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CONGRESSO NAZIONALE
SOCIETÀ ITALIANA DI PEDIATRIA
PREVENTIVA E SOCIALE



BAMBINI DI IERI, OGGI E DOMANI...
LA NOSTRA CARE, IL NOSTRO CUORE

TEATRO DI SIRACUSA • 7 - 10 GIUGNO 2018

SIRACUSA

IL RUOLO della VITAMINA D e dei
PROBIOTICI nella
IMMUNO-MODULAZIONE della RISPOSTA
ALLERGICA

APPLICABILITÀ SU BASI SCIENTIFICHE

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Università della Campania "Luigi Vanvitelli"



Università
degli Studi
della Campania
Luigi Vanvitelli

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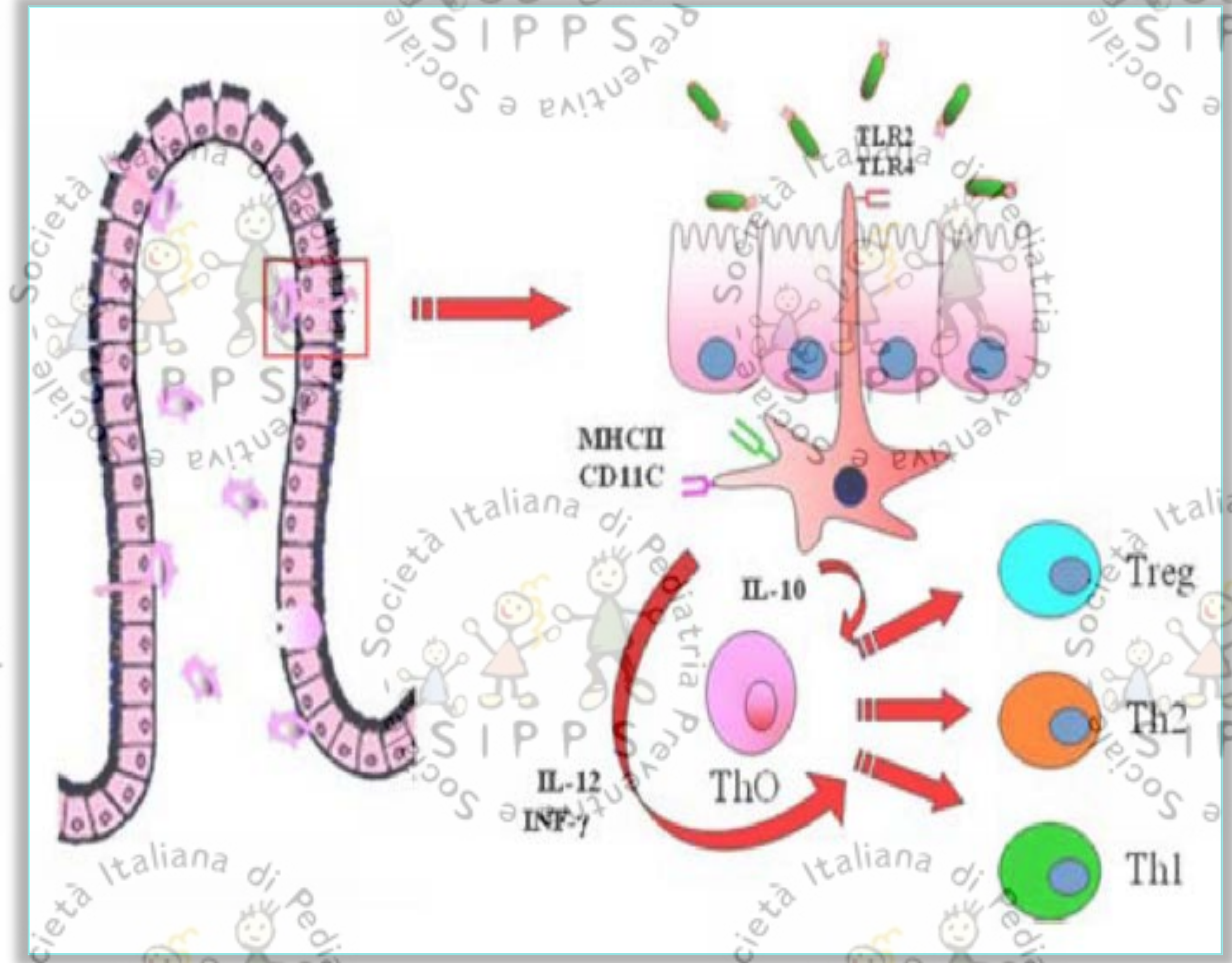
APPLICABILITÀ SU BASI SCIENTIFICHE

PROBIOTICI

The influence of bacterial colonization on intestinal function. Walker WA *Ann Nutr Metab* 2013;63::8–15

Tolleranza Orale Indotta dal Microbiota Intestinale

Nel lume intestinale, il microbiota intestinale attiva le cell. dendritiche attraverso l'interazione con i TLR2/TLR4.

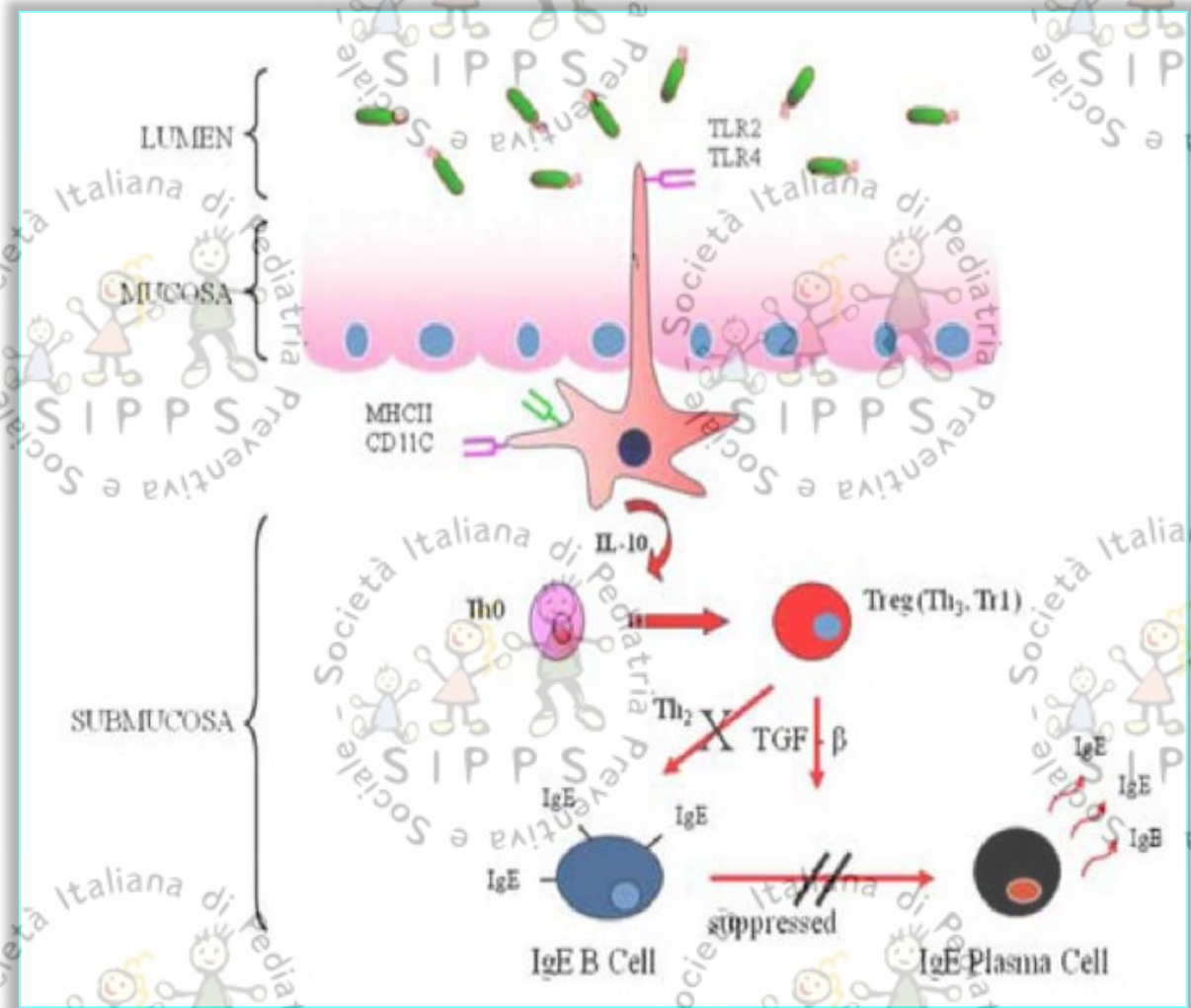


The influence of bacterial colonization on intestinal function. Walker WA *Ann Nutr Metab* 2013;63::8–15

Tolleranza Orale Indotta dal Microbiota Intestinale

Nel lume intestinale, il microbiota intestinale attiva le cell. dendritiche attraverso l'interazione con TLR2/TLR4.

Le cell. dendritiche attivate causano la maturazione del subset Th0 (Th3 - Tr1) delle cell. Treg attraverso il rilascio di IL-10 stimolando il rilascio TGF- β e quindi sopprimendo la produzione di IgE.



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PROBIOTICI

➔ **Probiotici e ALLERGIA**

L. reuteri supplementation induces a low level of TGF- β 2 in breast milk and it is associated with a reduced risk of sensitization in childhood.

Bottcher MF et al Pediatr Allergy Immunol 2008; 19:497-504



The aim of this study was to evaluate the effect of probiotic **Lactobacillus reuteri** supplementation on the immunological composition of **breast milk** in relation to sensitization and eczema in the babies.

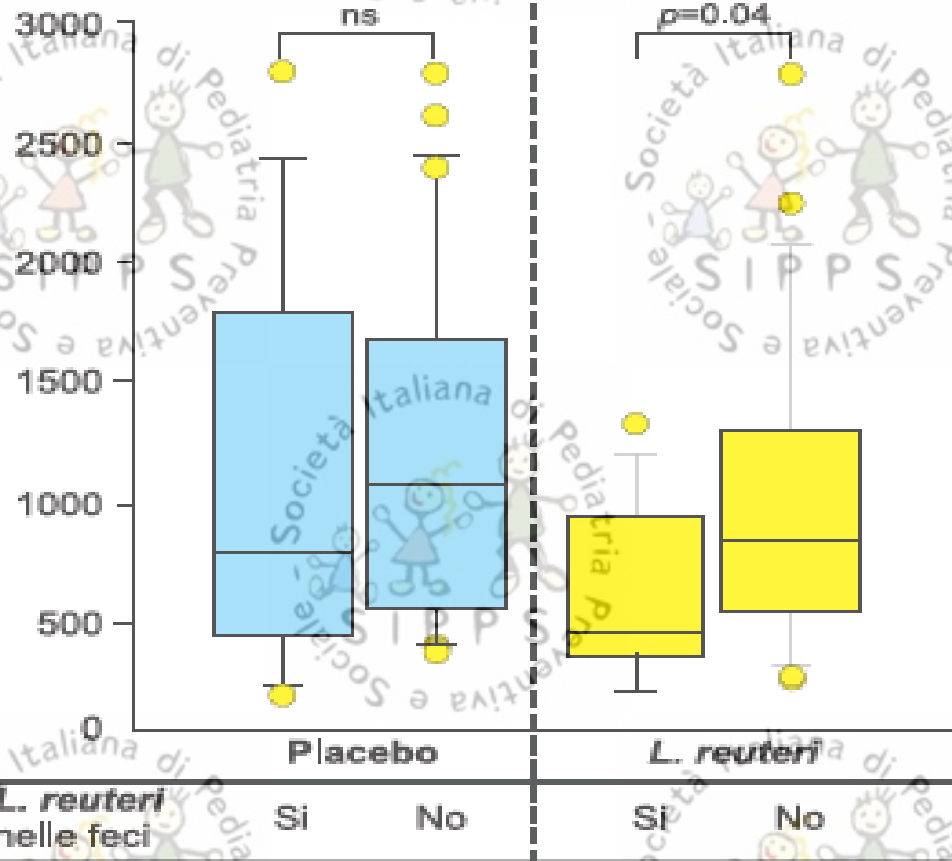
- Total IgA, secretory IgA (SIgA), TGF-beta1, TGF-beta2, IL-10, TNF, soluble CD14 (sCD14), and Na/K ratios were analyzed in **colostrum** and **mature milk** obtained from **women treated with L. reuteri** (n = 54) or **placebo** (n = 55) from gestational week 36 until delivery.

L. reuteri supplementation induces a low level of TGF- β 2 in breast milk and it is associated with a reduced risk of sensitization in childhood.

Bottcher MF et al Pediatr Allergy Immunol 2008; 19:497-504



TGF- β 2
(pg/ml)



There were significantly **lower levels of TGF- β 2** and slightly **higher IL-10** in the colostrum of mothers who received **L. Reuteri** from the 36th gestational week

L. reuteri supplementation induces a low level of TGF- β 2 in breast milk and it is associated with a reduced risk of sensitization in childhood.

Bottcher MF et al Pediatr Allergy Immunol 2008; 19:497-504

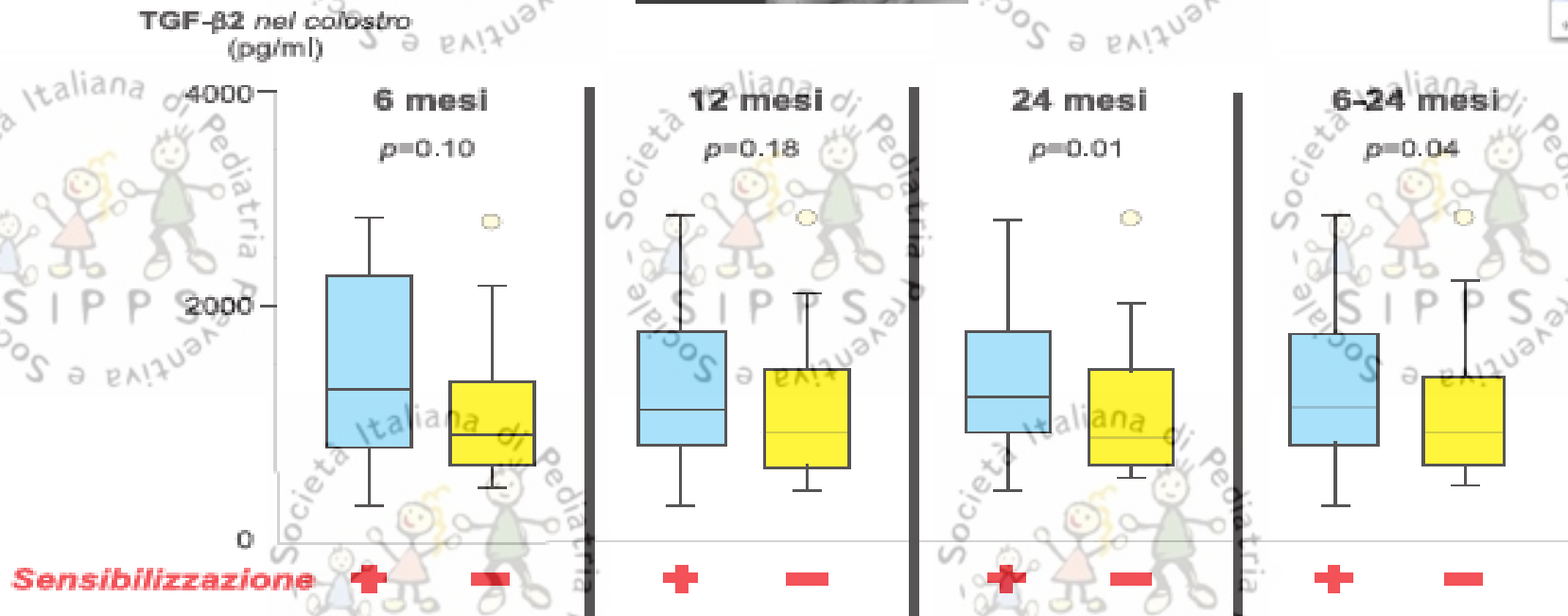


The aim of this study was to evaluate the effect of probiotic *Lactobacillus reuteri* supplementation on the immunological composition of breast milk in relation to sensitization and eczema in the babies.

- ◆ The infants were followed prospectively for 2 yr regarding **development of eczema and sensitization** as defined by a positive skin prick test and/or circulating allergen-specific IgE antibodies at 6, 12, and **24 months of age.**

L. reuteri supplementation induces a low level of TGF- β 2 in breast milk and it is associated with a reduced risk of sensitization in childhood.

Bottcher MF et al Pediatr Allergy Immunol 2008; 19:497-504



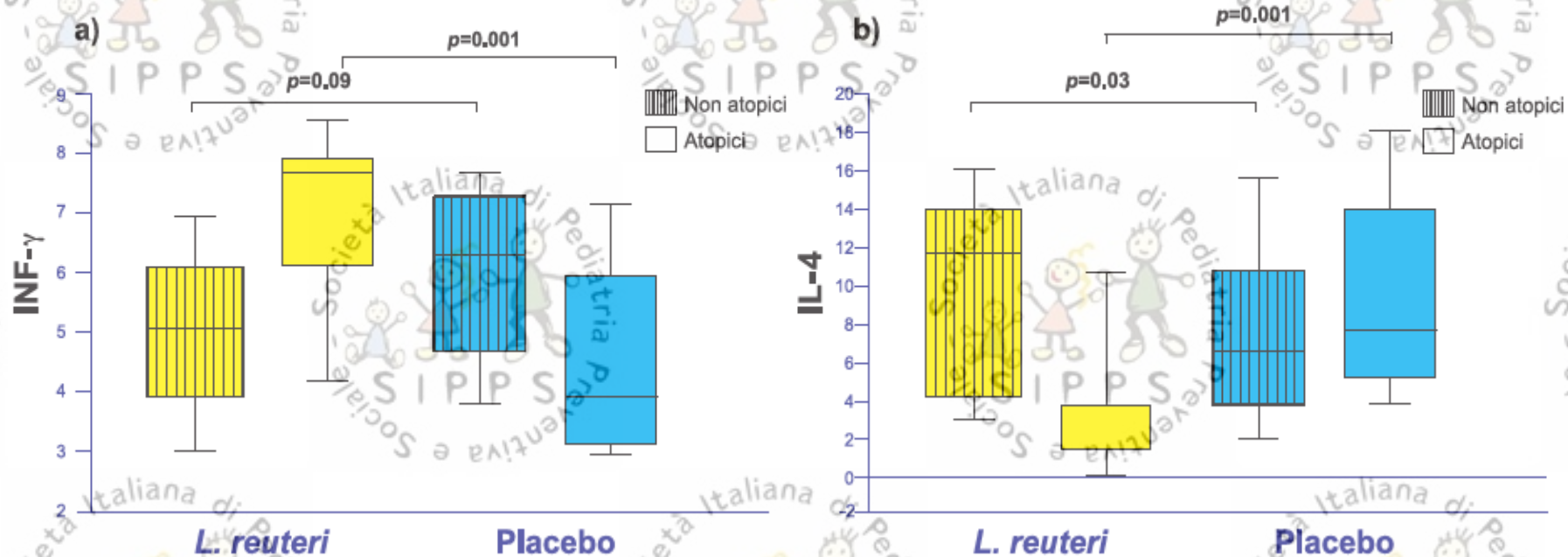
Children born from women of the first group showed a **reduced allergic sensitization** and a **less Ig-E associated eczema** within the first 2 years of life

L. reuteri modulates cytokines expression in patients with DA

Miniello VL, et al. *J Ped Gastroenterol Nutr*, 2010;50(5):573-6

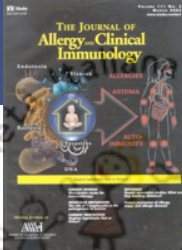


IFN- γ and IL-4 levels in 50 children (2-10 years) with **IgE-associated eczema** who received supplementation with the probiotic **L. reuteri** (5 drops/day) or placebo for 8 weeks.



Probiotics in prevention of IgE-associated eczema: a randomized double-blind placebo-controlled clinical trial

T. R. Abrahamsson, *J Allergy Clin Immunol*, 2007;119:1174-80



232 donne gravide con storia familiare di allergia
Supplementazione di *L. reuteri* o placebo per 4 settimane preparto

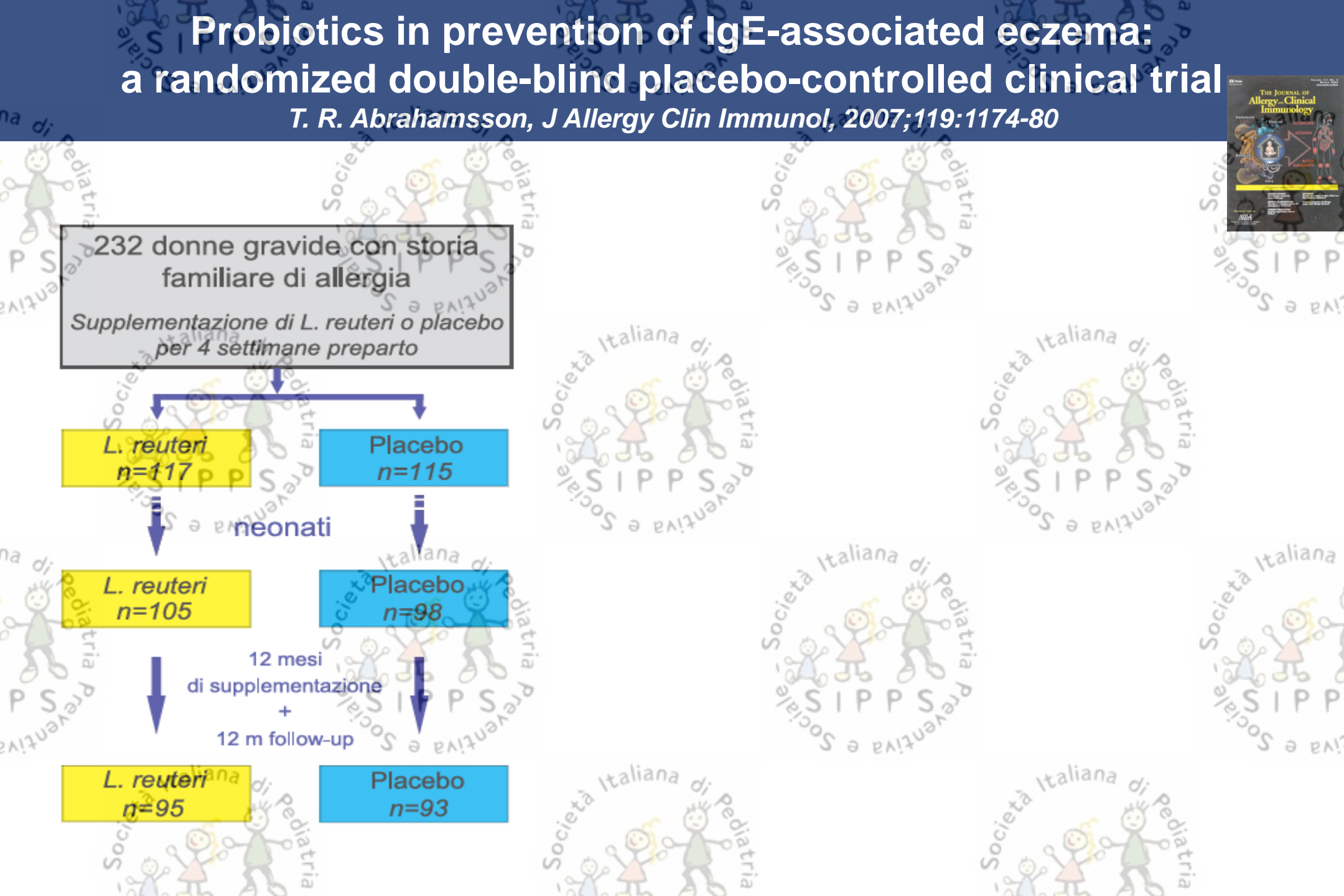
L. reuteri n=117 Placebo n=115

neonati

L. reuteri n=105 Placebo n=98

12 mesi di supplementazione +
12 m follow-up

L. reuteri n=95 Placebo n=93



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PROBIOTICI

Probiotici e ALLERGIA

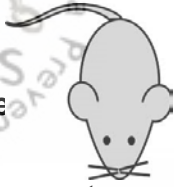
➔ Probiotici e ASMA

ORAL TREATMENT WITH LIVE LACTOBACILLUS REUTERI INHIBITS THE ALLERGIC AIRWAY RESPONSE IN MICE

Forsythe P. et al. American J Respiratory Critical Care Med 2007;175:561-569



Animals received 1×10^9 live, heat-killed, or irradiated *L. reuteri*, *L. salivarius*, or equivalent isolated *L. reuteri* DNA via gavage for 9 consecutive day before antigen challenge.



After antigen challenge, airway responsiveness to methacholine, influx of inflammatory cells to the lung, and cytokine levels in bronchoalveolar lavage fluid were assessed.

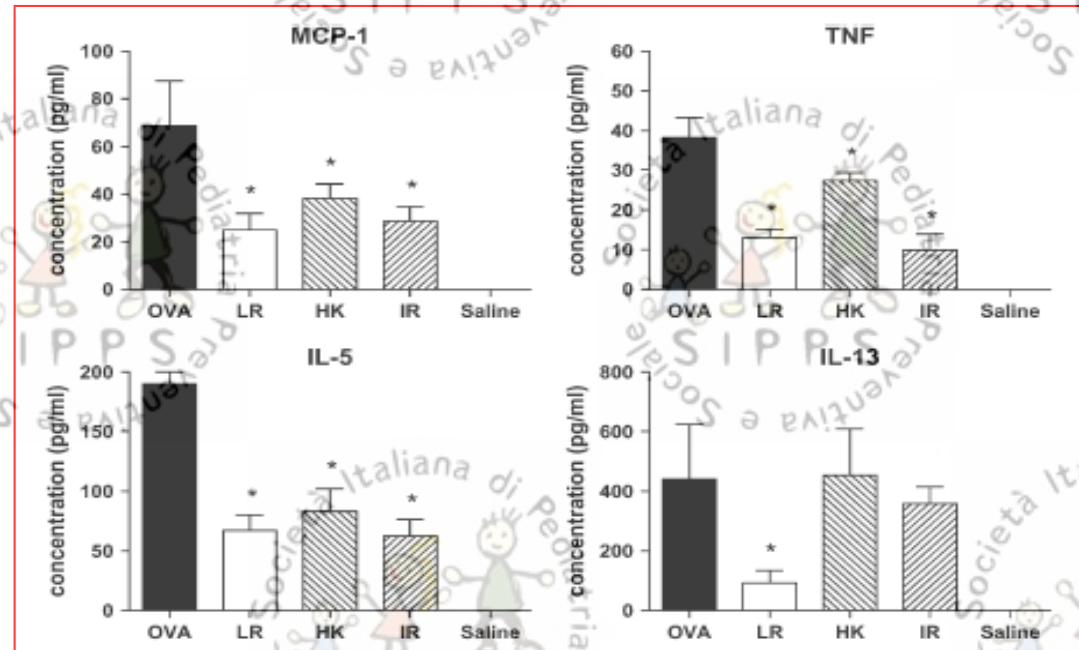
RESULTS

Cytokine Levels were significantly increased in BAL fluid after OVA challenge compared with saline-challenged animals.

Treatment with *L. reuteri* significantly attenuated the increase in Tumor necrosis factor, monocyte chemoattractant protein-1, IL-5, and IL-13.

Treatment with either heat-killed or irradiated *L. reuteri* also significantly attenuated the increase in MCP-1 TNF and IL-5 after antigen challenge but did not significantly alter levels of IL-13

- **CONCLUSION:** *L. reuteri* but not *L. salivarius* also decreased allergen-induced airway hyperresponsiveness. These responses were dependent on Toll like receptor 9.



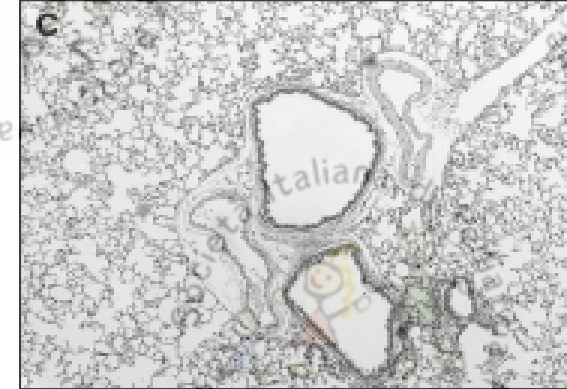
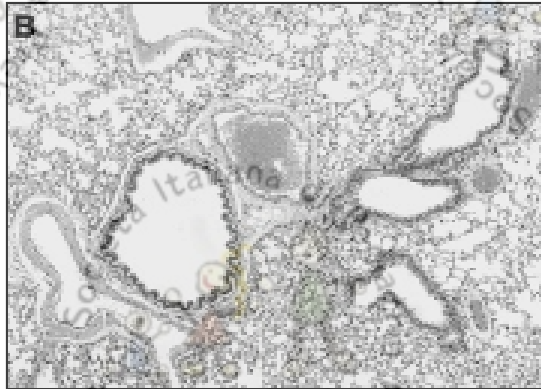
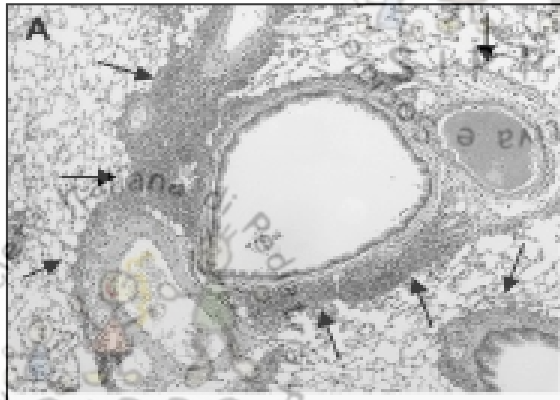
LR= live *Lactobacillus Reuteri*

HK= heat killed LR

IR= γ irradiated LR

ORAL TREATMENT WITH LIVE LACTOBACILLUS REUTERI INHIBITS THE ALLERGIC AIRWAY RESPONSE IN MICE

Forsythe P. et al. American J Respiratory Critical Care Med 2007;175:561-569



Representative sections of lung tissue from **L. reuteri-treated (A)** and **untreated (B)** OVA-sensitized mice after antigen challenge. A section from a saline-challenged control animal is shown for comparison (C). Arrows indicate inflammatory cell influx to the parenchyma. This influx was markedly reduced by treatment with live *L. reuteri*.

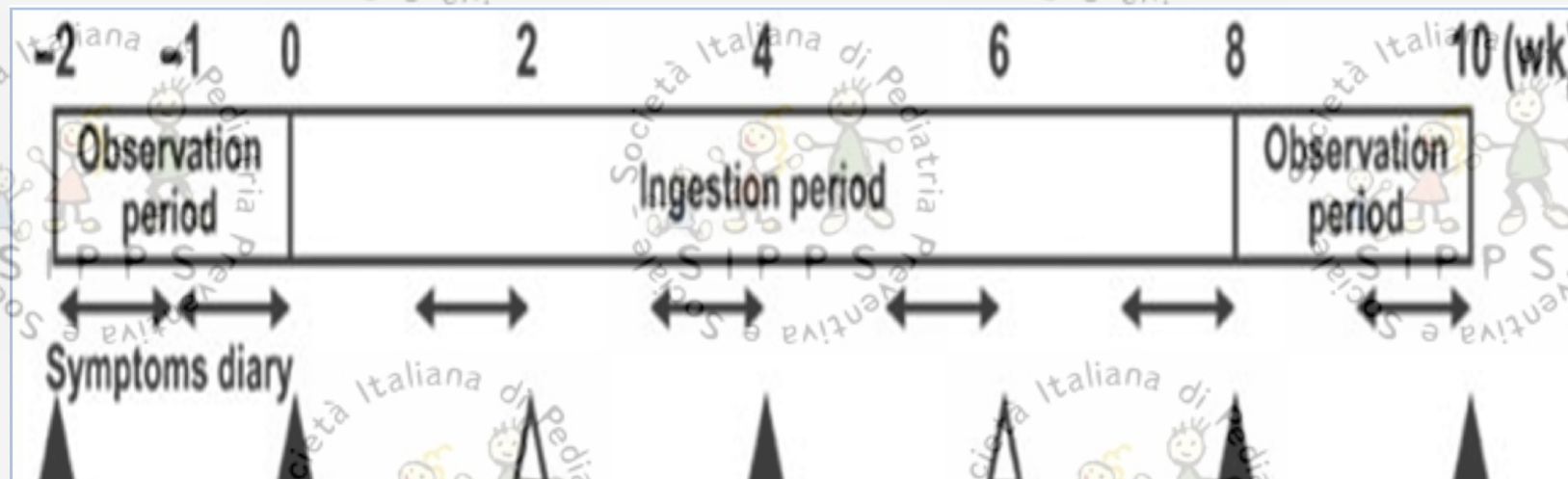
Oral treatment with live *L. reuteri* can attenuate major characteristics of an asthmatic response in a mouse model of allergic airway inflammation. Oral treatment with specific live probiotic strains may have therapeutic potential in the treatment of allergic airway disease.

Airways allergic inflammation and *L.reuteri* treatment in asthmatic children

M. Miraglia del Giudice et al. Journal of Biolog. Regulators 2012;26:35-40

Aim:

- To evaluate the beneficial effects of the oral administration of the probiotic *L. Reuteri* (1×10^8 CFU) on the airways allergic inflammation in mild persistent asthmatic children allergic to HDM .
- We selected 50 children with well controlled asthma (C-ACT >19 and FEV₁ > 80%)



★ spirometria



★ spirometria, determinazione del FeNO e analisi del condensato esalato.



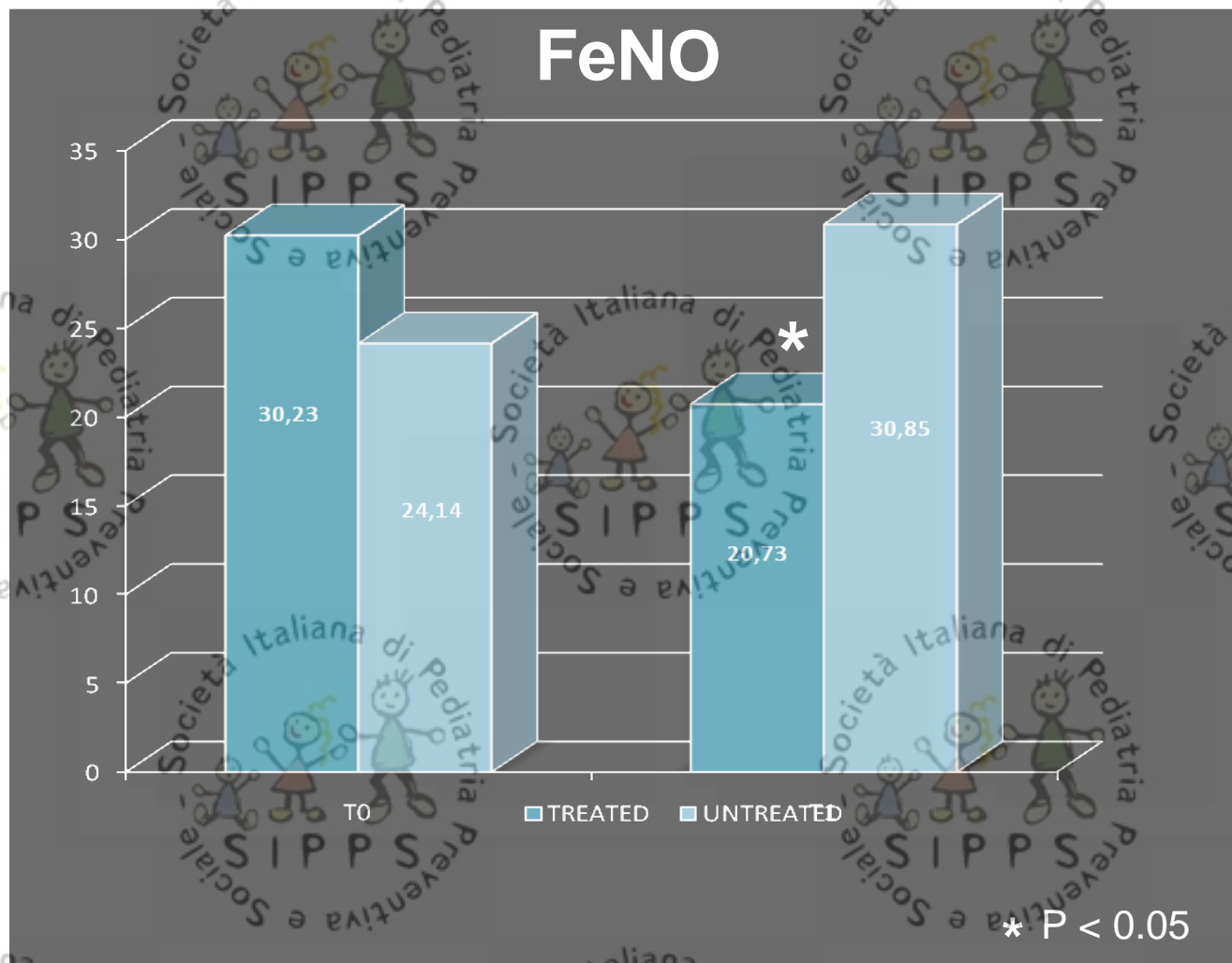
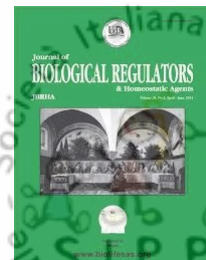
▲ C-ACT



★ spirometria, determinazione del FeNO

Airways allergic inflammation and L.reuteri treatment in asthmatic children

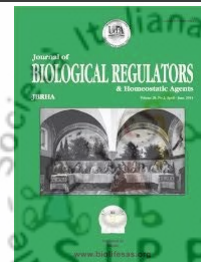
M. Miraglia del Giudice et al. *Journal of Biolog. Regulators* 2012;26:35-40



The FeNO values showed a significant reduction ($p=0,045$) in L. reuteri group but not in the placebo group at the end of the treatment

Airways allergic inflammation and L.reuteri treatment in asthmatic children

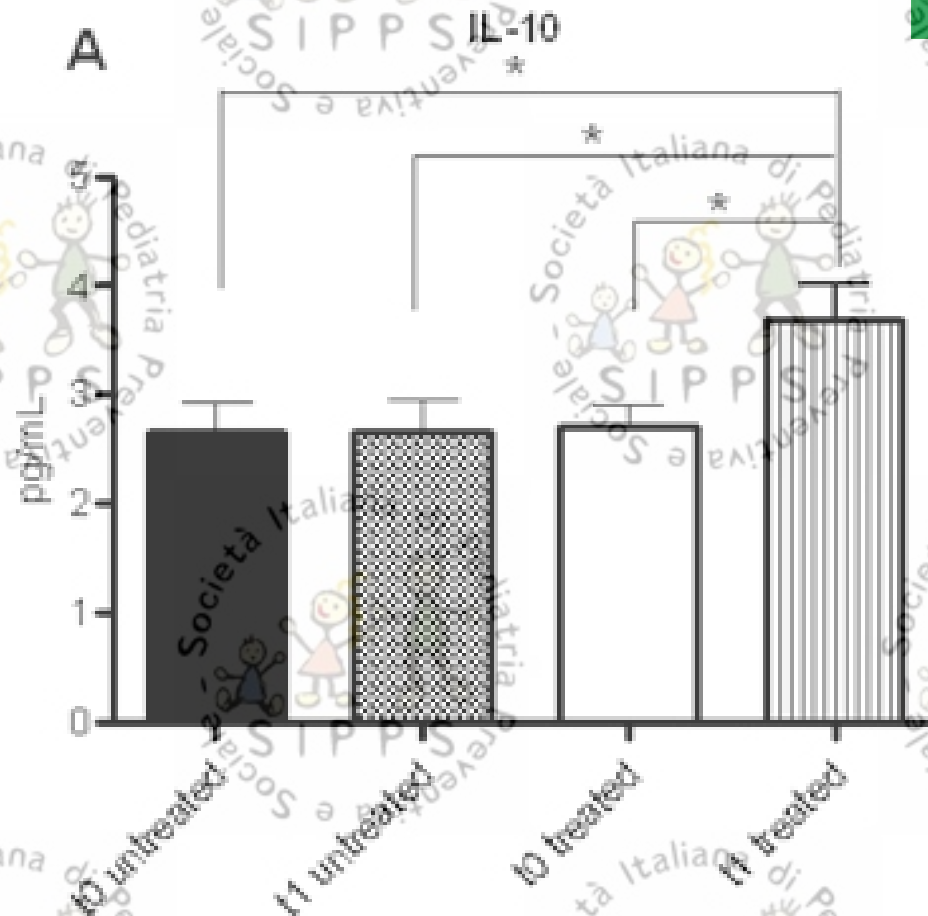
M. Miraglia del Giudice et al. *Journal of Biolog. Regulators* 2012;26:35-40



Reuteri Group:

Significant increase of IL-10 levels vs T₀ and vs T₁ of the untreated patients

Control Group: no significant difference between T₀ and T₁ values



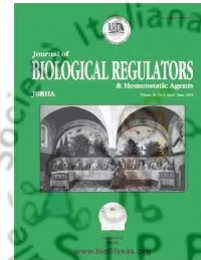
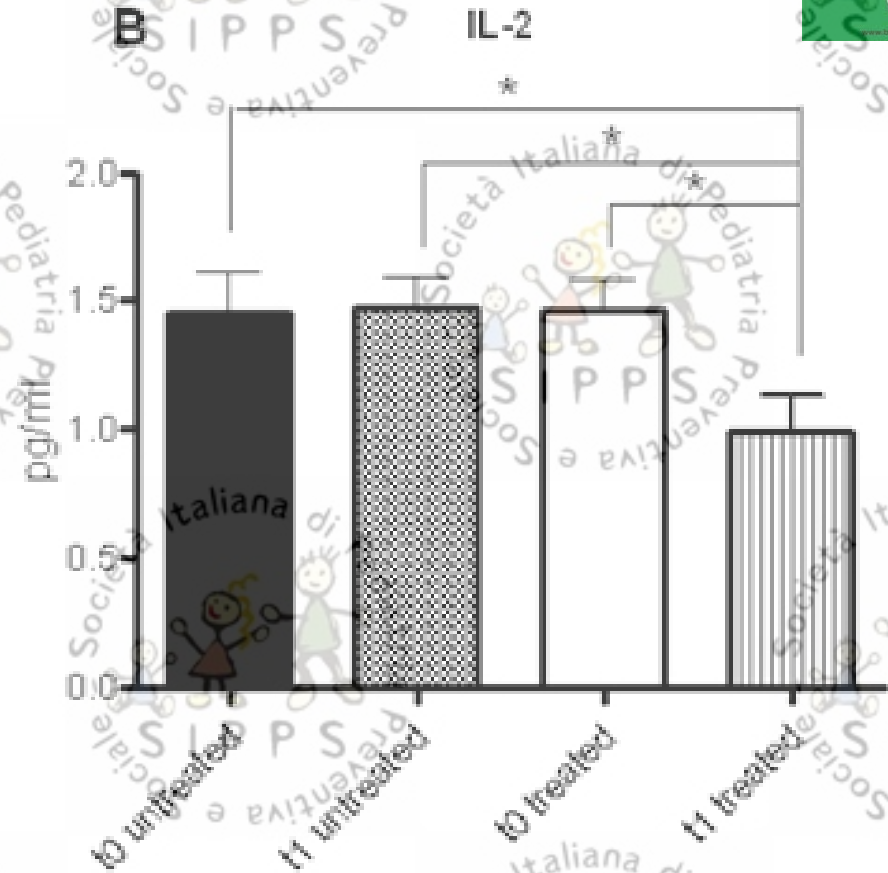
Airways allergic inflammation and L.reuteri treatment in asthmatic children

M. Miraglia del Giudice et al. *Journal of Biolog. Regulators* 2012;26:35-40

Reuteri Group:

Significant reduction of IL-2 levels vs T0 and vs T1 of the untreated patients

Control Group: no significant difference between T0 and T1 values

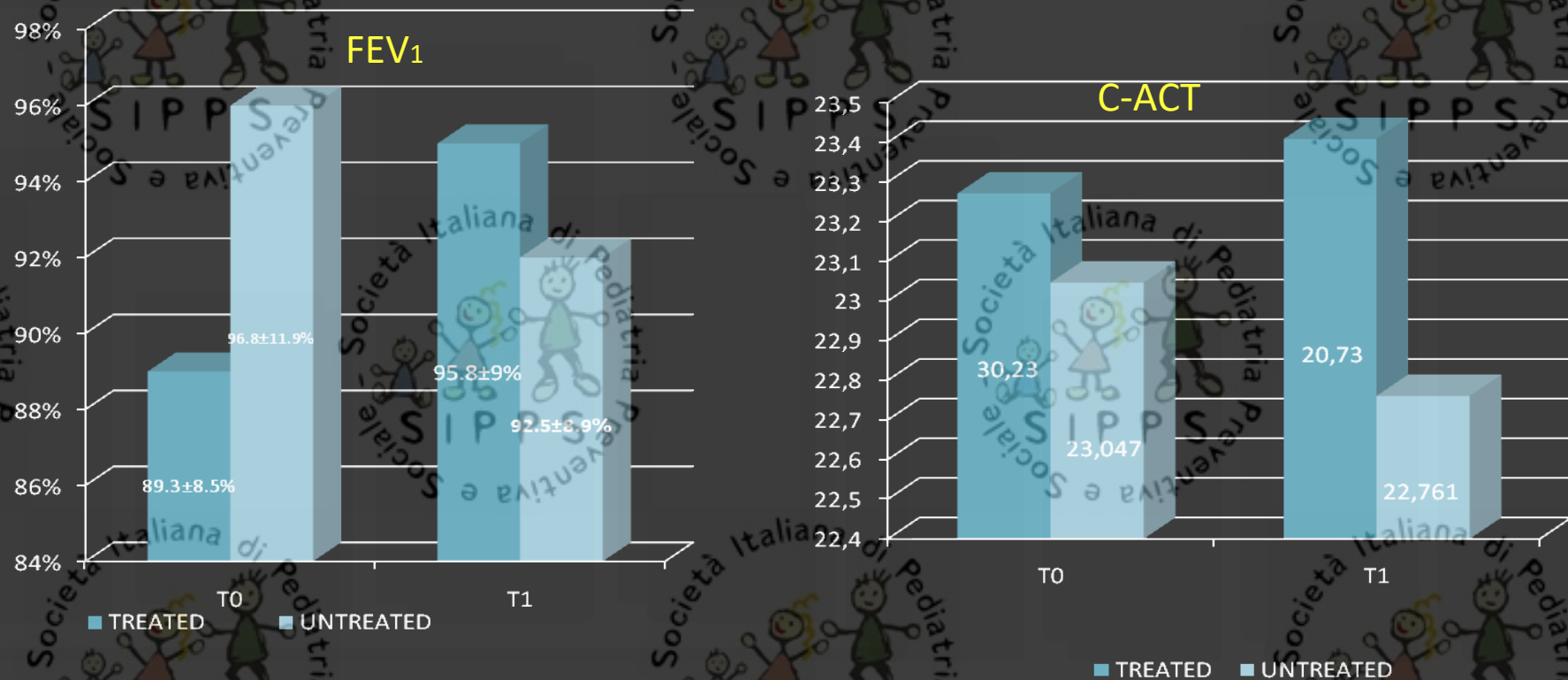


Airways allergic inflammation and L.reuteri treatment in asthmatic children

M. Miraglia del Giudice et al. *Journal of Biolog. Regulators* 2012;26:35-40

FEV₁: no significant increase in the average values of FEV₁ between T0 and T1

C-ACT: no statistically significant differences into the groups and between the two groups before and after treatment

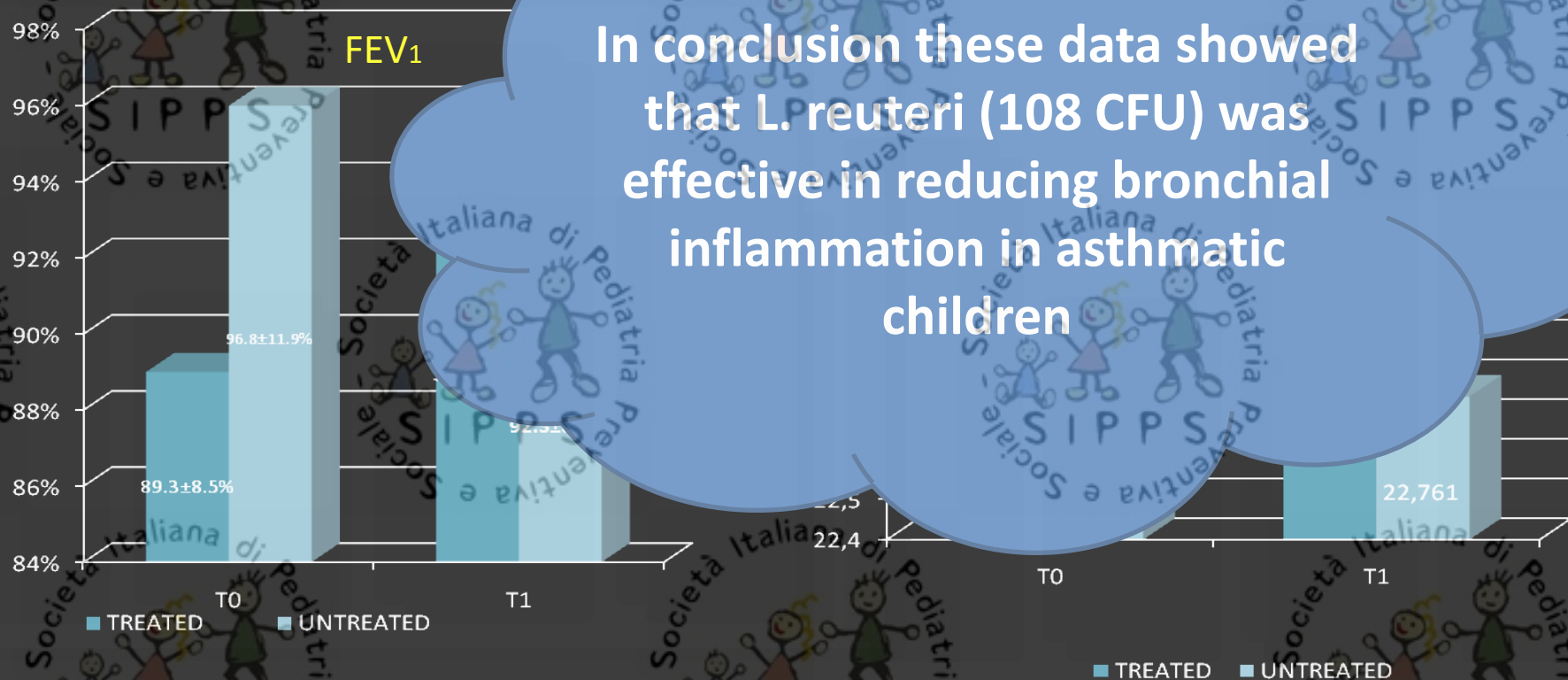


Airways allergic inflammation and L.reuteri treatment in asthmatic children

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FEV₁: no significant increase in the average values of FEV₁ between T0 and T1

C-ACT: no statistically significant difference between the two groups



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PROBIOTICI

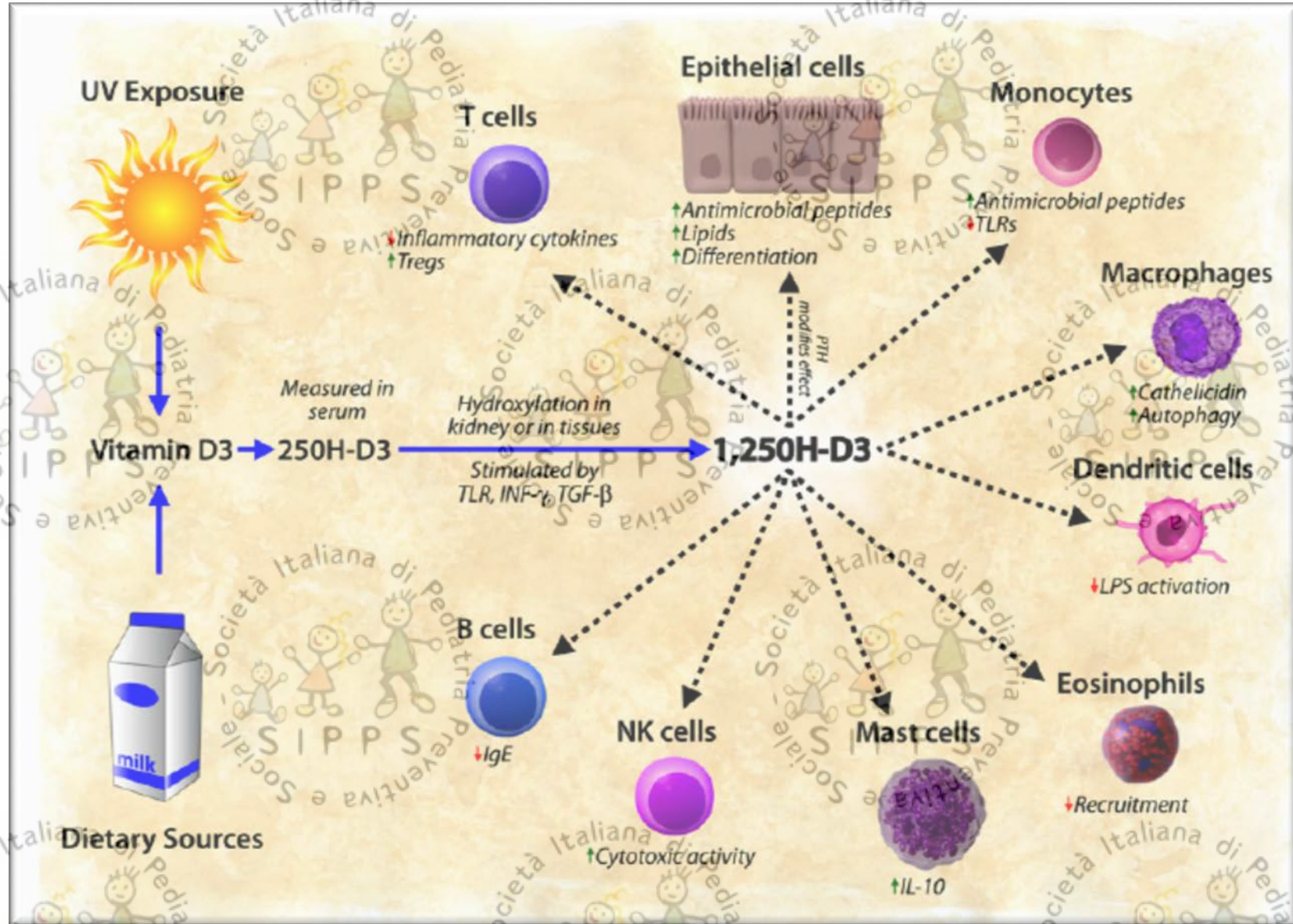
Probiotici e ALLERGIA
Probiotici e ASMA

VITAMINA D

➡ Vitamina D e ASMA

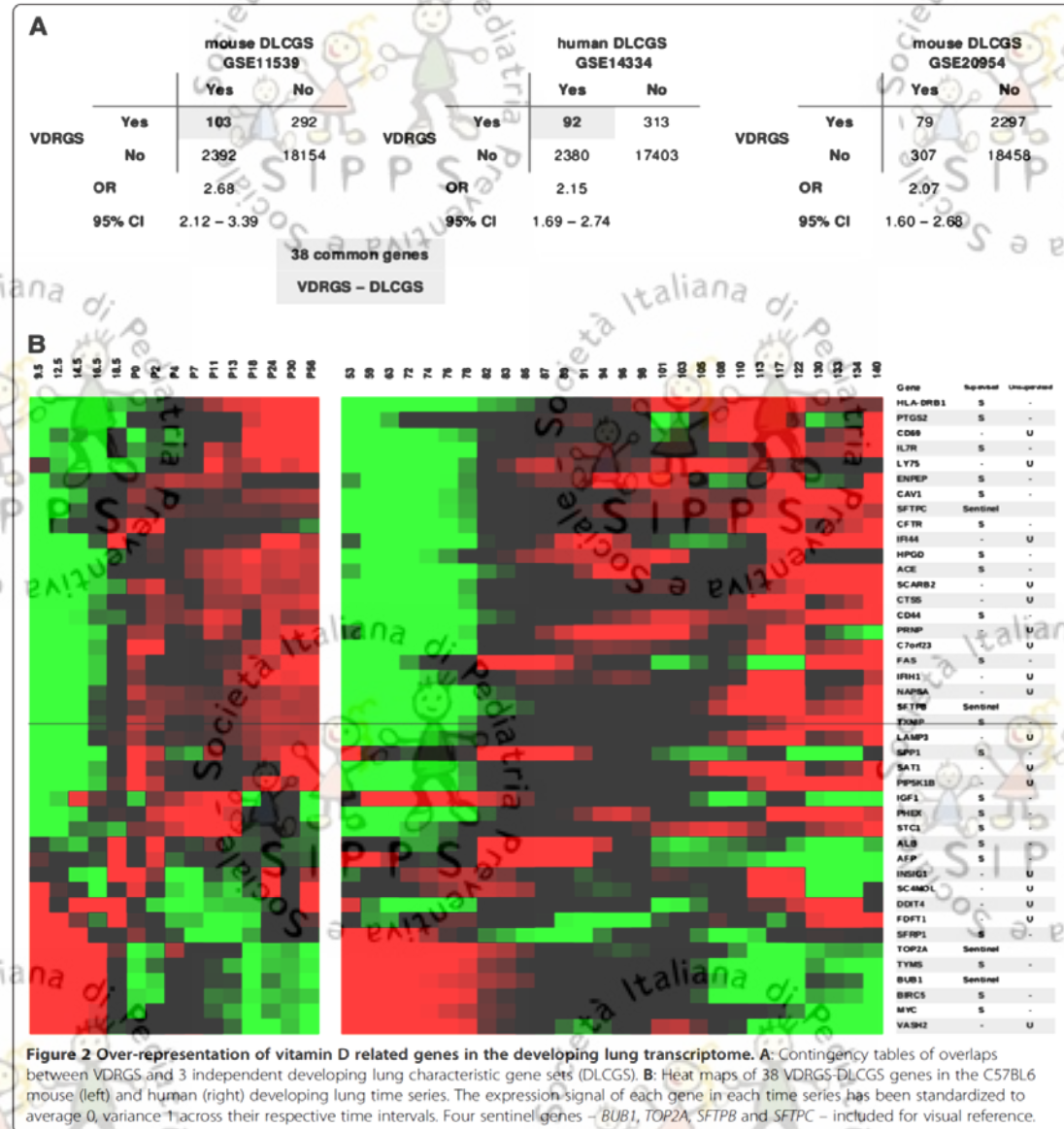
Vitamin D in allergic disease: Shedding light on a complex problem

Muehleisen B et al. *J Allergy Clin Immunol* 2013;131:324-9



Vitamin D related genes in lung development and asthma pathogenesis

- ✓ La vit D influenza la trascrizione di alcuni geni durante lo sviluppo del polmone.
- ✓ Questi geni vitamina D responsivi aumentano l'espressione durante lo sviluppo del polmone fetale, con picco di espressione appena prima della nascita.
- ✓ Gli AA hanno dimostrato che circa 1/3 (12 di 38) dei geni vitamina D sensibili che influenzano congiuntamente sia lo sviluppo del polmone umano e murino sono espressi in modo differente in cellule derivate da bambini asmatici rispetto ai controlli non asmatici.

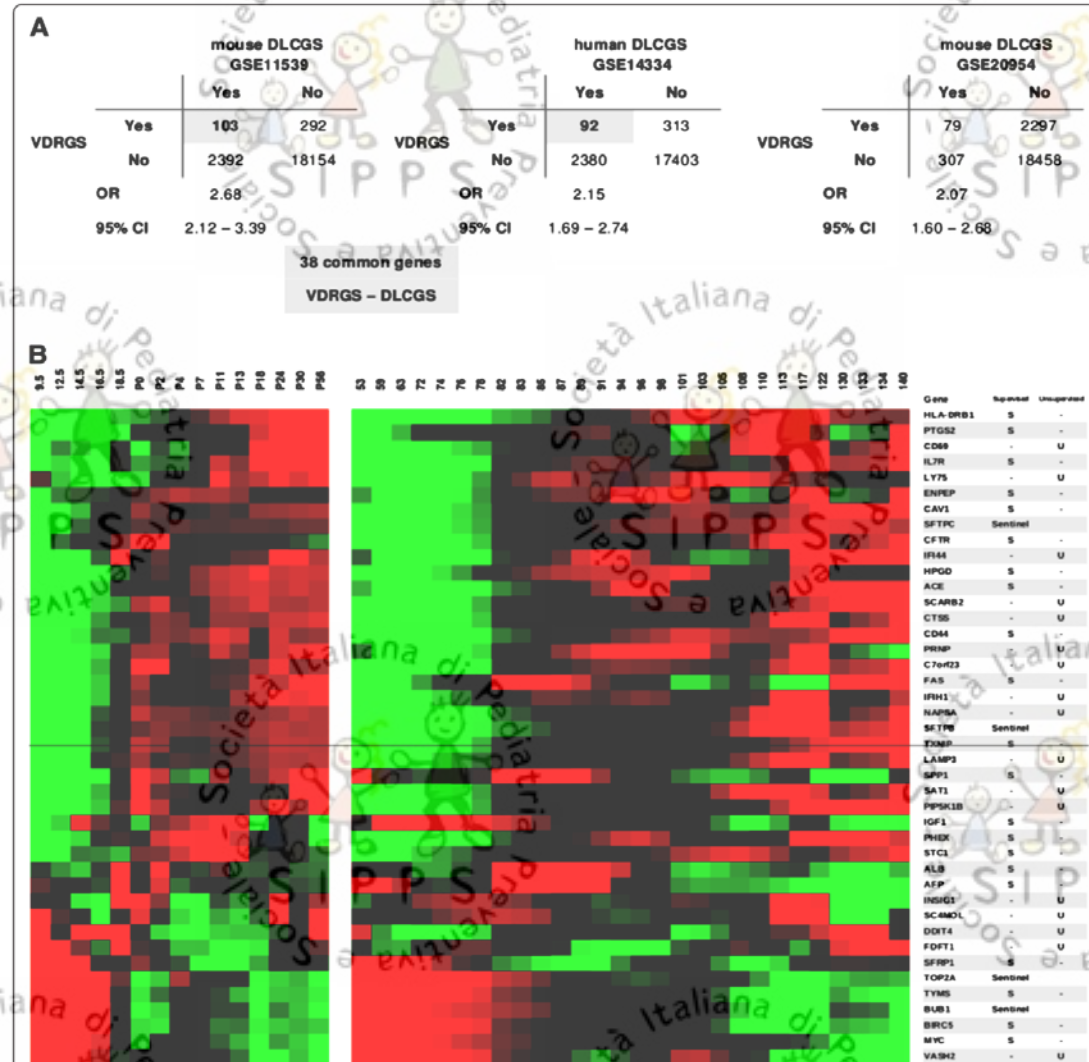


Vitamin D related genes in lung development and asthma pathogenesis

Kho et al. BMC Medical Genomics 2013, 6:47

✓ Inoltre, dei 12 geni trascrizionalmente attivi nello sviluppo polmonare e correlati allo sviluppo di asma, 4 - LAMPADA1, PIP5K1B, SCARB2, e TXNIP – venivano espressi in maniera significativamente differente in seguito alla somministrazione di vitamina D nelle cellule derivate da bambini asmatici.

✓ In conclusione l'insufficienza materna di vitamina D può portare a una differenziale regolazione dello sviluppo di geni chiave vitamina D sensibili nello sviluppo del polmone fetale e, quindi, aumentare il rischio di asma infantile.

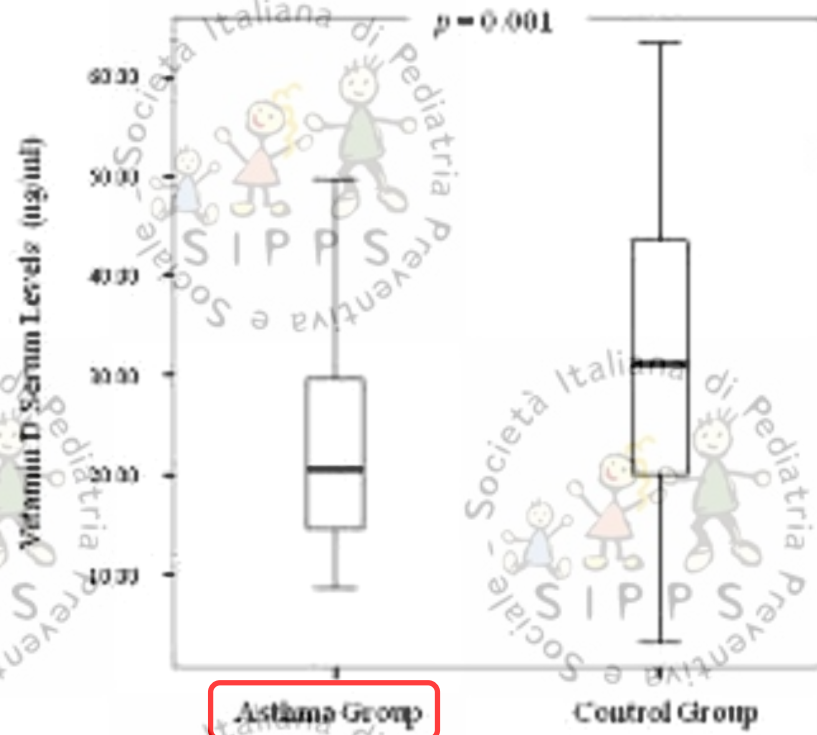


Effects of vitamin D levels on asthma control and severity in pre-school children

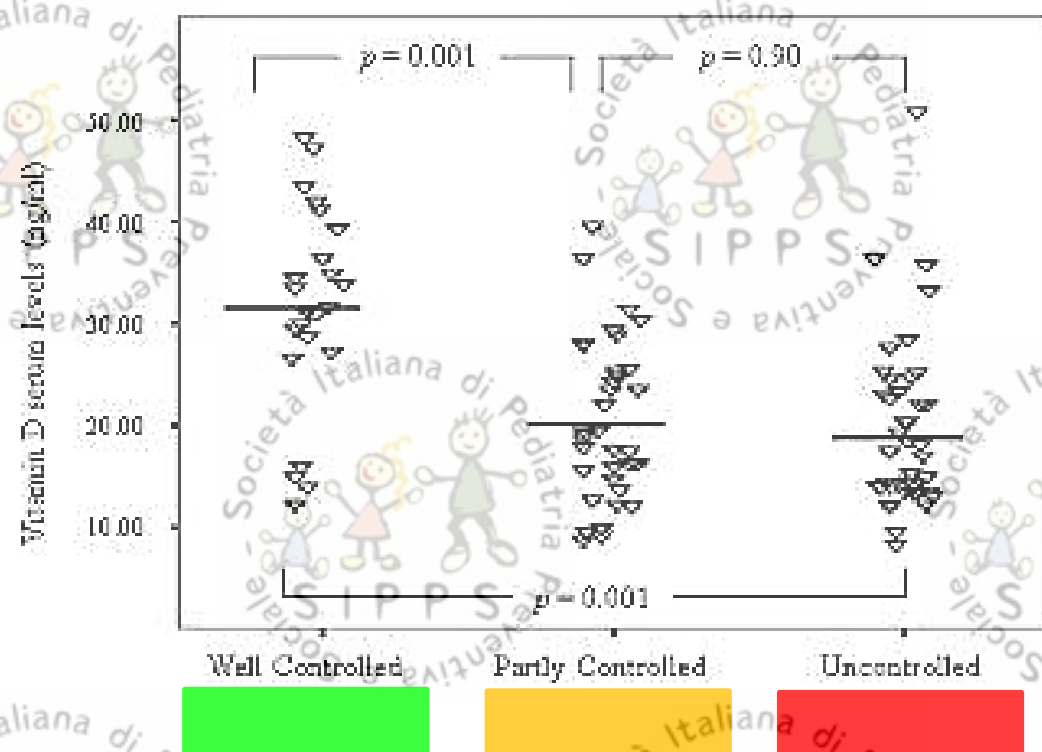
Turkeli A et al *Eur Rev Med Pharmacol Sci* 2016; 20: 26-36

Vitamin D levels were measured in 102 preschool ch., aged 1-4 years with asthma and 102 healthy controls in winter.

Vitamin D levels



Asthma control



There was a positive correlation between serum vitamin D levels and asthma control.

Vitamin D level and brochial inflammation in asthmatic children

M. Miraglia del Giudice et al JBRHA 2015; 2: 125-29



66 bambini asmatici (45 M e 21 F) di età compresa tra 4 e 19 anni.

I bambini, sono stati divisi in due gruppi:

> **Gruppo 1** : bambini (n° 46) con livelli ematici di 25 OH vitamina D < 30 ng/ml .

> **Gruppo 2** : bambini (n° 20) con livelli ematici di 25 OH vitamina D > 30 ng/ml.

Parametri	Gruppo 1 (Vit.D<30 ng/ml)	Gruppo 2 (Vit.D>30 ng/ml)	Valore di P*
Vit D (ng/ml)	21,1 ± 6	32 ± 1	<0,0001
FeNO in ppb	32,3 ± 31,8	10,1 ± 3,6	0,0018
C-ACT	20,3 ± 4,3	20,9 ± 3,6	0,9

I b. asmatici con livelli di 25 OH Vit. D > 30 ng/ml mostravano una riduzione significativa nei livelli di di FeNO(p = 0,0018) rispetto ai quelli con livelli di 25 OH Vit. D < 30 ng/ml.

In conclusione, i nostri dati evidenziano un effetto antinfiammatorio della Vit.D sull'infiammazione bronchiale misurata con il FeNO in b. asmatici

Effect of Vitamin D and Inhaled Corticosteroid Treatment on Lung Function in Children

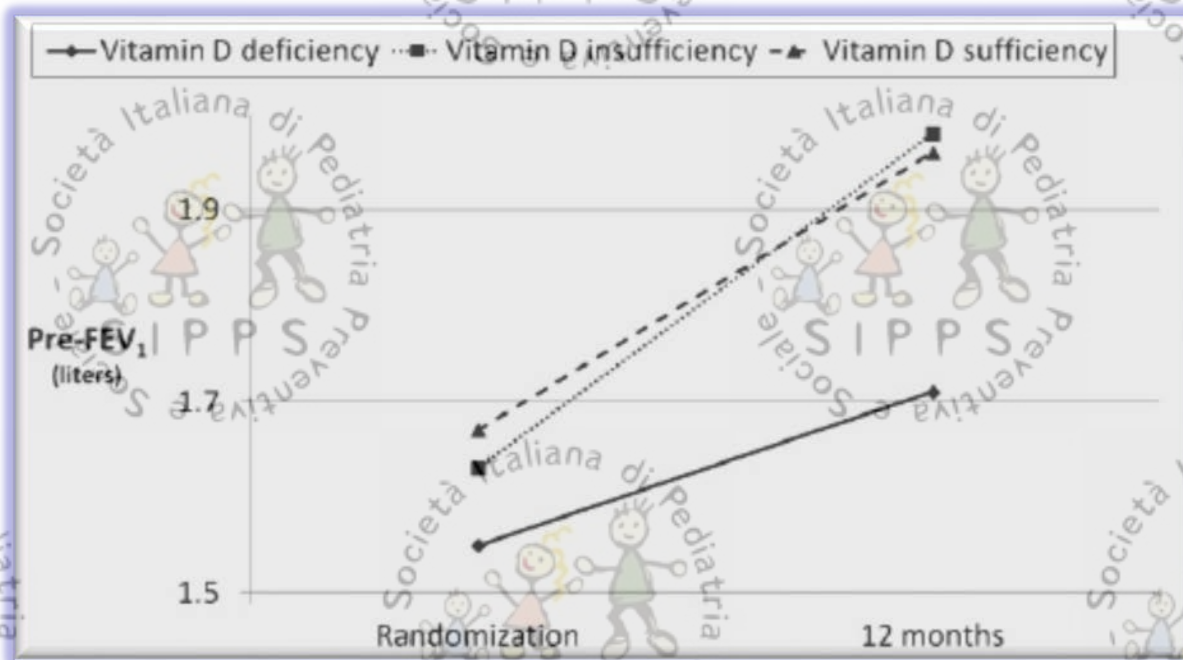
Wu AC et al *Am J Respir Crit Care Med* 2012; 186:508–513

■ Bambini asmatici (n° 1024) con bassi livelli di Vit D hanno un ridotto miglioramento del FEV1 dopo 1 anno di trattamento con CSI rispetto a quelli con livelli sufficienti di vitamina D.

■ Questi risultati supportano l'ipotesi che la **supplementazione di vitamina D può aumentare l'azione antiinfiammatoria dei CSI** nei bambini con asma.



Vit. D sufficiency (>30 ng/ml), insufficiency (20–30 ng/ml), and deficiency (<20 ng/ml)



Change in prebronchodilator FEV1 predicted from randomization to 12 months for vitamin D deficiency (solid line, n 110), insufficiency (dotted line, n 260), and sufficiency (dashed line, n 663) groups, while adjusting for age, sex, race, body mass index, history of emergency department visit, and season

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Probiotici e ALLERGIA

Probiotici e ASMA

Probiotici e RINITE

VITAMINA D

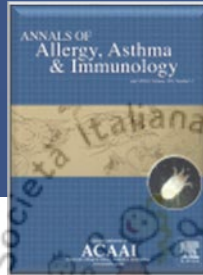
Vitamina D e ASMA


PROBIOTICI + VIT. D



Lactobacillus reuteri DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma.

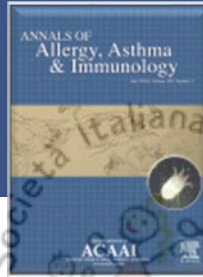
Miraglia Del Giudice M, et al Ann Allergy Asthma Immunol. 2016 Dec;117(6):710-712



- ➡ Randomized DBPCT, 32 ch. age 6-14 years
- ➡ **Inclusion criteria** were as follows:
 - (1) diagnosis of **mild persistent asthma** well controlled with montelukast (5 mg/d).
 - (2) allergy to **HDMs**. 
 - (3) serum **Vit. D level less than 30 ng/mL**.

Lactobacillus reuteri DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma.

Miraglia Del Giudice M, et al Ann Allergy Asthma Immunol. 2016 Dec;117(6):710-712



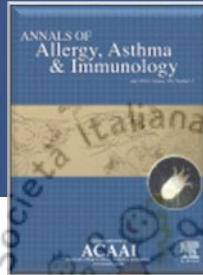
➡ **Group A** : L. Reuteri + Vitamin D3 (400 IU)

➡ **Group B** : placebo

- Children were revisited after 90-days, at the end of treatment (T1) and after a 30-days follow-up (T2).
- Montelukast treatment was continued throughout the study. Salbutamol spray was used as symptomatic treatment.

Lactobacillus reuteri DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma.

Miraglia Del Giudice M, et al Ann Allergy Asthma Immunol. 2016 Dec;117(6):710-712

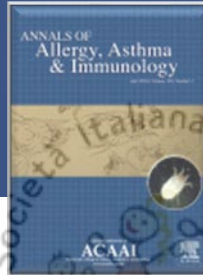


- **infiammazione allergica bronchiale** → **FeNO (primary outcome)**
- **funzionalità polmonare** → **ΔFEV₁**
- **controllo dell'asma** → **C-ACT**

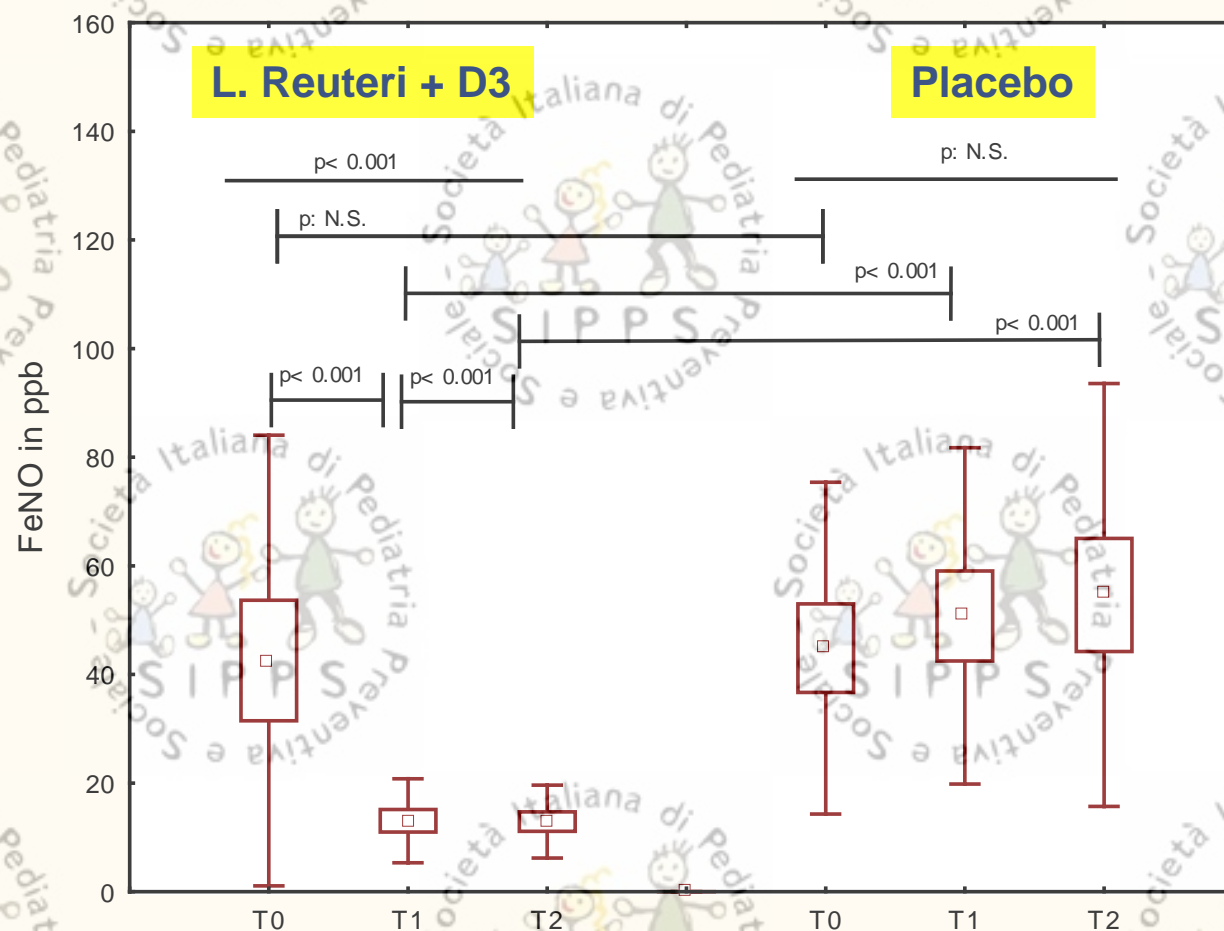
Variable	T0			T1			T2		
	Group A	Group B	P Value ^b	Group A	Group B	P Value ^b	Group A	Group B	P Value ^b
Serum vitamin D, ng/mL	18.3 (5)	17.8 (4)	NS	35.8 (8)	16.9 (7)	<.001			
FeNO, ppm	42.5 (3.9)	44 (3.6)	NS	13 (7)	51 (40)	<.001	12.9 (6.7)	55 (47)	<.001
ΔFEV ₁ , % of predicted	9.8 (6)	8.7 (7)	NS	6.1 (4)	8.5 (5)	.02	5.6 (3.6)	9.3 (5)	<.05
C-ACT	23 (2)	21 (3.6)	NS	24 (4)	16 (4.6)	<.001			

Lactobacillus reuteri DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma.

Miraglia Del Giudice M, et al *Ann Allergy Asthma Immunol.* 2016 Dec;117(6):710-712

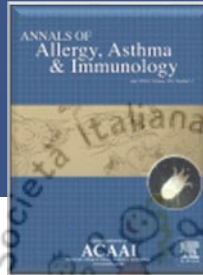


infiammazione allergica bronchiale → FeNO (primary outcome)

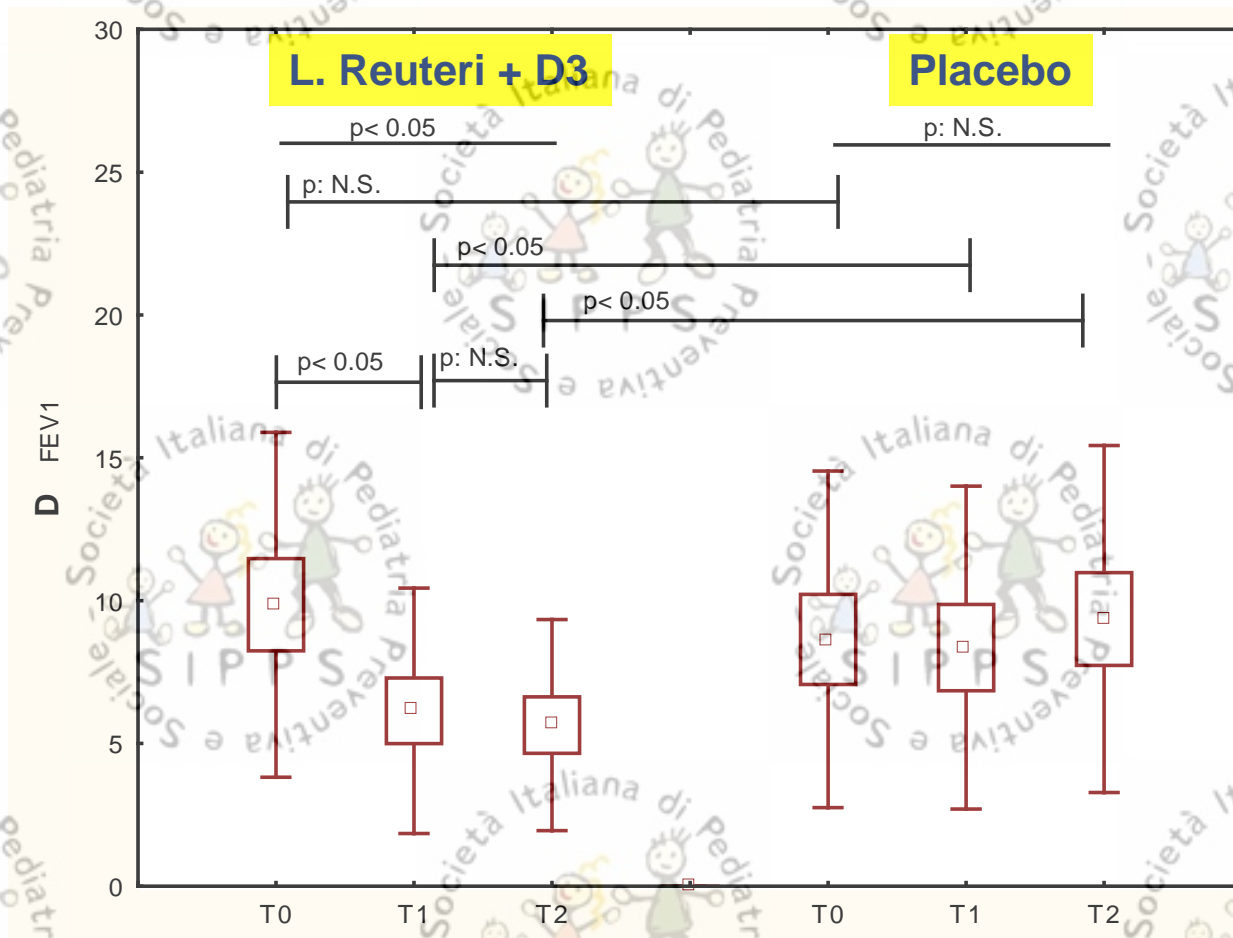


Lactobacillus reuteri DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma.

Miraglia Del Giudice M, et al *Ann Allergy Asthma Immunol.* 2016 Dec;117(6):710-712

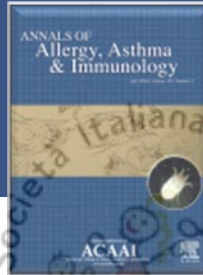


Funzionalità polmonare → Δ FEV₁

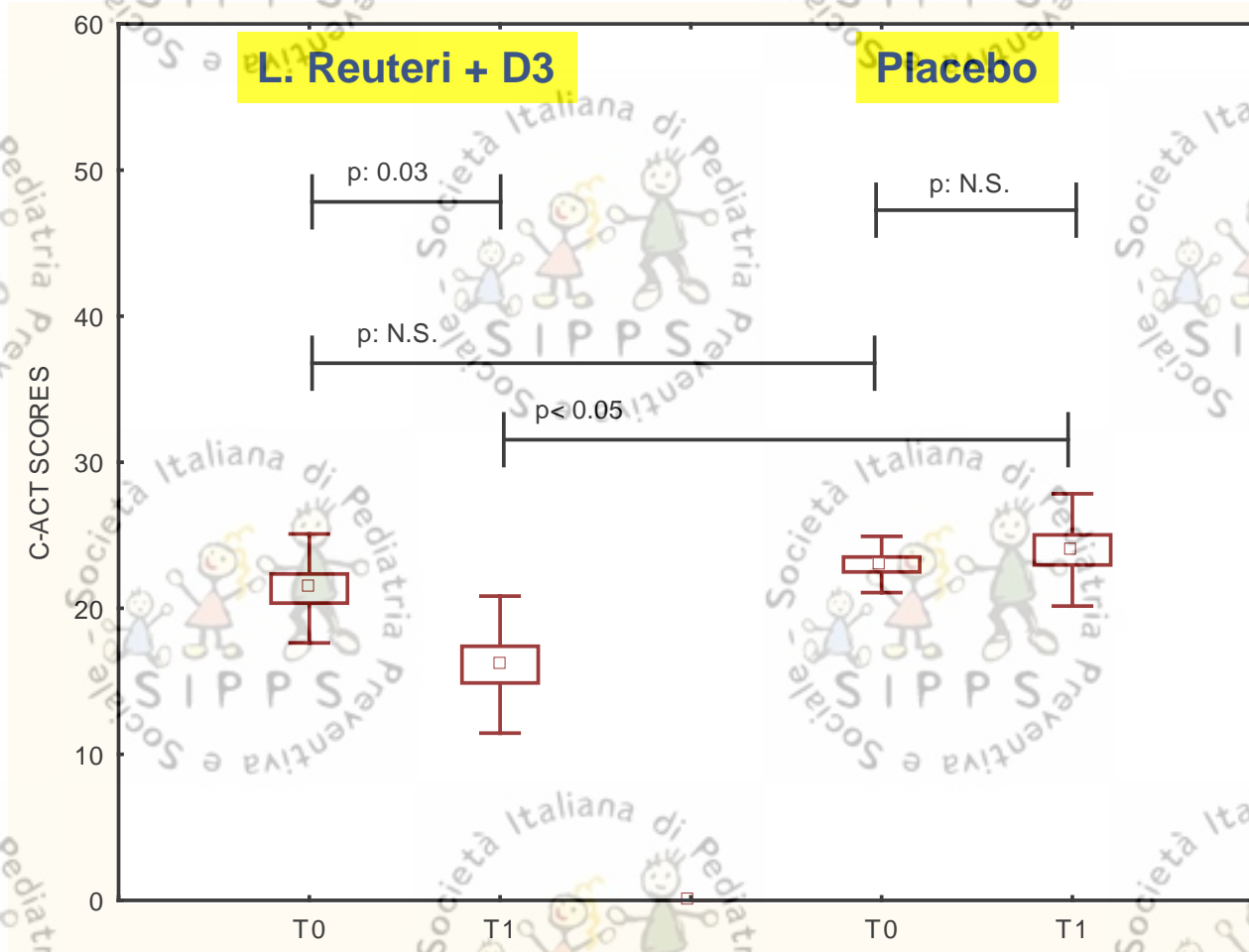


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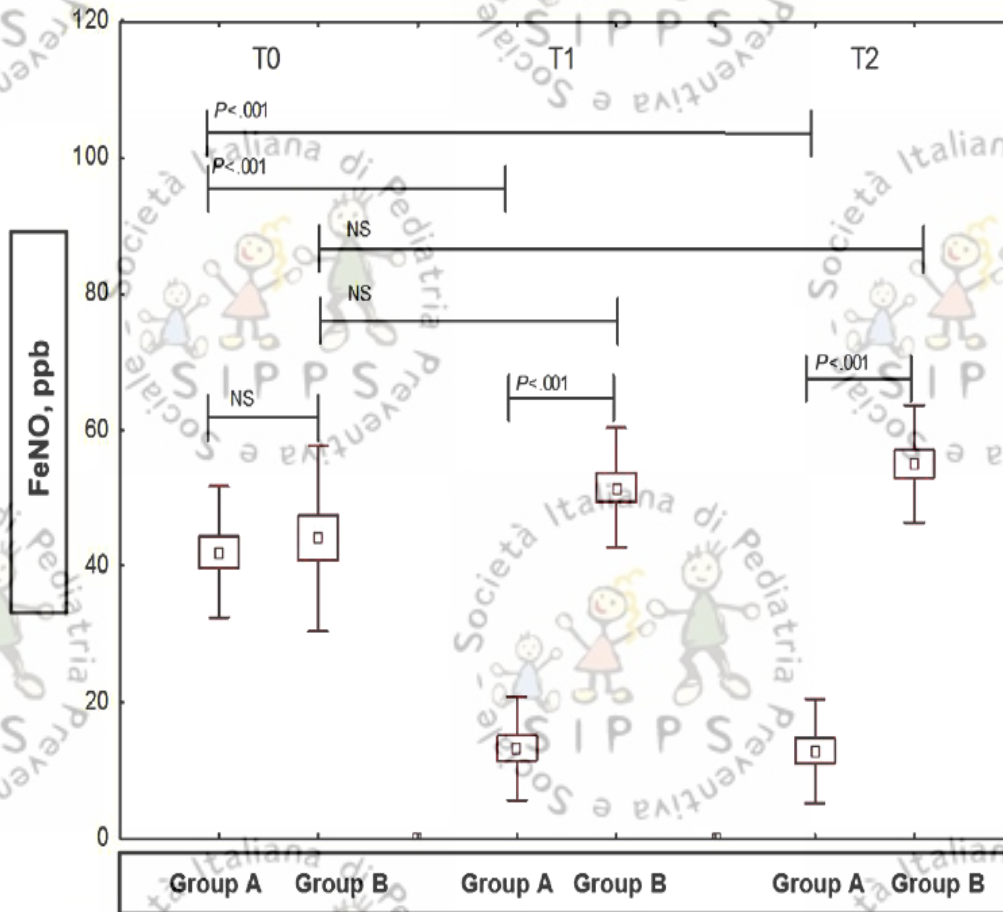
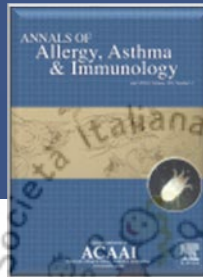


controllo dell'asma → C-ACT



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➡ The present pilot study found that the food supplement that contained **L reuteri DSM 17938 (108 CFU) plus vitamin D3 (400 IU)** was effective in reducing bronchial inflammation.

➡ In addition, there was a **better asthma control** and a **reduced response to bronchodilation** in actively-treated children.

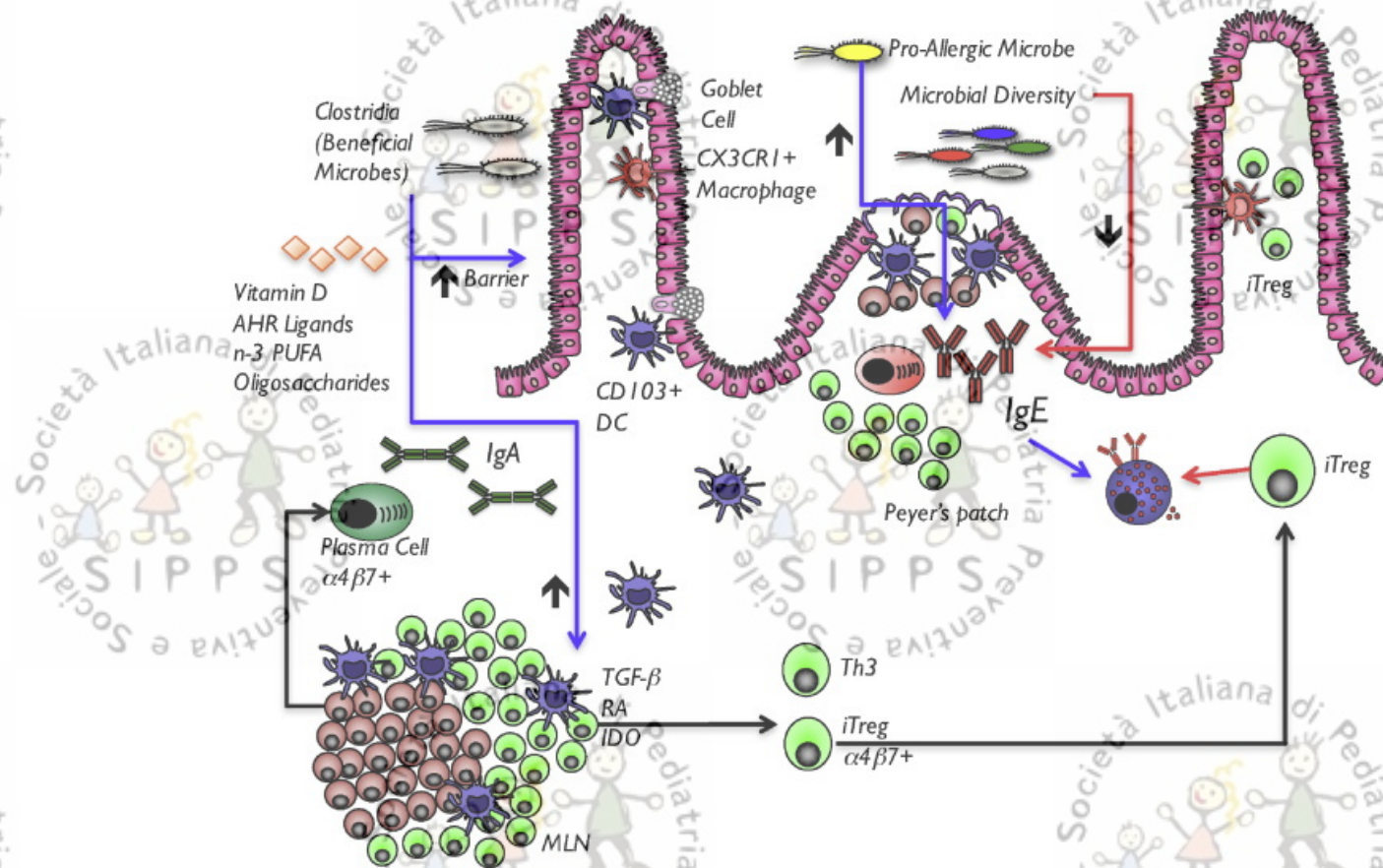
➡ These findings were associated with significant increase in serum vitamin D3 concentration



WINE

The rise of food allergy: Environmental factors and emerging treatments

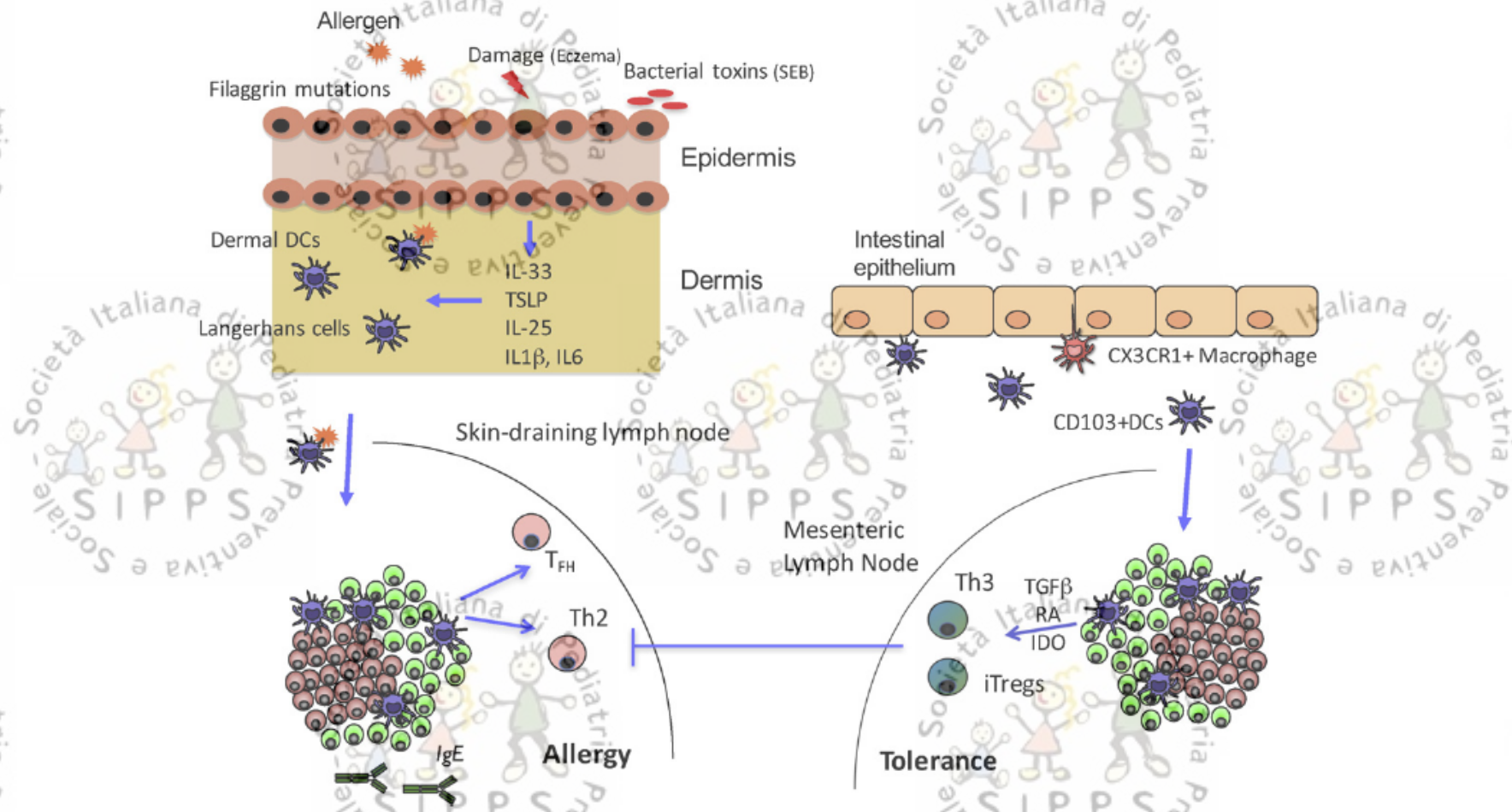
Sara Benedé et al *EBioMedicine* 7 (2016) 27–34



Microbiota and diet influence the development of allergy and tolerance. Microbial diversity suppresses IgE class-switching, which occurs within the Peyer's patch. Strains of bacteria including Clostridia have been shown to suppress allergy, and to enhance the generation of Tregs and improve epithelial barrier function. There is also evidence that microbial composition can promote food allergy, suggesting the role of pro-allergic bacteria. Nutrients including vitamin D, aryl hydrocarbon receptor (AHR) ligands, polyunsaturated fatty acids (PUFA) and oligosaccharides can also suppress food allergy through enhancement of regulatory responses

The rise of food allergy: Environmental factors and emerging treatments

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Allergen exposure through the skin in the presence of skin damage, filaggrin mutation or bacterial toxins (SEB) promotes sensitization. Depending on the nature of the allergen and adjuvant, epithelial cells produce cytokines that instruct dendritic cells on the skin. They transport the antigen to the skin-draining lymph nodes, where Th2 and T follicular helper (T_{FH}) cells are generated and promote IgE class-switching. Antigen exposure by oral route leads to tolerance. CX3CR1+ macrophages sample antigen from the lumen and transfer it to CD103+DCs that transport the antigen to the mesenteric lymph nodes and promote the induction of Tregs. Oral tolerance can prevent the development of sensitization through the skin.