

Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects

Shinichi Watanabe, MD,^a Yutaka Narisawa, MD,^b Seiji Arase, MD,^c
Hiroshi Okamatsu, MS,^d Takeshi Ikenaga, MS,^d Yoshito Tajiri, MS,^d and
Megumi Kumemura, MS^d Tokyo, Saga, Tokushima, and Otsu, Japan

Background: The prevalence of allergic diseases, such as atopic dermatitis (AD), has been increasing. However, few investigations have been made of the intestinal microflora in Japanese patients with AD.

Objective: The purpose of this study was to determine the differences in microflora, fecal serum IgA concentrations, and skin IgA contents between patients with AD and healthy control subjects.

Methods: This trial was conducted as a case-control study using 30 minor patients with AD and age- and sex-matched healthy control subjects (n = 68). One week after a questionnaire was administered, fecal specimens and 24-hour skin secretion specimens were collected from all subjects. Fecal microflora, fecal IgA concentrations, and IgA contents on the skin surface were analyzed.

Results: The counts of *Bifidobacterium* (in log₁₀ colony-forming units per gram) were significantly lower in patients with AD than in healthy control subjects (9.75 ± 0.68 vs 10.10 ± 0.50 log₁₀ colony-forming units/g, $P < .05$). In particular, percentages of *Bifidobacterium* were significantly lower in patients with severe skin symptoms than in those with mild skin symptoms ($40\% \pm 6\%$ vs $19\% \pm 6\%$, $P < .05$). In addition, the frequency of occurrence of *Staphylococcus* was significantly higher in patients with AD than in healthy control subjects (83% vs 59% , $P < .05$). There were no significant differences in fecal IgA content or IgA content on the skin between the 2 groups.

Conclusion: Patients with AD had lower counts of *Bifidobacterium* than healthy control subjects, and the frequency of *Staphylococcus* was higher in patients with AD than in control subjects. Disorder of the intestinal microflora might play a role in the onset of AD and the aggravation of skin symptoms. (J Allergy Clin Immunol 2003;111:587-91.)

Key words: Allergy, atopic dermatitis, microflora, Bifidobacterium, Bacteroides, Staphylococcus, fecal IgA, skin IgA, minority, case-control study

The prevalence of allergic diseases has been continuously on the increase in many countries.^{1,2} Recent epidemiologic studies have shown that the prevalence of atopic dermatitis (AD) is much higher among children in urban areas than among those in suburban areas.³ AD is a chronically relapsing skin disorder that most commonly arises during early infancy, childhood, or adolescence. It is frequently associated with increased serum IgE levels and a personal or family history of at least one of the following: AD, allergic rhinitis, and asthma.

Recently, a new theory, the hygiene hypothesis, has been proposed, according to which the prevalence of allergic disorders is increasing in industrialized countries partly because human subjects there are less likely to be exposed to a variety of pathogenic and nonpathogenic microorganisms in the gastrointestinal tract during infancy.⁴⁻⁶ In particular, the relationship between intestinal microflora and the cause of allergic diseases has received considerable research and clinical attention. Sudo et al⁷ conducted in vivo studies using germ-free mice and demonstrated that the presence of microflora in the gut is necessary for the establishment of oral immune tolerance to IgE. Recently, several investigators examined the intestinal microflora in allergic infants and young children between 1 and 2 years of age who had positive reactions to skin prick tests and allergic symptoms, such as AD and food allergy, and found that allergic infants had low levels of lactic acid-producing bacteria in the feces.⁸⁻¹⁰ Taken together, these findings indicate that intestinal microflora is associated with development of the immune system in the gut during infancy and early childhood. Kalliomäki et al¹⁰ have emphasized the importance of administration of probiotic bacteria for the primary prevention of atopic disease by means of regulation of intestinal microflora.

The prevalence of allergic diseases, such as AD, is increasing in Japan, possibly as a result of improvements in health and hygiene.¹¹ Few studies have examined the composition of intestinal microflora in patients with AD less than 20 years of age in Japan. Therefore we conducted a case-control study to compare the composition of fecal

From ^athe Department of Dermatology, Teikyo University School of Medicine, Tokyo; ^bthe Division of Dermatology, Department of Internal Medicine, Saga Medical School, Saga; ^cthe Department of Dermatology, School of Medicine, the University of Tokushima, Tokushima; and ^dOtsu Nutraceuticals Research Institute, Otsuka Pharmaceutical Co, Ltd, Otsu. Received for publication May 7, 2002; revised October 14, 2002, and October 29, 2002; accepted for publication October 30, 2002.

Reprint requests: Megumi Kumemura, MS, Otsu Nutraceuticals Research Institute, Otsuka Pharmaceutical Co, Ltd, 31-13-3, Saigawa Otsu-Shi, Shiga 520-0002 Japan.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.105

Abbreviations used

AD: Atopic dermatitis
CFU: Colony-forming units
SCB: Sodium carbonate buffer

microflora, fecal IgA levels, and IgA content on the skin surface in patients with AD who were less than 20 years of age with those in age- and sex-matched healthy control subjects. The results of this clinical trial are presented here.

METHODS**Subjects**

The study cohort comprised 30 patients with AD less than 20 years of age and age- and sex-matched control subjects (68 healthy individuals). All 30 patients were given a diagnosis of AD in accordance with the criteria for the diagnosis of AD established by the Japanese Dermatological Association.¹² Skin manifestations of patients included erythema, papules, scaling, excoriation, lichenification, and itching. The severity of skin symptoms was rated by using the following 4-point scale: 0, absence; 1, mild; 2, moderate; and 3, severe. Control subjects were selected from among healthy volunteers in accordance with the following criteria. First, control subjects and their families did not have a past history of any of the following diseases: bronchial asthma, allergic rhinitis, allergic conjunctivitis, and AD. Second, control subjects had no skin symptoms at the time of the study. We excluded subjects who had been treated with antibiotics or antifungal agents within 1 month before the start of the trial and those who had eaten yogurt or foods containing live bacteria within 7 days before initiation of the study. We explained the details of the trial to each subject and his or her guardians and obtained informed consent to participate in the trial (ie, the written informed consent form was signed by the subject or his or her guardians) before participation in the trial. This study was conducted in accordance with the spirit of the Helsinki Declaration under the supervision of physicians.

Study design

This trial was conducted as a case-control study with age- and sex-matched patients and healthy control subjects. A questionnaire survey was conducted for 1 week, and then fecal specimens and 24-hour skin secretion specimens were collected from all subjects. Fecal microflora, fecal IgA levels, and IgA concentrations in the skin were analyzed. Statistical analysis was performed, and the data for the 2 groups (ie, patients with AD and healthy control subjects) were compared.

Collection of fecal samples

Fecal specimens were collected on day 7 after initiation of the questionnaire survey. Approximately 2 g of a fresh fecal sample was collected in an anaerobic transport container (seed tube, Eiken Chemical Co, Ltd), and approximately 10 g of a fresh stool sample was collected in a plastic cup. Fecal specimens collected in anaerobic transport containers were stored in a refrigerator and used for a bacterial culture test within 24 hours. Fecal specimens collected in plastic cups were stored frozen at -80°C until use (determination of IgA concentrations in stool).

Collection of dermal secretion product

On day 5 after initiation of the questionnaire survey, a 24-hour skin secretion product was harvested. After rinsing and drying the skin on the inner side of the forearm, excluding atopic lesions, a disc for col-

lection of skin secretion product was patched on the skin for 24 hours. The disc was prepared with 10×10 mm nitrocellulose membrane (Bio-Rad, 162-0115), 12×12 mm Whatman filter paper (Whatman, 60×60 conical filter paper), and 20×30 mm adhesive tape (Transpore, 3M). The disc was collected 24 hours later and stored frozen at -80°C until analysis (determination of IgA level in skin).

Bacteriologic analyses

Determination of fecal microflora was performed according to the method of Mitsuoka.¹³ Four nonselective media (Brucella HK agar, BL agar, sheep blood agar medium M58, and CLED medium) and 8 selective media (BBE agar, Kanamycin CW agar, LBS-modified agar, Villonella agar, mannitol salt agar, EF agar, DHL agar, and potato dextrose agar) were used for isolation of intestinal bacteria. Approximately 1.0 g of fresh fecal specimen was weighed and suspended in 9 mL of anaerobic diluent. Then a series of 10-fold dilutions (10^1 - 10^8) was prepared with diluent in an atmosphere of carbon dioxide gas. A given amount of each dilution (0.05 mL) was inoculated onto each of the 4 nonselective media and 8 selective media. After inoculation, plates containing Brucella HK agar, BL agar, BBE agar, Kanamycin CW agar, LBS-modified agar, and Villonella agar were placed in a plastic anaerobic jar filled with an atmosphere of oxygen-free CO_2 gas and incubated in an anaerobic environment at 37°C for 72 hours. Plates containing sheep blood agar M58, CLED medium, mannitol salt agar, EF agar, DHL agar, and potato dextrose agar were incubated at 37°C for 48 hours. After incubation, identification of the genus of bacteria was performed on the basis of the number of colonies on each medium, characteristics of colonies, Gram reaction (Gram staining), spore formation, aerobic growth, and cellular morphologies, as determined by means of light microscopy. Bacterial counts per gram of wet feces were calculated and transformed to logarithmic values ($\log_{10}$ colony-forming units [CFU] per gram of wet feces).

Measurement of fecal IgA

Fecal IgA concentrations were determined according to the method of Tsuyuki et al.¹⁴ Fecal specimens were freeze-dried, and 1.0 mL of 0.1 mol/L bicarbonate buffer solution (pH 9.6) was added to 0.1 g of freeze-dried fecal specimens. After suspending the fecal samples in bicarbonate buffer solution thoroughly, the fecal suspension was centrifuged at 15,000 rpm for 10 minutes. The supernatant was used for determination of fecal IgA concentrations. Fecal IgA concentrations were measured with an ELISA, according to the method of Tsuyuki et al.

Ninety-six-well microtiter ELISA plates (Nunc-immuno plate II, Nunc) were coated with 50 μL per well of polyclonal mouse anti-human IgA antibody (Biosource) diluted 1:40 in sodium carbonate buffer (SCB; 0.1 mol/L, pH 9.6) and incubated at 4°C for overnight. After removing the solution, each well was blocked with 200 μL of 1% BSA. The plates were then incubated at 37°C for 60 minutes. After washing with buffer (SCB-Tween 0.05%), 40 μL of standard solution and sample solution was added to the wells in duplicate. As standard solution, human IgA (ICN Pharmaceuticals, Inc) diluted 2-fold (range, 1.25-80 ng/mL) in SCB-BSA was used. After washing, the plates were incubated with 40 μL of horseradish peroxidase-conjugated mouse anti-human IgA antibody (Biosource) diluted 1:1000 in SCB-BSA. To develop the reaction, 200 μL of *p*-nitrophenyl phosphate (FAST pNPP, Sigma-Aldrich) was used as a substrate. The reaction was stopped after 30 minutes by adding 50 μL of 3 mol/L sodium hydroxide. The OD was read at 490 nm.

Measurement of IgA on the skin surface

The skin IgA level was determined according to the method of Imayama et al.¹⁵ The disc was thawed, and the nitrocellulose mem-

brane was peeled off. The discs were initially incubated with PBS (pH 7.2) containing 1% BSA to block nonspecific reactions for 2 hours. After thorough rinsing with 50 mmol/L Tris buffer (pH 7.4) containing 150 mmol/L sodium chloride and Tween 20 (Merck), the disks were incubated for 1 hour with a 1:1000 dilution of peroxidase-conjugated anti-human IgA goat polyclonal antibody (Cappel). Specimens were again rinsed with Tris buffer solution, and color reaction was initiated with a horseradish peroxidase conjugate substrate kit (Bio-Rad). Finally, the amount of immunoreactive product per square millimeter was determined with a densitometer (AE-6900, Atto)

Statistical analysis

First, we performed regression analysis to determine whether age or sex affected the counts of bacteria in the gut, frequencies of occurrence, IgA concentrations in the skin, fecal IgA concentrations, or ratings on the severity scale. If the data exhibited a normal distribution, a *t* test was applied to compare values between the 2 groups. If the data did not exhibit a normal distribution, the Mann-Whitney *U* test was applied to compare values between the 2 groups. The rates of detection of intestinal microflora were compared by using the χ^2 test.

Ethical considerations

Written informed consent was obtained from the parents of the children. The study was approved by the Ethical Committee of Teikyo University School of Medicine.

RESULTS

Clinical characteristics of the subjects

Thirty children with AD (7.6 ± 5.0 years, 16 boys and 14 girls) and 68 age- and sex-matched control subjects (6.5 ± 4.4 years, 31 boys and 37 girls) participated in this study. Most patients had mild-to-moderate skin symptoms. The median values of severity scores in the AD group were as follows: erythema, 1; papules, 1; scaling, 2; excoriation, 1; lichenification, 1; and itching, 1. The mean values were as follows: erythema, 1.5 ± 0.6 ; papules, 1.3 ± 0.5 ; scaling, 1.2 ± 0.5 ; excoriation, 1.7 ± 0.5 ; lichenification, 1.3 ± 0.5 ; and itching, 1.7 ± 0.5 . Duration of illness in the AD group was 6.6 ± 5.3 years. Sixteen of the 30 patients had received topical steroid therapy. Twelve patients had been treated with emollients alone.

In the present study there was no significant correlation between age and bacteria counts in the gut, frequency of occurrence, IgA concentrations in the skin, or fecal IgA concentrations. There were no sex-related differences in those parameters. Therefore statistical analysis was performed to compare the 2 groups without age- and sex-based compensation.

Fecal microflora

The composition of the fecal microflora in atopic patients and healthy individuals is shown in Table I. The table shows the counts and the frequency of occurrence of intestinal microorganisms. The counts of *Bifidobacterium* were significantly ($P = .006$) lower in patients with AD (9.75 ± 0.68 log CFU/g wet feces) than in healthy individuals (10.10 ± 0.50 log CFU/g wet feces). *Bifidobacterium* was not detected in feces collected from

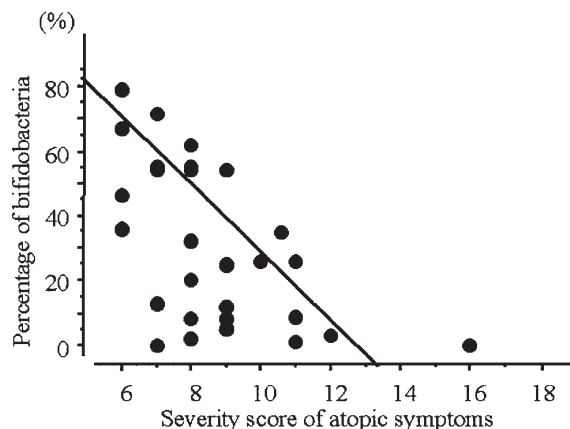


FIG 1. Relationship between the percentage of *Bifidobacterium* species and the severity score of atopic symptoms of individual patients with AD ($R = 0.470$, $P < .001$).

the 2 patients with AD who had the most severe symptoms of the 30 patients.

The total fecal microflora count tended to be lower in patients with AD (10.54 ± 0.46 log CFU/g wet feces) than in healthy individuals (10.70 ± 0.40 log CFU/g wet feces, $P = .097$). The counts of *Clostridium*, *Streptococcus*, and *Enterobacteriaceae* also tended to be lower in patients with AD than in healthy individuals. The frequency of occurrence of *Staphylococcus* was significantly higher in patients with AD (83%) than in healthy individuals (59%, $P = .0209$).

We also examined the relationship between the severity of skin symptoms and fecal microflora. The severity rating of skin symptoms of patients with AD (erythema, papules, itching, scaling, excoriation, and lichenification) was scored. The counts and percentages of fecal microflora in the mild group (total score, 1-8 points; 18 patients) and the moderate or severe group (more severe group: total score, ≥ 9 points; 12 patients) were compared. The counts of *Bifidobacterium* were significantly ($P = .0406$) lower in the more severe group (9.52 ± 0.51 log CFU/g wet feces) than in the mild group (9.90 ± 0.74 log CFU/g wet feces). Moreover, the percentage of *Bifidobacterium* was significantly ($P = .0038$) lower in the more severe group ($19\% \pm 6\%$) than in the mild group ($40\% \pm 6\%$). On the other hand, the percentage of *Bacteroides* was significantly ($P = .0443$) higher in the more severe group ($62\% \pm 7\%$) than in the mild group ($37\% \pm 7\%$). There was a significant negative correlation ($R = -0.472$, $P = .0077$) between the percentage of *Bifidobacterium* and the individual subject severity score (Fig 1).

Fecal IgA and skin IgA concentrations

There was no significant difference in fecal IgA content between patients with AD (2.61 ± 2.41 mg/g) and healthy control subjects (1.78 ± 1.12 mg/g) nor was there a significant difference in secretion-type IgA concentrations in nonatopic skin lesions (patients with AD, 2.17 ± 4.61 pg/mm²; healthy control subjects, 1.69 ± 5.02 pg/mm²).

TABLE I. Fecal microflora in the AD and control groups

	Atopic dermatitis group (n = 30)		Control group (n = 68)		P value
	Fecal microflora count*	Frequency of occurrence	Fecal microflora count*	Frequency of occurrence	
<i>Staphylococcus</i>	4.04 ± 1.22	83%	4.25 ± 1.21	59%†	.497
<i>Enterococcus</i>	6.75 ± 2.10	93%	7.17 ± 1.64	97%	.298
<i>Streptococcus</i>	7.60 ± 1.18	47%	8.33 ± 1.14	47%	.055
<i>Enterobacteriaceae</i>	7.23 ± 1.32	97%	7.69 ± 0.94	100%	.052
Yeast-like organism	3.04 ± 0.83	43%	3.34 ± 0.89	41%	.306
<i>Bifidobacterium</i>	9.75 ± 0.68	93%	10.10 ± 0.50	100%	.006
<i>Eubacterium</i>	9.50 ± 0.75	93%	9.68 ± 0.69	99%	.269
<i>Lactobacillus</i>	5.27 ± 1.59	77%	4.89 ± 1.80	76%	.387
<i>Clostridium perfringens</i>	4.57 ± 1.80	50%	5.36 ± 1.53	40%	.142
<i>Clostridium</i>	7.10 ± 1.04	77%	7.47 ± 0.71	88%	.071
<i>Peptostreptococcus</i>	8.63 ± 1.20	57%	8.97 ± 0.61	78%	.124
<i>Veillonella</i>	6.71 ± 1.69	53%	6.35 ± 1.78	51%	.492
<i>Bacteroides</i>	10.02 ± 0.71	100%	10.19 ± 0.63	100%	.241
Total counts	10.54 ± 0.46		10.70 ± 0.40		.097

*Results are presented as means ± SD and as log CFU per gram of wet feces.

†P < .05.

DISCUSSION

In the present study we compared the composition of intestinal microflora in patients with AD (all were minors) with that in age- and sex-matched healthy control subjects and confirmed that the counts of *Bifidobacterium* were significantly lower in patients with AD than in healthy individuals. Furthermore, we performed a stratified analysis on the basis of the severity rating of symptoms and found that the counts and percentages of *Bifidobacterium* were significantly lower in those with severe AD than in patients with mild atopic symptoms. In addition, the frequency of occurrence of *Staphylococcus* was significantly higher in patients with AD than in healthy individuals.

Björkstén et al⁸ investigated intestinal microflora in 62 allergic Estonian and Swedish 2-year-old children, including children with AD, and found that in both countries allergic children were less often colonized with *Lactobacillus* than nonallergic children. They also demonstrated that the prevalence of colonization with *Bifidobacterium* was lower during the first year of life in infants who had allergy than in those who did not.¹⁶ Böttcher et al⁹ studied the composition of intestinal microflora-derived volatile fatty acids in feces collected from 25 allergic infants (including infants with AD) and 47 nonallergic infants and found that the fecal level of *Clostridium difficile*-derived I-caproic acid was significantly higher in allergic than in nonallergic infants. Kalliomäki et al¹⁰ conducted a prospective study to determine the composition of intestinal microflora in infants with a family history of atopic disorders by means of fluorescence in situ hybridization and demonstrated that counts of *Clostridium* were significantly higher at week 3 after birth in infants who had AD by 13 months after birth than in those who did not have AD. In contrast, counts of *Bifidobacterium* tended to be lower at week 3 after birth in infants who had AD than in those who did

not have AD. Our results were in accordance with those reported by Kalliomäki et al in that counts of *Bifidobacterium* were lower in children who had AD than in those who did not have AD. Moreover, this difference in counts was more striking in patients who had severe atopic symptoms. Intradermal administration of killed *Mycobacterium vaccae* suspension suppressed T_H2-type allergic response and decreased the severity of AD. In addition, oral administration of *Bifidobacterium* stimulates the production of T_H1-type cytokines and leads to T_H1-dominant immunity.¹⁷ Therefore the amount of *Bifidobacterium* in the intestine might be related to the onset of allergic disease in the host. It has been suggested that the prevalence and counts of *Bifidobacterium* are decreased with aging^{18,19} and by stress,²⁰ inflammatory bowel disease,²¹ cancer,²² antibiotics, and other factors and that this disturbance of microflora affects host conditions, such as digestion of nutrients, drug efficacy, carcinogenesis, and immune response.¹⁹

In the present study the frequency of occurrence of *Staphylococcus* was significantly higher in patients with AD than in healthy individuals. The frequency and counts of *Staphylococcus aureus* were higher in the skin lesions of patients with AD than in healthy individuals.²³ Recently, many investigators have demonstrated that dermal *Staphylococcus aureus*-derived exotoxins modulate the severity of AD.²⁴⁻²⁶ Our results suggest that colonization with *Staphylococcus aureus* in the intestine could influence skin symptoms in patients with AD.

The immunologic abnormalities observed in patients with AD might be related to the onset of AD. Decreased levels of secretion-type IgA contribute to increased susceptibility to infection and increased permeability of allergens through the skin barrier. Iikura et al²⁷ analyzed fecal IgA levels in children with mild AD and patients with intractable AD and found that fecal levels of secretion-type IgA were lower in cases of serious AD than in those of mild or moderate AD. In addition, Imayama et

al²⁸ compared skin IgA concentrations in nonatopic lesions of adult patients with AD with those in healthy individuals and found that skin IgA levels were significantly lower in patients with AD than in healthy control subjects. In the present study, however, there were no significant differences in skin or intestinal IgA levels (secretion-type IgA) between patients with AD and healthy individuals. All patients who participated in this study had mild-to-moderate AD symptoms, and all were minors, possibly accounting for the discrepancy between the results obtained by Imayama et al²⁸ and our own results. In addition, we did not forbid eating lactic acid before collection of fecal specimens in the present study, and the IgA concentration in the gut is probably affected by dietary antigens, such as lactic acid bacteria. Care is needed in future studies as well in this respect.

In the present study we found that counts of *Bifidobacterium* were significantly lower in patients with AD less than 20 years of age than in age- and sex-matched healthy individuals and that the counts and frequency of *Bifidobacterium* were lower in atopic patients with more severe skin symptoms. Recently, Kalliomäki et al²⁹ conducted a randomized, placebo-controlled, double-blind comparative study in an attempt to prevent the onset of allergic disorders by use of probiotics in neonates in Finland. In their study the prevalence of allergic disorder was significantly lower in infants inoculated with probiotics during the first 6 months after birth than in those administered placebo capsules.

In the near future, the relationship between intestinal microflora and immunity-allergy in the gut will doubtless receive increased attention. Despite recent advances in this field, the cause of AD is still largely unknown. Because some bacteria in the gut are uncultivable, further studies will be needed to elucidate the relationship between AD and intestinal microflora.

REFERENCES

- Larssen, FS, Holm, NV, Henningsen K. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1986;15:487-94.
- Lasen FS, Diepgen T, Svensson A. The occurrence of atopic dermatitis in North Europe: an international questionnaire study. *J Am Acad Dermatol* 1996;34:760-4.
- Holgate ST. The epidemic of allergy and asthma. *Nature* 1999;402(suppl):B2-4.
- Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;354(suppl 2):12-5.
- Holt PG, Macaubas C, Prescott SL, Sly PD. Microbial stimulation as an aetiological factor in atopic disease. *Allergy* 1999;54:12-6.
- Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998;53(suppl 46):20-5.
- Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal flora for the development of an IgE production system fully susceptible to pral tolerance induction. *J Immunol* 1997;159:1739-45.
- Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999;29:342-6.
- Böttcher MF, Nordin EK, Sandin A, Midtvedt T, Björkstén B. Microflora-associated characteristics in faeces from allergic and nonallergic infants. *Clin Exp Allergy* 2000;30:1590-6.
- Kalliomäki M, Kirjavainen P, Eekki E, Kero P, Salonen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001;107:129-34.
- Takeuchi M, Ueda H. Increase of adult atopic dermatitis (AD) in recent Japan. *Environ Dermatol* 2000;7:133-6.
- Tagami H. Japanese Dermatological Association Criteria for the Diagnosis of Atopic Dermatitis. *J Dermatol* 1995;22:966-7.
- Mitsuoka T. A color atlas of anaerobic bacteria. Tokyo: Sobunsha; 1980. p. 341.
- Tsuyuki S, Yamazaki S, Kamimura H, Kimura M, Kawashima T, Ueda K. An enzyme-linked immunosorbent assay for measuring IgA antibody and total IgA in bile and small intestinal contents of mice. *Bifidus* 1988;2:9-13.
- Imayama S, Shimozono Y, Urabe A, Hori Y. A simple method for measuring the amount of immunoglobulin A secreted onto the skin surface. *Acta Derm Venereol* 1995;75:212-7.
- Björkstén B, Sepp E, Julge K, Boor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;108:516-20.
- Arkwright PD, David TJ. Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J Allergy Clin Immunol* 2001;107:531-4.
- Benno Y, Mitsuoka T. Development of intestinal microflora in humans and animals. *Bifidobacteria Microflora* 1986;5:13-25.
- Mitsuoka T. Intestinal flora and aging. *Nutr Rev* 1992;50:438-46.
- Holdeman LV, Good IJ, Moore WEC. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl Environ Microbiol* 1976;31:359-75.
- Levitan MC, Marco OP. Intestinal microflora in patients with Crohn's disease and ulcerative colitis. *Am J Proctology* 1976;27:49-54.
- Kanazawa K, Konishi F, Mitsuoka T, Terada A, Itoh K, Narushima S, et al. Factors influencing the development of sigmoid colon cancer, bacteriologic and biochemical studies. *Cancer* 1996;77:1701-6.
- Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974;90:525-30.
- Bunikowski R, Mielke MEA, Skarabis H, Worm M, Anagnostopoulos J, Kolde G, et al. Evidence for a disease promoting effect of *S. aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:814-9.
- Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860-76.
- Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. *J Allergy Clin Immunol* 2002;109:379-92.
- Iikura Y, Ebisawa H, Akazawa A, Akigai K, Iikura N. Study on infantile atopic dermatitis. Immunological background of atopic dermatitis. *Prog Med* 1989;9:1803-7.
- Imayama S, Shimozono Y, Hoashi M, Yasumoto S, Ohta S, Yoneyama K, et al. Reduced secretion of IgA to skin surface of patients with atopic dermatitis. *J Allergy Clin Immunol* 1994;94:195-200.
- Kalliomäki M, Salminen S, Arvilommi H, Kero P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076-9.