

Prophylactic Probiotics Reduce Cow's Milk Protein Intolerance in Neonates after Small Intestine Surgery and Antibiotic Treatment Presenting Symptoms That Mimics Postoperative Infection

Shoichi Ezaki¹, Kanako Itoh¹, Tetsuya Kunikata¹, Keiji Suzuki¹,
Hisanori Sobajima¹ and Masanori Tamura¹

ABSTRACT

Background: To examine occurrence of cow's milk protein intolerance (CMPI) in newborns that underwent small intestine surgery and the clinical profiles of those newborns with postoperative CMPI, and to evaluate the preventive effects of probiotics on CMPI.

Methods: We retrospectively reviewed from 2000 to 2009, a total of 30 newborns required surgery on their small intestines. All of these patients had received antibiotics to prevent postoperative infection. Since 2005 we adopted a protocol of targeted probiotic therapy prophylaxis.

Results: Eighteen patients received probiotic therapy, while twelve did not. One infant among those eighteen patients and eight patients among those twelve developed CMPI, a significantly lower rate for the group with probiotic therapy than that without it ($p < 0.001$). Patients with positive cultures for gram positive and gram negative organisms increased in number before and after surgery but then decreased after probiotics treatment. Poor weight gain, gastrointestinal symptoms, and rise in C reactive protein (CRP) levels were observed in all of those nine CMPI patients. Specific IgE antibodies were elevated in four of the nine subjects, and total IgE levels were elevated in seven of them. All CMPI patients had increased level of CRP without proven infections.

Conclusions: CMPI was induced in newborns after surgery on their small intestines and antibiotics treatment with presentation of symptoms that mimic postoperative infection. Development of CMPI in this population possibly involves disruption of intestinal flora. Administration of probiotics can reduce the incidence of CMPI after small intestine surgery. The elevated CRP level may be useful in the diagnosis of CMPI.

KEY WORDS

cow's milk protein intolerance, CRP, newborn, probiotics, small intestine surgery

INTRODUCTION

Intestinal microbial flora plays important roles in their host organism. They can defend against pathogenic bacteria, mediate interactions between bacteria present in the intestines and contribute to gastroin-

testinal immunity.¹ Therefore, it is essential for newborns to acquire flora to gain mature intestinal immunity.

Normal development of intestinal flora can be disrupted by antibiotic therapy, which may cause abnormal immune function. When Oyama, *et al.*, adminis-

¹Division of Neonatal Medicine, Center for Maternal, Fetal and Neonatal Medicine, Saitama Medical Center, Saitama Medical University, Saitama, Japan.

Correspondence: Shoichi Ezaki, MD, PhD, Division of Neonatal Medicine, Center for Maternal, Fetal and Neonatal Medicine, Sai-

tama Medical Center, Saitama Medical University, 1981 Kamoda-Tujido, Kawagoe, Saitama 350-8550, Japan.

Email: s_ezaki@saitama-med.ac.jp

Received 9 February 2011. Accepted for publication 13 July 2011.

©0000 Japanese Society of Allergology

tered oral antibiotics to newborn mice, their intestinal flora was disrupted, Th2 cell dominant responses were induced and allergies were developed.² Establishment of immune tolerance to food antigens ingested orally has been blocked in adult mice when intestinal flora was removed at the age of their newborns (germ-free mice).³ When intestinal flora is disrupted or a delay occurs in acquiring normal intestinal flora, gastrointestinal immune functions do not mature, resulting in food intolerance.

Intestinal flora begins to develop immediately after birth and is fully formed within 2 days to a week.⁴ Newborns who require surgery on the small intestine soon after birth are subject to various conditions that can hinder them from acquiring intestinal flora. One of the problems is the delayed start of nutrition. However, the major problem of surgery concerning the effect on intestinal flora is the administration of antibiotics to prevent postoperative infections, because the existing flora can easily be disrupted by the use of systemic antibiotics.⁵

Bifidobacterium breve (*B. breve*) is used for probiotic treatment in neonates. *B. breve* can colonize the immature bowel of humans very effectively, and it is associated with fewer abnormal abdominal signs and better weight gain in newborns.⁶ *B. breve* is used in newborns to prevent neonatal necrotizing enterocolitis.⁷ For these reasons we have administered *B. breve* routinely to neonates after gastrointestinal surgery since 2005 under permission from the ethics committee of our university.

Based on our experience we considered that some newborns who undergo small intestine surgery develop intolerance to cow's milk protein, because they have not acquired intestinal flora or their existing intestinal microbial colonization is disrupted. Then we hypothesized that intolerance against cow's milk protein could be prevented by administering *B. breve*. The purpose of the present retrospective study was two-fold. Our first objective was to examine onset of cow's milk protein intolerance (CMPI) in newborns who underwent small intestine surgery, and the preventive effects of *B. breve* on the onset of milk intolerance. The second objective was to clarify the clinical profile of newborns with postoperative milk intolerance.

METHODS

We retrospectively reviewed the charts of infants who were admitted to the Neonatal Intensive Care Unit (NICU) at Saitama Medical Center at Saitama Medical University who underwent surgery of the small intestine between January 2000 and December 2009. We identified all infants who had surgery on the small intestine and used the discharge database (search keywords 'small intestine' or 'surgery') to determine further eligibility. The study was approved by the Institutional Review Board of the Human In-

vestigation Committee of Saitama Medical University, and informed consent was received from all of the participants.

TREATMENT PROTOCOL AFTER SURGERY OF THE SMALL INTESTINE

In our NICU we have implemented a standardized treatment protocol after small intestine surgery; cefotaxime sodium (CefotaxTM, Sanofiaventis, Tokyo, Japan) and gentamicin sulfate (GentacinTM, MSD, Tokyo, Japan) are the first line therapy for prevention of post-operative infection. Antibiotics were administered until white blood cell counts and CRP values returned to normal values after surgery. The normal CRP value for a newborn is less than 1 mg/dL. Complete blood count and CRP tests are performed routinely at least twice each week, while samples are taken three or four times each week if abnormal values are noted.

The surgeon and attending physician checked clinical findings of bowel peristalsis, flatulence, disappearance of bile from gastric aspiration and defecation.

X-ray findings included disappearance of postoperative ileus and intestinal swelling, and uniform gastrointestinal gas distribution. When both physicians judged that nutrition could be initiated, enteric feeding was begun after confirming good gastrointestinal motility on a gastroenterogram.

Mother's milk was basically given as enteric nutrient, unless otherwise it was not available, formula was given. The initial amounts given were 1, 3 or 5 ml each time for birth weights of <1, <1.5 or greater than 1.5 kg, respectively. Feeding was done every 3 hr after aspiration of gastric contents. Feeding amounts were increased once a day by the same amount as the initial one when the total amount of gastric residuals was less than 10% of the total amount of milk or formula given in the previous day. Feeds may have been withheld if there was abdominal distention, vomiting or repeated large amount of gastric aspirates. Parenteral nutrition was ceased when full oral feeds reached the amount of 100 ml/kg/day. These methods were not changed throughout the ten years of study period.

PROBIOTIC THERAPY

Since January 2005 we adopted a protocol of targeted probiotic therapy prophylaxis for all infants after small intestine surgery who were at risk for intestinal hypoperistalsis, detrimental intestinal flora, malfunctioning gastrointestinal tract, invasive bacterial infection and necrotizing enterocolitis until full enteral feeding (100 ml/kg/day) is reached.

A probiotic (*B. breve* M-16V; Morinaga Milk Industry, Tokyo, Japan) was administered simultaneously with either breast milk or infant formula. *B. breve* (1 × 10⁹ cells in a package of 1 g) was suspended in 2 mL

Table 1 Comparison of newborns who did and did not receive probiotic therapy after small intestine surgery

	Probiotics (+) <i>n</i> = 18	Probiotics (-) <i>n</i> = 12	<i>p</i> -value
Gestational age, weeks	34.5 (23.5-38.4)	34.4 (26.4-40.0)	0.67
Birth weight, g	1978 (296-3646)	2004 (542-3522)	0.82
Age at surgery, days	4 (0-64)	1.5 (0-16)	0.13
Disease requiring surgery			
Small intestinal atresia	12	10	0.42
Localized intestinal perforation	6	2	
Surgical procedure			
Development of fistulae	11	6	0.71
Anastomosis	7	6	
Nutrition			
Age after surgery when enteral feeding was initiated, days	7.5 (3-13)	6.0 (3-10)	0.06
Type of nutrition			
Breast milk	7	5	1.0
Breast milk and formula or Artificial milk	11	7	
Family history of allergic disease	2 (11)	2 (17)	1.0
Cases of milk intolerance	1 (6)	8 (67)	<0.001

Data are shown as median (range) or *n* (%).

of sterilized water and 0.5 mL (2.5×10^8 *B. breve*) of the suspension was administered enterally 3 times a day (7.5×10^8 cells/day). We adopted the dosage of *B. breve* determined and reported by Kitajima, *et al.*⁶

As for the newborns who were treated with prophylactic probiotic therapy, stool cultures were obtained before surgery, before the commencement of, and a week after the probiotic therapy was completed.

DIAGNOSIS OF CMPI

We diagnosed CMPI with respect to the guidelines from Europe⁸ and Japan.⁹ By those guidelines, CMPI is diagnosed when; symptoms disappear after two doses of milk with eliminated cow's milk protein; other digestive disorder of the lactose intolerance and the infection, etc were excluded; and recurrence of identical symptoms were noted after one challenge of normal formula.

Based on the diagnostic standards of food allergy in Europe⁸ and Japan⁹ in our facility, we first suspected CMPI from symptoms such as weight loss, weight gain, lethargy, abdominal distension, vomiting, decreased feeding intake, diarrhea and bloody stool.

Next, we conducted various allergy tests including peripheral eosinophil count, a level of total immunoglobulin E, a level of specific IgE antibodies (against cow's milk, α -lactalbumin, β -lactoglobulin, and casein), fecal eosinophil, and other tests such as blood count, biochemical screening, C reactive protein (CRP) values, stool culture, X-ray examination, and Clinitest. Using the results of these tests, we eliminated milk if all other diseases other than CMPI were ruled out. We often experience the patients with CMPI accom-

panied with the rise of CRP values which resembles postoperative infection.

The milk protein tolerance test was performed after the patient was fasted in order to eliminate milk protein and two weeks after symptoms relieved. During the fasting period, nutrition was managed by parenteral nutrition. There are no unified opinion concerning particular method of milk protein tolerance test for newborn, such as the amount of milk to give and time interval of milk feeds. Therefore, we conducted the tests using formulas or mother's milk which contains the same milk protein which patients were given when they expressed the symptoms that were suspected of CMPI, with different loading doses by bodyweight. With respect to the nutrition management protocol for post small intestine operation in our facility, initial loading dose were assigned according to body weight at the time when milk protein tolerance test was performed. For bodyweight of less than 1 kg, 1 ml (<1 ml/kg/dose), for 1-1.5 kg, 1.5 ml (1-1.5 ml/kg/dose), and for over 1.5 kg, 5 ml (<3.3 ml/kg/dose) were the initial loading dose. The loads were given 8 times per day and the amounts were doubled every day.

These diagnostic standards were similar to those of "Food Protein-Induced Enterocolitis Syndrome: Consensus Recommendations for Diagnosis and Treatment" (Japanese Research Group for Neonatal, Infantile Allergic Disorders; http://www.nch.go.jp/imal/FPIES/FPIES_eng.pdf). However, the milk-specific lymphocyte stimulation responses recommended by this research group required a large quantity of blood collection, and since the intestinal mucosal biopsy is invasive, it could not be performed.

Table 2 Fecal culture of newborns who received probiotic therapy after small intestine surgery

Pathogens	Before operation	Before probiotics treatment	After probiotics treatment
Gram positive organisms	9	19	13
<i>Enterococcus spp.</i>	2 (11)	5 (28)	4 (22)
<i>Corynebacterium spp.</i>	2 (11)	4 (22)	1 (6)
<i>Staphylococcus-coagulase-negative</i>	5 (28)	8 (44)	8 (44)
<i>Staphylococcus aureus</i> (MRSA)	0 (0)	2 (11)	0 (0)
Gram negative organisms	1	9	2
<i>Escherichia coli</i>	1 (6)	3 (17)	1 (6)
<i>Klebsiella spp.</i>	0 (0)	4 (22)	1 (6)
<i>Acinetobacter spp.</i>	0 (0)	1 (6)	0 (0)
<i>Pseudomonas spp.</i>	0 (0)	1 (6)	0 (0)
Fungi	0	3	2
<i>Candida spp.</i>	0 (0)	3 (17)	2 (11)
Two or more types of pathogens	4 (22)	9 (50)	6 (33)
Free of pathogens	13 (72)	2 (11)	6 (33)

n (%). Numbers for gram positive organisms, gram negative organisms, and fungi include those with multiple pathogens.

Eosinophilia is defined in neonates as an eosinophil count ≥ 700 cells/mm³ and has been further subclassified as mild (700 to 999 cells/mm³), moderate (1000 to 2999 cells/mm³), or severe (>3000 cells/mm³).^{10,11}

STATISTICAL ANALYSIS

Software of SPSS 14.0 J (SPSS, Chicago, IL, USA) was used to generate summary statistics (mean, standard deviation, median and range values) for clinical variables. A group of patients with CMPI without probiotic therapy was compared with the group who used probiotic therapy by the Mann-Whitney U test. Proportions and continuous variables were compared using Fisher's exact two-tailed test.

RESULTS

DEMOGRAPHICS

Among all infants screened, 30 required surgery on their small intestine. 12 infants in the group with probiotic treatment and 18 infants in the group without probiotic treatment were included during this study. No differences in baseline demographics were noted. But, as for the initiation of the milk feeding, non probiotic group tended to be earlier ($p = 0.06$, Table 1).

INCIDENCE OF CMPI AND EFFICACY OF PROBIOTIC THERAPY

One infant among those eighteen patients who received probiotics and eight infants out of twelve that did not developed CMPI, a significantly lower rate for the patients with probiotic therapy than those without probiotic therapy (Table 1, $p < 0.001$). No adverse effect such as bacteremia, sepsis, and infection with anaerobic bacteria were observed in patients who were given probiotics.

The number of neonates who had positive stool

cultures for either of gram positive or gram negative organisms or both increased after surgery before probiotics treatment was started, but decreased after a week of probiotics treatment. Moreover, after initiation of probiotics therapy, the number of patient with negative culture increased from two to six (Table 2).

CHARACTERISTICS OF CMPI PATIENTS

Clinical symptoms, including poor weight gain and gastrointestinal symptoms such as diarrhea and bloody stool, were observed in all of nine milk intolerance subjects. All patients except patient # 2 did not use probiotics (Table 3). CMPI was diagnosed at the time range of 4 to 61 days (median 31) after the start of enteric nutrition.

Among 8 patients who did not use probiotics and diagnosed as CMPI, two patients, patients #4 and #8, were fed by breast milk, patient #3 was fed by artificial formula and other 5, patients #1, 5, 6, 7 and 9 were fed by both breast milk and formula. On the other hands, patient #2 who had probiotics but established CMPI was fed by both breast milk and formula (Table 3).

IgE levels specific for major bovine milk proteins as well as total IgE were elevated in four patients diagnosed as CMPI including patient #2. Other three infants had increased levels of total IgE but had no rise of IgE specific for major bovine milk antigens tested (Table 3).

Although CMPI was suspected, the total IgE values in both the samples negated in the food tolerance test were less than 2 kU/L, that is, below the detection sensitivity.

All patients with CMPI increased CRP values without aberrant value of white blood cell count and ratios of bands to segment forms were also within normal range.

Table 3 Profile of the patients who developed milk intolerance after small intestine surgery

Individual no.	Sex	Period from		Symptoms	Age at diagnosis (days)	Specific IgE					Eosinophil in blood (μL , %)	Fecal eosinophils	Symptoms in challenge test	Dietary treatment
		operation to start of enteral nutrition (days)	start of enteral nutrition to diagnosis (days)			Total IgE (kU/L)	$\alpha\text{-La}$ (kU/L)	$\beta\text{-Lg}$ (kU/L)	Casein (kU/L)	Cow's milk (kU/L)				
1	m	4	61	F, D, Pwg	68	29	1.39	0.63	0.43	0.86	2352, 24	+	D	HA
2 [†]	m	6	31	D, Pwg	42	8.69	<0.35	<0.35	0.65	0.38	4379, 29	-	D	A
3	f	6	23	F, E	35	272	0.54	0.48	<0.35	0.55	980, 14	-	E, U, S	H
4	m	6	5	B, Pwg, E	13	23	<0.35	<0.35	<0.35	<0.2	1078, 14	-	B	A
5	f	6	38	D, Pwg, E	59	26	<0.35	<0.35	<0.35	<0.2	1729, 13	-	U	A
6	m	3	32	B, D	35	<2	<0.35	<0.35	<0.35	<0.2	1768, 15	+	D	HA
7	m	3	4	D, Pwg	21	<2	<0.35	<0.35	<0.35	<0.2	969, 8.5	-	B, D	H
8	m	10	14	D, Pwg, P	12	16	<0.35	<0.35	<0.35	<0.2	3502, 17	-	D	A
9	m	5	32	D, Pwg	37	23	1.84	0.96	4.5	2.49	3588, 26	-	D	A

f, female; m, male; B, bloody stool; D, diarrhea; E, emesis; F, fever; Pwg, poor weight gain; P, psychoresthesis; S, shock; $\alpha\text{-La}$, α lactalbumin; U, urticaria; $\beta\text{-Lg}$, β lactoglobulin; +, fecal eosinophils were found; -, fecal eosinophils not found; H, hyper hydrolyzed milk; A, amino acid-milk formula. No effects on HA or hydrolyzed milk, patients used amino-acid milk formula.

[†] Patients except individual # 2 did not use probiotics.

DISCUSSION

The incidence of CMPI among newborns has been reported previously as 2-5%.¹² In this present study, newborns who received small intestine surgery and antibiotics showed a high incidence (67%) of CMPI when they were not given probiotic treatment. However, incidence of CMPI became 6% by prophylactic probiotic administration. This incidence rate of CMPI is similar to that for the patients without operation.

In a similar research, milk hypersensitivity incidence rate after gastrointestinal tract surgery reported by EL. Hassani¹³ was 4.3%, which was evidently lower than the CMPI incidence rate of 67% observed in our research in cases where probiotics were not used. The reason for this could be that the concept of post gastrointestinal tract surgery milk hypersensitivity is just not prevalent in France, as pointed out in the thesis by EL. Hassani, as well as in many other countries. The second reason could be the difference in target organs. Our research only dealt with post small intestinal surgery cases, whereas theirs targeted post gastrointestinal tract surgery of stomach, small intestine, and large intestine.

Another possible reason is the presence of allergic colitis, along with neonatal necrotizing enterocolitis (NEC) and CMPI in inflammatory intestinal mucosal lesions occurring in neonates.¹⁴ Interestingly, there have been very few cases of NEC incidence in Japan as compared to other countries.¹⁵ From the fact that the symptoms of CMPI are very similar to the symptoms of NEC, probably CMPI is included in the diagnostic symptoms of NEC in other countries, and that Japan is more prone to suspecting CMPI as compared to other countries. Moreover, one of the triggers of NEC is bacterial infection. The variation in the use of antibiotics in Japan and other countries may be decreasing the incidence rate of NEC while increasing the incidence of CMPI. In either of the cases, there is variation in the data of inflammatory gastrointestinal mucosal lesions in Japan and other countries, requiring more research.

Oral tolerance is defined as a state of immunological unresponsiveness to a specific antigen after exposure to that antigen.¹⁶ It is now widely accepted that mechanisms of oral tolerance include anergy, apoptosis (deletion) of exogenous antigen-specific T cells in the gut and active suppression through induction of exogenous antigen-specific regulatory T cells. IL-10 and TGF- β -producing regulatory T cells play important roles in maintaining intestinal homeostasis.¹⁷ The microbiota in the gut can play an important role in generating fully functional regulatory T cells.¹⁸ The possibility that *B. breve* can help inhibiting inflammation and controlling the onset of allergies has been predicted, because it induces TGF- β 1 and adjusts the balance between Th1 and Th2 cells.¹⁹ Immune toler-

ance to food antigens ingested orally has not been established in mice with treatment to eliminate intestinal flora in neonatal period.³

In the present investigation the high incidence of milk intolerance in newborns who did not receive probiotic therapy appeared to be due to direct surgical invasion to the intestines. It has been reported that milk intolerance is apt to occur after colorectal surgery.¹³ Surgery of small intestine results in keeping the intestinal immune system away from food antigens for particular period of time, possibly losing a chance to induce oral tolerance to food antigens, some of which are milk antigens for infants. In addition, we assumed that colorectal surgery disrupted their intestinal flora, leading to CMPI. Use of antibiotics delayed the start of enteric nutrition and may have disrupted intestinal flora without inducing oral tolerance by anergy or apoptosis of exogenous antigen-specific T cells or activation of regulatory T cells, as described previously.¹⁷

Conversely, newborns who were given probiotics showed normal or strengthened intestinal immunity and improved oral tolerance to milk, most likely because the profile of microbiota in their gastrointestinal tract was rectified. Patients with positive cultures for gram positive and gram negative organisms increased in number before and after surgery but then decreased after probiotics treatment (Table 2). From the fact that probiotics are known to be efficient in improving the microbial environment in the gastrointestinal tract, there is a possibility that improvement of the intestinal environment by administration of probiotics may improve the incidence rate of CMPI. Furthermore, in animal experiments, the probiotic MV16 used in this research was found to possess anti-allergic properties,²⁰ which may help to inhibit the incidence of CMPI.

A recent randomized study showed that supplementing extensively hydrolyzed formula with probiotics does not decrease cow's milk intolerance in infants with milk allergy.²¹ In that study,²¹ probiotics was given after they were diagnosed of CMPI. However, in this study we applied probiotics before the diagnosis of CMPI was made. Probiotics may be effective not for treatment of, but prevention from developing CMPI. Also probiotic therapy probably had preventive effects on intolerance only in newborns with disrupted intestinal flora such as newborn treated with antibiotics.

B. breve was used in the present study because this bacteria is derived from human neonatal intestine and its bacterial translocation ability was extremely low compared with other probiotic bacteria.²² No adverse reactions, such as sepsis, have been reported in the use of *B. breve*. This bacterium is considered very safe for use in probiotic treatment. No adverse reactions were observed in any of the newborns in the present study.

The symptoms of CMPI in newborns are usually gastrointestinal and the mechanism of food allergy is independent of IgE.⁹ However, it has been shown that patients allergic to cow's milk had serum IgE specific for bovine milk proteins.²³ In the present study, the symptoms were also mainly gastrointestinal. IgE, RAST, eosinophil counts and neutrophil counts in the feces were examined in all subjects with CMPI. It was interesting that IgE antibodies specific for milk components were elevated in four of nine subjects. At the time of CMPI diagnosis, out of the 5 cases where there was no rise in specific IgE antibodies, there was 1 case with positive milk-specific IgE antibodies when evaluated at 2 months (patient #5).

In our hospital the total IgE is less than 5 kU/L in normal newborns. In this study the total IgE was 5 kU/L or higher in seven of nine patients with milk intolerance. When the intestinal flora of newborns was disrupted by antibiotics, increase in the production of IgE occurs,² and so we can speculate this response may contribute to form milk intolerance.

Except for 2 samples of eosinophil count, which is a factor in the determination of CMPI, the samples were classified between moderate to severe. The 2 samples classified as mild also showed values close to moderate (Table 2).

What makes the result even more interesting is that the patient of all CMPI showed the rise of CRP level. It has been reported that the amount of IL-6 in the duodenum increases with the development of CMPI,²⁴ and we herein focused our attention on its mediator, CRP.^{25,26} Serum CRP is easier to measure than IL-6, with the advantage that it takes a shorter time to obtain the test results. Rise in serum CRP can be used as one of a supportive factor in diagnosis of CMPI.

In conclusion, CMPI was induced in newborns after small intestine surgery and it is possible that disruption of intestinal flora is involved. Administration of *B. breve* reduced the incidence of CMPI after small intestine surgery. In the NICU, efforts should be made to improve intestinal flora in consideration of the risk of onset of CMPI after small intestine surgery. The measurement of CRP level seems to be effective for the diagnosis of CMPI.

ACKNOWLEDGEMENTS

The first author expresses his gratitude to the Dr. Yunosuke Ogawa, Dr. Akira Nishida, and Dr. Kazuo Itabashi for stimulating his interest in neonatology. The first author also thanks Dr. Yuko Hayase and Munenori Ezaki for valuable assistance and support throughout this study.

CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

REFERENCES

1. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003;**361**:512-9.
2. Oyama N, Sudo N, Sogawa H, Kubo C. Antibiotic use during infancy promotes a shift in the T(H)1/T(H)2 balance toward T(H)2-dominant immunity in mice. *J Allergy Clin Immunol* 2001;**107**:153-9.
3. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997;**159**:1739-45.
4. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999;**69**:1035S-45.
5. Madden JA, Plummer SF, Tang J *et al*. Effect of probiotics on preventing disruption of the intestinal microflora following antibiotic therapy: a double-blind, placebo-controlled pilot study. *Int Immunopharmacol* 2005;**5**:1091-7.
6. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 1997;**76**:F101-7.
7. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. *Lancet* 2007;**369**:1614-20.
8. The European Society for Paediatric Gastroenterology and Nutrition Working Group for the Diagnostic Criteria for Food Allergy. Diagnostic criteria for food allergy with predominantly intestinal symptoms. *J Pediatr Gastroenterol Nutr* 1992;**14**:108-12.
9. Ebisawa M. Management of food allergy in Japan "food allergy management guideline 2008 (revision from 2005)" and "guidelines for the treatment of allergic diseases in schools". *Allergol Int* 2009;**58**:475-83.
10. Gibson EL, Vaucher Y, Corrigan JJ Jr. Eosinophilia in premature infants: relationship to weight gain. *J Pediatr* 1979;**95**:99-101.
11. Juul SE, Haynes JW, McPherson RJ. Evaluation of eosinophilia in hospitalized preterm infants. *J Perinatol* 2005;**25**:182-8.
12. Host A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. *Pediatr Allergy Immunol* 1994;**5**:1-36.
13. El Hassani A, Michaud L, Chartier A *et al*. [Cow's milk protein allergy after neonatal intestinal surgery]. *Arch Pediatr* 2005;**12**:134-9 (in French).
14. Fell JM. Neonatal inflammatory intestinal diseases: necrotising enterocolitis and allergic colitis. *Early Hum Dev* 2005;**81**:117-22.
15. Kawase Y, Ishii T, Arai H, Uga N. Gastrointestinal perforation in very low-birthweight infants. *Pediatr Int* 2006;**48**:599-603.
16. Weiner HL, Friedman A, Miller A *et al*. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994;**12**:809-37.
17. Tsuji NM, Kosaka A. Oral tolerance: intestinal homeostasis and antigen-specific regulatory T cells. *Trends Immunol* 2008;**29**:532-40.
18. Ishikawa H, Tanaka K, Maeda Y *et al*. Effect of intestinal microbiota on the induction of regulatory CD25+ CD4+ T cells. *Clin Exp Immunol* 2008;**153**:127-35.
19. Fujii T, Ohtsuka Y, Lee T *et al*. Bifidobacterium breve enhances transforming growth factor beta1 signaling by regulating Smad7 expression in preterm infants. *J Pediatr Gastroenterol Nutr* 2006;**43**:83-8.
20. Hougee S, Vriesema AJ, Wijering SC *et al*. Oral treatment with probiotics reduces allergic symptoms in ovalbumin-sensitized mice: a bacterial strain comparative study. *Int Arch Allergy Immunol* 2010;**151**:107-17.
21. Hol J, van Leer EH, Elink Schuurman BE *et al*. The acquisition of tolerance toward cow's milk through probiotic supplementation: a randomized, controlled trial. *J Allergy Clin Immunol* 2008;**121**:1448-54.
22. Deitch EA. Role of bacterial translocation in necrotizing enterocolitis. *Acta Paediatr Suppl* 1994;**396**:33-6.
23. Lin J, Sampson HA. The role of immunoglobulin E-binding epitopes in the characterization of food allergy. *Curr Opin Allergy Clin Immunol* 2009;**9**:357-63.
24. Paajanen L, Vaarala O, Karttunen R, Tuure T, Korpela R, Kokkonen J. Increased IFN-gamma secretion from duodenal biopsy samples in delayed-type cow's milk allergy. *Pediatr Allergy Immunol* 2005;**16**:439-44.
25. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med* 2002;**252**:283-94.
26. Bennet AM, Prince JA, Fei GZ *et al*. Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction. *Atherosclerosis* 2003;**171**:359-67.