Effect of *Lactobacillus rhamnosus* GG on rBet v1 and rMal d1 specific IgA in the saliva of patients with birch pollen allergy

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Background: *Lactobacillus rhamnosus* GG (LGG) has demonstrated promising results in the treatment and prevention of atopic eczema.

Objective: To study the effects of LGG on the oral immune response in adolescents and adults with birch pollen allergy combined with oral allergy syndrome.

Methods: Patients received either LGG (n = 19) or a placebo (n = 19) for 5.5 months (from February 8 to August 6, 1999), starting 2.5 months before the birch pollen season. An oral apple challenge test was performed before, during, and after the pollen season. Saliva samples were collected before and after the challenges, and serum samples were collected before the challenges. Total IgA, IgG, and IgM and rBet v1 and rMal d1 specific IgA, IgG, IgG1, and IgG4 levels were measured from saliva with an enzyme-linked immunosorbent assay (ELISA). Serum rBet v1 specific IgE ELISA and birch radioallergosorbent testing were performed.

Results: After 5.5 months, rBet v1 and rMal d1 specific IgA levels had increased from baseline in the LGG compared with the placebo group (Δ rBet v1 IgA, 0.319 vs −0.136 relative units; $P = .02$; Δ rMal d1 IgA, 0.097 vs −0.117, $P = .02$). rBet v1 specific IgE serum levels did not differ between the groups. In the LGG group, rBet v1 specific IgE levels correlated positively with stimulated total IgA ($P = .04$) and IgG ($P = .003$) in saliva. In the placebo group, rBet v1 specific IgE levels correlated negatively with stimulated rBet v1 and rMal d1 IgA levels ($P = .009$ for both) and IgG ($P = .02$ and $P = .03$, respectively).

Conclusion: LGG showed immunostimulating effects on oral mucosa seen as increased allergen specific IgA levels in saliva.

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INTRODUCTION

The intestinal microbiota plays a crucial role in the programming and development of systemic immunity in early life. Direct and indirect epidemiologic data have indicated that microbiota alterations in the gut in childhood are linked to a risk of developing atopic sensitization and allergies. Some clinical trials with small children have demonstrated the beneficial effects of probiotics in promoting oral allergen tolerance in food allergy[1–3] and in allergy prevention.[4–7] Possible mechanisms include the induction of beneficial low-grade inflammation on the intestinal mucosa seen as an increase in sensitive C-reactive protein (CRP).[8–10]

Oral allergy syndrome (OAS) has been recognized clinically for several decades. It is characterized by itching or swelling of the mouth and sometimes edema of the tongue, immediately after contact of specific foods with the oral mucosa.[11] A preferential association has been found between some OAS-causing vegetables and rhinitis-causing pollens, such as that between apple and birch.[12]

Evidence of the prevention and treatment of birch pollen allergy and OAS with probiotics is scarce. In a murine model, a probiotic bacter, *Enterococcus faecalis* F23, showed mild anti-inflammatory effects, tending to increase pollen allergen specific IgG2 and decrease the pollen specific IgE/IgG2 ratio in sensitized mice.[13]

We have previously described the allergic symptoms discussed in this study, and LGG had no effect on the nasal, eye, or lung symptoms of adolescents and young adults with birch pollen allergy combined with OAS.[14] Herein, we aim to evaluate the effect of LGG supplementation on the oral mucosal immunoprofile (ie, saliva of these patients) in an acute oral apple challenge conducted before, during, and after the birch pollen season.

METHODS

Patients

The design, the patients, and the allergic symptoms of the patients during the study have been previously described.[14] The study comprised 38 adolescents and young adults recruited from the allergy outpatient unit of Helsinki University Hospital (9 male, 27 female; mean age, 27 years; range, 14–36 years). In addition to allergic respiratory and eye symptoms, caused by birch pollen, the patients had OAS

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caused by apple. Exclusion criteria included other pollen allergies, smoking, pregnancy, lactation, specific immunotherapy, current long-term medication, and intake of antibiotics 2 weeks and of probiotics 4 weeks before the study. A total of 33 patients completed the study: 4 did not consume the study capsules, and 1 moved from the area. The study protocol was accepted by the local ethics committee. Informed, written consent was obtained from all the study participants.

Design

The study was double-blind and placebo controlled and took place from February 8 to August 6, 1999. After a 1-week run-in period, the patients were randomized to consume daily either 2×10^{10} CFU of LGG (ATCC 53103, supplied by Valio Ltd, Helsinki, Finland) or placebo. The birch pollen season in the Helsinki area covers approximately the month of May. The patients ingested capsules for 5.5 months, beginning 2.5 months before the birch pollen season. The after season samples were gathered in August (ie, 2 months after the birch pollen season in Helsinki).

Apple Challenge

Both patients and personnel were blinded for the intervention, but the oral apple challenges were open. The challenge was performed 3 times, before, during, and after the pollen season, with a 60-g slice of apple (Golden Delicious), chewed for 1 minute before swallowing. Symptoms induced by the challenge (from 0 "no symptoms" to 10 "very severe") were evaluated by the same person (S.H.). At each challenge, a serum sample was taken before the challenge, and saliva samples were gathered before and 20 minutes after the challenge. Saliva samples were gathered as follows: first the patients chewed Parafilm for 1.5 minutes and swallowed all saliva, then they chewed Parafilm for 5 minutes and all the saliva was gathered. Serum and saliva samples were centrifuged and frozen at −20°C until analyzed.

Laboratory Methods

Total immunoglobulins were analyzed by a modified enzyme-linked immunosorbent assay (ELISA) according to Helenius et al.[15] The antibodies used for the primary coating were rabbit anti-human IgG, IgA, or IgM (DakoCytomation, Glostrup, Denmark), and the secondary antibodies were peroxidase-conjugated rabbit anti-human IgG, IgA, or IgM (DakoCytomation). The immunoglobulin concentrations were calculated from the control curve made from the human serum standard with known amounts of IgG, IgA, and IgM (Roche Diagnostics GmbH, Mannheim, Germany). Allergen specific immunoglobulins were measured by ELISA. The antibodies used for the primary coating were recombinant Bet v1, rMal d1 (Alk-Abelló, Hørsholm, Denmark) or κ-casein (Sigma, St Louis, MO), and the secondary antibodies were conjugated IgG (Zymed, San Francisco, CA) or biotinylated IgA, IgG1, or IgG4 (Vector Lab, Burlingame, CA) with AP-Streptavidin (Zymed). Reaction was measured at 405 nm, and optical densities, proportioned to saliva total protein, were considered as relative units. Total protein was measured with Micro Lowry (Bio-Rad Laboratories, Hercules, CA), birch pollen specific radioallergosorbent testing (RAST) measuring specific IgE with ImmunoCAP (Phadia, Uppsala, Sweden), and CRP with Instant ELISA (Bender MedSystems, Vienna, Austria). All determinations were performed as duplicates, and controls and standards were included throughout, except for the allergen specific measurements for which standards were unavailable.

Statistical Analysis

The main variables were the saliva antibodies that were compared between the LGG and placebo group. The effect of LGG on saliva antibodies on long-term challenge (ie, birch pollen season) and acute challenge (ie, oral apple challenge) were compared using the nonparametric Mann-Whitney test. Correlations were analyzed with Pearson bivariate correlations.

RESULTS

In the LGG group, levels of birch pollen specific rBet v1 IgA and apple specific rMal d1 IgA measured before the apple challenge increased from the baseline to the autumn, whereas in the placebo group they did not (LGG vs placebo: Δ rBet v1 IgA, 0.319 vs −0.136 relative units, $P = .02$; and Δ rMal d1 IgA, 0.097 vs −0.117, $P = .02$; Fig 1). No difference between the groups in κ-casein specific IgA response was seen (Fig 1).

No difference was found between the LGG and the placebo groups at baseline, in the birch pollen season or the autumn, in rBet v1 specific IgE in serum (median, 0.47 vs 0.43 relative unit, $P = .66$; median, 0.42 vs 0.34, $P = .66$; and median, 0.26 vs 0.25, $P = .59$), and in birch RAST measuring specific IgE (median, 35 vs 34 kU/L, $P = .52$; median, 29 vs 24 kU/L, $P = .26$; and median, 26 vs 22 kU/L, $P = .30$). After the pollen season, in the placebo group, high rBet v1 specific IgE levels in serum were associated with low rBet v1 and rMal d1 specific IgA ($r = -0.583$, $P = .009$; and $r = -0.580$, $P = .009$) and IgG ($r = -0.533$, $P = .02$; and $r = -0.498$, $P = .03$) levels in saliva after the apple challenge (Fig 2). In the LGG group, on the other hand, high rBet v1 specific IgE levels in serum were associated with high total IgA ($r = 0.514$, $P = .04$, birch pollen season) and IgG ($r = 0.703$, $P = .003$, after pollen season) levels in the saliva in response to allergen exposure. Birch RAST showed similar but more constricted correlations (Fig 2). There was already some difference in the correlations between the groups at baseline.

Median symptom scores induced by the apple challenge before, during, and after the pollen season were 3, 4.5, and 2 in the placebo group and 4, 3, and 3 in the LGG group, respectively. The differences were not statistically significant. In addition, no difference was found in CRP levels between the groups (data not shown).

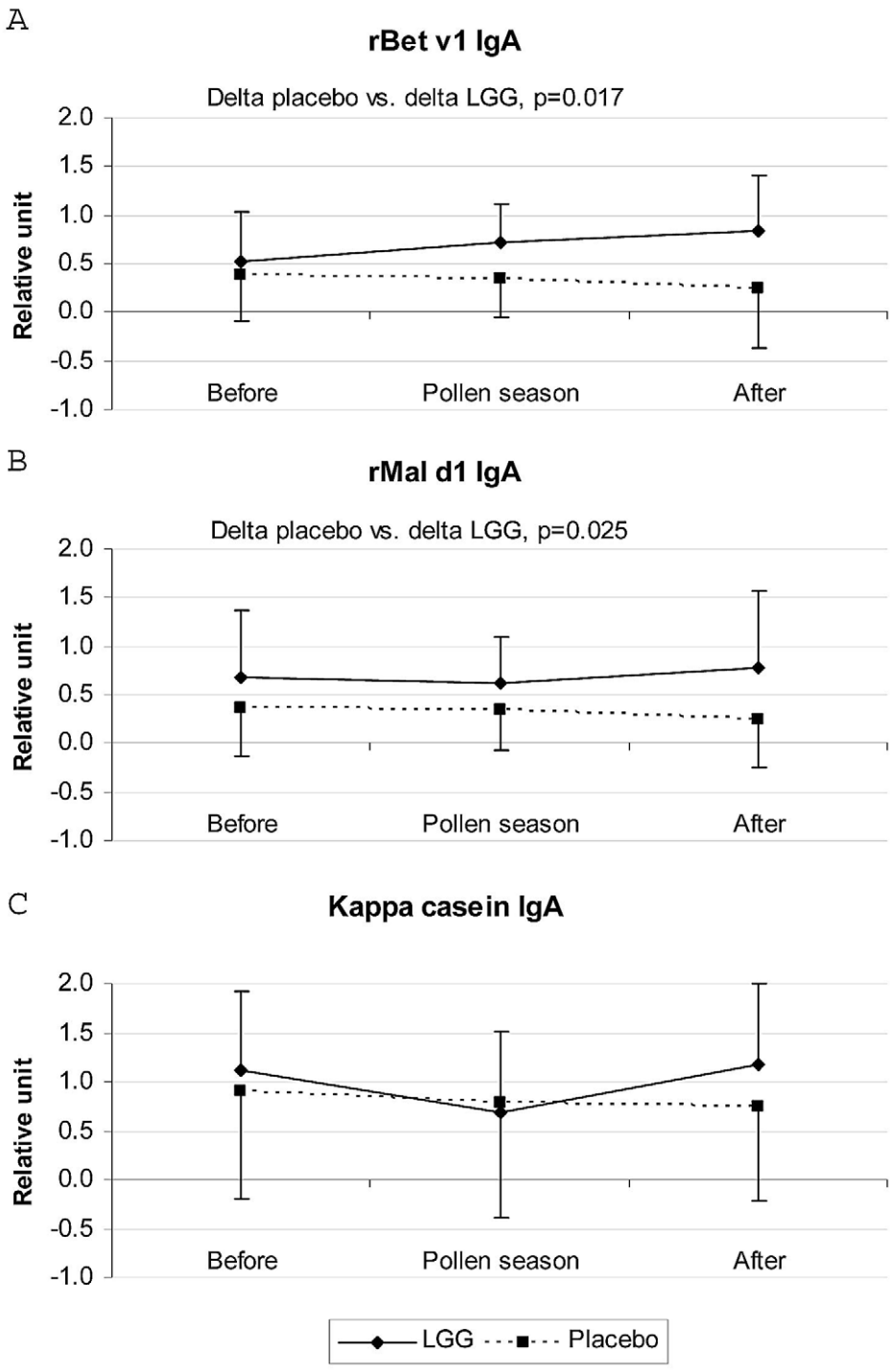

Figure 1. Specific IgA to rBet v1, rMal d1, and κ-casein in saliva before, during, and after the birch pollen season in patients receiving either *Lactobacillus rhamnosus* GG (LGG; n = 19) or placebo (n = 19). The saliva samples were unstimulated (ie, collected before acute apple challenge). The numbers are relative units with standard deviations (ie, median values of optical densities proportioned to saliva total protein). The effect of intervention (ie, difference between before season and after season) is considered as Δ and compared between the groups with the Mann-Whitney test.

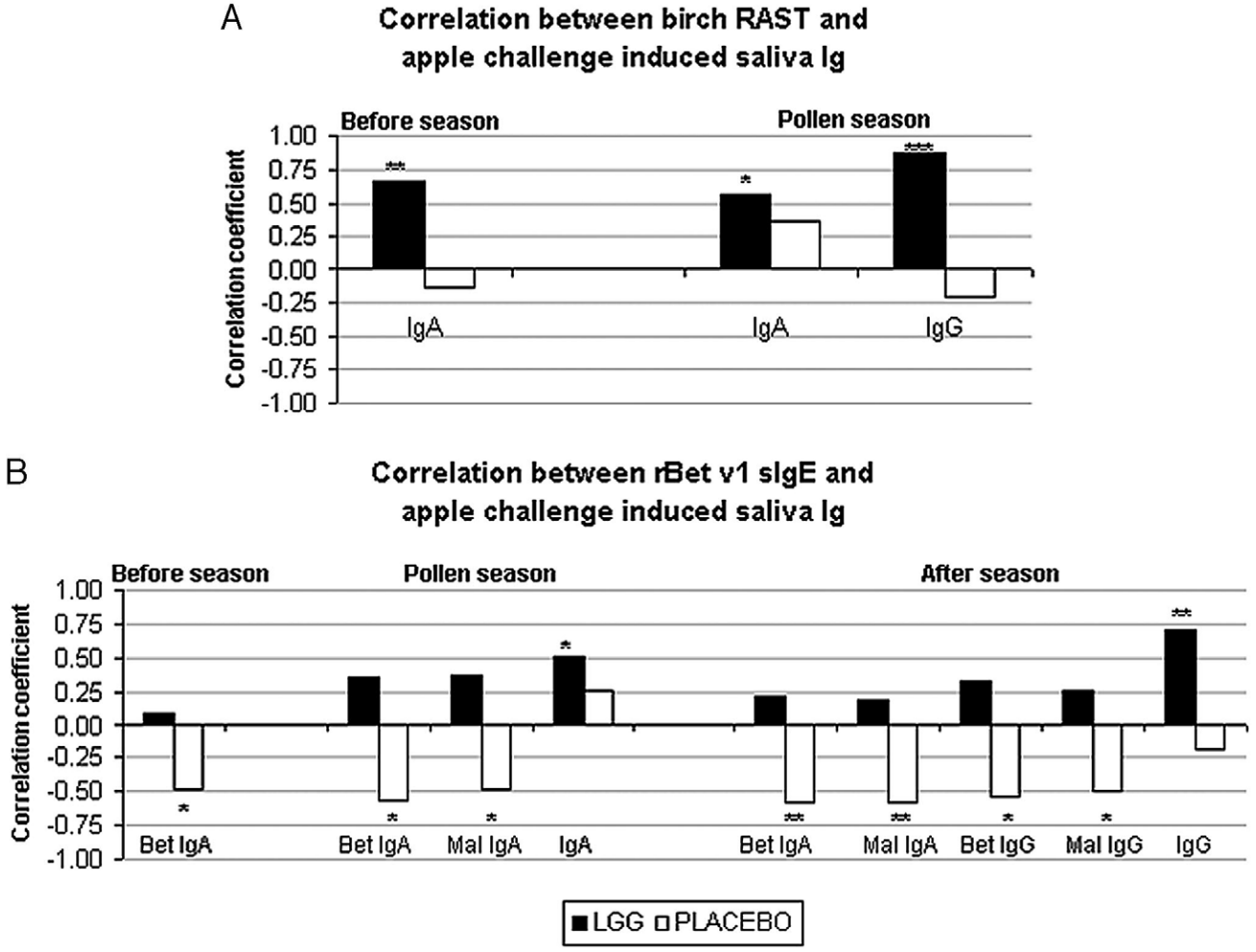

Figure 2. Statistically significant correlation coefficients between serum birch radioallergosorbent testing and rBet v1 specific IgE and apple challenge induced saliva immunoglobulins in the groups receiving either *Lactobacillus rhamnosus* GG (LGG; n = 19) or placebo (n = 19) at different time points (before, during, and after the birch pollen season). *$P < .05$, **$P < .01$, ***$P < .001$.

DISCUSSION

We found that LGG stimulated the secretion of birch pollen and apple specific IgA to saliva in patients with birch pollen allergy and OAS. No increase was seen in control κ-casein specific saliva IgA.

In the placebo group, low secretion of allergen specific IgA and IgG to saliva was associated with high rBet v1 specific IgE levels in serum. Instead, in the LGG group, high rBet v1 IgE levels in serum correlated positively with saliva total IgA and IgG levels, indicating that the stimulating effect of LGG was dominant in the most sensitive patients with high serum allergen specific IgE levels. LGG has earlier been shown to stimulate antigen specific fecal and/or serum IgA production ie, to have an immunostimulating effect in children with atopic eczema and IgE-mediated cow's milk allergy,[9] in formula-fed infants,[16] and when combined with an oral rotavirus[17] or *Salmonella*[18] vaccine. In the present study, LGG showed similar stimulation of mucosal allergen specific IgA and IgG, for the first time, in saliva.

We found an increase in symptom score during the birch pollen season, induced by the apple challenge, in the placebo group, whereas a decrease was seen in the LGG group. These clinical observations, although not statistically significant, are in close agreement with our findings on local allergen specific responses. However, LGG had no relieving effect on the symptoms of allergy during the birch pollen season, as we reported earlier.[14] Not all studies have found that probiotics have clinical benefits in the prevention of allergic diseases.[19] In children with atopic dermatitis, ingestion of LGG showed clinical benefits, and the treatment induced low-grade inflammation seen as elevated CRP levels.[10] We did not find any increase in CRP in the LGG group, indicating a less pronounced inflammation response to LGG in adults. This lack of systemic effect could explain why LGG did not signifi-

cantly relieve the symptoms of allergy in adults despite the local effect on oral antibody response. To conclude, in this study, ingestion of LGG stimulated the oral mucosal immune system in patients with birch pollen allergy and OAS, seen as increased allergen specific IgA in the saliva.

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REFERENCES

1. Isolauri E, Arvola T, Sütas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy*. 2000;30:1604–1610.
2. Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. *J Pediatr Gastroenterol Nutr*. 2003;36:223–227.
3. Viljanen M, Savilahti E, Haahtela T, et al. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy*. 2005;60:494–500.
4. Kalliomäki 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.
5. Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361:1869–1871.
6. Abrahamsson TR, Jakobsson T, Böttcher MF, et al. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2007;119:1174–1180.
7. Kukkonen K, Savilahti E, Haahtela 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.
8. Pohjavuori E, Viljanen M, Korpela R, et al. *Lactobacillus* GG effect in increasing IFN-gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol*. 2004;114:131–136.
9. Viljanen M, Kuitunen M, Haahtela T, Juntunen-Backman K, Korpela R, Savilahti E. Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants. *Pediatr Allergy Immunol*. 2005b;16:65–71.
10. Viljanen M, Pohjavuori E, Haahtela T, et al. Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. *J Allergy Clin Immunol*. 2005;115:1254–1259.
11. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. *Ann Allergy*. 1988;61:47–52.
12. de Groot H, de Jong NW, Vuijk MH, Gerth van Wijk R. Birch pollinosis and atopy caused by apple, peach, and hazelnut; comparison of three extraction procedures with two apple strains. *Allergy*. 1996;51:712–718.
13. Shimada T, Cheng L, Yamasaki A, et al. Effects of lysed *Enterococcus faecalis* FK-23 on allergen-induced serum antibody responses and active cutaneous anaphylaxis in mice. *Clin Exp Allergy*. 2004;34:1784–1788.
14. Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *Allergy*. 2002;57:243–246.
15. Helenius LM, Meurman JH, Helenius I, et al. Oral and salivary parameters in patients with rheumatic diseases. *Acta Odontol Scand*. 2005;63:284–293.
16. Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. *Pediatr Res*. 2006;60:221–224.
17. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine*. 1995;13:310–312.
18. He F, Tuomola E, Arvilommi H, Salminen S. Modulation of humoral immune response through probiotic intake. *FEMS Immunol Med Microbiol*. 2000;29:47–52.
19. 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.

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