

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/301584161>

The potential immunonutritional role of parmigiano reggiano cheese in children with food allergy

Article in *Progress in Nutrition* · March 2016

CITATIONS

0

READS

105

8 authors, including:



[Rita Nocerino](#)

University of Naples Federico II

82 PUBLICATIONS 298 CITATIONS

[SEE PROFILE](#)



[Lorella Paparo](#)

University of Naples Federico II

35 PUBLICATIONS 92 CITATIONS

[SEE PROFILE](#)



[Rosita Aitoro](#)

University of Naples Federico II

35 PUBLICATIONS 70 CITATIONS

[SEE PROFILE](#)



[Roberto Berni Canani](#)

University of Naples Federico II

288 PUBLICATIONS 4,315 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



MATFA (Gut Microbiota as Target for Food Allergy) [View project](#)

All content following this page was uploaded by [Roberto Berni Canani](#) on 26 April 2016.

The user has requested enhancement of the downloaded file. All in-text references [underlined in blue](#) are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.

PROGRESS IN NUTRITION

JOURNAL OF NUTRITIONAL AND INTERNAL MEDICINE

Organo Ufficiale della Società Italiana di Scienza dell'Alimentazione (S.I.S.A.)
Con il patrocinio dell'Associazione Ricercatori di Nutrizione e Alimenti (A.R.N.A.)

FONDATORE / FOUNDING EDITOR

Massimo Cocchi
Scottish Agricultural College, Edinburgh

PRESIDENTE / PRESIDENT

Andrea Strata
Università di Parma

DIRETTORE SCIENTIFICO / EDITOR

Leone Arsenio
Azienda Ospedaliera Universitaria di Parma

DIRETTORE RESPONSABILE /
JOURNAL DIRECTOR

Federico Cioni

COMITATO SCIENTIFICO / EXECUTIVE EDITORS

F. Arfini

Università di Parma

D. Atkinson

Scottish Agricultural College, Edinburgh

G. Ballarini

Università di Parma

S. Bernasconi

Università di Parma

R. Berni Canani

Università di Napoli

G. Bertoni

Università di Piacenza

S.E. Carlson

Kansas City University

F. Di Lisa

Università di Padova

G. Fatati

Università di Terni

N.G. Frega

Università di Ancona

C. Galli

Università di Milano

C. Giacomini

Università di Parma

M. Gualerzi

Direttore Sanitario Terme di Salsomaggiore e Tabiano, Parma

G.M. Halpern

Hong Kong Polytechnic University

E.L. Iorio

*Presidente Osservatorio Internazionale dello
Stress Ossidativo, Salerno*

T. Leighton

Berkeley University

M.C. Mancini

Università di Parma

R. Marchelli

Università di Parma

P. Migliaccio

Università Sapienza di Roma

A.L. Mordenti

Università di Bologna

K. Mullis

Premio Nobel per la Chimica 1993

S.M. Nabavi

Baqiyatallah University of Medical Sciences, Tehran, Iran

F. Nicastro

Università di Bari

R.C. Noble

Scottish Agricultural College of Edinburgh

G. Palmieri

Primario Emerito Medicina Interna, Azienda Ospedaliera

Ospedale Niguarda Ca' Granda, Milano

G. Riccardi

Università di Napoli

C.M. Rotella

Università di Firenze



PUBLISHER

Mattioli 1885 srl Casa Editrice
Strada di Lodesana, 649/sx, Loc. Vaio
43036 Fidenza (PR), Italy
Tel. ++39 0524 530383
Fax ++39 0524 82537
E-mail: edit@mattioli1885.com
www.progressinnutrition.it



Mattioli 1885

srl- Strada di Lodesana 649/sx
Loc. Vaio - 43036 Fidenza (Parma)
tel 0524/530383
fax 0524/82537
www.mattioli1885.com

DIREZIONE GENERALE
Direttore Generale
Paolo Cioni
Vice Presidente e Direttore Scientifico
Federico Cioni

DIREZIONE EDITORIALE
Editing Manager
Anna Scotti
Editing
Valeria Ceci
Foreign Rights
Nausicaa Cerioli

MARKETING E PUBBLICITÀ
Direttore Commerciale
Marco Spina
Responsabile Area ECM
Simone Agnello
Project Manager
Natalie Cerioli
Massimo Radaelli
Responsabile Distribuzione
Massimiliano Franzoni

PROGRESS IN NUTRITION
Registrazione Tribunale di Parma
N. 4 del 21/1/1999

Spedizione in abbonamento postale
Abbonamento annuale euro 57

I dati sono stati trattati elettronicamente e utilizzati dall'editore Mattioli 1885 spa per la spedizione della presente pubblicazione e di altro materiale medico scientifico. Ai sensi dell'Art. 13 L. 675/96 è possibile in qualsiasi momento e gratuitamente consultare, modificare e cancellare i dati o semplicemente opporsi all'utilizzo scrivendo a: Mattioli 1885 srl - Casa Editrice, Strada della Lodesana 249/sx, Loc. Vaio, 43036 Fidenza (PR) o a direct@mattioli1885.com

Progress in Nutrition is indexed by: Science Citation Index Expanded (SciSearch®); Journal Citation Reports/Science Edition; Excerpta Medica/Embase, ISI Web of Science; Scopus

Impact Factor (released in June 2015): 0,143

INDEX

Volume 18 / n. 1

March 2016

Reviews

- 3 *Giusi Grimaldi, Rita Nocerino, Lorella Paparo, Linda Cosenza, Rosita Aitoro, Giovanna Trinchese, Antonio Amoroso, Roberto Berni Canani*
The potential immunonutritional role of Parmigiano Reggiano cheese in children with food allergy
- 8 *Roberta Altomare, Giuseppe Damiano, Maria Concetta Gioviale, Vincenzo Davide Palumbo, Carla Maione, Gabriele Spinelli, Emanuele Sinagra, Alida Abruzzo, Giulia Lo Monte, Giovanni Tomasello, Attilio Ignazio Lo Monte*
The intestinal ecosystem and probiotics

Original articles

- 16 *Mahdieh Abbasalizad Farhangi*
Nutritional status and feeding practices in pre-school children aged 1-5 years in rural and urban areas of East Azerbaijan - Iran
- 22 *Seyed Mohammad Hosein Mousavi Jazayeri, Alireza Ostadrahimi, Abdolrasoul Safaiyan, Shahryar Hashemzadeh, Firouz Salehpour*
Standard enteral feeding improves nutritional status compared with hospital-prepared blended formula among Intensive Care Unit (ICU) patients
- 26 *Ilknur Aydin Avci, Burcu Nal, Mustafa Ayyildiz*
Assessment of chronic disease prevalence, nutritional habits and healthy lifestyle behaviors in elderly patients
- 32 *Pınar Sökülmez Kaya, Ali Eba Demirbağ, Meral Akdoğan Kayhan*
Nutritional problems of liver transplant candidates
- 39 *Mahmoud Mustafa Ali Abulmeaty, Ali Madi Almajwal, Mohammed Abdullah Alsaif, Heba Mohammed Zaher Hassan, Sarah Khalid Almansour*
Impedancemetry vs. anthropometry in the prediction of body adiposity and obesity diagnosis
- 47 *Abmet Gultekin, Dilek Memis, Mehmet Turan Inal, Osman Uzundere, F. Nesrin Turan*
The impact of nutritional risk screening 2002 and subjective global assessment upon prognosis for intensive care patients
- 56 *Abdullah Aslan, Didem Boydak, Muhammed Ismail Can, Tuncay Kuloglu, Serpil Baspinar*
Black cumin may be a potential drug for development of carbontetrachloride-induced lung damage in rats
- 63 *Khalid Mohammed Elbindi, Salah El-Hendawy, Eslam Abdel-Salam, Urs Schmidhalter, Shafiq ur Rahman, Al-Adl Hassan*
Foliar application of potassium nitrate affects the growth and photosynthesis in coriander (*Coriander sativum* L.) plants under salinity
- 74 *Seyed Fazel Nabavi, Seyed Mohammad Nabavi, Mohammad Ali Ebrahimzadeh*
Antioxidant activity of hydro-alcoholic extracts of 4 citrus species flower
- 81 *Fetbi Ahmet Ozdemir, Omer Kilic, Ekrem Atalan*
In vitro callus propagation and antibacterial activities of callus an edible endemic and medicinal plant *Scutellaria orientalis* L. subsp. *bicolor*
- Case Report**
- 87 *Maurizio Mennini, Diletta Valentini, Chiara Di Camillo, Anna Chiara Vittucci, Andrzej Krzysztofkiak, Alberto Villani*
Children's environmental health: a target for all Pediatricians

The potential immunonutritional role of parmigiano reggiano cheese in children with food allergy

Giusi Grimaldi¹, Rita Nocerino¹, Lorella Paparo¹, Linda Cosenza¹, Rosita Aitoro¹, Giovanna Trinchese¹, Antonio Amoroso¹, Roberto Berni Canani^{1,2,3}

¹Department of Translational Medical Science; ²European Laboratory for The Investigation of Food Induced Diseases; ³CEIN-GE Advanced Biotechnologies, University of Naples "Federico II", Naples, Italy - E-mail: berni@unina.it

Summary. Parmigiano Reggiano (PR) is a ripened cheese with high nutritional value. Throughout ripening the bacteria contained in PR promote an extensive hydrolysis of cow's milk proteins resulting in peptides that exhibit positive immunoregulatory activities. Additional modulatory activities on immune system are induced by butyrate, a short chain fatty acid widely expressed in PR. These findings suggest a possible immunonutritional role for PR able to stimulate oral tolerance in children with food allergy (FA).

Keywords: cow's milk allergy, oral tolerance, microbiota, extensively hydrolysed casein formula, short chain fatty acids, butyrate.

The Food Allergy Scenario

Food allergy (FA) is one of the most common chronic diseases in Italian children. There is no cure and the strict exclusion of dietary allergens together with the ready availability of life-saving drugs are the only therapeutic options available (1). Prevalence, persistence and severity of FA increased significantly over the last two decades in all industrialized countries under the pressure of the interaction gene-environment able to determine a dysfunction of the immune system, mediated, at least in part, by epigenetic mechanisms. This changing pattern of FA is promoting research efforts on the identification of possible strategies to stimulate oral tolerance in children with FA.

Primary aim for treatment and prevention of food allergy: oral tolerance

Oral tolerance consists in the well modulated suppression of the immune response to dietary antigens

(2). The mechanisms involved in the acquisition of oral tolerance are various and not yet fully defined. An essential role is played by gut microbiota and epithelium, dietary proteins, dendritic cells and activated regulatory T lymphocytes (Treg) (2) (Fig. 1). Recent evidence has emphasized the role of a healthy gut microbiota in maintaining the balance of microbial signals necessary to prevent or treat the FA. Changes in microbial exposure pattern (dysbiosis) in early life are now considered a critical factor for FA development (3).

Potential benefits of immunonutrition in patients with food allergy

Through the intake of specific nutrients, immunonutrition offers the possibility to regulate directly or indirectly (through a modulation of gut Microbiota composition and function) the activity of the immune system (4). A better understanding of the diet's effect on gut microbiota composition and function is very important to develop innovative preventive and thera-

peutic strategies against FA. Recent example of the potential importance of immunonutrition against FA has been obtained in cow's milk allergy (CMA), the most frequent FA in Italian children aged less than 3 years. In these subjects specific peptides derived from cow's milk proteins and a specific bacterial strain are able to regulate the immune mechanisms involved in the allergic response, at least in part through an epigenetic modulation of gene expression, leading to a positive influence on oral tolerance acquisition (5). We showed that it is possible to stimulate oral tolerance acquisition in children with CMA through the administration of extensively hydrolyzate casein formula containing the probiotic *L. rhamnosus* GG (LGG) (5). This derives from a combination of direct immunomodulator action induced by small peptides derived from beta-casein hydrolysis and by the action of this particular probiotic strain (6). We have also shown that LGG is able to regulate the composition and function of gut microbiota in children with CMA and to regulate directly some immunological mechanisms involved in the pathogenesis of this condition (7). At the same time it has been shown that a percentage of CMA subjects is able to tolerate food containing cow's milk hydrolyzed protein by enzymatic degradation and heat treatment. Both conditions determine not only a reduced risk of allergic reaction to cow's milk proteins, but they can also lead to the formation of peptides and substances with intrinsic immunoregulatory activities, that are able to facilitate the acquisition of oral tolerance in patients with CMA (2). In fact it has been demonstrated a significant effect elicited by beta-casein-hydrolysis derived peptides on the differentiation of Treg and the production of interleukin (IL)-10 (a potent cytokine with anti-inflammatory and anti-allergic activity) (8).

Potential immunonutritional role of Parmigiano Reggiano cheese in children with food allergy

Parmigiano Reggiano cheese (PR), thanks to the fermentation processes (maturation) is characterized by an extensive hydrolysis of cow's milk proteins resulting in the production of high quantities of peptides and free amino acids (9). It has also been demonstrated

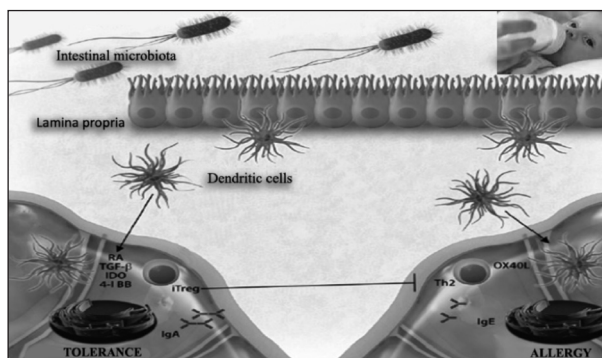


Figure 1. Oral tolerance: suppression of the immune response to food antigens mediated by the combined action of the intestinal microbiota, dietary factors, dendritic cells and antigen-specific T regulator lymphocytes.

the formation of non proteolytic amino acid derivatives (NPAD), produced by the recombination of single amino acids, that are able to stimulate the production of peptides of innate immunity (9). It has been demonstrated that children with CMA can tolerate oral intake of ripened PR (24–40 months) in over 50% of cases (10). During the PR's ripening process the lipidic component is also modified: a discrete amount of short-chain fatty acids (SCFAs) are synthesized with a considerable production of butyrate (average concentration in 36-month PR= 120mg/100g). It is important to highlight that PR contains 100% more butyrate than other fermented cheeses, such as Grana Padano (11). The *L. rhamnosus* contained in PR seems to play a crucial role in producing high levels of enzymes responsible for the proteolytic activity, fatty acids metabolism and butyrate release (12). By analyzing the PR's composition and considering its high content in hydrolyzates peptides, the presence of butyrate and other bacterial components, it is possible to hypothesize that this cheese has a high potential immunoregulatory role (Fig. 2).

Role of the bacterial component of Parmigiano Reggiano cheese

The PR has a specific microbial structure that seems to play a crucial role in determining the characteristics that make it an unique cheese. The starter cultures are strains of lactic acid bacteria (SLAB) that are added to the milk to induce the fermentation process

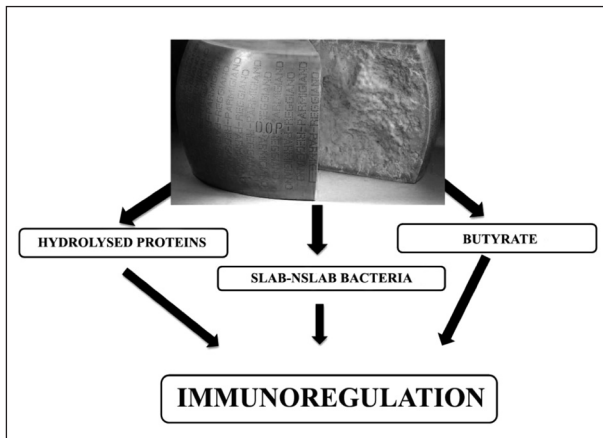


Figure 2. The three essential components of Parmigiano Reggiano: hydrolysed peptides, bacterial products and butyrate. All these components induce regulator effects of the function of the immune system, with anti-inflammatory and anti-allergic activities.

(producing lactic acid through the complete degradation of lactose) and, at the same time, to give the sensory characteristics of the cheese (improving flavor and texture). These bacteria are also important for the consumer safety since they are able to inhibit the growth of undesired microorganisms. However, during the ripening process the variation of temperature, the salt concentration, the content of water and the pH progressively reduce the SLAB population. The release of intracellular enzymes in the cheese matrix is crucial in the final process of degradation of caseins (13). Among the SLAB contained in PR, various studies have highlighted the dominant presence of thermophilic bacte-

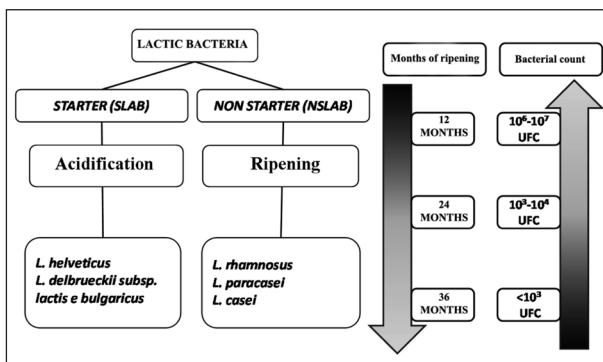


Figure 3. Bacterial component of Parmigiano Reggiano (PR): starter lactic acid bacteria (SLAB) contribute to acidification, non starter lactic acid bacteria (NSLAB) promote the ripening process. The total bacterial count decreases progressively with increasing months of maturation.

ria: *L. helveticus*, *L. delbrueckii subsp. lactis e bulgaricus*, however, they tend to decrease, progressively, from the first month of ripening (14) (Fig. 3). During ripening, the reduction of SLAB is associated with a progressive increase of non-starter mesophilic lactobacilli (NSLAB) of which the *L. rhamnosus* and *L. paracasei* species represent the major component. The NSLAB play a significant role in the biochemical process of cheese ripening (15). Recently it has been demonstrated the possible probiotic role of some NSLAB: *L. casei* and *L. rhamnosus* (15). We have also demonstrated that the LGG has an important immunoregulatory activity in the control of IgE-mediated response (6,7). This immunomodulator activity is induced at least in part by the lipoteicoic acid of the cell membrane (LTA) and by specific bacterial DNA sequences that act upon different intracellular pathways of signal transduction. LTA binds to TLR2 / 6 cell receptor and activates signaling pathways that have a co-stimulatory function in inducing tolerance: a gene response Th1-like, which suppresses the allergic response mediated by Th2 cells and the subsequent production of IgE. The second transduction pathway is activated by bacterial DNA found in PR. 163 micrograms of DNA are contained in 1 mg of PR, in the form of CpG oligodeoxynucleotides (CpG ODN). They are small sequences of DNA single chain, containing unmethylated CpG nucleotides, that act as potent stimulators of the immune response, resulting in overproduction of IL-10 (Fig. 4).

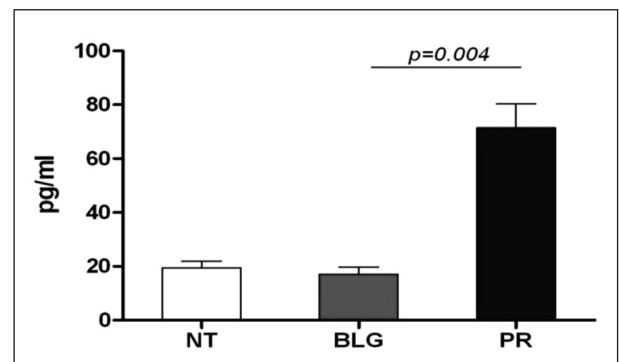


Figure 4. Production of interleukin (IL)-10, cytokine with anti-inflammatory and anti-allergic effects, by peripheral blood lymphocytes of patients with IgE-mediated cow's milk allergy after 24 hours of incubation with beta-lactoglobulin (BLG), Parmigiano Reggiano (PR) or unstimulated (NT) (n=3 experiments)

Role of the proteic component of Parmigiano Reggiano

A further immunoregulatory effect of PR seems to be induced by peptides produced during fermentation: higher is the ripening time more massive is the proteic hydrolysis (Tab. 1). It has been shown that among all the peptides obtained, two fragments resulting from β -casein have strong immunoregulatory activities: Gly-Leu-Phe (GLF) and Val-Glu-Pro-Ile-Pro-Tyr (VEPIPYPY), that are located in specific regions 60-69 and 193-209 (2). The two peptides are able to bind different receptors: GLF recognizes the receptor expressed on the surface of monocytes and polymorphonuclear leukocyte while VEPIPYPY recognizes the

receptors of monocytes and macrophages inducing potent anti-inflammatory and anti-allergic effects (2).

Role of butyrate contained in Parmigiano Reggiano

The immunonutritional properties of PR can also be attributed to the high concentration of butyrate. The use of 1-2 teaspoons of PR (about 6 g) in fact allows to assimilate about 7 mg of butyrate, the same quantity contained in 100 ml of breast milk. The transduction pathway implicated in this case seems to involve the pathway of IL-10: through the activation of Treg cells the butyrate induces a suppression of Th2 cell response, responsible for allergy outbreak. The epigenetic

Table 1. Main peptides obtained by bacterial proteolysis during ripening of PR (Modified from reference 9)

Peptide	MW	Peptide	MW	Peptide	MW
Lac-Val •	189	α S1 CN f (1-9)	1140	α S1 CN f (10-16)	754
Lac-Ile •	203	β CN f (199-209)	1151	β CN f (47-52)	755
Lac-Leu •	203	β CN f (17-25)2P	1154	β CN f (1-6)	787
Lac-Met •	221	α S1 f (169-179)	1198	α S1 CN f (18-23)	791
Lac-Phe •	237	S1 CN f (24-34)	1237	α S1 CN f (24-30)	805
Pyr-Ile •	242	S1 CN f (14-23)	1246	α S1 CN f (1-7)	874
Pyr-Leu •	242	β CN f (16-25)3P	1348	α S1 CN f (17-23)	905
γ Glu-Vat •	246	β CN 1 (71-83)	1403	β CN f (60-68)	1001
Lac-Tyr •	253	S1 CN f (24-36)	1495	β CN f (111-119)	1122
γ -Glu-Ile •	260	β CN f (82-95)	1510	α S2 CN f (154-162)	1132
γ -Gtu-Leu •	260	α S1 CN f (1-13)	1535	α S1 CN f (115-123)1P	1138
Pyr-Phe •	276	β CN f (195-209)	1589	β CN f (13-28) 4P	2098
γ -Glu-Met •	278	β CN f (17-28) 3P	1590	β CN f (12-28) 4P	2212
γ -Glu-Phe •	294	α S1CN f (1-14)	1664	β CN f (11-28) 4P	2340
γ -Gtu-Tyr •	310	β CN f (16-28)3P	1703	β CN f (142-161)	2340
α S1 CN f (10-13)	414	α S1 CN f (24-38)	1707	β CN f (159-183)	2746
β CN f (1-3)	416	β CN f (194-209)	1717	α S1 CN f (1-23)	2764
α S1 CN f (1-4)	536	β CN f (15-28)3P	1790	α S1 CN f (174-199)	2836
α S1 CN f (10-14)	542	β CN f (15-28) 4P	1870	β CN f (98-124)	3132
β CN f (1-4)	545	α S1 f (1-16)	1877	α S1 CN f (157-188)	3452
α S1 CN f (16-20)	601	β CN f (193-209)	1881	α S1 CN f (156-187)	3477
α S1 CN f (30-35)	707	α S1 f (1-17)	1991	β CN f (37-67)	3580
α S1 CN f (186-192)	734	β CN f (14-28) 4P	1999	α S1 CN f (85-114)	3601
α S1 CN f (1-6)	745	β CN f (103-119)	2042	α S1 CN f (155-187)	3605
β CN f (53-93)	4454	β CN f (57-93)	4065	α S1 CN f (83-114)	3860
β CN f (51-93)	4696	α S1 f (80-114)	4238	β CN f (59-96)	4024
β CN f (99-159)	7054	β CN f (55-93)	4253	β CN f (99-160)	7182

mechanisms regulated by butyrate are actually under investigation. By demethylation of histones, butyrate modulates the expression of FoxP3 gene, that is a transcription factor expressed by Treg lymphocytes. FoxP3 has a physiological function in the control of immune response, ensuring the immunological tolerance (16).

Conclusions

Parmigiano Reggiano cheese has not only favourable nutritional characteristics, but new data suggest the potential immunonutritional effect of this particular food which can modulate the function of the immune system and increase the immunological tolerance in children with food allergy. The mechanisms are varied and concern an epigenetic regulation of gene expression involved in inflammation and allergy.

Acknowledgements

This work was supported partially by a grant of Health Minister PE-2011-02348447 e Mother and Child Health Association (M.A.C.H.A.).

References

- Berni Canani R, Nocerino R, Terrin G et al. Hospital admissions for food-induced anaphylaxis in Italian children. *Clin Exp Allergy*. 2012;42:1813–1814. doi: 10.1111/cea.12036.
- Kiewiet MB, Gros M, van Neerven J, Faas M M, de Vos P. Immunomodulating properties of protein hydrolysates for application in cow's milk allergy. *Ped Allergy Immunol*. 2015;26:206–17. doi: 10.1111/pai.12354.
- Berni Canani R, Di Costanzo M. Gut microbiota as potential therapeutic target for the treatment of cow's milk allergy. *Nutrients*. 2013;5:651–62. doi: 10.3390/nu5030651.
- Berni Canani R, Costanzo MD, Leone L, et al. Epigenetic mechanisms elicited by nutrition in early life. *Nutr Res Rev*. 2011;24:198–205. doi: 10.1017/S0954422411000102.
- Berni Canani R, Nocerino R, Terrin G et al. Formula selection for management of children with cow's milk allergy influences the rate of acquisition of tolerance: a prospective multicenter study. *J Pediatr*. 2013;163:771–7.e1. doi: 10.1016/j.jpeds.2013.03.008.
- Berni Canani R, Di Costanzo M, Pezzella V. The Potential Therapeutic Efficacy of Lactobacillus GG in Children with Food Allergies. *Pharmaceuticals*. 2012;5:655–64. doi: 10.3390/ph5060655.
- Berni Canani R, Sangwan N, Stefka AT et al. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J*. 2016;10:742–750. doi:10.1038/ismej
- Lahart N, O'Collaghan Y, Aherne SA, O'Sullivan D, FitzGerald RJ, O'Brien NM. Extent of hydrolysis effect on casein hydrolysate bioactivity: Evaluation using the human jakart T cell line. *Int Dairy J* 2011;21:777–82.
- Sforza S, Cavatorta V, Lambertini F, Galaverna G, Dossena A, Marchelli R. Cheese peptidomics: a detailed study on the evolution of the oligopeptide fraction in Parmigiano-Reggiano cheese from curd to 24 months of aging. *J Dairy Sci*. 2012; 95:3514–3526.
- Alessandri C, Sforza S, Palazzo P, Lambertini F, Paoletta S, Zennaro D, Rafaiani C, Ferrara R, Bernardi ML, Santoro M, Zuzzi S, Giangrieco I, Dossena A, Mari A. Tolerability of a fully matured cheese in cow's milk allergic children: biochemical, immunochemical, and clinical aspects. *PLoS ONE* 2012;7:e40945; doi:10.1371/journal.pone.0040945
- Gatti M, Bottari B, Lazzi C, Neviani E, Mucchetti G. Invited review: Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. *J Dairy Sci*. 2014;97:573–91. doi: 10.3168/jds.2013-7187.
- Malacarne M, Summer A, Franceschi P. et al. Free fatty acid profile of Parmigiano-Reggiano cheese throughout ripening: Comparison between the inner and outer regions of the wheel. *Inter Dairy J* 2009;19:637–641. DOI: 10.1016/j.idair-yj.2009.04.004
- Neviani E, Bottari B, Lazzi C, Gatti M. New developments in the study of the microbiota of raw-milk, long-ripened cheeses by molecular methods: the case of Grana Padano e Parmigiano Reggiano. *Front Microbiol*. 2013;4:36. doi: 10.3389/fmicb.2013.00036.
- Gala E, Landi S, Solieri L, Nocetti M, Pulvirenti A, Giudici P. Diversity of lactic acid bacteria population in ripened Parmigiano Reggiano cheese. *Intern J Food Microbiol*. 2008; 125:347–51. doi:10.1016/j.ijfoodmicro.2008.04.008
- Solieri L, Bianchi A, Mottolose G, Lemmetti F, Giudici P. Tailoring the probiotic potential of non-starter Lactobacillus strains from ripened Parmigiano Reggiano cheese by in vitro screening and principal component analysis. *Food Microbiol*. 2014;38:240–9. doi: 10.1016/j.fm.2013.10.003.
- Berni Canani R, Di Costanzo M, Leone L. The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. *Clin Epigenetics* 2012; 4:4. doi: 10.1186/1868-7083-4-4

Correspondence:

Prof. Dr. Roberto Berni Canani, MD, PhD
University of Naples "Federico II", Naples, Italy
Tel. 0039 0817462680
E-mail: berni@unina.it