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An α-lactalbumin-enriched and symbiotic-supplemented *v.* a standard infant formula: a multicentre, double-blind, randomised trial

Jean-Christophe Rozé[1,2,3]*, Sébastien Barbarot[4], Marie-José Butel[5], Nathalie Kapel[5], Anne-Judith Waligora-Dupriet[5], Inès De Montgolfier[6], Magali Leblanc[7], Nathalie Godon[1], Pascale Soulaines[8], Dominique Darmaun[3], Montserrat Rivero[9] and Christophe Dupont[8]

[1]*Department of Neonatal Medicine, Nantes University Hospital, Boulevard Jean Monet, 44000 Nantes, France*
[2]*INSERM, CIC004, Nantes University Hospital, Nantes, France*
[3]*INRA UMR 1280, Physiologie des Adaptations Nutritionnelles, INRA and University of Nantes, Human Nutrition Research Centre (CRNH), Nantes, France*
[4]*Department of Dermatology, CHU Hôtel-Dieu, Nantes, France*
[5]*EA 4065, Ecosystème Intestinal, Probiotiques, Antibiotiques, Université Paris Descartes, Paris, France*
[6]*Department of Neonatal Medicine, Trousseau Hospital, Paris, France*
[7]*Department of Neonatal Medicine, Angers University Hospital, Angers, France*
[8]*Department of Paediatric Gastroenterology and Nutrition, Necker-Enfants Malades Hospital, Paris, France*
[9]*Research and Development Department, Ordesa Group, Barcelona, Spain*

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Abstract

The aim of the present study was to evaluate the safety, tolerance and preventive effect on atopic dermatitis of an experimental α-lactalbumin-enriched and symbiotic-supplemented infant formula. A total of ninety-seven non-breastfed term neonates were enrolled into a double-blind, multicentre, randomised controlled trial in which they received experimental (*n* 48) or standard formula (*n* 49) for 6 months. The primary outcome was weight at 6 months of age. Secondary outcomes were gastrointestinal tolerance and manifestation of atopic dermatitis. Faecal secretory IgA (SIgA) concentration and microbiota composition of forty-three infants were analysed at 1 and 6 months. Growth was similar in both groups. At 1 month, compared to those in the control group, infants in the experimental group exhibited less crying or agitation, and more quiet behaviour ($P=0{\cdot}03$). At 6 months, atopic dermatitis was less frequently observed in the experimental group ($P<0{\cdot}05$). Decrease of faecal SIgA concentration between 1 and 6 months was mainly observed in the control group. This decrease was significantly associated with atopic dermatitis ($P<0{\cdot}014$) and negatively correlated to the level of colonisation by bifidobacteria ($P<0{\cdot}005$). In conclusion, compared to the control formula, the experimental formula guaranteed a similar growth, was better tolerated at 1 month and had a protective effect against the development of atopic dermatitis.

Key words: Growth: Symbiotics: α-Lactalbumin: Faecal IgA: Microbiota: Neonates

Several modifications have been proposed to improve the quality of infant formulas. Enrichment in α-lactalbumin, the dominant whey protein in human milk, improves the amino-gram[(1)] while reducing the total protein content[(2,3)], and may have a positive effect on infant well-being and behaviour by reducing gastrointestinal side-effects[(1,3,4)]. α-Lactalbumin is a known immunomodulator present in human milk. This effect comes from its ability to form complexes with other glyco-proteins of the milk[(5)]. For example, α-lactalbumin alters the degradation of soluble CD14 in the gastrointestinal tract. This glycoprotein is an important immunomodulator that shows several beneficial effects, one of them being the reduction of the inflammatory response to pathogenic bacteria[(5)]. Supplementation with prebiotics, probiotics or symbiotics (mixtures of probiotics and prebiotics) has been proposed, based on clinical and laboratory observations of reduced atopic dermatitis, increased faecal IgA and modified composition of the microbiota[(6)] in adults and infants[(7–9)].

To determine the safety, tolerance and preventive effect on atopic dermatitis of an experimental α-lactalbumin-enriched and symbiotic-supplemented infant formula, we assessed in priority the growth of the infants by measuring their weight; then we observed the gastrointestinal tolerance of the formula, the occurrence of atopic dermatitis manifestations,

Abbreviations: CFU, colony-forming unit; SIgA, secretory IgA.

* **Corresponding author:** Professor J.-C. Rozé, fax +33 240 083 556, email jcroze@chu-nantes.fr

faecal secretory IgA (SIgA) concentration and microbiota composition in a double-blind, multicentre, randomised, controlled trial enrolling infants whom their mothers declined to breastfeed.

Materials and methods

Study population

Four centres participated in the present trial (Mother-and-Child Hospital, Nantes, France; Children's Hospital, Angers, France; Hospital Saint-Vincent de Paul and Hospital Salpêtrière, APHP, Paris, France). This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The protocol was approved by the Medical Ethics Committee of Nantes (CCPRB). Written informed parental consent was obtained for each infant before inclusion. To be eligible for enrolment in the present study, infants had to meet the following inclusion criteria: a gestational age >37 weeks, a postnatal age less than 3 d, fed from birth with probiotic- and prebiotic-free infant formula, and without human milk before inclusion. This trial was registered under number NCT00920166.

Formulas

The experimental formula was characterised by the presence of two strains of probiotics (*Lactobacillus rhamnosus* LCS-742 and *Bifidobacterium longum subsp infantis* M63) and by the addition of prebiotics: 96 % galacto-oligosaccharides and 4 % short-chain fructo-oligosaccharides.

This formula was also enriched with bovine α-lactalbumin, using a native whey protein concentrate with high α-lactalbumin concentration (34 % of soluble proteins) obtained using careful fractionation techniques (ultrafiltration). Whey protein concentrate was blended with all other macro- and micronutrients in the formula liquid phase and was finally spray-dried. Amounts of native or complexed α-lactalbumin in the final infant formula were not determined.

Randomisation, formula and compliance

After inclusion, infants were randomly assigned to the experimental or control formula (Table 1). Both patients and treating physicians were blinded to treatment group assignment. Each product was identified by a number randomly allocated using a software. This number was written on a double label: one remained on the can for identification and the other in the follow-up book. For each can, a sealed envelope with the corresponding formula was sent to the investigator and the coordinator. Envelopes could be opened if required for the baby's health. In this case, the reason, person and date had to be reported on the envelope itself and the baby was automatically removed from the trial. Parents received boxes of infant formula containing blinded experimental or control formula. The intervention ended at 6 months of age. Compliance was measured by counting the number of empty returned boxes at each clinic visit. Compliance was defined as good if the expected number of empty cans was returned and no change of formula was declared (see details in Fig. 1).

Clinical assessment

Anthropometric measurements, such as weight, length, BMI, cranial perimeter, were performed at birth. Two clinical visits were scheduled at 1 and 6 months of age. The following data were collected during these visits: anthropometric measurements, dermatological examination and scoring atopic dermatitis (SCORAD) index calculation, sleeping time and general well-being using structured interviews[10]. Data pertaining to formula intake for each meal, bowel habits and stool consistency over the 3 d before each visit were also collected from a home diary kept by the parents. For atopic dermatitis, we used the UK Working Party's diagnostic criteria and the SCORAD index was calculated to assess severity[11]. We used the WHO growth curves[12] to calculate *Z*-score according to the least mean squares method.

Biological assessment

In two centres located in Paris, stool samples were collected by parents at 1 and 6 months in sterile containers, immediately placed in an anaerobic atmosphere (GENbag anaer®-bioMérieux, Marcy l'Etoile, France), and transferred to the laboratory for bacterial analyses. Samples were frozen at −80°C immediately after collection during the clinical visit. Qualitative and quantitative analysis of the faecal microbiota allowing the isolation and quantification of the main genera were performed by spreading dilutions of the stools on various media, as described previously[13]. Bacterial counts were expressed as $\log_{10}$ colony-forming units (CFU)/g of faeces, and the count threshold was 3 $\log_{10}$ CFU/g of faeces. Identification of lactobacilli and bifidobacteria was performed

Table 1. Control and experimental formula compositions

	Control formula per 100 ml	Experimental formula per 100 ml
Energy		
kJ	17·2	16·2
kcal	72	68
Protein (g)	1·5	1·4
Casein (%)	50	40
Whey proteins (%)	50	60
α-Lactalbumin (g)	–	0·3
Carbohydrates (g)	8·9	7·6
Lactose (g)	6·3	5·3
Maltodextrin (g)	2·6	2·3
Fats (g)	3·4	3·5
Linoleic acid (mg)	550	540
α-Linolenic acid (mg)	51	53
AA (mg)	–	12·6
DHA (mg)	–	7
Symbiotics	–	
FOS (g)	–	0·02
GOS (g)	–	0·40
Lactobacillus rhamnosus LCS-742 (CFU)	–	$1{\cdot}4 \times 10^8$
Bifidobacterium longum subsp. *infantis* M63 (CFU)		$1{\cdot}4 \times 10^8$

AA, arachidonic acid; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides.

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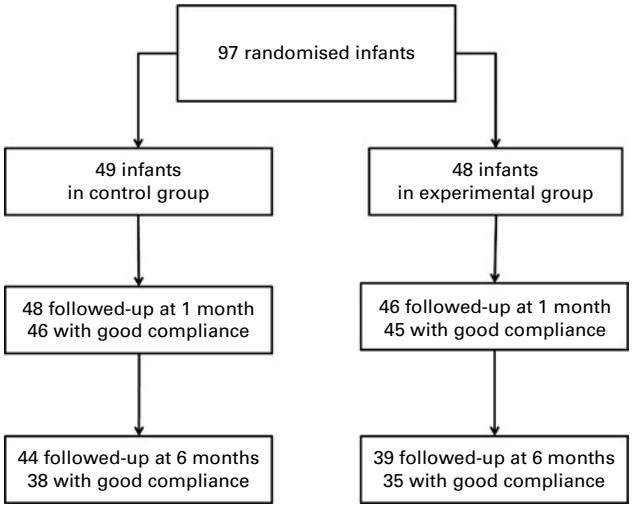

Fig. 1. Flow diagram of the progress of patients through the study.

using macroscopic aspects of colonies, microscopic characteristics and PCR using primers specific for genus and species, and 16S rDNA sequencing (Genome express, Meylan, France), as described previously[14].

Faecal SIgA were determined by ELISA using NUNC Immuno plates (Maxisorp F96, Roskilde, Denmark). Plates were coated for 1 h at 37°C and incubated overnight at 4°C with 1 mg/l antibody to human secretory component (Dako-Glostrup, Glostrup, Denmark) in veronal buffer (25 mmol/l; pH 8·6). Non-specific binding sites were blocked using PBS pH 7·2 containing 1 % bovine serum albumin. Serial two-fold dilutions of tested samples and purified human SIgA (Cappel, ICN, Aurora, CO, USA) were added to the wells. The plates were incubated for 1 h at 37°C under agitation, then washed, and incubated for 1 h under agitation with 0·25 μg/l horseradish peroxidase–conjugated rabbit antibody to human IgA (Dako-Glostrup). Addition of *O*-phenyl diamine triggered the reaction. Absorbance was read at 492 nm and quantitative results were determined by reference to the standard curves.

Statistical analysis

The primary outcome was weight at 6 months of age. The trial was a non-inferiority trial that compared the experimental formula to a control formula: a 300 g difference at 6 months between groups was deemed acceptable. The trial was designed to compare the experimental formula to a control formula. A sample size of forty-five infants in each group provided 80 % power to detect this non-inferiority difference of 300 g, at a 5 % one-sided significance level. Standard deviations of anthropometric *Z*-scores were calculated using the 2006 WHO growth standards. Data were analysed based on the intention-to-treat strategy. A per-protocol analysis was performed to confirm the intention-to-treat analysis. The final statistical analysis was performed using SPSS 15.0 software (Chicago, IL, USA). Student's *t* test, or the Mann–Whitney *U* test when appropriate, was used for the comparison of continuous variables, and a χ^2 test, or Fisher's exact test when appropriate, was used for comparison of categorical variables. A receiver operating characteristic curve was constructed to analyse the predictive relationship between the 1- to 6-month variation in SIgA concentrations and dermatological lesions at month 6. Logistic regression was performed to analyse the relationship between atopic dermatitis and formula after adjustment for family history of atopy and caesarean section.

Table 2. Characteristics at inclusion
(Number, percentage, mean values and standard deviations)

	Control group (*n* 49)		Experimental group (*n* 48)		
	Mean	SD	Mean	SD	*P*
Perinatal characteristics					
Female					0·09
n	19		27		
%	38·8		56·3		
Maternal antibiotherapy					0·40
n	8		5		
%	16·3		10·4		
History of allergic disease					0·18
n	25		18		
%	51·0		37·5		
Gestational age (weeks)	39·3	1·1	39·4	1·2	0·85
Mode of birth: Caesarean					
n	4		7		0·32
%	8·2		14·6		
Anthropometric measurements					
Weight (g)	3350	440	3300	390	0·66
Weight-for-age *Z*-scores	0·1	0·9	0·0	0·8	0·89
Height (cm)	49·6	1·8	49·3	1·9	0·52
Height-for-age *Z*-score	0·0	0·9	-0·1	1·0	0·75
Head circumference (cm)	34·7	1·2	34·4	1·2	0·26
Head circumference for age *Z*-score	0·3	0·9	0·2	1·0	0·49

Results

Intention-to-treat analysis

At birth, ninety-seven infants were enrolled, forty-eight in the experimental and forty-nine in the control group. No statistically significant differences for perinatal characteristics were observed (Table 2).

At 1 month (Table 3), forty-six out of forty-eight experimental and forty-eight out of forty-nine control infants were examined. Growth was not significantly different between the two groups. During the 3 d preceding the 1-month clinical visit, infants exhibited less crying or agitation, and more quiet behaviour in the experimental group than in the control group ($P=0{\cdot}03$). No other clinical differences were found. During the first month, one infant in the experimental group and two infants in the control group received antibiotherapy. Stools of twenty infants in the experimental group and twenty-three in the control group showed significantly lower incidence ($P=0{\cdot}02$) and level of colonisation ($P=0{\cdot}009$) by staphylococci in the experimental group. The level of colonisation by lactobacilli was higher in the experimental than in the control group (respectively, 8·0 (SD 1·0) *v.* 6·0 (SD 1·1) CFU/g of faeces, $P<0{\cdot}001$).

At 6 months (Table 4), thirty-nine out of forty-eight of experimental and forty-four out of forty-nine of control infants were seen. Weight was not statistically different between the groups, but a trend towards higher weight-for-age ($P=0{\cdot}053$) and head circumference-for-age ($P=0{\cdot}061$) *Z*-scores was observed in the experimental group (see Table 4). Atopic dermatitis was less frequently observed in the experimental group ($P<0{\cdot}05$). The association tended to be significant after adjustment for family history of allergic disease and caesarean section: OR 0·12 (95% CI 0·02, 1·04, $P=0{\cdot}05$). However, the SCORAD measured during the 6-month clinical visit was not significantly different between the groups ($P=0{\cdot}40$). No other clinical differences were observed except a trend to receive less frequently antibiotics in the experimental group: six out of thirty-nine (15·4%) *v.* fourteen out of forty-four (31·8%) in the control group, $P=0{\cdot}08$. The level of colonisation by staphylococci was significantly lower with the experimental formula (respectively, 1·4 (SD 2·6) *v.* 3·9 (SD 2·9) CFU/g of faeces, $P=0{\cdot}02$), while that of lactobacilli

Table 3. Outcome measurements at 1 month (intention-to-treat)
(Number, percentage, mean values and standard deviations)

	Control group		Experimental group		
	Mean	SD	Mean	SD	*P*
1 month (*n*)	45		43		
Anthropometric measurements					
Weight (g)	4390·4	624·7	4307·7	475·0	0·515
Weight-for-age *Z*-score	−0·1599	0·774	−0·1577	0·665	0·991
Height (cm)	53·73	2·06	53·78	2·02	0·917
Height-for-age *Z*-score	−0·4731	0·863	−0·3166	0·853	0·395
Head circumference (cm)	37·70	1·28	37·32	1·30	0·170
Head circumference for age *Z*-score	0·4649	0·8968	0·2774	0·8662	0·322
Gastrointestinal tolerance and atopic dermatitis					
Daily mean ingested volume (ml)	597	110	580	114	0·59
Number of regurgitations per d (*n*)	0·9	1·4	1·0	1·2	0·80
Number of stools a day (*n*)	1·5	1·0	2	1·1	0·05
Daily duration of crying or agitation (min)	89·3	33·2	75·6	22·3	0·03
Daily duration of sucking thumb (min)	190·0	42·8	214·6	38·8	0·03
Atopic dermatitis					0·435
n	5		2		
%	11·1		4·7		
Stool analysis (*n*)	23		20		
SIgA (μg/g)	1620	1137	1892	1635	0·53
Colonisation with *Staphylococci*					0·02
n	20		11		
%	87·0		55·0		
Colonisation with *Clostridium*					0·97
n	16		14		
%	69·6		70·0		
Colonisation with *Bacteroides*					0·82
n	13		8		
%	56·5		40·0		
Colonisation with *Lactobacilli*					0·93*
n	23		19		
%	100·0		95·0		
Colonisation with *Bifidobacteria*					0·14*
n	17		19		
%	73·9		95·0		

SIgA, secretory IgA.
* Fisher's exact test.

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and bifidobacteria tended to be higher (respectively, 6·8 (SD 2·9) *v.* 5·0 (SD 2·4) CFU/g of faeces, $P=0·06$, and 10·6 (SD 0·3) *v.* 9·0 (SD 3·3) CFU/g of faeces, $P=0·07$). The variation of SIgA between 1 and 6 months was significantly different between the groups. At 6 months, SIgA were at similar high levels to that found at 1 month in the experimental group, whereas they decreased in the control group. The SIgA decrease between 1 and 6 months was significantly associated with atopic dermatitis (Fig. 2) and negatively correlated to the level of colonisation by bifidobacteria (R^2 0·27, $P<0·005$). There was a trend towards an association between the level of colonisation by bifidobacteriae and a lower risk of atopic dermatitis (OR 0·60 per log CFU (95 % CI 0·34, 1·07)).

Per-protocol analysis

Among the ninety-seven infants enrolled, seventy-three had good compliance and these were equally distributed between the two groups: thirty-five in the experimental group and thirty-eight in the control group ($P=0·91$). The reasons for non-compliance were not significantly different between groups: poor digestive tolerance in four out of thirteen in the experimental group *v.* seven out of eleven of the control group ($P=0·11$), poor parental compliance in four out of thirteen *v.* one out of eleven ($P=0·43$). Per-protocol analysis showed the same main results as that found in the intention-to-treat analysis. At 1 month, infants exhibited less crying or agitation during the 3 d preceding the 1-month visit in the experimental group than in the control group ($P<0·02$). At 6 months, we did not observe any significant difference between the experimental and the control groups in weight (respectively, 8100 (SD 840) *v.* 7940 (SD 770) g, $P=0·40$), weight *Z*-score (0·46 (SD 0·89) *v.* 0·23 (SD 0·82), $P=0·26$), height (67·5 (SD 2·7) *v.* 67·2 (SD 2·5) cm, $P=0·64$), height *Z*-score (0·30 (SD 1·08) *v.* 0·10 (SD 1·08), $P=0·41$) or head circumference (44·0 (SD 1·5) *v.* 43·6 (SD 1·1) cm, $P=0·19$). The only difference observed was a trend towards a higher head circumference *Z*-score in the experimental group (0·96 (SD 0·92) *v.* 0·56 (SD 0·84), $P=0·05$). The daily mean ingested volume tended to be higher in the experimental group: 750 (SD 108) *v.* 690 (SD 120) ml/d, $P=0·08$. We observed 0/35 in the experimental group ($P=0·06$) *v.* five out of thirty-eight atopic dermatitis in the control group. After adjustment for family history of allergic disease and caesarean section,

Table 4. Outcome measurements at 6 months (intention-to-treat)

(Number, percentage, mean values and standard deviations)

	Control group		Experimental group		
	Mean	SD	Mean	SD	*P*
6 months (*n*)	45		39		
Anthropometric measurements					
Weight (g)	7769·8	918·1	8015·7	834·7	0·193
Weight-for-age *Z*-score	0·0981	0·8706	0·4715	0·8704	0·053
Height (cm)	66·72	3·31	67.07	2·67	0·980
Height-for-age *Z*-score	−0·0177	1·124	0·2647	1·0423	0·239
Head circumference (cm)	43·53	1·35	43·77	1·50	0·620
Head circumference for age *Z*-score	0·5614	0·8580	0·9374	0·9430	0·061
Gastrointestinal tolerance and atopic dermatitis					
Daily mean ingested volume (ml)	680	155	740	119	0·09
Number of regurgitations a day (*n*)	0·4	0·9	0·6	1·1	0·30
Number of stools a day (*n*)	1·2	0·9	1·5	1·1	0·28
Daily duration of crying or agitation (min)	48·8	23·4	42·5	23·8	0·25
Daily duration of sucking thumb (min)	185	32	172	41	0·28
Atopic dermatitis					0·03*
n	8		1		
%	17·8		2·6		
Stool analysis (*n*)	18		16		
SIgA (μg/g)	1322	840	1869	1624	0·21
Difference in SIgA between 1 and 6 months (%)	−80	145·0	−2	54·3	0·05
Colonisation with *Staphylococci*					0·02
n	12		4		
%	66·6		25·0		
Colonisation with *Clostridium*					0·29
n	16		12		
%	88·8		75·0		
Colonisation with *Bacteroides*					0·23
n	17		13		
%	94·4		81·3		
Colonisation with *Lactobacilli*					0·99
n	16		15		
%	88·8		93·8		
Colonisation with *Bifidobacteria*					0·99
n	17		16		
%	94·4		100·0		

SIgA, secretory IgA.
* Fisher's exact test.

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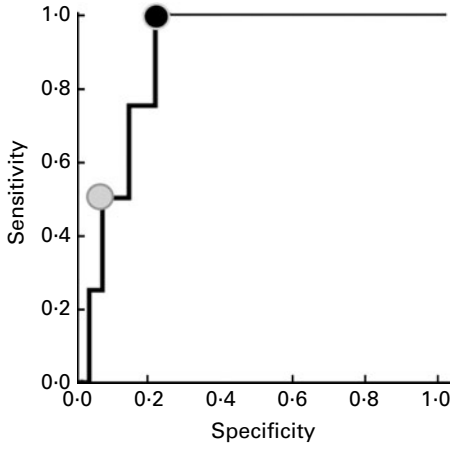

Fig. 2. Receiver operating characteristic curves of prediction of atopic dermatitis by decrease of faecal secretory IgA (SIgA) between 1 and 6 months. Results are expressed as ((SIgA at 1 month − SIgA at 6 months)/SIgA at 6 months) × 100. As indicated by the black circle, a decrease of 60 % predicts atopic dermatitis with a sensitivity of 100 % and a specificity of 79 %. The grey circle shows that a decrease of 150 % predicts atopic dermatitis with a sensitivity of 75 % and a specificity of 93 %. Area under the curve = 0·88 + 0·06; P=0·014.

feeding the experimental formula was associated with a reduction in the risk for atopic dermatitis: OR 0·11 (95 % CI 0·01, 0·94, $P<0·05$).

Discussion

In the double-blind, multicentre, randomised trial reported here, the experimental α-lactalbumin-enriched and symbiotic-supplemented infant formula ensured the same growth as a standard formula in terms of weight and height gain. This finding confirms the nutritional adequacy of the protein profile of the experimental formula[15]. In addition, in this unselected population, the α-lactalbumin-enriched and symbiotic-supplemented formula was better tolerated at 1 month of age, and had a protective effect against the occurrence of mild atopic dermatitis at 6 months of age.

The better gastrointestinal tolerance at 1 month of age could result from the α-lactalbumin enrichment of the formula. Such an effect has been reported in three earlier studies[2,3,4]. Lien *et al.*[3] speculated that this effect may result from the higher tryptophan content of α-lactalbumin, since tryptophan is a precursor of serotonin, a neurotransmitter that may regulate the sleep–wake rhythm. Nevertheless, in a previous study, Sandström *et al.*[15] failed to observe any difference in sleep pattern between infants receiving either standard formula, α-lactalbumin-enriched formula or breast-feeding. A possible role of prebiotics and probiotics should be considered as well. Indeed, improved gastrointestinal tolerance to feeding was observed in infants with colic when they received *Lactobacillus reuteri*[16] or prebiotics[17].

Prebiotics, and possibly probiotics, could protect against atopic dermatitis. This protection resulting from supplementation with fructo-oligosaccharides–galacto-oligosaccharides has already been observed in the recent MIPS1 study. This effect might be due to a mechanism involving bifidobacteria. Our data suggest a significant relationship between the level of colonisation by bifidobacteria and the variation in faecal SIgA, and a correlation of the latter parameter with the incidence of atopic dermatitis. The positive effect on atopic dermatitis may also come from the supplementation with probiotics, as their stimulatory role on the secretion of IgA has already been observed in other clinical studies[18,19]. Several clinical trials[7,20–22], aimed at the primary prevention of atopic dermatitis by probiotics, or prebiotics, have subsequently been published with conflicting results. Our results suggest an inverse association between atopic diseases and colonisation of the gut by probiotics, as already observed[22], with an effect on SIgA, significantly associated with a reduction of risk for atopic dermatitis. The mechanisms involved in the observed effects clearly warrant further investigation to confirm our interpretation.

This study has some limitations: only 80 % of enrolled infants had good compliance. Yet, similar conclusions were reached, whether results were analysed on an intention-to-treat or per-protocol basis. Moreover, the effects of the experimental formula on clinical symptoms were consistent with results from laboratory analysis regarding SIgA and microbiota, and corroborated by the observed relationship between these biological parameters and clinical symptoms.

In conclusion, α-lactalbumin enrichment and symbiotic supplementation may represent a significant advance in neonatal nutrition when the baby cannot be breastfed, as feeding such enriched formula ensured the same growth pattern as a standard formula with an improved tolerance and a protective effect against the development of atopic dermatitis.

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