Norwegian Scientific Committee for Food Safety

Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods (II)

The Norwegian Scientific Committee for Food Safety

Panel on biological hazards

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Panel on nutrition, dietetic products, novel food and allergy

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Contents

I- Abstract

On 10. March 2006 , The Norwegian Food Safety Authority (Mattilsynet) decided that, on the basis of VKM's previous risk assessment (2005), Nutramigen 1 with *Lactobacillus rhamnosus* GG (LGG) could not be marketed in Norway as medical foods for infants (0-4 months). In addition, The Norwegian Food Safety Authority (Mattilsynet) decided (08. November 2006) to withdraw permission for marketing of Nutramigen 2 with LGG, which is a milk supplement for infants aged between 4 and 6 months, with cow's milk and soy protein allergy. On 13. December 2006, Mead Johnson Nutritionals appealed against this decision from The Norwegian Food Safety Authority (Mattilsynet). The Norwegian Food Safety Authority forwarded the appeal from the companies, asked the VKM Panel on biological hazards and the VKM Panel on nutrition, dietetic products, novel food and allergy, for a new risk assessment including the new data provided in the appeal.

LGG is one of the most studied probiotic strains. Lactic acid produced by LGG in the human gut results in a decrease in faecal pH, which in turn inhibits colonisation by potentially pathogenic bacteria. Short-term beneficial effects from administration of LGG to infants and young children with infectious diarrhoea have been reported in a number of studies, but a prophylactic effect on diarrhoea has not been documented. Furthermore, whilst some studies have reported a prophylactic, or even a curative, effect of LGG on atopic eczema in young children, more recent studies do not report such effects. Some even suggest an increased incidence of allergic sensitization in children receiving LGG supplemented formula at an early age. There are no published data that demonstrate long-term clinical benefits of infant formula supplemented with LGG for children between 4 months and 3 years, although no immediate deleterious effects of LGG have been found. Possible long-term effects of LGG on intestinal colonisation, and its effects on long-term gastrointestinal and immune functions, are not known.

LGG, as an ingredient in infant formula and baby foods, is intended for daily use in the target group, and not for short-term, specific treatment. Furthermore, the targeted consumer group includes children below the age of twelve months. These two aspects demand particular consideration with regard to the unknown effects of long-term treatment with large doses of live bacteria on the ecology of the microbiota of the gastrointestinal tract and on the immune system. Neither of these systems is fully matured in infants and small children, and therefore may be particularly susceptible.

There is no documented prophylactic effect of LGG on any disease in children. The effect of treatment with LGG-supplemented formula in small children is questionable, except for a documented short-term effect on infectious diarrhoea. Panel on Biological Hazards and Panel on nutrition, dietetic products, novel food and allergy at the Norwegian Scientific Committee for Food Safety find that the data available are not sufficient to support the suggested beneficial effects, or the safety, of LGG in infant formula and baby foods for children aged between 4 months and 3 years, when the products are intended for daily use.

II- Sammendrag

Basert på VKMs tidligere risikovurdering fra 2005, bestemte Mattilsynet 10. mars 2006 at Nutramigen 1 med LGG ikke kunne markedsføres som næringsmiddel til spesielle medisinske formål (0-4 måneder) i Norge. I tillegg trakk Mattilsynet tilbake tillatelsen (08. november, 2006) til å markedsføre Nutramigen 2 med LGG, som er en melkeerstatning for spedbarn mellom fire og seks måneder som er allergiske mot kumelk og soyaproteiner. Den 13. desember 2006 Mead Johnson Nutritionals på vedtaket fra Mattilsynet. Mattilsynet videresendte klagen fra selskapene og ba VKMs faggrupper for hygiene og smittestoffer samt ernæring, dietetiske produkter, ny mat og allergi om å foreta en ny risikovurdering basert på nye data som er lagt frem i forbindelse med klagen.

Lactobacillus rhamnosus GG (LGG) er en av de mest studerte probiotiske bakteriestammene. Produksjon av melkesyre fra LGG reduserer pH i feces og kolonisering av potensielt patogene mikrober kan derved forhindres. Flere studier kan vise til en kortsiktig gunstig effekt av LGG på infeksiøs diaré hos sped- og småbarn, men en forebyggende effekt på diaré er ikke dokumentert. Det er studier som rapporterer en forebyggende og også behandlende effekt av LGG ved atopisk eksem hos sped- og småbarn. Nyere studier kan ikke reprodusere en slik effekt og enkelte nye studier viser til og med en øket allergisk sensibilisering hos spedbarn som får LGG-tilskudd. Det er ikke publisert data som støtter en lang tids gunstig klinisk effekt av morsmelkerstatninger med LGG for barn mellom 4 måneder og 3 år selv om ingen umiddelbare uheldige bivirkninger er funnet. Mulige langtids effekter på kolonisering i tarm og virkningen av slik kolonisering på tarmens funksjon og immunologisk funksjon generelt er ukjent. LGG som tilsats i morsmelkerstatninger og barnemat er ment for daglig bruk i målgruppen og ikke for spesifikk korttids behandling. Videre inkluderer målgruppen små barn <1 år. Dette er fakta som maner til spesiell aktsomhet når det gjelder ukjente virkninger av langtids behandling med store doser levende bakterier på økologien i tarmen og på immunsystemet. Verken tarmens mikrobiota eller immunsystemet generelt er ferdig modnet hos sped- og småbarn opp til 2–3 år. Virkningen av LGG supplementering i morsmelkerstatninger og småbarnmat er usikker bortsett fra en dokumentert korttids effekt på diaré. VKMs faggrupper for hygiene og smittestoffer samt ernæring, dietetiske produkter, ny mat og allergi konkluderer med at de foreliggende data vedrørende effekt og sikkerhet ikke er tilstrekkelige til å anbefale bruk av LGG i morsmelkerstatninger eller

småbarnmat til barn mellom 4 måneder og 3 år så lenge produktene er beregnet for daglig bruk.

III- Background

In 2003, The Norwegian Food Safety Authority (Mattilsynet) was asked by Nutri Konsult Täby (Finland) to permit the marketing of infant formula that had been supplemented with *Lactobacillus rhamnosus* (LGG) at a concentration of 10^8 CFU/g formula.

In March 2004, The Norwegian Scientific Committee for Food Safety (VKM) was asked by The Norwegian Food Safety Authority to address this issue (00/1956/touse and 2000/1956/gyomj). In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft a risk assessment regarding the use of LGG in infant formula and baby foods. In February 2005, the VKM Panel on nutrition, dietetic products, novel food and allergy performed a risk assessment, which concluded that:

The long-term effects on the immune function (immune defence, allergy, autoimmunity) of the gut, and systemically, when LGG is given to small children is unknown.

The long-term effects of a heavy, artificial, single-species bacterial load on the newborn infant intestine is unknown.

Since there is no documented prophylactic effect of LGG on any diseases in children, there is currently no medical indication for supplementing milk substitutes or children's food with LGG.

On 29.03.05, Mead Johnson Nutritionals commented on this risk assessment, provided some additional data, and asked for re-evaluation of the recommendations that had resulted from the risk assessment. The VKM panel on nutrition, dietetic products, novel food and allergy discussed the comments from the companies and made some modifications to the conclusion in a corrigendum (28.06.05). The risk assessment, including corrigendum, is available at www.vkm.no.

Based on this risk assessment, on 10. March 2006, The Norwegian Food Safety Authority decided that Nutramigen 1 with LGG could not be marketed as medical nutrition for infants (0-4 months) in Norway. In addition, The Norwegian Food Safety Authority decided (08. November, 2006) to withdraw permission for marketing of Nutramigen 2 with LGG, which is a milk supplement for infants aged between 4 and 6 months, with cow's milk and soy protein allergy. On 13. December 2006, Mead

Johnson Nutritionals appealed against the decision from The Norwegian Food Safety Authority. The Norwegian Food Safety Authority forwarded the appeal from the company, including new reports and scientific articles, to the VKM for further evaluation.

Based on the overall content of the appeal, The Norwegian Food Safety Authority asked the VKM Panel on biological hazards and the VKM Panel on nutrition, dietetic products, novel food and allergy, for a new risk assessment including the new data provided in the appeal. In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft the appeal from the Norwegian Food Safety Authority.

IV- Terms of Reference[1]

In collaboration with the Norwegian Scientific Committee for Food Safety (VKM), a mandate was prepared for the group reviewing the appeal.

The Norwegian Food Safety Authority requests VKM to address the following questions:

1. Has there been published, in the period since the preparation of the report 'Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods', including the associated corrigendum, up until today, any articles or reports that provide a basis for a re-evaluation of the above named risk assessment's conclusion, with respect to:
   1.1. Possible long-term effects on the immune system generally, and intestinal immune function in particular, from use of LGG in infant formula that are classified as medicinal foods for babies and infants over 4 months.

Mandat
I samarbeid med VKM er det blitt utarbeidet et mandat for gruppen som skal vurdere klagen.

Mattilsynet ber Vitenskapskomiteen for mattrygghet (VKM) vurdere følgende spørsmål:

1. Er det, i tidsrommet fra rapporten "Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingrediens in infant formula and baby foods", inkl. korrigendum, utarbeidet av VKM i 2004 og frem til i dag, blitt publisert artikler eller rapporter som gir grunnlag for å revurdere den ovenfor nevnte risikovurderingens konklusjon i forhold til;
   1. mulige langtidseffekter på immunforsvaret generelt og tarmens immunfunksjon spesielt ved bruk av LGG i morsmelkerstatninger som er klassifisert som medisinske næringsmidler til sped- og småbarn over 4 måneder
   2. mulige langtidseffekter på tarmkoloniseringen ved bruk av LGG i morsmelkerstatninger som er klassifisert som medisinske næringsmidler til sped- og småbarn over 4 måneder
   3. LGGs manglende profylaktiske effekt
2. Er det, i morsmelkerstaninger som er klassifisert som medisinske næringsmidler til sped- og småbarn over 4 måneder, sett noen gunstige effekter ved bruk av produkter tilsatt LGG sammenliknet med tilsvarende produkter uten tilsatt LGG?
3. Dersom VKM mener at det finnes tilstrekkelig med vitenskapelig dokumentasjon på, basert på generelt anerkjente data, at morsmelkerstatninger som er klassifisert som medisinske næringsmidler til sped- og småbarn over 4 måneder tilsatt LGG har enkelte eller flere gunstige effekter, vil VKM etter en helhetsvurdering (inkl. en vurdering av sikkerheten og eventuelle bivirkninger ved bruk av LGG til denne gruppen) anbefale bruken av morsmelkerstatninger som er klassifisert som medisinske næringsmidler tilsatt LGG til sped- og småbarn over 4 måneder?

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1.2. Possible long-term effects on intestinal colonisation from the use of LGG in infant formula classified as medicinal foods for babies and infants over 4 months.

1.3. LGG's lack of prophylactic effect.

2. Has there been observed, in infant formula that are classified as medicinal foods for babies and infants over 4 months, any beneficial effects from the use of products with added LGG, in comparison with similar products without added LGG?
3. If VKM should consider that there is sufficient scientific documentation, based upon generally accepted data, which indicates that infant formula with added LGG, classified as medicinal foods for babies and infants over 4 months, have individual or various beneficial effects, will VKM, after a complete assessment (including an assessment of the safety and possible side effects associated with use of LGG in this group of consumers), recommend the use of infant formula products that are classified as medicinal nutriments, with added LGG, for babies and infants over 4 months?

V- Opinion

One of our major concerns regarding supplementation of infant food with LGG, is the possible long-term effects from any factors that affect the establishment of intestinal microbiota in early infancy. The microbiota of the intestine are not fully established until after 2 years of age. Thus factors that influence the composition of the microbiota at an early stage might permanently affect the further development of the ecosystem. This is demonstrated by studies on mode of delivery and intestinal microbiota, which are outlined below. Caesarean delivery affects the composition of early microbiota, since infants that are delivered by a caesarean section do not have any input of microbes from the birth canal, and differences in intestinal microbiota have been reported in children delivered by a caesarean, compared with children delivered vaginally. Of more interest is the finding that children delivered by caesarean do not apparently attain over time the same flora as the babies that have been delivered vaginally (Bennet and Nord 1987). Differences in the composition of intestinal micobiota are still evident in children delivered by caesarean when they reach 6 months of age (Gronlund et al. 1999), and even at 7 years of age (Salminen et al. 2004).

While there is some limited information regarding the effects of single-species bacterial load on the short-term composition of the newborn infant intestinal

microbiota, there is no information at all on the effects of supplementing the diets of infants and small children with LGG over a prolonged period of time on the long-term composition of their intestinal microbiota. Such information is crucial.

In small children (<6 months) infant formula may represent their only food, and be a substantial part of their diet up to 1 year. If this infant formula is supplemented with a probiotic bacterial culture, then this culture will be present in large numbers in all, or most, of the food that they consume. This is an entirely different situation to the consumption of a probiotic yoghurt by an adult as a small part of their total diet. In comparing these circumstances it is obvious that the challenge to the resident flora is likely to be much greater in small children than in adults. Additionally, the counter-challenge from the resident flora is likely to be considerably less in small children than in adults, and this could allow greater proliferation of the given probiotic strain.

Furthermore, there is little knowledge of the enzymatic properties of the single-species bacterial load. Recent studies have shown that microbes have the ability to alter gene expression in enterocytes. There is, however, no available knowledge on how LGG alters gene expression in the enterocytes. These are factors that may have effects on the diversity of microbiota in the intestine, as well as other unwanted side effects. *In vitro* data presented by Yan et al., (Yan et al. 2007), showing that LGG may regulate intestinal epithelial cell survival and growth, strengthened our concerns about possible long-term effects of a dietary mono-bacterial supplement to the age-groups in question.

Several of the papers put forward show apparently favourable effects on some of the cytokines and immune markers studied. However, studies showing a favourable shift in one or two markers failed to convince us of the overall benefit of the supplementation, due to the well-known complexity of the immune system. This is illustrated by knowledge on allergic diseases, as outlined below. The immune response towards Th_2 in allergic diseases is documented as being skewed, and this was the basis for the Th_1/Th_2 paradigm, in which it was believed that treatment or prophylaxis of allergic diseases could be obtained if the balance could be shifted towards an increase in Th_1 responses. However, as autoimmune diseases are based on Th_1 responses, this balance must be very delicate. Furthermore, a multitude of

different disease phenotypes exist, which are associated with different complex patterns of cytokines, and not even the allergic phenotypes are limited to Th_2 types. Thus, a study that shows an increase in cytokines believed to be advantageous may be associated with an increase in less favourable cytokines, which may, or may not, be detected. Some studies have reported an increase in food allergy (Kalliomaki et al. 2003) and allergic sensitisation (Taylor et al. 2007) among children given LGG as part of clinical trials on the effect of LGG on atopic eczema, and these have added to our concern that this may actually be the case.

Increased insight into the fine-tuning of the immune system has recently been gained, and the crucial role of regulatory T-cells has been established. Rather than shifting the balance from Th_2 to Th_1, an independent down-regulation of the Th_2 responses may be needed in order to prevent allergy (and, correspondingly, a down-regulation in Th_1 responses may be needed in order to prevent autoimmune diseases). However, the characteristics of these regulatory T-cells still remain largely undefined (Woodfolk 2006).

The fact is, that we presently do not have the scientific knowledge necessary to start manipulating the immune system in a predicable manner by administration of probiotics, and manipulation at an early age is of particular concern as the effects may be non-reversible. Our lack of knowledge necessitates the application of precautionary principles.

Other comments:

Most of the papers provided by Mead Johnson and Valio support the use of LGG for the treatment of diarrhoea. As was noted in our previous risk assessment, LGG may have some beneficial effects in the treatment of viral infectious diarrhoea in infants and young children, and may shorten the period of illness by 1 or 2 days. However, the claim that incorporation of LGG into infant formula and baby foods may assist in the prophylaxis of diarrhoea lacks supportive data.

Furthermore, previous studies that suggested that LGG might have a moderately beneficial effect in the prevention of atopic eczema in some sub-populations of sensitized children, are not supported by data from a new study, although a probiotic supplement other than LGG was used in this study (Abrahamsson et al. 2007). Conflicting results have been obtained in studies on treatment of atopic eczema by

LGG administration. Those studies which indicated a beneficial effect (Kalliomaki et al. 2001)(Viljanen et al. 2005)(Weston et al. 2005), have been contradicted by data obtained in more recent studies (Brouwer et al. 2006;Folster-Holst et al. 2006;Taylor et al. 2007). Moreover, there is some evidence that administration of probiotic bacteria to pregnant women, and to children via infant formula, may increase the degree of allergic sensitization in children with atopic eczema (Kalliomaki et al. 2001;Kalliomaki et al. 2003;Taylor et al. 2007)

In nutritional, as well as pharmaceutical, studies, the value of any claim is strengthened if the compound under study is administered in a similar format to the one intended for the market. With the exception of one study (Rautava et al. 2006), a general weakness in nearly all of the new clinical papers that have been provided by Mead Johnson and Valio, is that they have not used infant formula or baby foods in which LGG is an ingredient. The LGG-preparations used in these studies differ in composition from that of infant formula with LGG, and therefore any effect of matrix (infant formula and baby foods without LGG) is unknown.
Of interest, however, is the finding of elevated levels of cow's milk-specific IgA associated with Enfamil feeding. However, the results are inconsistent as the expected corresponding result for TGF-β2 was not observed (Rautava et al. 2006), and this strengthens our concerns about the unknown effects on the total immune system. Furthermore, the study results should be confirmed in other studies, and the long-term effect(s) of LGG still need to be elucidated

The expert group, who performed the GRAS determination on request from Valio, Ltd and Mead Johnson, also did not confirm any beneficial effects of LGG with respect to allergic symptoms. Although many infants with impaired immune competence, heart defects, or central line requirements are probably not identified at birth, the expert group nevertheless suggested that the consumption of LGG is acceptable from birth onwards. Additionally, the expert group did not exclude translocation of LGG; this has previously been reported in 2 patients who received LGG and subsequently developed bacteraemia and sepsis, which was attributed to the LGG (Land et al. 2005). The expert group suggested that "the powder containing *Lactobacillus casei* ssp. *rhamnosus* strain (LGG) manufactured by Valio Ltd., is generally recognised as safe (GRAS) by scientific procedure for use under the supervision of a physician as a

source of LGG in formula intended for term infants from time of birth". In our opinion, by suggesting that product containing LGG is to be used under supervision of a physician, indicates that this product cannot be considered as food.

Effects on growth and behaviour

The number of individuals involved in published studies is often so low that it is not possible to draw any firm conclusions on infants/children as a population. Claims such as "Infants fed with LGG-enriched formulas are better than those fed with regular formula" are usually not substantiated by relevant facts. In general, infants receiving LGG had a slight, but not significant, increased tendency towards crying, vomiting, and loose stools. Possible mechanisms behind these slight differences are virtually unknown and deserve a more thorough investigation before LGG is introduced to the market in infant food.

In summary, it can be concluded that:

1. LGG has been widely studied and characterized in short-term trials.
2. No immediate deleterious effects of LGG have been found.
3. LGG seems to have some beneficial effects on infectious diarrhoea in infants and young children.
4. Some studies indicate that early inclusion of LGG into the diet may provoke allergic sensitization.
5. No scientific proof of a prophylactic effect on any disease is provided.
6. Data on the possible beneficial effect of LGG on atopic eczema in infants and small children is conflicting, and the most recent studies conclude that it has no effect.
7. Long-term effects on immune function in general, or of the gut in particular, when LGG is consumed on a daily basis is not known.

VI- Evaluation of reports and scientific papers

See Appendix I and Answers to the questions.

VII- Answers to the questions

The Norwegian Food Safety Authority requested VKM to address the following questions:

1. Has there been published, in the period since the preparation of the report 'Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods', and including the associated corrigendum, up

until today, any articles or reports that provide a basis for a re-evaluation of the above named risk assessment's conclusion, with respect to:

1.1. Possible long-term effects on the immune system generally, and intestinal immune function in particular, from use of LGG in infant formula products that are classified as medicinal nutriments for babies and infants over 4 months.

Although several studies addressing the inclusion of LGG, or other probiotic bacteria, into children's diets have been published between 2004 and today, none of these studies address the potential long-term effects of including LGG into the diet on the intestinal immune system or the immune system in general. Furthermore, in only one study has the LGG been administered as intended for the market, (i.e. in an infant formula), but this study also does not address long-term effects on immunity. Thus, our re-evaluation concludes that scientific knowledge on long-term effects of LGG on the intestinal and general immune system is still lacking.

1.2. Possible long-term effects on intestinal colonisation from the use of LGG in infant formula products that are classified as medicinal nutriments for babies and infants over 4 months.

The newest literature, from 2004 to date, does not provide information on the long-term effects on intestinal colonisation from the use of LGG in infant formula products for infants older than four months.

1.3. LGG's lack of prophylactic effect.

Recently published reports do not substantiate the claim that LGG supplemented formula will have a prophylactic effect on any disease, including atopic eczema, allergic sensitization, or viral or antibiotic-associated diarrhoea.

2. Has there been observed, in infant formula products that are classified as medicinal nutriments for babies and infants over 4 months, any beneficial effects from the use of the products with added LGG, in comparison with similar products without added LGG?

No obvious beneficial effects of LGG-supplemented formula have been observed compared with formula not supplemented with LGG. Again it should

be noted that only one study gives information on the use of LGG-supplemented formula.

3. **If VKM should consider that there is sufficient scientific documentation, based upon generally accepted data, which indicates that infant formula products with added LGG, that are classified as medicinal nutriments for babies and infants over 4 months, have individual or various beneficial effects, will VKM, after a complete assessment (including an assessment of the safety and possible side effects associated with use of LGG in this consumer group), recommend the use of infant formula products that are classified as medicinal nutriments, with added LGG, for babies and infants over 4 months?**

   After complete assessment of the scientific documentation, including an assessment of safety and possible side effects associated with the daily use of LGG in this consumer group (consisting of children aged between four months and 2-3 years), VKM does not recommend the use of infant formula products that are classified as medicinal nutriments with added LGG, for infants over four months.

VIII- Appendix I

Viljanen M, Savilahi E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. (2005). Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double blind placebo controlled trial. Allergy 60: 494-500.

This is a double-blind, placebo-controlled study of 230 infants with AEDS, with or without CMA, and with or without IgE involvement. The infants were randomised into three groups, one of which received LGG, one of which received a mix of LGG and other probiotic bacteria, and one of which received placebo. Administration was for four weeks following the first visit. SCORAD was assessed at the first visit, after 4 weeks, and at 8 weeks. Skin treatment with emollients and 1% hydrocortisone was instituted at the first visit and all children were kept on a CM-free diet, with supplementation with an extensively hydrolysed whey formula.
Symptoms of AEDS improved continuously, as indicated by decreasing SCORAD throughout the study period. No differences were detected in SCORAD decrease between the groups. However, a subgroup of infants with IgE-associated AEDS showed a significantly greater SCORAD decrease in the LGG group than in the mix or placebo group. Faecal prevalence of probiotic strains was high in the treatment groups, indicating that the protocol was followed.
Comments: The aim of this study was to investigate the effect of a 4-week treatment period with LGG on AEDS in infants. An overall improvement in symptoms during the treatment period in all groups was detected, although a somewhat greater improvement in a subgroup of infants with IgE-associated AEDS receiving LGG supplementation was reported. The study did not focus on safety and no side effects were reported. The treatment period was short, but considerable amounts of probiotic bacteria were administered to the infants in the treatment groups.

Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tittanen M, Vaarala O, Savilahti E. (2004). *Lactobacillus* GG effect in increasing IFN-γ production in infants with cow's milk allergy. J. Allergy Clin. Immunol. 114:131-6

230 infants participated in a double-blind, placebo-controlled study as described in the previous publication. This article reports experiments with *in vitro* stimulation of patient PBMC before and after the four-week treatment period with probiotic bacteria (protocol as described in the previous article). The main result was an increase in IFN- γ production in the subgroup of infants with IgE-associated AEDS who received LGG, but not in those receiving a mix of probiotic bacteria or in the placebo group. LGG did not influence the IFN- γ level in non IgE-associated AEDS. The increase corresponded with clinical improvement in the IgE-associated AEDS LGG group. The authors concluded that *Lactobacillus* strains might offer clinical benefits that are mediated by immunological mechanisms.
Comments: The article demonstrates that immunological changes may occur in a subgroup of infants whose diets are supplemented with LGG. There is no mention of side effects, but the study did not focus on safety. The results are interesting, but perhaps unsurprising, as immunological effects of an oral supplement of a live bacterial strain were demonstrated. The clinical benefits of this immunological phenomenon remain to be proven.

Viljanen M, Pohjavuori E, Haahtela T, Korpela R, Kuitunen M, Sarnesto A, Vaarala O, Savilahti E. (2005). Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. J. Allergy Clin. Immunol. 115:1254-9.

This is the same patient population as in the previous two articles. In this publication the effect of probiotics on infants' intestinal immune systems was studied by measuring the systemic concentrations of cytokines (IL-2, IL-4, IL-6, IL 10, IFN γ, TNF α, TGF β1, TGF β2), inflammatory marker (CRP), soluble adhesion molecule (E-selectin), and intercellular adhesion molecule (ICAM-1) both before and after LGG supplementation. A slight, but significant, up-regulation of IL-6 and CRP was demonstrated in IgE-associated AEDS infants who received LGG for four weeks. The other two treatment groups did not exhibit these changes, indicating a competition between probiotic strains in the gut flora. The authors discussed the possibility that probiotics stimulate the intestinal immune system, inducing low-grade inflammation, which alleviates allergic symptoms.
Comments: The study reports immunological changes in peripheral blood in a subgroup of infants with AEDS and suspected CMA. The changes corresponded with clinical improvement reported in another article (cited above). There is no focus on safety, and no mention of side effects. The probiotics were administered to the infants over a relatively short time period, but in considerable doses.

Laitinen K, Kalliomaki M, Poussa T, Lagstrøm H, Isolauri E. (2005). Evaluation of diet and growth in children with and without atopic eczema: follow up study from birth to 4 years. Br. J. Nutr. 94:565-74

This study aimed to evaluate nutritional factors and their impact on AD in a cohort of children described previously (Kalliomaki et al. 2001) whose mothers had received LGG supplementation during pregnancy, and the children continued to receive LGG postnatally. The method used was a four-day food diary, and associations between foods and AD were investigated by logistic regression models. It was concluded that retinol, Ca, and Zn, together with probiotics, reduced the risk of AD, whilst elevated intake of ascorbic acid increased the likelihood of AD. The authors concluded that perinatal administration of probiotics is safe as it did not influence the height, or the weight for height, of the children at 48 months, and that the combined effects of nutrients and probiotics should be considered in active prevention and management schemes for allergic diseases.
Comments: This study does not provide any new knowledge on the effect of probiotics, and repeats that which has already been published on the same cohort by (Kalliomaki et al. 2001). The administration of LGG was considered safe as it did not influence the growth of the children, but no mention was made of effects on gastrointestinal immunity or of potential long-term effects of probiotics.

Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. (1992). Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Pediatr. Res. 32:141-144.

The authors report on the effect of LGG administered during an acute rotavirus infection, and seeks to elucidate the mechanisms behind the clinical effect on the diarrhoea. LGG therapy was associated with a significant, non-specific humoral

response during the acute phase of the infection. During convalescence the study group had a significantly higher IgA specific antibody-secreting cell response to rotavirus, indicating that LGG augmented the local immune defence.
Comments: LGG and other probiotica seem to have a favourable effect on acute rotavirus diarrhoea. This means short-term use of the bacteria, which in many studies has proven to be safe, can be beneficial in particular circumstances. Our concerns are directed towards long-term effects and possible threats from prolonged, regular intake.

Taylor A, Dunstan J, Prescott S. (2007). Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: A randomized controlled trial. J. Allergy Clin. Immunol. 119:184-9.

This is an investigation of high-risk children receiving a *Lactobacillus* strain (other than LGG) or placebo for the first 6 months of life. No effect of the probiotic on the presence of AD was noted at 6 and 12 months. The rate of allergic sensitization was significantly higher at 12 months in the probiotic group than in the placebo group. The conclusion was that no effect on AD was noted, and thus the possible benefit of probiotics on AD is unclear.
Comments: The higher allergic sensitization rate, especially to cow's milk in the probiotic group, must be considered seriously. This finding was also apparent from the previous studies by Kalliomaki et al., (Kalliomaki et al. 2001). No side effects of the probiotic were noted, apart from the sensitization, but this study was not designed to investigate safety.

Gawronska A, Dziechciarz, P, Horvath, A, Szajewska H. (2007). A randomized double-blind placebo-controlled trial of *Lacobacillus* GG for abdominal pain disorders in children. Aliment Pharmacol. Ther. 25: 177-184.
In a randomised, placebo-controlled intervention trial, 104 children with abdominal pain, from 6-16 years, were randomised into two groups, one of which received LGG for 4 weeks, and the other placebo. Stratification was by initial diagnosis; either functional dyspepsia (n=20), irritable bowel syndrome (n=37), or functional abdominal pain (n=47). Treatment success was defined as no pain. In the IBS group, children who received LGG were more likely to experience treatment success (33% and 5%, treatment success among LGG and placebo-treated, respectively). In the children with functional dyspepsia or functional abdominal pain, there were no significant differences between the two groups. In the overall group there was an effect, primarily due to the children with IBS. Although the number of children with treatment success, (i.e. no pain), differed significantly between groups, there was no overall improvement in severity of pain. A number of other outcomes are also reported.
Comments: A well-designed study, although the number of participants was low. Despite power calculations being performed before beginning the study, the inclusion of subgroups, resulted in n being smaller than had been estimated as required. The difference in treatment results between the groups may therefore be coincidental, due to the small groups and the different outcome measures. Focus should therefore be on the whole group; this does show larger number of responders in the treatment group, but the results are inconsistent as there was no discernible effect on pain severity.

Gueimonde M, Kalliomaki M, Isolauri E, Salminen S. (2006). Probiotic intervention in neonates - will permanent colonization ensue? J. Pediatr. Gastroenterol. Nutr. 42: 604-606.
In this study the effect of maternal consumption of LGG for 2-4 weeks before delivery on neonatal microbiota was investigated in a group of 53 newborns with a family history of atopic disease, with focus directed particularly towards *Bifidobacteria* species. No differences in the concentrations of specific bifidobacterial species were detected at the different time points investigated (mother, before and after birth; infant, 5 days and 3 weeks). However, significant differences in the concentrations of *B. breve* and *B. adolescentis* were detected between infants belonging to the placebo and LGG group, at 5 days, but not at 3 weeks. Additionally, there was a non-significant trend towards increased diversity of species in the group of children whose mothers had taken LGG (1.22 versus 0.81).
Comments: This paper lacks crucial information on many aspects. There is an absence of information on randomisation process, and on the characteristics of the 2 groups. No effort has been directed towards optimising homogeneity between the groups (mode of delivery, feeding regimens, and gestational age are all factors that may influence microbiota, and should have been considered). The number of subjects in each group is small. As 6 different species were examined at 2 different age points for the infants alone, 2 out of 10 findings would be expected to be caused by random variation. The inconsistency over time for the 2 species that differed at 5 days, suggests that these results are perhaps only random findings, rather than true differences. The various attempts to place the non-significant finding of diversity into context (Tables 2 and 3 in the publication) seem neither relevant nor convincing.
It is not clear why LGG was not also monitored, in order to determine the rate of LGG colonisation in the infant, and whether this was a factor that affected the microbiota.
Conclusion: there may, or may not, be an effect of LGG administration to the mother on the microbiota in the child, but this paper is inconclusive. This is a poorly-designed study, and inadequately described, and the reported results could be chance findings.

Folster-Holst R, Muller F, Schnopp N, Abeck D, Kreiselmaier I, Lenz T, von Ruden U, Schrezenmeir J, Christophers E, Weichenthal M. (2006). Prospective randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. Br. J. Dermatol. 155:1256-61.

In this study the effect of LGG supplementation for 8 weeks on 54 infants aged 1 to 55 months with moderate to severe atopic eczema was examined in a randomised intervention trial. No significant differences on eczema were detected between the placebo and LGG treatment group during the treatment period, as evaluated by SCORAD, pruritus, sleeplessness, use of corticosteroids, and ECP levels in faeces. Among children with a positive test for IGE sensitization, there was a SCORAD reduction of 6.9 in the LGG treatment group, and of 8.6 in the placebo group.
Comments: Randomisation may have been unfortunate in this trial, with more allergic children placed in the placebo group, (particularly subjects with food allergy and rhinitis). This was a very small study, and therefore may have lacked the power to show significant differences. However, none of the non-significant trends in the data provide support for an effect of LGG; indeed, if there is any trend, it is in the opposite direction. The study seems to have been generally well conducted, but

although bottom-line characteristics are described, more information on the randomisation procedure could have been provided (children were "allocated" to receive LGG or placebo, they do not seem to have been allocated to each centre, which would have been a serious flaw in the study, but this is not totally certain from the information given). The dosage used in this study is higher and for a longer duration than in the last 2 studies.

Rautava S, Arvilommi H, Isolauri E. (2006). Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res. 60:221-224.

In this study the infants were randomised to receive either infant formula with LGG (Enfamil), or, if weaned early, placebo. IgA secreting cells, cow's milk specific IgA, sCD14, and TGF-β2 were measured in serum at 3, 7, and 12 months of age. There was a tendency towards higher levels of cow's milk specific IgA among the Enfamil-treated infants, as compared with the placebo group at all age points, and this was significantly so at 7 months. Additionally the Enfamil-treated infants had higher levels of sCD14, and this was significantly different at 12 months. The serum concentrations of TGF-β2 were similar between the two groups at 3 months, lower in the Enfamil-treated infants at 7 months, and higher in this group at 12 months, but none of the differences were significant. The total number of IgA secreting cells was higher in the Enfamil-treated infants at all age points, but not significantly so at any time point.
Comments: The findings of higher levels of cow's milk specific IgA associated with Enfamil, seem consistent. As the author points out, correspondingly elevated results would have been expected for TGF-β2, but this was not detected. There was also a significant difference in sCD14, although the change in concentrations seems minor. More information on the randomisation process should have been provided in order to explain why the number of subjects differs between the 2 groups before drop out. Furthermore, although it is claimed that intention to treat is followed, results on dropouts are not included. Were no blood tests available on the dropouts? It would also have been useful to have the analysis repeated, but with subjects that had developed CMA/CMPI excluded. Whilst the bottom-line finding of increased cow's milk specific IgA in the LGG treated group seems convincing, the investigation should be repeated in a larger study sample.

Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. (1999). Development of allergen-specific T-cell memory in atopic and normal children. The Lancet, 353; 196-200.

In this study allergen-specific T-cell responses were compared in 31 children divided into two groups. One group had no familial risk of atopy and also remained without such disease up to at least 2 years of age, whilst the other group consisted of infants with a familial risk of atopy, and who developed definite disease before the age of 2 years. The children were selected from a prospective cohort study that started follow-up at birth. The main finding is that the children who subsequently developed atopic diseases had lower allergen-specific responses of Th2 type in cord blood, than children who remained healthy and was demonstrated for IL-4, IL-6, IL-10, and IL-13. All children showed allergen-specific Th2 responses at birth, but in healthy children the responses were quickly down-regulated. Interferon gamma production was low in

both groups at birth but increased rapidly among the children who remained healthy. The authors proposed that the decreased capacity to produce high levels of interferon gamma was the reason for the persistent Th2 profile in cytokine patterns among the atopic children. In contrast to the general reduced capacity to produce interferon gamma, which is seen in all infants after birth and which is believed to be caused by inefficient co-stimulation of accessory cells, the decreased capacity in atopic individuals seems to be associated with an intrinsic defect in T-cells (shown in Fig 5 in the publication: Nearly no Interferon gamma production by CD4 T cells from atopic individuals). The authors believe that the period of infancy is critical; if the Th2 inhibitory signal is absent, early expansion and maturation of Th2 memory cells may occur.
Comments: A well-designed and important study that received considerable attention at publication.

Viljanen M, Kuitunen M, Haahtela T, Juntunen-Backman K, Korpela R, Savilahti E. **(**2005). Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants. Pediatr. Allergy Immunol. 16: 65-71.

This complex study is divided into several sections.
Children with AEDS and suspected CMA
First, 203 children with AEDS and ***suspected*** CMA, aged 1-12 months, were randomised to receive 1 of 3 treatment options: LGG, MIX (LGG + *L.rhamnosus* LC705 + *B. breve* + *P. freudenreichii*), or placebo for 1 month. However, curiously only 102 of the 203 participants had their samples analysed for the markers of interest. The children were also simultaneously put on an elimination diet, which means that reaching a conclusion by comparison of before and after values is complicated, and only differences between groups at individual time points can be considered. IgA values tended to be higher in the LGG and MIX group than in the placebo group after treatment. However, as the values in the placebo group were lower before treatment the authors adjusted the results for pre-treatment values. Following this adjustment, a non-significant tendency for higher faecal IgA concentrations in LGG and MIX group, compared with the placebo group, was detected. Corresponding adjustments for TNF-α, antitrypsin, and ECP demonstrated no differences between the groups.
Children with CMA/CMPI.
In the second section, CMA/CMPI was confirmed in 120 out of the 203 participating children, as based on DBPCFC-tests. However, only 67 of these had had their samples analysed. In comparing values before and after treatment (parallel to the previous section for all the participants), no significant differences in IgA, TNF-α, antitrypsin, and ECP were detected between the treatment groups.
In comparing values both before and after challenges, significantly higher faecal IgA levels were detected after challenge (as compared with before challenge) in the LGG group, but not in the MIX group or in the placebo group. Comparison of IgA levels after cow's milk challenge, adjusted for pre-treatment values, showed no significant differences between the treatment groups in IgA, TNF-α, antitrypsin, and ECP levels.
Children with CMA/CMPI and IgE sensitization to any antigen
27 out of the 102 children with adverse reactions to milk demonstrated signs of IgE-sensitization and had their values measured.

Significantly higher IgA levels, lower TNF-α levels, and borderline significantly higher antitrypsin levels were reported for the LGG group, but not for the MIX group. ECP was higher in the LGG group, but this was not significant.
Comments: In the last analysis the sample size was very low (n for each group was 14, 14, and 9). As well as the increase in IgA and decrease in TNF-α in the LGG group, which are assumed to be beneficial alterations, there were increases in antitrypsin and a non-significant increase in ECP; this is unexpected and increases the concern that these results may be random findings associated with the small sample size. In the definition of cases in the last section it is puzzling that the authors chose to include all types of IgE sensitizations, rather than only to milk, which would have provided a well-defined group of CMA children.
It is noteworthy that the findings differ considerably between the LGG and MIX treatment groups. What this could mean, and why this is not discussed in the publication, are subjects for speculation.

Blumer N, Sel S, Virna S, Patrascan CC, Zimmermann S, Herz U, Renz H, Garn H. (2007). Perinatal maternal application of *Lactobacillus rhamnosus* GG suppresses allergic airway inflammation in mouse offspring. Clin Exp Allergy 37: 348-357.

The authors studied the effects of a perinatal LGG supplementation in mice on the development of allergic disorders in their offspring. The authors concluded that LGG might exert beneficial effects on the development of experimental allergic asthma, when applied in a very early phase of life.
Comments: The number of mice in the experiment was low. High variations in individual results were obtained for some tests, and therefore the standard deviations were high. It is not clear whether the results can be extrapolated to humans.

Isolauri E, Joensuu J, Suomalainen H, Luomala M, and Vesikari T. (1995) Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine 13: 310-312

Effects of orally-administrated LGG in conjunction with live oral rotavirus vaccine were tested in 2-5 month-old infants. Infants who received LGG showed an increased responsiveness of rotavirus-specific IgM-secreting cells, as measured with an ELISPOT technique on day 8 after vaccination. Both IgM and IgA seroconversion were higher in infants receiving LGG, as compared with the placebo group.
Comments: The authors did not study the duration of protection against rotavirus, and the IgM and IgA seroconversions against serogroups of rotavirus other than those used in the study were not measured. Concentrations of IgA and IgM in the intestinal mucus layer were not measured and studies on cellular immunity were not performed.

Kajander K, and Korpela R. (2006). Clinical studies on alleviating the symptoms of irritable bowel syndrome with probiotic combination. Asia Pac. J. Clin. Nutri. **15**, 576-580.

The authors screened for optimal strains, and developed a multi-species probiotic combination consisting of LGG and several other probiotic microorganisms. A randomised, double-blinded, placebo-controlled, six-month trial was used to study the therapeutic value of the probiotic combination in IBS patients. The authors found

that the multispecies probiotic combination, including LGG, seemed to alleviate IBS symptoms significantly.
Comments: This is an interesting paper. However the age distribution of the patients is not clear, and the authors have not indicated whether any of the individuals with IBS included in the study were children.

Wenus C, **Goll R**, **Loken EB**, **Biong AS**, **Halvorsen DS**, **Florholmen J.** (2007). Prevention of antibiotic-associated diarrhoea by a fermented probiotic milk drink. Eur J.Clin. Nutr.

87 eligible patients (selected from 853) were randomly divided into two groups (46 test patients and 41 controls) and participated in a double-blind, placebo-controlled study. 8 patients (27.6%) in the control group and 2 (5.9%) in the test group experienced AAD. The study group was given 250mL Biola Surmelk per day, which contained LGG, *L. acidophilus* LA5, and *Bifidobacterium* Bb12. The aetiolgoical agents of the AAD were not identified, but one patient had *Clostridium difficile* and another had *Yersinia enterocolitica*. The authors rightly concluded that the results were promising, but that the study should be repeated with a larger group. They cited that the use of multistrain probiotics has been recommended, and also that they are more efficient when ingested as a fermented milk product, rather than as a freeze-dried powder.

Katz JA. (2006). Probiotics for the prevention of antibiotic-associated diarrhea and *Clostridium difficile* diarrhea. J. Clin. Gastroenterol. 40:249-55

This is a comprehensive review, published in 2006, and submitted for publication Sept. 2005. It has 54 references, of which 55% are from 2000 or later. The author is considered to be eminent in the field of gastroenterology and probiotics. The conclusions were as follows:
Probiotics for prevention/treatment of *C. difficile* diarrhoea:
A recent systematic review of the literature suggested that studies published to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat *C. difficile* diarrhoea.
Probiotics in antibiotic-associated diarrhoea: The final verdict on the role of probiotics in antibiotic-associated diarrhoea awaits further large, well-designed, and well-executed clinical trials, including dose-range studies, comparative trials, and formal cost-benefit analysis.
Comments: The review is a balanced and unbiased assessment of the problems associated with the use of probiotics for prevention of diarrhoea.

Yli-Knuuttila H, Snäll J, Kai K, Meurman H. (2006). Colonization of *Lactobacillus rhamnosus GG in the oral cavity*. Oral Microbiol. Immunol. 21:129-131

The aim of this study was to investigate whether LGG could “be detected in the oral cavity after discontinuation of a product prepared with this bacterium.” Fifty-six volunteers consumed Gefilus R juice for 14 days and saliva samples were collected daily on MRS agar. LGG-like colonies were analysed and characterized by both classical methods and PCR. LGG was only detected temporarily and the authors concluded that “permanent colonization of LGG in the oral cavity is improbable”,

Comments: This is a short communication and details on selection, age, and gender of volunteers, daily amount of Gefilus consumed, duration of the study period etc, were not provided. Nevertheless, it seems reasonable to concur with the authors' conclusion.

Guiemonde M, Kalliomäki M, Isolauri E, and Salminen S. (2006). Probiotic intervention in neonates - will permanent colonisation ensue? J. Pediatric Gastroentol. Nutri. 42: 604-606.

The purpose of the study was to assess whether LGG can be detected in the gut microbiota in an infant population beyond the period of administration of LGG. 128 infants, whose mothers were given daily capsules containing 1x 10^{10} CFU of LGG (63 subjects) or placebo (65 subjects) for 4 weeks before delivery, participated in the study. After delivery, LGG or placebo was administered either directly to the infant (in 38 cases receiving LGG) or to the breast-feeding mother (25 cases receiving LGG) for 6 months. Faecal samples were collected at 6 and 12 months of age. In addition, the infants with samples positive for LGG at 12 months were also analysed at 24 months.
Results: 58% of the samples in the LGG treated group and 28% of the samples in the placebo group were positive for presence of LGG by colony identification after 6 months (78% and 43% respectively by PCR).
After 12 months, the occurrence of LGG was much less common, with 24% (treated group) and 14% (placebo group) positive by colony identification (26% and 20% respectively by PCR).
Only two samples from individuals, who were positive at 12 months, were also positive at 24 months.
Comments:
A detailed description of materials and results were not presented in this "short communication". The presence of LGG in infants in the placebo-group was high. This is probably because LGG was extensively used in Finland (where this study was conducted) during the study period. This confounder was not discussed in relation to the treated group. The term "colonisation" was not defined. The high presence of LGG in faeces at the first sampling occasion does not necessarily indicate high "colonisation"-rate in infants, since this sampling was immediately after the termination of the LGG administration period. The study demonstrated that LGG does not permanently colonise the intestine of infants.

Petschaw P, Figueroa R, Harris CL, Beck LB, Ziegler E, Goldin B. (2005).
Effects of feeding an infant formula containing *Lactobacillus* GG on the colonization of the intestine. J. Gastroenterol. 39: 786-790.

This publication describes a multicentre, double-blind, randomised, parallel group study comprised of 3 study periods: a 7-day baseline period, during which all infants received the baseline/control formula; a 14-day test period, during which eligible infants were randomised and received either the control formula or 1 of the 3 supplemented study formulas containing LGG at 3 different concentrations (low, medium, and high); a 14-day follow-up period, during which all infants received the baseline/control formula. The purpose of the study was to determine whether feeding LGG at varying concentrations (10^8 to 10^{10} cfu / day) would result in colonisation, defined as $\geq$ 1,000 cfu per gram of stool in 3 of the 5 samples collected during the

feeding period. Median stool counts of LGG (Log_{10} cfu/g) in colonised infants were 5.24 (low), 6.05 (medium), and 5.97 (high). LGG was detected in stool samples for between 7 to 14 days after discontinuing LGG.
Comments: This was a well-designed study, intended to determine whether feeding LGG at varying concentrations affected the "colonisation" of the infants' intestines. The results obtained are similar to those previously reported by others. The limited number of participants (n=55) and the short study period (4 weeks) mean that it is not possible to draw any firm conclusions. Although colonisation is probably not an appropriate term to use for LGG, some survive passage through the gastrointestinal tract.

Bier DM, Doyle MP, Borzelleca JF, Kolezko B, Clemens RA, O'Sullivan DJ. (2006). Conclusions of the expert panel: Generally Regarded as Safe (GRAS)-Determination for the use of *Lactobacillus casei* spp. *rhamnosus* strain GG in exempt infant formula. Prepared for Mead Johnson Nutritionals-Evansvielle-Indiana.

Members of the expert group evaluated the available publicity information on the proposed use of LGG in exempt infant formula. The evaluation included a review of the starting materials, production methods, and genetic stability of the LGG; the effects of LGG on the gastrointestinal and immune systems; the history of apparent safe use of lactic acid bacteria, the genus *Lactobacillus*, and the specific strain LGG; and the apparent safety of administering LGG to newborn infants. The expert group concluded that the powder containing *Lactobacillus casei* spp. *rhamnosus* strain GG, manufactured by Valio Ltd., could be generally regarded as safe (GRAS) by scientific procedure, for use under the supervision of a physician as a source of a LGG in formula intended for term infants from time of birth.
Comments: The phrase that "LGG appears to confer **possible** beneficial effects with respect to allergic symptoms" does not concur with the published data that claim beneficial effects on allergic symptoms (Kalliomaki et al., 2001; Kalliomaki et al., 2003). The term "possible" suggests that the expert group was not wholly confident with the published articles regarding beneficial effects on allergic symptoms.
The expert group did not confirm the colonisation of LGG on the intestinal surface, which has been published several times, but used the phrase "can adhere to intestinal surfaces".
According to the expert group LGG may inhibit translocation of intestinal pathogens, and translocation of LGG itself has been only rarely observed in controlled studies. The expert group suggested use of LGG under supervision of a physician from time of birth. Many infants with impaired immunocompetence, heart defects, or central lines are probably not identified at birth.

Land MH, Rouster-Stevnes K, Woods CR, Cannon ML, Conta J, Shetyy KS. (2005). *Lactobacillus* sepsis associated with probiotic therapy. Pediatrics 115: 178-181.
This paper reports on 2 patients who received *Lactobacillus* LGG and subsequently developed bacteraemia and sepsis attributable to the LGG. DNA-fingerprinting revealed that the implicated LGG strain was similar to the probiotic strain ingested by the patients. Both patients were children (one aged 6-weeks, the other aged 6-years). The first patient had been admitted for scheduled repair of a double-outlet right ventricle and pulmonis stenosis. The other patient, who had cerebral palsy,

microcephaly, mental retardation, and a seizure disorder that required feeding through a gastrojejunostomy tube, had been admitted for treatment of a urinary tract infection.
Comments: This report confirms that LGG can cause invasive disease in certain individuals.

Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. (2007). Soluble protein produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology. 132: 562-575.

Two novel proteins (75 and 40 kD) secreted by LGG were purified and characterized and shown to prevent cytokine-induced apoptosis in human and mouse epithelial cells. Polyclonal antibodies against these proteins were used to prepare LGG culture media depleted of these proteins (= control). The proteins were shown to activate Akt (which inhibits apoptotic processes), inhibit cytokine-induced epithelial cell apoptosis, and promote cell growth in human and mouse colon epithelial cells and cultured mouse colon explants. The proteins also significantly reduced damage to the colon epithelium by tumour necrosis factor (TNF). A weak reaction with the antisera to p75 was also found with the supernatant from another *Lactobacillus* strain (three other strains were investigated, two *L. casei* and one *L. acidophilus*). There was also a relationship between p40 from LGG and from a strain of *L. casei.*
The authors proposed that supernatant-derived proteins from LGG could be used to regulate intestinal inflammatory responses. LGG has been shown to induce remission and prevent recurrence of IBD in patients and animals. However this effect was not shown in a trial with children with Crohn's disease. The authors also noted concerns regarding treating the very young, following the cases of bacteraemia associated with probiotic therapy (Land et al, 2005). They therefore suggested that the use of bacteria-derived proteins could be an alternative for treatment of IBD and other inflammation-derived disorders. Reference is made to communication between intestinal flora and epithelial cells; apparently some probiotic strains need direct cell contact with the epithelium in order for an effect to occur, whereas the strain investigated here (LGG) has an active secreted protein.

Zhang L, Li N, des Robert C, Fang M, Liboni K, McMahon R, Caicedo RA, Neu J. (2006). *Lactobacillus rhamnosus* GG decreases lipopolysaccharide-induced systemic inflammation in a gastronomy-fed infant rat model. J. Pediatr. Gastroenterol. Nutr. 42: 545-552.

Two groups of rat pups (6-7 day old) were fed a rat milk substitute combined with *E. coli* lipopolysaccharide (LPS). One group was also given a supplement of LGG (10^8cfu/L milk). Mother-fed rat pups were used as control.
Mother-fed pups grew better than the pups on substitute diet, and in this respect the two experimental groups did not differ. However, the experimental group without LGG showed striking metaplasia of the villous epithelium, which was absent in the mother-fed pups and was attenuated in the LGG-supplemented pups. Assay of markers of inflammation (CINC-1, TNF-α, MPO and cytokine multiplex assay) showed that supplementation of the milk with LGG markedly reduced the inflammatory response to LPS, although the response was higher than in the control group. LGG had therefore attenuated or reduced the response, but did not totally

abrogate it. The authors proposed potential mechanisms for the observed effects and suggested that these results may be of importance for the treatment of critically underweight infants, who may suffer from systemic infections which may result in systemic inflammation, leading to necrotizing conditions. Should this state occur in the intestine, the inflammatory response can cause damage in distal organs? Various articles are cited reporting studies on anti-inflammatory actions of other probiotics. This article provides new information about effects of LGG – *in rats.*
This study was funded by Mead Johnson.

Apostolou E, Pelto L, Kirjavainen PV, Isolauri E, Salminen SJ, Gibson GR. (2001). Differences in the gut bacterial flora of healthy and milk-hypersensitive adults, as measured by in situ hybridization. FEMS Immunol. Med, Microbiol. 30:217-221.

Predominant gut flora (genera) were determined in two groups of adults (9 healthy and 8 milk-hypersensitive) both before and after 4 weeks of LGG intervention. It was concluded that the gut flora in both groups were similar and that intervention with LGG resulted in an increase in *Bifiobacteria* in healthy, but not milk-hypersensitive, adults. Pathogenic types were not reduced by LGG intervention. These results therefore indicate that the gross gut flora is not directly associated with milk hypersensitivity.
Comments: The number of participants in this study was low, and calculations based on the results show that it would be necessary to have between 70 and 200 individuals per group. The bacteria genera were identified by the use of 4 oligonucleotide probes. Considering the enormous variation of the gut flora, this is a very coarse study. In addition the bacteria were detected in faecal samples, which may not be representative of the flora in the small and large intestine. The authors concluded that intervention with LGG does not down-regulate the milk induced inflammatory response by altering the microbial gut flora. It is questionable whether this proposition is valid, given the small size of the study. This study was published 6 years ago; as the pace of development in this field is rapid, this study can be considered old.

Roselli M, Finamore A, Britti MS, Mengheri E. (2006). Probiotic bacteria *Bifidobacterium animalis MB5* and *Lactobacillus rhamnosus GG* protect intestinal Caco-2 cells from the inflammation response induced by enterotoxinogenic *Escherichia coli* K88. Br. J. Nutr. 95:1177-1184.

Two probiotic strains, LGG and MB5, were investigated for their ability to prevent adhesion of *E. coli* K88 *in vitro*. Numbers of adhering EC appeared to be affected by the numbers of MB5 added, but were not number-dependent with respect to LGG. When spent supernatant was added, this also reduced the adhesion. Supernatant treated with proteolytic enzymes had the same effect, indicating that the soluble active component was not a protein. It was suggested that the effect could be due to competition for binding sites. Both the strains studied (when living, not when dead) also reduced neutrophil migration induced by K88, and the supernatant also had this effect. LGG, but not MB5, induced IL-1ß indicating variation in action of different probiotic strains. The authors rightly suggested that *in vivo* studies must be conducted before either MB5 or GG can be suggested as being of use in the prevention or alleviation of ETEC-induced intestinal disorders.

Vendt N, Grünberg H, Tuure T, Malminiemi O, Wuolijoki E, Tillmann V, Sepp E, Korpela R. (2006). Growth during the first 6 month of life in infants using formula enriched with *Lactobacillus rhamnosus GG:* double-blind, randomized trial. J. Hum. Nutr. Dietet. 19:51-58.

This is a prospective, randomised, double-blind, placebo-controlled clinical study conducted in Tartu, Estonia. A cohort of 120 healthy, full-term children aged between 0 and 2 months, and on formula for at least half their daily feeding, participated. The formula that had been given seemed to be Tutteli ®. When enrolled, the infants were randomised into two group, one of which continued on the same unchanged formula, whilst the other received the same formula, to which had been added *L. rhamnosus* (at the end of shelf life of formula, the concentration of bacteria was claimed to be log 7 CFU per gram). 105 of the participating infants completed the study.
Findings: Slight, but not statistically significant, increases in the LGG group were reported for the following parameters: defecation frequency, number of loose stools, infectious periods, and crying behaviour. Growth rates (increase in length and weight) were approximately the same in both groups. The authors claimed that "infants fed with LGG-enriched formula grew better than those fed with regular formula". However, this claim is based upon a calculation based on "data of normal Estonian infants" and no reference to this material is provided.
Comments: Many of the points raised by the authors in this publication are controversial.

Rinne M, Kalliomäki M, Salminen S, Isolauri E. (2006). Probiotic intervention in the first months of life: short-term effects on gastrointestinal symptoms and long-term effects on gut microbiota. J. Pediatr, Gastroent. Nutr.43:200-205

This is a double-blind, placebo-controlled trial, following a protocol published some years ago by the same group of investigators. It may be reasonable to assume that the cohort described in this paper, is a part of the cohort previously described.
The aim of this study was to investigate whether administration of LGG affected particular selected symptoms and signs in the infants at 7 and 12 weeks old, and the composition of a defined part of the gut microbiota at 6, 12, 18, and 24 months of age.
In total, for the data obtained at 7 and 12 weeks of age, the values given for vomiting, loose stools, watery stools, crying and fussing were higher in the group that received LGG than in the group that received placebo. However, at both time points the differences were small. The authors concluded that LGG "was well tolerated". Based upon these data, it might have been more appropriate to state that LGG did not cause any major harm.
Further, the authors concluded that LGG "did not significantly interfere with long-term composition or quantity of gut microbiota". In considering these data it should be noted that over 20% of the faecal samples were missing (113 out of 528) and that only a minor proportion (at least at 18 and 24 months of age) was investigated.

Rinne M, Kalliomaki M, Arvlommi H, Salminen S, Isolauri M. (2005). Effect of probiotics and breasfeeding on the bifidobacterium and *Lactobacillus* / *Enterococcus* microbiota and humoral immune responses. J. Pediatr. 147: 186-191.

This is a double blind, placebo-controlled trial; apparently following a protocol published 6 years ago by the same group of investigators, and presumably using the same group of subjects as described in previous papers.
The aim of this part of the study was to assess the impact of probiotics and breastfeeding on gut microbiota composition, as characterised by bifidobacteria and lactobacilli/enterococci, and humoral immune response as indirectly assessed by circulating immunglobulin-secreting cells. Additionally, it was intended to investigate the impact of sCD14 in colostrum.
The impact of sCD14 is somewhat difficult to evaluate in this paper. The authors claimed that "again, the CD14 in colostrums correlated with number of IgM and IgA cells; P=0.05 on both". However, colostrum was investigated in less than 50% of the mothers, and the relative distribution between two groups is not given.
If the data collected at 3 and 6 months of age are considered together, the number of IgA, IgG, and IgM cells were higher in the group receiving LGG than in the group receiving placebo at two out 6 measurement points. However, at all points, the differences were small. At 12 months of age, the number was higher in the group receiving LGG. The authors found a correlation between duration of breastfeeding and number of immunglobulin-secreting cells and claimed that "The results presented here suggest that some human-derived compounds may be mandatory for probiotics to stimulate humoral immune response …." If this statement is accepted, then the effect of probiotics in infants should be properly investigated.
Taken together, the data from 3, 6, and 12 months of age, the number of bifidobacteria and lactobacilli/enterococcus were lower at 5 out of 6 measurement points in the group receiving LGG than in the group receiving placebo. The effect of breastfeeding on the presence of bifidobacteria at 3 and 6 months of age was clearly shown.
Conclusion: the results reinforce current knowledge on the impact of breastfeeding upon composition of the microbiota and development on Ig-secreting cells in infants.

Maija-Liisa Saxelin. Note Dated 31.3.2005**.** Enzyme activities of *Lactobacillus* GG with particular emphasis on beta-glucuronidase
This is not a scientific report or article, but provides useful information regarding the strain.

APIZYM profile
The profile is provided for LGG and *L.rhamnosus* type strain (type strain), and the values are relatively similar.

Valio. (2005). Safety of LGG administration for infants and children in controlled clinical studies.
This is not a scientific report or article, but a list of published articles, in which LGG has been administered to infants and young children in clinical and human intervention studies. It is claimed that none of these studies report the occurrence of any adverse effects.

Valio. (2004). Identification of *Lactobacillus* GG
Valio. (2003). *Lactobacillus* GG- qualification analysis.

These two attachments are not scientific reports or articles, but provide useful information regarding the strain, genetic and biochemical properties, antimicrobial susceptibility, etc.

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