ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial

R. J. Boyle[1,2,3], I. H. Ismail[1,3], S. Kivivuori[1], P. V. Licciardi[1], R. M. Robins-Browne[4], L.-J. Mah[1], C. Axelrad[1,5], S. Moore[1,5], S. Donath[3,6], J. B. Carlin[3,6], S. J. Lahtinen[1,7,8] & M. L. K. Tang[1,3,5]

[1]Allergy and Immune Disorders, Murdoch Children's Research Institute; [2]Department of Paediatrics, Imperial College London, London, UK; [3]Departments of Paediatrics and [4]Microbiology and Immunology, University of Melbourne, Melbourne, Australia; [5]Department of Allergy and Immunology, Royal Children's Hospital, Parkville, Australia; [6]Clinical Epidemiology and Biostatistics Unit, Murdoch Children's Research Institute, Parkville, Australia; [7]Functional Foods Forum, University of Turku, Turku, Finland; [8]currently at Danisco Health and Nutrition, Kantvik, Finland

To cite this article: Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah L-J, Axelrad C, Moore S, Donath S, Carlin JB, Lahtinen SJ, Tang MLK. *Lactobacillus* GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. *Allergy* 2011; **66**: 509–516.

Keywords
breast milk; cord blood; eczema; immune; pregnancy; probiotic.

Correspondence
M. Tang, Department of Allergy and Immunology, Royal Children's Hospital, Flemington Road, Parkville, Vic. 3052, Australia
Tel.: +613 9345 5733
Fax: +613 9345 4848
E-mail: mimi.tang@rch.org.au

Accepted for publication 15 October 2010

DOI:10.1111/j.1398-9995.2010.02507.x

Edited by: Werner Aberer

Abstract

Background: Probiotic supplementation in early life may be effective for preventing eczema. Previous studies have suggested that prenatal administration may be particularly important for beneficial effects.
Objective: We examined whether prenatal treatment with the probiotic *Lactobacillus rhamnosus* GG (LGG) can influence the risk of eczema during infancy.
Methods: We recruited 250 pregnant women carrying infants at high risk of allergic disease to a randomized controlled trial of probiotic supplementation (LGG 1.8×10^{10} cfu/day) from 36 weeks gestation until delivery. Infants were assessed during their first year for eczema or allergic sensitization. Immunological investigations were performed in a subgroup. Umbilical cord blood was examined for dendritic cell and regulatory T cell numbers and production of TGFβ, IL-10, IL-12, IL-13, IFN-γ and TNFα. Maternal breast milk was examined for total IgA, soluble CD14 and TGFβ.
Results: Prenatal probiotic treatment was not associated with reduced risk of eczema (34% probiotic, 39% placebo; RR 0.88; 95% CI 0.63, 1.22) or IgE-associated eczema (18% probiotic, 19% placebo; RR 0.94; 95% CI 0.53, 1.68). Prenatal probiotic treatment was not associated with any change in cord blood immune markers, but was associated with decreased breast milk soluble CD14 and IgA levels.
Conclusions: Prenatal treatment with *Lactobacillus rhamnosus* GG was not sufficient for preventing eczema. If probiotics are effective for preventing eczema, then a postnatal component to treatment or possibly an alternative probiotic strain is necessary.

Allergic diseases are the most common causes of chronic childhood illness in many parts of the world. The prevalence of these diseases increased significantly during the 20th century, and they represent a major burden to human health. Several environmental factors have been associated with protection against developing allergic disease, including altered microbial exposure (1). Exposure to these factors in early life is critical, and prenatal exposure may provide the greatest protection (2, 3). These observations have led to the hypothesis that allergic diseases might be prevented by the administration of microbial supplements such as probiotic bacteria during pregnancy or early life.

Abbreviations
APC, allophycocyanin; CBMC, cord blood mononuclear cells; CI, confidence interval; DC, dendritic cell; EEF1A1, eukaryotic translation elongation factor 1 alpha; ELISA, enzyme-linked immunosorbent assay; FoxP3, forkhead box P3; IgA, total immunoglobulin A; LGG, *Lactobacillus rhamnosus* GG; LPS, lipopolysaccharide; LTA, lipoteichoic acid; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; sCD14, soluble CD14; SCORAD, scoring atopic dermatitis; SPT, skin prick test.

A number of randomized controlled trials have now been completed evaluating the effects of early life probiotic treatment for the prevention of eczema (4–16). Meta-analysis of such studies suggests that probiotic treatment may be effective, although there is significant heterogeneity between study findings (17). Such heterogeneity may be explained by differences in the selection of probiotic strain(s) and differences in the timing of probiotic use, particularly the application of probiotics during the prenatal versus postnatal period (18). Those studies without a prenatal component to treatment have usually failed to demonstrate prevention of eczema (9, 11, 13). This suggests that it may be necessary, and perhaps sufficient, to administer probiotic treatment during pregnancy for effective eczema prevention (2). A prevention approach that only involves treatment in the prenatal period would be more acceptable and feasible as a public health intervention.

We have previously reported that prenatal administration of a probiotic *Lactobacillus rhamnosus* GG (LGG) to mothers in the final weeks of pregnancy resulted in altered development of *Bifidobacterium* populations in the infant intestinal microbiota, with greatest effects observed in infants delivered by Caesarean section (19). In this study we examined the effects of prenatal probiotic supplementation on the risk of eczema development or allergic sensitization during infancy. Various immunological parameters were also examined to investigate potential mechanisms for any clinical effects. We used the probiotic bacterium LGG because this has been reported in some studies to reduce the risk of allergic disease when administered pre and postnatally (6, 16, 20).

Methods

Subject recruitment, treatment and sample collection

We enrolled 250 pregnant women in a randomized controlled trial of prenatal LGG for the prevention of eczema in their infant between January 2006 and February 2008 (Probiotic Eczema Prevention Study registered with Cochrane Skin Group http://www.nottingham.ac.uk/ongoingskin trials Trial No. 36). Participants were recruited at antenatal clinics of the Mercy Hospital for Women in metropolitan Melbourne, Australia and through community advertising in Melbourne. Inclusion criteria were that the participant, their partner or a previous child was affected by a doctor-diagnosed allergic disease including asthma, eczema, food allergy or allergic rhinitis. Multiple pregnancies, those with known fetal abnormality or maternal immune deficiency, and women already taking probiotic supplements were not eligible. Royal Children's Hospital and Mercy Hospital for Women Research Ethics Committees approved this study, and all participants gave written informed consent. Treatment was allocated by a hospital pharmacist at enrolment according to the order in which subjects were recruited, using a computer generated randomization list stratified by number of parents affected by allergic disease ('2' versus '1 or 0'). Participants were allocated to take 1.8×10^{10} cfu LGG (American Type Culture Collection 53103; Dicofarm, Italy) each morning from 36 weeks gestation until delivery, or maltodextrin placebo. Participants, clinical trial and laboratory staff were blinded to treatment allocations throughout the study.

Clinical outcomes

The primary outcome measure was cumulative incidence of eczema during the first year. Secondary outcome measures were allergic sensitization, IgE-associated eczema, eczema severity, gastrointestinal and respiratory symptoms. Infants were examined at 3, 6 and 12 months and completed a questionnaire about allergy and eczema symptoms. Eczema was defined according to the UK Eczema Working Party criteria: that is, a history of itchy skin, scratching or rubbing plus at least three of the following: family history of atopic disease; history of generally dry skin; history of skin rash affecting the flexures, cheeks or outer surfaces of the limbs; onset of rash under the age of 2 years; visible dermatitis at any study visit affecting the flexures, cheeks or outer surfaces of the limbs (21). As it has been argued that the use of 'onset under 2 years' may reduce the specificity of these criteria in infants (22), we also analysed our data excluding 'onset under 2 years' from the criteria. Skin prick testing (SPT) was performed on the back at 12 months (positive control 10% histamine chloride; negative control glycerin-saline) to house dust mite, cat, ryegrass pollen, cow's milk, egg and peanut (Stallergens, Antony, France). Atopy was defined as a SPT wheal diameter ≥3 mm greater than the negative control to any single allergen tested. IgE-associated eczema was defined as the presence of both a positive SPT and eczema at any time during the first 12 months. Eczema severity was assessed using the Scoring Atopic Dermatitis (SCORAD) scale. Colic was defined as crying or fussing without obvious reason for ≥3 h on ≥3 days/week for ≥3 consecutive weeks. A positive asthma predictive index was defined according to the 'loose' index proposed by Castro-Rodriguez (23). Assessments were undertaken by a research nurse (CA), or pediatric allergist (RJB) trained in eczema diagnosis using the UK Eczema Working Party criteria, and in SCORAD assessment using online training packages. Blinded parallel evaluation of the presence of eczema and eczema severity in 24 participants was closely correlated between these two investigators (κ 0.88; estimated 95% of SCORAD scores differing by between −4.0 and +4.9 points).

Immunological and microbiological methods

These are described in detail in the Supporting information.

Statistics

Assuming eczema prevalence of 50% and 20% loss to follow-up, recruitment of 250 participants would provide 80% power to detect 20% absolute difference in eczema risk between treatment groups using a two-group continuity-corrected χ^2 test with two-sided significance of 0.05. Primary analyses were by intention to treat, with subjects without

follow-up data excluded from analysis. For subjects with incomplete follow-up data, last observation carried forward was used for cumulative incidence of eczema. A second analysis using only subjects with complete follow-up data was also performed. The *t*-test was used for normally distributed continuous data, rank sum test for skewed data. Cytokine levels were $\log_{10}$ transformed for analysis. For categorical data we used chi-square test, or Fisher's exact test where expected frequencies were ≤5. Analyses were performed using STATA 11 (Stata Corp. LP, College Station, TX, USA).

Results

Comparison of treatment groups

The characteristics of study participants are shown in Table 1. Known risk factors for allergy or eczema, birth weight and mode of delivery (vaginal versus Caesarean), were similar in each randomization group.

Loss to follow up, treatment compliance, product stability and efficacy of blinding

Figure 1 shows the flow of participants through the study. Two-hundred and twelve of 250 (85%) randomized participants completed 1 year follow up – 2 infants died perinatally (1 in probiotic group; 1 in placebo group); 30 were lost to follow up; 6 withdrew from the study – 2 due to adverse effects of treatment (1 in probiotic group; 1 in placebo group). Returned capsule counts from 195 participants (98 probiotic; 97 placebo) showed that 87% (84% placebo; 91% probiotic) took ≥80% of expected treatment doses. Viability of LGG measured every 6 months was consistent (range 2.0–6.0×10^{10} cfu per dose). Rectal swabs at randomization (study commencement) revealed LGG in 1 of 121 (0.8%) in the probiotic and 6 of 122 (4.9%) in the placebo group. Swabs at birth revealed LGG in 36 of 54 (67%) women in the probiotic group, and 5 of 50 (10%) in the placebo group. At the 3 month visit 58 of 112 (52%) placebo treated participants believed they had received placebo, compared with 60 of 113 (53%) in the probiotic group.

Effects of prenatal LGG on eczema and allergic sensitization during infancy

The prevalence of eczema and allergic sensitization during the first 12 months of life is shown in Table 2. There was no evidence of a difference in eczema prevalence between infants in probiotic and placebo groups – risk difference −4.7% (95% CI −16.9%, 7.4%). This result did not change when we applied different criteria for eczema diagnosis (22) (data not shown). There was no evidence of a difference in prevalence of IgE-associated eczema or allergic sensitization, nor in eczema severity, assessed as maximum SCORAD score recorded at any study visit in the first 12 months of life.

Effects of prenatal LGG on eczema and atopy in infants born by Caesarean section

Previous findings from our group and others have suggested that probiotics may have specific microbiological (and possibly clinical) effects in Caesarean born infants (8, 19). We therefore undertook a subgroup analysis of Caesarean born infants. The statistical power of this analysis was limited, but there was little evidence of any beneficial effect of prenatal LGG treatment in Caesarean delivered infants −12/33 (36%) eczema in probiotic arm, 14/32 (44%) in placebo arm; risk difference −7.4% (−31%–16%).

Effects of prenatal LGG on gastrointestinal and respiratory outcomes

The prevalence of gastrointestinal and respiratory outcomes during the first 12 months of life is shown in Table 3, together with the prevalence of adverse events in pregnant women during the intervention period. There was no evidence

Table 1 Characteristics of study participants

	Probiotic (*n* = 125)	Placebo (*n* = 125)
No. prior pregnancies, median (range)	1 (0–5)	1 (0–6)
Maternal age (years), median (range)	34 (24–45)	34 (19–45)
Paternal eczema	23.2%	22.8%
Maternal eczema	57.6%	55.2%
Sibling eczema	75.5%	69.4%
Antibiotics during pregnancy	25.6%	26.4%
Daily yoghurt intake during pregnancy (g/week), median (range)	300 (0–1400)	400 (0–1400)
Maternal tertiary education	72.8%	73.6%
Household smoker	20.8%	21.6%
Other children present in household	68.9%	69.2%
Infant sex – female	45.1%	42.1%
Gestation (weeks), median (range)	39.6 (35.4–42.0)	39.5 (36.0–42.3)
Birthweight (g), median (range)	3560 (2324–4970)	3615 (2105–5020)
Caesarean delivery	27.6%	26.4%
Duration of breastfeeding in first year (months), median (range)	12.0 (0–12.0)	12.0 (0–12.0)

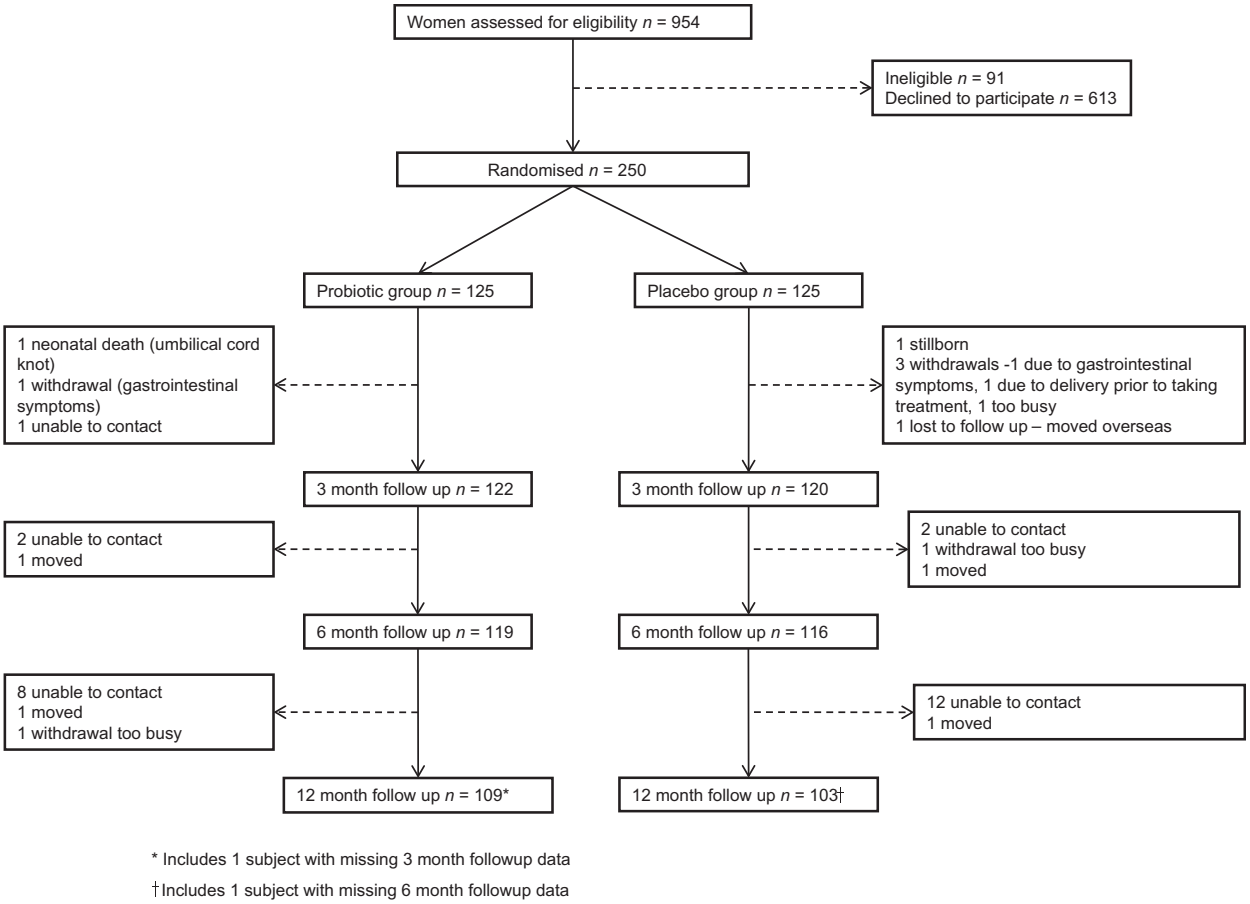

Figure 1 Flow of participants in the clinical trial.

Table 2 Eczema and allergic sensitization in the probiotic and placebo groups

Outcome measure	Probiotic	Placebo	*P*-value	Relative risk	Risk difference (%)
Eczema ever	42/122 (34%)	47/120 (39%)	0.44	0.88 (0.63, 1.22)	−4.7 (−16.9, 7.4)
Eczema ever (complete data)*	35/108 (32%)	43/102 (42%)	0.14	0.77 (0.54, 1.10)	−9.7 (−22.8, 3.2)
Atopic eczema	19/107 (18%)	19/101 (19%)	0.84	0.94 (0.53, 1.68)	−1.1 (−11.6, 9.5)
Any positive SPT	35/107 (33%)	33/101 (33%)	1.00	1.00 (0.68, 1.48)	0.0 (−12.7, 12.8)
Positive food SPT	31/107 (29%)	29/101 (29%)	0.97	1.01 (0.66, 1.53)	0.3 (−12.1, 12.6)
Positive aeroallergen SPT	11/107 (11%)	11/101 (11%)	0.89	0.94 (0.43, 2.08)	−0.6 (−9.0, 7.8)
Positive egg SPT	27/107 (25%)	22/101 (22%)	0.56	1.16 (0.71, 1.90)	3.5 (−8.1, 15.0)
Positive peanut SPT	7/107 (7%)	6/101 (6%)	0.86	1.10 (0.38, 3.17)	0.6 (−6.0, 7.2)
Positive cow's milk SPT	7/107 (7%)	7/101 (7%)	0.91	0.94 (0.34, 2.60)	−0.4 (−7.2, 6.4)
Maximum SCORAD 0	58/122 (48%)	58/120 (48%)	0.39		
Maximum SCORAD 1–25	50/122 (41%)	55/120 (46%)			
Maximum SCORAD 25–50	13/122 (11%)	7/120 (6%)			
Maximum SCORAD >50	1/122 (1%)	0/120 (0%)			

*Participants with missing data for ≥1 outcome assessment were excluded for this analysis. For all other analyses the 'last observation carried forward' method was used to impute missing data.

of any meaningful differences in any of the outcomes. Adverse events were reported by 50 (20%) study participants, mainly gastrointestinal symptoms during the prenatal intervention period (45 participants), with no difference between treatment groups in the adverse event rate (probiotic group 20/125 participants, placebo group 30/124; $P = 0.11$).

Table 3 Other health outcomes and adverse events in the probiotic and placebo groups

Outcome measure	Probiotic	Placebo	*P*-value	Relative risk	Difference
History of wheeze	27/122 (22%)	29/120 (24%)	0.71	0.92 (0.58, 1.45)	−2.0 (−12.7, 8.6)
Positive asthma predictive index	21/122 (17%)	19/120 (16%)	0.77	1.09 (0.62, 1.92)	1.4 (−8.0, 10.7)
Vomiting in first 3 months	51/121 (42%)	40/119 (34%)	0.17	1.25 (0.90, 1.74)	8.5 (−3.7, 20.8)
Colic at 3 months	16/120 (13%)	14/119 (12%)	0.71	1.13 (0.58, 2.22)	1.6 (−6.8, 10.0)
Gastroenteritis in first year	44/122 (36%)	43/120 (36%)	0.97	1.01 (0.72, 1.41)	0.2 (−11.0, 12.6)
Hospital admission in first year	9/122 (7%)	13/120 (11%)	0.35	0.68 (0.30, 1.53)	−3.5 (−10.2, 3.8)
Infant fecal frequency at 3 months*	1.9 (1.7)	2.1 (1.7)	0.29		0.2 (−0.2, 0.6)
Infant fecal consistency at 3 months†	6.1 (0.7)	6.1 (0.8)	0.72		0.0 (−0.2, 0.2)
Maternal adverse effects reported during treatment‡	20/125 (16%)	30/124 (24%)	0.11	0.66 (0.40, 1.10)	−8.2 (−18.1, 1.7)

Note: Last observation carried forward was used to impute missing outcome data for all categorical analyses.

*Fecal frequency is mean (SD) number of stools passed by infant in 24 h at 3 months postnatal age.

†Fecal consistency is mean (SD) parent-reported fecal consistency at 3 months using the Bristol Stool Form Scale.

‡Adverse events were increased frequency/looser consistency of maternal feces (n = 20 placebo, 14 probiotic); nausea/vomiting (n = 6 placebo, 4 probiotic), constipation (n = 1 placebo), increased blood glucose (n = 1 probiotic), migraine/dizziness (n = 2 placebo), eczema flare (n = 1 probiotic) and reduced fetal movements (n = 1 placebo).

Effects of prenatal LGG treatment on CBMC cytokine secretion, Tregs and DCs

CBMC samples from 77 participants were successfully obtained and cryopreserved within 12 h – of these 73 had cell counts $\geq 10 \times 10^6$ and were used for immune studies. Mean cell viability after thawing determined by trypan blue exclusion for all samples was 83.1% (SD 6.4). Table S1 shows the results of Treg and DC analyses in fresh thawed CBMC, and CBMC cultured for 48 h with LTA or LPS (n = 63; 32 placebo, 31 probiotic), with no evidence of a difference between groups in these immune parameters. CBMC culture supernatants obtained after 48 h culture with LTA, LPS or medium alone were evaluated for cytokines IL-10, IL-12p40, IL-13, IFN-γ, TNFα and TGF-β1. There was no evidence of a difference between groups in the levels of IL-10, IL-13, IFN-γ or TNFα after 48 h culture with LTA, LPS or medium alone (Fig. 2). Insufficient samples had detectable TGF-β1 or IL-12 levels to be included in these analyses. Production of IL-12 by CBMC after 24 h culture with IFN-γ and LPS was also determined, and no difference between treatment groups was found (mean $\log_{10}$ IL-12 level probiotic 1.87, placebo 1.87; P = 0.96).

Effects of prenatal LGG treatment on breast milk immune composition

Paired breast milk samples from day 7 and day 28 were analysed from 73 participants (38 placebo; 35 probiotic). Breast milk samples from participants in the probiotic group had lower levels of total IgA at day 28 and lower sCD14 at day 7 than the placebo group (Table S2). There was no difference in breast milk TGF-β1 levels at either time point between the treatment groups.

Discussion

The data from completed randomized controlled trials suggest that in infants at high risk of allergic disease, prenatal and/or postnatal administration of probiotic bacteria may be effective for eczema prevention (17). However, the heterogeneous nature of trial outcomes and the interventions used makes it difficult to translate this finding into a meaningful public health intervention. Two major sources of variation between studies are the selection of probiotic strain(s) and the timing of intervention. Five of the six trials that have shown a statistically significant reduction in eczema used a combined prenatal/postnatal intervention, and three of these used LGG either alone or in combination with other probiotics/prebiotic (6, 10, 15, 16, 20). Observational studies suggest the prenatal period is a time when microbial exposures can act to prevent development of the allergic phenotype (3). We conducted a randomized controlled trial to investigate whether prenatal LGG treatment could reduce eczema risk in infancy.

We found no evidence that prenatal treatment with LGG is effective for reducing eczema risk. This suggests that extension of treatment into the postnatal period is important for any beneficial effects and/or that a different probiotic strain may be needed. We also found that prenatal administration of LGG led to reduced sCD14 and total IgA levels in breast milk, without any change in TGFβ. These unexpected effects of prenatal LGG intake on postnatal breast milk regulatory factors are likely to be mediated indirectly, possibly through immune and/or microbiota changes. sCD14 is present at high levels in human breast milk, and may have an important role in permitting LPS-induced activation of intestinal epithelial cells in the neonatal intestine, which in turn is important for intestinal homeostasis (24). Reduced levels of sCD14 in breast milk and amniotic fluid have been associated with increased risk of eczema (25). IgA and TGFβ are important regulators of mucosal immunity with protective effects against allergic disease (26). The lack of clinical effect observed in our study may therefore relate to the unexpected effects of prenatal LGG on breast milk immune composition.

In contrast to our findings, the first study evaluating combined prenatal/postnatal LGG treatment reported a significant reduction in eczema prevalence (6) that was associated with increased breast milk TGFβ-2 (27). These differences in LGG effects on breast milk composition may relate to diffe-

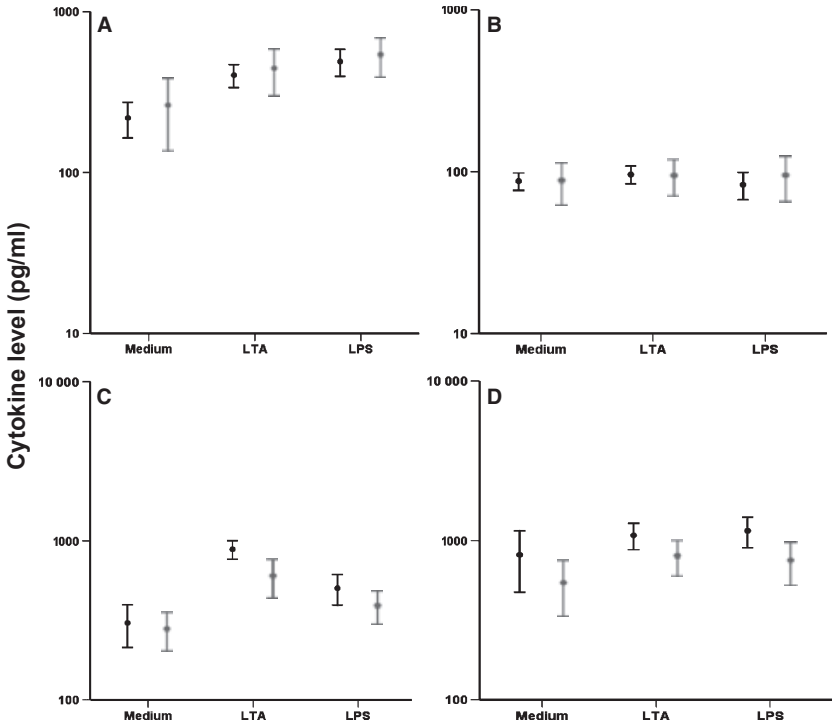

Figure 2 Secretion of IL-10 (A), IL-13 (B), TNF-α (C) and IFN-γ (D) in CBMC from infants whose pregnant mothers received probiotic ($n = 31$; grey circle/bar) or placebo ($n = 32$; black circle/bar). Means ± 1 SEM are shown. Culture supernatants were harvested after 48 h culture with LTA, LPS or medium alone. There was no significant difference between treatment groups for these outcomes.

rences in the timing of LGG administration – postnatal treatment of breastfeeding mothers may be important for beneficial effects on breast milk that contribute to protective clinical effects. However prenatal/postnatal LGG was found in a separate study to have no clinical effect on eczema despite treatment of breastfeeding mothers for 3 months (7). Nevertheless, the observation in two studies that administration of LGG alone or with other probiotics prenatally and then to breastfeeding mothers for the first 3 months reduced eczema risk (16, 27), suggests that postnatal administration of probiotic to the breastfeeding mother may be important for eczema prevention.

The immune effects necessary for eczema prevention in probiotic studies are not clear. Reduced cord blood IFN-γ is associated with increased risk for eczema in early life, suggesting prenatal interventions which modulate the developing fetal immune system may be effective for eczema prevention (28). Placento-fetal interactions may be an important mechanism by which prenatal probiotics mediate protective effects – for example in mice, prenatal LGG treatment led to altered placental cytokine expression and reduced allergic disease in offspring (29). A human study found that prenatal/postnatal administration of *Lactobacillus rhamnosus* HN001 reduced risk of eczema at 2 years and increased cord blood IFN-γ level (10, 30). These findings support the notion that the protective effects of prenatal/postnatal probiotic administration may in part be mediated through modulation of developing fetal immune responses, the effects of which can be demonstrated in cord blood at the time of birth. In our trial we previously reported that prenatal LGG failed to modulate newborn T helper or regulatory compartment responses to allergen (31) and now report a similar lack of effect on newborn Treg and T helper responses to Toll-like receptor agonists. Thus, the failure of prenatal LGG to prevent infant eczema may be explained in part by a lack of effect on fetal/infant immune responses as measured in cord blood. The use of a different probiotic strain may have modulated immune responses that are important for reducing risk of eczema. Indeed, other probiotic strains but not prenatal LGG have demonstrated immunological effects on breast milk and cord blood that might be expected to protect against eczema development (30). So it is possible that the strain of probiotic we used may be ineffective where others might have been effective when used prenatally.

We previously found prenatal LGG induced potentially beneficial effects on infant *Bifidobacterium* populations in the subjects within this trial (19). Infant eczema is associated with altered intestinal microbiota composition, particularly in *Bifidobacterium* populations, so it might have been expected that modulation of infant microbiota towards a more healthy profile similar to that observed in breast fed infants would lead to reduced eczema risk. However, our new findings suggest the microbiological effects we observed were not sufficient to influence infant immune development. Alternatively, it is possible that the association between altered intestinal microbiota and infant eczema reported in other studies is not causal.

A possible explanation for the differences in clinical outcomes between probiotic studies is differences in the genetic background, diet and intestinal microbiota of the study populations. For example the microbiota composition and its relationship with allergy differs between infants in New Zealand, Japan and Scandinavia (32, 33). The quality of probiotics used in clinical trials is important for *in vivo* activity, and the quality and viability of our study intervention were carefully monitored throughout our trial and treatment compliance was high. It is unlikely that these variables accounted for the lack of positive findings in our study. Our sample size was designed to detect a 20% absolute reduction in eczema prevalence, however there was no trend towards a protective effect with probiotic intervention, making a type II error unlikely.

Probiotic treatment during infancy has been associated with other beneficial health effects, including reduced rates of infection and gastrointestinal symptoms (34). We found no evidence that probiotic treatment during the prenatal period has any effect on infant health, including gastrointestinal symptoms. We also found no evidence of a beneficial effect on maternal intestinal symptoms during the prenatal period. LGG has been associated with adverse effects such as bacteraemia in rare cases, particularly in high risk individuals (35). There were no episodes of bacteraemia in this study during the intervention period, and probiotic treatment was not associated with increased risk of any adverse effect.

In summary we found that for infants at high risk of developing allergic disease, treatment of their pregnant mothers with LGG from 36 weeks gestation to delivery did not reduce eczema risk. Prenatal LGG did not have any effects on newborn immune responses, and led to reduced breast milk sCD14 and IgA levels. Our findings suggest a longer treatment duration that includes a postnatal component, or possibly the use of a different probiotic strain, is required for protective effects.

Sources of support

The studies were funded by grants from the Jack Brockhoff Foundation, the Murdoch Children's Research Institute, the Australian National Health and Medical Research Council and the Ilhan Food Allergy Foundation. Robert Boyle was supported by a University of Melbourne Baillieu Scholarship, a Murdoch Children's Research Institute Postgraduate Scholarship and a National Institute for Health Research Comprehensive Biomedical Research Centre. Intan Hakimah Ismail was supported by a scholarship from the Ministry of Higher Education, Malaysia and Universiti Putra Malaysia. Sampo Lahtinen was supported by Applied Biosciences Graduate School and Elintarvikkeiden Tutkimussaatio, Finland. LGG and placebo capsules were manufactured and supplied by Dicofarm ltd (Roma, Italy).

Author contributions

RJB established the clinical trial, undertook cord blood immunology assays and wrote the manuscript with contributions from all authors. IHI assisted with data analysis and contributed to the manuscript. SK set up the real time RT-PCR assays and contributed to the manuscript. PVL ran the breast milk assays and contributed to the manuscript. RMR-B supervised microbiology analyses, contributed to study design and to the manuscript. LJM ran the real time RT-PCR and Luminex assays and contributed to the manuscript. CA and SM recruited patients to the clinical trial and contributed to the manuscript. SD and JBC contributed to study design and analyses, and to the manuscript. SJL undertook microbiology analyses. MLT designed and oversaw the running of the study, and contributed to the manuscript.

Conflicts of interest

No author has stated that they have a conflict of interest with respect to this study.

Acknowledgements

We are grateful to the participating families and the midwifery staff of the Mercy Hospital for Women for their cooperation, to Michael Permezel, Kay Hynes, Christine Plover, Maggie Flood, Val Bryant and Noel Cranswick for their assistance in the conduct of this trial, to Frances Oppedisano for her assistance with immunological and microbiological studies, to John Su for advice regarding eczema assessment, and to Seppo Salminen for his careful review of the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article at www.wileyonlinelibrary.com:

Data S1. Immunological and microbiological methods
Table S1. Immune markers in uncultured CBMC and CBMC cultured for 48h with LTA or LPS
Table S2. Breast milk immune composition

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; **107**:129–134.
2. Boyle RJ, Tang ML. Can allergic diseases be prevented prenatally? *Allergy* 2006;**61**: 1423–1431.
3. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensiti-

zation in school-age children. *J Allergy Clin Immunol* 2006;**117**:817–823.
4. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B et al. Probiotics in prevention of IgE-associated eczema: A double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007;**119**:1174–1180.
5. Huurre A, Laitinen K, Rautava S, Korkeamaki M, Isolauri E. Impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization: a double-blind placebo-controlled study. *Clin Exp Allergy* 2008;**38**:1342–1348.
6. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;**357**:1076–1079.
7. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics* 2008;**121**:e850–856.
8. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;**123**:335–341.
9. Taylor AL, Dunstan JA, Prescott SL. 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* 2007;**119**:184–191.
10. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2008;**122**:788–794.
11. Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. *Pediatr Res* 2006;**60**:221–224.
12. Niers L, Martin R, Rijkers G, Sengers F, Timmerman H, van Uden N et al. The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy* 2009;**64**:1349–1358.
13. Soh SE, Aw M, Gerez I, Chong YS, Rauff M, Ng YP et al. Probiotic supplementation in the first 6 months of life in at risk Asian infants–effects on eczema and atopic sensitization at the age of 1 year. *Clin Exp Allergy* 2009;**39**:571–578.
14. West CE, Hammarstrom ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol* 2009;**20**:430–437.
15. Kim JY, Kwon JH, Ahn SH, Lee SI, Han YS, Choi YO et al. Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial. *Pediatr Allergy Immunol* 2010;**21**:e386–93.
16. Dotterud C, Oien T, Storro O, Johnsen R. Probiotic supplementation given to mothers in primary prevention of allergic diseases in early childhood – a randomised, double blind, placebo controlled trial in a nonselected population. *Allergy* 2009;**64**(S90):1–98.
17. Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev* 2007;**4**:CD006475.
18. Prescott SL, Bjorksten B. Probiotics for the prevention or treatment of allergic diseases. *J Allergy Clin Immunol* 2007;**120**:255–262.
19. Lahtinen SJ, Boyle RJ, Kivivuori S, Oppedisano F, Smith KR, Robins-Browne R et al. Prenatal probiotic administration can influence Bifidobacterium microbiota development in infants at high risk of allergy. *J Allergy Clin Immunol* 2009;**123**:499–501.
20. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007;**119**:192–198.
21. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994;**131**:406–416.
22. Fleming S, Bodner C, Devereux G, Russell G, Campbell D, Godden D et al. An application of the United Kingdom Working Party diagnostic criteria for atopic dermatitis in Scottish infants. *J Invest Dermatol* 2001;**117**:1526–1530.
23. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;**343**:538–543.
24. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004;**118**:229–241.
25. Jones CA, Holloway JA, Popplewell EJ, Diaper ND, Holloway JW, Vance GH et al. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J Allergy Clin Immunol* 2002;**109**:858–866.
26. Oddy WH, Rosales F. A systematic review of the importance of milk TGF-beta on immunological outcomes in the infant and young child. *Pediatr Allergy Immunol* 2010;**21**:47–59.
27. Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002;**109**:119–121.
28. Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R et al. Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *J Allergy Clin Immunol* 2003;**112**:740–746.
29. Blumer N, Sel S, Virna S, Patrascan CC, Zimmermann S, Herz U et al. Perinatal maternal application of Lactobacillus rhamnosus GG suppresses allergic airway inflammation in mouse offspring. *Clin Exp Allergy* 2007;**37**:348–357.
30. Prescott SL, Wickens K, Westcott L, Jung W, Currie H, Black PN et al. Supplementation with Lactobacillus rhamnosus or Bifidobacterium lactis probiotics in pregnancy increases cord blood interferon-gamma and breast milk transforming growth factor-beta and immunoglobin A detection. *Clin Exp Allergy* 2008;**38**:1606–1614.
31. Boyle RJ, Mah LJ, Chen A, Kivivuori S, Robins-Browne RM, Tang ML. Effects of Lactobacillus GG treatment during pregnancy on the development of fetal antigen-specific immune responses. *Clin Exp Allergy* 2008;**38**:1882–1890.
32. Gore C, Munro K, Lay C, Bibiloni R, Morris J, Woodcock A et al. Bifidobacterium pseudocatenulatum is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. *J Allergy Clin Immunol* 2008;**121**:135–140.
33. Suzuki S, Shimojo N, Tajiri Y, Kumemura M, Kohno Y. Differences in the composition of intestinal Bifidobacterium species and the development of allergic diseases in infants in rural Japan. *Clin Exp Allergy* 2007;**37**:506–511.
34. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics* 2005;**115**:5–9.
35. Boyle RJ, Robins-Browne RM, Tang ML. Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006;**83**:1256–1264.