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Immune effects of probiotics

By Agnes E. Wold

ABSTRACT

Twenty papers concerning the effects on the immune system of ingestion of probiotic bacteria in humans have been reviewed. Several studies report that intake of probiotics stimulate cell-mediated immune effector functions. Thus, enhanced production of interferon-γ by blood cells, enhanced phagocytosis by polymorphonuclear leukocytes (PMN) and to a lesser extent monocytes, and enhanced expression of complement receptors on PMNs are effects quite consistently seen in subjects consuming probiotic bacteria. It is likely that this is the result of probiotic bacteria being taken up across the small intestinal mucosa and being ingested by macrophages, leading to the production of cytokines and other mediators stimulating cell-mediated immunity.

Probiotics have also been suggested to function as adjuvants, i.e. agents that increase immune responses to other antigens administered concomitantly. However, responses to vaccination are generally only mildly increased during probiotic consumption and most likely depend on the occurrence of cross-reactive antibodies that are induced by the probitic bacteria but also bind to structures on unrelated antigens. A few studies have tested the clinical effect of probiotics on allergy, but to date no effect has been convincingly proven.

In conclusion, probiotic treatment could be beneficial in conditions where stimulation of cell-mediated effector functions is desired. *Key words: clinical studies, humans, immune, probiotics*

Introduction

Microbes and other particles that are ingested are taken up in the Peyer's patches. These are lymphoid nodules in the wall of the small intestine containing macrophages and other antigen-presenting cells, B cells and T cells. The Peyer's patches serve as inductive sites for mucosal immune responses. Usually, invasive and otherwise pathogenic microbes are the best inducers of immunity, which is probably due to their superior capacity to multiply in the intestine and penetrate across the mucosal barrier, a prerequisite in order to stimulate the immune system (1). However, dead microbes also induce immunity, provided that they are given in high enough doses (2,3).

Animal experiments document that the commensal microflora is the major stimulus to the gut immune system and is also a potent regulator of the innate immune system (reviewed in 4). The presence in healthy individuals of break-down products of bacteria in spleen macrophages and their secretion in the urine, is testimony to the constant influx of bacterial products across the mucosal surfaces (5,6).

The small intestine, where the Peyer's patches are situated, is relatively sparsely populated by bacteria (from 10^3 CFU/g in the proximal duodenum to between 10^5 and 10^8 in the distal ileum). Thus, if one assumes that the bacterial population density in the small intestine is 1/10 000 of that in the colon, the entire small intestine probably harbours no more that 10^{10} bacteria. It is thus feasible that ingestion of some 10^{10}-10^{11} bacteria daily can have a significant impact on the gut immune system, even if this dose is small compared to the 10^{14} bacteria inhabiting the colonic microflora. Since the Peyer's patches are designed to take up any particles present in the small intestinal lumen, probiotic bacteria will probably not have to colonise in order to stimulate immune effector functions.

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Aim

In spring 2000 an *ad hoc* committee of Swedish scientists was formed jointly by the Expert Group of Diet and Health of the National Food Administration and the Research Board of SNF Swedish Nutrition Foundation. The task was to review and evaluate human clinical studies on the health effects of probiotics and prebiotics (7). This paper examines clinical trials concerning immune effects of probiotics. References were collected from Medline and from available reviews on clinical immune effects of probiotics, until September 2000.

Some studies are listed in Table 1 on pages 79-85. Those references are marked (*) in the following text and in the reference list. Articles are evaluated in the Tables (column 13) as +low quality; ++ medium quality; +++ high quality.

Triggering of IFN–γ production and stimulation of cell-mediated immune effector functions

Several studies report that ingestion of probiotic bacteria or fermented milk products triggers spontaneous (8*) and enhances mitogen-induced (9*,10*) production of interferon–γ by blood leukocytes. The cells responsible for the interferon production have not been identified in these studies, but are likely to be NK cells and/or T cells (11,12). Enhanced production in blood cells of the antiviral enzyme 2'-5'A synthase has been observed in volunteers consuming yoghurt (13*). This enzyme is induced by interferons. One study reports increased production of interferon-α after PHA stimulation of blood leukocytes in persons consuming lactobacilli, but the IFN-α levels in both groups were very low (14*)

A single study reports increased levels of IFN–γ in serum after intake of yoghurt supplemented with lactobacilli and streptococci (15*), whereas other studies are negative (8*,13*).

Changes in blood cell population levels were also noted in the former study, which is not seen in other studies (9*,16*). The exceptionally high dose given ($3x10^{12}$ CFU/day) may be one explanation for the deviating results of this study.

Intake of probiotics has also been shown to affect blood lymphocytes so that they display enhanced IL-2 responses after stimulation of T cell mitogens (8*,9*,10*). There are, however, also studies that report lack of effect on IFN–γ or IL-2 production after ingestion of probiotics, e.g. *Lactobacillus casei* strain Shirota (17*) or yoghurt (18*). The volunteers in the former study had remarkably high counts of lactobacilli in faeces prior to the study (10^7 CFU/g), while the participants in the latter study showed high spontaneous activation of their blood lymphocytes.

Ingestion of probiotic bacteria enhances the phagocytic capacity of blood polymorphonuclear leukocytes (14*,16*, 19*) triggers respiratory burst (9*,19*), and increases their expression of receptors involved in phagocytosis, especially complement receptor 3 (CR3) (20*,21*). Blood monocytes are similarly affected, although to a lesser extent (16*, 20*).

This stimulation of cell-mediated effector functions (IFN–γ) production and enhanced phagocyte function) probably results from the production of immune-stimulating cytokines and other mediators when probiotic bacteria interact with monocytes/ macrophages in the Peyer's patches, intestinal mucosa, or other sites. Lactobacilli, as well as other Gram-positive bacteria, are very efficient in inducing the production of IL-12 (22 ,23). IL-12 is the major stimulator of cell-mediated immune effector functions. Thus, IL-12 stimulates IFN–γ production in T cells and NK cells and increases their cytotoxic potential (24). Needless to say, many other cytokines and mediators will be triggered when probiotic bacteria interact with macrophages and other cells in the innate immune system, many of which might be involved in the enhancement of phagocytic function after intake of probiotics.

Although many types of probiotic bacteria – *Lactobacillus rhamnosus, Lactobacillus johnsonii* and yoghurt cultures – appear to activate phagocytes, He and coworkers compared up-regulation of complement receptors on blood phagocytes after feeding *L. rhamnosus* and *Lactococcus lactis* and found the latter to be superior (21*). Tentatively, this might be explained by the induction of relatively large amounts of IL-10 by *L. rhamnosus* compared to other *Lactobacillus* species (22). IL-10 is a macrophage-derived cytokine that down-regulates IFN–γ production, thus opposing the action of IL-12 on T cells and NK cells (25).

Local IgA responses

Probiotic bacteria, like any other microbes that are taken up from the intestinal lumen, will induce a specific immune response to themselves. In addition, microbes also stimulate production of antibodies that have other, unknown, specificities (26,27). The function of such non-specific antibodies is unknown, as are the mechanisms for their induction. However, it is conceivable that ingestion of probiotics by this mechanism could stimulate, in a non-specific manner, enhanced production of secretory IgA on mucosal surfaces, or serum IgA in the circulation.

Four studies have examined the local production of secretory IgA in response to probiotic treatment. Fukushima et al. reported increased levels of total faecal IgA and faecal IgA anti-poliovirus antibodies after feeding formula supplemented with bifidobacteria (28). The infants had been vaccinated with attenuated poliovirus as part of the regular vaccination scheme which had been completed several months before the study. Statistically significant differences in faecal IgA levels were, however, only demonstrated at one time point but not the others. Moreover, it cannot be ruled out that the survival of secretory IgA in the intestinal milieu is affected differences in faecal pH, water content or transient time, factors that may all be affected by ingestion of bifidobacteria. Quantification of immunoglobulins in faeces has been strongly discouraged due to large variations in recovery rates between samples (29).

In an elegant study, Marteau et l. used jejunal perfusion to investigate whether ingestion of fermented milk containing a *Lactobacillus johnsonii* strain would alter secretion of IgA or other immunoglobulin isotypes in the small intestine. No increased secretion of IgA (nor of IgG or IgM) was noted (30*). Lactating mothers who were given *L. rhamnosus* GG ($4x10^{10}$ CFU/day) did not increase their milk IgA levels (31*).

In conclusion, evidence for increased local IgA responses in humans after probiotic intake are still awaiting.

Serum IgA

Two studies (30*,32*) report an increase in serum IgA concentrations of approximately 10% after consumption of the same probiotic bacterial strain, *Lactobacillus johnsonii* La1 from the Nestlé company. Although other studies show no increase of serum IgA concentrations after intake of other probiotic strains or yoghurt products (9*,17*), it is possible that this particular strain is more efficient than others in inducing serum IgA. Serum IgA is produced in the bone marrow and is regulated independently from the mucosal IgA system.

Adjuvant function

Adjuvants are substances that enhance immune responses to other, unrelated antigens, with which they are mixed. Adjuvants function by stimulating antigen presentation to T cells by enhancing the production of T cell-stimulating cytokines and expression of accessory molecules by antigen-presenting cells. This is the basis for the fact that microbial antigens, which possess conserved structures with capacity to activate monocytes, macrophages and dendritic cells, are very good immunogens. In contrast, food proteins have no capacity to activate the antigen-presenting cell and are poorly immunogenic. It has been suggested that lactobacilli may function as adjuvants, which means that they would enhance immune responses to other, unrelated, antigens administered concomitantly.

Several studies have examined the antibody response to peroral (9*,32*,33*) or systemic (9*) vaccination, or to natural infection (34*,35*) or dietary components (36*) in volunteers consuming probiotic bacteria and their controls. No increased reponse to oral poliovirus or parenteral pneumococcal vaccination was noted in one study (9*), but Link-Amster et al. reported that the serum IgA antibodies in serum to *Salmonella typhi* increased 4.1 x after vaccination in volunteers consuming lactobacilli, compared with a 2.5 x increase in the control group ($p=0.04$) (32*). On the other hand, the control group responded better in saliva than the group consuming probiotics. Similarly, the IgA response to rotavirus in serum 3 weeks after rotavirus infection was 2.3 times higher in a group of children consuming *L. rhamnosus* than in control children (35*), but in another study by the same authors (34*), there was no difference in serum antibody responses between the groups. In a study of the response to vaccination with rotavirus, seroconversion was more frequent in volunteers consuming lactobacilli than in controls, but the definition of "seroconversion" was an increase of >20% in antibody levels (33*). At least a doubled or tripled antibody response is more generally accepted as a definition of seroconversion. Similarly, studies that have used enumeration of antibody-producing cells in blood as a measure of the immune response have employed an abnormally low cut-off levels for defining "responders" (0.5 antibody-secreting cell per 10^6 blood cells) (21*,35*). Usually, at least 5 or 10 antibody-producing

cells/10^6 blood cells is considered a relevant response to vaccination, whereas the response to natural infection is usually 10–100 times higher (37-40).

It is important to realize that the presence of cross-reacting antibodies, induced by and against the probiotic strain, but reacting with the vaccine strain, does not mean that the probiotic displayed an adjuvant effect. Antibodies to lactobacilli may cross-react with structures on *E. coli*, which are not at all closely related (41). In accordance, increased amounts of antibodies to completely unrelated antigens were noted in one study of volunteers consuming probiotics (32*).

Thus, in order to prove an adjuvant effect, sera must be absorbed with the probiotic bacteria before being assayed for antibody activities against the vaccine strain. No study has employed such measures. It can be concluded that adjuvanticity of probiotic bacteria on immune responses to infection or vaccination has not been proven. Actually, it could be quite hazardous if ingestion of yoghurt or other probiotic products stimulated immune responses to completely unrelated antigens, which could include autoantigen, food proteins etc. However, animals reared devoid of bacterial flora respond equally well, or better, to vaccination, food antigens and colonisation by microbes, compared to animals that already harbour a full microflora (42). This speaks against an adjuvant effect by intestinal bacteria on immune responses to unrelated antigens.

Effects on allergies

Three studies have examined the effect of consumption of probiotics on asthma and allergic symptoms. Majamaa and Isolauri (31*) gave an extensively hydrolysed formula containing *L. rhamnosus* GG to infants with atopic eczema and clinical evidence of cow's milk allergy, control infants received the hydrolysed formula without bacteria. The severity of the atopic eczema, as measured by SCORAD, was improved more in the GG group than in the control group after one month's feeding, but the former group had a higher value before initiation of treatment. After treatment, the median SCORAD value was, in fact, 19 in the control group and 20 in the GG group. One may, thus, conclude that the study failed to demonstrate any clinical effect of the treatment. In the GG group, faecal α-1-anti-trypsin concentrations and TNF-α concentrations fell sharply after treatment, whereas serum ECP was unaltered. This is an interesting indication of a reduced intestinal inflammatory response, but it needs to be confirmed that alterations in the intestinal contents due to the presence of lactobacilli (lowered pH, increased water content, increased content of organic acids) simply have not rendered the environment more hostile to the survival of these molecules.

Trapp and coworkers (18*) performed a very large study in which young and elderly people consumed yoghurt, inactivated yoghurt, or no yoghurt, for one year. The authors found fewer symptoms of nasal allergies and lower total serum IgE levels in volunteers who consumed either of the yoghurt preparations than in the control group. Unfortunately, randomisation was only performed between people drinking live or inactivated yoghurt, while the non-yoghurt control group consisted of people not wanting to consume yoghurt products. It cannot be excluded that the latter individuals have a less healthy lifestyle or are less willing to medicate adequately than the volunteers who complied with taking yoghurt. The differences between the groups was present in the first sample already and persisted throughout the one-year study period. It is therefore possible that the differerences were already present before treatment.

Wheeler and coworkers (10*) gave yoghurt with live lactobacilli to 15 adults with moderate asthma and yoghurt without lactobacilli for another month in a double-blind crossover study. No differences in spirometric functions could be detected relating to the consumption of lactobacilli.

In conclusion, more studies are needed to elucidate whether probiotics might have beneficial clinical effects on established allergy. This is an extremely dynamic area, and more data can be expected within the next few years.

Conclusion

In conclusion, there is relatively good evidence that intake of probiotics transiently activates cell-mediated immune effector functions, such as interferon–γ production and phagocyte function. An adjuvant effect *vis-à-vis* unrelated antigens administered concomitantly has not been proven, and the clinical effects on allergy have been too little studied to date in order for any conclusions to be drawn.

REFERENCES

References listed in Tables (pages 78-85)

1. Hohmann A, Schmidt G, Rowley D: Intestinal and serum antibody responses in mice after oral immunization with *Salmonella, Escherichia coli*, and *Salmonella-Escherichia coli* hybrid strains. Infect Immun 1979; 25:27-33.
2. Ogra PL, Karzon DT, Righthand F, MacGillivray M: Immunoglobulin response in serum and secretions after immunization with live and inactivated poliovaccine and natural infection. N Engl J Med 1968; 279: 893-900.
3. Moreau MC, Ducluzeau R, Guy-Grand D, Muller MC: Increase in the population of duodenal immunoglobulin A plasmocytes in axenic mice associated with different living or dead bacterial strains of intestinal origin. Infect Immun 1978;21:532-9.
4. Wold AE, Adlerberth I. Pathologic effects of commensalism. In: Nataro JP, Blaser MJ, Cunningham-Rundles S, editors. Persistent Bacterial Infections: ASM Publishing Co.2000;pp115-144.
5. Hoijer MA, Melief MJ, van Helden-Meeuwsen CG, Eulderink F, Hazenberg MP: Detection of muramic acid in a carbohydrate fraction of human spleen. Infect Immun 1995;63:1652-7.
6. Johannsen L, Krueger JM: Quantification of diaminopimelic acid in human urine. Adv. Biosci. 1988;68:445-9.
7. Andersson H, Asp NG, Bruce Å, Roos S, Wadström T, Wold AE: Health effects of probiotics and prebiotics. A literature review on human studies. Scand J Nutr 2001;45:58.75.
8. * Halpern GM, Vruwink KG, van der Water J, Keen CL, Gershwin ME: Influence of long-term yoghurt consumption in young adults. Int J Immunother 1991;VII:207-10.
9. * Wheeler JG, Bogle ML, Shema SJ, Shirrell MA, Stine KC, Pittler AJ, et al: Impact of dietary yogurt on immune function. Am J Med Sci 1997;313:120-3.
10. *Wheeler JG, Shema SJ, Bogle ML, Shirrell MA, Burks AW, Pittler A, et al: Immune and clinical impact of *Lactobacillus acidophilus* on asthma. Ann Allergy Asthma Immunol 1997;79:229-33.
11. Haller D, Blum S, Bode C, Hammes WP, Schiffrin EJ: Activation of human peripheral blood mononuclear cells by non pathogenic bacteria *in vitro*: evidence of NK cells as primary targets. Infect Immun 2000;68:752-9.
12. Hessle C, Andersson B, Wold AE: Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production. Infect Immun 2000;68:3581-6.
13. *Solis-Pereyra B, Aattouri N, Lemonnier D: Role of food in the stimulation of cytokine production. Am J Clin Nutr 1997;66:521S-525S.
14. *Arunachalam K, Gill HS, Chandra RK: Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). Eur J Clin Nutr 2000;54:263-7.
15. *De Simone C, Vesely R, Bianchi Salvadori B, Jirillo E: The role of probiotics in modulation of the immune system in man and in animals. Int J Immunother 1993;IX:23-8.
16. *Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A: Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J Dairy Sci 1995;78:491-7.
17. *Spanhaak S, Havenaar R, Schaafsma G: The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans. Eur J Clin Nutr 1998;52:899-907.
18. *Trapp CL, Chang CC, Halpern GM, Keen CL, Gershwin ME: The influence of chronic yoghurt consumption on populations of young and elderly adults. Int J Immunother 1993;IX:53-64.
19. *Donnet-Hughes A, Rochat F, Serrant P, Aeschlimann JM, Schiffrin EJ: Modulation of non specific mechanisms of defense by lactic acid bacteria: effective dose. J Dairy Sci 1999;82:863-9.
20. *Pelto L, Isolauri E, Lilius EM, Nuutila J, Salminen S: Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clin Exp Allergy 1998;28:1474-9.
21. *He F, Tuomola E, Arvilommi H, Salmonen S: Modulation of humoral immune response through probiotic intake. FEMS Immunol Med Microbiol 2000;29:47-52.
22 Hessle C, Hanson LÅ, Wold AE: Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. Clin Exp Immunol 1999;116:276-82.

23. Hessle C, Hanson LÅ, Wold AE: Interleukin-10 produced by the innate immune system masks in vitro evidence of acquired T-cell immunity to E. coli. Scand J Immunol 2000;52:13-20.
24. Trinchieri G: Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. Blood 1994;84:4008-27.
25. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G: Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041-8.
26. Quan CP, Berneman A, Pires R, Avrameas S, Bouvet JP: Natural polyreactive secretory immunoglobulin. A autoantibodies as a possible barrier to infection in humans. Infect Immun 1997;65:3997-4004.
27. Cebra JJ: Influences of microbiota on intestinal immune system development. Am J Clin Nutr 1999;69:1046S-1051S.
28. *Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T: Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. Int J Food Microbiol 1998;42:39-44.
29. Ferguson A, Humphreys KA, Croft NM: Technical report: results of immunological tests on faecal extracts are likely to be extremely misleading. Clin Exp Immunol 1995;99:70-5.
30. *Marteau P, Vaerman JP, Dehennin JP, Bord S, Brassart D, Pochart P, et al: Effects of intrajejunal perfusion and chronic ingestion of Lactobacillus johnsonii strain La1 on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. Gastroenterol Clin Biol 1997;21:293-8.
31. *Majamaa H, Isolauri E. Probiotics: A novel approach in the management of food allergy. J Allergy Clin Immunol 1997;99:179-85.
32. *Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM: Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 1994;10:55-63.
33. *Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T: Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by Lactobacillus casei GG. Vaccine 1995;13:310-2.
34. *Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain. Pediatr Res 1992;32:141-4.
35. *Kaila M, Isolauri E, Saxelin M, Arvilommi H, Vesikari T: Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhoea. Arch Dis Child 1995;72:51-3.
36. *Malin M, Suomalainen H, Saxelin M, Isolauri E: Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG. Ann Nutr Metab 1996;40:137-45.
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37. Czerkinsky C, Svennerholm AM, Quiding M, Jonsson R, Holmgren J: Antibody-producing cells in peripheral blood and salivary glands after oral cholera vaccination of humans. Infect Immun 1991;59:996-1001.
38. Kantele A, Arvilommi H, Jokinen I: Specific immunoglobulin-secreting human blood cells after peroral vaccination against *Salmonella typhi. J* Infect Dis 1986;153:1126-31.
39. Kantele AM, Takanen R, Arvilommi H: Immune response to acute diarrhea seen as circulating antibody-secreting cells. J Infect Dis 1988;158:1011-6.
40. Wennerås C, Qadri F, Bardhan PK, Sack RB, Svennerholm AM: Intestinal immune responses in patients infected with enterotoxigenic *Escherichia coli* and in vaccines. Infect Immun 1999;67:6234-41.
41. Herías MV, Midtvedt T, Hanson LÅ, Wold AE: Increased antibody production against gut-colonizing *Escherichia coli* in the presence of the anaerobic bacterium *Peptostreptococcus*. Scand J Immunol 1998;48:277-82.
42. Wold AE, Dahlgren UI, Hanson LÅ, Mattsby-Baltzer I, Midvetdt T: Difference between bacterial and food antigens in mucosal immunogenicity. Infect Immun 1989;57:2666-73.

Table 1. Immune effects of probiotics, columns 1-6.

1. Ref.	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
Halpern et al (8)	Yoghurt consumption increases IFN-γ production by blood cells	1) 450 g yoghurt/d 2) 450 g heat-treated yoghurt/d 3) No yoghurt products	RCT	Students and university employees and other people 20-40 years of age	1) Medications that would alter measured parameters 2) Extreme diets 3) Recent illness
Wheeler et al (9)	Yoghurt consumption increases IFN-γ and IL-2 production by blood lymphocytes and response to systemic and peroral vaccination	1) Yoghurt (Danone) 225 g/d containing 3x10e8 CFU/g of *Lactobacillus bulgaricus* (7 x10^{10} CFU/d) and 4 x 10^8 *Streptococcus thermophilus* (9 x 10^7/d) 2) 225 g milk/d	RCT Cross-over	2) Clinical history of asthma or rhinoconjunctivitis 3) Positive skin prick test (>3x3 mm greater than saline control) 31 years of age (21-47)	Allergy or other intolerance to milk products
Wheeler et al (10)	Consumption of yoghurt with *L. acidophilus* improves clinical symptoms in asthma	1) 450g/d live yoghurt (3x10^8 *S. thermophilus*/g and 3x10^8 *L. bulgaricus*/g) with 8x10^8 CFU/g *L. acidophilus* (1.5 x 10^{11} *L. acidophilus*/d) 2) 450 g/d of live yoghurt	RCT Cross-over, double-blind	1) Clinical history of asthma and/or rhinitis 2) Positive prick test (>3x3 mm larger than saline control) 3) $FEV_{1.0}$ 40-80% of normal and >15% improvement upon β1 stimulant inhalation 33 years of age (13-45)	1) Allergy or other intolerance to milk products now or previously 2) Active smoking last 3 months 3) Antibiotic treatment 4) Immunotherapy
Solis-Pereyra et al (13)	Yoghurt consumption increases production o 2'-5' A synthetase in blood cells	1) 250 g yoghurt (10^9 *S. thermophilus* and 10^9 *L. bulgaricus*/g) 2) 250 g milk 3) 250g yoghurt/d, 15 days 4) 250 g milk/d, 15 d	RCT Parallel group (group 1 and 2) Cross-over (group 3 and 4)	25±7 years of age	1) Somatic disease 2) Medication
Arunachalam et al (14)	Intake of *Bifidobacterium lactis* indreases IFN-α production, phagocytic capacity and bactericidal activity by blood cells	1) 180 ml x 2/day milk containing *Bifidobacterium lactis* (daily dose 3x10^{11}) 2) 180 ml x 2 milk	RCT	60-83 years healthy	1) History of chronic or debilitating illness 2) Milk intolerance

cont.

Table 1. Immune effects of probiotics, columns 1-6 *(cont).*

1. Ref.	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
De Simone et al (15)	Yoghurt consumption increases serum IFN-γ and affects cell populations in blood	1) Yoghurt supplemented with lyophilized *L. bulgaricus* and *S. thermophilus* (3 x 10^{12} in 200 g!) 2) 200 g skimmed milk		Medical students 20-47 years of age	
Schiffrin et al (16)	Ingestion of milk fermented by lactic acid bacteria increases phagocytic capacity of blood cells	1) 120 ml milk daily fermented by *Bifdobacterium bifidum* Bb12 (10^{10} CFU/d) 2) 120 ml milk daily fermented by *L. acidophilus* LA1 ($7x10^7$ CFU/d)	RT without placebo group	23-62 years of age	1) Low CD4/CD8 ratio 2) Activated T cells in blood before treatment
Spanhaak et al (17)	Fermented milk containing *L. casei* affects NK cell activity, phagocytosis and cytokine production in blood cells	1) Milk fermented by *L. casei* strain Shirota 100 ml x 3/d ($3x10^{11}$ lactobacilli per day) 2) Non-fermented milk 100 ml x 3	RCT Parallel groups	Male subjects 40-65 years of age	1) Obesity (BMI>30) 2) Abnormal blood pressure 3) Medication affecting normal flora or immune function
Trapp et al (18)	Yoghurt consumption affects vaccine response and allergic symptoms	1) 200 g low-fat yoghurt/d 2) 200 g heat-inactivated yoghurt/d 3) No yoghurt	Groups 1 and 2 randomized and double-blinded, but group 3 were those who did not want to eat yoghurt	College students 20-40 years of age Elderly adults 55-70 years of age	Pregnancy or nursing, serious illness past 5 years, active infection, autoimmunity cancer, liver disease, lactose intolerance, malabsorption. Intake of drugs that could affect parameters
Donnet-Hughes et al (19)	Aim to determine the effective dose of *L. johnsonii* La1 for increasing phagocytosis by blood cells and to determine whether the starter culture has stimulating effects	1) 150 ml milk product fermented with *S. thermophilus* daily with addition of *L. johnsonii* (1.5 x 10^9/d) 2) 150 ml aged product, containing $1.5x10^8$ *L. johnsonii*/d 3) 150 ml milk fermented with *S. thermophilus* only	RCT Double-blind Parallel groups	21-57 years	1) Antibiotic treatment 2) Metabolic disorders 1) Intestinal disorders
Pelto et al (20)	Ingestion of milk products fermented by *Lactobacillus rhamnosus* GG affects receptor expression on blood PMN and monocytes	2) 200 ml x 2 daily of milk containing *L. rhamnosus* GG ($3x10^8$/d) 3) Regular milk 200 ml x 2	RCT Cross-over, double-blind	Students and staff tolerant (9) or non-tolerant (8) to milk upon blinded challenge. All had normal lactose tolerance. Symptoms: bloating flatulence, pain and diarrhea, asthma and atopic dermatitis (1 pat) 28 years of age (22-50)	Acute infection
He et al (21)	Consumption of *Lactobacillus rhamnosus* GG or *Lactococcus lactis* increases antibody response to oral *S. typhi* vaccination and affects receptor expression on blood neutrophils	1) *L. rhamnosus* GG, lyophilized, $4x10^{10}$ CFU/d 2) *Lactococcus lactis*, lyohilized, $3.4x10^{10}$/d 3) Placebo: ethyl cellulose	RCT	Healthy volunteers not consuming fermented dairy products	1) Infections by *E. coli* or *Salmonella* 2) Antibiotic treatment last 2 months 3) Vaccination with *S. typhi* last 5 years
Fukushima et al (28)	Administration of forumula containing bifidobacteria increases fectal IgA levels and fecal antibody response to oral polio virus vaccination	>200 ml daily of formula NAN BF (Nestlé) containing *Bifidobacterium lactis* (daily dose $>10^9$ bifidobacteria)	OT Comparison before-during treatment, no control group	Japanese healthy children 15-31 months old. All had completed routine poliovirus vaccination by 12 months of age	

cont.

Table 1. Immune effects of probiotics, columns 1-6 *(cont).*

1. Ref.	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
Marteau et al (30)	Ingestion of milk fermented by lactobacilli increases jejunal secretion of IgA	1) 150 g x 2 of milk fermented by *L. johnsonii* L1 per day ($3x10^{10}$ CFU/d) 2) Acidified milk of indistinguishable appearance in same quantity	RCT double-blind	Healthy volunteers	1) History of immunologic, allergic or digestive disease 2) Seropositive for hepatitis B, C or HIV 3) Abnormal clinical examination
Majamaa et al (31)	Ingestion of *L. rhamnosus* GG ameliorates cow's milk allergy in children	1) Hydrolyzed milk formula with $5x10^{8}$ CFU/g *L. rhamnosus* GG (2-5 x 10^{11} CFU/d) 2) Hydrolysed milk formula	RCT		
Link-Amster et al (32)	Ingestion of yoghurt with *L. acidophilus* La1 and *Bifidobacterium bifidum* Bb12 increases response to oral vaccination with attenuated *Salmonella typhi*	1) 125g x 3 daily of milk fermented by mesophilic streptococci and *S. thermophilus* supplemented with *L. acidophilus* and *B. Bifidum* (10^{10}-10^{11} CFU/d) 2) No yoghurt	RCT No placebo in control group	19-54 years of age	1) Lactose intolerance 2) Recent antibiotic treatment 3) Previous *S. typhi* vaccination last 5 years
Isolauri et al (33)	*L. rhamnosus* GG improves vaccine response to rotavirus	1) 5 ml water with freeze-dried *L. rhamnosus* GG twice daily (10^{11} CFU/d) 2) Same amount of placebo (cellulose)	RCT	Children 60-150 days (mean 4.1 months)	Not stated
Kaila et al (34)	Intake of *L. rhamnosus* GG stumulates antibody response to rotavirus infection	1) 125 g x 2 per day of milk product fermented with *L. rhamnosus* GG 2) Fermented milk as above, but pasteurized	RCT	Children with acute gastroenteritis of <7 days' duration admitted to hospital during rotavirus epidemic	
Kaila et al (35)	Ingestion of live *L. rhamnosus* GG is better than ingestion of dead bacteria to stimulate antibody response to rotavirus in children with rotavirus diarrhoea	1) *L. rhamnosus* GG lyophilized 10^{10}-10^{11} CFU/d 2) Same as above but heat-treated	RCT Double-blind	1) Children with acute gastroenteritis of <7 days' duration admitted to hospital during rotavirus epidemic. 2) <4 years of age 3) rotavirus positive	Not stated
Malin et al (36)	Administration of freeze-dried *L. rhamnosus* GG increases circulation of cells producing IgA antibodies against β-lactoglobulin	10^{10} CFU freeze-dried *L. rhamnosus* GG in liquid once daily	OT Response compared before-during *L. rhamnosus* feeding	1) Crohn's disease 5-17 years of age of which half had active disease 2) Juvenile chronic arthritis 2-14 years of age, all taking NSAIDs 3) Surgery patients 4-15 years	

cont.

Table 1. Immune effects of probiotics, columns 7-10 *(cont).*

Ref.	7. Matching of groups	8. Treatment time	9. Follow up after treatment (if any, e.g. persistence of probiotic organism in faeces, recurrence of symptoms)	10. Number of subjects/patients		
				Number starting	**Number ending experimental period**	**Number followed up** (if any)
8		4 months		68		67
9	Nine persons were randomised to consuming milk first, while 11 were randomised to consuming yoghurt first	1 month on each diet, both preceded by 2 w wash-out period		21	1	20
10		1 month on each diet, both preceded by 2 wkwash-out period		16	1	15
13		1+2: 1 day 3+4: 15 days preceded by abstaining from yoghurt or cheese for 15 d		13 (group 1+2) 8 cross-over		
14		6 weeks	6 weeks	25		
15		28 d				
16	*L. acidophilus* group slightly higher phagocytic index before treatment (45% vs 42%, p=0.051)	3 weeks preceded by 3 weeks wash-out	6 weeks	28 (12 female, 16 male)		
17		4 weeks preceded by 8 weeks wash-out period	2 weeks follow-up	20		20
18	Good between 1 and 2, but group 3 may have different life-style	1 year		123 60 young, 63 elderly		98
20	Statistics used do not take advantage of cross-over design	1 week treatment preceded by 1 week wash-out		17		17
19	Randomisation procedure awkwardly described: "randomly distributed according to age, sex, and faecal lactobacilli into three groups" Treatment groups had lower initial phagocytic activity of blood cells	3 weeks preceded by 3 weeks wash-out period	6 weeks (150 ml milk per day, no fermented products)	42		42
21		7 days vaccination days 1,3,5	Blood samples day –1, +7	30		30
28		20 days preceded by 10 days without yoghurt or other foods containing viable microbes	7 days after ceased intake	7		
30	Serum IgA concentration before treatment twice as high in placebo as in intervention group	28 days		12 (6 intervention group, 6 control group)		
31	Treatment group had higher SCORAD of allergic symptoms before treatment	1 month	2 months	31	4 no positive reaction to provocation with cow's milk	27
32		3 weeks preceded by 2 weeks wash-out	3 weeks	30		
33	No data on breastfeeding. No data given on how many children that were examined!	5 days	30 days	60??	??	??
34	No data concerning breast-feeding!	5 days	3 weeks measurement of antibody response to rotavirus	44	5 who did not have rotavirus diarrhoea	39 22 treatment group 17 control group
35	No data on breast-feeding	5 days	1 month. Serum antibodies measured on admission and one month later	26		26 (13 expt group, 143 control group)
36	Much fewer control children than children with inflammatory diseases. Significances can, thus, not be compared as done	10 days		14 Crohn's 9 JCA 7 controls		

cont.

Table 1. Immune effects of probiotics, columns 11-14 *(cont).*

Ref.	11. Results						12. a. Side effects b. Compliance measure	13. Evaluation of quality	14. Concluding remarks – strength of evidence
	Main outcome		Other effects		Drop-outs				
	Treatment	Control	Treatment	Control	Treat-ment	Con-trol			
8	IFN-γ production by blood mononucl. cells: 1) 20±9 U/ml	2) 1.7 ±1.3 U/ml 3) 5.2 ± 3.1	No IFN-γ detected in sera. No difference in blood cell counts	Increased ionized calcium in both yoghurt groups		Flatul. 1 person in heat-treated yoghurt group	"90% compliance" Not further specified	++(+) No compliance measure	++(+) Consumption of live yoghurt enhances spontaneous production of IFN-γ by peripheral blood lymphocytes
9	Cytokine pouduction after 3 d PHA stimul: IL-2: 1) 88 (pre) --- 106 (post) 2) 94 (pre) --- 88 (post) 1vs2: p=0.09 IFN-γ: 1) 125 (pre) -- 165 (post) 2) 139 (pre) -- 154 (post) 1vs2: p=0.24 PMN oxidative burst: 1) 0.40 - 0.54 2) 0.50 - 0.53 p=0.16		No effect on serum IgG, IgM, IgA, IgE, salivary S-IgA. No effect on blood counts of CD4, CD8 or NK cells, NK cell function, PHA or ConA induced proliferation. No effect on response to parenteral pneumococcal or oral polio vacc.		1(due to scheduling conflicts)			+++ Beautiful study	+++ No effect on response to vaccination +(+) Discretely (statistically non-significant) enhanced IFN-γ and IL-2 production of blood lymphocytes and PMN oxidative burst
10	Eosinophils/µl 1) 224 (before)-- 209 (after) 2) 175---150 Total IgE: 1) 163 --- 187 2) 172 --- 159 Cytokine prod after ConA stimulation(ng/ml) IL-2: IFN-γ 1)107-252 1) 48-61 2) 120–296 2) 30-46		No effect on clinical asthma symptoms or spirometry values		1 (due to scheduling conflicts)			++(+) No group not eating yoghurt	+++ No clinical effect on asthma by either yoghurt +(+) Increase (statistical significance not tested) in ConA-stimulated production of IL-2 and IFN-γ by blood lymphocytes after yoghurt consumption
13	2'-5' A synthetase in blood cells (24h after ingestion): 1) 84 (13) before --- 196 (12) after 2) 89 (16) before -- 102 (62) after (p=0.002 for 1 vs 2) 3) 265 (33) yoghurt phase 4) 192 (milk phase) p<0.05		No increase of IFN-γ, IL-1ß or TNF-α in plasma					++	++ Ingestion of yoghurt induces 2'-5' A synthetase in blood mononuclear cells
14	% PMN ingesing bacteria: 2x by 3 weeks 3x by 6 weeks IFN-α production (PHA stimulation): 3U/ml before-10U (3w)	1.4x by 3 weeks 1.3x by 6 weeks 4 U/ml before – 5 U/ml (3w)	Phagocytosis still increased 2.7x 6 weeks after ceased treatment				Strain detected in stools using DNA probe, but no data shown "Compliance was confirmed by the subjects"(!)	+ Poorly explained statistics (effect in treatment group compared "either" with control group or with pre-study values)	+(+) Ingestion of yoghurt with *Bifidobacterium lactis* enhances phagocytosis by PMN. Interferon-α values extremely low – biologically insignificant?
15	IFN-α in blood: 1) 43 (U/ml?) before, 70 (U/ml?) after B cells in blood: increase from 370 to 500 (unit?) NK cells: 450 to 650	Control group not shown						+ Very high lactobacillus dose	+ Questionable if IFN-γ can be measured in serum

cont.

Table 1. Immune effects of probiotics, columns 11-14 *(cont).*

16	% of blood PMN ingesting *E. coli*: 1) 38% before 87% after 2) 46% before 84% after Increase also in monocyte phagocytic capacity		Appr. 50% of effect still evident 6 weeks after ceased intake. No change in lympho-cyte subsets (T, B, NK)				Increased faecal bifido-bacterial counts in group 1) and faecal lacto-bacillus counts in group 2). Strain with API pattern as LA1 detected in faeces of all subjects in group 2)	++ No placebo group	++ Consumption of milk fermented by bifidobacteria or lactobacilli enhances phagocytic capacity of blood PMN and monocytes
17	No effect on serum IgG, IgA, IgM, IgD, IgE, C3, C4, factor B. No effect on NK cell function or production of IFN-γ or IL-2 after ConA stimulation, or IL-1 after LPS stimulation		High levels of total lactobacilli in both groups: 10^7/g faeces				*L. casei* shirota-like colonies: 10^2/g faeces before treatment, $10^{7.5}$ after treatment	+++	+++ No effect on serum immuno-globulin levels, NK cell function and lymphocyte cytokine production. Can possibly depend on already high levels of lactobacilli in this population.
18	Groups 1 and 2 had lesser allergic symptoms and lower total serum IgE than group 3		No effect on IFN-γ pro-duction, but high base-line values. No effect on counts of PMN,lymph eos.,basophils				Diet recorded monthly	++ large study, good follow-up, but control group consisted of people not wanting to eat yoghurt	(+) Yoghurt consumption reduces allergic symptoms
19	Phagocytosis (before-after), fluorescence int. 1) 390---530 *** 2) 430 ---520 ns 3) 450---460 ns Respiratory burst: 1) 67—84 *** 2) 69—84 NS 3) 67—77 NS No difference in bactericidal activity		6 weeks after treatment, phagocytos-is decreased below pre-treatment values				La1 detected in stools (hybridisation of colonies with specific probe) 1) <3.3 – 4.8 2) <3.3 – 4.3 3) <3.3 –<3.3	++ No control group. Increases seen in all three groups. No comparisons between groups, only within. How can 67 - 84 be p<0.001 and 69 - 84 be ns?	(+) Increased phagocytosis of PMN after ingestion of fermented product with La1, compared to pre-treatment values, but probably not compared to control group
20	Expression after/before % CR1: 128 CR3: 133 FcγR1: 109 FcγRII: 110 FcγRIII: 108 FcγR: 110	 104 95 104 106 104 102	Milk challenge per se upregulated CR1, CR3, FcγRI, FcγR on PMN in allergic subj					+(+) Good design, faulty statistics (experimental group not compared with control group)	++ Upregulation of CR1 and CR3 on PMN in healthy persons - "Downregulating effect" on allergic individuals not substantiated
21	IgA ASC day 7: 1) 21 (17-116) 2) 18 (7-79) no difference between groups 3) 28 (17-44) CR1 expression, CR3 expression on PMN (SD) 1) 98 (25) 98(17) 2)120 (30) 150(42) *L. lactis* enhances 3) 96 (31) 110(30)							++	++ No difference in response to oral *Salmonella typhi* vaccine ++ Increased expression of CR3, and to a lesser extent CR1 on PMN after *Lactococcus lactis* administration
28	Faecal IgA: 1.5 (d0)--4.3* (d8)—3.0 (d20) –2.4 (d 27) Anti-poliovirus titres in feces increased from 0.1 (absorbance) to 0.2.						Administered strain detected in faeces during treatment $10^{8.5}$ by colony morphology. Vanished 7 days after feeding	+	- Measurements of IgA in faeces can be affected by pH, transit time etc.

Table 1. Immune effects of probiotics, columns 11-14 *(cont).*

30	Serum IgA increased from 1.76 to 1.85 g/l in treatment group (but control group had 2.4 before and after). No increased secretion of IgA, SC, IgG or IgM in jejunum		Perfusion of intestine with 10^7 lactobacilli/ml gave higher exsudation of serum proteins (ns.)					++(+) Elegant protocol, Small group	++ No increased production of secretory IgA in intestine after consumption of lactic acid bacteria
31	SCORAD: 22 (before)—18 (1 mo) α1-AT in feces: 1.4---0.5 TNF-α in feces: 710---34 ECP in feces: 71—48	27---21 1.7---1.7 630--490 77—47						- Comparisons not made between intervention and control group, only before-after treatment	- No proven effect on clinicals symptoms + Decrease of TNF-α in faeces, but survival in faeces might be influenced by diet (pH, water content, transient time)
32	Serum IgA titer Pre: 25 2 w: 102 42 d: 37 Diff betw. groups (2w): p=0.04 Control group had better response in saliva. Increased antibody response also to unrelated antigens	25 62* 28	Total serum IgA increased by 10% (1.8—2.0 g/l) during yoghurt consumption				Faecal bifidobacterial and lactobacillus counts increased by 1 log in treatment group	+ lousy vaccine response	No adjuvant effect proven. Only minimal increase in antibody titres and minimal difference between yoghurt and control. Increase also to unrelated antigens. Increase in total serum IgA after youghurt consumption
33	Postvaccination titres: IgM: 67 (33-136) IgA: 22(11-43) IgM ASC before: 0.3 (0.1-1.1) day 8: 4.3 (1.5-12)	26 (7-97) p=0.19 6 (1-27) p= 0.10 0.7 (0.1-3.1) 0.6 (0.1-2.7) p=0.02			Fever >38 17% vomiting: 2 children	14%			(+) Minimal effect on response to oral rotavirus vaccination. Only IgM response affected. No evidence of adjuvant effect (non-specific stimulation).
34	Non-specific immunoglobulin secreting cells twice as many in GG-fed group Only 1 in treatment and 2 in control group had >10 ASC against rotavirus				6 no information			++ for study - for statistical evaluation. Too low cut-off for response (0.5 antibody-secreting cells/10^6)	++ Increase in immunoglobulin-secreting cells in blood (non-specific) - Increase in response to rotavirus (no response in either group).
35	Antibody response in serum Acute: 0.04(0.01-0.3) Conval:51(29-87) "5 times more IgA-secreting cells in convalescence in group 1, but no data given.	0.1 (0.01-0.5) 22 (12-43) p=0.04						++ for study (+) for statistical evaluation	- Very weak antibody response in both groups. Too low cut-off for definition of response (0.5 cells per 10^6).
36	Minimal increase in IgA ASC to food antigens in Crohn group with lactobacillus treatment: BLG: 0.2-1.4 spots/10^6 Casein: 0.3-1.0 Gliadin 0.7-1.6							+ for study - for statistical evaluation	- Minimal increase in IgA-secreting cells producing antibodies to food proteins. Clinical significance?