Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant

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Background: Probiotics have shown promising potential in reducing the risk of eczema in infants. Optimal probiotic intervention regimen remains to be determined.
Objective: We investigated whether maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of developing eczema in high-risk infants.
Methods: This was a parallel, double-blind placebo-controlled trial of 241 mother-infant pairs. Mothers with allergic disease and atopic sensitization were randomly assigned to receive (1) *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 (LPR+BL999), (2) *L paracasei* ST11 and *B longum* BL999 (ST11+BL999), or (3) placebo, beginning 2 months before delivery and during the first 2 months of breast-feeding. The infants were followed until the age of 24 months. Skin prick tests were performed at the ages of 6, 12, and 24 months.
Results: Altogether 205 infants completed the follow-up and were included in the analyses. The risk of developing eczema during the first 24 months of life was significantly reduced in infants of mothers receiving LPR+BL999 (odds ratio [OR], 0.17; 95% CI, 0.08-0.35; $P < .001$) and ST11+BL999 (OR, 0.16; 95% CI, 0.08-0.35; $P < .001$). The respective ORs for chronically persistent eczema were 0.30 (95% CI, 0.12-0.80; $P = .016$) and 0.17 (95% CI, 0.05-0.56; $P = .003$). Probiotics had no effect on the risk of atopic sensitization in the infants. No adverse effects were related to the use of probiotics.
Conclusion: Prevention regimen with specific probiotics administered to the pregnant and breast-feeding mother, that is, prenatally and postnatally, is safe and effective in reducing the risk of eczema in infants with allergic mothers positive for skin prick test. (J Allergy Clin Immunol 2012;130:1355-60.)

Key words: *Eczema, probiotics, breast-feeding, infant*

Abbreviations used
BL999: *Bifidobacterium longum* BL999
LPR: *Lactobacillus rhamnosus* LPR
OR: Odds ratio
ST11: *Lactobacillus paracasei* ST11

Several clinical trials associate specific probiotic supplementation in early life with decreased risk of developing eczema.[1-7] Nonetheless, it is currently not possible to devise recommendations for routine clinical use of probiotics for this purpose because the studies published thus far are heterogeneous for inclusion criteria for study subjects, the probiotic strains used, as well as the probiotic preparation methods, matrix, and delivery vehicles. Data about breast-feeding and hereditary risk of developing atopic disease are variably reported. Perhaps most importantly, the timing and duration of probiotic supplementation, which is likely to have a significant effect on efficacy as well as feasibility of the intervention, varies between the studies. Thus, the optimal probiotic strains, mode of administration, and objectively defined target population remain to be determined.

On the basis of available evidence, it appears that probiotic intervention is most effective in reducing the risk of eczema in the infant if started during pregnancy. All 7 published trials[1-7] that showed efficacy in reducing disease risk include both prenatal maternal and postnatal probiotic supplementation. Only one study with both prenatal and postnatal interventions shows lack of effect,[8] whereas both of the 2 published trials in which probiotic supplementation is given only postnatally and directly to the infants have negative results.[9,10] According to a recent study, maternal prenatal probiotic supplementation alone may not be sufficient to achieve the desired clinical effects.[11] We have previously provided data to suggest that *Lactobacillus rhamnosus* GG administered to the pregnant and breast-feeding mother significantly reduces the risk of developing eczema in high-risk infants.[12] The purpose of the present study was to investigate whether exclusively maternal probiotic intervention without direct probiotic supplementation to the infant during the last 2 months of pregnancy and the first 2 months of breast-feeding is effective in reducing the risk of developing eczema in high-risk infants identified objectively as those with mothers with allergic disease and atopic sensitization.

METHODS

A double-blind, randomized, placebo-controlled trial was devised to assess the effects of maternal probiotic administration on the risk of eczema in the infant. Pregnant women with atopic sensitization and either a history of or active allergic disease and the intention to breast feed for a minimum of 2 months were considered eligible for the study. The assessment of maternal allergic disease was based on reported clinical history of atopic eczema, allergic rhinoconjunctivitis, food allergy, or asthma. Sensitization was verified

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by skin prick testing. Women with immune-mediated disease other than atopic or allergic disease were excluded from the study. Infants born of multiple pregnancies were excluded from the analyses to ensure independence of the study subjects.

The study was conducted in a single tertiary center in Turku, Finland. Recruitment took place between August 2005 and April 2009. Information about the study was distributed to pregnant women at prenatal care centers in the Turku region in Southwest Finland. All families who were interested in participating and contacted the research nurse during the recruitment period were assessed for eligibility. Altogether 241 pregnant women were randomly assigned in a parallel design to receive a dietary food supplement that contained minerals, including calcium, vitamins, including vitamins B12, A, and D, folic acid and other micronutrients, including iron, zinc, and iodine, with composition and dosage in compliance with recommended daily allowances supplemented with either the combination LPR and BL999 (LPR+BL999) consisting of *Lactobacillus rhamnosus* LPR (CGMCC 1.3724) and *Bifidobacterium longum* BL999 (ATCC: BAA-999) or the combination ST11 and BL999 (ST11+BL999) consisting of *L paracasei* ST11 (CNCM 1-2116) and *B longum* BL999. Daily dose for each probiotic was 1×10^9 cfu provided in 1 sachet of 7 g/d (powder form) which was diluted in a glass of water. The same dietary supplement without probiotics served as placebo. The study preparations were provided by Nestlé S.A. and were similar in appearance. Viable probiotics or placebo was prepared within the powder by the manufacturer. Control of preparation quality was conducted by the supplier, and fresh new sachets were provided at frequent intervals to ensure viability. Maternal probiotic supplementation was started 2 months before the expected day of delivery and continued during breast-feeding until the child was 2 months of age. Information about products that contained probiotics available in the market during the study period was given when the intervention was started and subsequently at 5 scheduled study clinic visits 1, 3, 6, 12, and 24 months after delivery. Use of such products was discouraged. Compliance with the intervention was controlled by interview during the scheduled visits.

The number of subjects enrolled in the study is based on an assumed 50% prevalence of the primary outcome measure eczema up to the age of 2 years and a clinically relevant reduction of 25% by intervention. Assuming a type I error of 2.5% and a power of 80%, the required number of subjects to be analyzed per group should be 64 and in total 192 (Fisher exact test, 1-sided). The random allocation was computer-generated independently from the investigators by the manufacturer of the study products. All investigations were performed double-blind, and the code was opened after all the infants had completed the follow-up, outcomes had been assessed, and the data had been finalized. The study was deemed ethically acceptable by the ethics committee of the Intermunicipal Hospital District of Southwest Finland and registered (NCT00167700). Oral and written informed consent was obtained from the mothers.

Outcome measures

The infants were followed until the age of 24 months. Clinical examination of the infants was performed at scheduled visits at the ages of 1, 3, 6, 12, and 24 months. The primary outcome measure of the trial was cumulative incidence of eczema in the infant up to the age of 2 years. Eczema in early life is one of the strongest risk factors for subsequent development of atopic diseases such as allergic rhinoconjunctivitis or asthma in later life.[13] Cumulative incidence of eczema was considered to be the most relevant and reliable outcome measure because the chronic and relapsing course of the affliction may render point prevalence unreliable.[14] Eczema was diagnosed according to the criteria introduced by Hanifin,[15] based on the following features: pruritus, typical morphology and distribution, and a chronic relapsing course. The last criterion was fulfilled if the infant had 2 episodes of eczema with duration of at least 1 month each during the first 2 years of life. If the skin condition persisted without periods of remission, the eczema was considered chronically persistent. All adverse effects possibly related to the study products were systematically monitored and recorded.

To objectively assess atopic sensitization in the infants, skin prick tests were performed at the ages of 6, 12, and 24 months as described previously.[16] Skin prick testing was used because of its high sensitivity and equal or superior accuracy compared with serum allergen-specific IgE antibody concentrations in predicting allergic reactions.[16-18] The antigens tested included cow's milk, egg white, and wheat and rice flour both diluted 1/10 (w/v) with 0.9% (w/v) sodium chloride, gliadin diluted 1 mg/mL with an ethanol/glyceroleum/ALK-diluent (Allergologisk Laboratorium A/S, Hørsholm, Denmark) mixture, cod, soy bean, birch, 6 grasses, cat, dog, *Dermatophagoides pteronyssimus* allergen Der p1 (Allergologisk Laboratorium A/S), latex (Starallergens S.A., Anthony Cedex, France), potato, carrot, and banana by prick-prick technique. The chosen panel of allergens represents the most common sources of allergic reactions in the target population. The testing was performed on the volar side of the forearm with a 1-mm, 1-peak lancet (Allergologisk Laboratorium A/S) with a shoulder to prevent deeper penetration. Histamine dihydrochloride (10 mg/mL; Allergologisk Laboratorium A/S) was used as the positive control, and the negative control solution was provided by the same manufacturer. Reactions were read at 10 to 15 minutes. Reactions with a mean diameter of the wheal of at least 3 mm were considered positive on the condition that the mean diameter of the wheal to the positive control was at least 3 mm and the negative control reaction was 0 mm.

Statistical analyses

Continuous background data are expressed as means with range and categorical data as percentages. Differences between the groups were assessed with ANOVA for continuous variables and χ^2 test for categorical variables. To assess the effects of the interventions on outcome, comparisons between groups were conducted with logistic regression analysis and expressed as odds ratios (ORs) with 95 % CI. A P value <.05 was considered statistically significant. All analyses were conducted with intention to treat.

RESULTS

Altogether 205/241 (85%) mother/infant pairs completed the follow-up (Fig 1). The rate of discontinuing the study or lost to follow-up was similar in the 3 study groups. The background characteristics of the infants in the study groups are presented in Table I. Altogether 10 mothers in the study (2 mothers receiving placebo, 3 mothers receiving LPR+BL999, and 5 mothers receiving ST11+BL999) ceased to breast feed before the infant was 2 months of age. The infants of these mothers were included in the analyses according to the intention-to-treat principle.

Eczema was detected during the first 24 months of life in 85/205 (41%) infants who completed the follow-up, and chronically persistent eczema was diagnosed in 27/205 (13%) infants. Maternal consumption of either LPR+BL999 or ST11+BL999 were both associated with a statistically significant reduction in the risk of developing eczema and chronically persistent eczema during the first 24 months of life compared with infants whose mothers received placebo (Table II). Atopic sensitization was detected in 53/214 (25%) of the infants by skin prick testing during the follow-up. The rate of skin prick test positivity was comparable between infants born to mothers receiving LPR+BL999 or ST11+BL999 or placebo (Table II). Atopic sensitization was significantly associated with the risk of developing eczema or chronically persistent eczema, because 54/155 (35%) of skin prick test–negative infants who completed the follow-up developed eczema compared with 31/50 (62%) skin prick test–positive infants ($P = .001$). The respective rates for chronically persistent eczema were 12/155 (8%) and 15/50 (30%) ($P < .001$). However, no statistically significant interactions between status of skin prick testing and the probiotic interventions for the risk of eczema ($P = .328$) or chronically persistent eczema ($P = .283$) were detected with logistic regression analysis.

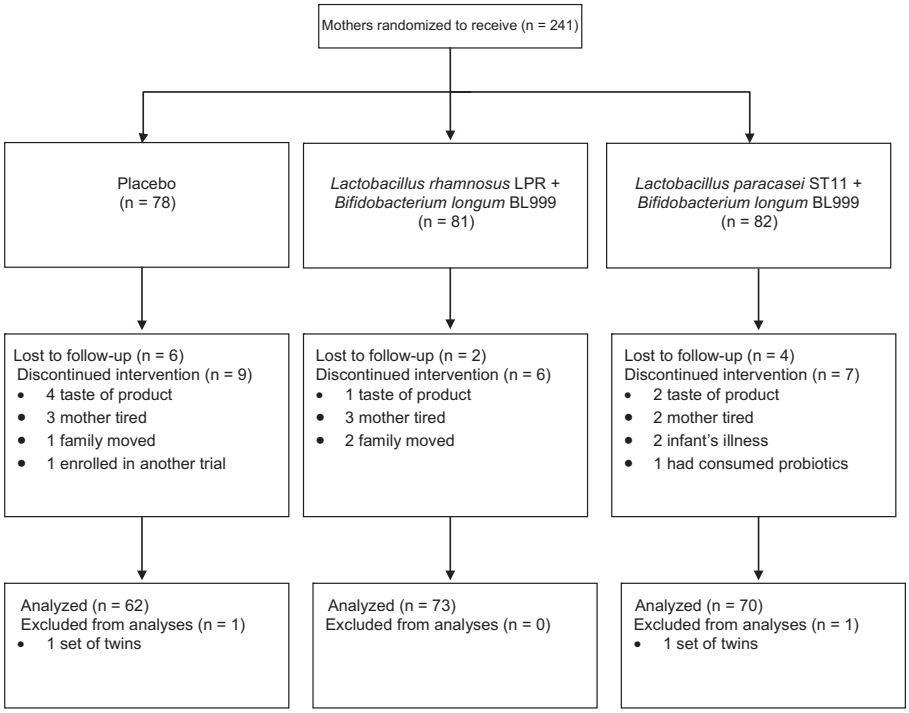

FIG 1. Flow diagram of the trial.

TABLE I. Background characteristics of mother-infant pairs receiving placebo, the combination of *Lactobacillus rhamnosus* PR and *Bifidobacterium longum* BL999 (LPR+BL999), or the combination of *Lactobacillus paracasei* ST11 and *B longum* BL999 (ST11+BL999)

	Placebo	LPR+BL999	ST11+BL999	*P* value
Maternal age (y), mean (range)	30 (22-42)	31 (22-43)	30 (22-40)	.97
Gestational age (wk), mean (range)	39.4 (34.5-41.0)	39.8 (34.6-41.0)	39.8 (33.5-41.5)	.25
Birth weight (g), mean (range)	3561 (2380-4670)	3558 (2580-4800)	3582 (1910-4660)	.94
Cesarean section, ratio (%)	14/73 (19)	4/80 (5)	9/78 (12)	.024
Boys, ratio (%)	40/74 (54)	39/80 (49)	37/79 (47)	.69
Older siblings, ratio (%)	27/75 (36)	31/81 (38)	34/81 (47)	.74
Pets, ratio (%)	25/75 (33)	27/81 (33)	30/81 (37)	.85
Exclusive breast-feeding (mo), mean (range)	3.3 (0.0-6.0)	3.2 (0.0-7.0)	3.1 (0.0-11.0)	.93
Total breast-feeding (mo), mean (range)	9.5 (1.5-24.0)	9.1 (1.0-24.0)	10.0 (0.0-24.0)	.59

Groups were compared with ANOVA for continuous variables and χ^2 test for categorical variables.

Because the rate of cesarean section delivery was different between the study groups (Table I), further analyses were conducted. The difference in cesarean section rate between mothers receiving LPR+BL999 (5%) and placebo (19%) was statistically significant ($P = .011$), but that between mothers receiving ST11+BL999 (12%) and placebo was not ($P = .20$). Of note, mode of delivery did not interact with the effect of the interventions on the occurrence of eczema ($P = .94$) or chronically persistent eczema ($P = .46$) as assessed with logistic regression analysis.

No adverse effects related to probiotic supplementation were detected in the study subjects. Gastrointestinal symptoms were reported in 4/70 (6%) mothers receiving placebo, in 6/80 (8%) mothers receiving LPR+BL999, and in 3/74 (4%) mothers receiving ST11+BL999 ($P = .66$). One mother receiving placebo and 1 mother receiving LPR+BL999 experienced aggravation of eczema during supplementation. Gastrointestinal symptoms were observed in 87/223 (39%) of the infants during the first 2 months of life when the study products were administered to the breast-feeding mothers. The rates of gastrointestinal symptoms among infants whose mothers received placebo, LPR+BL999, or ST11+BL999 were 24/70 (34%), 35/79 (44%), and 28/74 (38%), respectively ($P = .44$).

DISCUSSION

Maternal probiotic supplementation during the last 2 months of pregnancy and the first 2 months of breast-feeding significantly reduced the risk of developing eczema in high-risk infants in this study. Note that the probiotics were administered exclusively to the mothers, whereas the infants did not receive probiotics. The results of this randomized, controlled trial corroborate our initial observation from a subgroup of 62 breast-fed infants[12] from a clinical study in which 159 high-risk mother–infant pairs received the probiotic *L rhamnosus* GG or placebo.[1] Recently, similar data have been published from trials conducted in Norway[6] and Korea[7] which used different combinations of probiotic lactobacilli and

TABLE II. Occurrence of eczema, chronically persistent eczema, and skin prick test positivity in infants whose mothers received placebo, the combination of *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 (LPR+BL999), or the combination of *Lactobacillus paracasei* ST11 and *B longum* BL999 (ST11+BL999)

	Placebo	LPR+BL999	ST11+BL999
Eczema			
Ratio (%)	44/62 (71)	21/73 (29)	20/70 (29)
OR (95% CI)*		0.17 (0.08-0.35)	0.16 (0.08-0.35)
P value†		<.001	<.001
Chronically persistent eczema			
Ratio (%)	16/62 (26)	7/73 (10)	4/70 (6)
OR (95% CI)*		0.30 (0.12-0.80)	0.17 (0.12-0.80)
P value†		.016	.003
Skin prick test positive			
Ratio (%)	17/65 (26)	17/76 (22)	19/73 (26)
OR (95% CI)*		0.81 (0.38-1.76)	0.99 (0.46-2.13)
P value†		.60	.99

*Compared with placebo group.
†Corresponds to logistic regression analyses.

bifidobacteria, but these studies had high rates of subjects lost to follow-up. We interpret our data to suggest a feasible and effective, relatively short-term, exclusively maternal intervention to reduce the risk of eczema in the infant. Importantly, we describe objective criteria for a clearly defined target population, which may be identified by maternal allergic disease and skin prick test positivity.

Interestingly, both the combination of LPR and BL999 and the combination of ST11 and BL999 appeared to be effective in reducing the risk of eczema in this study. The use of combinations of 2 probiotic strains leaves room for speculation whether a single strain might be equally effective. Nonetheless, *in vitro* and clinical studies on the effects of probiotic bacteria suggest that lactobacilli and bifidobacteria may have synergistic effects. We have previously reported that, although *Lactobacillus* GG and *Bifidobacterium lactis* Bb12 have similar clinical efficacy in treatment of eczema, they appear to have distinct immunologic effects.[19] We have also published data showing that probiotic lactobacilli increase mucus binding of bifidobacteria.[20] These observations combined with the present data suggest potentially synergistic mechanisms underlying the clinical effects of the present probiotic combinations.

The use of viable bacteria to reduce the risk of disease in early infancy has raised obvious safety concerns related to risk of bacterial translocation and to development of septicemia. Instances of probiotic-induced sepsis have been reported in infants,[21,22] but no serious adverse effects have to our knowledge been observed in clinical trials that assessed the effects of probiotics in neonates or infants. It is vital to acknowledge, however, that clinical trials may not have sufficient numbers of subjects to detect rare but significant adverse effects because statistical power is typically calculated on the basis of the primary outcome measures of the study. Recent reports from our unit[23] and Italy[24] indicate that routine use of the probiotic lactobacilli in a neonatal intensive care setting has been safe and well tolerated over a period of several years. In the present study, we observed no adverse effects associated with probiotics in the pregnant or breast-feeding mothers or their infants. The fact that the infants received no direct intervention and that the probiotics were solely administered to the mothers may be interpreted to further improve the safety profile of the intervention. Thus, according to our results, probiotics administered to the pregnant and breast-feeding mother appear to be safe and effective in reducing the risk of eczema in infants with high hereditary risk.

Notwithstanding the accumulating evidence of health benefits of specific probiotics in neonates and infants, relatively little is known about the mechanisms that mediate these effects. Conventionally, it has been assumed that probiotics exert their effects by modulating intestinal microbiota composition or by directly stimulating the intestinal immune system.[25] Interestingly, however, careful review of the published clinical trials[1-11] that aimed to reduce the occurrence of eczema in infants suggests that the intervention is effective only if the probiotics are administered prenatally to the mother. Our present data are also consistent with the notion of prenatal probiotic effects. Prenatal probiotic supplementation may modulate the composition of maternal vaginal and intestinal microbiota, which provide an important colonizing inoculum to the newborn infant and thus have an effect on neonatal gut colonization. However, the mechanisms of prenatal probiotic effects may also be more indirect. Epidemiologic data suggest that maternal contact with farm animals and thus presumably increased microbial exposure during pregnancy reduces the risk of eczema in infancy, which in turn is associated with alterations in innate immune gene expression.[26] In line with these observations, maternal mucosal contact with the microbe *Acinetobacter lwoffii* during pregnancy reportedly modulates placental innate immune gene expression and protects the offspring from asthma in a murine model.[27] Given that maternal immune cells were found to cross the placenta and to induce tolerogenic immune responses in the human fetus,[28] it is conceivable that maternal probiotic supplementation modulates immune physiology in the fetoplacental unit. To support this notion, we have recently observed that maternal prenatal probiotic intervention significantly affects immune gene expression in the placenta and in the fetal gut in humans.[29]

Even though maternal probiotic supplementation during pregnancy appears necessary for reducing eczema risk in the infant, prenatal intervention alone is not sufficient to achieve the desired clinical effects according to a recent report.[11] On the basis of our present data and those from previously published trials,[6,7,12] we conclude that, in addition to prenatal intervention, maternal probiotic supplementation during breast-feeding may modulate disease risk in the infant. Although nonspecific microbial transfer from the mother through nursing and skin-to-skin contact undoubtedly occurs, we suggest that maternal probiotic intervention exerts its effect also via specific modulation of the immunologic properties of breast milk. We have previously shown that maternal consumption of *L rhamnosus* GG during pregnancy and breast-feeding increases the concentration of the immunomodulatory cytokine transforming growth factor (TGF)-β2 in breast milk and is associated with reduced eczema risk in the infant.[12] TGF-β2 has been found to be necessary for breast milk–induced tolerance in an animal model,[30] and we have provided experimental evidence to suggest that TGF-β2 at a concentration comparable with that in breast milk modulates immune responses in the immature human gut[31] and promotes immune maturation.[32]

In addition to its obvious nutritional function and immunomodulatory properties, breast milk has a profound effect on the composition of neonatal gut microbiota.[33] Human milk oligosaccharides promote the growth of intestinal bifidobacteria, and breast milk has also been discovered to be a source of potentially colonizing bacteria to the newborn infant.[34] Both cells in human breast milk and maternal peripheral blood mononuclear cells have recently been shown to contain bacteria and bacterial DNA.[35] Live *Lactobacillus reuteri* have been recovered in breast milk after maternal supplementation with the probiotic in an intervention study that failed to reduce eczema risk but showed decreased risk of IgE-mediated eczema after subgroup analysis.[2,36] Experimental animal models found increased intestinal bacterial translocation during pregnancy and lactation which results in the presence of bacteria within dendritic cells in the mammary gland.[35] Taken together, these observations suggest a unique mechanism by which breast milk serves as a vehicle to introduce maternal gut microbiota to the infant in a tolerogenic immune milieu.[37] We hypothesize that probiotic intervention during lactation modulates this process and supports healthy immunologic maturation and intestinal microbiota development.[37] Collectively, the data reviewed above suggest that maternal probiotic supplementation during pregnancy and breast-feeding exerts its effect via several unconventional, indirect mechanisms, including modulation of placental and fetal immunophysiology and by promoting the protective potential of breast milk.

In conclusion, our data suggest that maternal supplementation with either the combination of the probiotics LPR and BL999 or the combination of ST11 and BL999 during the last 2 months of pregnancy and the first 2 months of breast-feeding may reduce the risk of infant eczema in a clinically and statistically significant manner in infants with mothers with allergic disease positive on skin prick testing. Maternal probiotic intervention appears to be safe, inexpensive, and relatively easy to implement even during exclusive breast-feeding without need to administer probiotics to the infant.

Johanna Hvitfelt-Koskelainen, RN, cared for the infants participating in the study. Statistical consultation was provided by Tuija Poussa, MSc. The probiotic strains were provided by Nestlé S.A. without compensation; Nestlé S.A. had no influence on the design or conduct of the study, data management and analysis, or writing of the report.

Clinical implications: Maternal supplementation with the probiotics LPR and BL999 or ST11 and BL999 during pregnancy and breast-feeding safely reduces the risk of eczema in infants with allergic mothers positive for skin prick test.

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