Review

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

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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).

Key words: 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 an 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 therapeutic 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 hydrolysate 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).

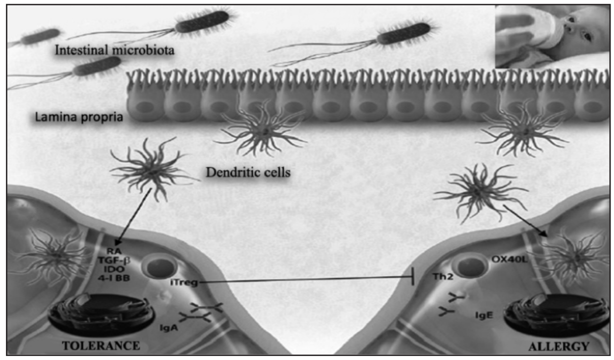

Figure 1. Oral tolerance: suppression of the immune response to food antigens mediated by the combined action of the intestinal microbiota , dietary factors, dendritic cells and antigen-specific T regulator lymphocytes.

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 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 hydrolysates 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

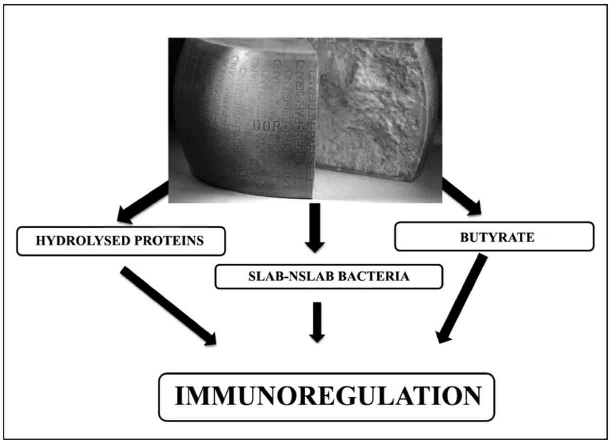

Figure 2. The three essential components of Parmigiano Reggiano: hydrolysed peptides, bacterial products and butyrate. All these components induce regulator effects of the function of the immune system, with anti-inflammatory and anti- allergic activities.

(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 bacteria: *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 lipotecoic 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).

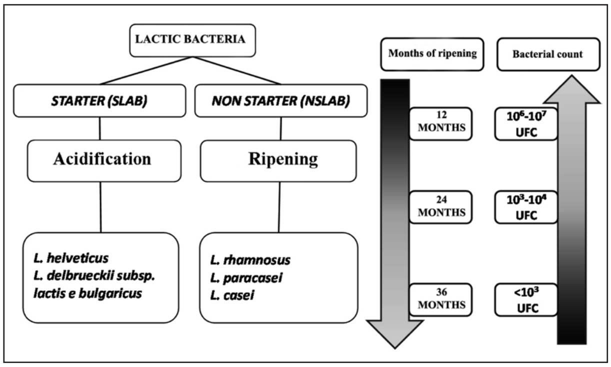

Figure 3. Bacterial component of Parmigiano Reggiano (PR): starter lactic acid bacteria (SLAB) contribute to acidification , non starter lactic acid bacteria (NSLAB) promote the ripening process. The total bacterial count decreases progressively with increasing months of maturation.

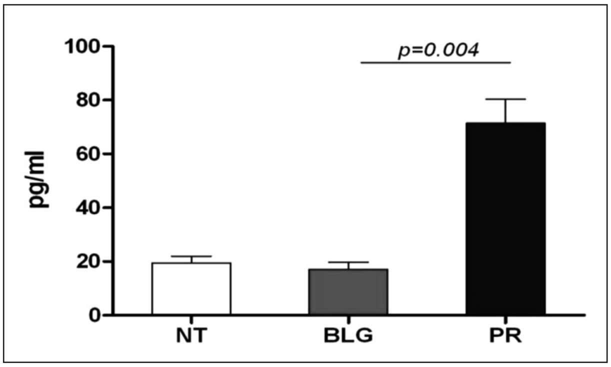

Figure 4. Production of interleukin (IL)-10, cytokine with anti - inflammatory and anti – allergic effects, by peripheral blood lymphocytes of patients with IgE-mediated cow's milk allergy after 24 hours of incubation with beta-lactoglobulin (BLG), Parmigiano Reggiano (PR) or unstimulated (NT) (n=3 experiments)

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 (VEPIPY), 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 VEPIPY 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	αS1 CN f (1-9)	1140	αS1 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	αS1 f (169-179)	1198	αS1 CN f (18-23)	791
Lac-Phe •	237	S1 CN f (24-34)	1237	αS1 CN f (24-30)	805
Pyr-Ile •	242	S1 CN f (14-23)	1246	αS1 CN f (1-7)	874
Pyr-Leu •	242	β CN f (16-25)3P	1348	αS1 CN f (17-23)	905
γGlu-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	αS2 CN f (154-162)	1132
γ-Gtu-Leu •	260	αS1 CN f (1-13)	1535	αS1 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	αS1CN 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
αS1 CN f (10-13)	414	αS1 CN f (24-38)	1707	β CN f (159-183)	2746
β CN f (1-3)	416	β CN f (194-209)	1717	αS1 CN f (1-23)	2764
αS1 CN f (1-4)	536	β CN f (15-28)3P	1790	αS1 CN f (174-199)	2836
αS1 CN f (10-14)	542	β CN f (15-28) 4P	1870	β CN f (98-124)	3132
β CN f (1-4)	545	αS1 f (1-16)	1877	αS1 CN f (157-188)	3452
αS1 CN f (16-20)	601	β CN f (193-209)	1881	αS1 CN f (156-187)	3477
αS1 CN f (30-35)	707	αS1 f (1-17)	1991	β CN f (37-67)	3580
αS1 CN f (186-192)	734	β CN f (14-28) 4P	1999	αS1 CN f (85-114)	3601
αS1 CN f (1-6)	745	β CN f (103-119)	2042	αS1 CN f (155-187)	3605
β CN f (53-93)	4454	β CN f (57-93)	4065	αS1 CN f (83-114)	3860
β CN f (51-93)	4696	αS1 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

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