Patrizia Vigneri, Gaetano Cecere, Pasqualina Ferri, Paola Roggero, Giorgio Bedogni, Fabio Mosca, Lorella Paparo, Rita Nocerino, and Roberto Berni Canani

Numero totale di infezioni: riduzione del **30%**

Numero di episodi di diarrea acuta: riduzione del **32%**

Numero di episodi di infezioni a carico delle alte vie del respiro: riduzione del **29%**

Numero di terapie antibiotiche: riduzione del **31%**

Giorni di assenza da scuola: riduzione del **75%**

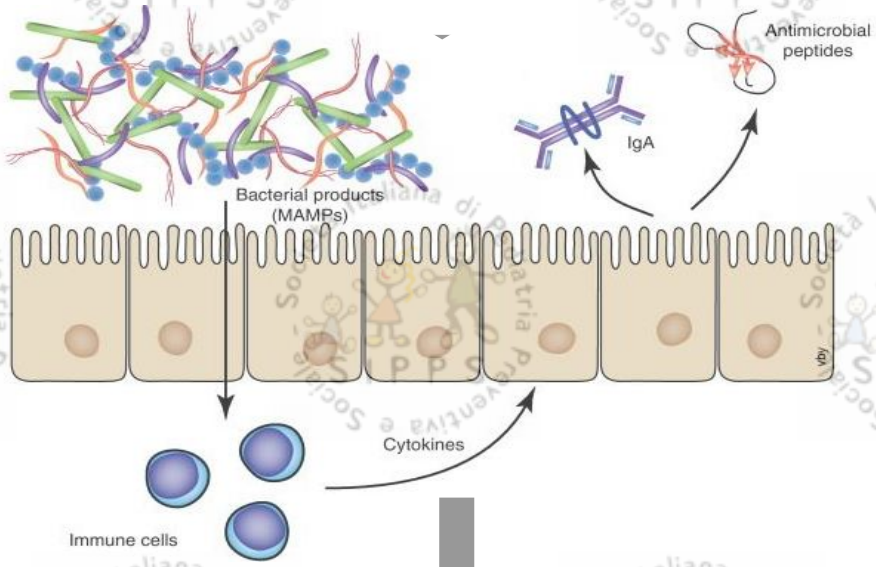
Giorni di assenza da lavoro: riduzione dell' **80%**

Berni Canani R. 2017, submitted

Approccio post-biotico con latte fermentato e riso fermentato con CBA-L74

Peptidi

Prodotti batterici



Stimolazione dei meccanismi di difesa immunologici e non-immunologici

Punti di forza dell'approccio "post-biotico" con FM CBA L74

+ Probiotico di origine umana, ben caratterizzato
+ Probiotico inattivato = No traslocazione batterica/sepsi
+ Elevata facilità di conservazione e utilizzo
+ Monitoraggio preciso del processo tecnologico e identificazione precisa della dose da somministrare = Elevata riproducibilità degli effetti
+ Positiva modulazione del sistema immunitario e del microbiota intestinale

= **Dimostrata efficacia clinica nella prevenzione delle infezioni del bambino**

L’attività di ricerca continua…..

- Attività biologiche/meccanismi di azione
- Caratterizzazione molecolare (lipidi, nucleotidi, piccoli peptidi termostabili derivanti dalle PLV e dal batterio, ecc)
- Nuove applicazioni cliniche
- Trial clinici randomizzati controllati

Subjects with ≥1 CID

56%

81%

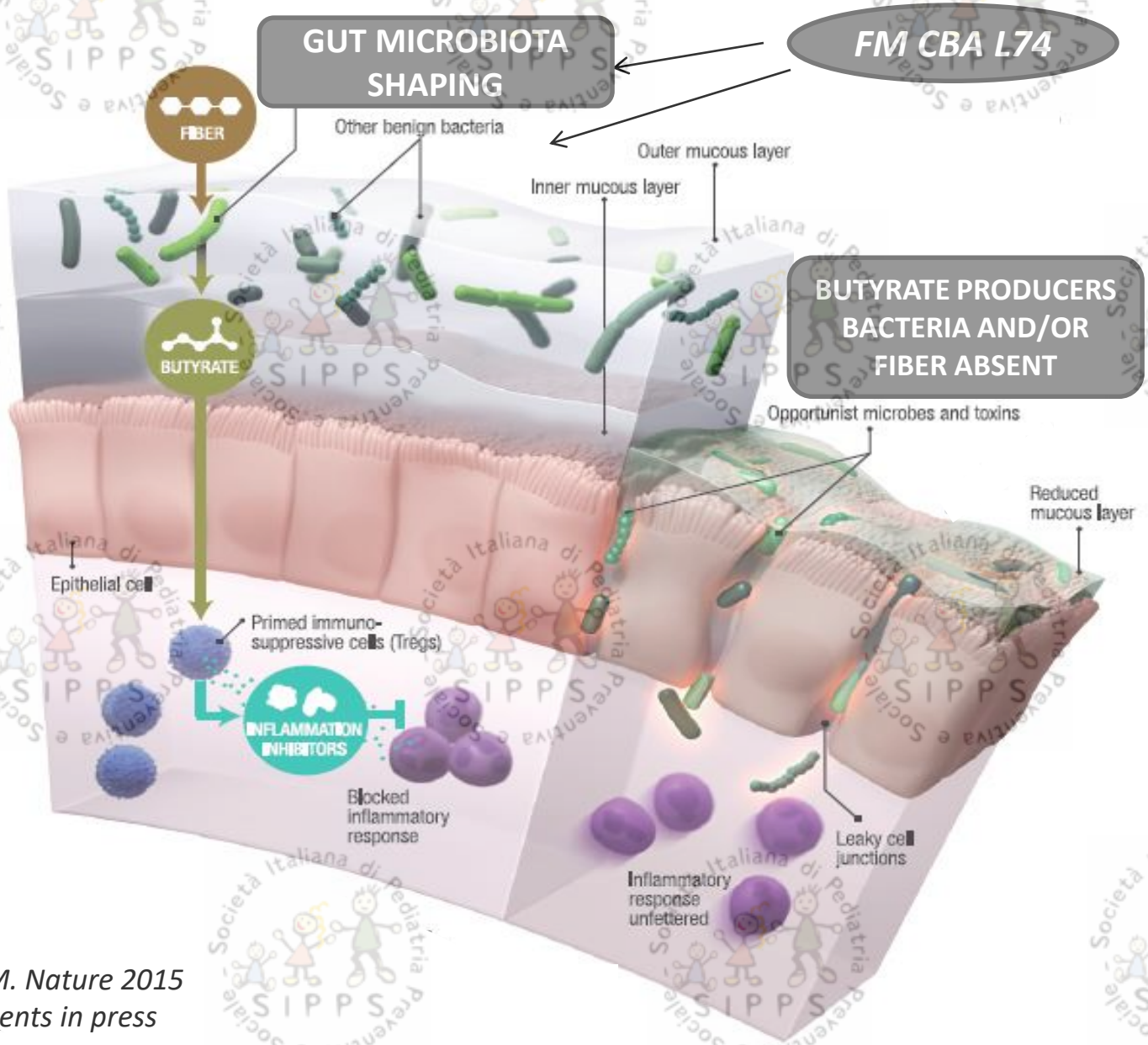
ITT analysis, p=0.002

Total number CIDs

Fermented milk vs Placebo: 119 vs 169, *p<0.05*

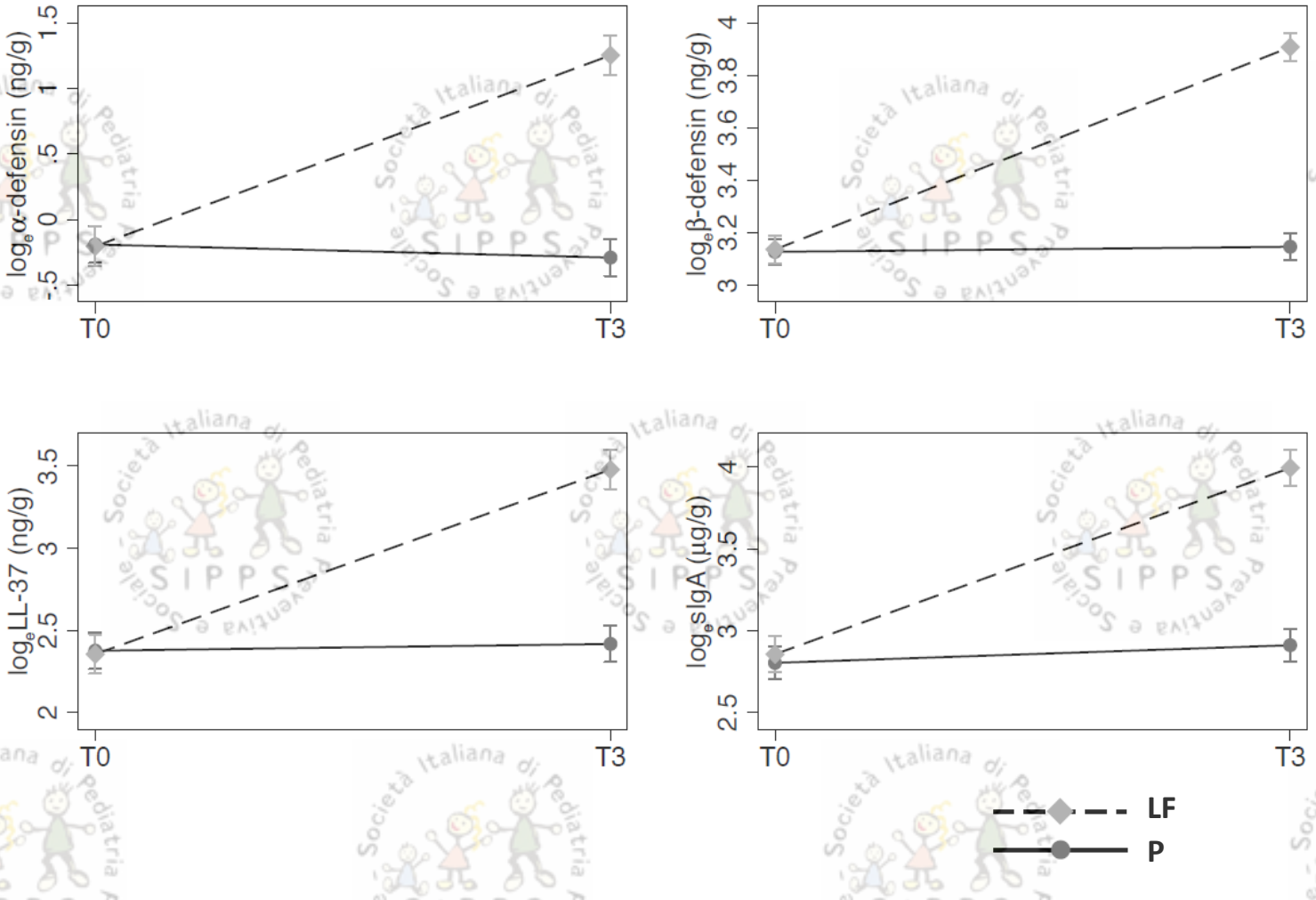
-30%

FM CBA L74: The Peace-keeper for GI tract

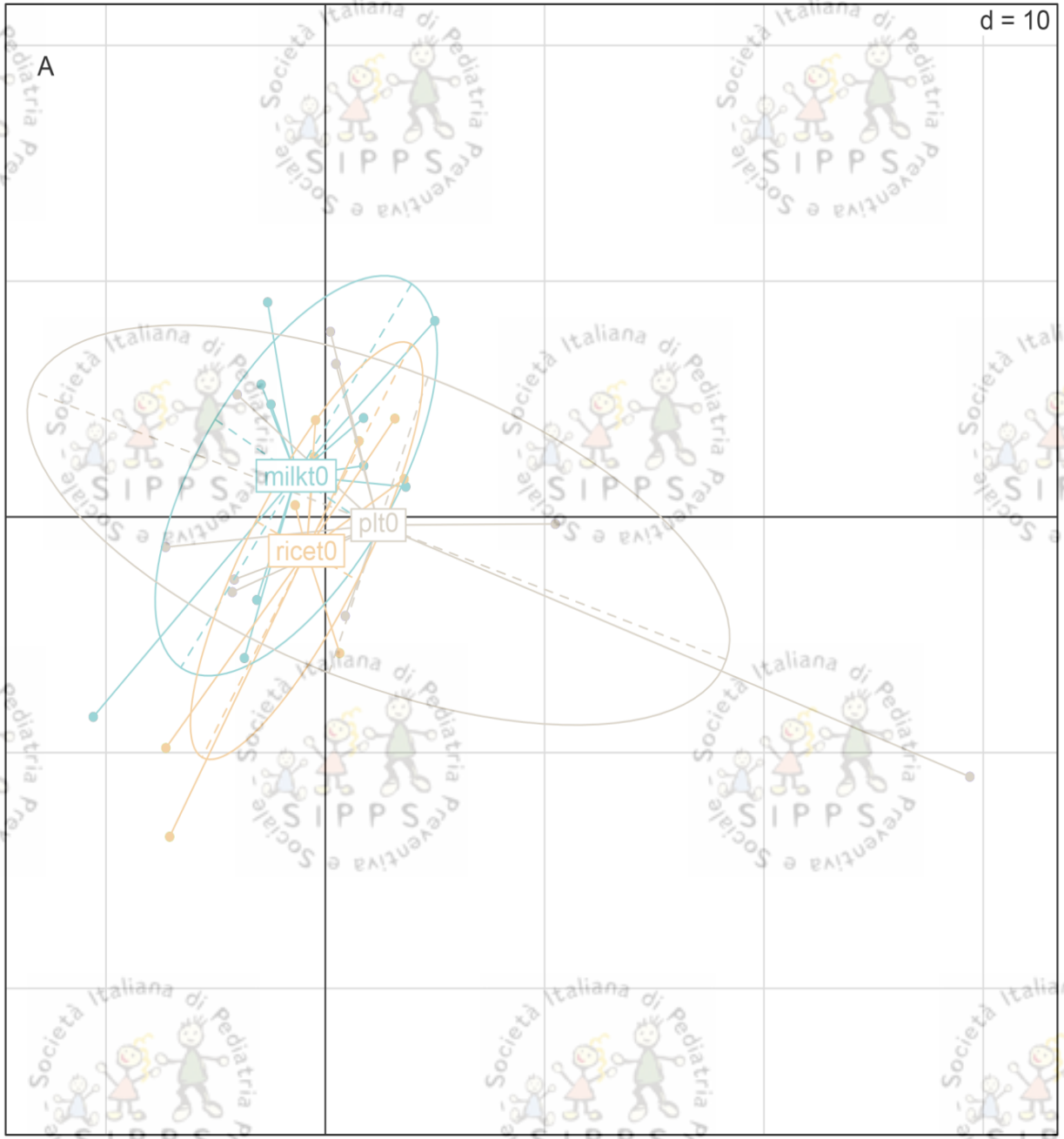


Modified from
Velasquez-Manoff M. Nature 2015
Paparo L et al. Nutrients in press

Stimolazione dell'immunità innata ed acquisita dopo 3 mesi di trattamento



Berni Canani R. 2017, submitted

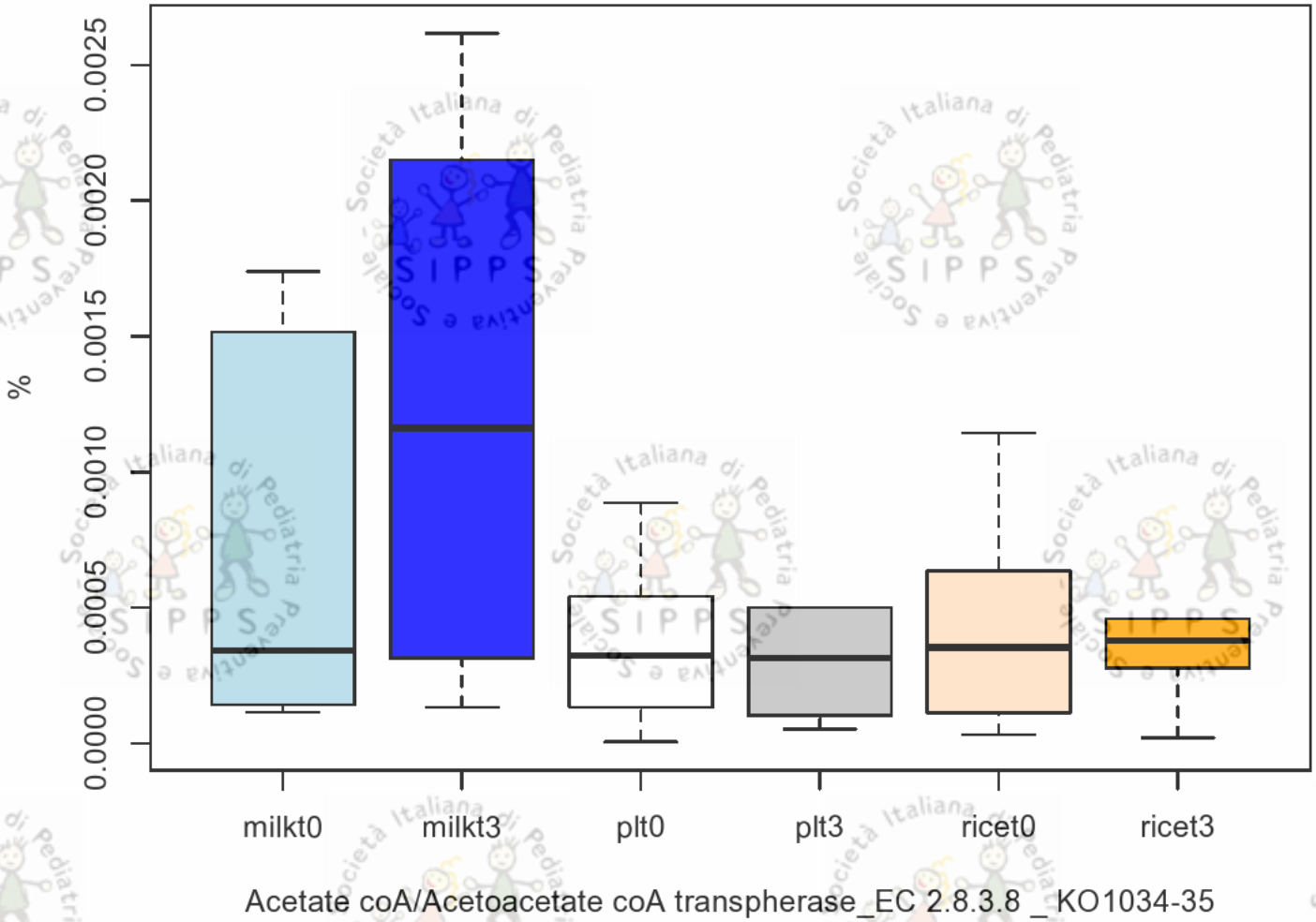


Although a great variability was observed, the gut microbiota composition of treated children was different from that of the placebo group.

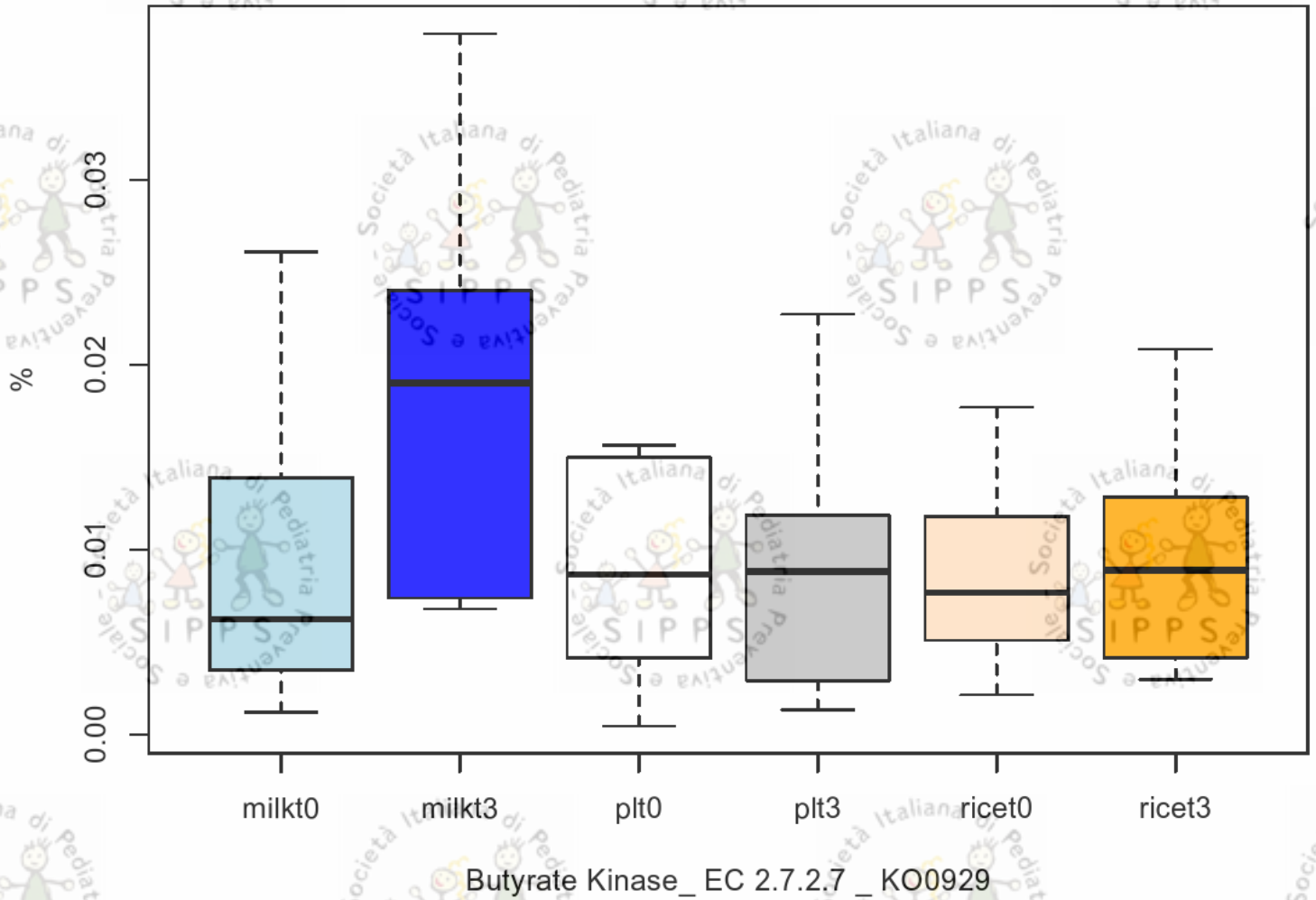
Significant differences between PL and rice/milk treated children ($P<0.05$)

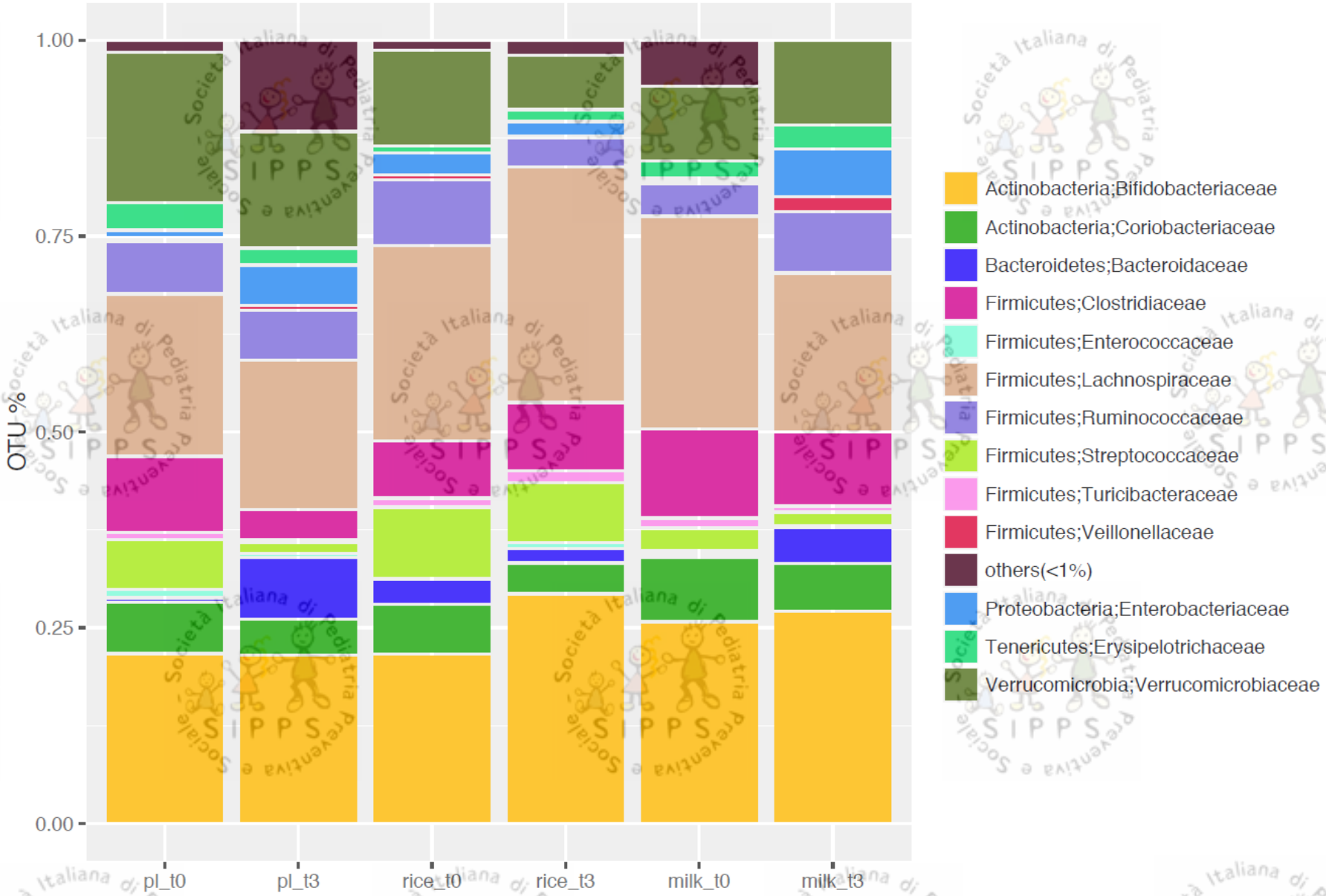
EC 2.8.3.8 : acetate CoA-transferase (butyryl coenzyme A transferase)

a) **K01034** : acetate CoA/acetoacetate CoA-transferase alpha subunit
b) **K01035** : acetate CoA/acetoacetate CoA-transferase beta subunit



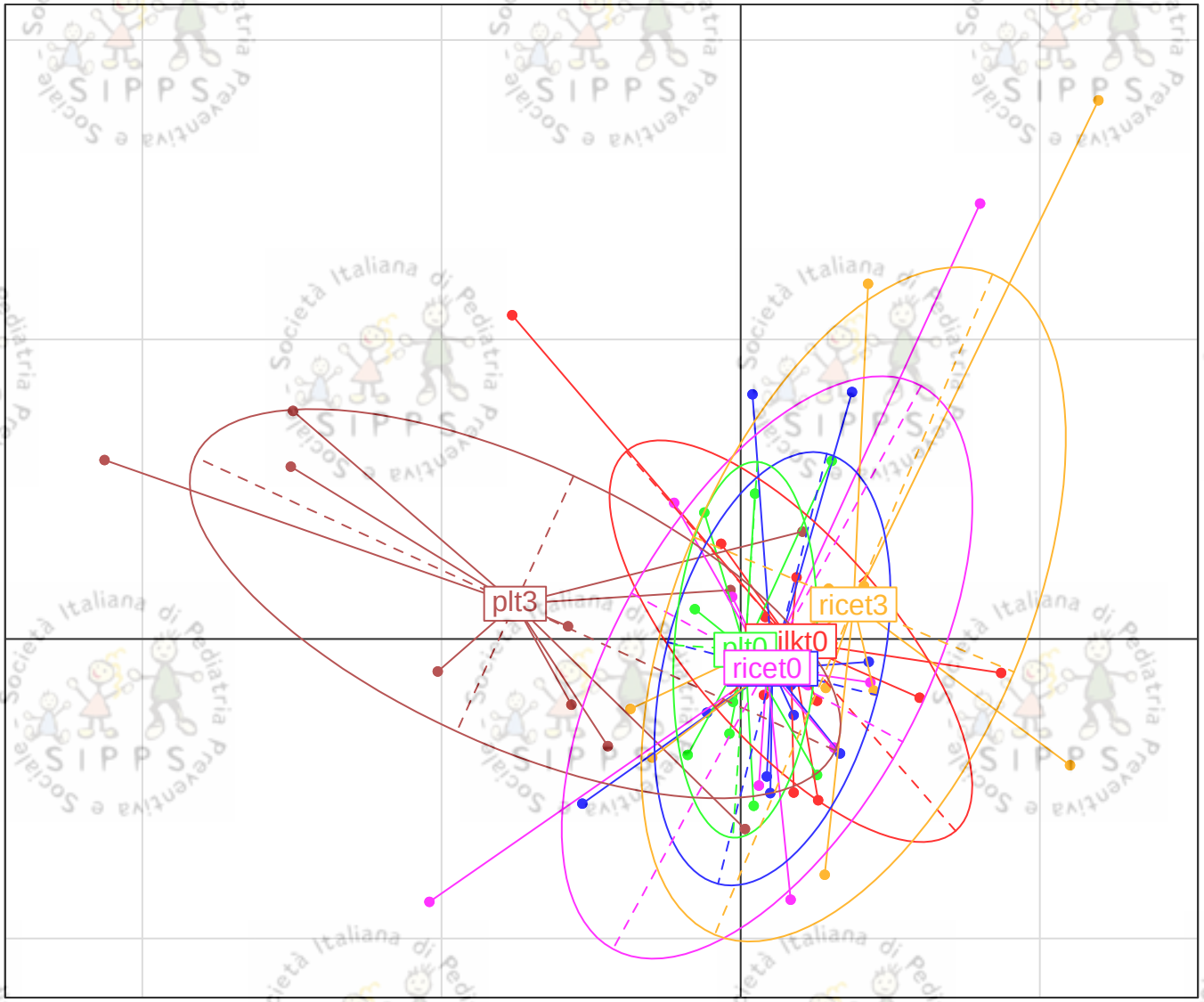
EC 2.7.2.7 : butyrate kinase (K00929)

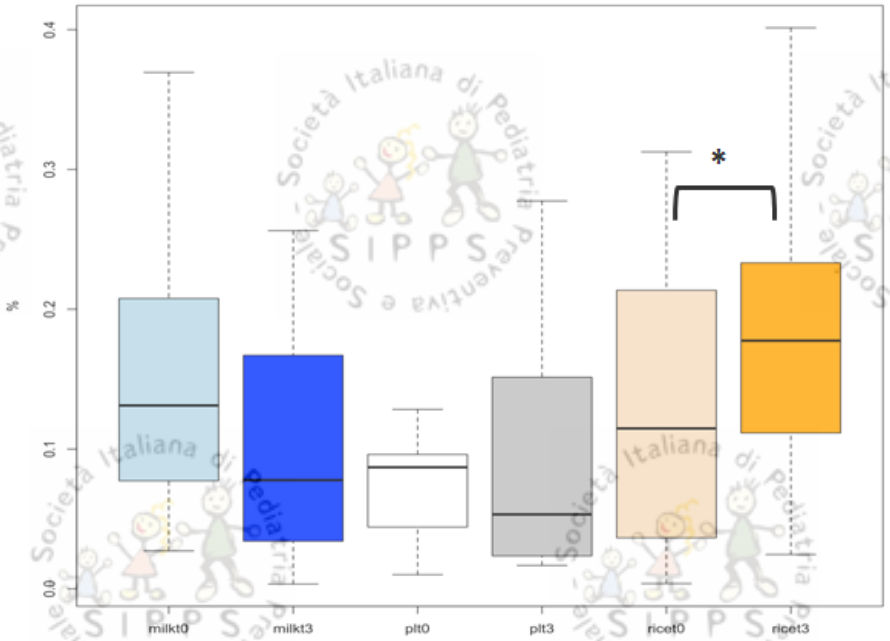




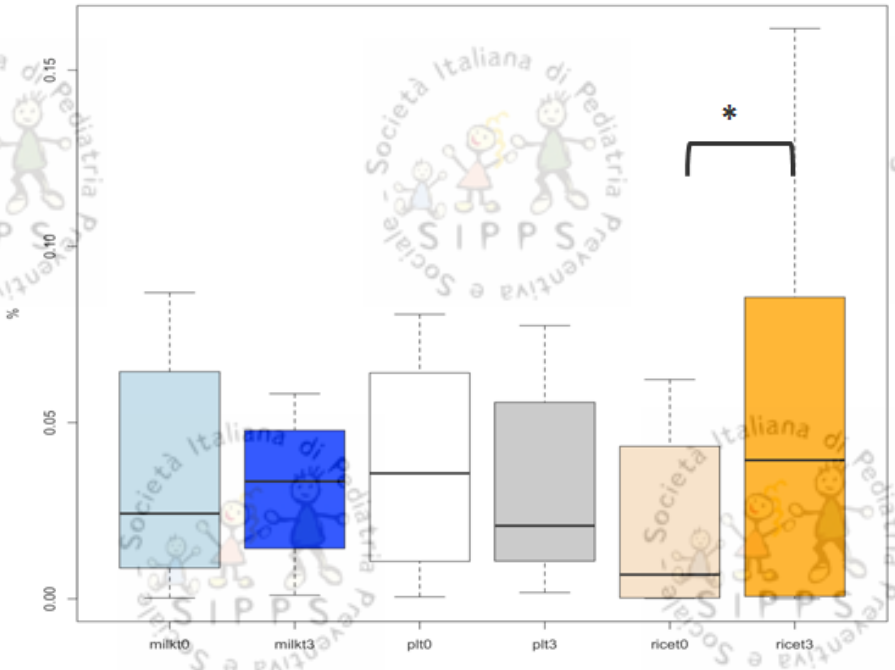
- *Lachnospiraceae* increased after 3-months treatment with fermented rice
- *Ruminococcaceae* were boosted by fermented milk (P<0.05)

PCA

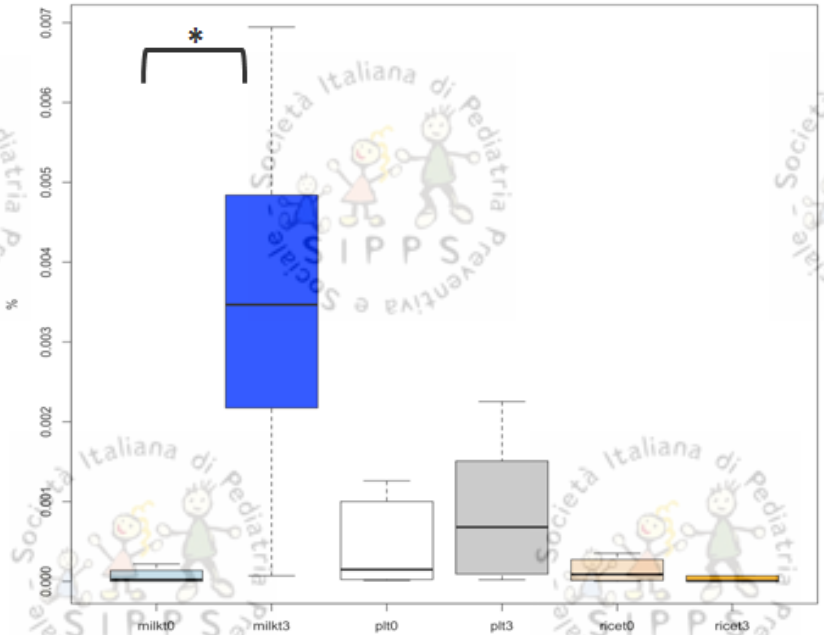




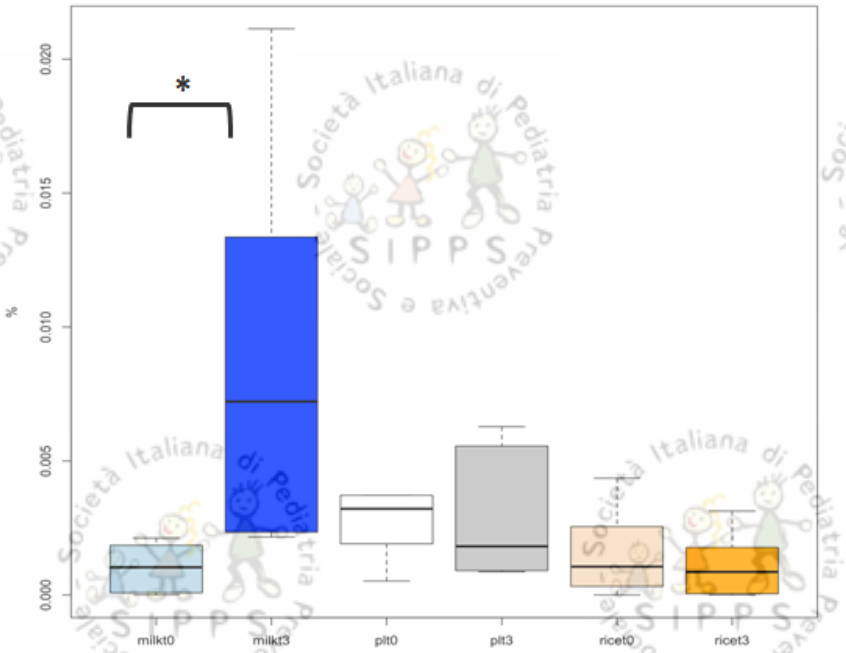
BLAUTIA



COPROCOCCUS

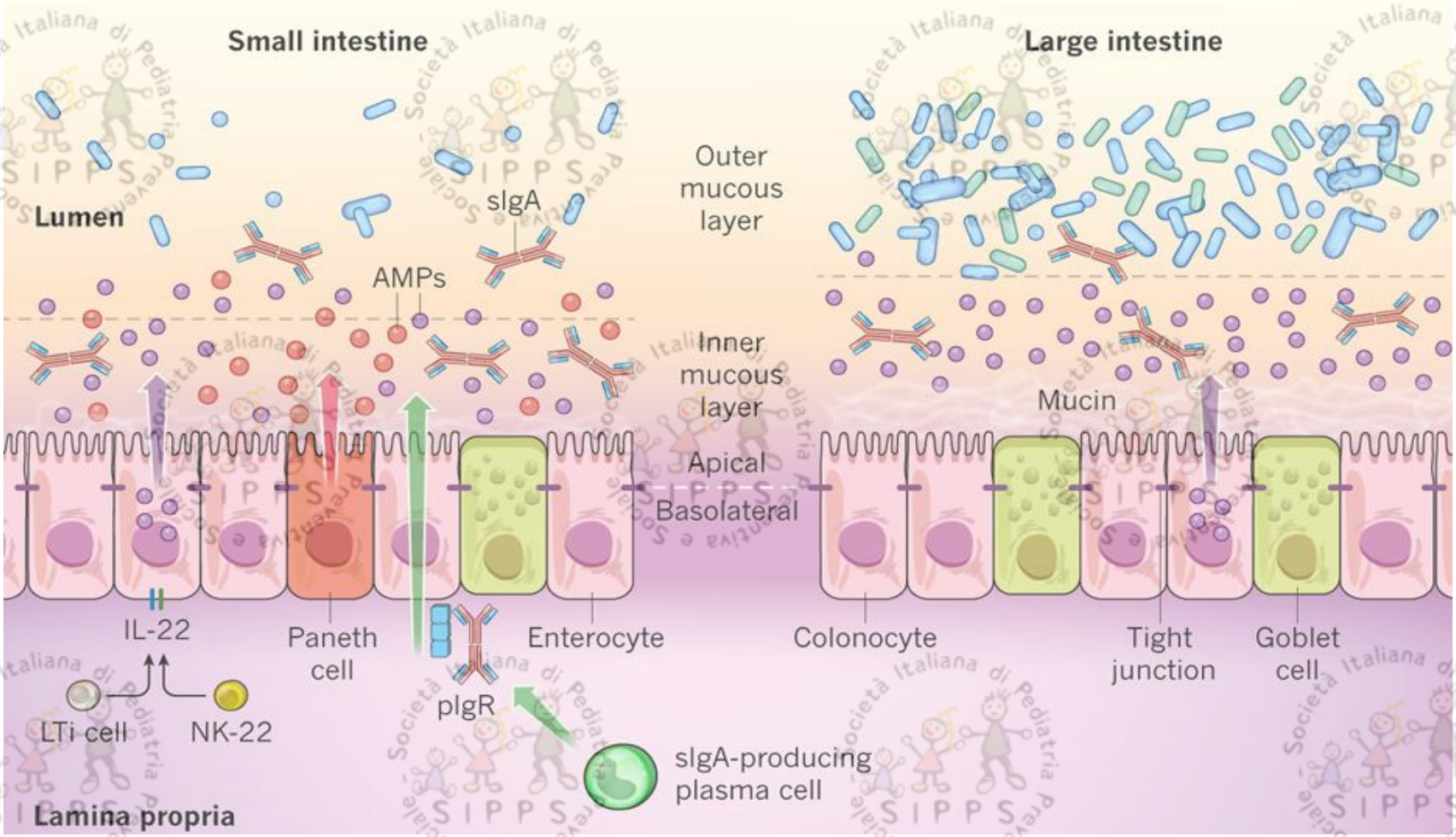


OSCILLOSPORA

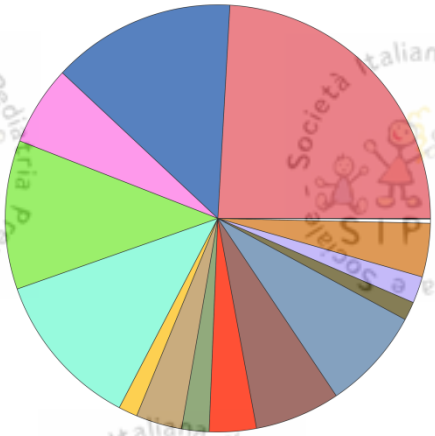


FAECALIBACTERIUM

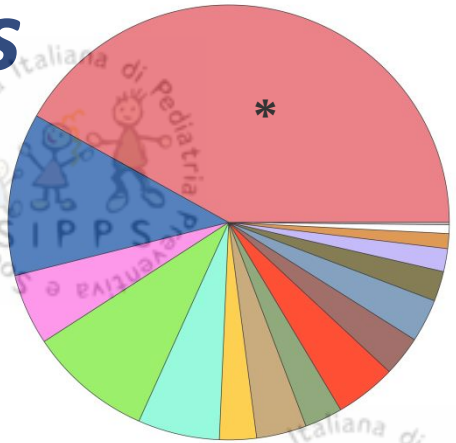

Gut microbiota
Gut microbiota products (SCFAs)
Nutrients


Small intestine
Large intestine
Lumen
sIgA
AMPs
Outer mucous layer
Inner mucous layer
Mucin
Apical
Basolateral
IL-22
LTi cell
NK-22
Paneth cell
Enterocyte
pIgR
sIgA-producing plasma cell
Colonocyte
Tight junction
Goblet cell
Lamina propria

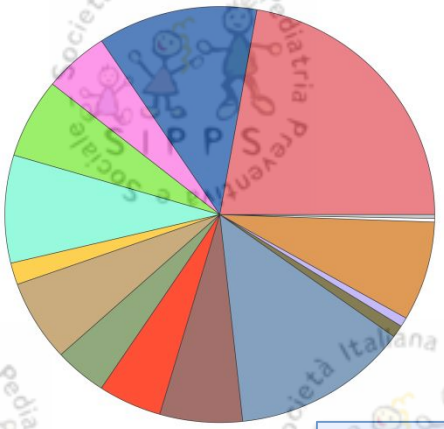
Roseburia Oligotypes



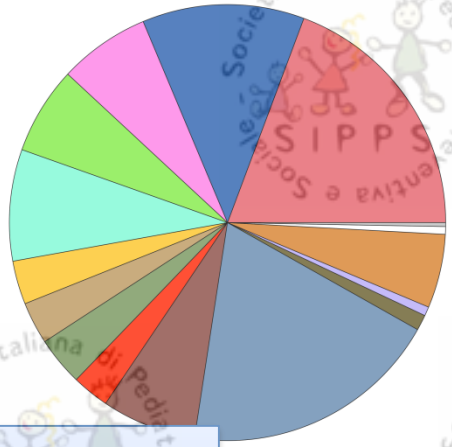
Milk t0



Milk t3



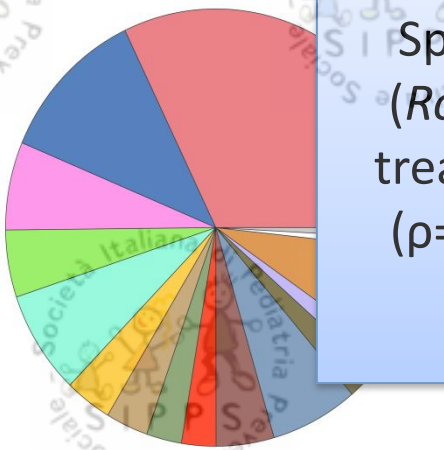
Placebo t0



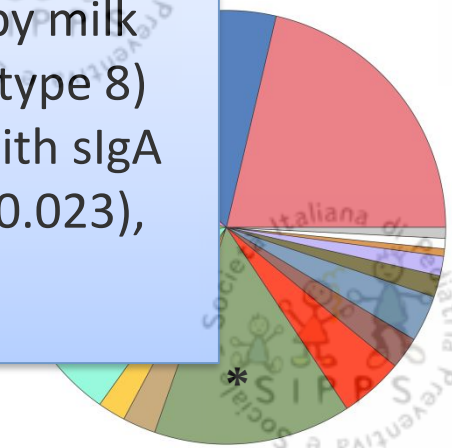
Placebo t3

Ros1 Ros2 Ros3 Ros4 Ros5 Ros6 Ros7 Ros8 Ros9 Ros10 Ros11 Ros12 Ros13 Ros14 Ros15 Ros16

Specific *Roseburia* oligotypes were promoted by milk (*Roseburia* oligotype 1) or rice (*Roseburia* oligotype 8) treatments and showed positive correlations with sIgA (ρ=0.63, P=0.038) or beta-defensin (ρ=0.87, P=0.023), respectively.

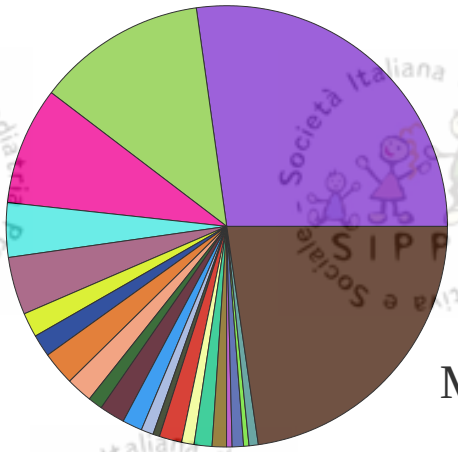


Rice t0

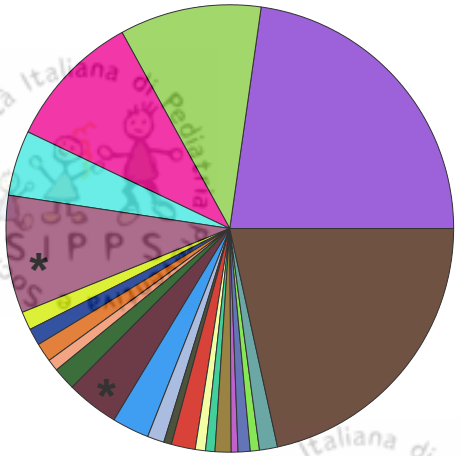


Rice t3

Blautia Oligotypes



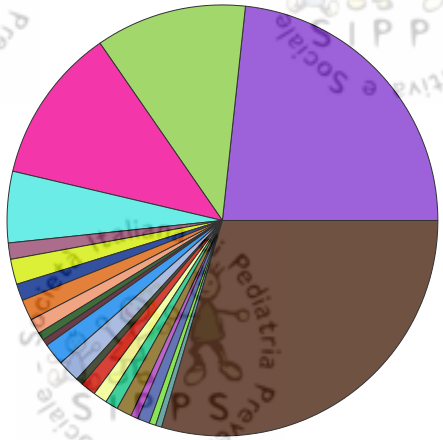
Milk t0



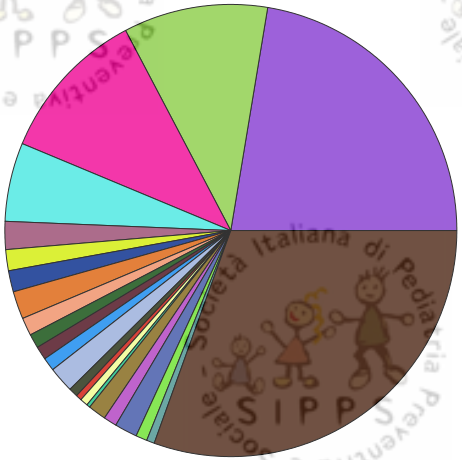
Milk t3

Bla1 Bla9 Bla27

Blautia oligotype pattern was affected only by milk treatment, that promoted an increase in oligotype 5 and 13, that were also positively correlated with alpha-defensin (ρ=0.84, P=0.007; ρ=0.58, P=0.040)

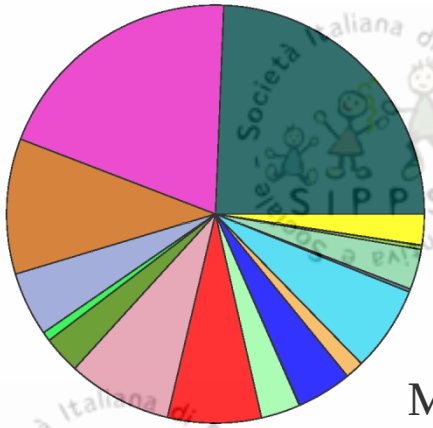


Rice t0

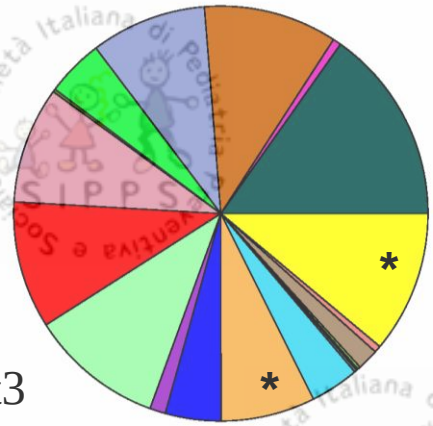


Rice t3

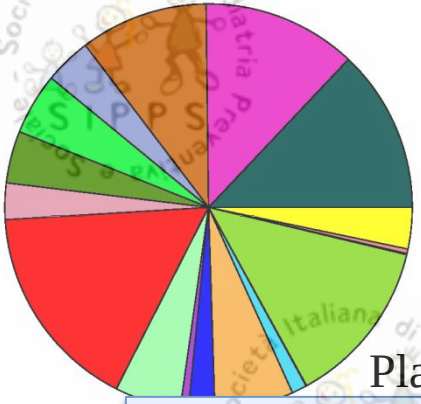
Bacteroides oligotypes



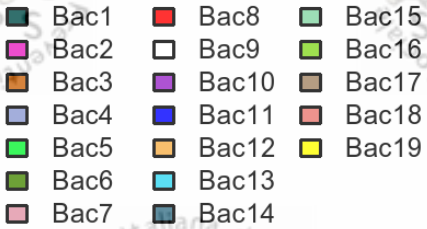
Milk t0

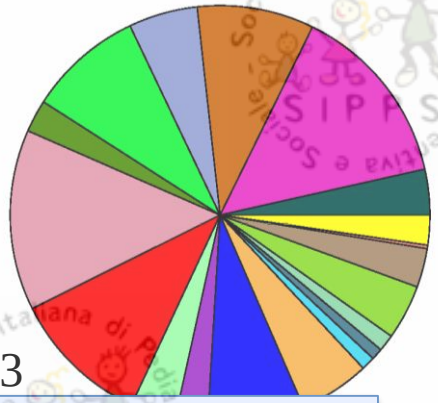


Milk t3

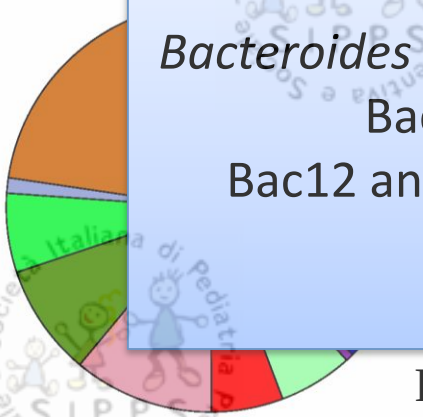


Placebo t0

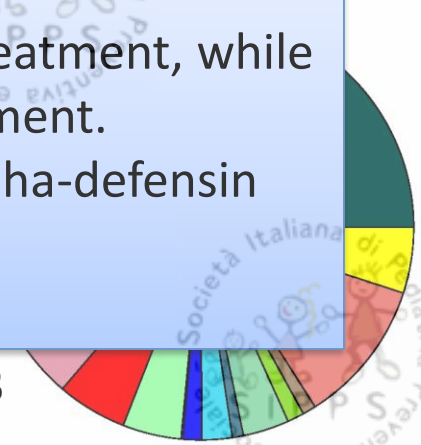




Placebo t3

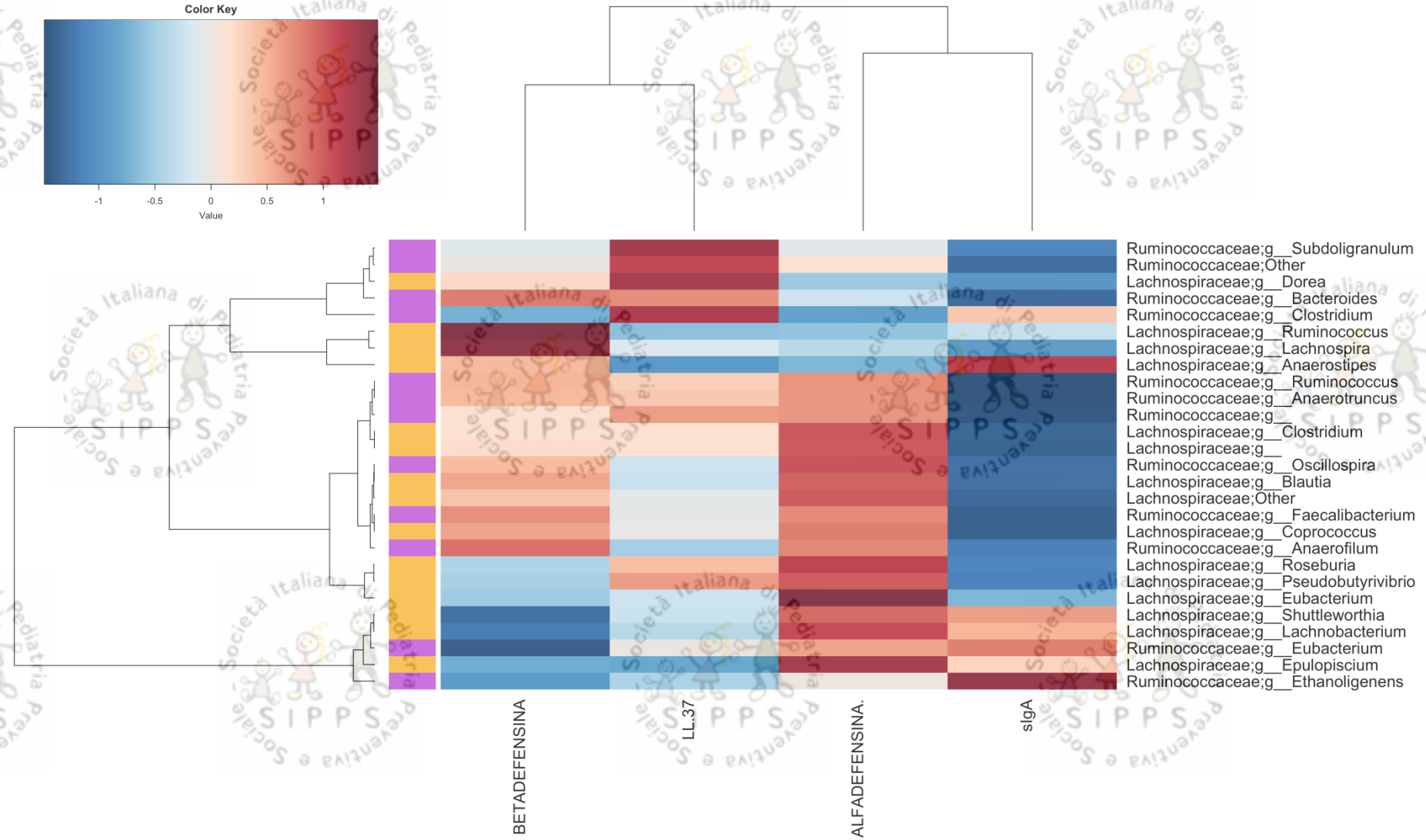


Rice t0



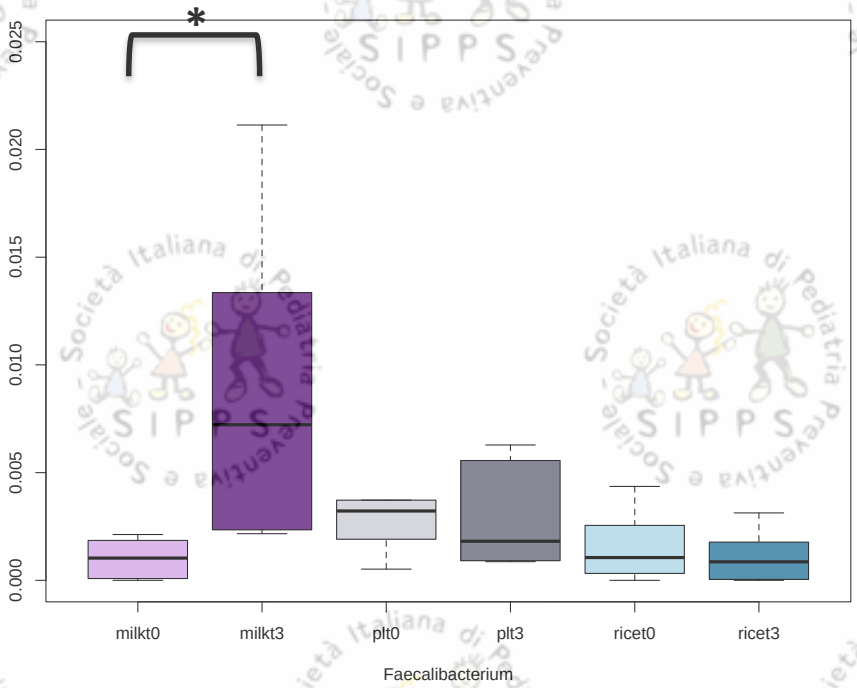
Rice t3

Bacteroides oligotypes 12 and 19 increased after milk treatment, while Bac18 was apparently stimulated by rice treatment.
Bac12 and 19 were also positively correlated with alpha-defensin levels, while Bac18 correlated with LL37



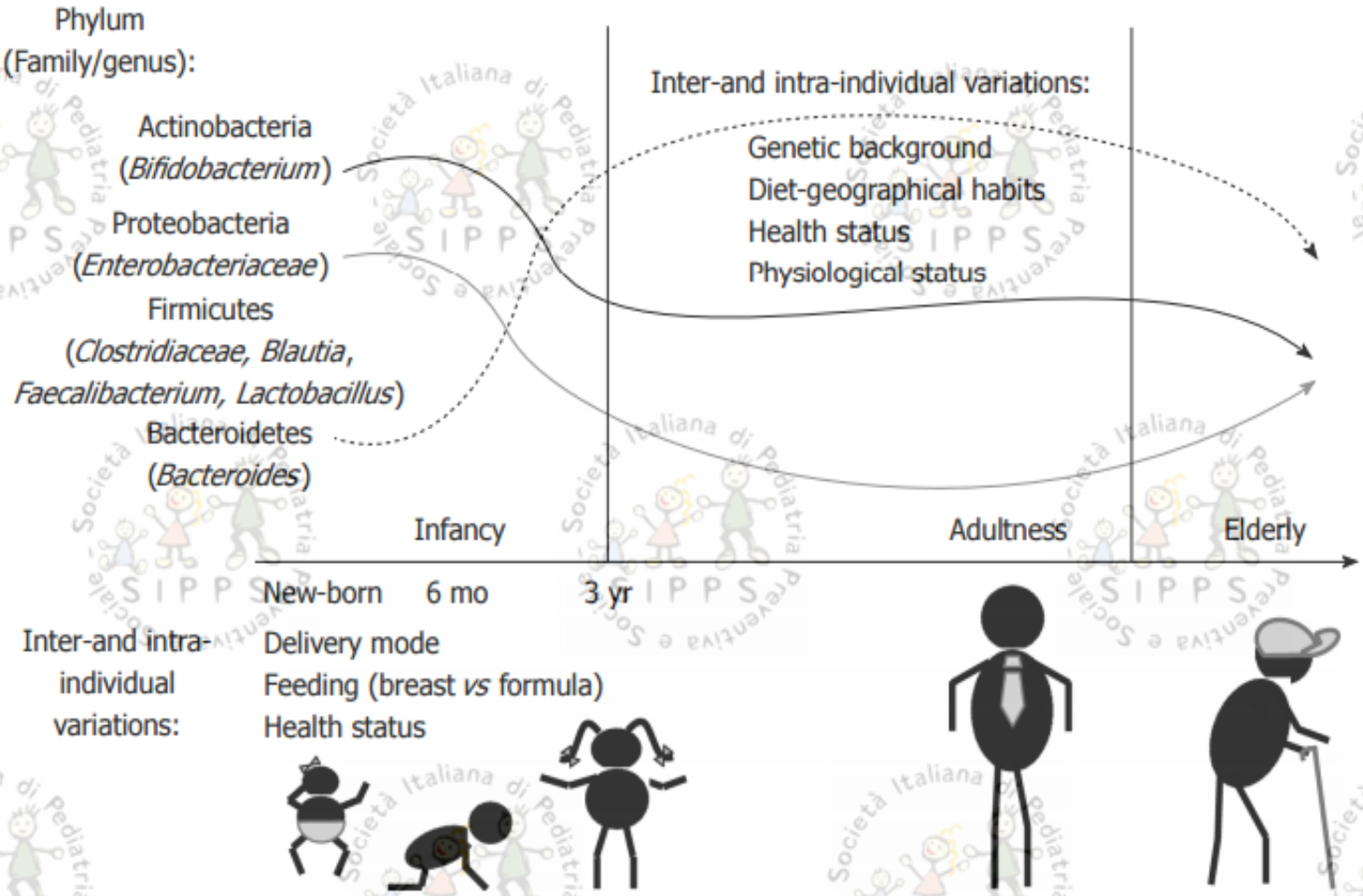
- Positive correlations between *Lachnospiraceae* and alpha-defensin
- *Ruminococcaceae* correlated to LL-37
- *Lachnospira* and *Ruminococcus* correlated with beta-defensin levels

Ruminococcaceae family i**ncreased by fermented milk matrix**



*(P<0.05)

Development of gut microbiota

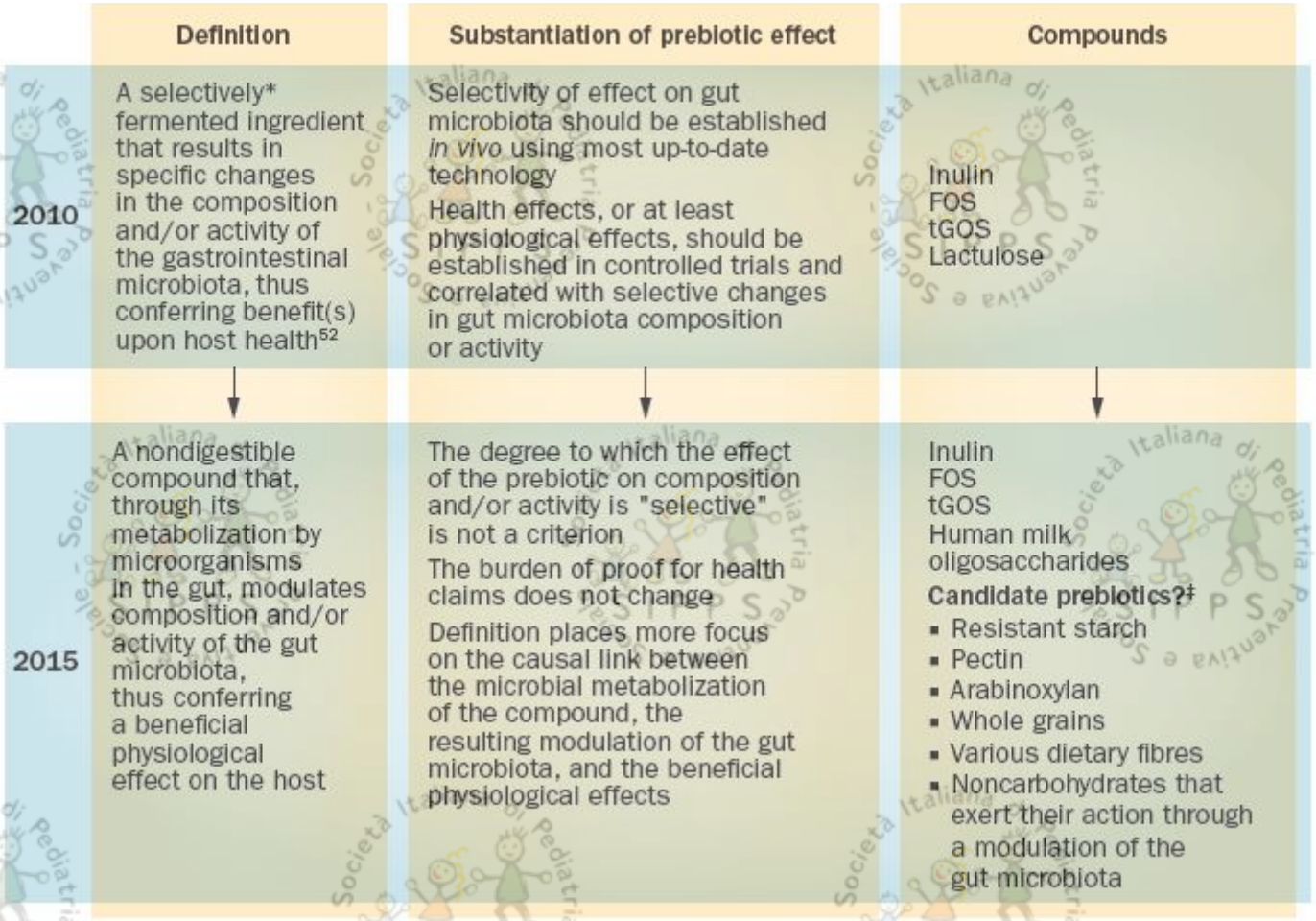


Tojo R et al. World J Gastroenterol. 2

OPINION

Towards a more comprehensive concept for prebiotics

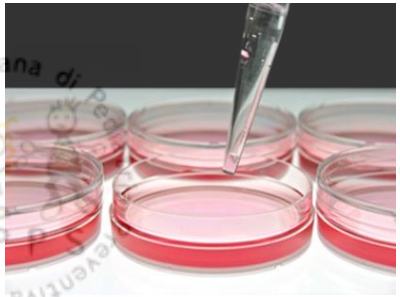
Laure B. Bindels, Nathalie M. Delzenne, Patrice D. Cani and Jens Walter

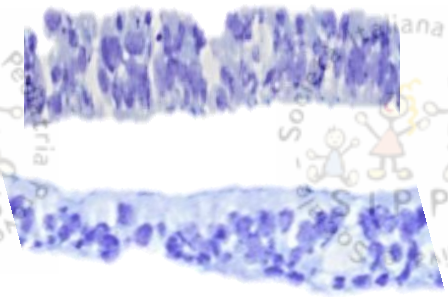


	Definition	Substantiation of prebiotic effect	Compounds
2010	A selectively* fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health[52]	Selectivity of effect on gut microbiota should be established *in vivo* using most up-to-date technology Health effects, or at least physiological effects, should be established in controlled trials and correlated with selective changes in gut microbiota composition or activity	Inulin FOS tGOS Lactulose
2015	A nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host	The degree to which the effect of the prebiotic on composition and/or activity is "selective" is not a criterion The burden of proof for health claims does not change Definition places more focus on the causal link between the microbial metabolization of the compound, the resulting modulation of the gut microbiota, and the beneficial physiological effects	Inulin FOS tGOS Human milk oligosaccharides **Candidate prebiotics?‡** ▪ Resistant starch ▪ Pectin ▪ Arabinoxylan ▪ Whole grains ▪ Various dietary fibres ▪ Noncarbohydrates that exert their action through a modulation of the gut microbiota

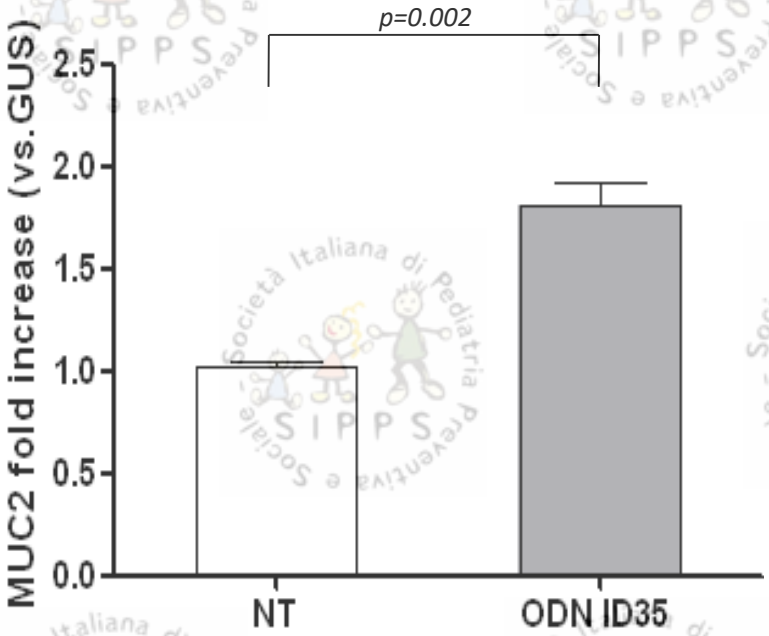
Nat. Rev. Gastroenterol. Hepatol. **12**, 303–310 (2015)

LGG ODN ID35 stimulates mucus production in human enterocytes: non-immune protective mechanism





Dose	Tickness (µm)
CTRL	-
1 µM	1±2



Paparo L et al. ESPGHAN Meeting 201

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

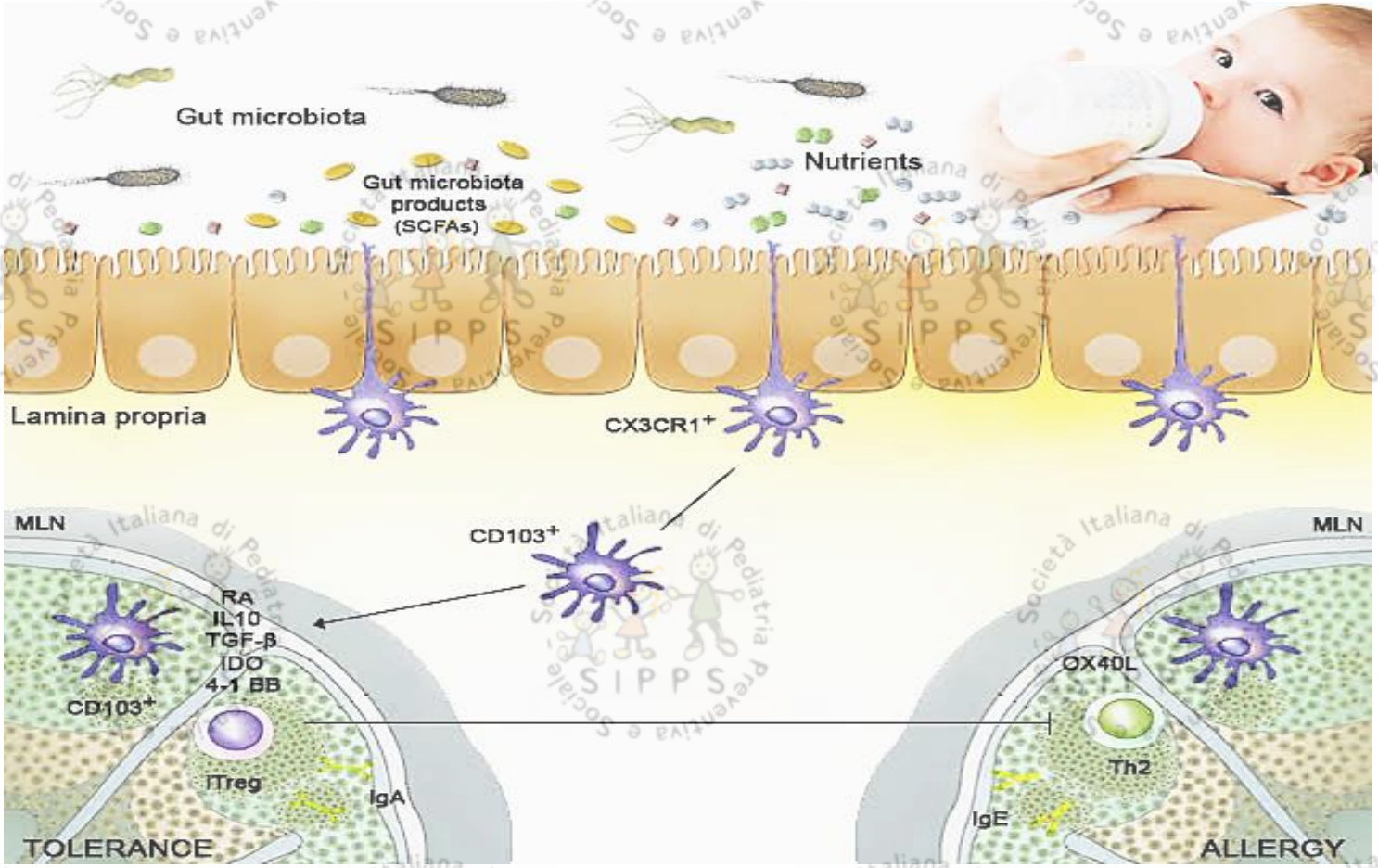
Widespread

Among studied probiotics

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

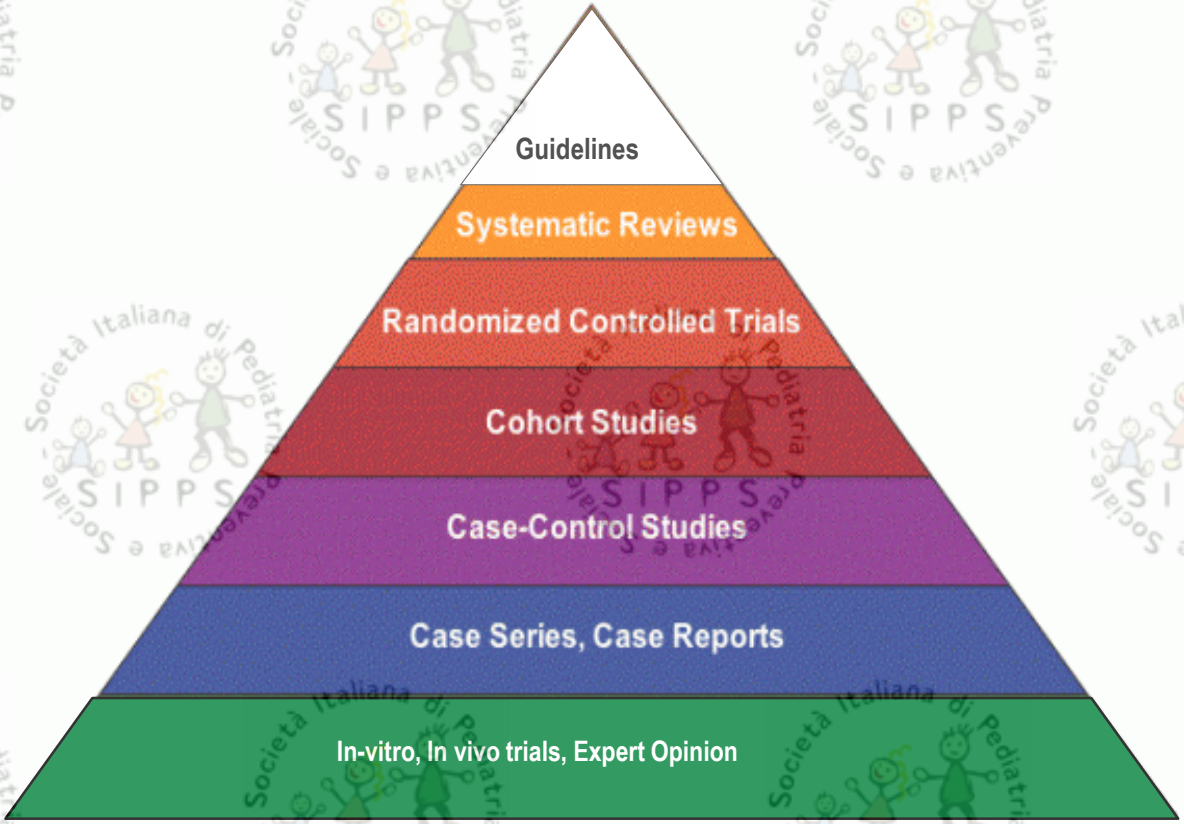
NATURE REVIEWS | GASTROENTEROLOGY & HEPATOLOGY 2014;11:506–14

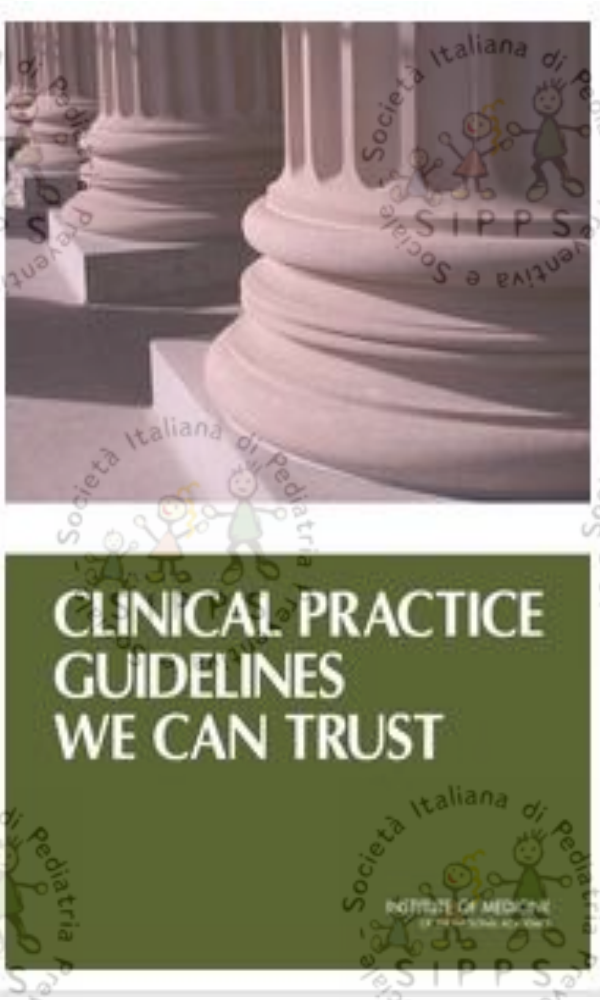
Tolleranza orale: soppressione risposta immunitaria ad Ag della dieta mediata da cellule T regolatorie Ag-specifiche



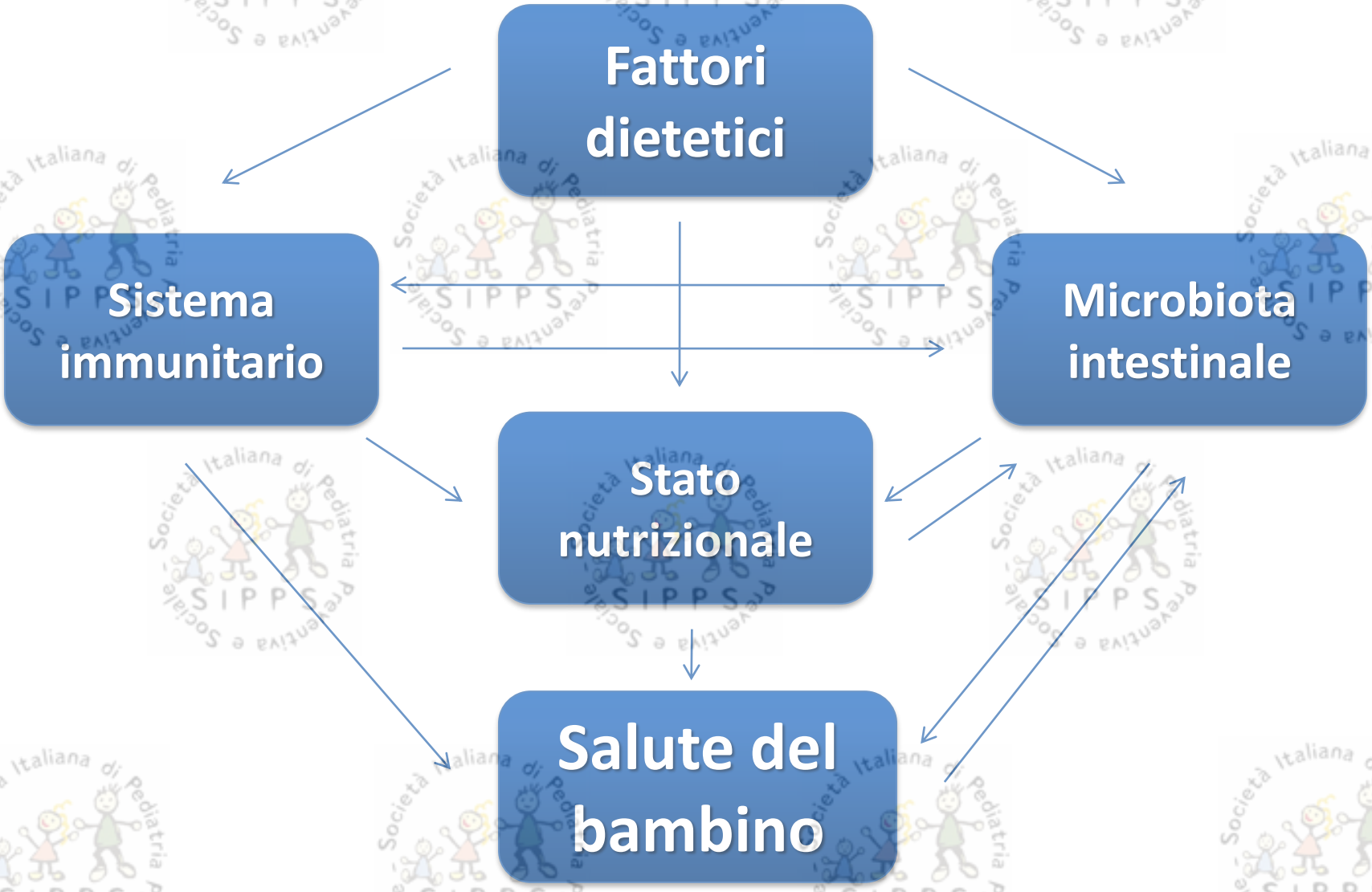
Modulazione dinamica indotta sin dalle prime epoche della vita dalla esposizione a fattori ambientali (dieta) e dallo sviluppo del microbiota intestinale

Di Costanzo M and Berni Canani R JPGN 2016


Guidelines
Systematic Reviews
Randomized Controlled Trials
Cohort Studies
Case-Control Studies
Case Series, Case Reports
In-vitro, In vivo trials, Expert Opinion


CLINICAL PRACTICE
GUIDELINES
WE CAN TRUST

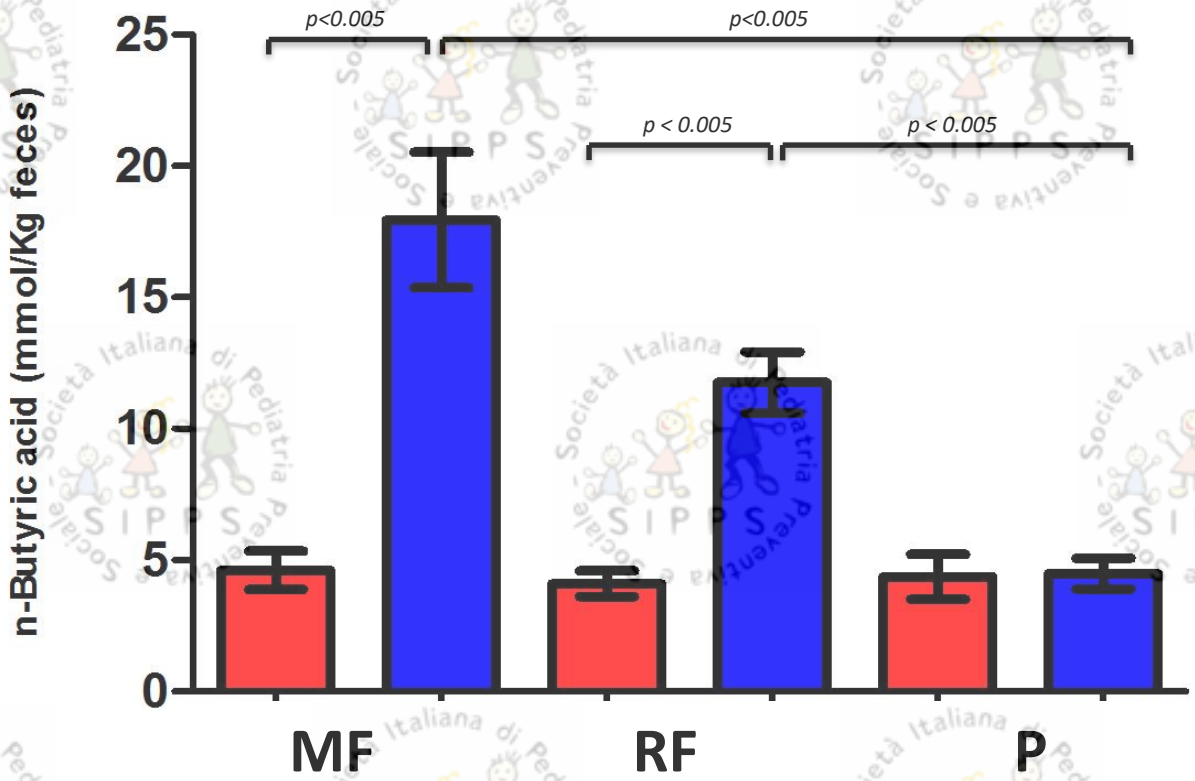
Immunonutrizione



Indications to probiotics in pediatric gastroenterology

- **Acute gastroenteritis**
- **Nosocomial diarrhea**
- **Antibiotic-associated diarrhea**
- ***C.difficile* infection**
- *H.pylori* infection
- FGIDs
- IBD
- NEC

Stimolazione della produzione di butirrato a livello intestinale



Berni Canani R, et al. submitted

Conclusioni

- Scarse evidenze su utilizzo “clinico” di prebiotici
- Uso raccomandato di particolari ceppi di probiotici a determinate dosi per alcune condizioni patologiche (GEA, AAD, CDD)
- Per altre indicazioni utilizzare ceppi, dosi e modalità di somministrazione riportate nei RCTs
- Autoprescrizione
- Ben tollerati e sicuri
- Controllo di qualità/efficacia
- Approccio “post-biotico” promettente

OPEN ACCESS Freely available online

PLOS ONE

Lactobacillus paracasei CBA L74 Metabolic Products and Fermented Milk for Infant Formula Have Anti-Inflammatory Activity on Dendritic Cells *In Vitro* and Protective Effects against Colitis and an Enteric Pathogen *In Vivo*

Elena Zagato[1], Erika Mileti[1], Lucia Massimiliano[1], Francesca Fasano[2], Andrea Budelli[2], Giuseppe Penna[1]*, Maria Rescigno[1]*

1 Department of Experimental Oncology, European Institute of Oncology, Milan, Italy, 2 R&D, Heinz Italia S.p.A., Latina, Italy

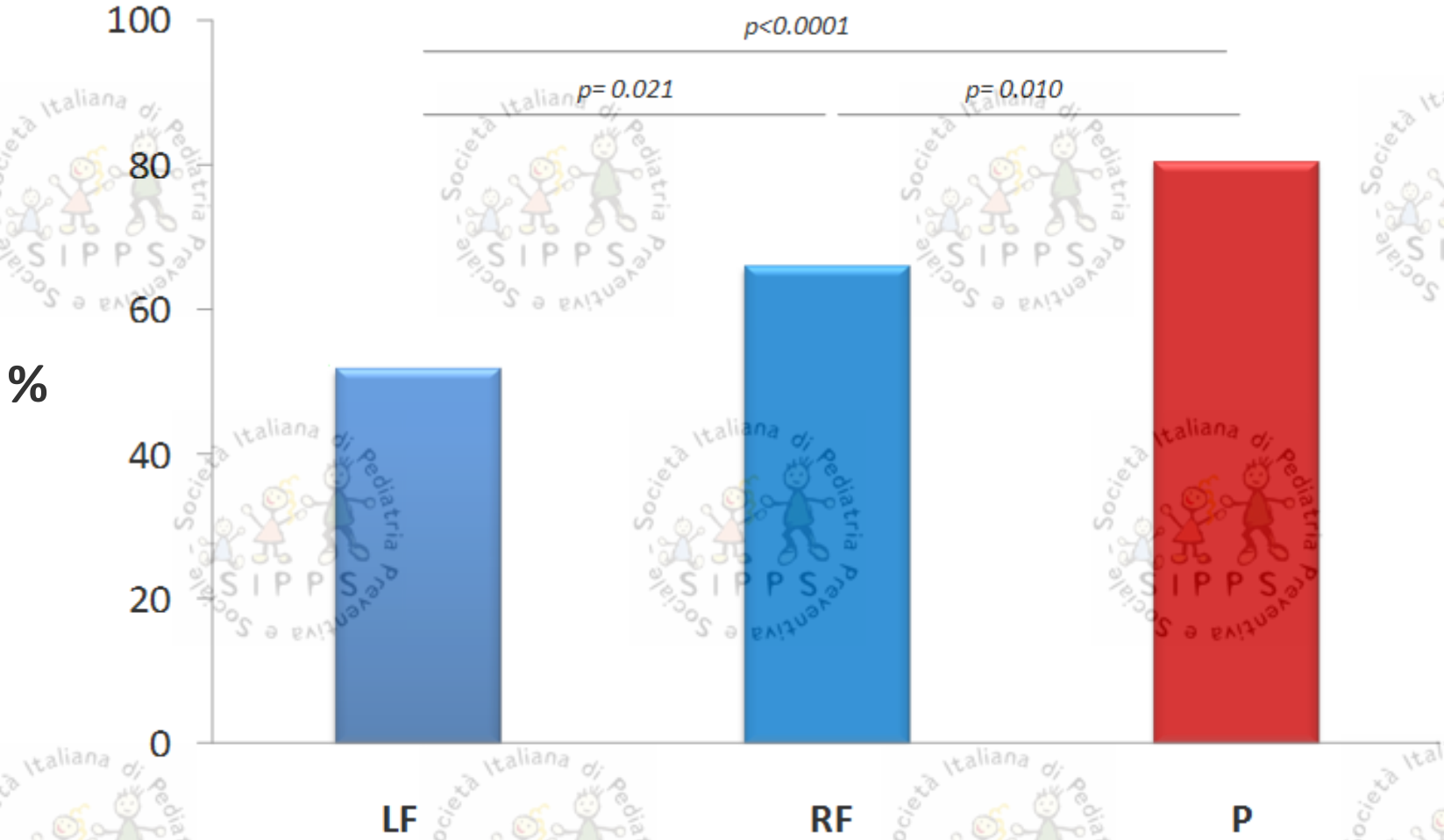
Abstract

The rapid expansion of commercially available fermented food products raises important safety issues particularly when infant food is concerned. In many cases, the activity of the microorganisms used for fermentation as well as what will be the immunological outcome of fermented food intake is not known. In this manuscript we used complex *in vitro*, *ex vivo* and *in vivo* systems to study the immunomodulatory properties of probiotic-fermented products (culture supernatant and fermented milk without live bacteria to be used in infant formula). We found *in vitro* and *ex vivo* that fermented products of *Lactobacillus paracasei* CBA L74 act via the inhibition of proinflammatory cytokine release leaving anti-inflammatory cytokines either unaffected or even increased in response to *Salmonella* typhimurium. These activities are not dependent on the inactivated bacteria but to metabolic products released during the fermentation process. We also show that our *in vitro* systems are predictive of an *in vivo* efficacy by the fermented products. Indeed CBA L74 fermented products (both culture medium and fermented milk) could protect against colitis and against an enteric pathogen infection (*Salmonella* typhimurium). Hence we found that fermented products can act via the inhibition of immune cell inflammation and can protect the host from pathobionts and enteric pathogens. These results open new perspectives in infant nutrition and suggest that *L. paracasei* CBA L74 fermented formula can provide immune benefits to formula-fed infants, without carrying live bacteria that may be potentially dangerous to an immature infant immune system.

Caratteristiche della popolazione di studio

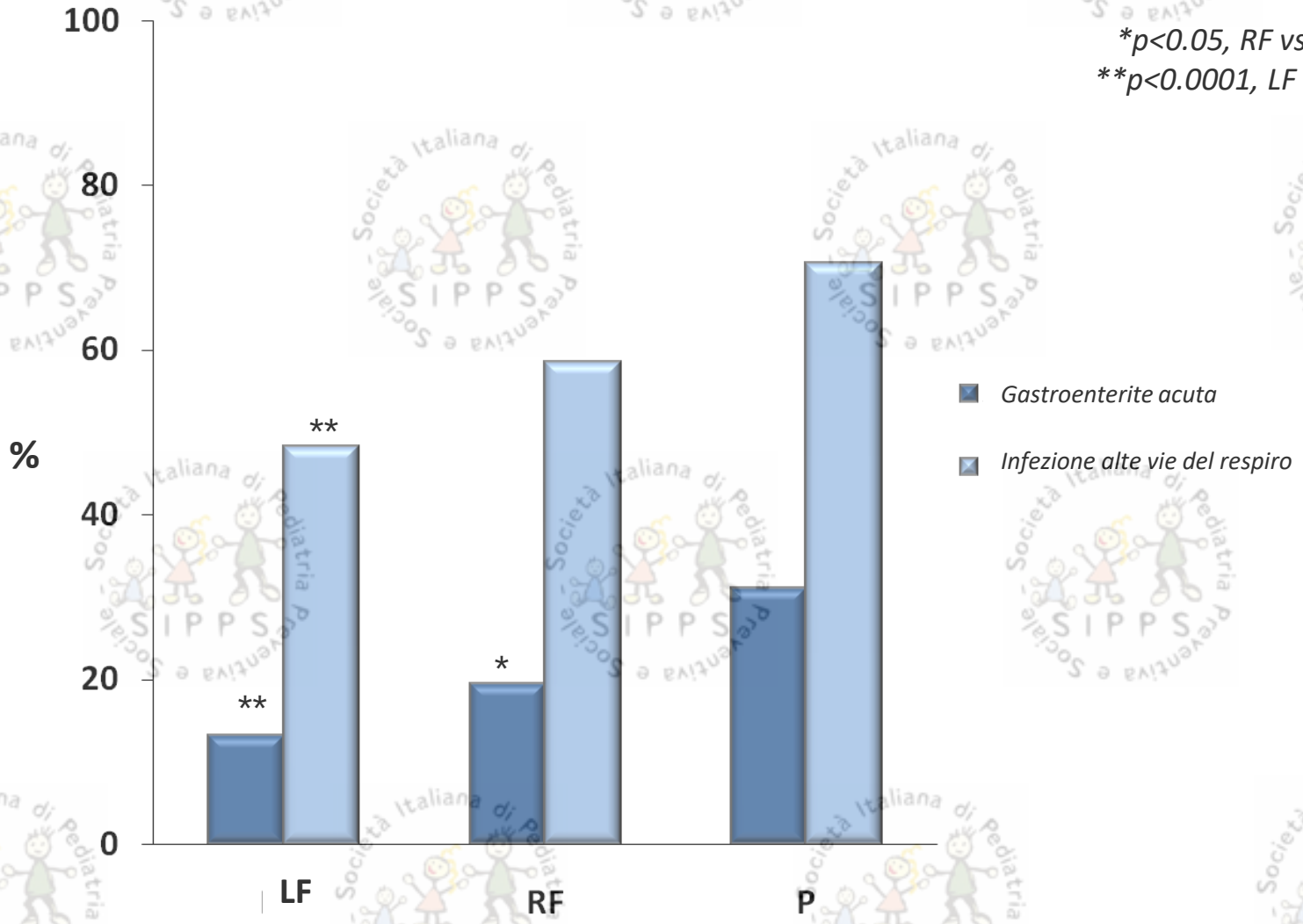
	LF	RF	P
	n=141	*n=123*	*n=127*
Maschi, n (%)	72 (51.1)	64 (52)	65 (51.2)
Età, mesi (±DS)	32 (10)	31 (11)	34 (9)
Peso, kg (±DS)	14.6 (2.8)	14.5 (2.7)	14.8 (2.9)
Altezza, cm (±DS)	93.3 (9)	92.7 (9)	94.3 (7.2)
Allattamento materno, n (%)	99 (70.2)	88 (71.5)	97 (76.4)
Durata dell'allattamento, mesi (±DS)	7.6 (6.1)	6.2 (4)	6.4 (5.1)
Età alla scolarizzazione, mesi (±DS)	13.4 (2.4)	12.9 (2.2)	12.8 (2.3)
Fratelli, n (%)	108 (76.6)	96 (78)	100 (78.7)
N. di fratelli, (±DS)	1.30 (0.6)	1.4 (0.6)	1.4 (0.6)
Fumo passivo, n (%)	65 (46.1)	56 (45.5)	59 (46.5)

Soggetti che hanno presentato ≥ 1 episodio infettivo



Nocerino R, et al. Clin Nutr 2015

Soggetti che hanno presentato ≥1 episodio di gastroenterite acuta o infezione alte vie del respiro



*p<0.05, RF vs P
**p<0.0001, LF vs P

Nocerino R, et al. Clin Nutr 2015

I bambini che hanno assunto latte o riso fermentato hanno presentato meno episodi infettivi

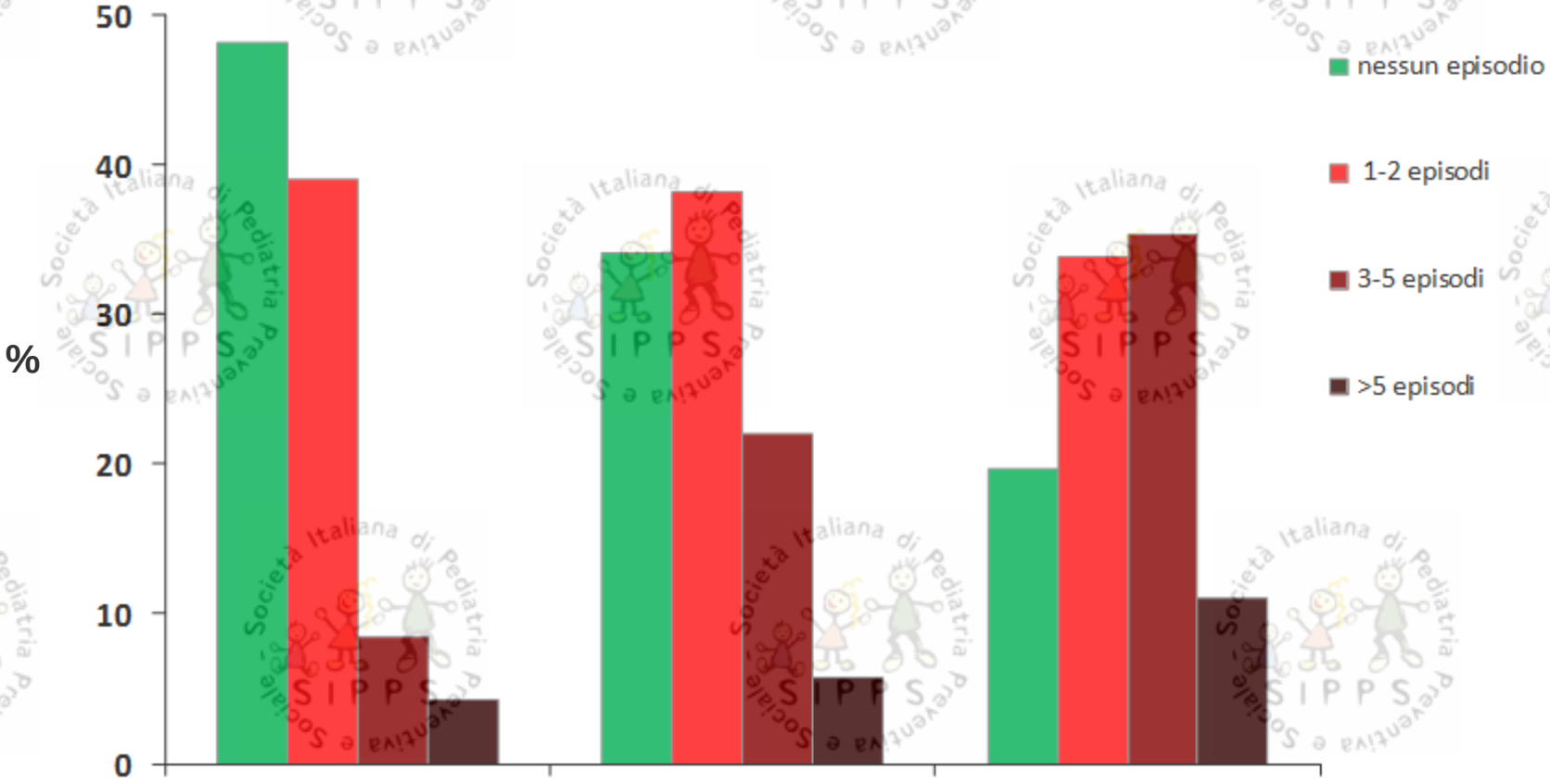
Numero totale di infezioni
Riduzione del 60% nei bambini che assumevano latte fermentato e del 40% nei bambini che assumevano riso fermentato

Numero di episodi di diarrea acuta
Riduzione del 55% nei bambini che assumevano latte fermentato e del 45% nei bambini che assumevano riso fermentato

Numero di episodi di infezioni a carico delle alte vie del respiro
Riduzione del 60% nei bambini che assumevano latte fermentato e del 40% nei bambini che assumevano riso fermentato

Nocerino R, et al. Clin Nutr 2015

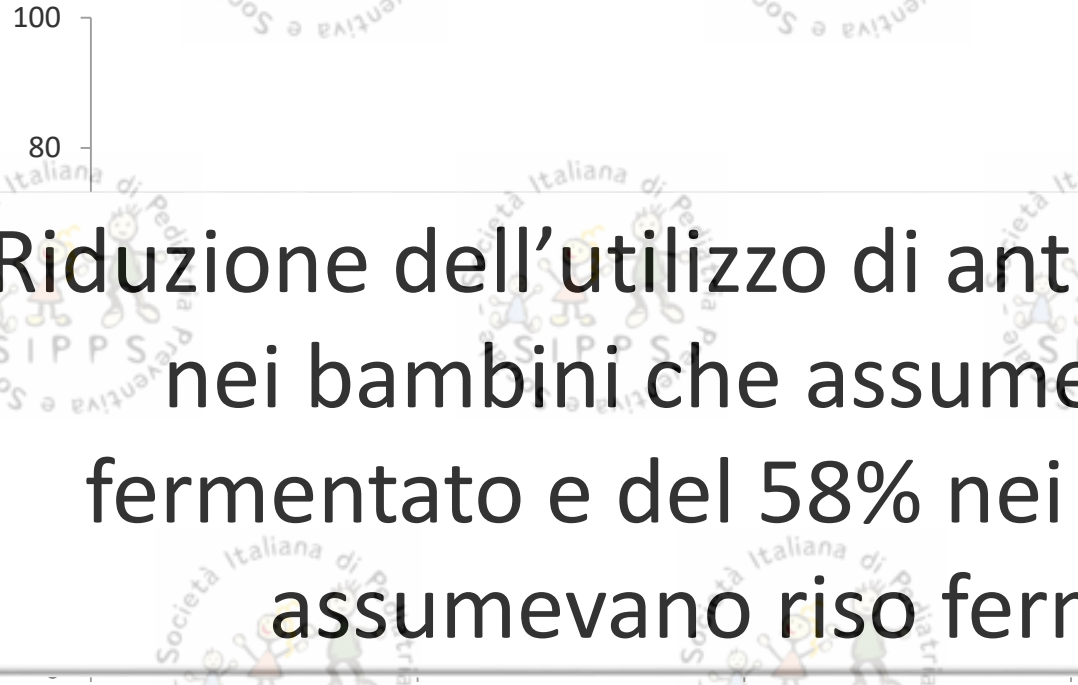
Soggetti che hanno presentato episodi infettivi ricorrenti



LF vs RF	LF vs P	RF vs P
p	*P*	*p*
0.010	*<0.0001*	*0.010*

Nocerino R, et al. Clin Nutr 2105

Utilizzo di farmaci



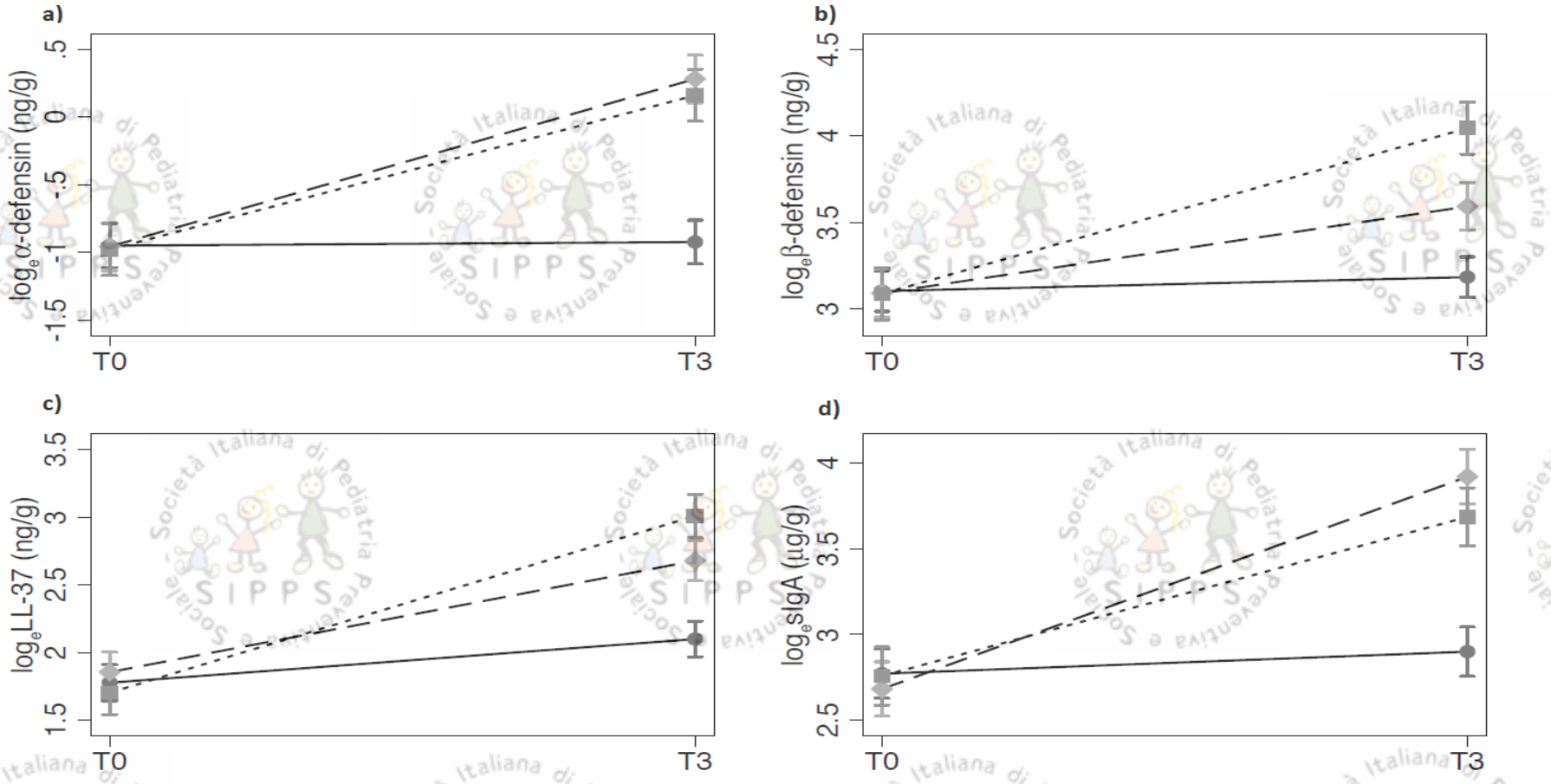
Riduzione dell'utilizzo di antibiotici del 75% nei bambini che assumevano latte fermentato e del 58% nei bambini che assumevano riso fermentato

	LF	RF	P
	LF vs RF *p*	LF vs P *p*	RF vs P *p*
Almeno 1 farmaco	*0.004**	*<0.0001**	*0.021*
Antibiotici	*0.003**	*<0.0001**	*0.001**
Antipiretici	*0.163*	*0.001**	*0.058*
Steroidi	*0.141*	*0.001**	*0.070*

**I valori di p restano significativi dopo la correzione di Bonferroni*

Nocerino R, et al. Clin Nutr 2015

Latte fermentato e riso fermentato con *L.paracasei* CBA L74 esercitano una significativa immunomodulazione

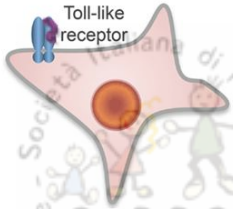


Nocerino R, et al. Clin Nutr 2015

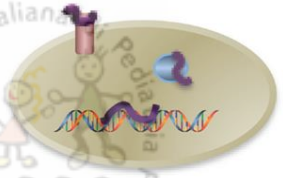
Peptidi dell'immunità innata

Inibizione legame patogeno cellule bersaglio

Attivazione sistema immunitario



Interazione diretta con espressione dei geni del patogeno

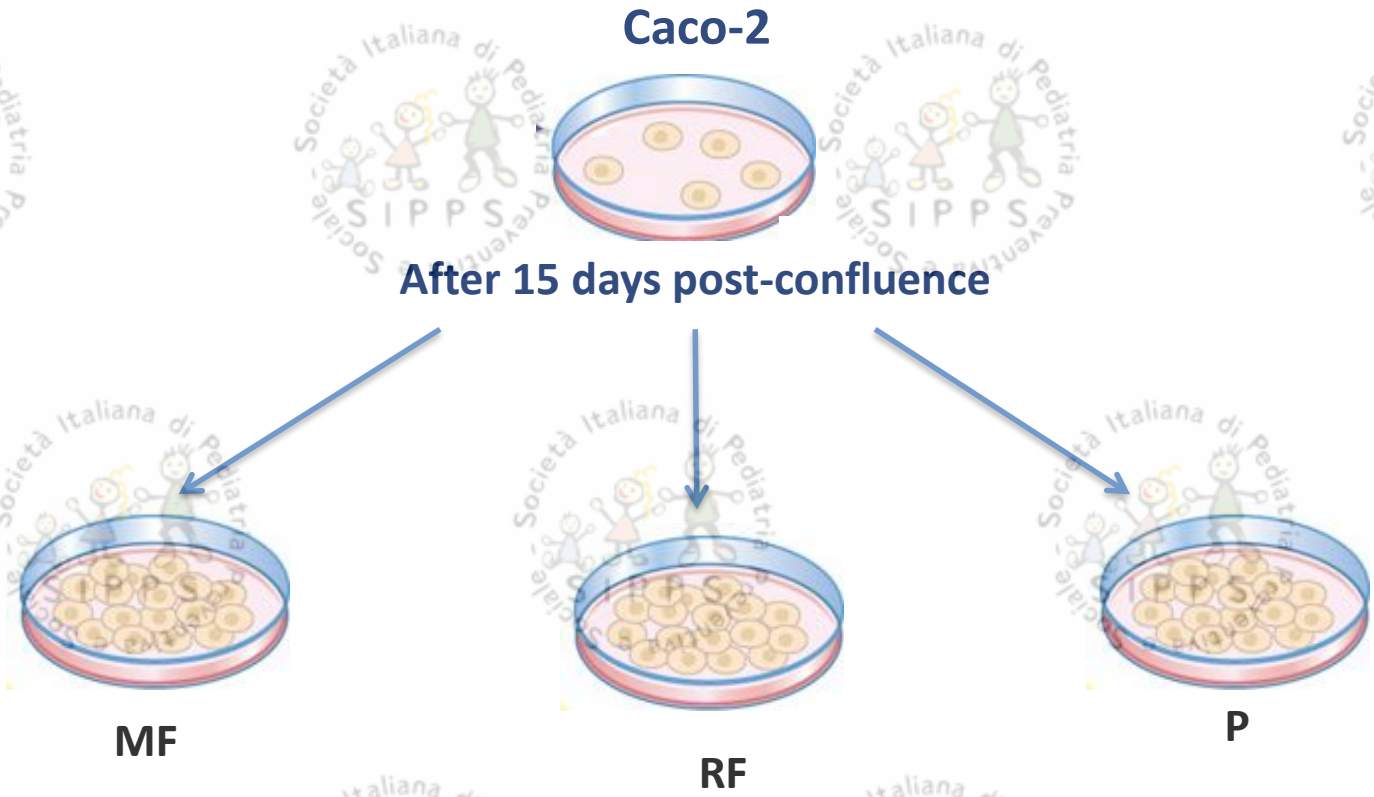


Alterazione membrana cellulare

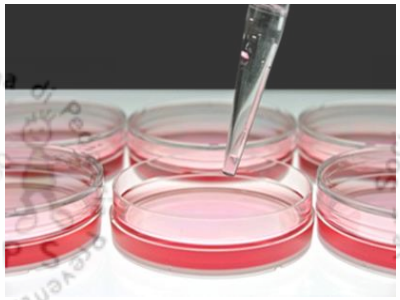
Modified from Duplantier AJ Front Immunol 2013

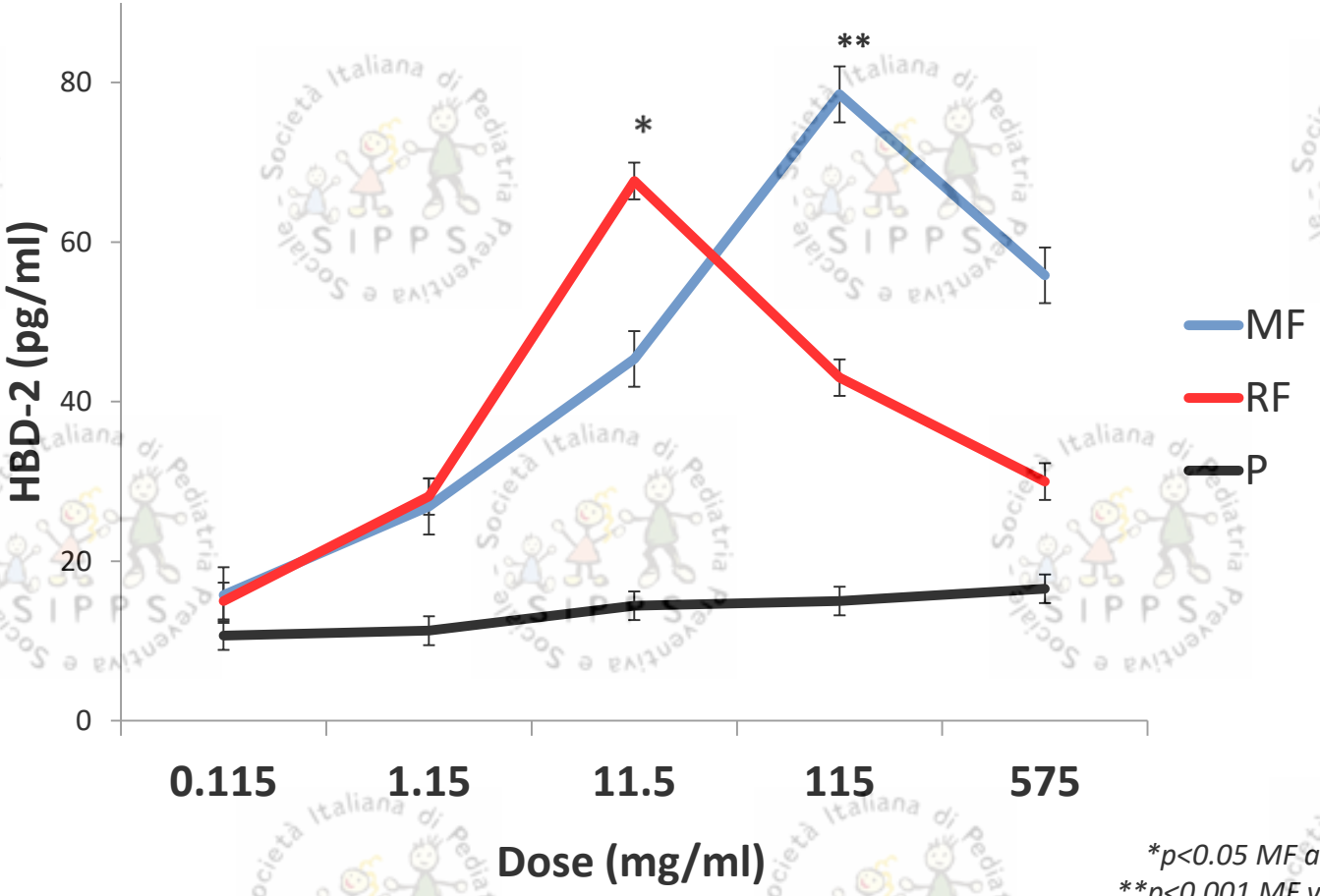
Mechanisms of Action

Exploring the direct interaction with human enterocytes



1. Protection against infection: innate immunity stimulation in human enterocytes

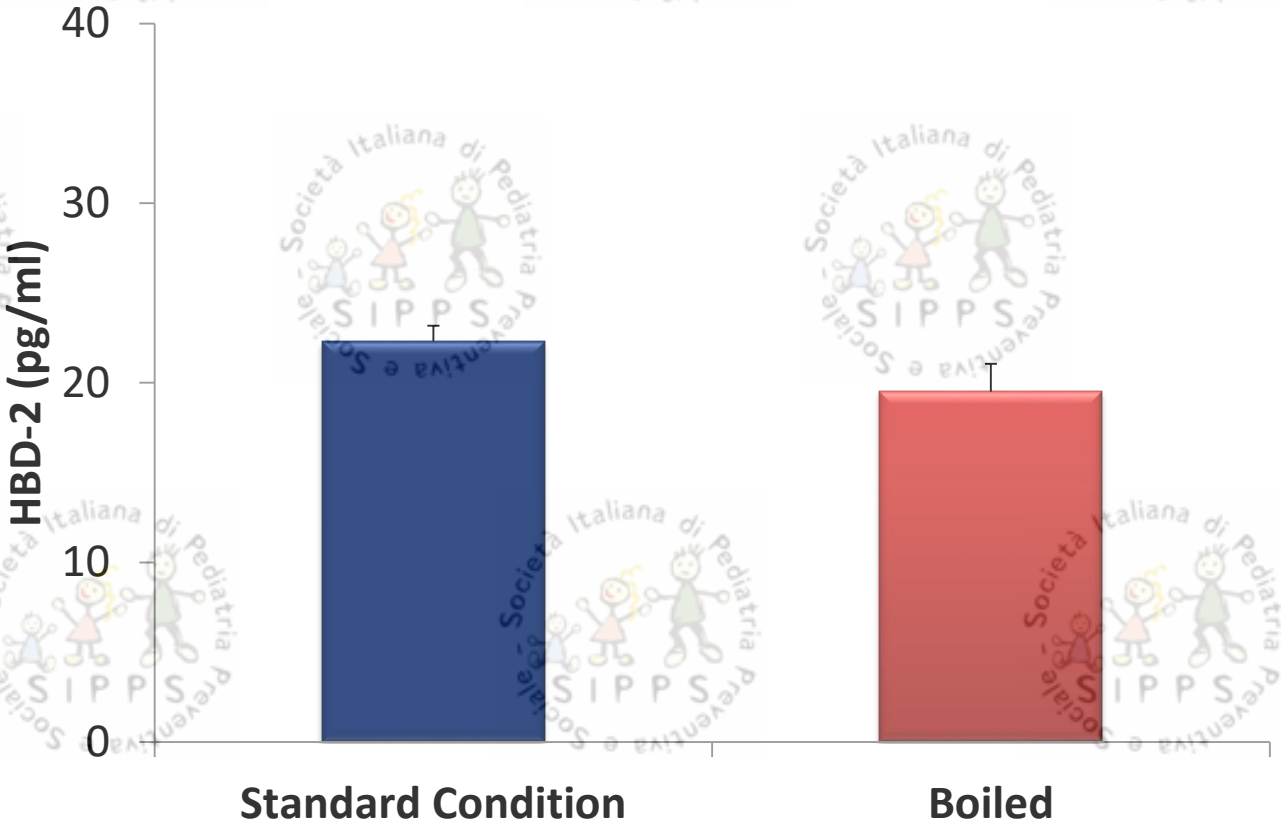




*p<0.05 MF and RF vs P
**p<0.001 MF vs P

Berni Canani R, data on file

The effect is heat-stable

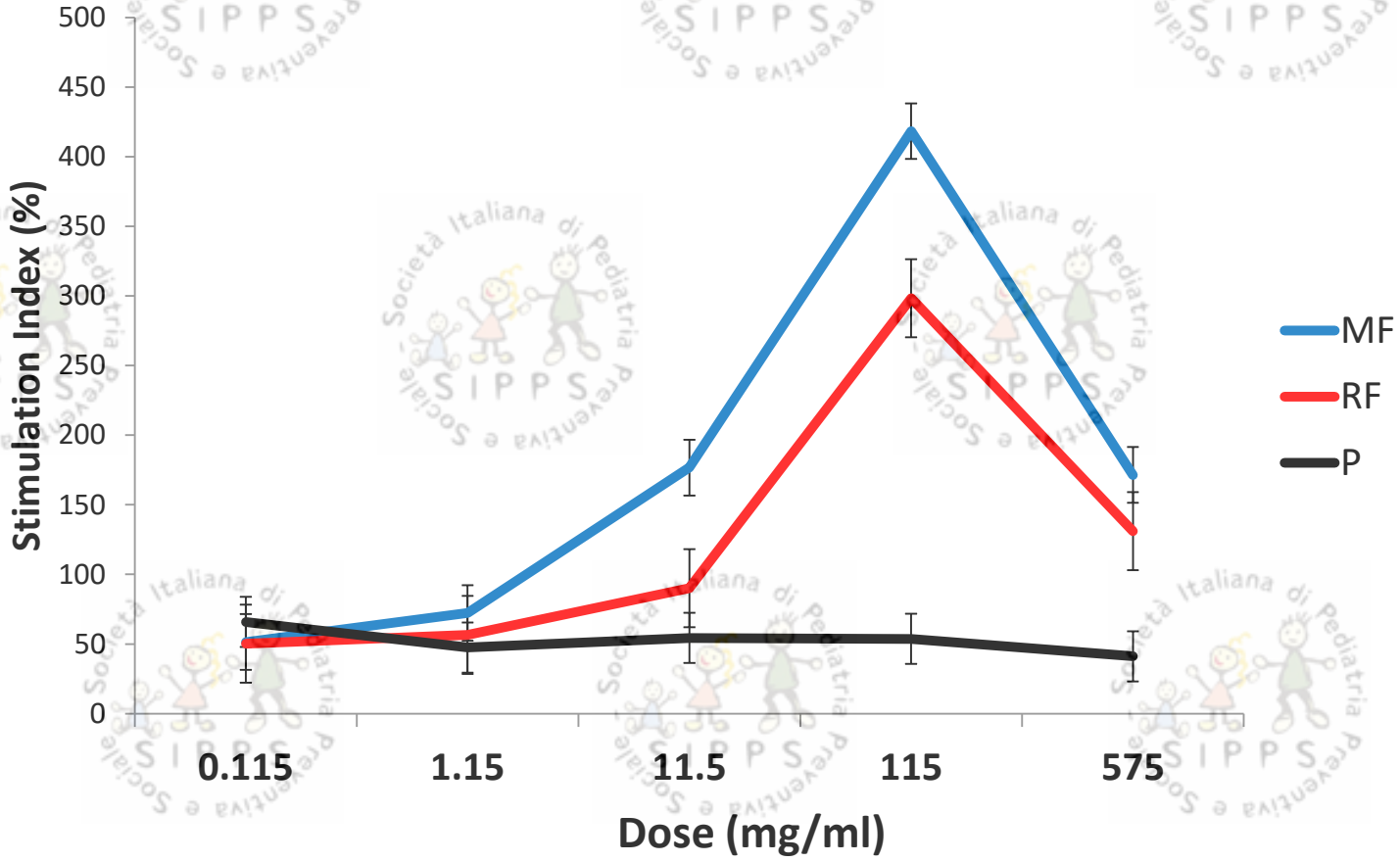


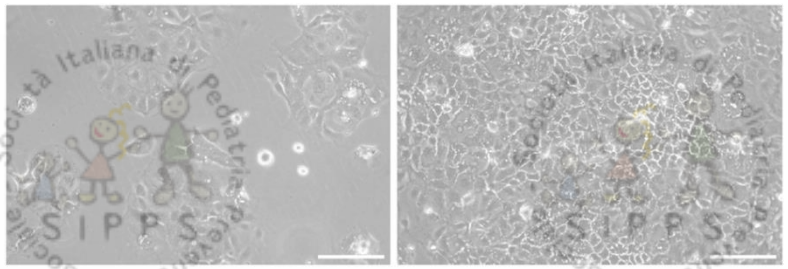
Fermented milk product (11.5 mg/ml) treated at 95°C for 10 min

Berni Canani R, data on file

2. Protection against infection:non-immune mechanisms

Effect on cell proliferation

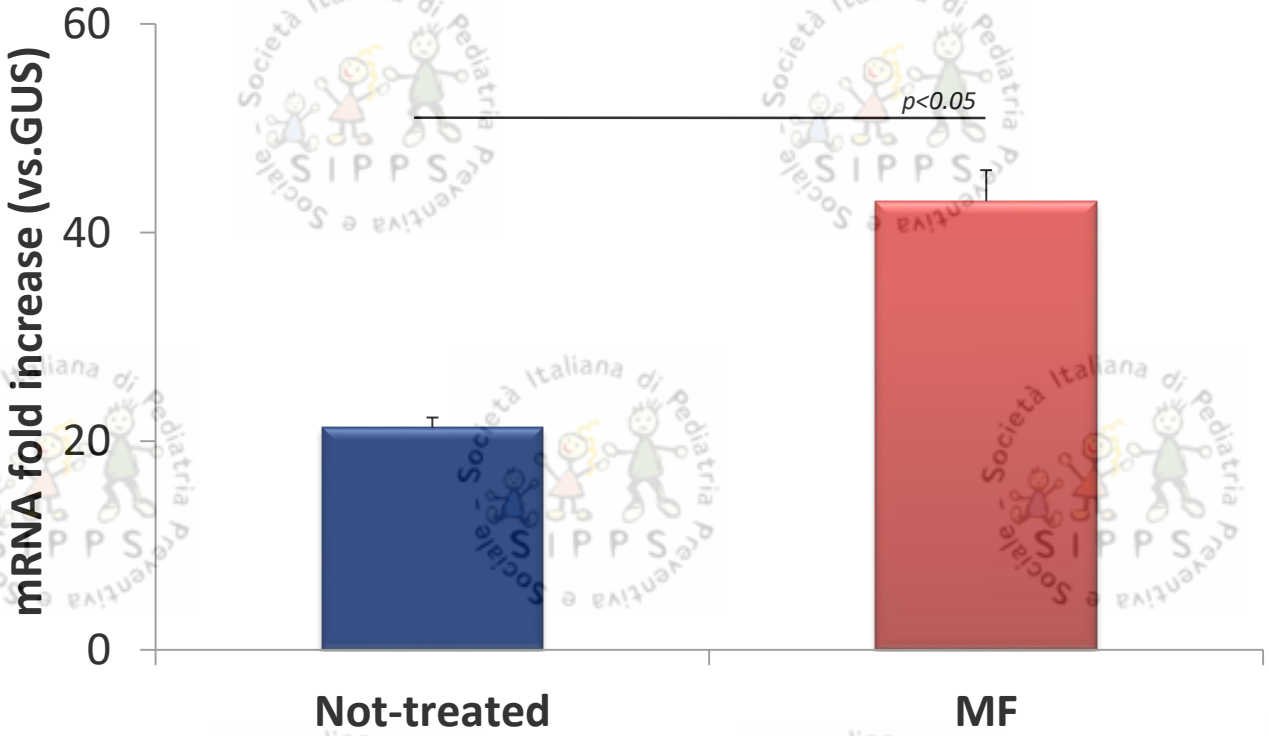




Berni Canani R, data on file

Effects on cell differentiation

Lactase

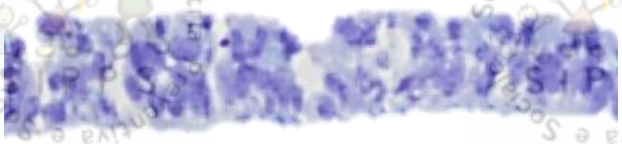


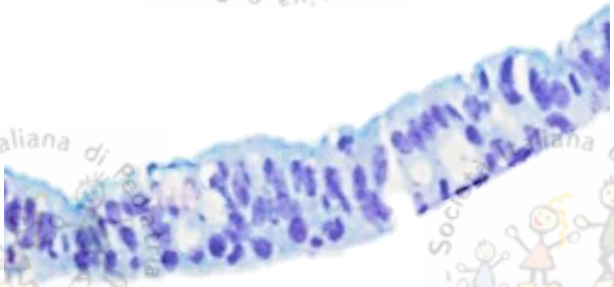
***MF dose:11.5 mg/ml**

Berni Canani R, data on file

Effect on mucus production

48h incubation

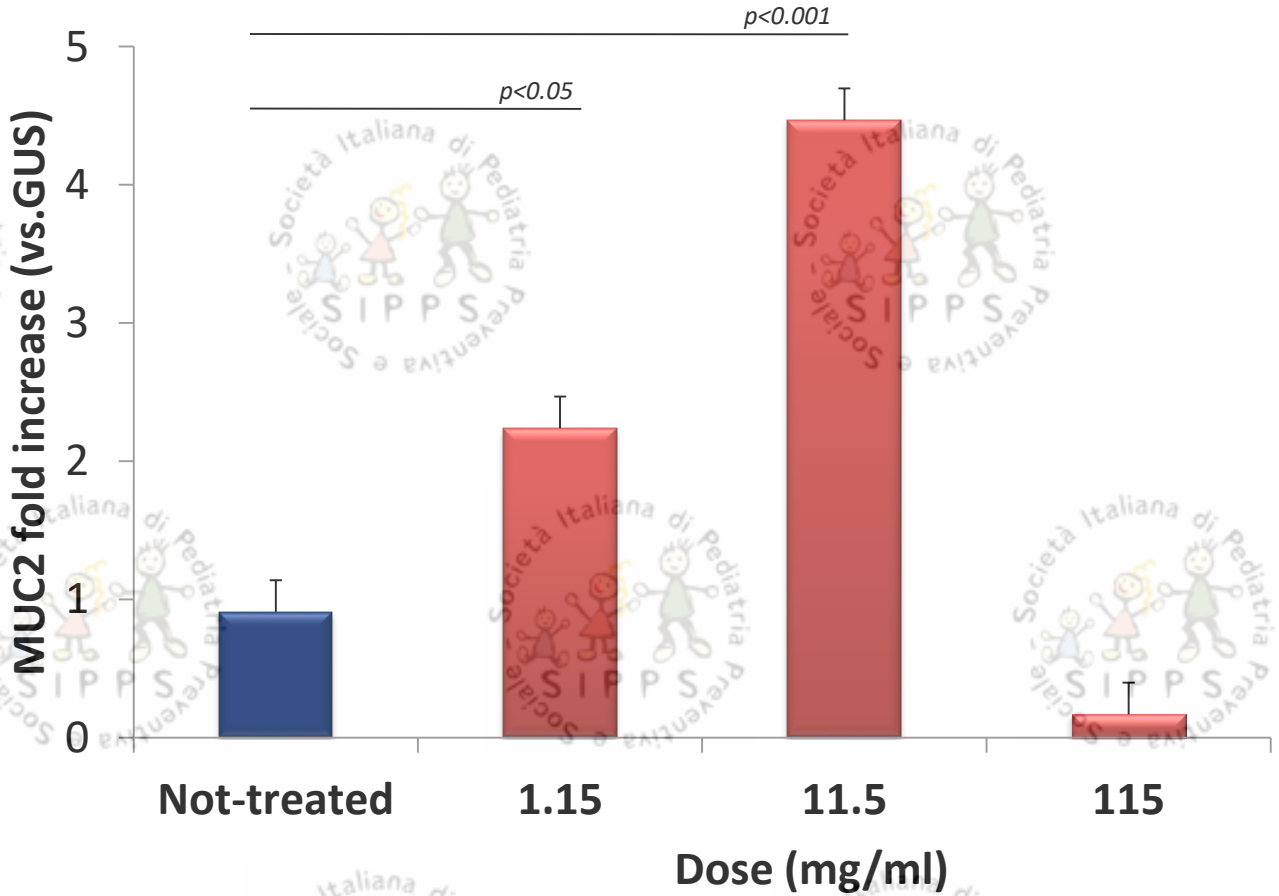




Dose (mg/ml)	Layer (µm)
Not-treated	-
1.15	3±2
11.5	6±2

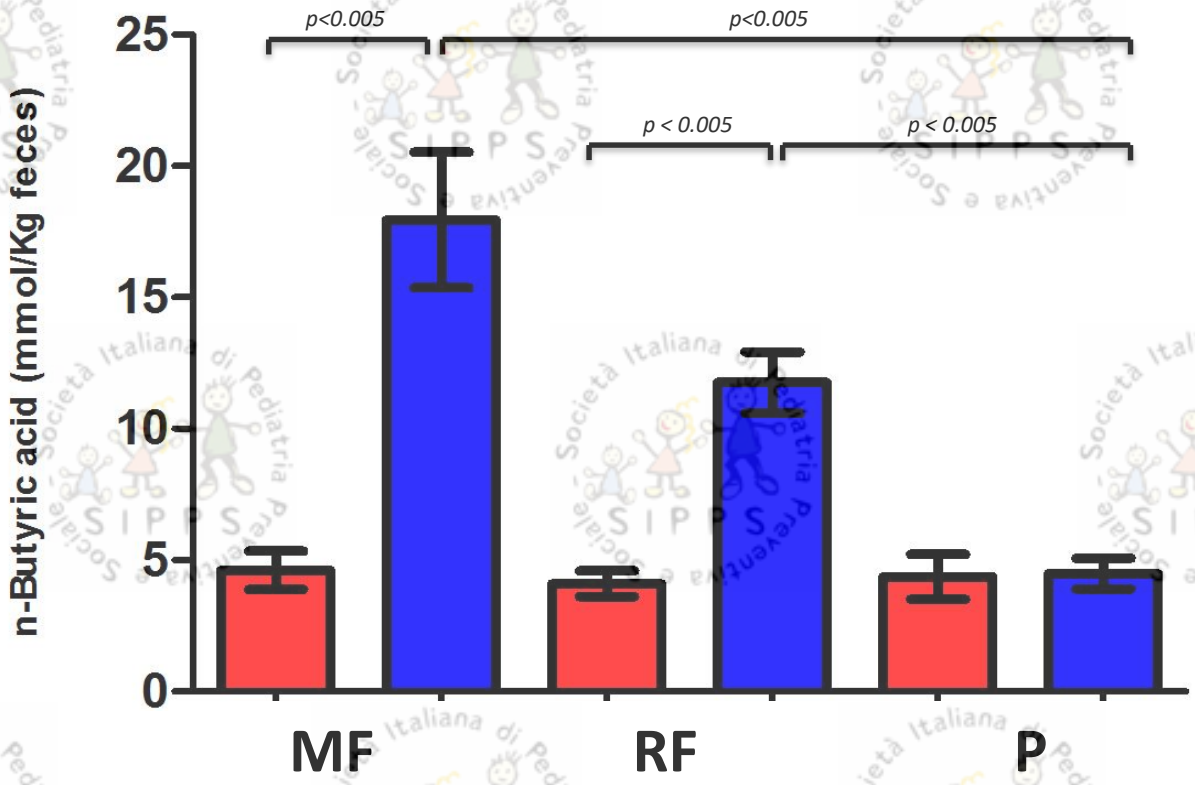
Berni Canani R, data on file

Effect on Mucin 2 expression



Berni Canani R, data on file

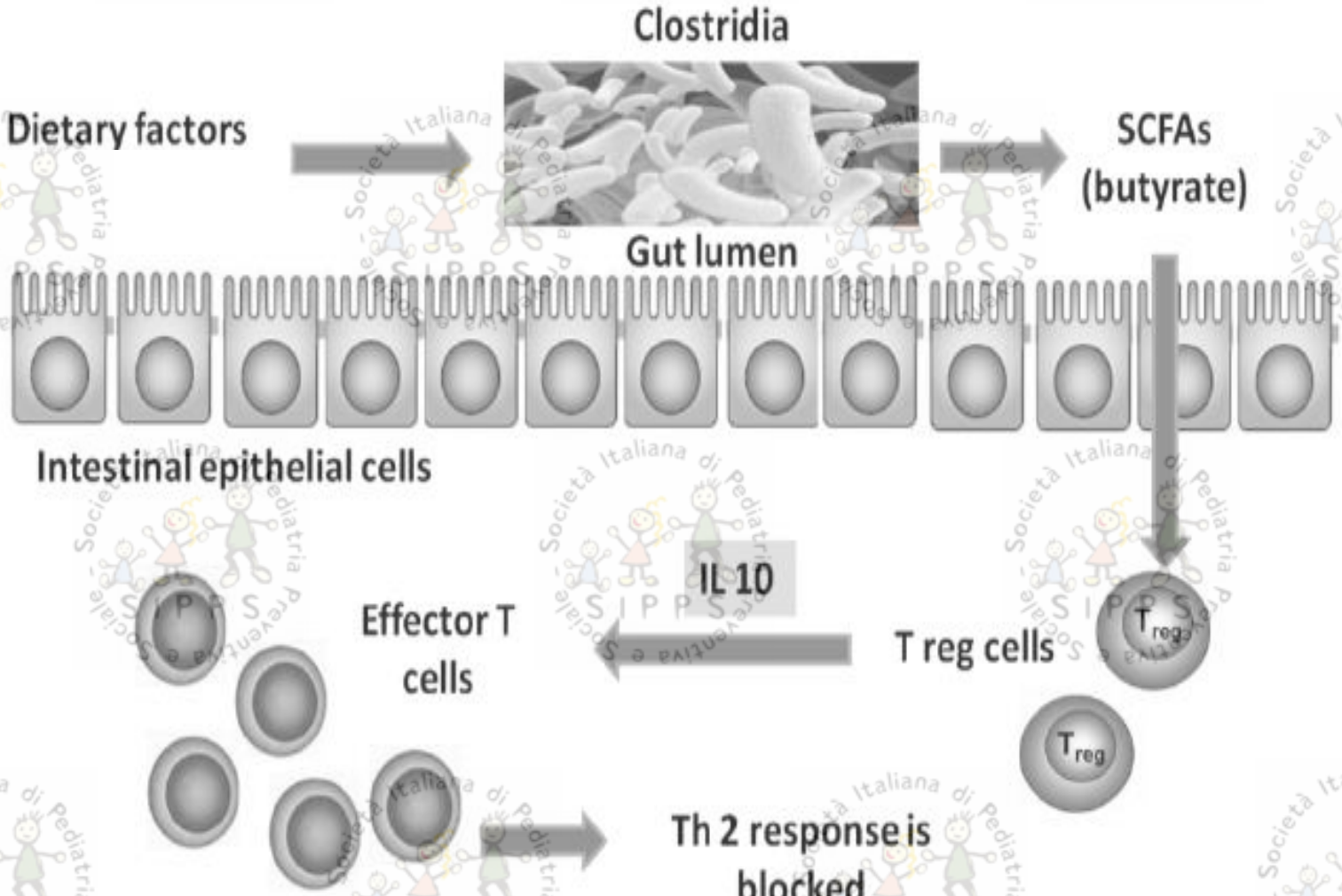
FM and FR stimulate butyrate production in human gut



Berni Canani R, data on file

POTENTIAL BENEFICIAL EFFECTS OF BUTYRATE

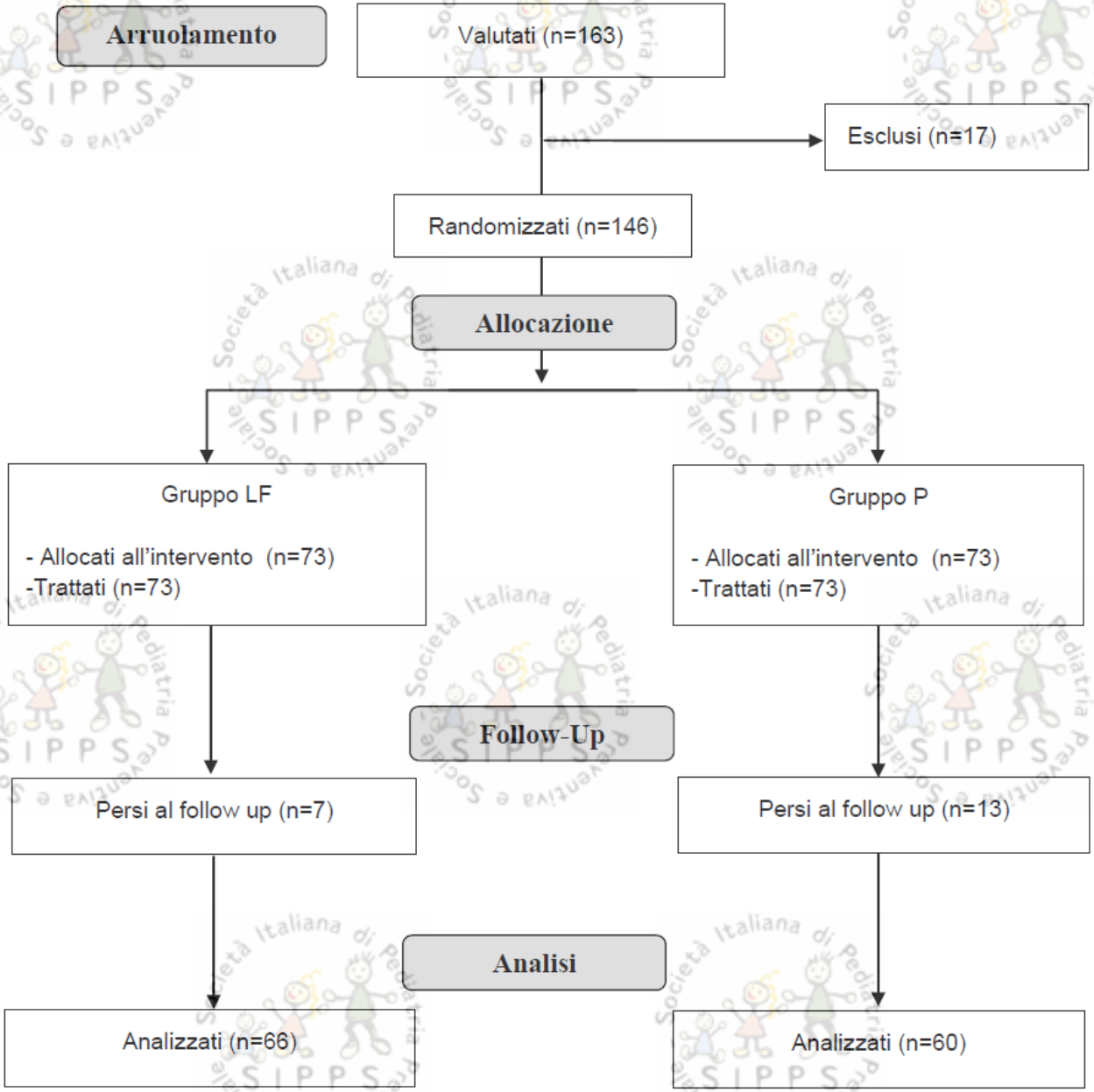
Margherita Di Costanzo[1], Lorella Paparo[1], Rosita Aitoro[1], Linda Cosenza[1], Rita Nocerino[1], Tommaso Cozzolino[1], Vincenza Pezzella[1], Gianfranco Vallone[2] and Roberto Berni Canani[1,3,]*



In: Butyrate: Food Sources, Functions and Health Benefits

Centri partecipanti

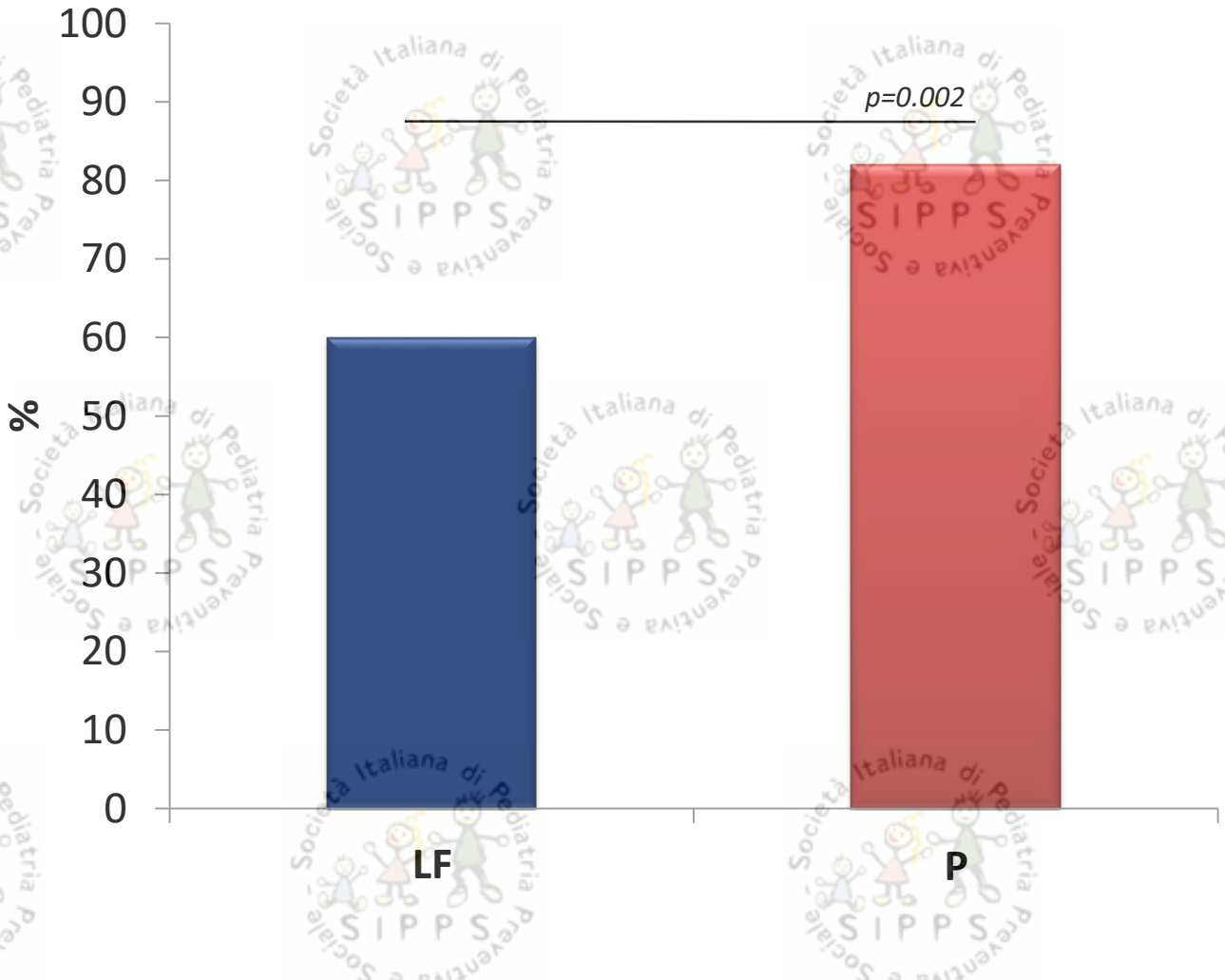
Flusso dei soggetti durante le fasi dello studio



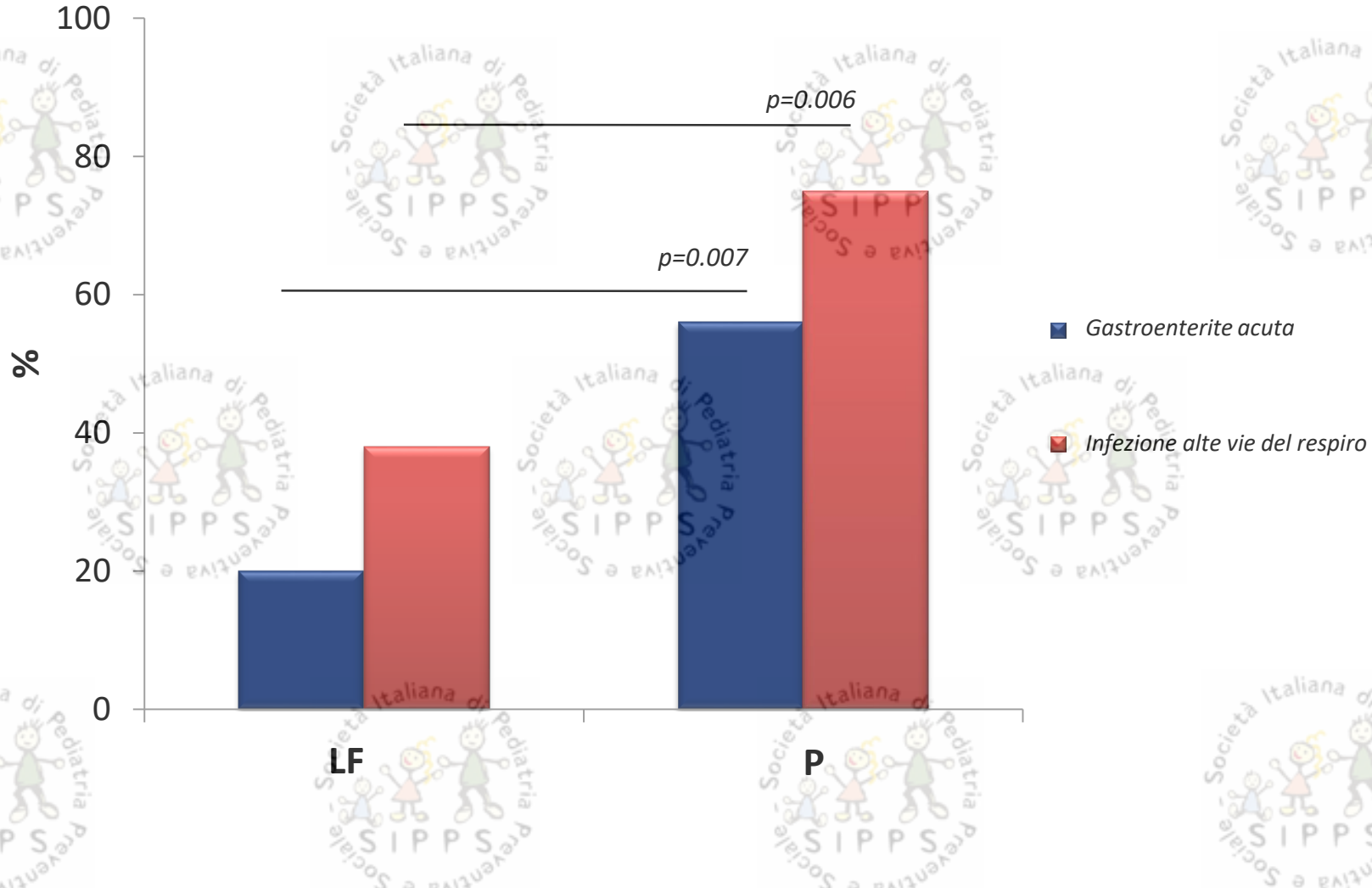
Principali caratteristiche della popolazione di studio all'arruolamento

	LF	P
	n=73	*n=73*
Maschi, n (%)	39 (53.4)	45 (61.6)
Età, mesi (±DS)	32.5 (9.7)	33.7 (8.6)
Peso, kg (±DS)	14.8 (3.2)	15 (3)
Altezza, cm (±DS)	92 (8.1)	94 (7.6)
Allattamento materno, n (%)	50 (68.5)	41 (56.2)
Durata dell'allattamento, mesi (±DS)	7.1 (6)	7 (8.5)
Età alla scolarizzazione, mesi (±DS)	23.2 (9)	25.9 (8.5)
Fratelli, n (%)	60 (82.2)	52 (71.2)
N. di fratelli, (±DS)	1.4 (0.6)	1.4 (0.6)
Fumo passivo, n (%)	28 (38.4)	30 (41.1)

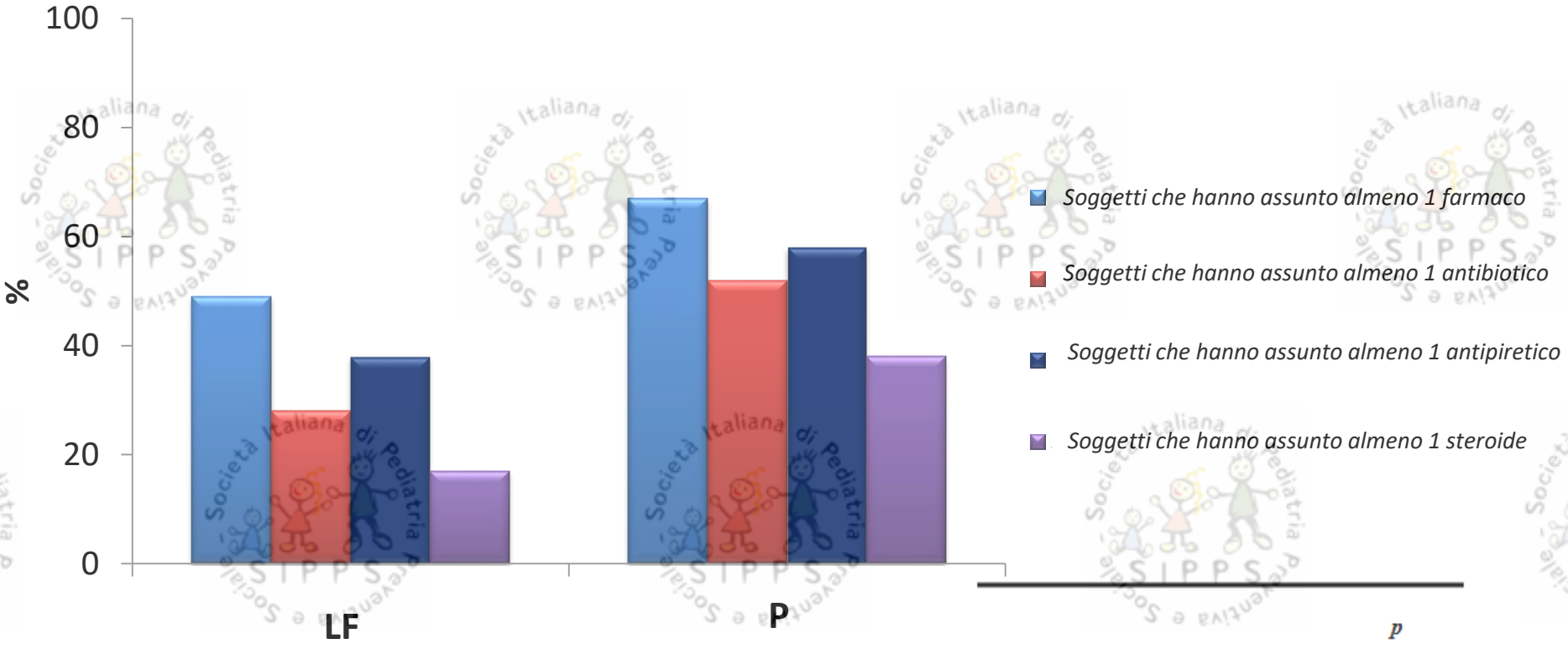
Soggetti che hanno presentato ≥1 episodio infettivo *(analisi ITT)*



Soggetti che hanno presentato ≥1 episodio di gastroenterite acuta/infezione alte vie del respiro



Utilizzo di farmaci

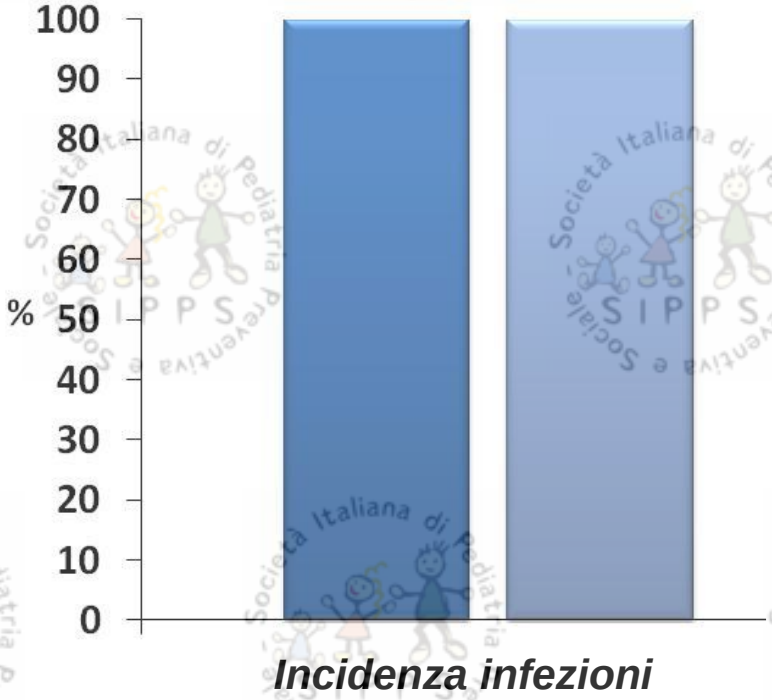


	p
Almeno 1 farmaco	***0.019***
Antibiotici	***0.03***
Antipiretici	***0.044***
Steroidi	***0.027***

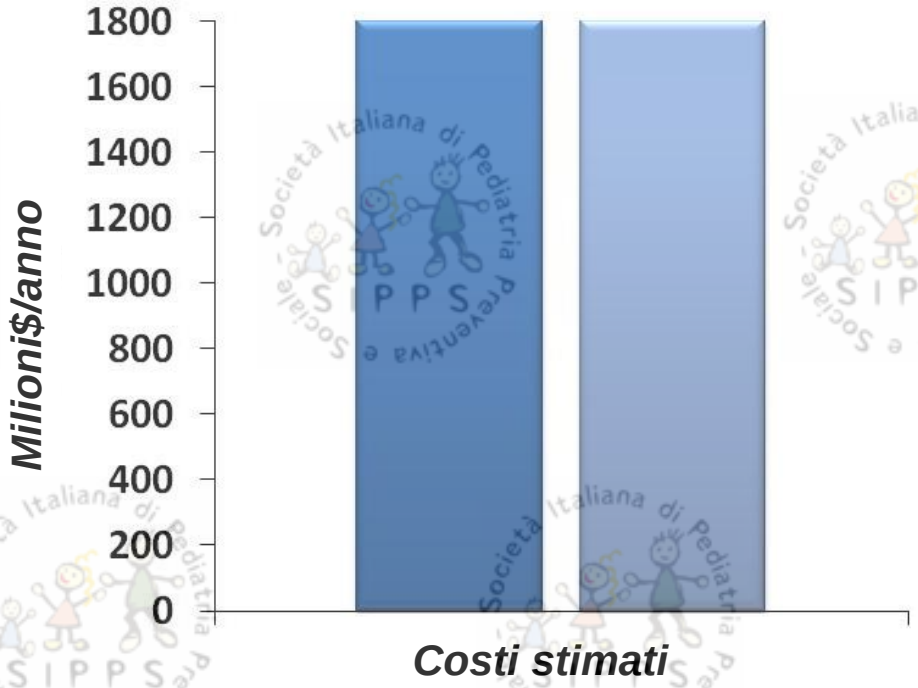
Punti di forza degli alimenti funzionali fermentati

- Contengono probiotici di origine umana
- Contengono probiotici inattivati
- Nessun rischio di traslocazione batterica
- Elevata facilità di conservazione e trasporto
- Elevata riproducibilità (monitoraggio preciso del processo tecnologico)

L'impatto



Incidenza infezioni



Costi stimati

LF

RF

- Spesa sanitaria per consumo antibiotici in Italia nel 2015 in età : **15.661.863 €**
- Utilizzo di latte fermentato con CBA-L74 riduce del 75% l'utilizzo di antibiotici

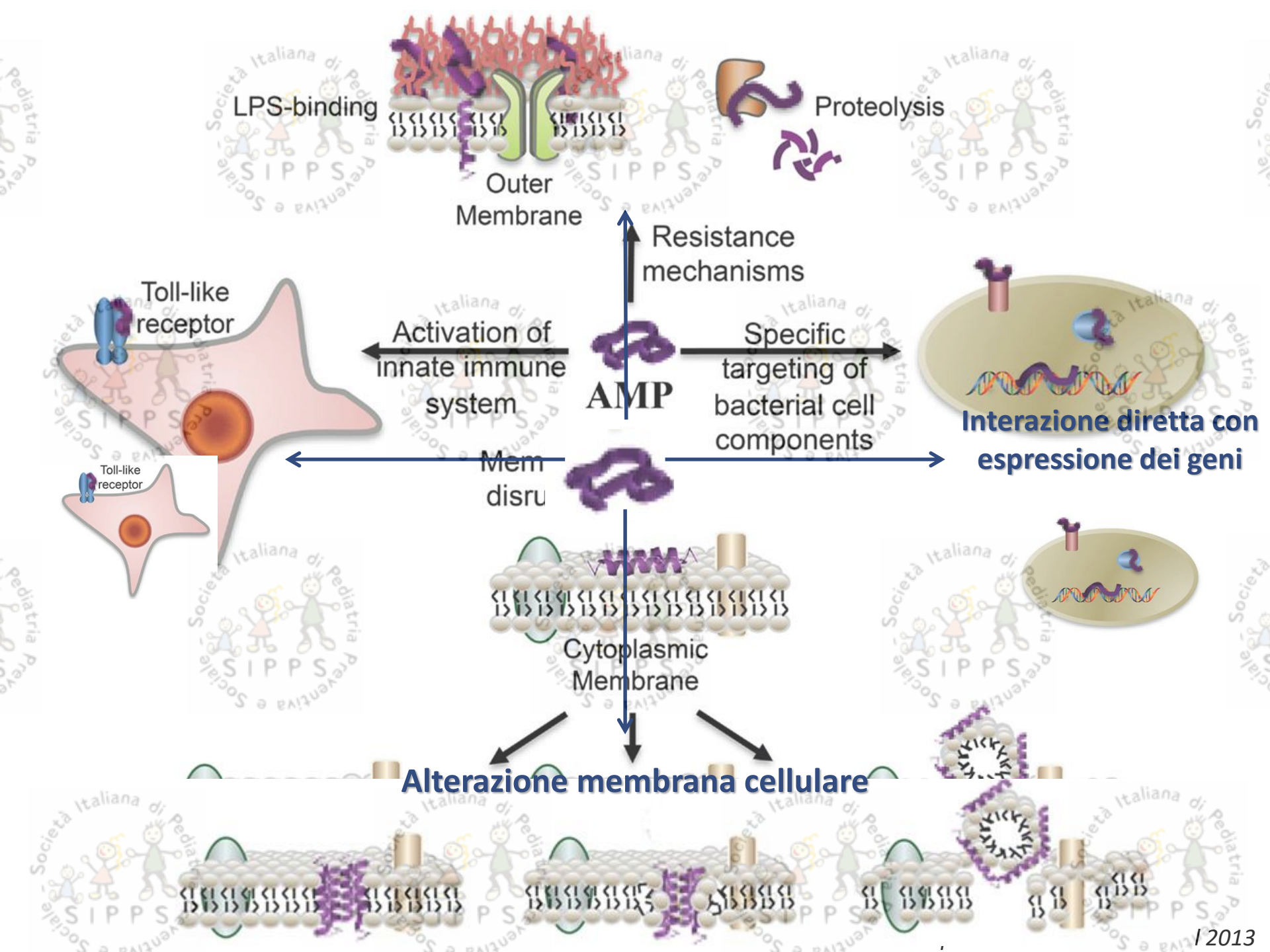
Spesa per consumo di antibiotici: 3.915.715 €

Risparmio di € 11.746.397

ISTAT 2015

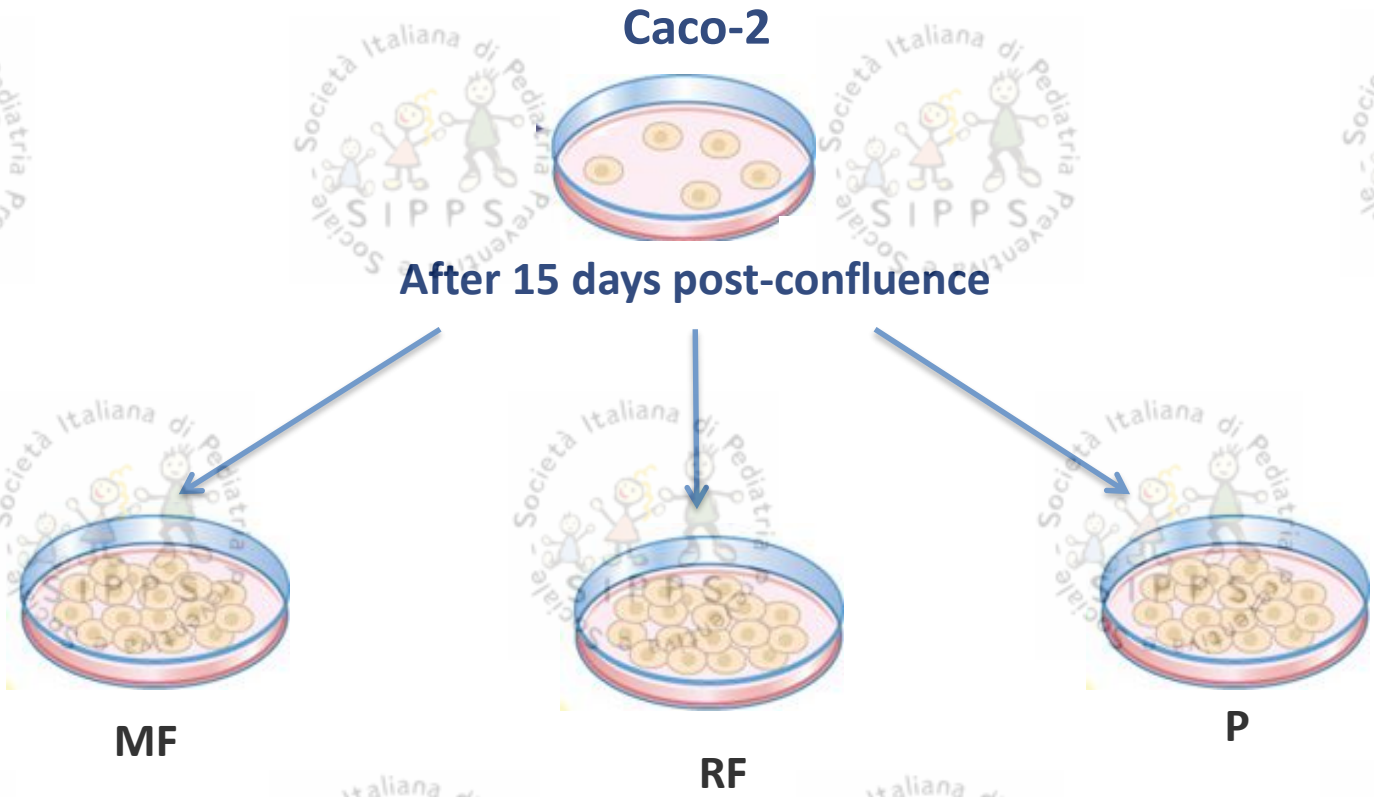
1. Segui sempre seguire le tue passioni
2. Vivi in una dimensione internazionale
3. Sii sempre utile e collaborativo
4. Raccogli le sfide e ascolta gli altri
5. Impara dai pazienti: sono il centro di tutto
6. Condividi sempre idee e risultati

A.Mantovani - Corriere della Sera 2015

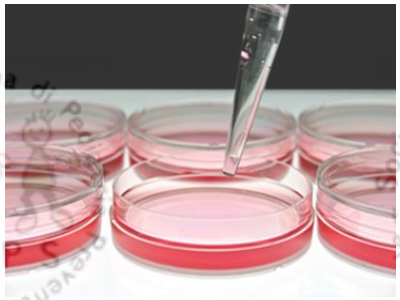

LPS-binding
Outer Membrane
Proteolysis
Resistance mechanisms
Toll-like receptor
Activation of innate immune system
AMP
Specific targeting of bacterial cell components
Interazione diretta con espressione dei geni
Toll-like receptor
Mem
disru
Cytoplasmic Membrane
Alterazione membrana cellulare

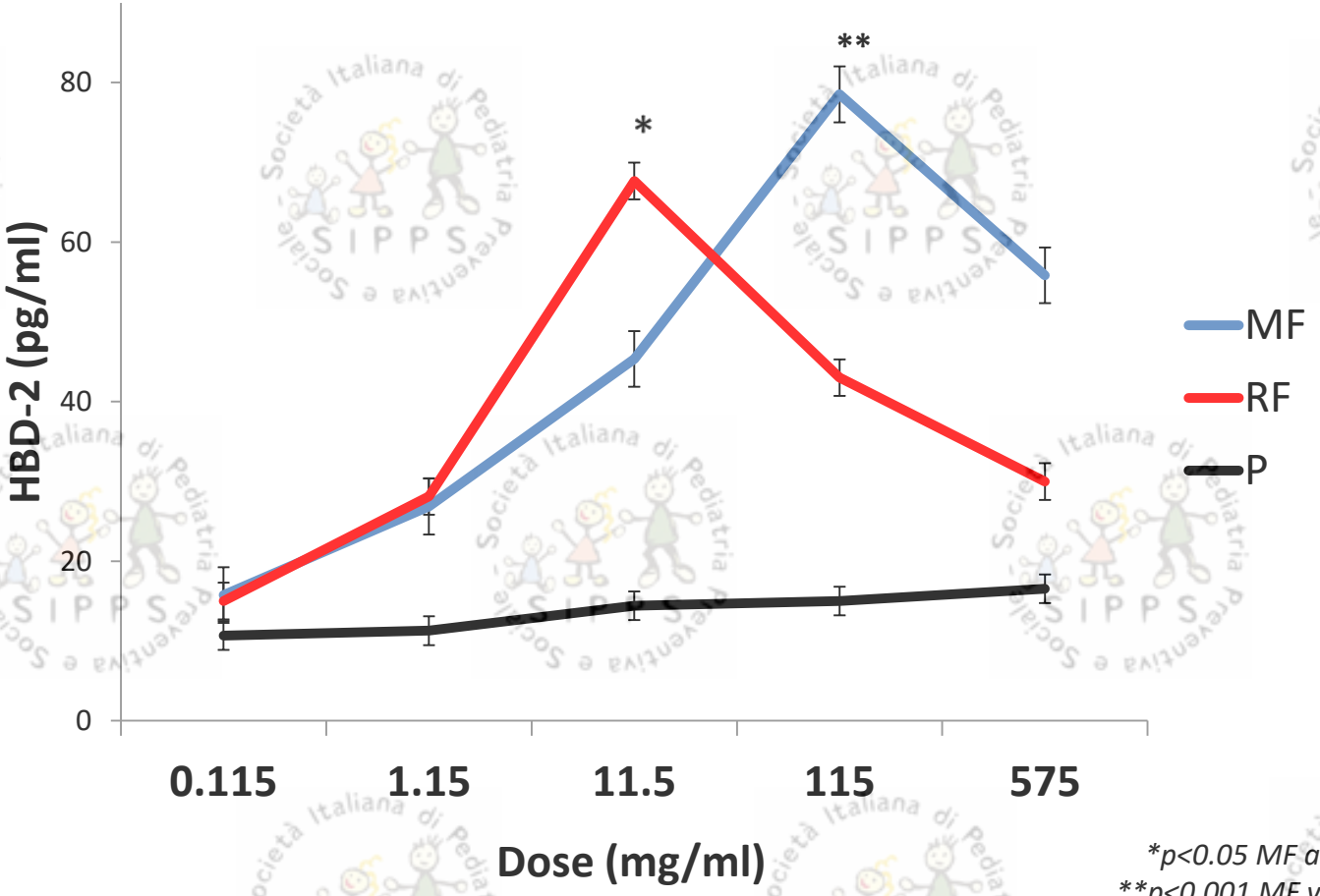
Mechanisms of Action

Exploring the direct interaction with human enterocytes



1. Protection against infection: innate immunity stimulation in human enterocytes

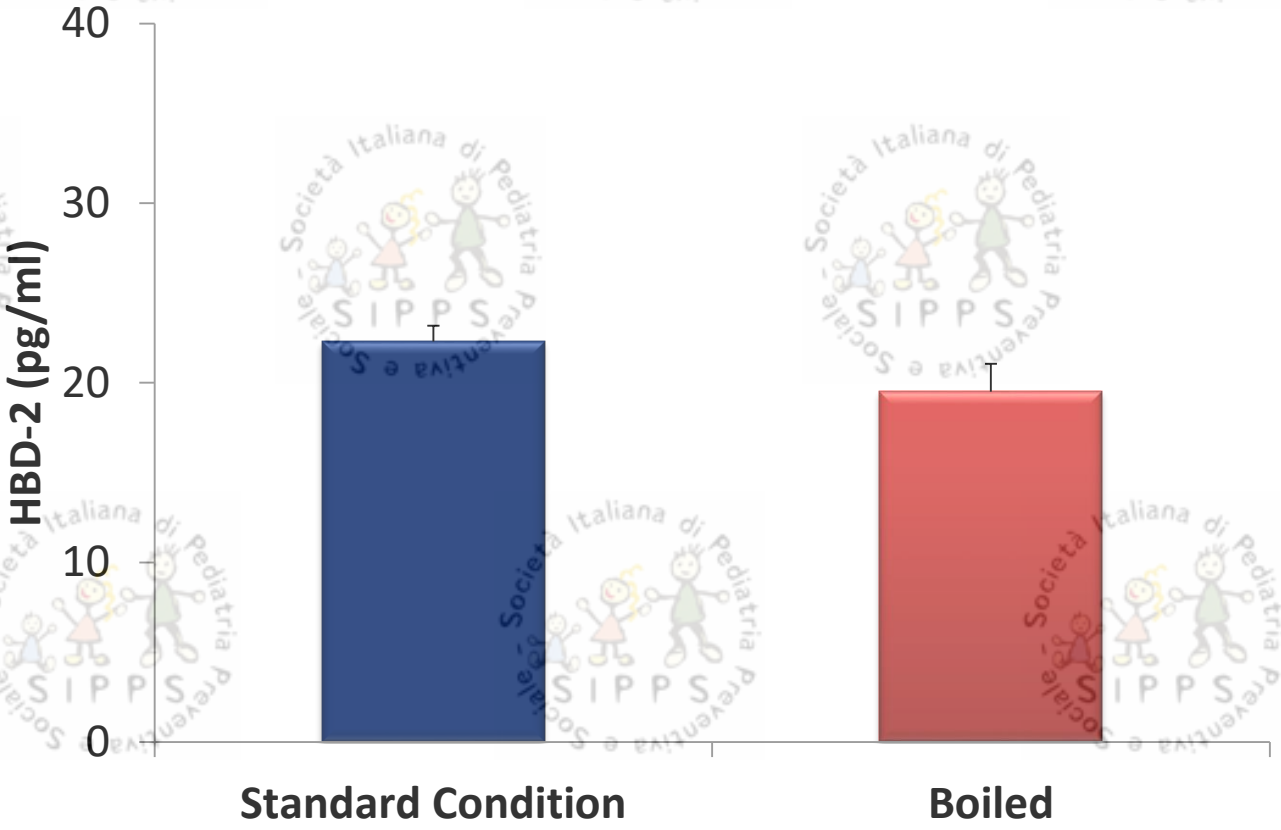




*p<0.05 MF and RF vs P
**p<0.001 MF vs P

Berni Canani R, data on file

The effect is heat-stable

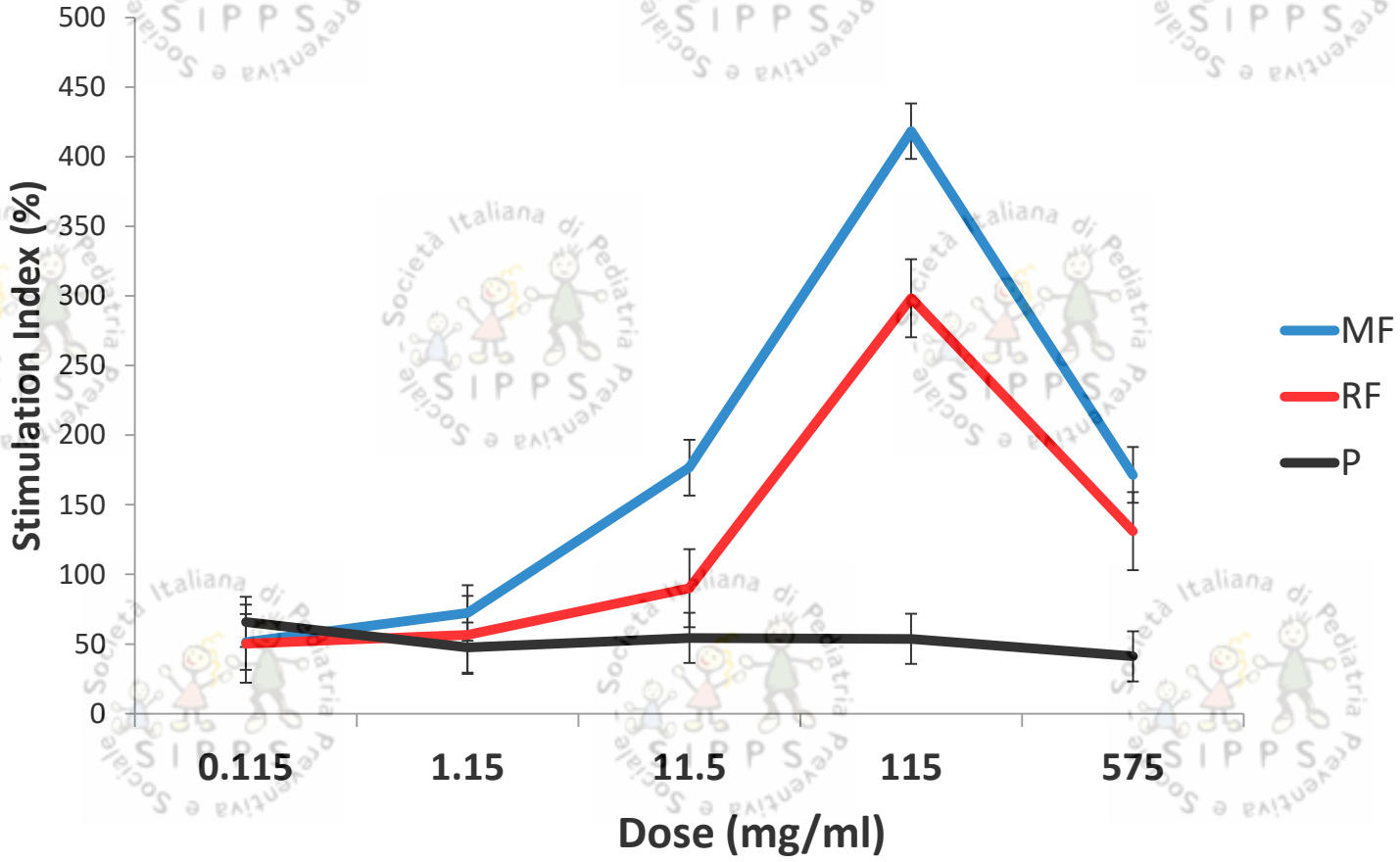


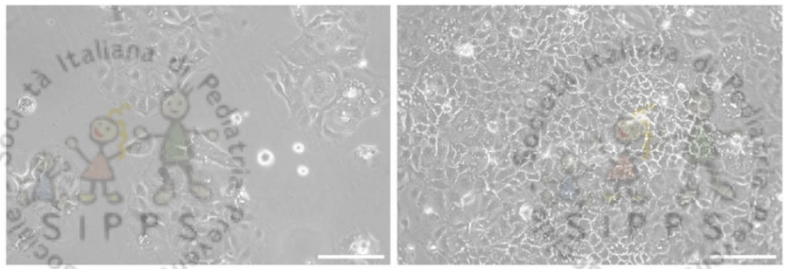
Fermented milk product (11.5 mg/ml) treated at 95°C for 10 min

Berni Canani R, data on file

2. Protection against infection:non-immune mechanisms

Effect on cell proliferation

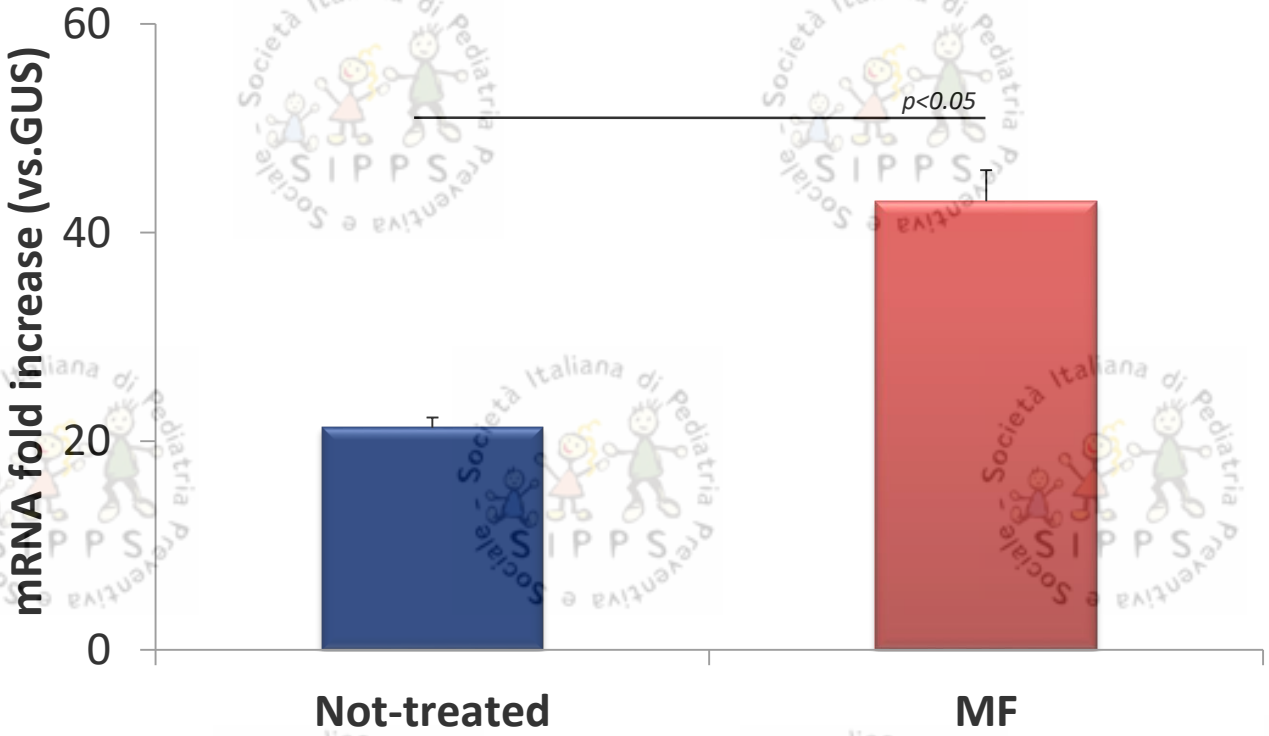




Berni Canani R, data on file

Effects on cell differentiation

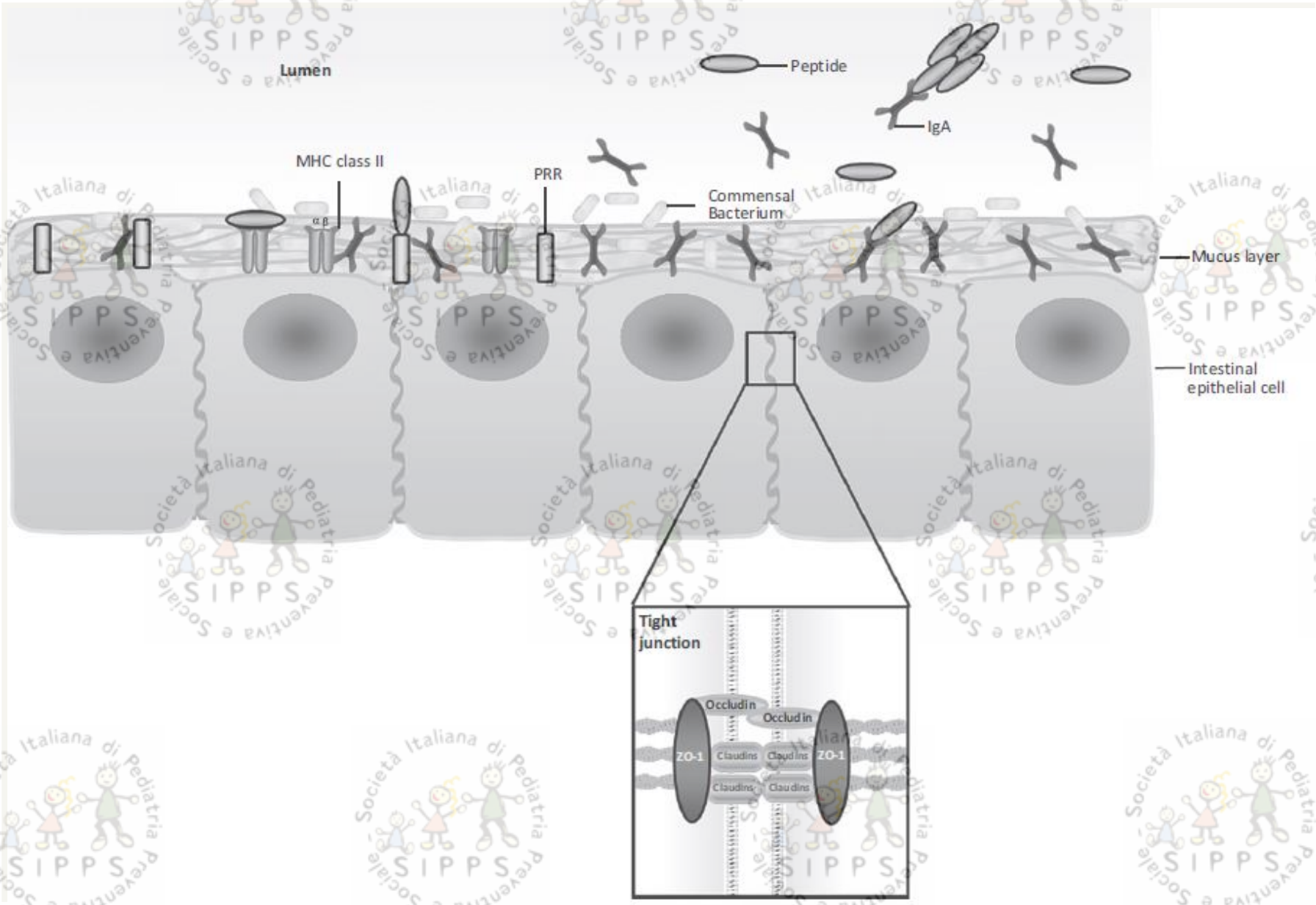
Lactase



***MF dose:11.5 mg/ml**

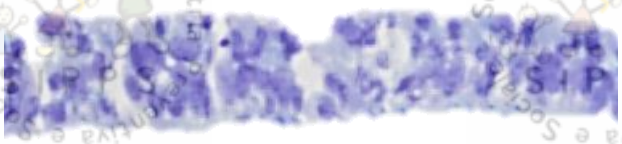
Berni Canani R, data on file

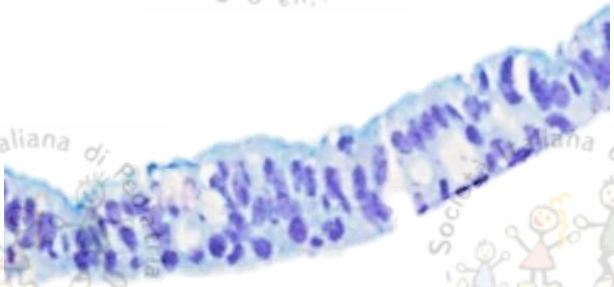
Effects on gut permeability



Effect on mucus production

48h incubation

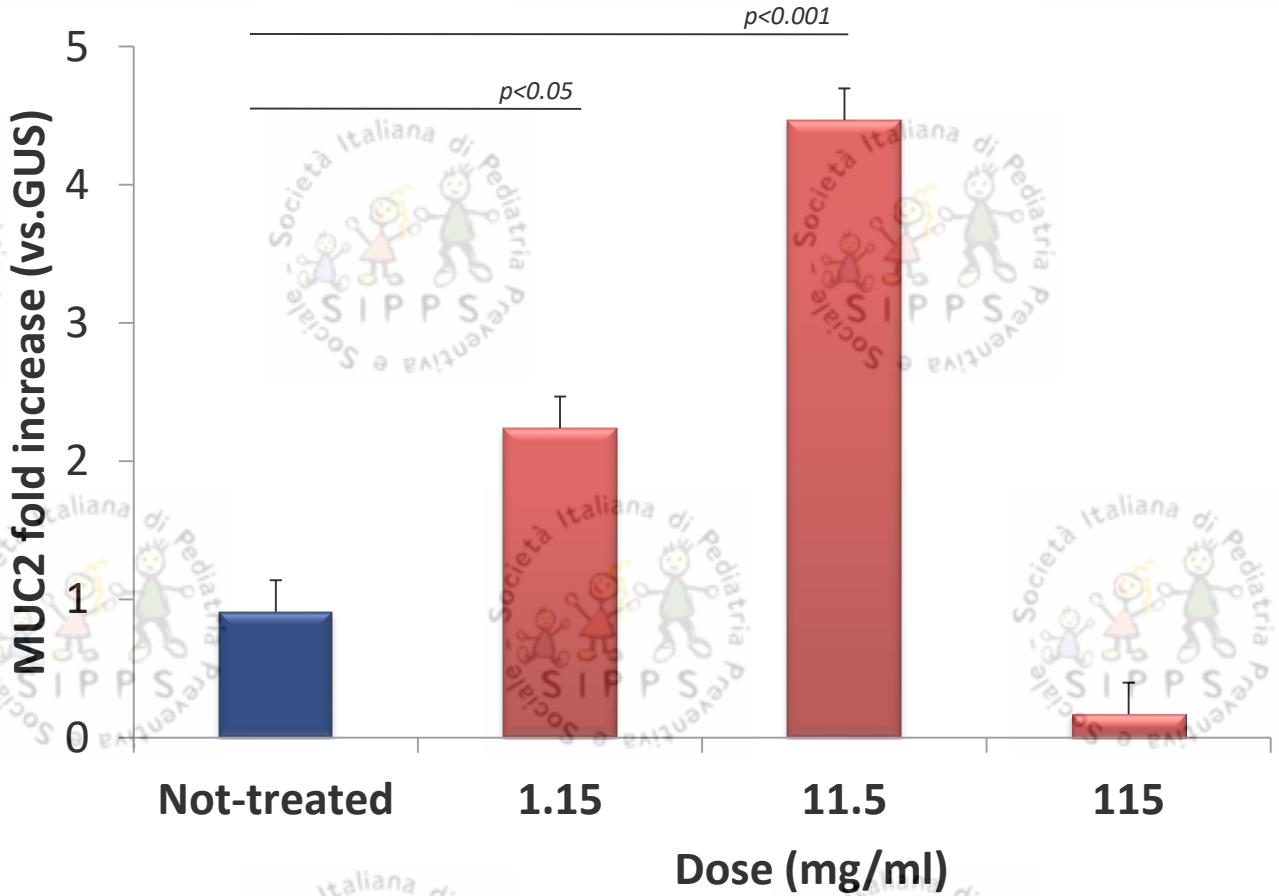




Dose (mg/ml)	Layer (µm)
Not-treated	-
1.15	3±2
11.5	6±2

Berni Canani R, data on file

Effect on Mucin 2 expression

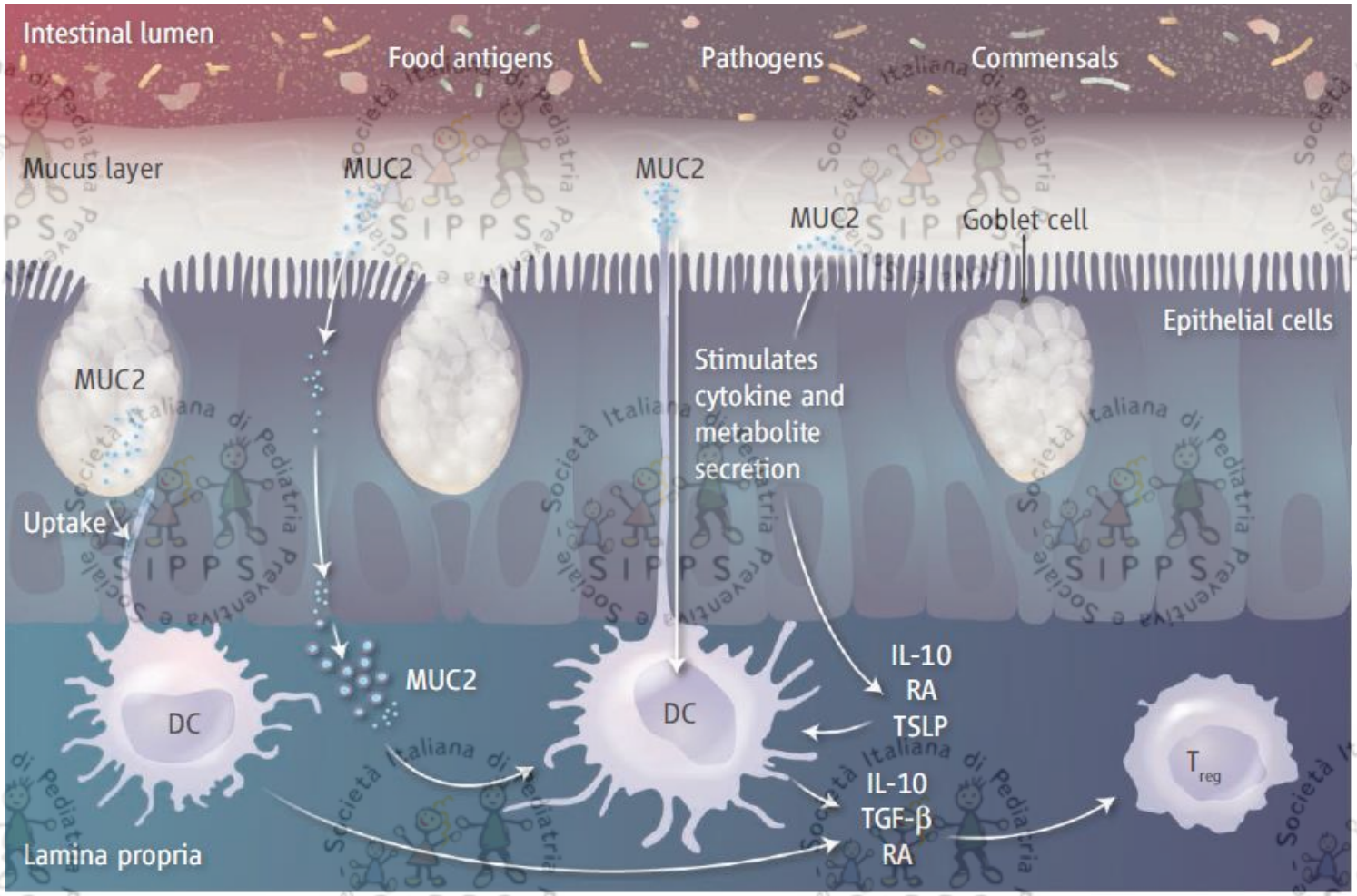


Berni Canani R, data on file

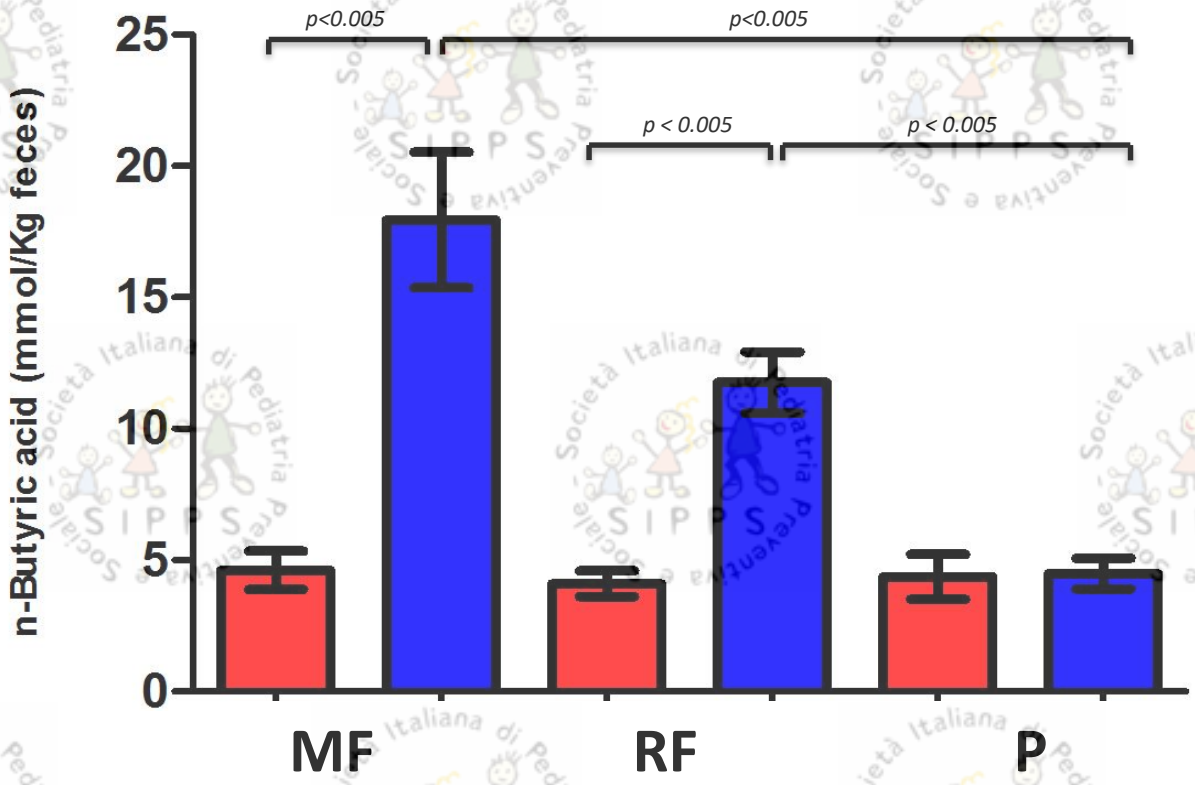
Mucus Coat, a Dress Code for Tolerance

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

Yasmine Belkaid and John Grainger



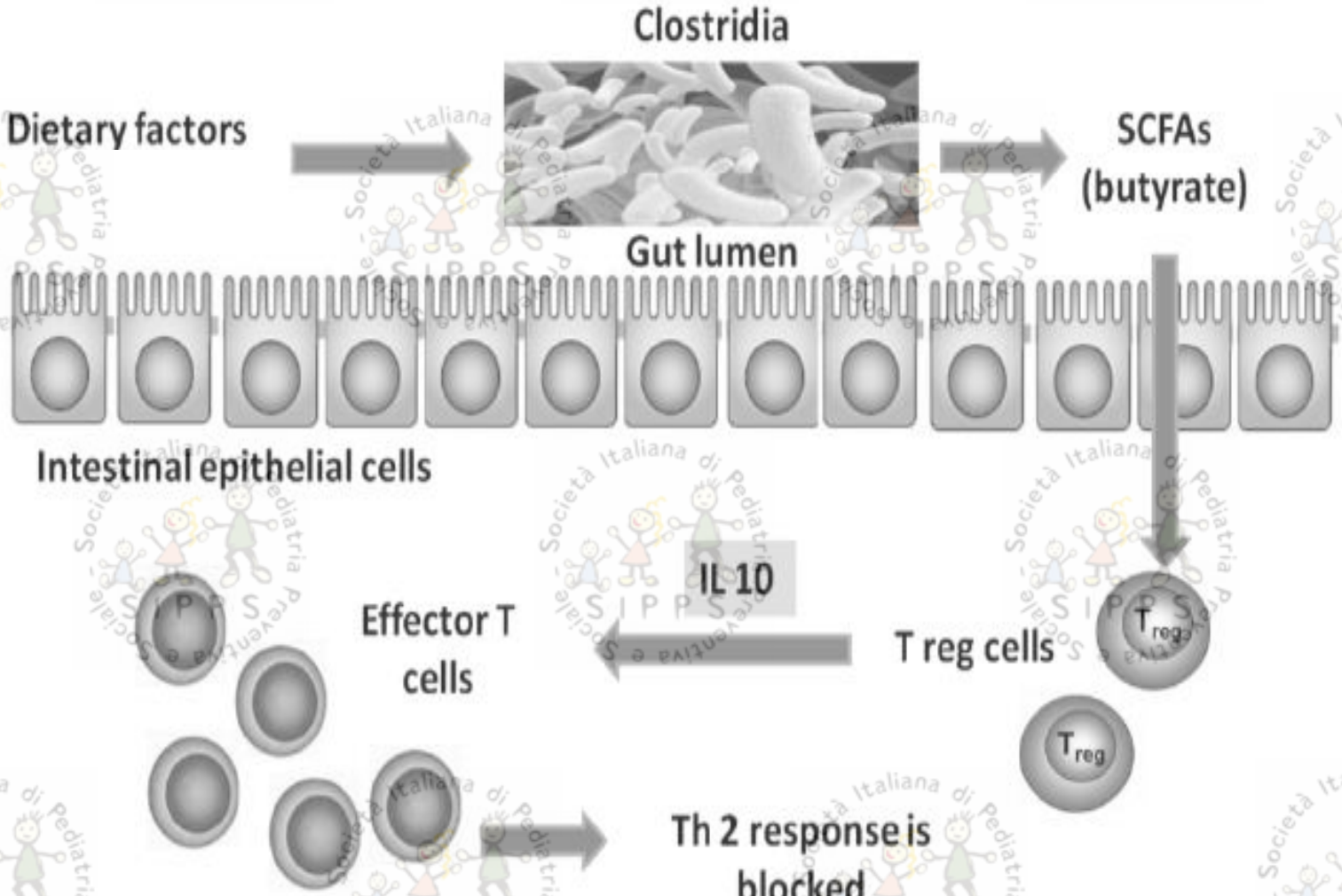
FM and FR stimulate butyrate production in human gut



Berni Canani R, data on file

POTENTIAL BENEFICIAL EFFECTS OF BUTYRATE

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

IgE-mediated CMA children (1-4 yrs) all positive for SPT and specific IgE against β-lactoglobulin (BLG)

PBMCs

CTRL

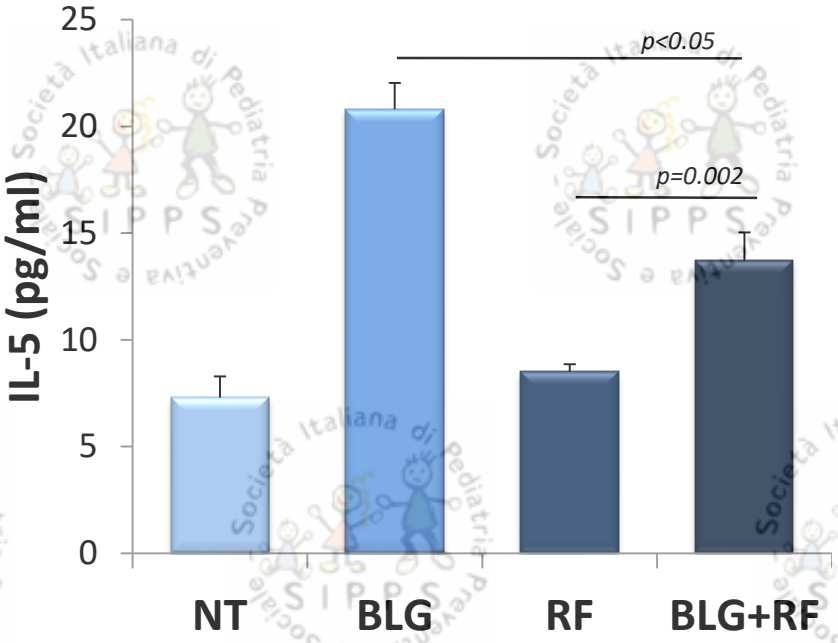
BLG

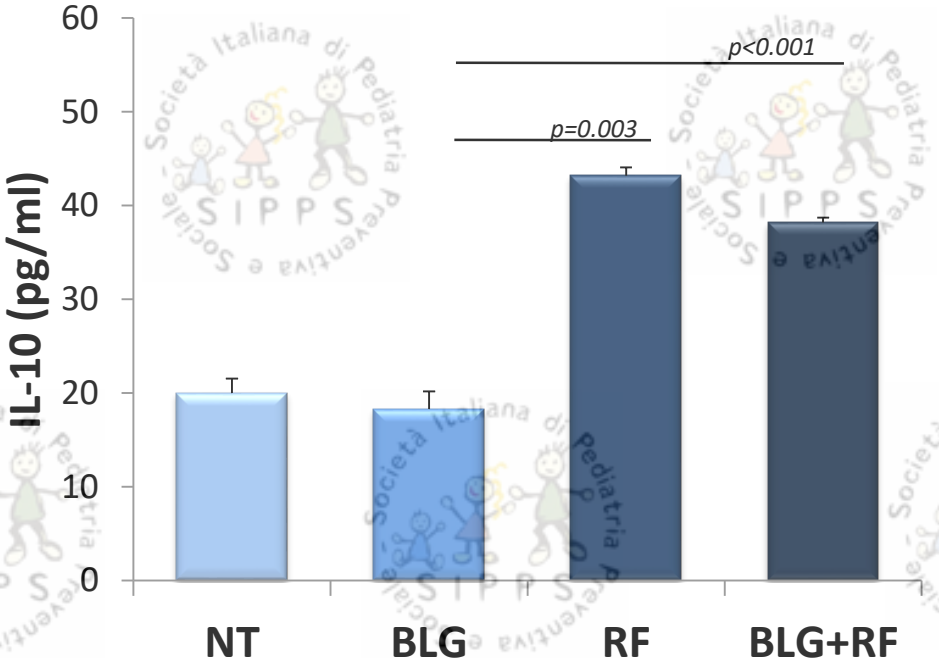
100 µg/ml

RF

BLG +RF

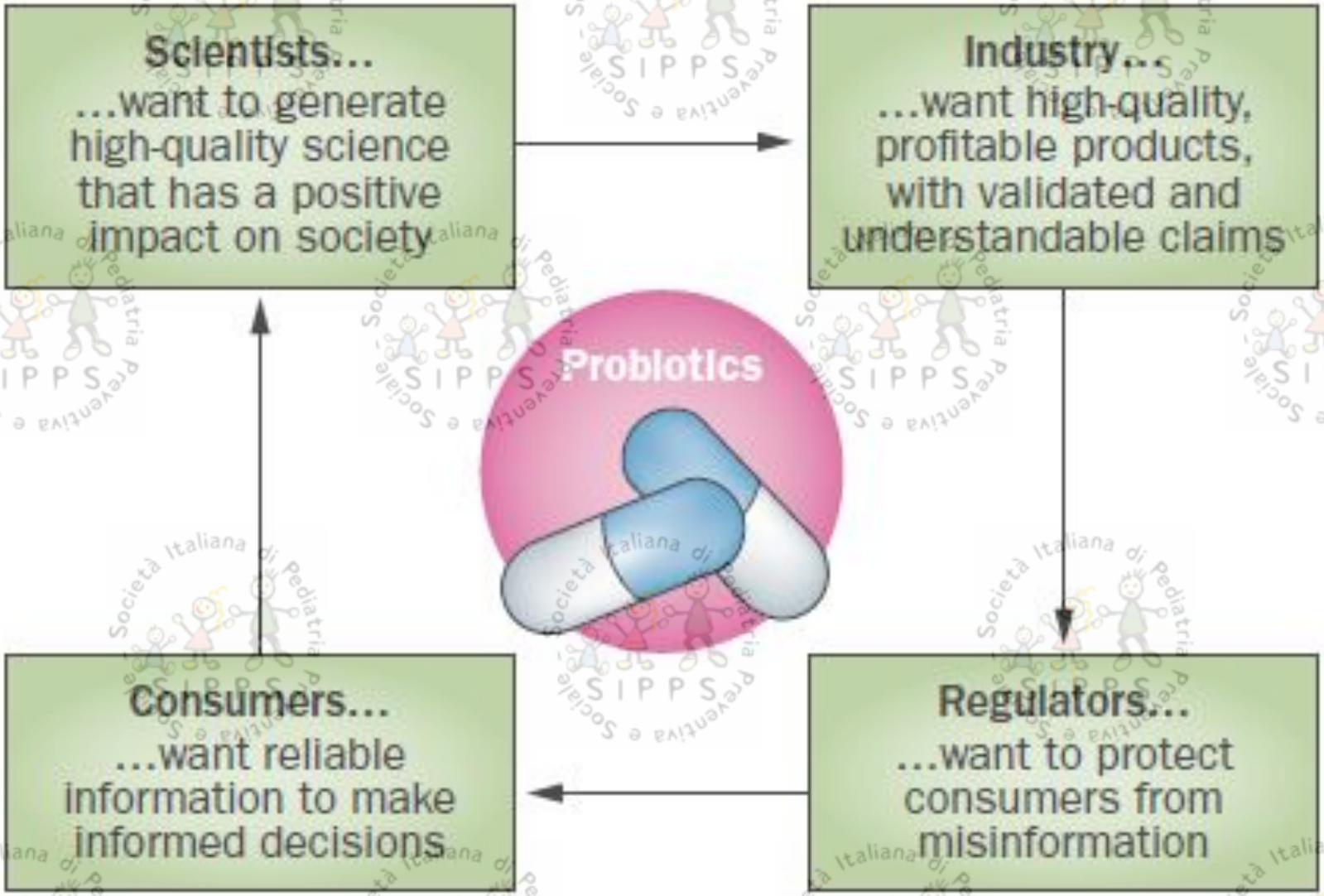
…after 48h of incubation…





***RF dose: 11.5 mg/ml**

Berni Canani R, data on file



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

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

Common infectious diseases observed during the study period

Disease	Group A	Group B	*p*
Acute gastroenteritis, n (%) [number of episodes]	12 (18.2) [19]	24 (40) [28]	*0.007*
Rhinitis, n (%) [number of episodes]	22 (33.3) [44]	24 (40) [50]	*0.438*
Otitis, n (%) [number of episodes]	8 (12.1) [11]	13 (21.7) [17]	*0.151*
Pharyngitis, n (%) [number of episodes]	13 (19.7) [22]	25 (41.7) [30]	*0.007*
Laringitis, n (%) [number of episodes]	6 (9.1) [7]	14 (23.3) [14]	*0.029*
Tracheitis, n (%) [number of episodes]	11 (16.7) [16]	19 (31.7) [30]	*0.048*

Study design

Visit 1 (Enrollment) → **Visit 2 (1 months)** → **Visit 3 (2 months)** → **Visit 4 (3 months)**

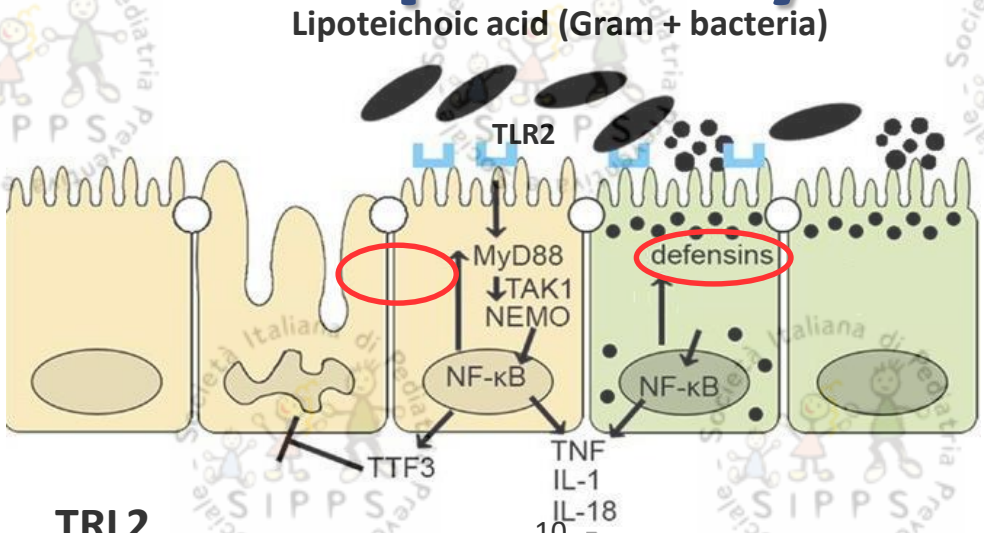
- Anamnestic and clinical evaluation
- Informed consent
- Randomization
- Start study products
- Clinical diary instruction
- Stool sampling

- Clinical evaluation
- Clinical diary checking
- Compliance evaluation

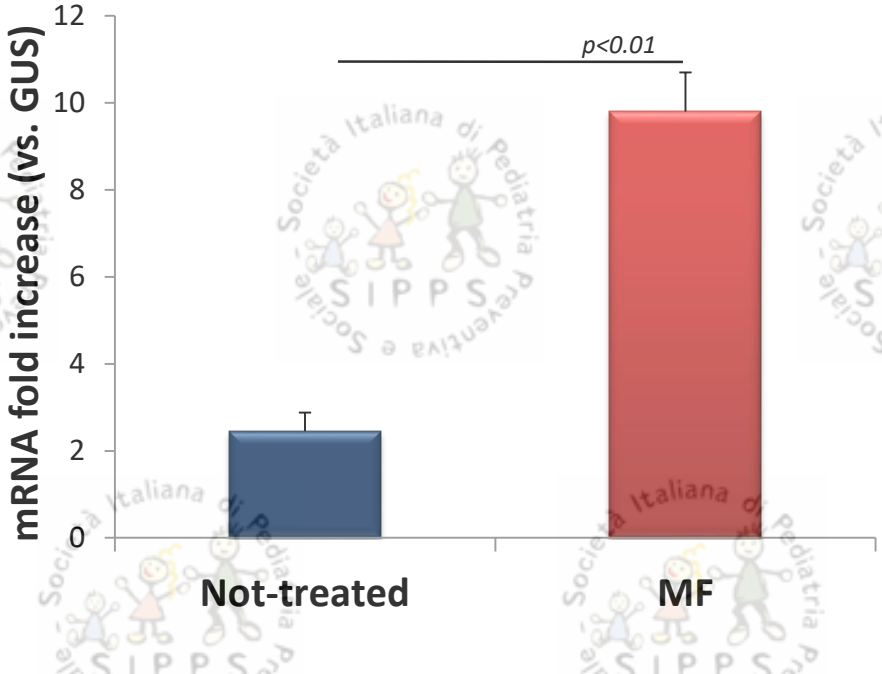
- Clinical evaluation
- Clinical diary collection
- Compliance evaluation
- Stool sampling

A visit by the FP was planned whenever it was necessary because infectious diseases or other morbidities

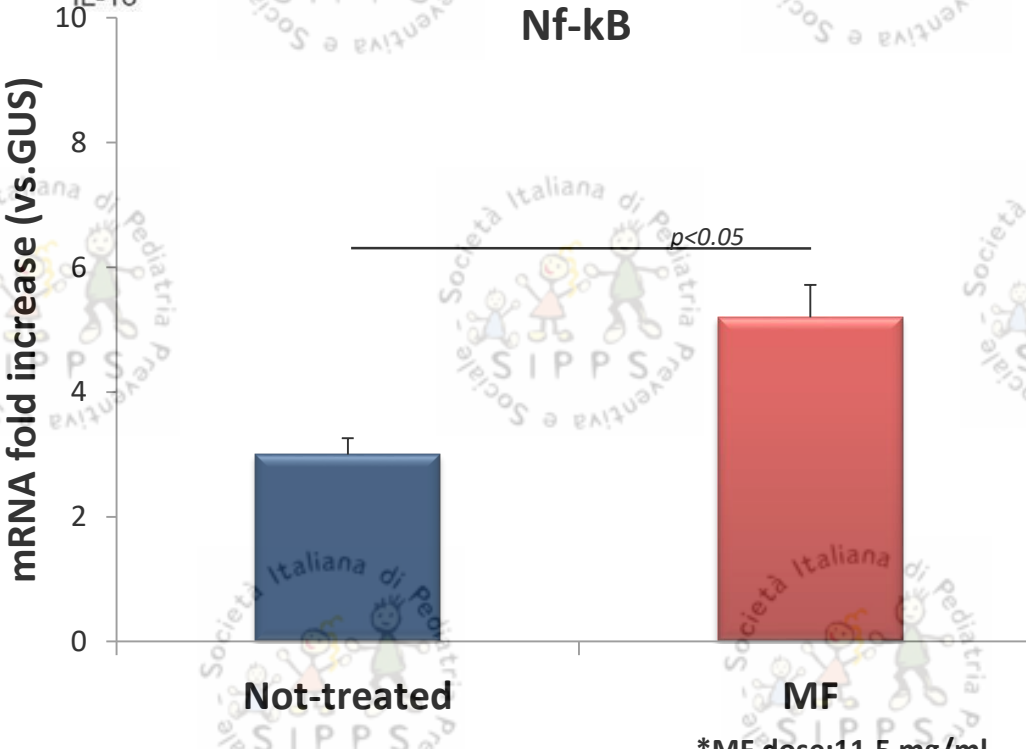
Beta-defensins pathway



TRL2

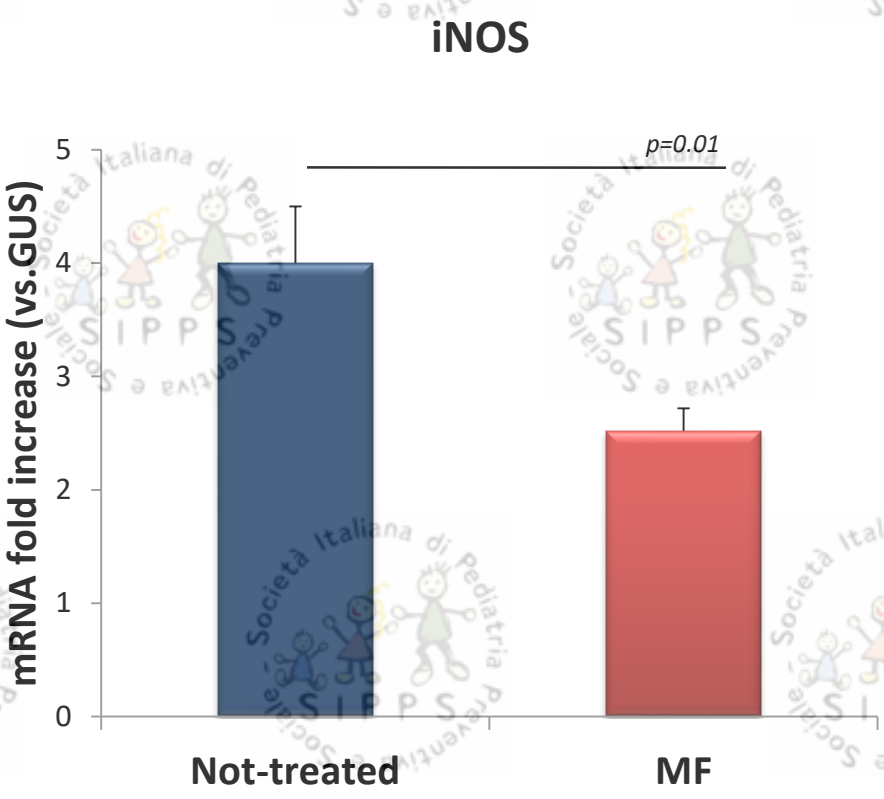


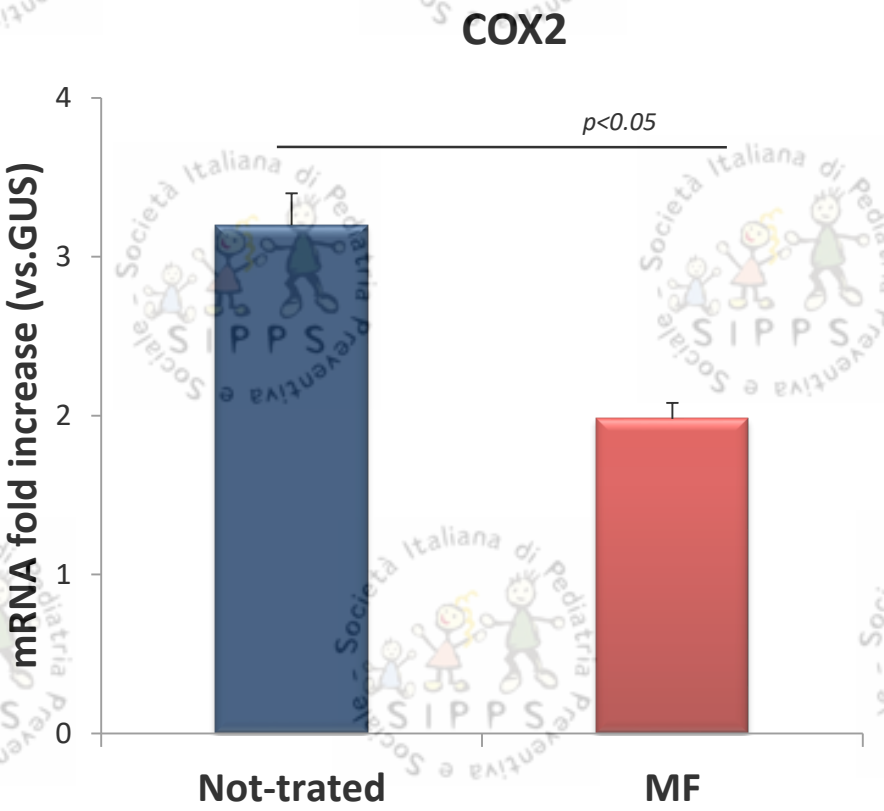
Nf-kB



*MF dose:11.5 mg/ml

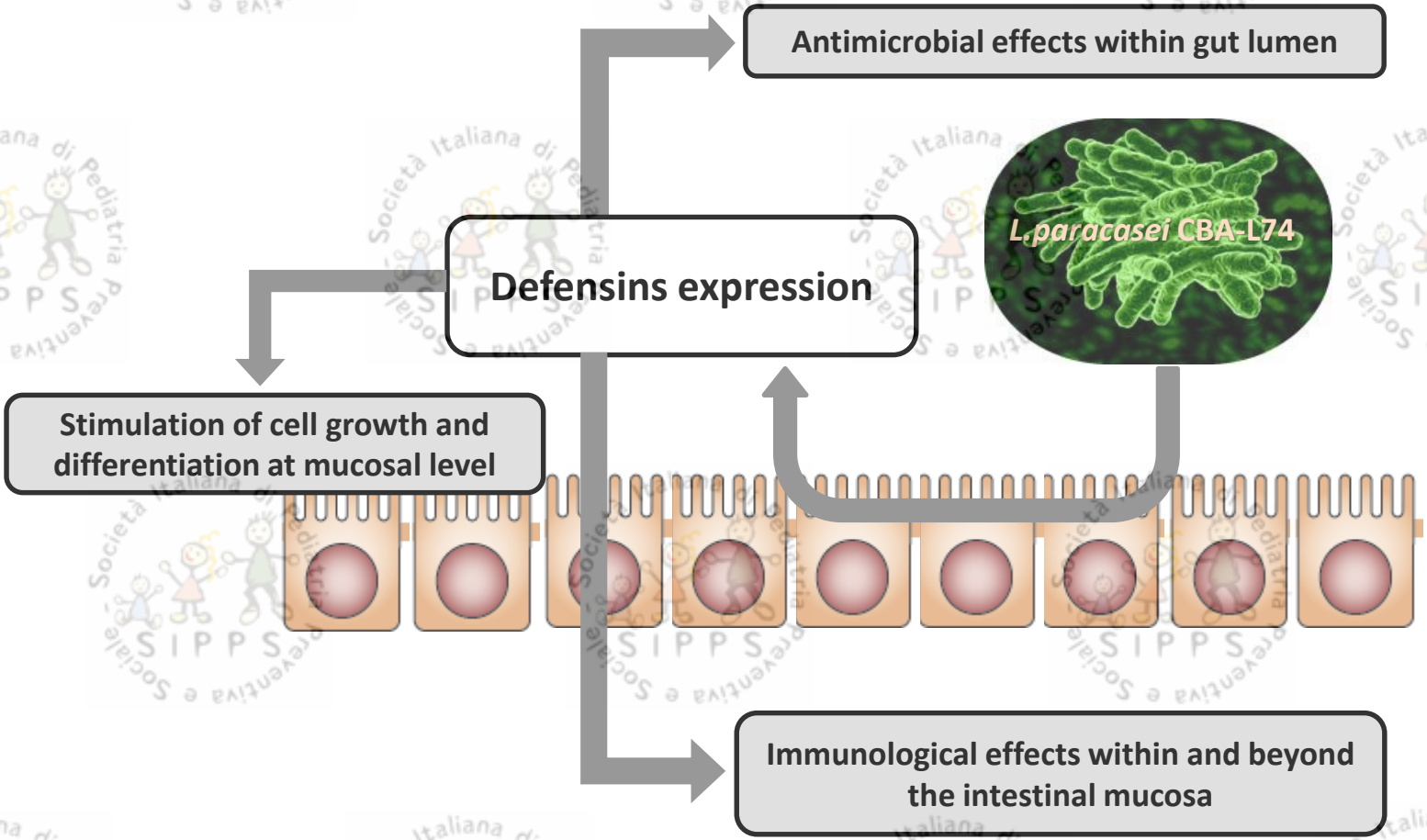
Anti-inflammatory effects





*MF dose:11.5 mg/ml

Mechanisms of action



Modified from Berni Canani R. et al. CML–Gastroenterology 2012


Argonne National Laboratory campus

Prof. C.Nagler – U.Chicago, IL
Dr. D.Antonopoulos – ANL, IL
Prof. Jack Gilbert – ANL, IL and U.Chicago

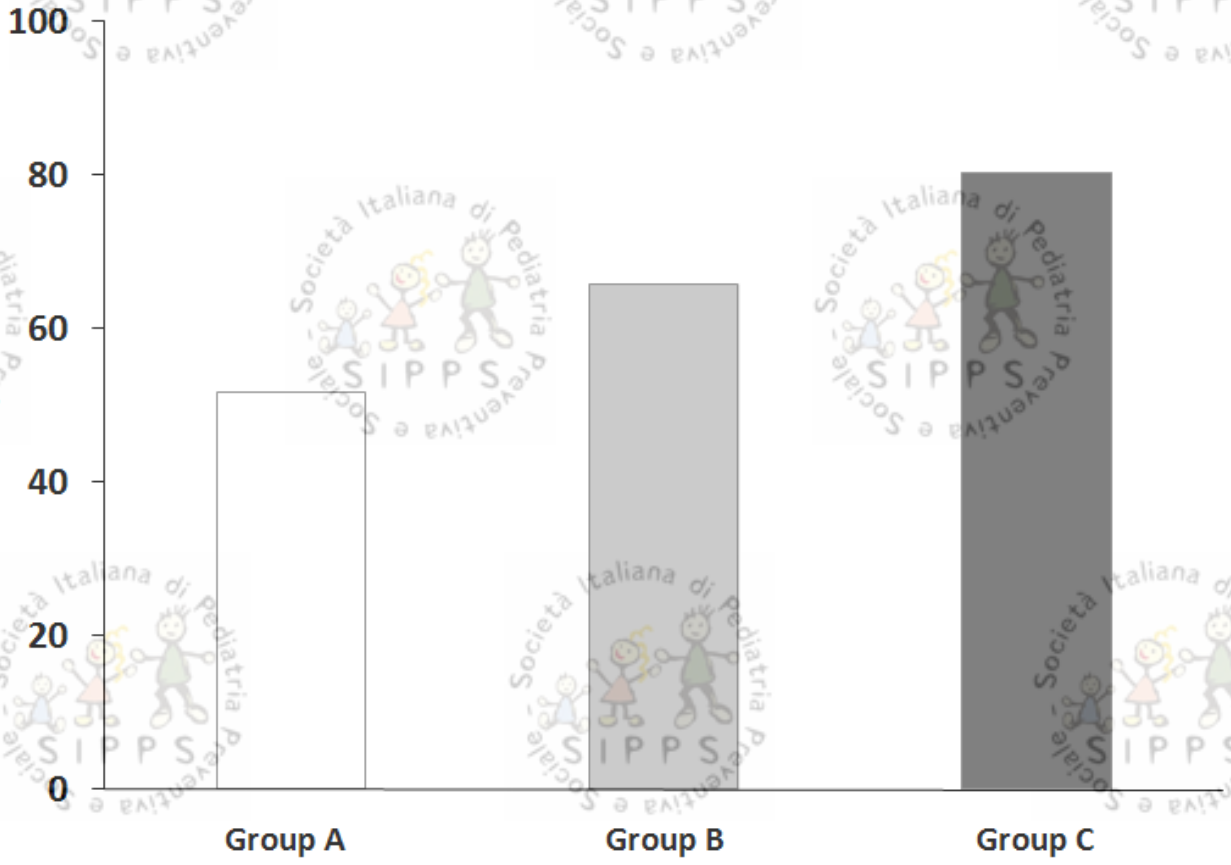
- No significant effect on OTUs count, Shannon diversity Index, Bacterial load (16s RNA copies/gr of stool)
- Significant increase in Firmicutes phyla in FM-treated subjects
- Significant increase of a particular OTU falling in Bifidobacterium species in FM>FR-treated subjects
- Significant increase in Lachnospiraceae in FM>FR-treated subjects
- Eubacterium increase in FR-treated children

Fermented Functional Foods

- Positive influence on gut microbiota
- Intestinal discomfort/GER
- Immune response to vaccinations
- Intestinal sIgA production
- Thymus size
- Acute gastroenteritis prevention
- CMP sensitization
- Allergy prevention

Mullie C, et al. Pediatr Res 2004
Roy P et al. Arch Ped 2004
Thibault H, et al. JPGN 2004
Indrio F, et al. Pediatr Res 2007
Chouraqui JP, et al. Am J Clin Nutr 2008
Campeotto F, et al. Br J Nutr 2011
Morrisset M, et al. Eur J Clin Nutr 2011
Granier A et al. Pediatr Res 2011
Van de Heijning BJ et al. Nutrients 2014
Szajewska H et al. Eur J Pediatr 2015

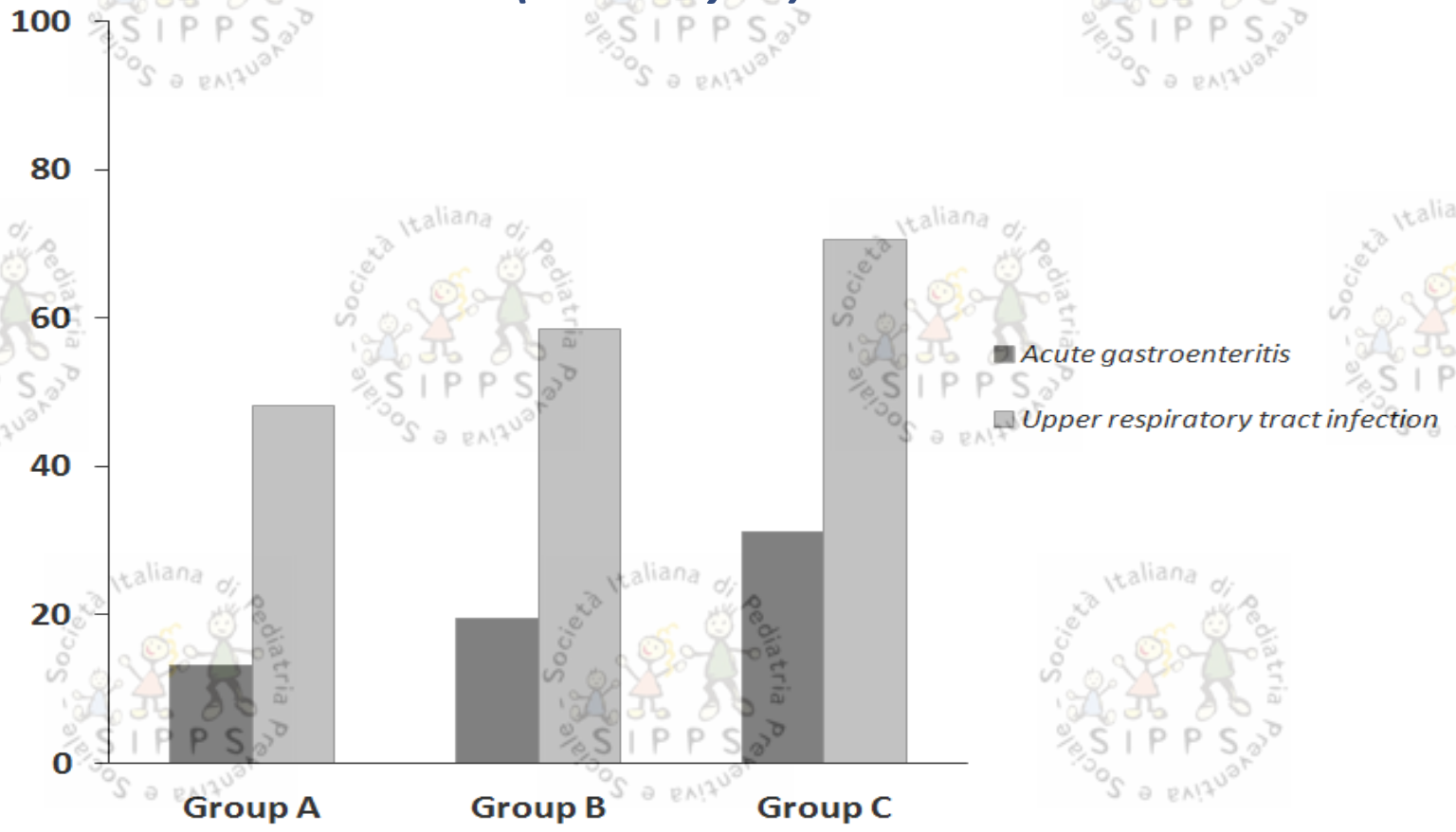
Proportion of children presenting at least one common infectious disease *(ITT analysis)*



Disease	Group A vs Group B *p*	Group A vs Group C *p*	Group B vs Group C *p*
CID, n (%)	*0.021*	*<0.0001*	*0.010*

Proportion of children presenting at least one episode of acute gastroenteritis or at least one episode of upper respiratory tract infection

(PP analysis)



Disease	Group A vs Group B *p*	Group A vs Group C *p*	Group B vs Group C *p*
Acute gastroenteritis, n (%)	*0.169*	*<0.0001*	*0.038*
Upper respiratory tract infection, n (%)	*0.100*	*<0.0001*	*0.052*

Common infectious diseases observed during the study period

Disease	Group A	Group B	Group C	Group A vs Group B *p*	Group A vs Group C *p*	Group B vs Group C *p*
Acute gastroenteritis, n (%) [number of episodes]	18 (13.1) [21]	23 (19.5) [26]	38 (31.1) [47]	*0.169*	*<0.0001**	*0.038*
Rhinitis, n (%) [number of episodes]	19 (13.9) [23]	31 (26.3) [47]	35 (28.7) [46]	*0.013*	*0.003**	*0.675*
Otitis, n (%) [number of episodes]	3 (2.2) [3]	5 (4.2) [5]	18 (14.8) [31]	*0.477*	*<0.0001**	*0.006**
Pharyngitis, n (%) [number of episodes]	21 (15.3) [28]	41 (34.7) [57]	53 (43.4) [84]	*<0.0001**	*<0.0001**	*0.168*
Laringitis, n (%) [number of episodes]	9 (6.6) [10]	11 (9.3) [13]	22 (18) [40]	*0.415*	*0.005**	*0.05*
Tracheitis, n (%) [number of episodes]	36 (26.3) [44]	32 (27.1) [41]	49 (40.2) [76]	*0.880*	*0.018*	*0.033*

**the p-values remain significant after Bonferroni correction*

Study design

Visit 1 (Enrollment) → **Visit 2 (1 months)** → **Visit 3 (2 months)** → **Visit 4 (3 months)**

- Anamnestic and clinical evaluation
- Informed consent
- Randomization
- Start study products
- Clinical diary instruction
- Stool sampling

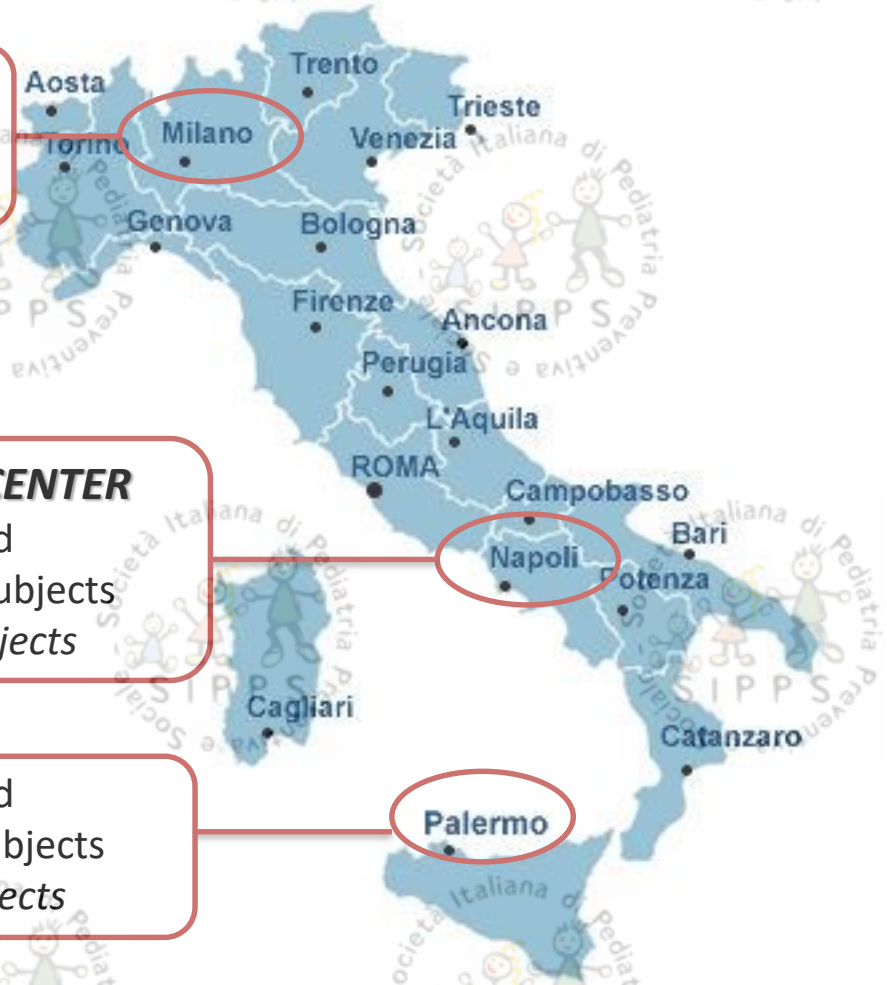
- Clinical evaluation
- Clinical diary checking
- Compliance evaluation

- Clinical evaluation
- Clinical diary collection
- Compliance evaluation
- Stool sampling

A visit by the FP was planned whenever it was necessary because infectious diseases or other morbidities

Centers

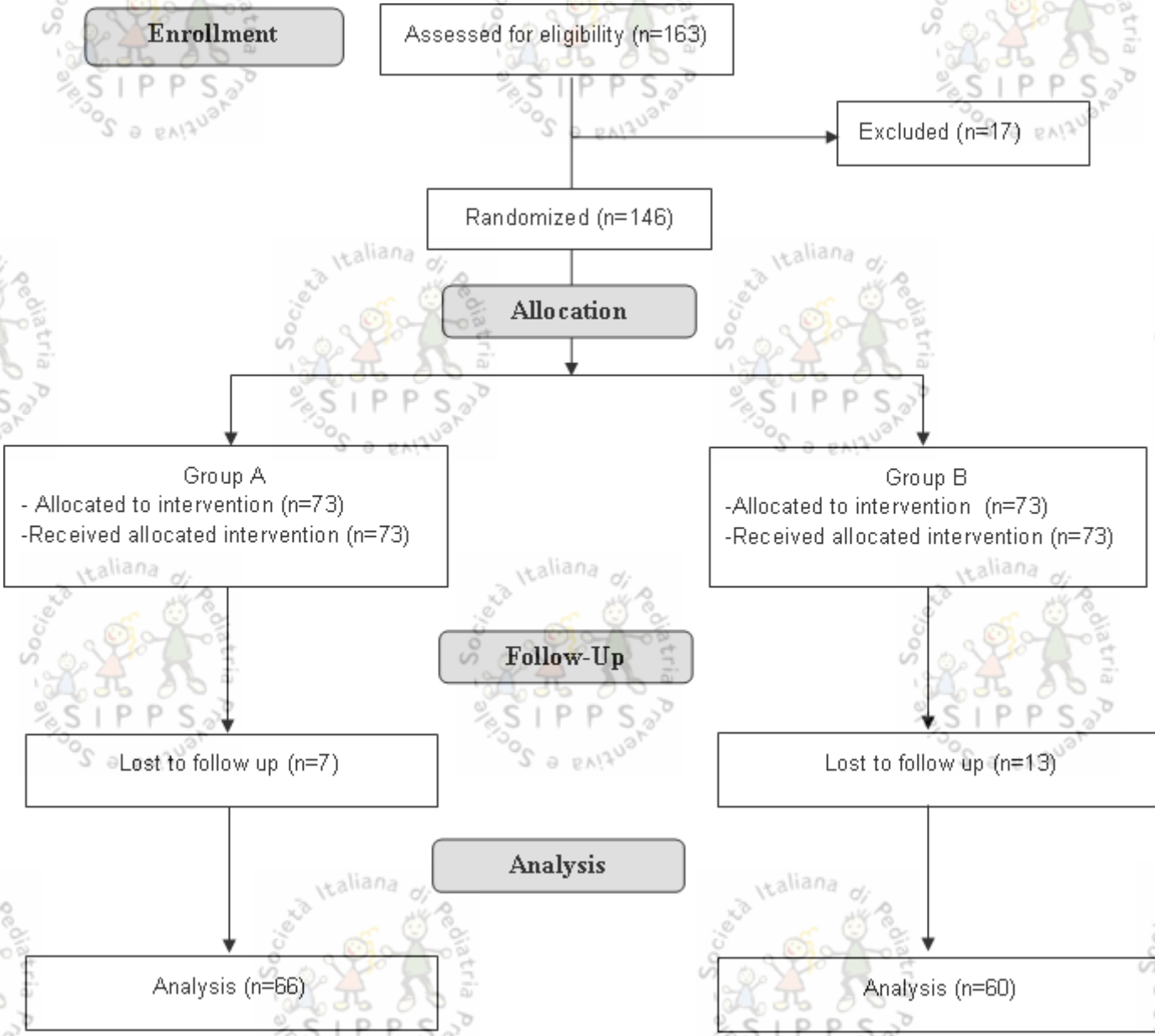
5 FP involved
17 randomized subjects
2 drop-out subjects



COORDINATOR CENTER
7 FP involved
105 randomized subjects
13 drop-out subjects

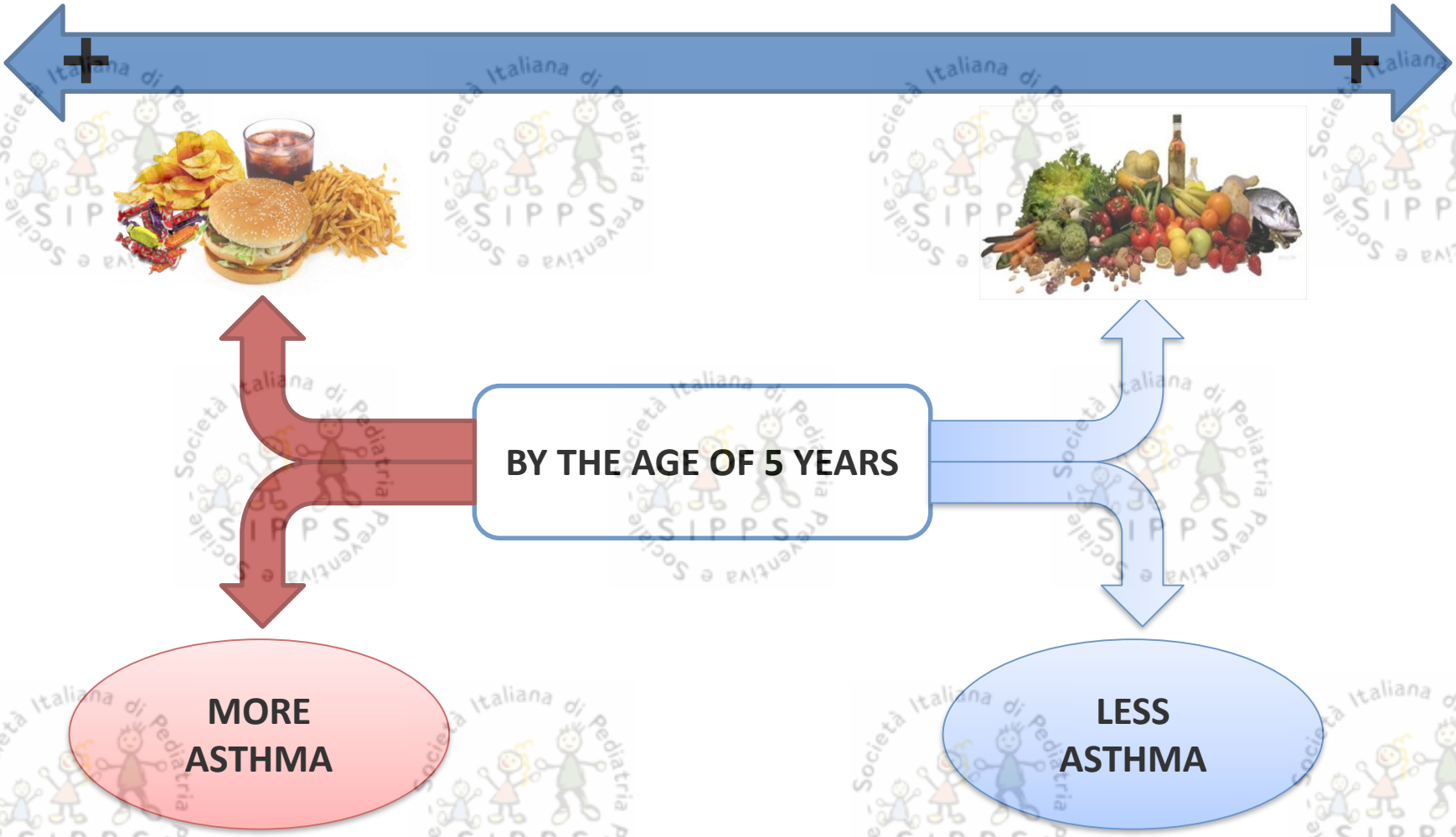
7 FP involved
24 randomized subjects
5 drop-out subjects

Flow of children through the study



Prenatal and childhood Mediterranean diet and the development of asthma and allergies in children

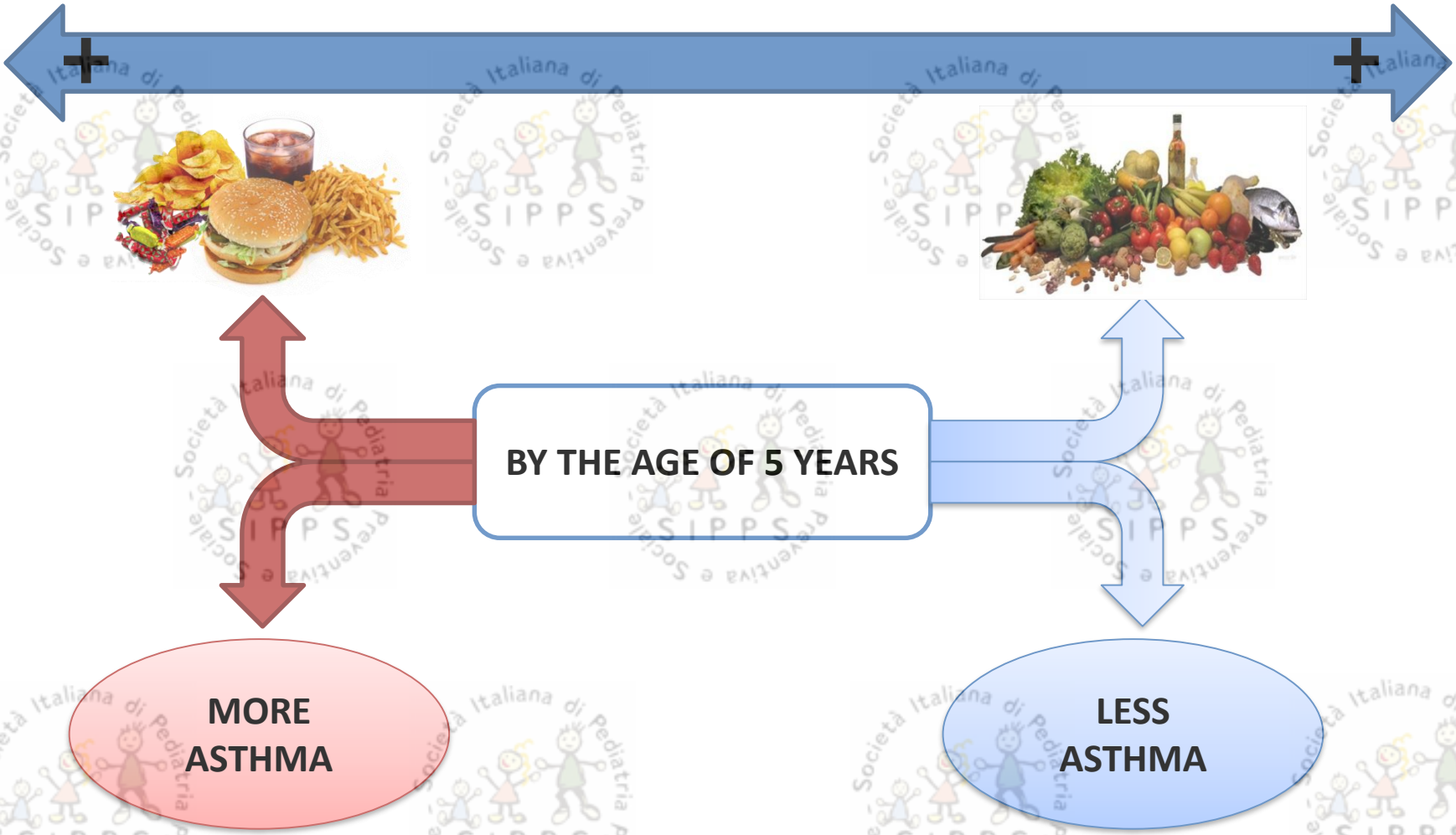
Leda Chatzi[1,*] and Manolis Kogevinas[1,2,3,4]



Public Health Nutrition: 12(9A), 1629–1634

Risk of asthma and allergic outcomes in the offspring in relation to maternal food consumption during pregnancy: A Finnish birth cohort study

Maijaliisa Erkkola[1], Bright I. Nwaru[2], Minna Kaila[3], Carina Kronberg-Kippilä[4], Jorma Ilonen[5,6], Olli Simell[7], Riitta Veijola[8], Mikael Knip[3,9,10] & Suvi M. Virtanen[2,4,11]

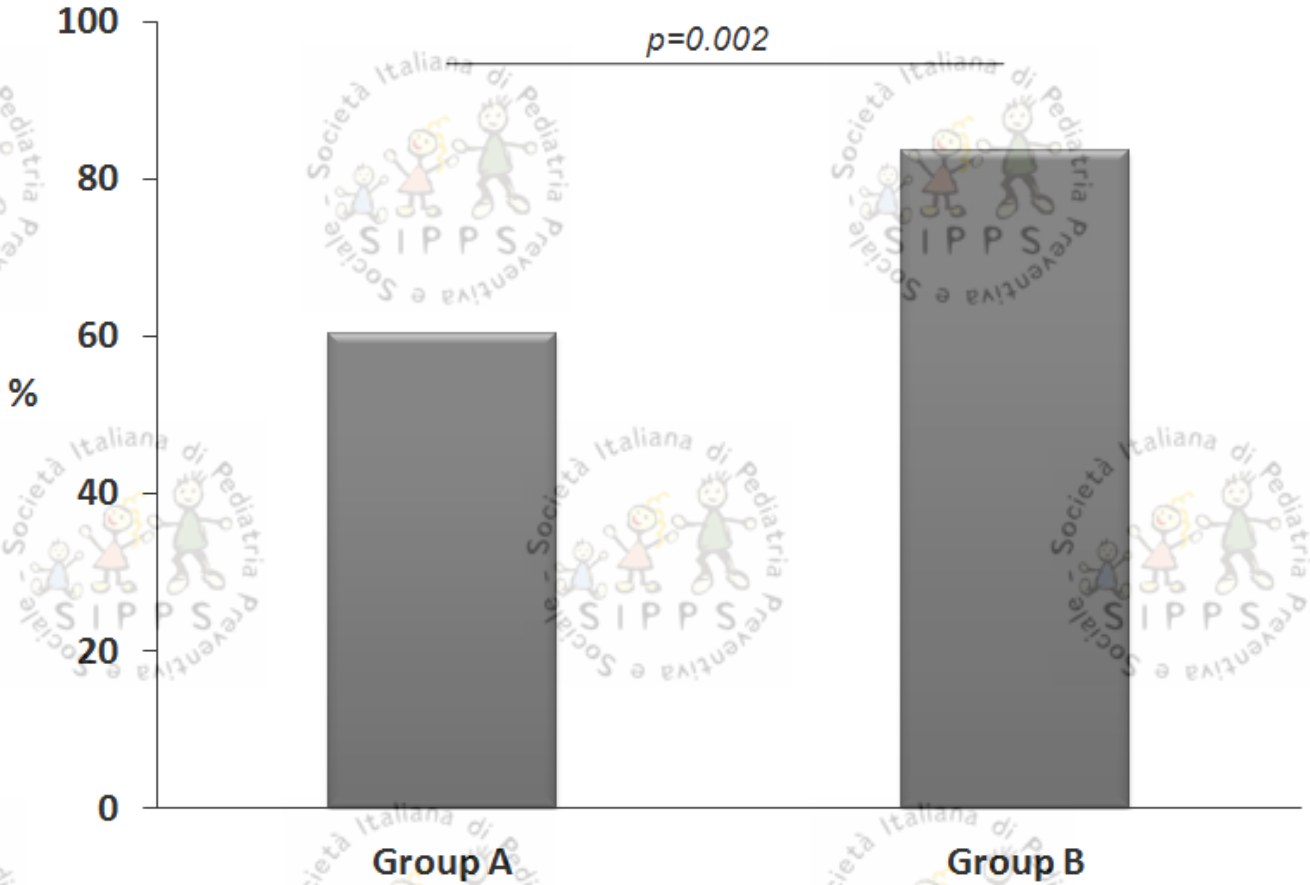


Pediatric Allergy and Immunology 23 (2012) 186–194

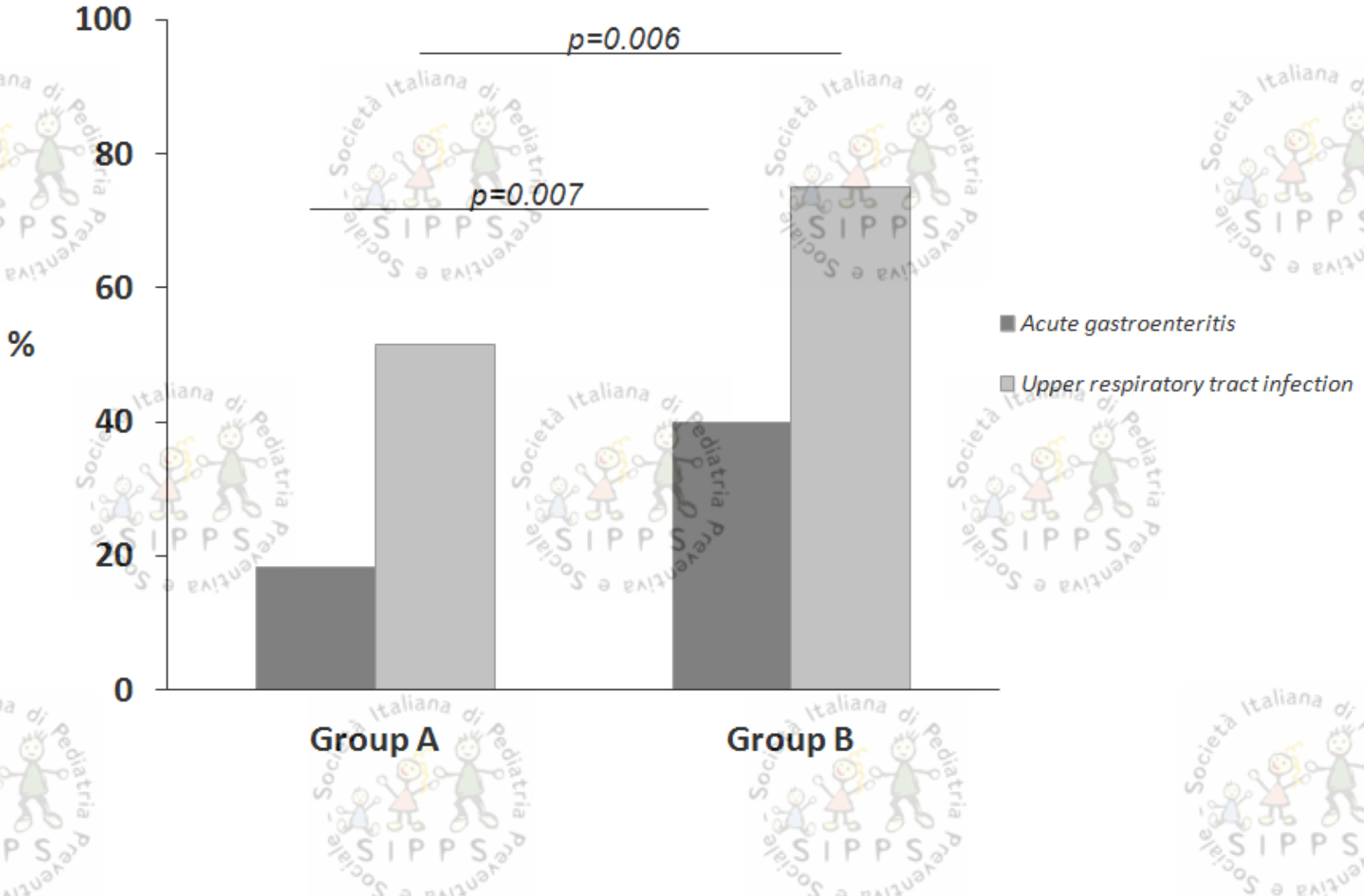
Mean features of the study population at enrollment

	Group A *n=73*	Group B *n=73*
Male, n (%)	39 (53.4)	45 (61.6)
Age, months (±SD)	32.5 (9.7)	33.7 (8.6)
Weight, kg (±SD)	14.8 (3.2)	15 (3)
Height, cm (±SD)	92 (8.1)	94 (7.6)
Breastfeeding, n (%)	50 (68.5)	41 (56.2)
Duration of breastfeeding, months (±SD)	7.1 (6)	7 (8.5)
Age at schooling, months (±SD)	23.2 (9)	25.9 (8.5)
Siblings, n (%)	60 (82.2)	52 (71.2)
N. of siblings, (±SD)	1.4 (0.6)	1.4 (0.6)
Passive smoking, n (%)	28 (38.4)	30 (41.1)

Proportion of children presenting at least one common infectious disease *(ITT analysis)*



Proportion of children presenting at least one episode of acute gastroenteritis or at least one episode of upper respiratory tract infection *(PP analysis)*

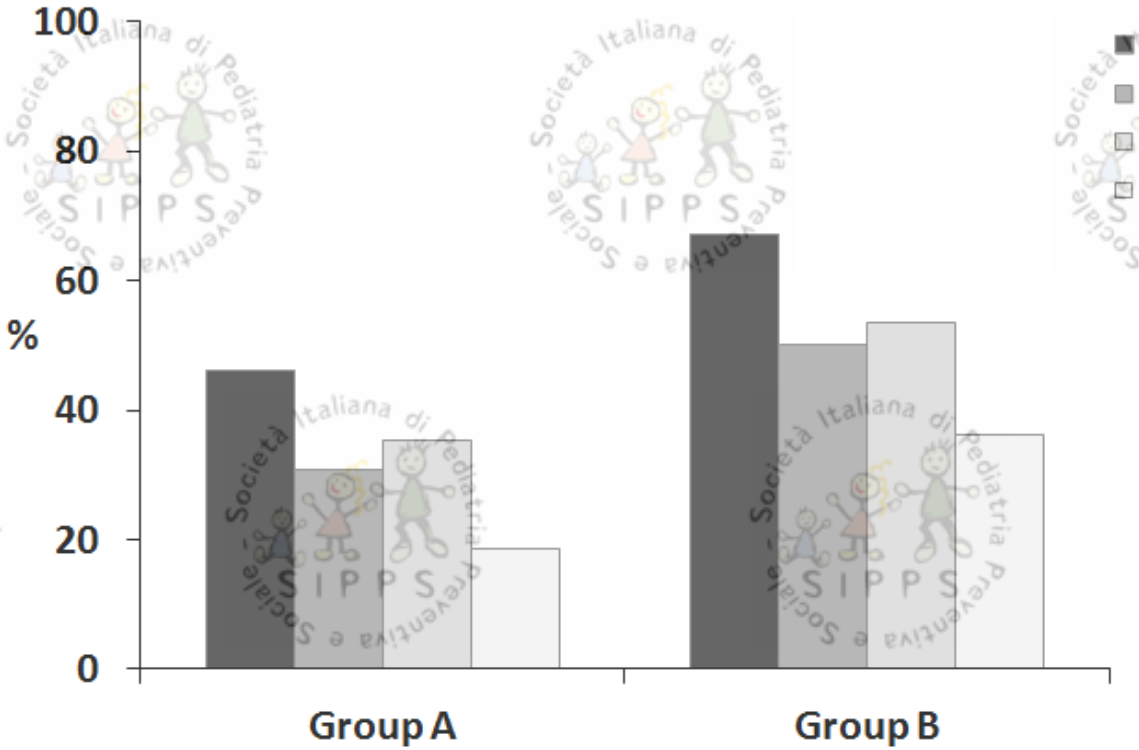


Common infectious diseases observed during the study period

Disease	Group A	Group B	*p*
Acute gastroenteritis, n (%) [number of episodes]	12 (18.2) [19]	24 (40) [28]	*0.007*
Rhinitis, n (%) [number of episodes]	22 (33.3) [44]	24 (40) [50]	*0.438*
Otitis, n (%) [number of episodes]	8 (12.1) [11]	13 (21.7) [17]	*0.151*
Pharyngitis, n (%) [number of episodes]	13 (19.7) [22]	25 (41.7) [30]	*0.007*
Laringitis, n (%) [number of episodes]	6 (9.1) [7]	14 (23.3) [14]	*0.029*
Tracheitis, n (%) [number of episodes]	11 (16.7) [16]	19 (31.7) [30]	*0.048*

Rate of subjects requiring medication use during the study period*

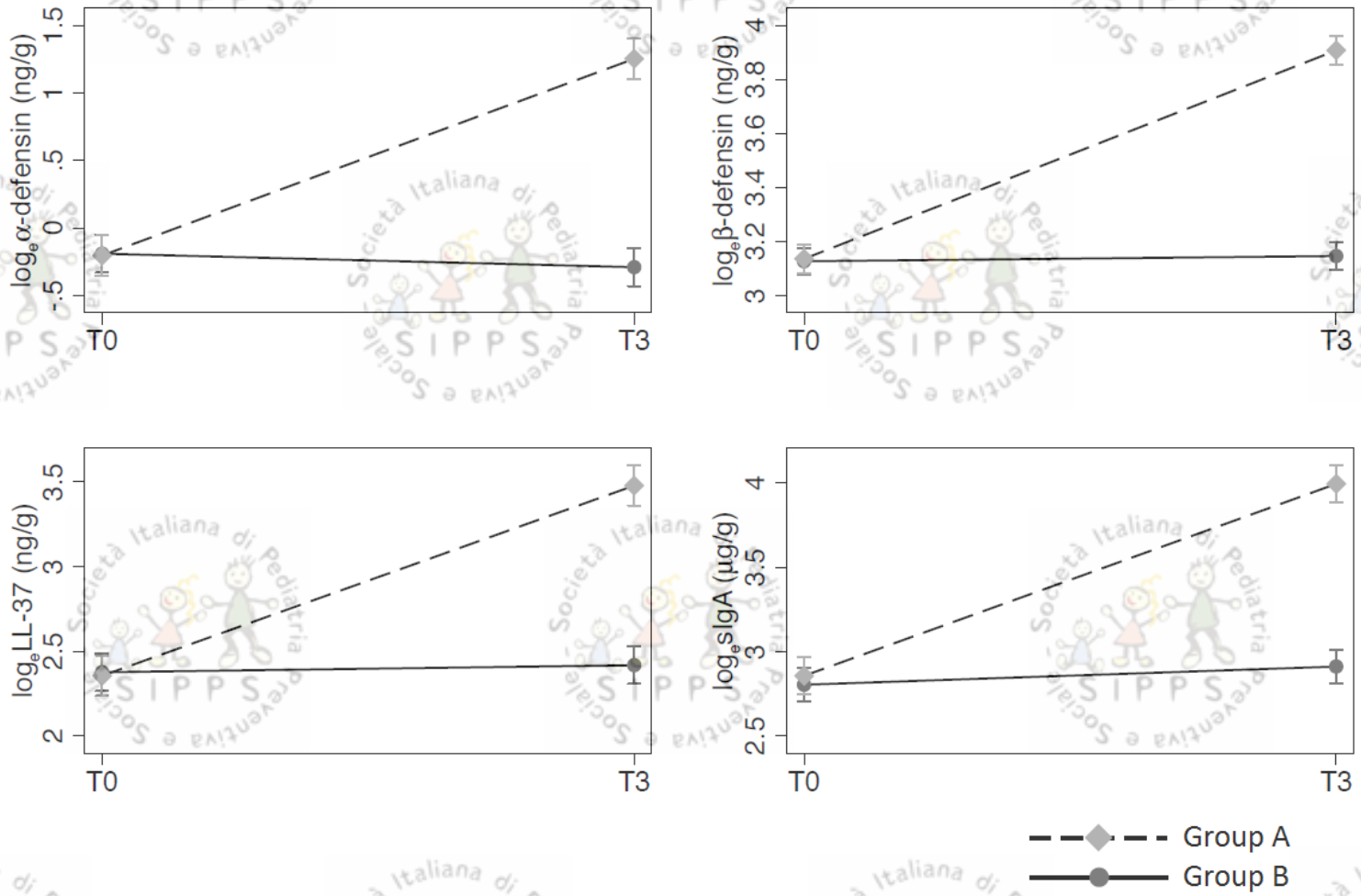
(PP analysis)



	p
At least one medication course	*0.019*
Antibiotics	*0.03*
Antipyretics	*0.044*
Steroids	*0.027*

**the analysis has been made only on 123 out 126 subjects for incomplete data for 3 subjects*

Determination of innate and acquired immunity biomarkers at enrollment and after 3-month treatment in children evaluated in the two study groups



Values are means and 95% confidence intervals from random effect linear regression with correction for baseline

Road Map

- Biological activities/mechanisms of action characterization
- Molecular characterization (lipids, nucleotides, small heat-stable peptides, etc.)
- New clinical applications
- Well designed RCTs

Cow's milk and fermented rice with *L.paracasei* CBA L74 prevent common winter infectious diseases in children

Nocerino R, et al. 2015 submitted

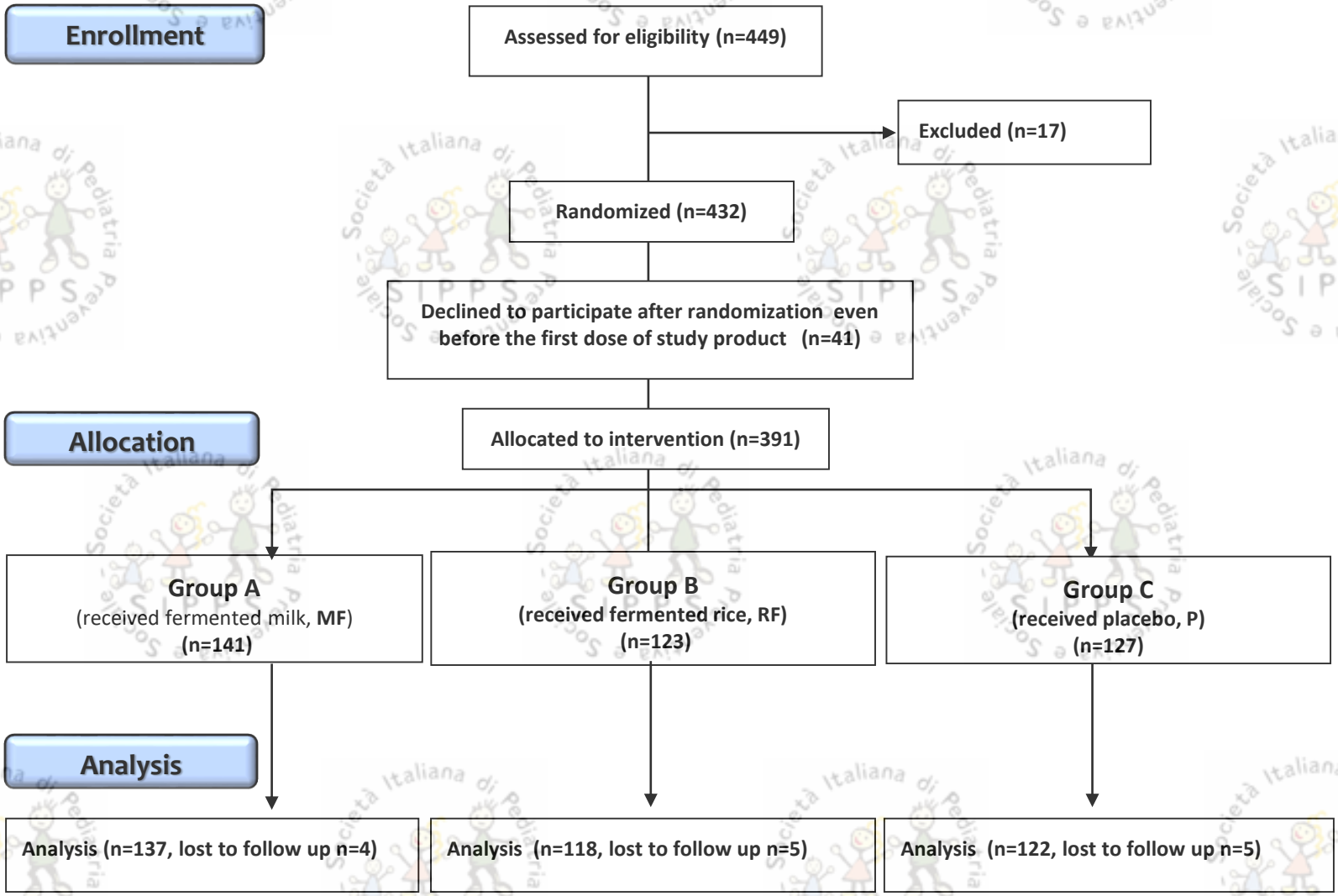
Visita 1 (arruolamento) → **Visita 2 (1 m)** → **Visita 3 (2 m)** → **Visita 4 (3 m)**

- Valutaz. anamestica e clinica
- Consenso informato
- Randomizzazione
- Consegna dei prodotti
- Consegna diario clinico
- Raccolta feci

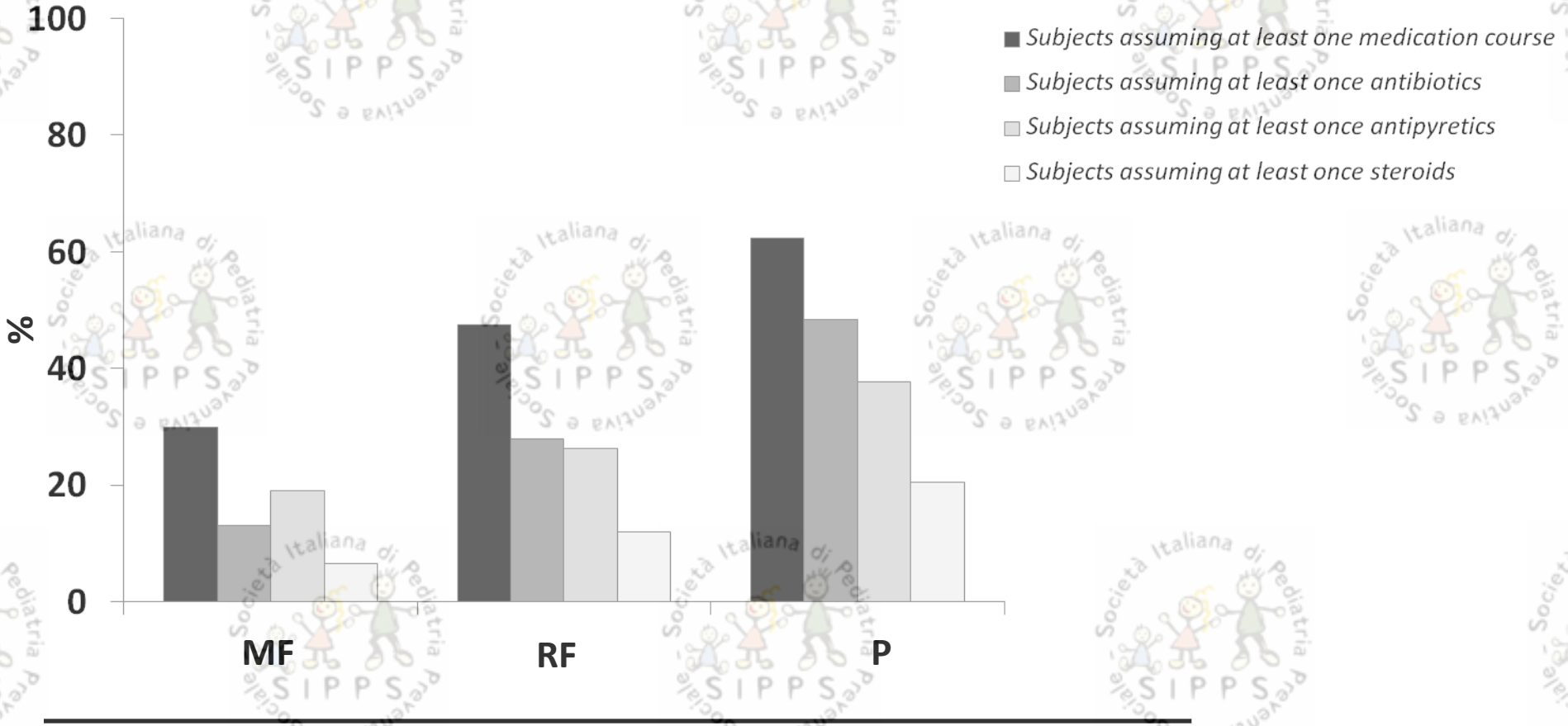
- Valutazione clinica
- Raccolta e valutazione del diario clinico
- Valutazione aderenza

- Valutazione clinica
- Raccolta e valutazione del diario clinico
- Valutazione aderenza
- Raccolta feci

Flow of children through the study



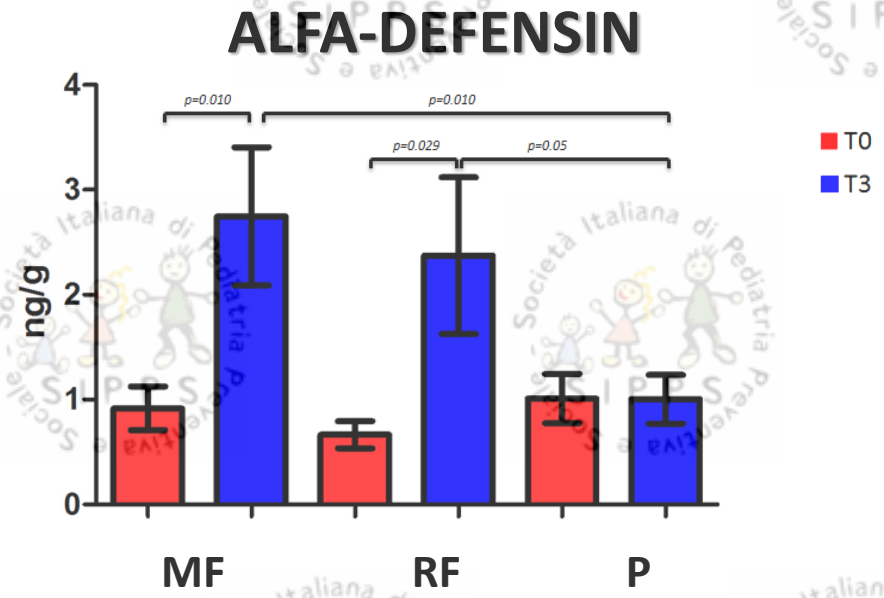
Rate of subjects requiring medication use

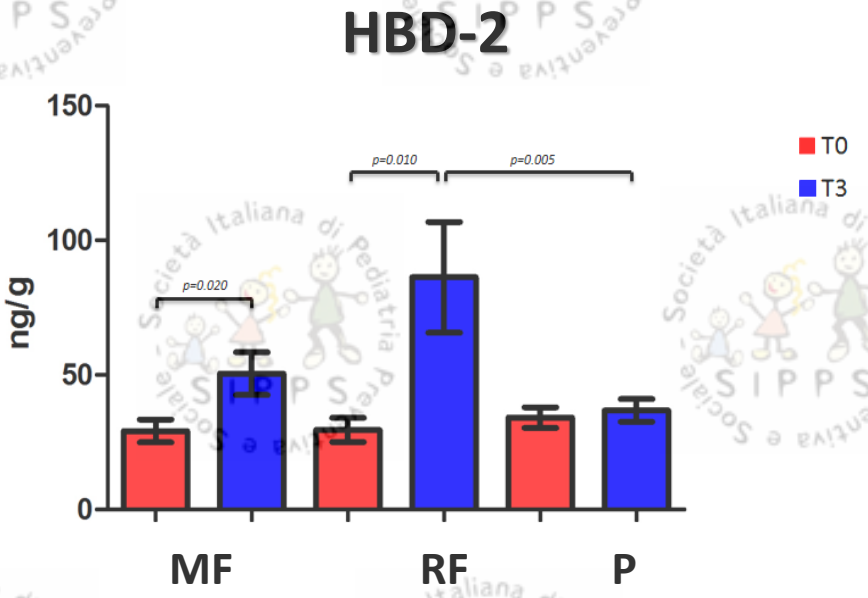


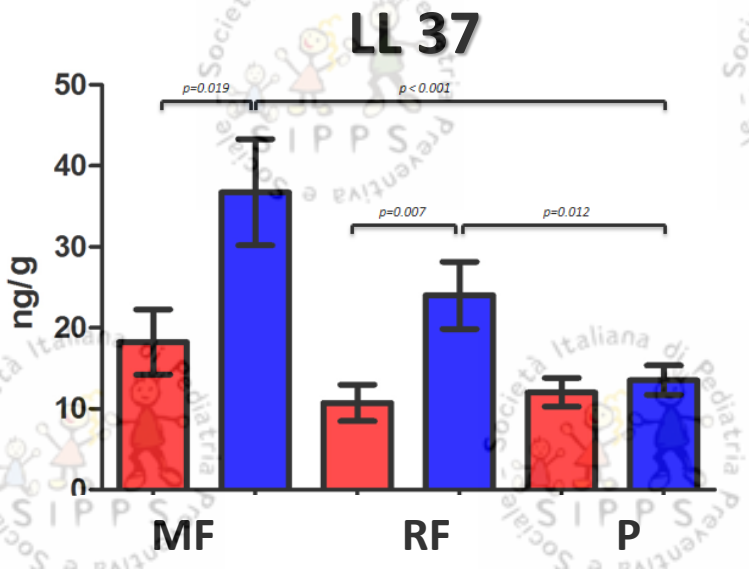
	MF vs RF *p*	RF vs P *p*	MF vs P *p*
At least one medication course	*0.004**	*<0.0001**	*0.021*
Antibiotics	*0.003**	*<0.0001**	*0.001**
Antipyretics	*0.163*	*0.001**	*0.058*
Steroids	*0.141*	*0.001**	*0.070*

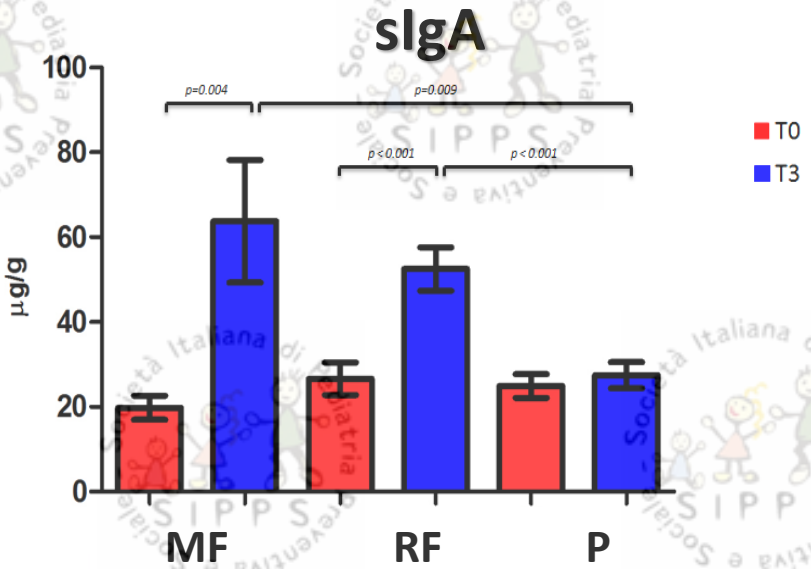
**the p-values remain significant after Bonferroni correction*

Milk and Rice fermented with *L.paracasei* CBA L74 elicit a potent immunomodulation

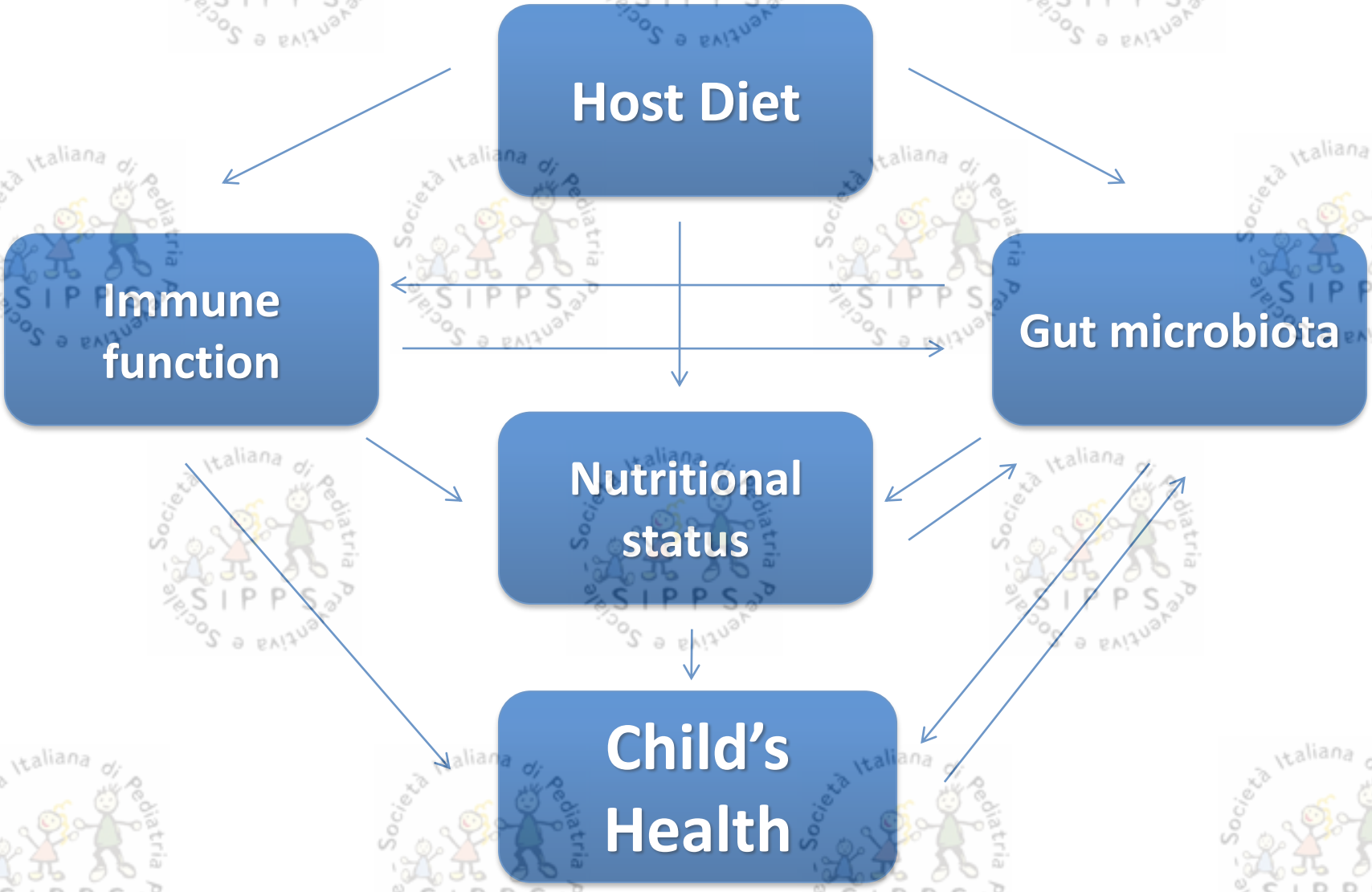








Nutritional Immunology



Strengths of Fermented Functional Foods

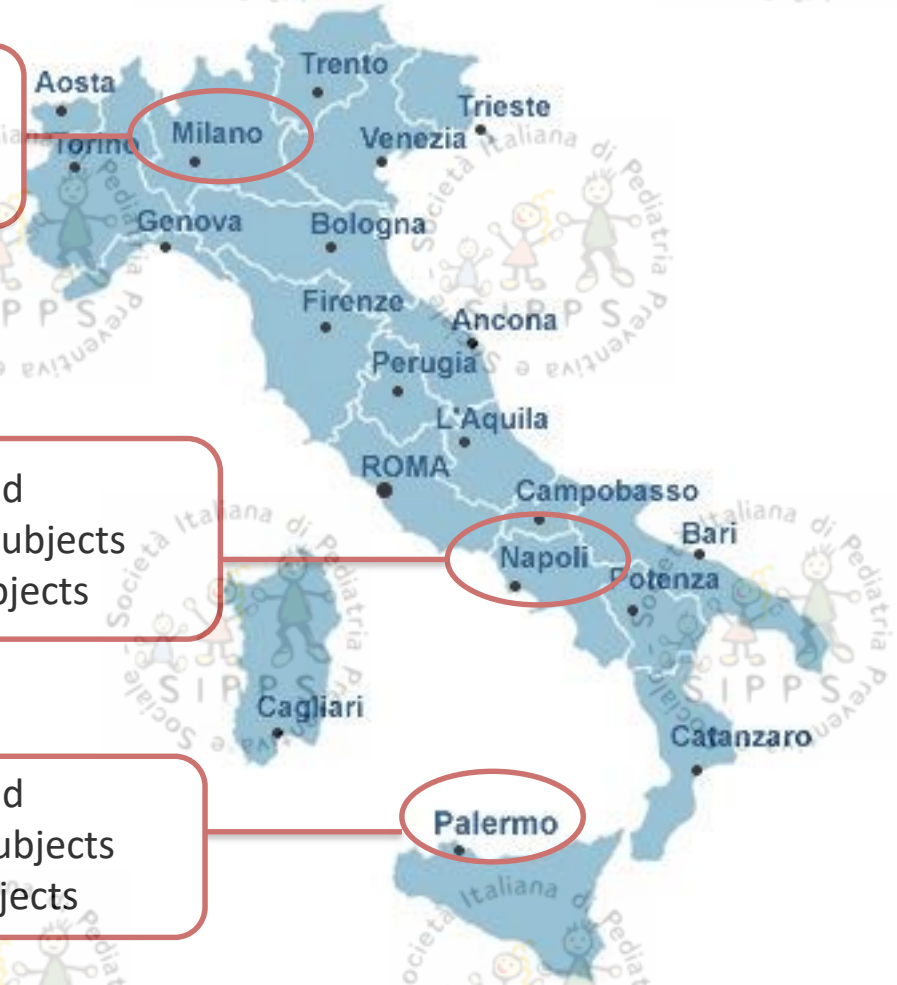
- Preparations contain safe probiotic
- No living organisms
- No risk of bacterial translocation
- Easy storage
- Reproducibility (precise monitoring of the technological process)

Centers

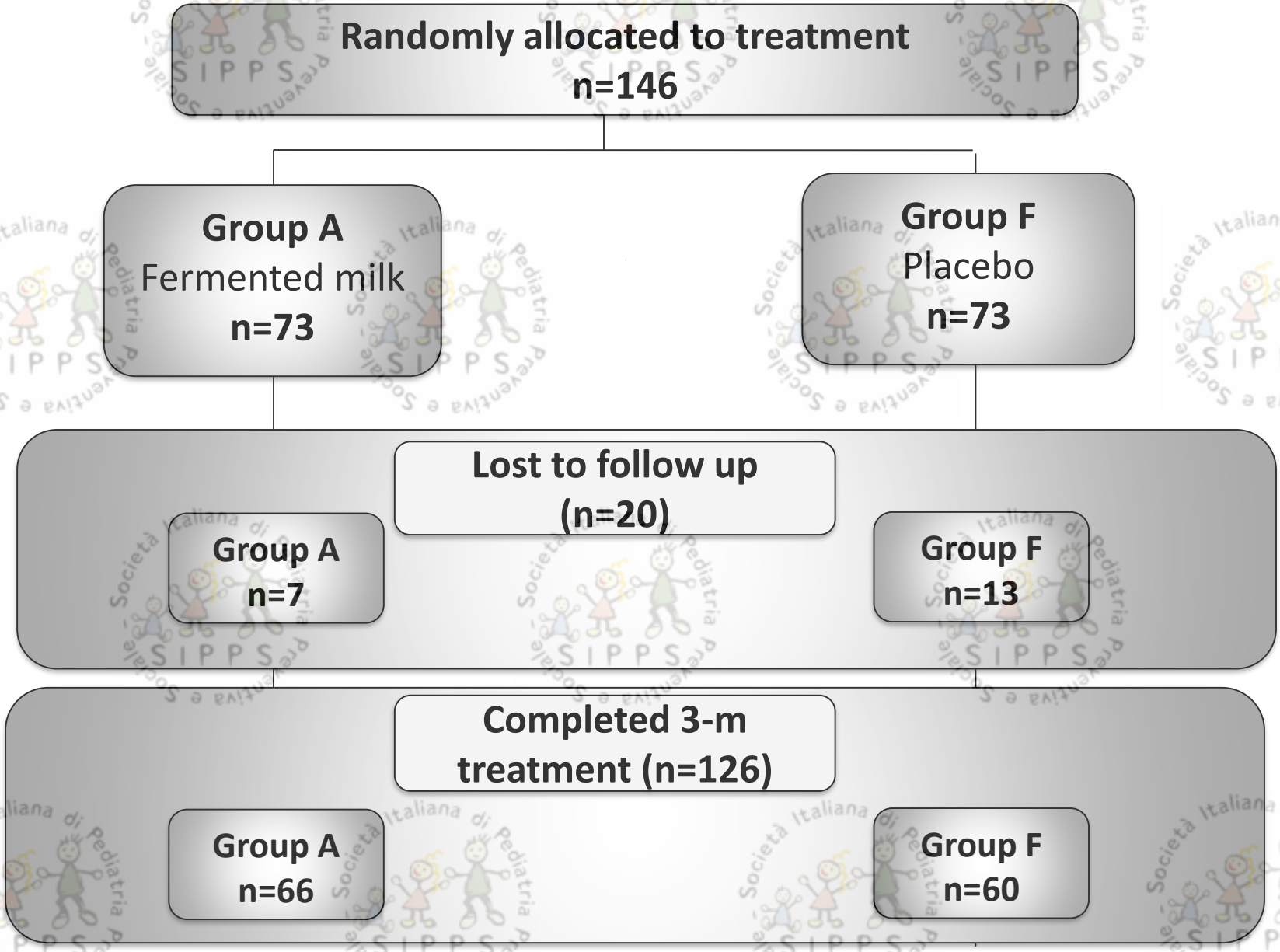
5 FP involved
17 randomized subjects
2 drop-out subjects

7 FP involved
105 randomized subjects
13 drop-out subjects

7 FP involved
24 randomized subjects
5 drop-out subjects



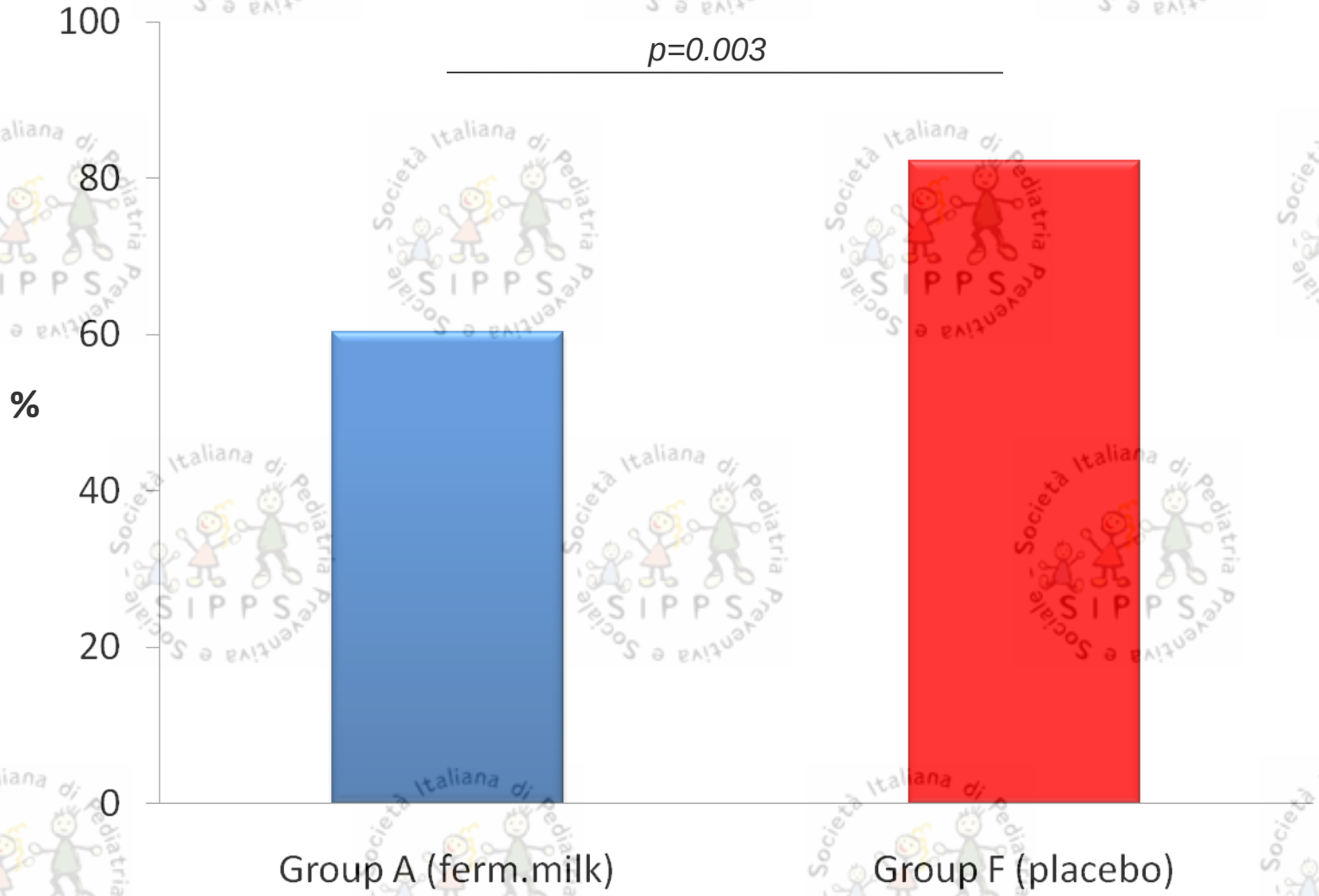
Flow of participants throughout the study



Main features of the study population

	Group A	Group F
Male, n (%)	37 (53.6)	32 (46.4)
Age, months (±SD)	32.89 (9.66)	33.6 (8.53)
Weight, kg (±SD)	14.85 (3.21)	14.9 (3)
Height, cm (±SD)	92.35 (8.17)	93.42 (7.7)
Breastfeeding, n (%)	48 (69.6)	38 (54.3)
Duration of breastfeeding, months (±SD)	7.14 (6)	6.6 (8.38)
Age at schooling, months (±SD)	23.49 (9.05)	25.87 (8.49)
Siblings, n (%)	56 (81.2)	50 (71.4)
N. of siblings, (±SD)	1.37 (0.61)	1.34 (0.55)
Passive smoking, n (%)	26 (41.4)	29 (37.7)

Rate of children presenting at least one infectious disease *(ITT analysis)*



Main features of the study population

	MF *n=137*	RF *n=118*	P *n=122*
Male, n (%)	70 (51.1)	61 (51.7)	62 (50.8)
Age, months (±SD)	32.32 (10.23)	31.28 (10.95)	33.79 (9.17)
Weight, kg (±SD)	14.55 (2.75)	14.53 (2.74)	14.88 (2.93)
Height, cm (±SD)	93.48 (8.99)	92.92 (9.04)	94.47 (7.22)
Breastfeeding, n (%)	97 (70.8)	86 (72.9)	94 (77)
Duration of breastfeeding, months (±SD)	7.33 (5.63)	6.38 (3.98)	6.51 (5.03)
Age at schooling, months (±SD)	12.90 (2.56)	12.55 (2.32)	12.79 (2.34)
Siblings, n (%)	106 (77.4)	92 (78)	96 (78.7)
N. of siblings, (±SD)	1.30 (0.57)	1.33 (0.58)	1.40 (0.57)
Passive smoking, n (%)	64 (46.7)	56 (47.5)	56 (45.9)

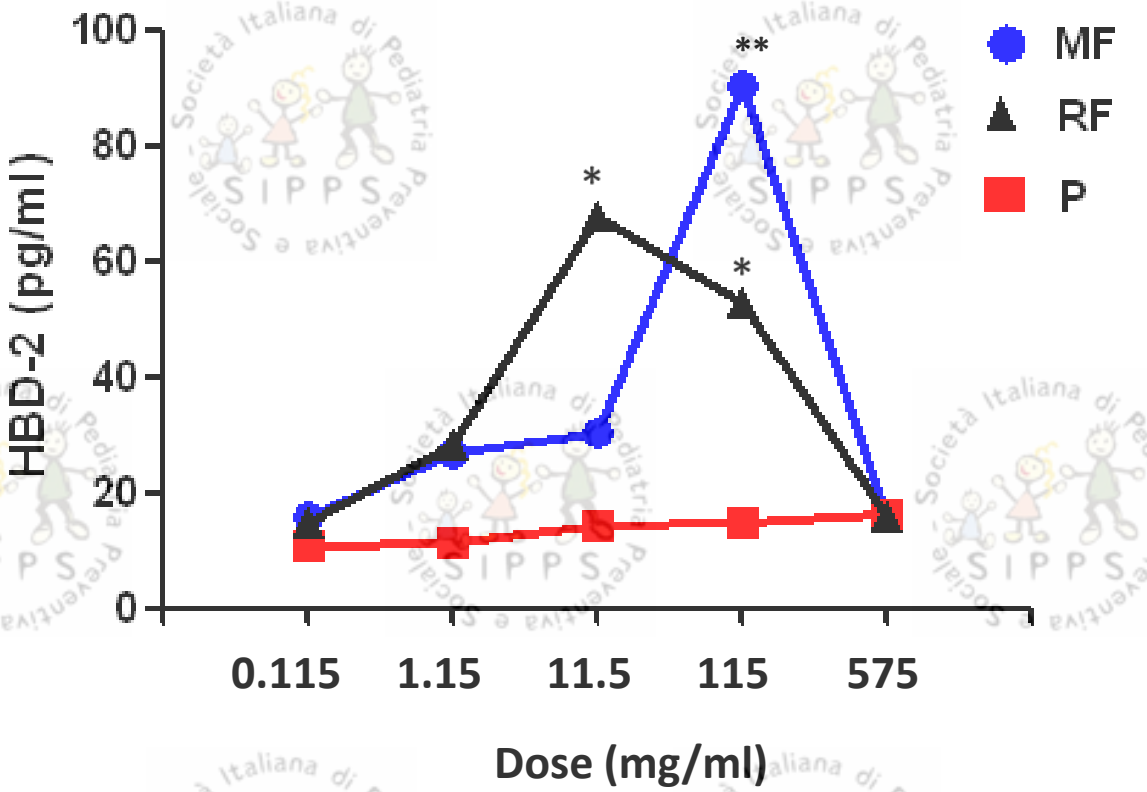
Nocerino R, et al. 2015 submitted

Number of children experienced at least one CWID during the study period and total number of episodes

Disease	MF	RF	P	MF vs RF *p*	MF vs P *p*	RF vs P *p*
Acute gastroenteritis, n (%) [n. episodes]	18 (13.1) [21]	23 (19.5) [26]	38 (31.1) [47]	*0.169*	*<0.0001**	*0.038*
Rhinitis, n (%) [n. episodes]	19 (13.9) [23]	31 (26.3) [47]	35 (28.7) [46]	*0.013*	*0.003**	*0.675*
Otitis, n (%) [n. episodes]	3 (2.2) [3]	5 (4.2) [5]	18 (14.8) [31]	*0.477*	*<0.0001**	*0.006**
Pharyngitis, n (%) [n. episodes]	21 (15.3) [28]	41 (34.7) [57]	53 (43.4) [84]	*<0.0001**	*<0.0001**	*0.168*
Laryngitis, n (%) [n. episodes]	9 (6.6) [10]	11 (9.3) [13]	22 (18) [40]	*0.415*	*0.005**	*0.05*
Tracheitis, n (%) [n. episodes]	36 (26.3) [44]	32 (27.1) [41]	49 (40.2) [76]	*0.880*	*0.018*	*0.033*

**the p-values remain significant after Bonferroni correction*

Dose-dependency of the effect on HBD-2 synthesis in human enterocytes



*p<0.05, MF and RF vs P
**p<0.001 MF vs P

Study design

Visit 1 (Enrollment) → **Visit 2 (1 months)** → **Visit 3 (2 months)** → **Visit 4 (3 months)**

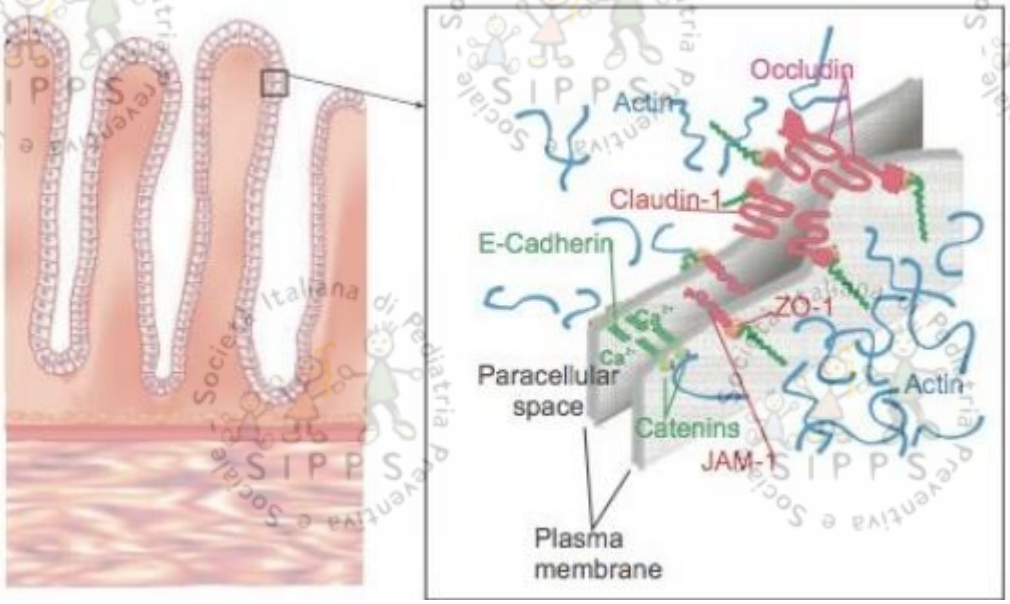
- Anamnestic and clinical evaluation
- Informed consent
- Randomization
- Start study products
- Clinical diary instruction
- Stool sampling

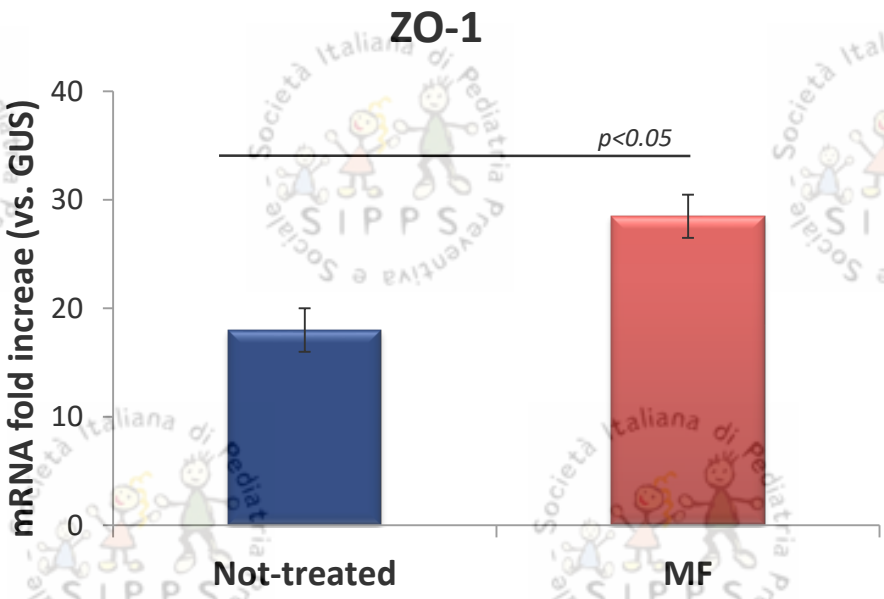
- Clinical evaluation
- Clinical diary checking
- Compliance evaluation

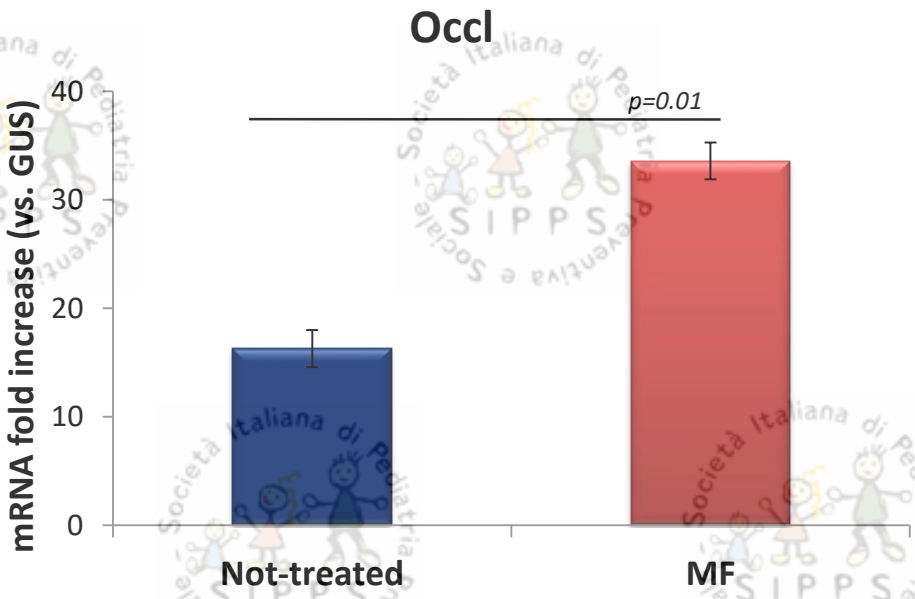
- Clinical evaluation
- Clinical diary collection
- Compliance evaluation
- Stool sampling

A visit by the FP was planned whenever it was necessary because infectious diseases or other morbidities

Effect on TJ expression

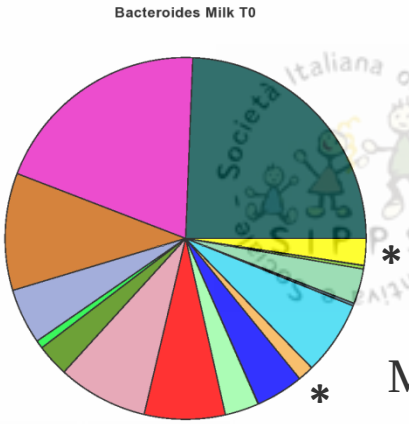




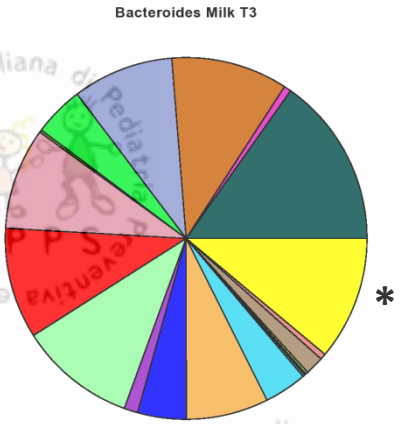


Berni Canani R, data on file

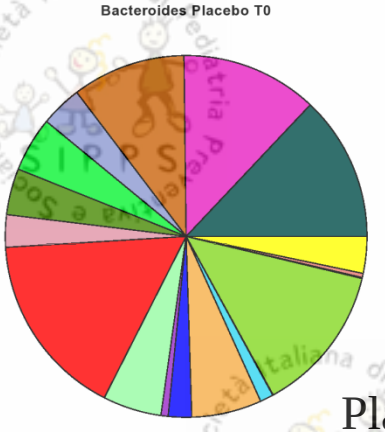
Bacteroides Oligotypes *



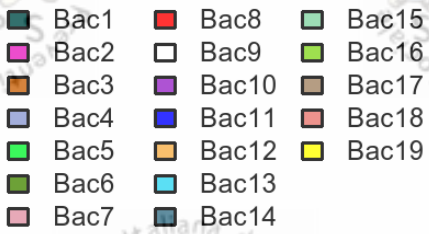
Milk t0

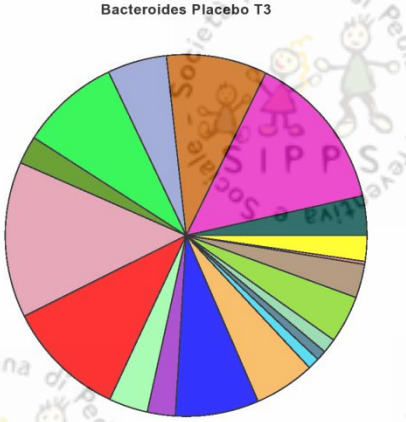


Milk t3



Placebo t0





Placebo t3

Bac 12 ↑ **in milk t3**

Bac 19 ↑ **in milk t3**

Bac 18 ↑ **in rice t3**

Bacteroides Oligotypes *

1_Bacteroides dorei	(419/420)	
2_Bacteroides fragilis strain NCTC 9343	(419/420)	
3_Bacteroides fragilis strain JMC 11019	(418/420)	
4_Bacteroides dorei strain 175	(420/420)	
5_Bacteroides fragilis ATCC 25285	(420/420)	
6_Bacteroides fragilis strain NCTC, ATCC, JMC	(419/420)	
7_Bacteroides uniformis strain JCM 5828	(417/420)	
8_Bacteroides vulgatus strain ATCC 8482	(419/420)	
9_Bacteroides caccae strain JCM 9498	(418/420)	
10_Bacteroides vulgatus strain ATCC 8482	(402/420)	
11_Bacteroides uniformis strain JCM 5828	(418/420)	
12_Bacteroides vulgatus strain ATCC 8482	**(420/420)**	↑ Milk treatment
13_Bacteroides caccae strain ATCC 43185	(419/420)	
14_Bacteroides dorei strain 175	(405/420)	
15_Bacteroides uniformis strain JCM 5828	(404/420)	
16_Bacteroides xylanisolvens strain XB1A	(419/420)	
17_Bacteroides vulgatus strain JCM 5826	(418/420)	
18_Bacteroides faecis strain MAJ27	**(419/420)**	
19_Bacteroides vulgatus strain JCM 5826	**(417/420)**	↑ Milk treatment

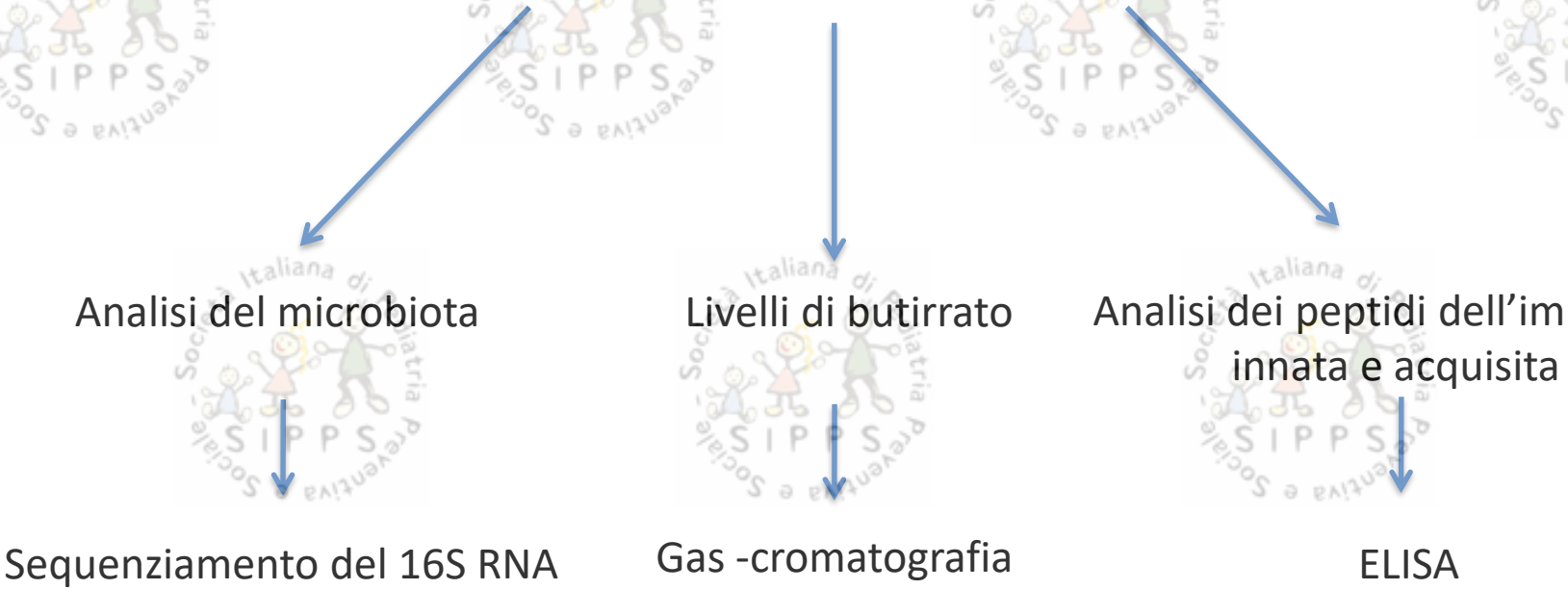
Faecalibacterium Oligotypes

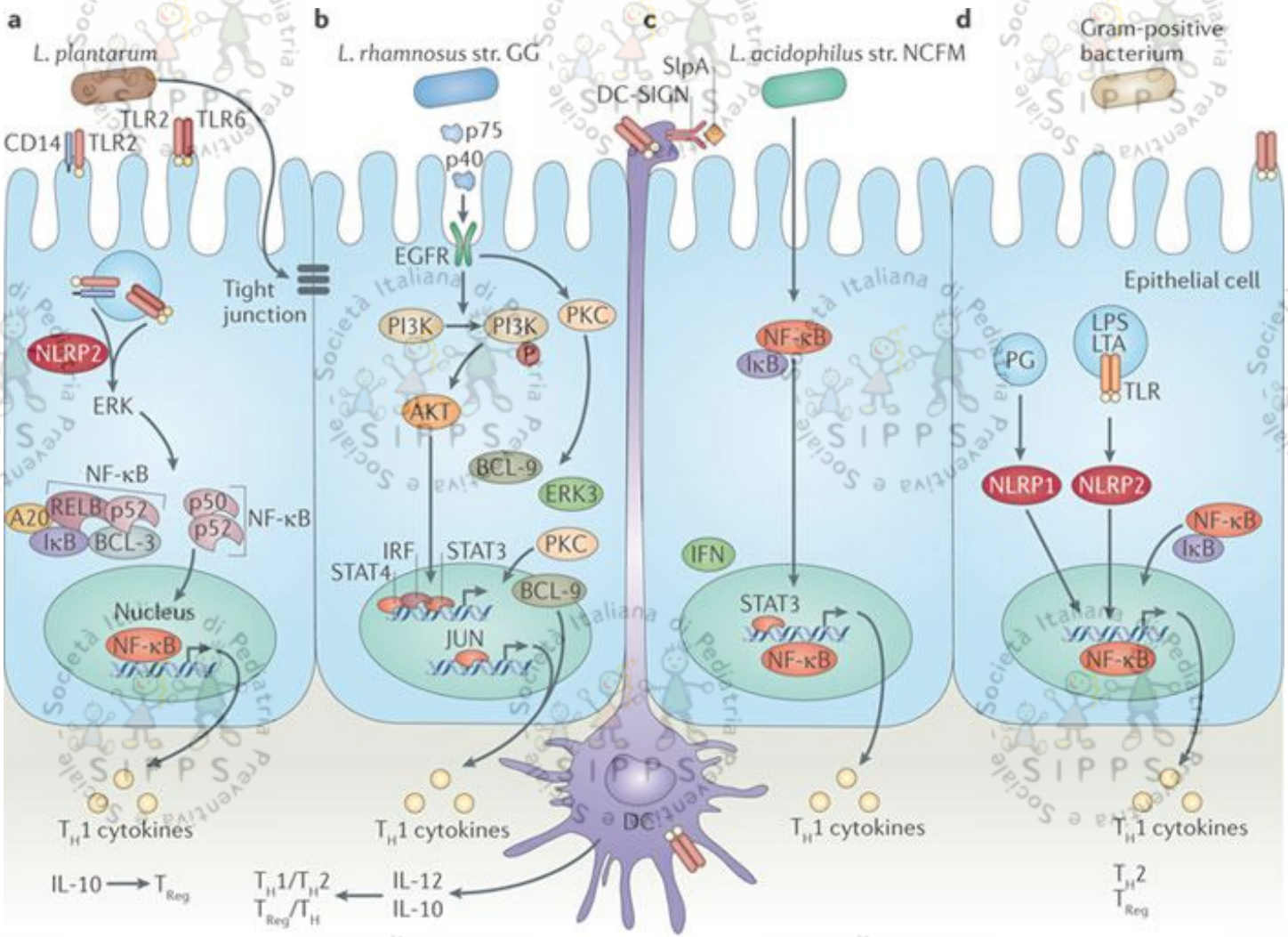
1. **Faecalibacterium prausnitzii strain ATCC 27768 (416/422)**
2. Faecalibacterium prausnitzii strain ATCC 27768 (422/430)
3. Faecalibacterium prausnitzii strain ATCC 27768 (422/430)
4. **Faecalibacterium prausnitzii strain ATCC 27768 (416/420)**
5. Faecalibacterium prausnitzii strain ATCC 27768 (423/420)
6. Faecalibacterium prausnitzii strain ATCC 27768 (416/422)
7. Faecalibacterium prausnitzii strain ATCC 27768 (422/430)
8. Faecalibacterium prausnitzii strain ATCC 27768 (416/422)
9. Faecalibacterium prausnitzii strain ATCC 27768 (395/430)
10. Faecalibacterium prausnitzii strain ATCC 27768 (389/422)
11. **Faecalibacterium prausnitzii strain ATCC 27768 (422/430)**
12. Faecalibacterium prausnitzii strain ATCC 27768 (389/422)

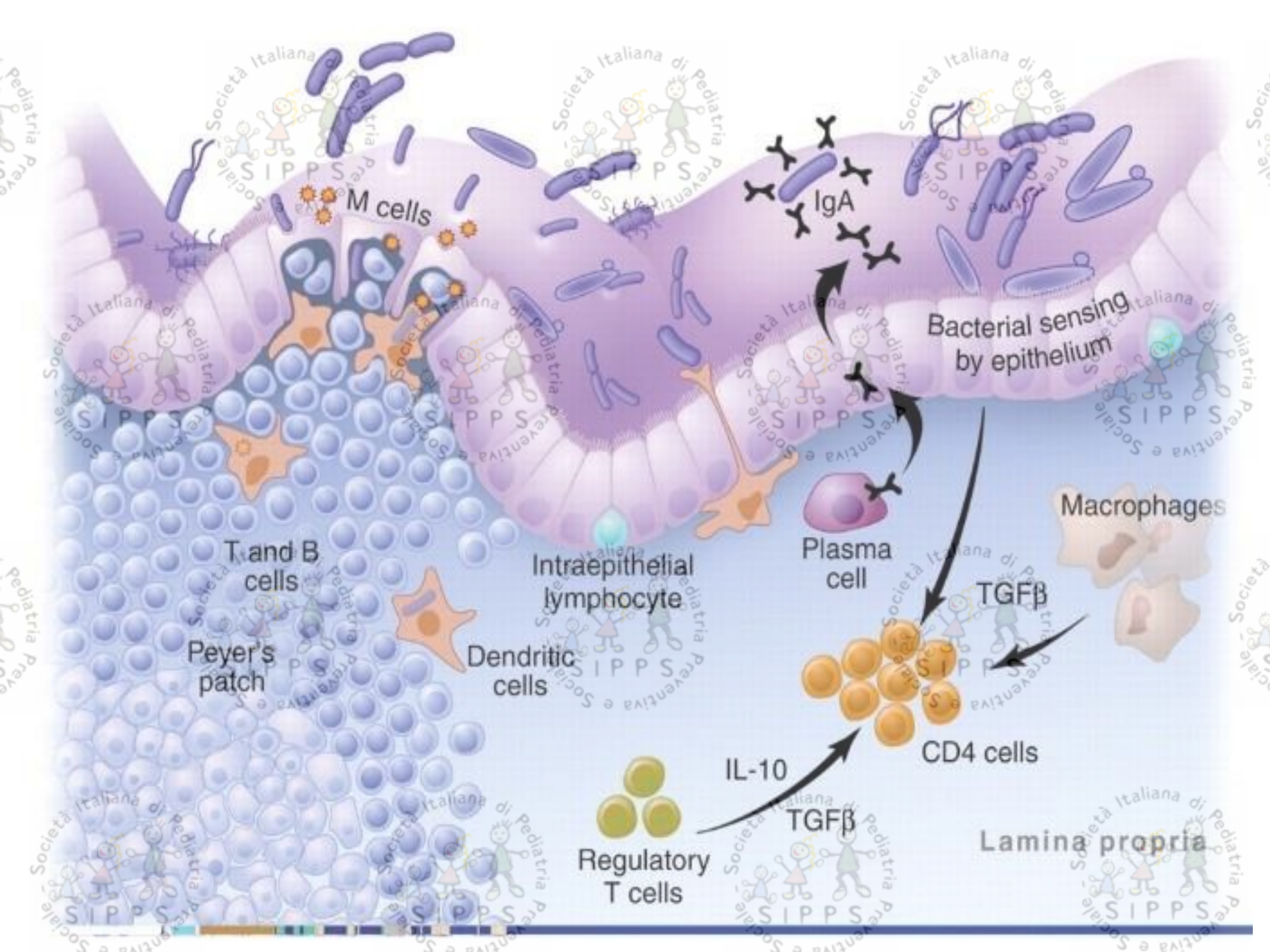
Progetto#5

Analisi del microbiota intestinale in bambini trattati con matrici fermentate di latte e riso con L. *paracasei* CBA L74

- Valutazione della modificazione della composizione del microbiota intestinale
- Correlazione con livelli di butirrato fecale
- Correlazione con stimolazione peptidi dell'immunità innata e acquisita
- Correlazione con insorgenza episodi infettivi

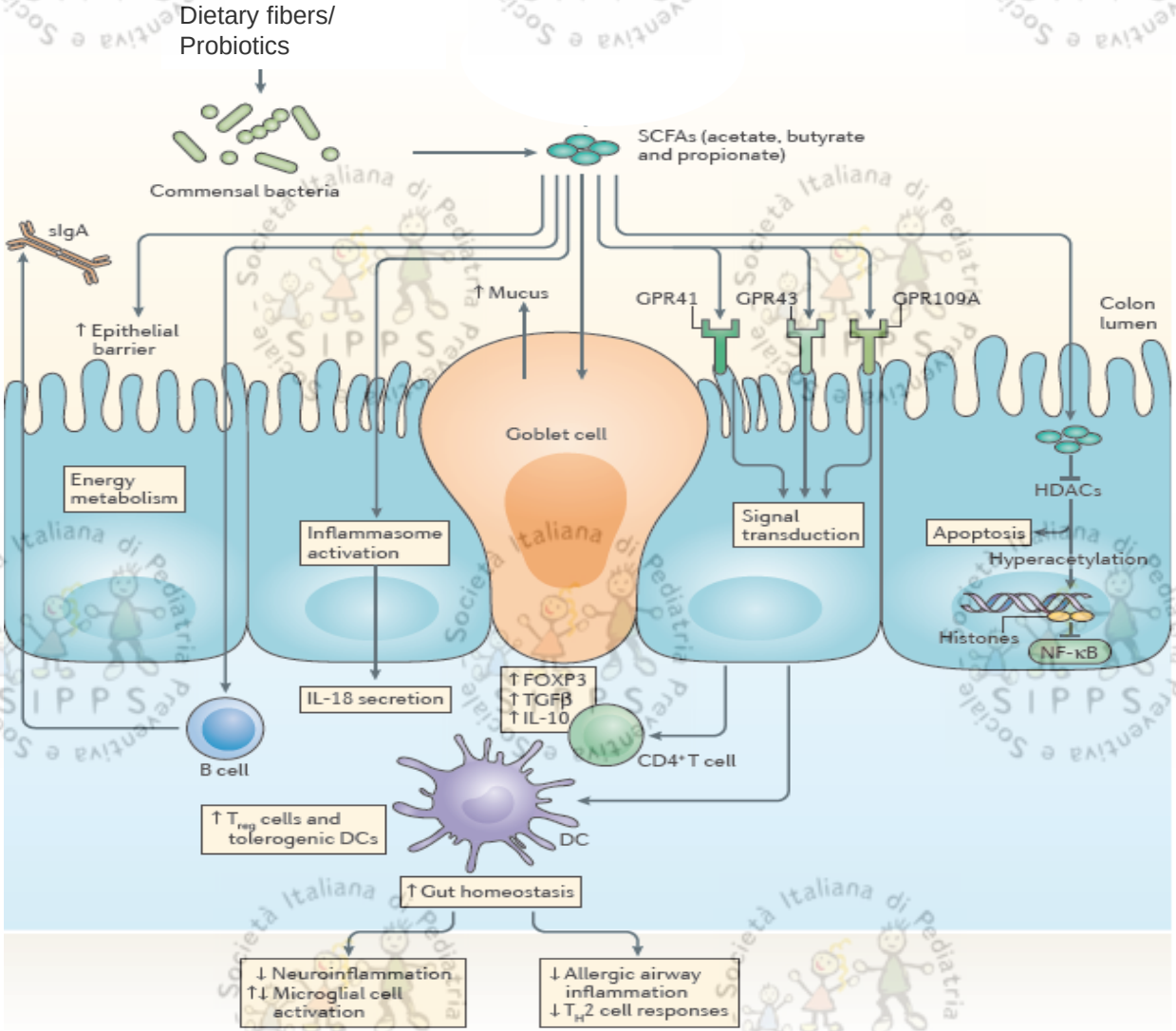

Analisi del microbiota
Livelli di butirrato
Analisi dei peptidi dell'immunità innata e acquisita
Sequenziamento del 16S RNA
Gas -cromatografia
ELISA

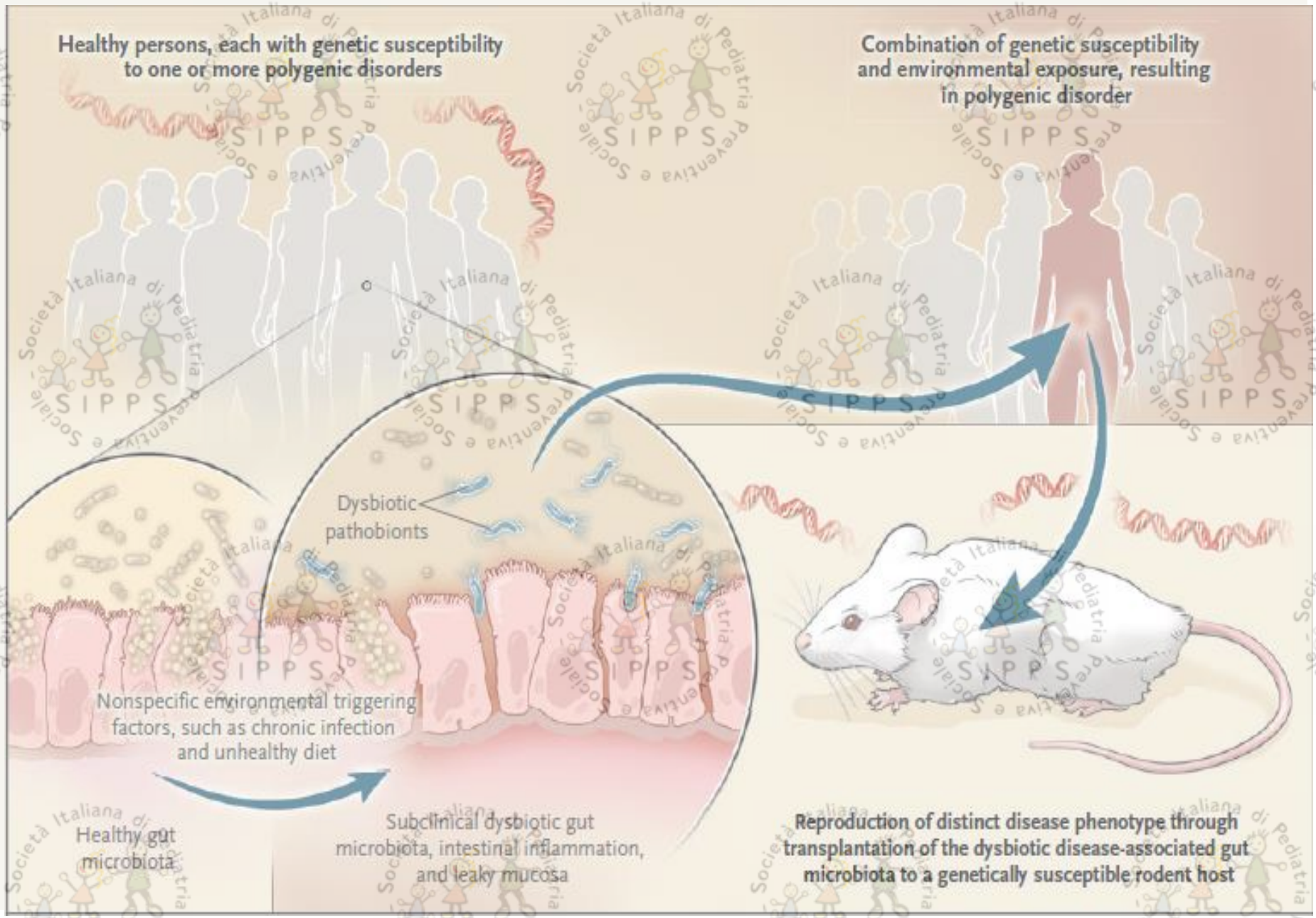

a
L. plantarum
TLR2
TLR6
CD14
TLR2
Tight junction
NLRP2
ERK
NF-κB
A20
RELB
p52
IκB
BCL-3
p50
p52
NF-κB
Nucleus
NF-κB
T_H1 cytokines
IL-10 → T_Reg
b
L. rhamnosus str. GG
p75
p40
EGFR
PI3K
PI3K
P
PKC
AKT
BCL-9
ERK3
IRF
STAT3
STAT4
PKC
BCL-9
JUN
T_H1 cytokines
T_H1/T_H2
T_Reg/T_H
IL-12
IL-10
c
SlpA
DC-SIGN
L. acidophilus str. NCFM
NF-κB
IκB
IFN
STAT3
NF-κB
T_H1 cytokines
DC
d
Gram-positive bacterium
Epithelial cell
PG
LPS
LTA
TLR
NLRP1
NLRP2
NF-κB
IκB
NF-κB
T_H1 cytokines
T_H2
T_Reg


M cells
IgA
Bacterial sensing by epithelium
T and B cells
Peyer's patch
Intraepithelial lymphocyte
Dendritic cells
Plasma cell
Macrophages
TGFβ
CD4 cells
IL-10
TGFβ
Regulatory T cells
Lamina propria

Gut microbiota, metabolites and host immunity

Michelle G. Rooks[1] and Wendy S. Garrett[1–4]

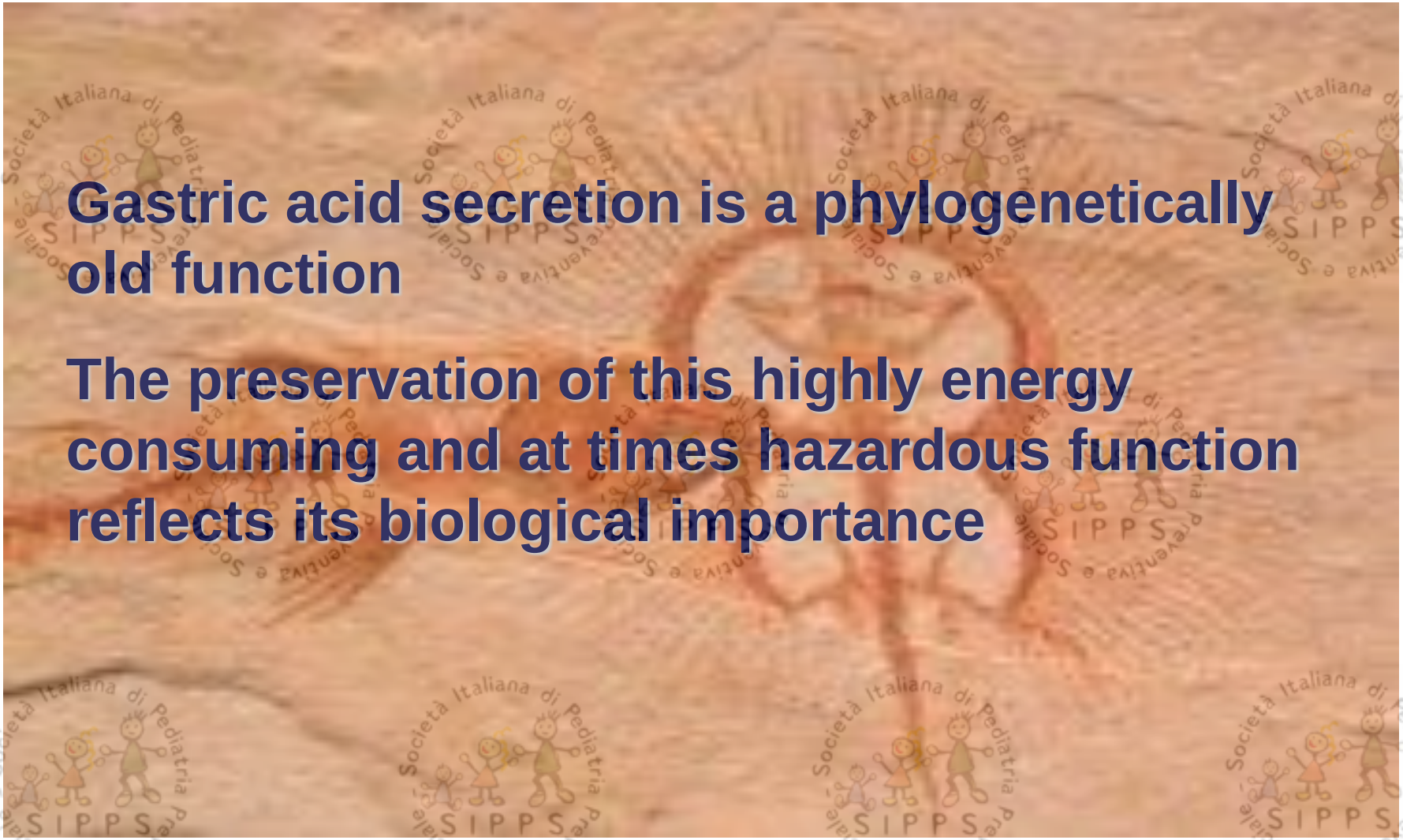


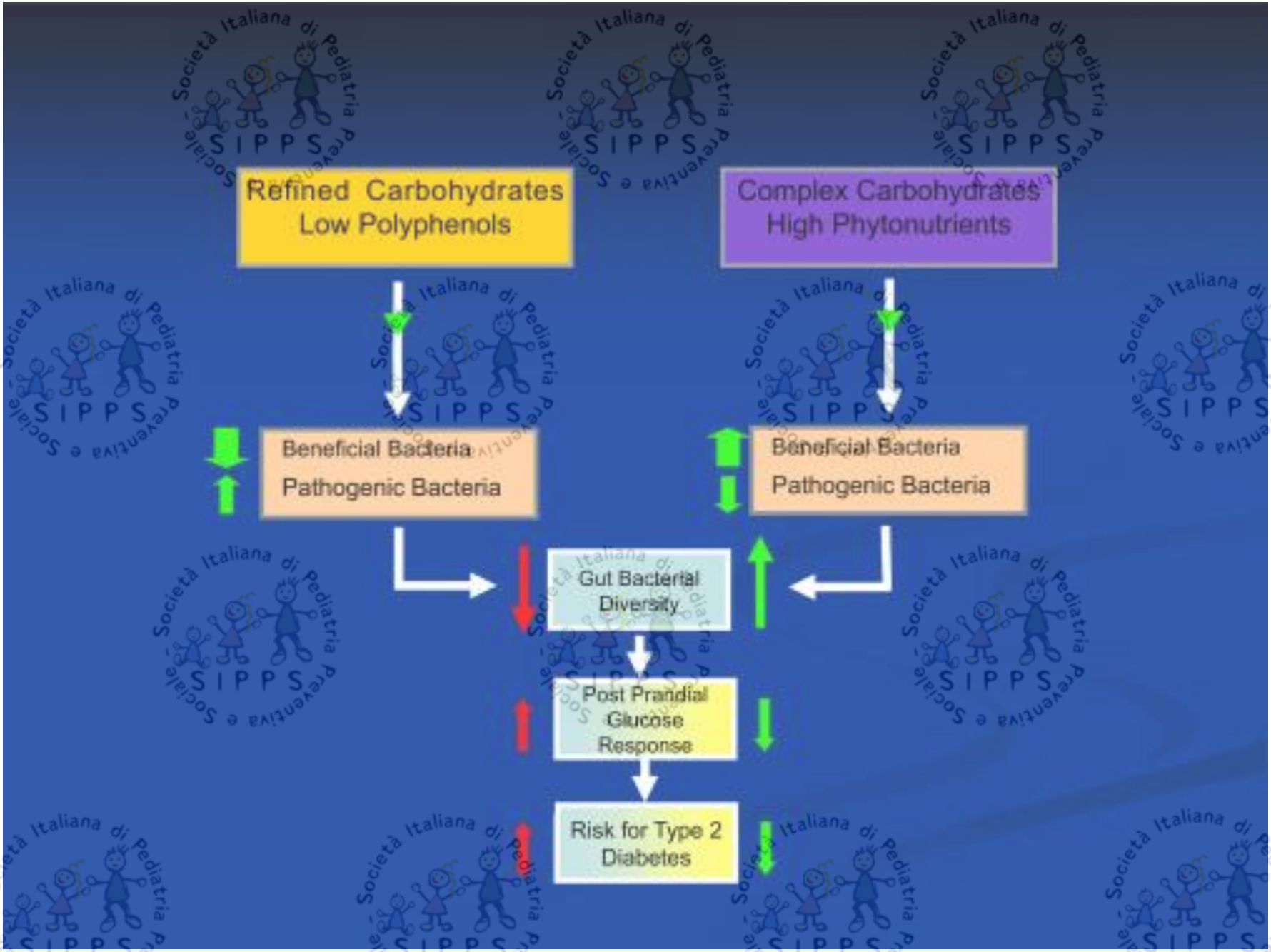


Lynch VS and Pedersen O. N Eng J Med 2016

Rare Cretaceous Stingray Fossil Cyclobatis major from the sublithographic limestone deposits of Haqel, Lebanon

Cretaceous (146 to 65 mya)





Vanamala JKP Cell 2015