most cases first develop as infantile eczema with severe pruritus at predilection sites such as the face, neck, and perioral and flexural regions, and they are closely related with asthma and hay fever [5, 8]. Since then, the term "prurigo Besnier" has often appeared in literature, especially in Europe. In 1923, Coca and Cooke [9] first reported that asthma and hay fever are specifically compartmentalized in individuals or family members who were hypersensitive to various external antigens, and they proposed the use of the term "atopy" for these mysterious phenomena. Meanwhile, the close association of certain types of itchy eczema with asthma and hay fever again drew special attention by Low as an eczema-asthma-prurigo complex or by Drake as an asthma-eczema-prurigo complex in 1928 [8, 10], leading to the proposal of "atopic dermatitis/eczema" [5, 11, 12]. Even in the early 1930s, basic concepts for AD had been postulated: 1) family history of atopy; 2) antecedent infantile eczema; 3) preponderance on antecubital and popliteal fossae, anterior neck, chest, face and eyelids; 4) skin hyperpigmentation; 5) no appreciable vesicle formation as is usually clinically and histologically found in contact dermatitis; 6) unstable or hypersensitive vasomotor neuron reaction; 7) negative patch test reaction to most contact allergens; 8) positive skin test reaction to various environmental and food allergens; and 9) positive serum reaginic activity (=IgE) as detected by Prausnitz-Küstner reaction [11-13].

Genetic and environmental factors are apparently involved in the onset and exacerbation of AD in a mutually interactive manner. In 1993, Schultz Larsen et al. [14] reported that the pairwise concordance rate of AD in monozygotic twin pairs was 72% while it was only 23% in their dizygotic counterparts. They also noted that the prevalence of AD increased sharply from 3.2% in 1960-1964 to 10.2% in 1970-1974. Interestingly, the results of a similar study published in 1971 indicated that the concordance rate seemed to be affected by the prevalence rate even in genetically identical twins [15]. In the 6996 pairs of twins with a 4.3% AD prevalence rate, the pairwise concordance rate of AD was only 15.4% and 4.5% in the monozygotic and dizygotic twins, respectively [15]. AD-inducing environmental factors, as yet unknown, had increased their power in industrialized and developing countries and seemed to trigger the onset of AD in almost all individuals who were genetically predisposed to the disease.

Incidence of AD in dermatological clinics in Japan

Masuda reported that the incidence of AD in outpatient clinics was 4.1% in 36,233 patients who visited the Dermatology Clinic of the University of Tokyo from 1959 to 1961. In the Branch Hospital of the University of Tokyo, the incidence of AD in our first-visit patients was 5.6% (195/3,491) in 1967, 8.7% in 1976 (277/3,188), 7.7% (224/2,913) in 1986 and 10.1% (261/2,586) in 1996 [16]. Considering the results of previous reports on the incidence of AD in dermatological outpatient clinics [2.3% (Drake, 1928, London), 0.6% (Nexmand, 1926-1935, Copenhagen), 1.4% (Nexmand, 1936-1945, Copenhagen), 0.32-1.53% (Iijima & Saito, 1938-1955, Sendai), 1.4% (Ofuji, 1954-1956, Kyoto), 7% (Hellerström & Rajka, 1953-1959, Karolinska), and 8.3% (Uehara & Ofuji, 1977, Kyoto)], AD had become a very common skin disease during the 1950s through 1970s in industrialized countries [16]. Another interesting point was the age distribution of AD patients. In 1967, 73.9% of AD patients were 0-9 years old at the Branch Hospital of the University of Tokyo, but this figure gradually dropped to 23.4% by 1996. In contrast, the percentage of AD patients 20-29 years old was 3.1% in 1967, and markedly increased to reach 38.7% by 1996 [16]. Adult-type AD patients at dermatological clinics have clearly increased recently (Fig. 1) as pointed out by Sugiura et al. [4].

In order to clarify the current prevalence of skin disorders among dermatology patients in Japan, the Japanese Dermatological

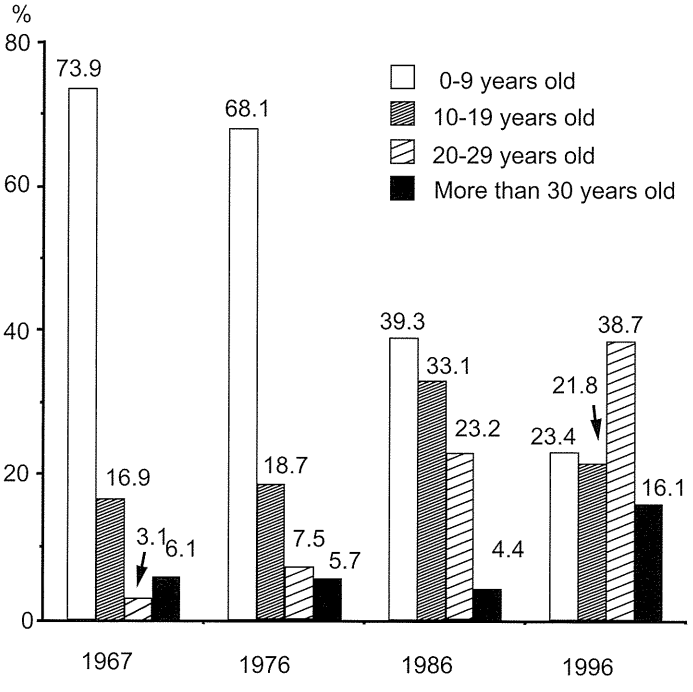

Fig. 1. Age distribution of outpatients with atopic dermatitis at the Branch Hospital, University of Tokyo.

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Association conducted a nationwide, cross-sectional, seasonal, multicenter study in 2007-2008 [17]. Among the 67,448 cases, the top twenty skin disorders were, in descending order of incidence, miscellaneous eczema, AD, tinea pedis, urticaria/angioedema, tinea unguium, viral warts, psoriasis, contact dermatitis, acne, seborrheic dermatitis, hand eczema, miscellaneous benign skin tumors, alopecia areata, herpes zoster/postherpetic neuralgia, skin ulcers (nondiabetic), prurigo, epidermal cysts, vitiligo vulgaris, seborrheic keratosis, and drug eruption/toxicoderma (Table 1). The incidence of AD was 9.8% without any gender difference. The age distribution of AD was biphasic, peaking at 0-5 years old and 21-35 years old (Fig. 2) [17]. Patients older than 46 years comprised 9.6% (649/6,733) of all AD patients [17].

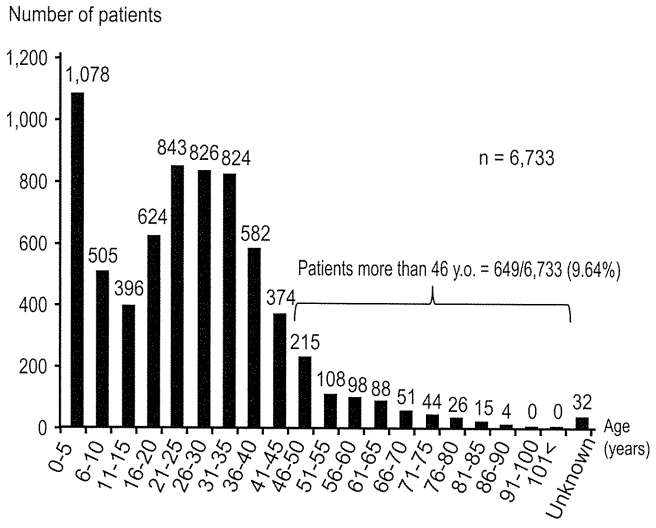

Fig. 2. Age distribution of atopic dermatitis in dermatology clinics. Report from the Japanese Dermatological Association (n = 6,733).

Incidence of AD in the general population in Japan

The incidence of AD in Japanese elementary school students was around 3% in 1981-1983 but increased to around 6-7% in the 1990s [3]. Yura and Shimizu conducted a questionnaire survey of health symptoms in four million school children (aged 7-12 years) in Osaka and reported that the lifetime prevalence of AD increased from 15.0% in 1985 to 24.1% in 1993 but leveled off thereafter (22.9% in both 1995 and 1997) [18]. Their subsequent study also confirmed the consecutive decrease of AD prevalence from 1993 to 2006 (16.5%) [19]. Meanwhile, the prevalence of wheezing was constant at 3.0 ± 0.1% between 1975 and 1983, and then increased to 4.7% in 1993. It restabilized at 4.4 ± 0.3% between 1993 and 2006 [19]. The prevalence of rhinitis increased from 12.3% in 1983 to 16.7% in 1991, increased further to 25.4% in 2003, and then leveled off at 24.7% in 2006 [19]. The reported prevalence of AD of Japanese children is summarized in Table 2.

From 2000 to 2002, the research team of the Japanese Ministry of Health, Labour and Welfare (chief researcher: Dr. S. Yamamoto) examined 48,072 children living in Asahikawa, Iwate, Tokyo, Gifu, Osaka, Hiroshima, Kochi, and Fukuoka [20]. They reported that the national average prevalence rate of AD was 12.8% in 4-month-old children, 9.8% in 18-month-olds, 13.2% in 3-year-olds, 11.8% in 6- to 7-year-olds, 10.6% in 12- to 13-year-olds, and 8.2% in 18-year-olds [20]. In 2001, we started a population-based survey of children aged 5 years and younger in Ishigaki Island, Okinawa, Japan (Kyushu University Ishigaki Dermatitis Study; KIDS). The prevalence of childhood AD in this southern subtropical island was as low as around 6% [21, 22] and did not increase during the period from 2001 to 2010 (unpublished data).

Table 1. Top 20 diseases in dermatology clinics in Japan (n = 67,448)

1	Eczema (miscellaneous)	12,590	18.67%
2	Atopic dermatitis	6,733	9.98%
3	Tinea pedis	4,379	6.49%
4	Urticaria / angioedema	3,369	4.99%
5	Tinea unguim	3,231	4.79%
6	Viral warts	3,028	4.49%
7	Psoriasis	2,985	4.43%
8	Contact dermatitis	2,643	3.92%
9	Acne vulgaris	2,430	3.60%
10	Seborrheic dermatitis	2,213	3.28%
11	Hand eczema	2,024	3.00%
12	Benign skin tumors (miscellaneous)	1,666	2.47%
13	Alopecia areata	1,653	2.45%
14	Herpes zoster / Postherpetic neuralgia	1,609	2.39%
15	Skin ulcer (non-diabetic)	1,334	1.98%
16	Prurigo	1,229	1.82%
17	Epidermoid cysts	1,194	1.77%
18	Vitiligo vulgaris	1,134	1.68%
19	Seborrheic keratosis	1,095	1.62%
20	Drug eruption / Toxicoderma	1,018	1.51%
	Total	57,557	85.34%

Japanese Dermatological Association (2007).

Regression rate of AD

It is well documented that AD regresses spontaneously, especially in infants and children. Kawaguchi et al. [23] reported that out of 102 4-month-old patients, 83 regressed spontaneously

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Table 2. Prevalence of atopic dermatitis in Japan

		6-10 years	12-13 years	14-15 years
1981-83	Aichi	3.85%		1.96%
1990	Osaka, questionnaire	19%	9.2%	9.0%
1992	West Japan (11 Prefs., questionnaire)	17.3%		
1993	Hirosaki	9%	9.2%	
1993	Hamamatsu	24.3%		
1995	Nagasaki	8.5%		
1996	Ibaragi	7.6%		
1992-97	Hiroshima, Asa area	13.7%		
2001	Hiroshima	10.9%		
2001	Maebashi	9.9%		
2002	Isahaya	10.1%		
2002	West Japan (11 Prefs., questionnaire)	13.8%		
2000-02	All Japan, 8 cities	11.8%	10.6%	

by 18 months old. Illi et al. [24] reported that the cumulative prevalence of AD in the first 2 years of life was 21.5%. Among these children with early AD, 43.2% were in complete remission by the age of 3 years, 38.3% had an intermittent pattern of disease, and 18.7% had symptoms of AD every year. Severity (adjusted cumulative odds ratio, 5.86; 95% CI, 3.04-11.29) and atopic sensitization (adjusted cumulative odds ratio, 2.76; 95% CI, 1.29-5.91) were major determinants of prognosis of AD [24]. In our KIDS cohort, 71.6% (53/74) of AD patients under 5 years old regressed spontaneously, whereas 5.5% (44/795) of non-AD individuals developed AD during the 3-year followup. Increases in total IgE levels were greater and more rapid in children with long-term AD compared to those who had spontaneously regressed, had newly developed AD or did not have AD [22]. Anan and Yamamoto reported that 287 out of 1,072 6-year-old children in Nagasaki had a present (91) or past history (196) of AD in 1995, that is, 68.3% (196/287) of AD children experienced spontaneous regression by age 6 [25]. They also followed up on 50 AD children of 6 years old and found that 30 children had spontaneously regressed within the subsequent 6 years [25]. In Hiroshima, mean prevalence of AD children of 6 years old from 1992 to 1997 was 13.6% (male: 13.6%, female: 13.7%; total number 1,327) [26]. Among 121 children of 6 years old with AD, 61 were cured and 22 had improved 5 years later. Among 464 non-AD pupils of 6 years old, 6 (1.3%) had AD 5 years later [26]. Williams and Strachan analyzed the age of onset and clearance rates for examined and/or reported eczema in 6,877 children born during the period 3-9 March 1958 for whom linked data was available at birth and at the ages of 7, 11, 16 and 23 years. Of the 870 cases with examined or reported eczema by the age of 16 years, 66% had experienced onset by the age of 7 years. Of the 571 children with reported or examined eczema by the age of 7 years, the proportion of children who were clear in terms of examined or reported eczema in the last year at ages 11 and 16 years was 65% and 74%, respectively. These 'apparent' or short-term clearance rates fell to 53% and 65%, respectively, after allowing for subsequent recurrences in adolescence and early adulthood [27]. Uehara et al. [28] examined the regression rate of 266 AD cases (older than 2 years of age at the first visit in 1953 to 1961) in 1966 (5 to 13 years later) by questionnaire. They reported that AD was cured in 76 cases (29%). In the 190 uncured cases, 40 cases experienced a short regression of more than a year's duration. Of these 40 cases, 36 (90%) reported that their transient regression appeared within 15 years after the onset of AD, and 29 cases (79%) reported that their transient regression did not last more than 5 years [28]. Another questionnaire study revealed that either transient or complete remission occurred in 33.3% of 794 AD patients (10-20 years old) [29]. In a mail-in questionnaire study, 220 AD patients (mean age: 21.3 ± 11.8) were monitored every 3 months by for 3 years from 2006 to 2009. Thirty-six (16.4%) patients reported having at least a year's remission. Four cases developed atopic cataract (12, 13, 54 and 59 years old) during this period and none of the four had experienced any remission. Patients older than 30 years had a significantly lower remission rate compared to those younger than 30 years [30].

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The regression of adult-type AD tends to occur more slowly. Our 95 cases of adult AD (mean age 23.9 ± 11.5 in 1991-1992, male: 45, female: 50) reported the following in 1996: AD was cured (10.5%), much improved (32.6%), slightly improved (34.7%), and unchanged or worse (22.1%). Eleven patients reported that the onset of AD was as late as 18 years or older. Association with other allergic disease significantly lowered the regression/improvement rate compared to the pure AD group [31]. The severity of AD also increased according to age. Yamamoto demonstrated that the percentage of mild cases decreased gradually with age (84.3% at 1.5 years old, 85% at 3 years old, 75.9% at 6 years old, 71.6% at 12 years old and 72.7% at 18 years old), while the percentage of moderate cases increased inversely (12.4% at 1.5 years old, 11.8% at 3 years old, 22.4% at 6 years old, 26.3% at 12 years old and 21.9% at 18 years old) [20]. This phenomenon can be caused by numerous aggravating factors including hormonal imbalance in adolescence, increased physical and schoolwork stress, and emotional stress related to social and family issues. Decreased compliance with treatment regimens may also contribute to the deterioration of AD.

Topical steroid therapy with special reference to steroid phobia in Japan

Although topical steroids, emollients and oral antihistamines are used as the first-line therapy for AD, long-term application, even with intermittent use, induces local and unavoidable adverse effects such as skin atrophy and telangiectasia in a substantial percentage of patients. These adverse effects and the emotional fear of long-term use of topical steroids have induced a topical steroid phobia in patients throughout the world [32].

Before the topical tacrolimus was commercially available in Japan, we collected clinical data on 1,271 AD patients who had been followed for at least 6 months in outpatient clinics [33]. The check sheet for each patient included the following items: age; gender; duration of AD; global severity before treatment; global severity after 6 months of conventional topical steroid therapy; evaluation of clinical improvement; total dose of each rank of topical steroids per 6 months' therapy on the face, scalp, trunk and extremities; association with herpes simplex infection and/or Kaposi's varicelliform eruption; association with molluscum contagiosum; and adverse effects of topical steroids (telangiectasia on cheeks, skin atrophy of antecubital/popliteal fossae, acne and folliculitis, hypertrichosis, bacterial infection, dermatomycosis, rosacea-like dermatitis, contact dermatitis caused by topical steroids, and steroid-induced striae atrophicae). Global clinical severity was classified as "very severe", "severe", "moderate" and "mild". The ranking of topical steroids was "strongest", "very strong", "strong", "mild" and "weak" in Japan.

The 1,271 patients with AD were divided into 3 groups according to age: 210 infantile (0-2 year old) cases, 546 childhood (≥2 and <13 years old) cases, and 515 adolescent and adult (≥13 years old) cases. All of the patients were treated with topical steroids and moisturizing emollients. The clinical severity of AD in the majority of patients improved or was unchanged after 6 months of conventional therapy ("controlled" group). However, 7% of infantile AD, 10% of childhood AD and 19% of adolescent and adult AD patients remained in a very severe or severe state or experienced exacerbation ("uncontrolled" group) (Table 3) [33]. This data suggested that the incidence of very severe and severe AD was significantly higher in the adolescent/adult AD group than in the infantile and childhood groups. Concordantly, Brunsting pointed out in 1936 that the recurrent lesions of adolescent and adult AD were resistant to treatment by local measures [34]. These

Table 3. Change of clinical severity pre- and post-treatment

		Pre-treatment											
		Infantile AD*				Childhood AD				Adolescent & Adult AD			
		Very severe	Severe	Moderate	Mild	Very severe	Severe	Moderate	Mild	Very severe	Severe	Moderate	Mild
post-treatment	Very severe					3	2			15	2		
	Severe		8		1	5	27	3		6	65	6	
	Moderate	2	9	41	6	5	44	155	11	7	58	161	4
	Mild		6	57	76	1	17	141	117	2	21	92	64
	undescribed			1			1	2	6	1	1		
	Total	(207) 2	23	99	83	(540)14	91	301	134	(505)31	147	259	68

☐ Uncontrolled patients per 6 months. Infantile: 7%. Childhood: 10%. Adolescent & Adult: 19%. *AD: atopic dermatitis.

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uncontrolled patients were considered as "intractable" and they received much attention as a social problem in Japan.

The total doses of topical steroids used during the 6-month treatment period are listed in Table 4 as median, 75 percentile and 90 percentile doses. During the 6 months, 90% of AD patients used less than 89.5 g, 135 g and 304 g of topical steroids on the entire body in the infantile, childhood, and adolescent/adult groups, respectively. The 90 percentile doses applied on the face during the 6 months were 10 g, 15 g and 35 g in the infantile, childhood and adolescent/adult AD groups, respectively. The amount of topical steroids applied in this survey was much less than that reported by Wilson et al. and Munro et al. [35, 36]. Association with herpes simplex virus infection and/or Kaposi's varicelliform eruption was found in 2.4% of infantile AD patients, 2.5% of childhood AD patients and 3.5% of adolescent and adult AD patients during the 6-month treatment period. Association with molluscum contagiosum was detected in 7% of infantile AD patients, 9% of childhood AD patients, and 0.2% of adolescent and adult AD patients. The cumulative incidence of adverse effects was assessed (Table 5) and as expected, it was much higher in the adolescent and adult AD patients than in the infantile AD patients. Telangiectasia on the cheeks and skin atrophy of antecubital/popliteal fossae were frequently observed as the predilection sites of AD (face and flexure areas). There was a small but appreciable percentage of patients with adverse effects such as local skin atrophy and telangiectasia [33]. The incidence of these adverse effects might be predicted by age, sex, and strength and quantity of topical steroids. In addition, these adverse effects were reversible [37].

Table 4. Clinical doses of topical steroids during 6 months of treatment (g)

		Infantile	Childhood	Adolescent & Adult
	No. of cases	210	546	515
Face	50% dose	1	0	0
	75% dose	5	5	15
	90% dose	10	15	35
Scalp	50% dose	0	0	0
	75% dose	0	0	0
	90% dose	10	10	65
Body	50% dose	21	45	80
	75% dose	40	80	160
	90% dose	74.5	130	280
Total	50% dose	25	45	95
	75% dose	43	80	180
	90% dose	89.5	135	304

We next examined the topical steroid doses used in the "controlled" and "uncontrolled" groups (Table 6) [33]. The total usage of topical steroids was unexpectedly higher in the "uncontrolled" group than in the "controlled" group. The statistical difference became more obvious in the adolescent/adult group than in the childhood group (Table 3). Topical steroids are useful for treating AD, but there appears to be a subgroup of patients who remain severe despite increasing applications of topical steroids. Nevertheless, all of the patients in the "uncontrolled" group may not have been "uncontrollable", because the total application dose per 6 months in 50% of the "uncontrolled" patients was very small (Table 6, undertreatment state). Undertreatment may cause or enhance the development of the "uncontrolled" group. The most popular tube size of topical steroids is 5 g in Japan, which is much smaller than European and American tubes (Fig. 3). This may, at

Table 5. Adverse effects of topical steroids

	Infantile	Childhood	Adolescent & Adult
Teleangiectasia of cheeks	0%	2.3%	13.3%
Skin atrophy of antecubital fossae	1.5%	5.2%	15.8%
Skin atrophy of popliteal fossae	1.9%	4.1%	9.8%
Acne / folliculitis	0%	1.3%	8.2%
Hypertrichosis	0.5%	1%	2.7%
Bacterial infection	1.4%	2.1%	2.5%
Fungal infection	1.9%	0.6%	1.2%
Rosacea-like dermatitis	0%	0.4%	3.1%
Contact dermatitis	0%	0.4%	0.8%
Striae cutanea	0%	0%	1%

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Table 6. Dose of topical steroids between controlled and uncontrolled groups

		Total doses		"Strongest + very strong + strong" dose		"Mild + weak" dose	
		Controlled group	Uncontrolled group	Controlled group	Uncontrolled group	Controlled group	Uncontrolled group
Infantile	No	191	15	191	15	191	15
	50% dose	20 (g)	30 (g)	5 (g)	0 (g)	5 (g)	20 (g)
	75% dose	40	60	25	15	20	40
	90% dose	70	90	45.8	72	40	59
	Mann-Whitney's U-test	0.3002		0.3427		0.0496	
Childhood	No	481	51	481	51	481	51
	50% dose	40 (g)	60 (g)	25 (g)	24 (g)	0 (g)	10 (g)
	75% dose	75	120	60	75	20	40
	90% dose	120	207.8	100	151	51.6	99
	Mann-Whitney's U-test	0.0018		0.2895		0.0003	
Adolescent & Adult	No	406	98	406	98	406	98
	50% dose	75 (g)	140 (g)	60 (g)	117.5 (g)	0 (g)	0 (g)
	75% dose	150	252.5	120	230	0	0
	90% dose	250	400.5	225	245	56.5	75.5
	Mann-Whitney's U-test	0.0000		0.0000		0.1918	

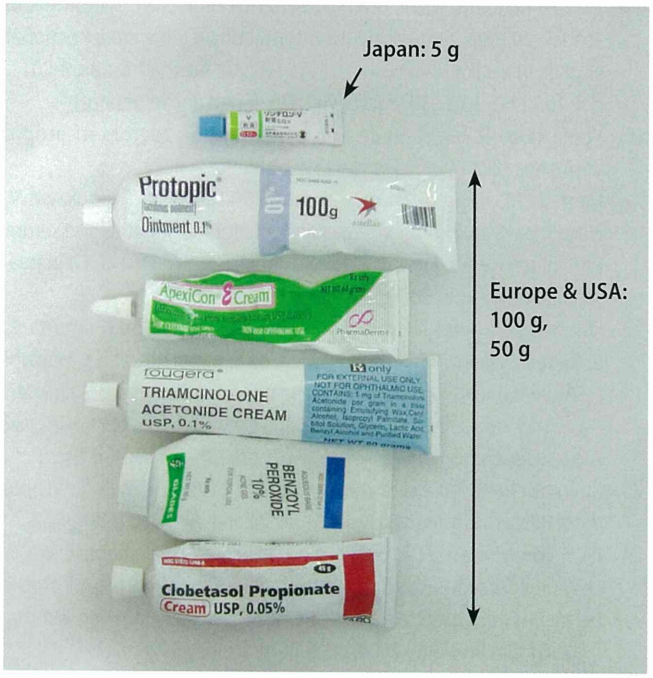

Fig. 3. In Japan, 5-g tubes are typically used in daily clinics. Larger tubes (100 g or 50 g) are more commonly used in Europe and the USA.

least in part, contribute to topical steroid undertreatment in Japan. Suitable amounts of topical steroid application, such as the finger-tip-unit dose [38], may markedly decrease the percentage of the "uncontrolled" group. Various social, economic and ecological factors together with the medical insurance system may affect "steroid phobia"-related undertreatment.

Atopic cataract is another important issue that may be misunderstood by AD patients. Brunsting first described atopic cataract in detail in 1936 [34]. He demonstrated the frequent association of juvenile cataract with AD in 10 out of 101 AD patients (mean age: 22 years old) [34]. He then performed an ophthalmological check in 1,158 AD patients from 1940 to 1953, and found typical atopic cataract in 136 patients (11.7%) including 79 cases of visual disturbance [39]. He also pointed out that the rapid progression of atopic cataract in adolescent- and adult-type AD was usually associated with the exacerbation of skin eruption [34, 39]. The incidence of atopic cataracts in Japanese AD patients is also around 10-15% in the literature. Importantly, steroids were not available as medication until 1949 to 1952 [40]. These facts emphasize that atopic cataract is a distinct clinical sign of AD, and is likely induced by repeated scratching/rubbing/patting of facial and periocular lesions and is not directly related to steroids [41-44]. Retinal detachment and subsequent blindness are also serious ophthalmological complications [42].

CONCLUSION

AD is a very common skin disease in dermatology clinics in

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Japan. The English version of the guidelines for management and severity of AD by the Japanese Dermatological Association have been published in literature [45-47], the principals of which are identical to those of other academic societies. AD affects patients across several generations with different disease activity. Genetic, environmental and emotional factors are variably involved in the onset and clinical course of AD. It is also a challenging task to fight against topical steroid phobia in daily clinics. Therefore, the Japanese guideline for AD includes many practical instructions for the patients [45]. Education, understanding and cooperation of patients and their guardians are a key issue for the successful treatment of AD.

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REFERENCES

1. Williams HC. Is the prevalence of atopic dermatitis increasing? Clin Exp Dermatol 1992;17:385-91.
2. Williams HC, Robertson C, Stewart A. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J Allergy Clin Immunol 1999;103:125-38.
3. Takeuchi M, Ueda H. Increase of adult atopic dermatitis (AD) in recent Japan. Envir Dermatol 2000;7:133-6.
4. Sugiura H, Umemoto N, Deguchi H, Murata Y, Tanaka K, Sawai T, Omoto M, Uchiyama M, Kiriyama T, Uehara M. Prevalence of childhood and adolescent atopic dermatitis in a Japanese population: comparison with the disease frequency examined 20 years ago. Acta Derm Venereol 1998;78:293-4.
5. Sulzberger MB. Historical notes on atopic dermatitis: Its names and nature. Semin Dermatol 1983;2:1-4.
6. Hill LW, Sulzberger MB. Evolution of atopic dermatitis. Arch Dermatol Syph 1935;32:451-63.
7. Wise F. The neurodermatoses and pseudo-lichens: a consideration of their nosological and clinical features. J Cutan Dis 1919;37:590-620.
8. Low RC. The eczema-asthma-prurigo-complex. Br J Dermatol 1928;40:389-406.
9. Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 1923;8:163-82.
10. Drake JA. The asthma-eczema-prurigo complex. Br J Dermatol 1928;40:407-14.
11. Sulzberger MB, Spain WC, Sammis F, Shahon HI. Studies in hypersensitiveness in certain dermatoses. I. Neurodermatitis (disseminated type). J Allergy 1932;3:423-37.
12. Hill LW. Chronic atopic eczema (neurodermatitis) in childhood. JAMA 1934;103:1430-4.
13. Sulzberger MB, Rostenberg A Jr. Practical procedures in the investigation of certain allergic dermatoses. J Allergy 1935;6:448-59.
14. Schultz Larsen F. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. J Am Acad Dermatol 1993;28:719-23.
15. Edfors-Lubs ML. Allergy in 7000 twin pairs. Acta Allergol 1971;26:249-85.
16. Furue M. History of atopic dermatitis. In: Tamaki K, Furue M, Nakagawa H, editors, Atopic dermatitis and topical steroid therapy. Shinjuku: Chugai-Igakusha; 1998. p. 1-19.
17. Furue M, Yamazaki S, Jimbow K, Tsuchida T, Amagai M, Tanaka T, Matsunaga K, Muto M, Morita E, Akiyama M, Soma Y, Terui T, Manabe M. Prevalence of dermatological disorders in Japan: a nationwide, cross-sectional, seasonal, multicenter, hospital-based study. J Dermatol 2011;38:310-20.
18. Yura A, Shimizu T. Trends in the prevalence of atopic dermatitis in school children: longitudinal study in Osaka Prefecture, Japan, from 1985 to 1997. Br J Dermatol 2001;145:966-73.
19. Yura A, Kouda K, Iki M, Shimizu T. Trends of allergic symptoms in school children: large-scale long-term consecutive cross-sectional studies in Osaka Prefecture, Japan. Pediatr Allergy Immunol 2011. doi: 10.1111/j.1399-3038.2011.01159.x. [Epub ahead of print]
20. Yamamoto S. Prevalence and exacerbation factors of atopic dermatitis. Skin Allergy Frontier 2003;1:85-90.
21. Hamada M, Furusyo N, Urabe K, Morita K, Nakahara T, Kinukawa N, Nose Y, Hayashi J, Furue M. Prevalence of atopic dermatitis and serum IgE values in nursery school children in Ishigaki Island, Okinawa, Japan. J Dermatol 2005;32:248-55.
22. Fukiwake N, Furusyo N, Kubo N, Takeoka H, Toyoda K, Morita K, Shibata S, Nakahara T, Kido M, Hayashida S, Moroi Y, Urabe K, Hayashi J, Furue M. Incidence of atopic dermatitis in nursery school children - a follow-up study from 2001 to 2004, Kyushu University Ishigaki Atopic Dermatitis Study (KIDS). Eur J Dermatol 2006;16:416-9.
23. Kawaguchi H, Takeuchi M, Tanaka Y, Ishii N, Ikezawa Z. Atopic dermatitis at 18-months physical examination. Arerugi 2000;49:653-7.
24. Illi S, von Mutius E, Lau S, Nickel R, Grüber C, Niggemann B, Wahn U; Multicenter Allergy Study Group. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. J Allergy Clin Immunol 2004;113:925-31.
25. Anan S, Yamamoto N. Spontaneous regression of atopic dermatitis. Hifu 1996;38 (suppl 18):13-15. (in Japanese)
26. Okano S, Takahashi H, Yano T, Yamura M, Niimi N, Tanaka K, Iguchi H, Nakayama S, Horie M, Kuwahara M, Oda K, Hashimoto N, Doi T, Okino H, Imoo K, Doki M. Prevalence of childhood atopic dermatitis in first-year pupils of elementary school and its follow-up: Asa district in Hiroshima. Nihon Ishikai Zasshi 2006;135:97-103. (in Japanese)
27. Williams HC, Strachan DP. The natural history of childhood eczema: observations from the British 1958 birth cohort study. Br J Dermatol

1998;139:834-9.
28. Uehara M, Otaka T, Ogawa Y, Ofuji K. Clinical course of atopic dermatitis. Hifuka-Kiyo 1970;65:1-6.
29. Furue M, Kawashima M, Furukawa F, Iiduka H, Ito M, Shiohara T, Shimada S, Takigawa M, Takehara K, Miyaji Y, Katayama I, Iwatsuki K, Hashimoto K. Questionnaire study for chronological change in atopic dermatitis. Rinshohifuka 2007;61:286-295.
30. Furue M, Kawashima M, Furukawa F, Iiduka H, Ito M, Shiohara T, Shimada S, Takigawa M, Takehara K, Miyaji Y, Katayama I, Iwatsuki K, Hashimoto K. A prospective questionnaire study for atopic dermatitis. Rinshohifuka 2011;65:83-92.
31. Furue M, Hayashida S, Uchi H. Natural history of atopic dermatitis. Rinshomeneki-Arerugika 2009;52:320-325.
32. Charman CR, Morris AD, Williams HC. Topical corticosteroid phobia in patients with atopic eczema. Br J Dermatol 2000;142:931-6.
33. Furue M, Terao H, Rikihisa W, Urabe K, Kinukawa N, Nose Y, Koga T. Clinical dose and adverse effects of topical steroids in daily management of atopic dermatitis. Br J Dermatol 2003;148:128-33.
34. Brunsting LA. Atopic dermatitis (disseminated neurodermatitis) of young adults. Arch Derm Syphilol 1936;34:935-57.
35. Wilson L, Williams DI, Marsh SD. Plasma corticosteroid levels in out-patients treated with topical steroids. Br J Dermatol 1973;88:373-80.
36. Munro DD, Clift DC. Pituitary-adrenal function after prolonged use of topical corticosteroids. Br J Dermatol 1973;88:381-5.
37. Furue M, Terao H, Moroi Y, Koga T, Kubota Y, Nakayama J, Furukawa F, Tanaka Y, Katayama I, Kinukawa N, Nose Y, Urabe K. Dosage and adverse effects of topical tacrolimus and steroids in daily management of atopic dermatitis. J Dermatol 2004;31:277-83.
38. Long CC, Finlay AY. The finger-tip unit--a new practical measure. Clin Exp Dermatol 1991;16:444-7.
39. Brunsting LA, Reed WB, Bair HL. Occurrence of cataracts and keratoconus with atopic dermatitis. AMA Arch Dermatol 1955;72:237-41.
40. Goldman L, Thompson RG, Trice ER. Cortisone acetate in skin disease; local effect in the skin from topical application and local injection. AMA Arch Derm Syphilol 1952;65:177-86.
41. Haeck IM, Rouwen TJ, Timmer-de Mik L, de Bruin-Weller MS, Bruijnzeel-Koomen CA. Topical corticosteroids in atopic dermatitis and the risk of glaucoma and cataracts. J Am Acad Dermatol 2011;64:275-81.
42. Taniguchi H, Ohki O, Yokozeki H, Katayama I, Tanaka A, Kiyosawa M, Nishioka K. Cataract and retinal detachment in patients with severe atopic dermatitis who were withdrawn from the use of topical corticosteroid. J Dermatol 1999;26:658-65.
43. Nagaki Y, Hayasaka S, Kadoi C. Cataract progression in patients with atopic dermatitis. J Cataract Refract Surg 1999;25:96-9.
44. Matsuo T, Saito H, Matsuo N. Cataract and aqueous flare levels in patients with atopic dermatitis. Am J Ophthalmol 1997;124:36-9.
45. Saeki H, Furue M, Furukawa F, Hide M, Ohtsuki M, Katayama I, Sasaki R, Suto H, Takehara K; Committee for Guidelines for the Management of Atopic Dermatitis of Japanese Dermatological Association. Guidelines for management of atopic dermatitis. J Dermatol 2009;36:563-77.
46. Yoshida H, Aoki T, Furue M, Tagami H, Kaneko F, Ohtsuka F, Nishioka K, Toda K, Mizoguchi M, Nakayama H, Ikezawa Z, Takigawa M, Arata J, Yamamoto S, Tanaka Y, Ishigaki M, Kusunoki T, Yoshikawa K. English version of the interim report published in 1998 by the members of the Advisory Committee on Atopic Dermatitis Severity Classification Criteria of the Japanese Dermatological Association. J Dermatol 2011;38:625-31.
47. Aoki T, Yoshida H, Furue M, Tagami H, Kaneko F, Ohtsuka F, Nishioka K, Toda K, Mizoguchi M, Ichihashi M, Ueki H, Nakayama H, Ikezawa Z, Takigawa M, Arata J, Koro O, Yamamoto S, Tanaka Y, Ishigaki M, Kusunoki T, Yoshikawa K. English version of the concluding report published in 2001 by the Advisory Committee on Atopic Dermatitis Severity Classification Criteria of the Japanese Dermatological Association. J Dermatol 2011;38:632-44.

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ORIGINAL ARTICLE

Beneficial effect of a diet containing heat-killed *Lactobacillus paracasei* K71 on adult type atopic dermatitis

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ABSTRACT

The purpose of this study was to investigate the clinical effect of a supplementary diet containing heat-killed lactic acid bacterium *Lactobacillus paracasei* K71 (LAB diet) on adult patients with atopic dermatitis (AD). A randomized, double-blind, placebo-controlled study was conducted in 34 adult type AD subjects who were treated with conventional topical corticosteroid and tacrolimus. LAB diet or placebo was added over 12 weeks. The primary end-point was the clinical severity of AD which was evaluated by a severity scoring system proposed by the guideline of the Japanese Dermatological Association. The effect was also secondarily evaluated by itch scores of visual analog scales (VAS), quality-of-life (QOL) impairment scores of Skindex 16 and consumption amounts of topical therapeutics. Data on these four assessment variables were collected at baseline and at week 4, 8 and 12. Within the study population, the skin severity scores were significantly decreased from baseline at week 8 ($P < 0.05$) and at week 12 ($P < 0.01$) in the LAB diet group but not in the placebo group. Influence of LAB diet on itch scores or QOL impairment scores was not evident. The consumption of topical therapeutics in the placebo group was 1.9-times greater in total amount compared with the corresponding value in the LAB diet group during the intervention period, although there was no significant difference. No LAB diet- or placebo-related adverse events were observed. We concluded that the LAB diet may have some benefits as a complementary therapy for adult AD patients who are managed with the conventional treatment.

Key words: atopic dermatitis, complementary therapy, heat-killed probiotic bacteria, *Lactobacillus paracasei*, randomized controlled trial.

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic and refractory skin disease manifesting as eczema and pruritus with repeated exacerbations and regressions.[1] Although AD was originally known to be mainly prevalent among infants and children, its incidence in adults has increased worldwide over the past decade.[2] The same trend is also observed in Japan, and according to a currently conducted nationwide epidemiological surveillance, the prevalence of AD in the Japanese populations in their 20s and 30s reached 10.2% and 9.0%, respectively.[3,4] As AD enormously interfere with individuals' daily life

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socially and psychologically, the adequate management of AD should be considered an important medical and health issue.

Because the exact pathogenesis of AD remains obscure, the current management of the disorder aims to relieve repeatedly-occurring dermal inflammation and prevent its flare-up. Topical corticosteroids and tacrolimus are widely accepted as the standard treatment options for AD.[1,5] Although these treatment options might control the symptoms, relapses are not infrequent. Moreover, extensive and prolonged use of corticosteroid implies a risk of local side-effects potentially causing skin atrophy and there are substantial numbers of AD patients who have corticosteroid phobia.[6] Therefore, novel approaches to the management of AD are urgently needed to enhance efficacy of the ordinary pharmacological treatment.

Of a number of complementary measures thus far studied to control AD and other allergic responses, probiotic supplementation seems to be the most promising.[7,8] A probiotic is defined by the World Health Organization and the Food and Agriculture Organization of the United Nations as ''live microorganisms which, when administered in adequate amounts, confer a beneficial health effect on the host''. Probiotic microorganisms most often belong to the genera *Bifidobacterium* and *Lactobacillus*.[9,10] Majamaa and Isolauri[11] first demonstrated the effectiveness of the probiotic lactobacilli to reduce the severity of eczema in children with AD. Also, most studies on the effects of probiotic bacteria on AD have been conducted in neonates and infants.[12–15] Recently, Michail *et al.*[16] performed a meta-analysis of 10 randomized controlled trials (RCT) that were conducted in children aged 2.5 months to 13 years to determine whether probiotics are efficacious in treating AD. The results showed a modest role for probiotics in these pediatric AD populations. In contrast, there are much fewer reports which dealt with the effect of probiotics in adult AD patients,[17,18] and none of these papers provided any convincing results.

The impact of probiotics on allergic diseases has been studied in several animal models. These experimental studies have found that probiotics exert strain-specific effects in the intestinal lumen on epithelial cells and immune cells with anti-allergic potential.[19,20] It has been also shown that inactivated (heat-killed) lactobacilli have similar beneficial immunomodulatory effects in experimental animals[21,22] and humans.[23] There is the possibility, therefore, that viability may not be an essential property for the probiotic activity of lactobacilli in the management of AD.

Lactobacillus paracasei K71 is an isolate from *sake* lees, a Japanese traditional fermented product made from polished rice. We recently found that a heat-killed *L. paracasei* K71 bacterium was effective in downregulating immunoglobulin (Ig)E synthesis *in vitro* and *in vivo* (Dr Takashi Hara *et al.*, pers. comm., 2008). A preliminary clinical study showed that a supplementary diet containing heat-killed bacteria of this *Lactobacillus* strain (LAB diet) was a safe agent that may have potential to exert a beneficial effect in adult type AD patients (Dr Nobuyuki Shimizu, unpubl. data, 2008). This tempted us to conduct a double-blind RCT aiming to investigate the clinical usefulness of the LAB diet in adult patients with AD. Because topical corticosteroid and tacrolimus is recommended as the standard treatment of AD in various guidelines, evaluation of efficacy of probiotics and any other complementary therapy should be performed in those AD patients who are managed with the guideline-based treatment. However, almost all such RCT have been conducted using untreated AD patients for the control arm. As far as we are aware, the only exception is an RCT reported by Kobayashi *et al.*[24] who evaluated efficacy and safety of a prescribed traditional herbal medicine in a special population of AD adults managed with the standard treatment.

Taking these situations into consideration, the present double-blind RCT was undertaken to investigate whether the supplementation with the LAB diet or placebo can affect the clinical course of adult type AD patients who are managed with the conventional treatment.

METHODS

Study diets

A supplementary diet containing a lactic acid bacterium used as the active diet was manufactured in powder form consisting of (in a daily dose of 500 mg) 100 mg ($\sim 2 \times 10^{11}$ bacteria) of heat-killed *L. paracasei* K71 and 400 mg of dextrin NSD300 (Nissi, Chita,

Japan). Dummy placebo contained (in a daily dose) 500 mg of dextrin NSD300 and 0.45 mg of carotene base to make the placebo indistinguishable from the LAB diet in color. Both LAB diet and placebo were given once a day after being dissolved in approximately 100 mL of water, coffee or tea.

Subjects

Male and female Japanese adult subjects, aged 20–65 years, with diagnosed mild or moderate AD were recruited. The diagnosis of AD was made according to criteria of the Japanese Dermatological Association.[1] Those subjects with a fairly stable symptomatic status who had received guideline-based standard treatment with topical corticosteroid and tacrolimus prior to the study and were expected to continue the same regimen after the start of the intervention participated in the study. Exclusion criteria were: routine use of health food(s) containing some constituent(s) with AD-affecting potential within 2 weeks before the study and expected to be continued; use of oral corticosteroid; potential allergy to the LAB diet; pregnant women, nursing mothers or women of child-bearing potential; and presence of any clinically significant medical condition judged by the Medical Investigator to preclude the subjects' inclusion in the study.

Study design

A prospective, randomized, double-blind, placebo-controlled, parallel-group comparative study took place from August to December 2008 and involved the participation of two clinical service organization centers in Japan. The study protocol was approved by the local ethics committee, and was conducted in accordance with the principles of the amended Declaration of Helsinki and Ethical Guideline for Epidemiological Research. Written informed consent was obtained from all participants prior to enrollment in the study. Thirty-four subjects eligible for the study were randomly assigned to receive LAB diet or placebo. All enrolled subjects were seen on day 0 (baseline) and weeks 4, 8 and 12 (four visits in all) to receive medical examinations and laboratory tests and to collect blood/serum samples. At each visit, the Medical Investigator examined usages of topical therapeutics (corticosteroid and tacrolimus), consumption of allotted study diets (LAB diet and placebo) and occurrence of adverse events based on the individual's study diary. All subjects were asked not to change the kinds of topical therapeutics as far as possible through the intervention period. The used amount of topical therapeutics was expressed as scores for the total equivalent amount (TEA) by multiplying potency equivalent factors according to Kobayashi *et al.*[24]

Evaluation of outcome

Intervention outcome of symptomatic changes was evaluated primarily by skin severity scores and secondarily by itch scores and quality of life (QOL) impairment scores. These outcome measures were collected at baseline and at weeks 4, 8 and 12 after the onset of intervention. Evaluations were performed at each time point.

Skin severity scores were measured using the criteria of the Japanese Dermatological Association.[1] In this scoring system, the disease severity is assessed and scored on the basis of eruption intensity and affected skin areas. The eruption intensity was scored from 0–4 (0 = no symptom, 1 = mild, 2 = moderate, 3 = severe, 4 = extremely severe). The sum of scores for each of five sections of the body surface (i.e. [i] head and neck; [ii] anterior trunk; [iii] posterior trunk; [iv] upper limbs; and [v] lower limbs) was used as the outcome measure for skin severity.

Itch scores of 100 mm-visual analog scales (VAS) were measured and assessed in the daytime and at night. VAS were scored from 0 (0 mm, the left end) to 100 (100 mm, the right end), where 0 indicates no itch and 100 indicates the worst itch imaginable.

The Japanese edition of Skindex 16 questionnaires,[25] a dermal disease-specific, self-administered questionnaire, were administered at each visit to evaluate the intensity of QOL impairment. The Skindex 16 comprises three subscales (physical symptom, feeling and daily functioning) and 16 component items, and each item is rated on an ordinal scale of 0–6, with higher scores indicating lower QOL. The sum of scores for all of the 16 items was used as the parameter for evaluation of QOL impairment.

Evaluation of safety

Tolerability and safety were evaluated on the basis of the incidence and severity of study diet-related adverse events experienced throughout the study in

comparison between the LAB diet group and placebo group. All adverse events occurring in the two groups were analyzed for their frequency, severity, relatedness with intervention and efficacy outcome.

Statistics

Baseline characteristics were compared between the two groups with the two-sample Student's *t*-test. Changes in symptomatic scores over time were also compared with baseline by the paired Student's *t*-test. Intergroup comparison of the changes (Δ-values) during intervention was made by the two-sample Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

RESULTS

Baseline characteristics

Table 1 presents the baseline characteristics of 17 subjects in each of the LAB diet and placebo groups. The baseline variables included: demographic characteristics (age, men/women ratio); physical and physiological parameters (height, bodyweight, body mass index, systolic/diastolic blood pressures, pulse rate); subject distribution on the severity of AD; and symptomatic scores (skin severity scores, itch scores of VAS, QOL impairment scores of Skindex 16). There were no significant differences between the LAB diet group and placebo group in any of these variables.

Among a total of 17 subjects in the LAB diet group, one (a 30-year-old woman with mild AD) was aware of having been pregnant on day 53 after the start of intervention. She was immediately released from the study and excluded from all outcome evaluations. Adherence to the allotted study diet was: 100% and 90–99% for 13 and three subjects, respectively, in the LAB diet group and for 10 and seven subjects, respectively, in the placebo group. Thus, in both groups, there was no subject with a value below 80% that was considered a protocol violation. Based on these results, outcome evaluations based on symptomatic scores were performed with 16 subjects in the LAB diet group (10 with mild AD and six with

Table 1. Baseline characteristics of LAB diet and placebo groups

Variables	LAB diet group (n = 17)	Placebo group (n = 17)
Age (years)	29.4 ± 5.7	31.6 ± 10.1
Sex (M : F)	5:12	5:12
Height (cm)	162.45 ± 6.75	160.08 ± 7.92
Bodyweight (kg)	55.41 ± 8.44	55.41 ± 11.79
AD severity distribution (subjects)		
Mild	11	12
Moderate	6	5
Disease duration (years)	17.4 ± 9.7	17.2 ± 10.2
Skin severity scores		
Head and neck	0.82 ± 0.64	0.76 ± 0.66
Anterior trunk	0.65 ± 0.70	0.76 ± 0.66
Posterior trunk	0.53 ± 0.72	0.94 ± 0.56
Upper limbs	1.00 ± 0.50	1.06 ± 0.66
Lower limbs	0.71 ± 0.69	0.94 ± 0.75
Total	3.71 ± 1.76	4.47 ± 2.00
Itch scores of VAS (mm)		
In daytime	37.38 ± 20.47	32.97 ± 16.97
At night	28.18 ± 24.63	24.28 ± 25.86
Total	65.56 ± 38.67	57.25 ± 38.90
QOL impairment scores of Skindex 16		
Physical symptom	35.05 ± 22.00	37.25 ± 25.62
Feeling	41.32 ± 29.12	53.46 ± 24.11
Daily functioning	11.96 ± 13.54	17.84 ± 20.38
Total	30.58 ± 19.82	38.36 ± 16.26

Values are the mean ± standard deviation except in sex and AD severity distribution. AD, atopic dermatitis; LAB diet, *Lactobacillus paracasei* K71; QOL, quality of life; VAS, visual analog scales.

Table 2. Usage of topical therapeutics during 12-week intervention period

Group	Scores for TEA of topical therapeutics used			
	Weeks 0–4	Weeks 5–8	Weeks 9–12	Total
LAB diet (n = 16)	31.58 ± 33.44	32.69 ± 42.99	41.88 ± 50.49	106.14 ± 116.60
Placebo (n = 17)	57.86 ± 60.61	71.98 ± 96.55	70.08 ± 70.52	199.92 ± 206.72

Values are given as mean ± standard deviation. TEA, total equivalent amount; LAB diet, *Lactobacillus paracasei* K71.

moderate AD) and 17 subjects in the placebo group (12 with mild AD and five with moderate AD).

Table 2 shows the equivalent amounts of topical therapeutics measured every 4 weeks during the 12-week intervention period. In two groups, the amount for weeks 5–8 and 9–12 compared with the initial 4-week period appeared to be slightly increased. The extent of increase was approximately 33% (within weeks 9–12) in the LAB diet group and approximately 24% (within weeks 5–8) in the placebo group at the maximum, indicating that the dosage of topical therapeutics was kept almost constant in the two groups over the intervention period. By intergroup comparison, the consumption of topical therapeutics in the placebo group was 1.7–2.2-times greater at each measurement time point (1.9-times greater in total amount) compared with the corresponding value in the LAB diet group during the intervention period, although there was no significant difference.

Effect on skin severity scores

Figure 1 shows the time-course changes in skin severity scores during the 12-week intervention period, along with the magnitude of changes from the baseline to each of three assessment time points (weeks 4, 8 and 12), in both groups. By within-group comparison, scores for the LAB diet group were decreased from baseline by 10.2%, 18.6% and 27.1% at weeks 4, 8 and 12, respectively, achieving statistical significance at the latter two time points ($P < 0.05$ and <0.01, respectively). In contrast, scores for the placebo group were decreased to a lesser extent without statistical significance (5.3% at week 8 and 15.8% at week 12). By intergroup comparison, although Δ-values of scores changed from baseline for the LAB diet group at week 8 (−0.69 ± 1.14) and at week 12 (−1.00 ± 1.21), they appeared to be greater than the corresponding values for the placebo group (−0.24 ± 2.08 and −0.71 ± 1.99, respectively), none of the differences reached a significant level.

Effect on itch scores and QOL impairment scores

The efficacy to relieve itch and that to improve QOL were evaluated on the basis of itch scores of VAS and QOL impairment scores of Skindex 16, respectively. A trend toward reduction in itch scores was seen in both groups (Fig. 2). Compared with baseline, the values for the LAB diet group were reduced by 23.0% ($P = 0.032$) at week 4, 32.9% ($P = 0.064$) at week 8 and 17.8% ($P = 0.348$) at week 12. However, a similar trend of score reductions was also seen for the placebo group at week 4 (6.8%, $P = 0.538$), week 8 (27.5%, $P = 0.059$) and week 12 (39.2%, $P = 0.059$). Thus, both groups had the significant reduction or the likelihood of reduction in itch scores at two of the three assessment time points. No intergroup significant difference in Δ-values was observed at any time point.

As illustrated in Figure 3, both groups showed a trend toward the substantial reduction in QOL impairment scores over the intervention period. The extent of score reduction compared with baseline at weeks 4, 8 and 12 were 28.0%, 36.1% and 29.3%, respectively, in the LAB diet group and 28.3%, 42.5% and 41.9%, respectively, in the placebo group. All these changes in scores were statistically significant ($P < 0.01$ or <0.05), while there was no significant intergroup difference in Δ-values at any time point.

Tolerability and safety

During the 12-week intervention period, one of 16 subjects in the LAB diet group experienced two adverse events (nausea and headache) and four of 17 subjects in the placebo group had six adverse events (headache, toothache, diarrhea, stomach ache, nausea and vomiting). None of these adverse events

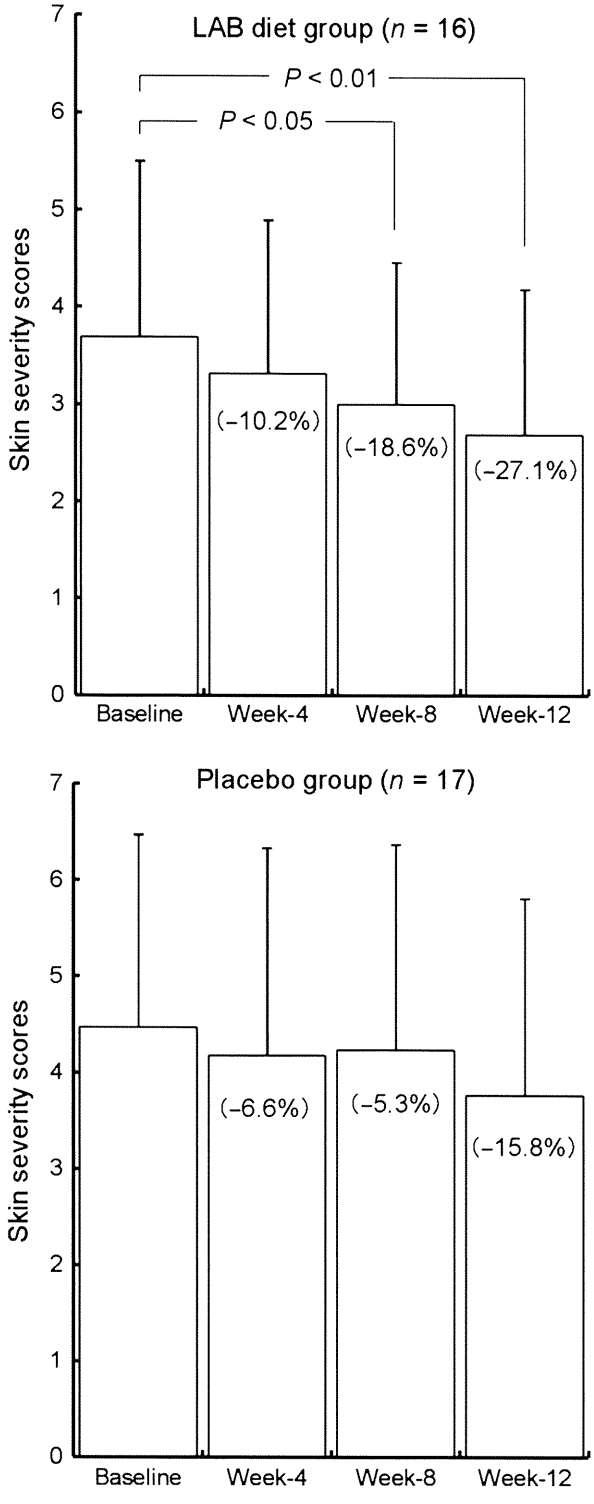

Figure 1. Time-course changes in skin severity scores during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71.

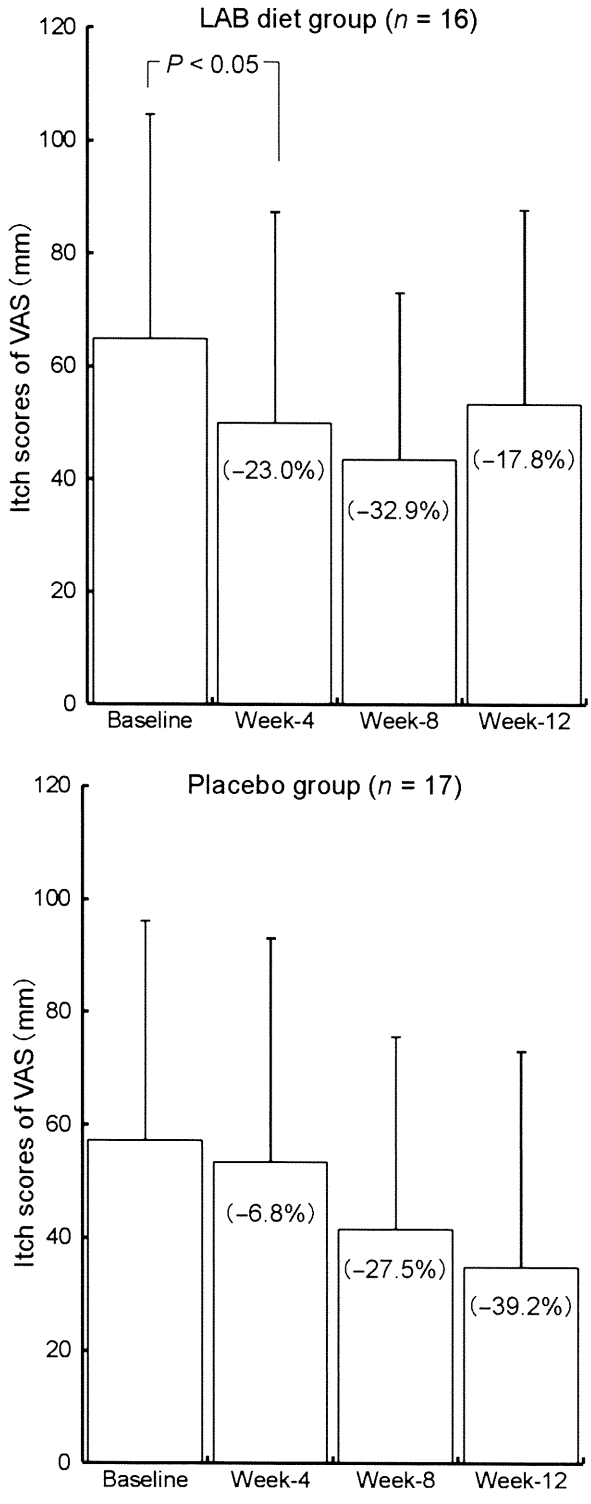

Figure 2. Time-course changes in itch scores of VAS during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71; VAS, visual analog scales.

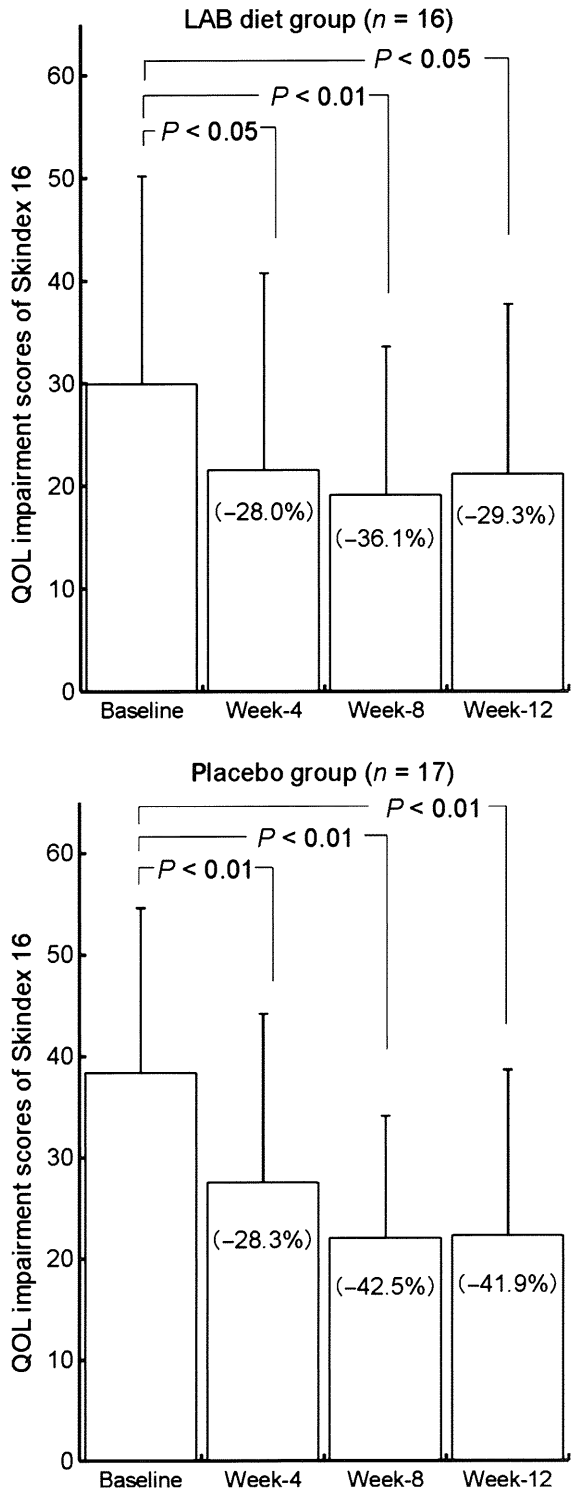

Figure 3. Time-course changes in QOL impairment scores of Skindex 16 during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71; QOL, quality of life.

were severe and were unrelated to the study diet taken.

DISCUSSION

There are a substantial number of reports which demonstrate the beneficial effect of probiotics, mostly live lactobacilli, in the management of pediatric AD.[13–15,26] In contrast, much fewer data are available on the effect of viable or non-viable probiotic bacteria in the prevention or treatment of AD in adult patients. With respect to probiotic effect of inactivated bacteria, several experimental studies with mice showed that p.o. administration of heat-killed bacteria of certain *Lactobacillus* strains are effective in protecting against intestinal inflammation,[27] reducing contact hypersensitivity reaction[28] and preventing spontaneous development of atopic skin lesions.[22] The use of inactivated instead of viable probiotic bacteria would have an advantage of lacking in infectivity and having technical merits in the form of longer shelf-life and reduced requirements for refrigerated storage. For these reasons, we have been very eager to investigate the clinical benefits of heat-killed probiotic bacteria, particularly those of *L. paracasei* K71, in the management of adult patients with AD.

In Japan, like almost all other developed countries, treatment with topical corticosteroid and tacrolimus has currently been recommended by the authorities as the standard treatment option for AD patients, although its therapeutic efficacy still has limitations.[1] Taking this medication status into consideration, the present clinical study was attempted to investigate potential usefulness of heat-killed probiotic lactobacilli administrated p.o. as a complementary therapy for adult AD patients who are managed with the conventional treatment.

In this double-blind RCT conducted in such patients, intake of a supplementary diet containing a heat-killed *L. paracasei* K71 bacterium (LAB diet) was shown to cause statistically significant decrease from baseline to weeks 8 and 12 in the mean skin severity scores, whereas no significant changes were observed after placebo intake. It appears, therefore, that the LAB diet may have some additive effect to improve the skin severity in adult AD subjects receiving standard treatment. However, the effect would be

considerably limited because there was no intergroup significant difference in Δ-values of skin severity scores after the start of intervention. Moreover, no substantial effect of LAB diet to improve itch or QOL impairment was demonstrated in this study. In interpretation of the results, it should be noted that the used amounts of topical therapeutics over the intervention period in the placebo group were 1.9-times greater than those in the LAB diet group. It suggests the possibility that the disease severity in the former group might be better controlled by the basal topical treatment than in the latter.

The patients with AD are known to have a decreased gut mucosal barrier function that permits the frequent transfer of exogenous antigens with resultant induction of symptomatic AD.[29–31] Matsumoto *et al.*[17] also reported that probiotic lactobacilli were useful in the management of adult patients with intractable AD through reversing increased intestinal permeability. These findings suggest that either viable or inactivated probiotic lactobacilli may exhibit beneficial activities in the management of symptomatic AD. Further studies on the clinical usefulness of a LAB diet as a complementary modality for AD patients with standard treatments are warranted to be continued in more detail.

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REFERENCES

1 Saeki H, Furue M, Furukawa F *et al.* Guidelines for management of atopic dermatitis. *J Dermatol* 2009; **36**: 563–577.
2 Galli E, Cicconi R, Rossi P, Casati A, Brunetti E, Mancino G. Atopic dermatitis: molecular mechanisms, clinical aspects and new therapeutical approaches. *Curr Mol Med* 2003; **3**: 127–138.
3 Takeuchi M, Ueda H. Increase of adult atopic dermatitis (AD) in recent Japan. *Environ Dermatol* 2000; **7**: 133–136.
4 Saeki H, Tsunemi Y, Fujita H *et al.* Prevalence of atopic dermatitis determined by clinical examination in Japanese adults. *J Dermatol* 2006; **33**: 817–819.
5 Ellis C, Luger T, Abeck D *et al.* International consensus conference on atopic dermatitis II (ICCAD II): clinical update and current treatment strategies. *Br J Dermatol* 2003; **148** (Suppl 63): 3–10.
6 Charman CR, Morris AD, Williams HC. Topical corticosteroid phobia in patients with atopic eczema. *Br J Dermatol* 2000; **142**: 931–936.
7 Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek* 2002; **82**: 279–289.
8 Kalliomäki MA, Isolauri E. Probiotics and down-regulation of the allergic response. *Immunol Allergy Clin North Am* 2004; **24**: 739–752.
9 Salminen S, Bouley C, Boutron-Ruault MC *et al.* Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998; **80**: S147–S171.
10 Sanders ME. Probiotics: considerations for human health. *Nutr Rev* 2003; **61**: 91–99.
11 Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997; **99**: 179–185.
12 Pessi T, Sütas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy* 2000; **30**: 1804–1808.
13 Rosenfeldt V, Benfeldt E, Nielsen SD *et al.* Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 2003; **111**: 389–395.
14 Weston S, Halbert A, Richmond P, Prescott SL. Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child* 2005; **90**: 892–897.
15 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.
16 Michail SK, Stolfi A, Johnson T, Onady GM. Efficacy of probiotics in the treatment of pediatric atopic dermatitis: a meta-analysis of randomized controlled trials. *Ann Allergy Asthma Immunol* 2008; **101**: 508–516.
17 Matsumoto M, Aranami A, Ishige A, Watanabe K, Benno Y. LKM 512 yogurt consumption improves the intestinal environment and induces the T-helper type 1 cytokine in adult patients with intractable atopic dermatitis. *Clin Exp Allergy* 2007; **37**: 358–370.
18 Roessler (nee Klein) A, Friedrich U, Vogelsang H *et al.* The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention. *Clin Exp Allergy* 2008; **38**: 93–102.
19 Fujiwara D, Inoue S, Wakabayashi H, Fujii T. The anti-allergic effects of lactic acid bacteria are strain-dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. *Int Arch Allergy Immunol* 2004; **135**: 205–215.
20 Shida K, Takahashi R, Iwadate E *et al. Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic

anaphylaxis in a food allergy model. *Clin Exp Allergy* 2002; **32**: 563–570.
21 Murosaki S, Yamamoto Y, Ito K *et al.* Heat-killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice. *J Allergy Clin Immunol* 1998; **102**: 57–64.
22 Sawada J, Morita H, Tanaka A, Salminen S, He F, Matsuda H. Ingestion of heat-treated *Lactobacillus rhamnosus* GG prevents development of atopic dermatitis in NC/Nga mice. *Clin Exp Allergy* 2007; **37**: 296–303.
23 Ishida Y, Nakamura F, Kanzato H *et al.* Clinical effects of *Lactobacillus acidophilus* strain L-92 on perennial allergic rhinitis: a double-blind, placebo-controlled study. *J Dairy Sci* 2005; **88**: 527–533.
24 Kobayashi H, Ishii M, Takeuchi S *et al.* Efficacy and safety of a traditional herbal medicine, *Hochu-ekki-to* in the long-term management of *Kikyo* (Delicate constitution) patients with atopic dermatitis: a 6-month, multicenter, double-blind, randomized, placebo-controlled study. *Evid Based Complement Alternat Med* Published online: 31 January, 2008; doi: 10.1093/ecam/nen003.
25 Higaki Y, Kawamoto K, Kamo T, Horikawa N, Kawashima M. Translation of Skindex 16 into Japanese, a disease-specific instrument to measure quality of life in patients with skin diseases. *Jpn J Clin Dermatol* 2002; **56**: 301–303.
26 Isolauri E, Arvola T, Sütas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000; **30**: 1604–1610.
27 Wagner RD, Pierson C, Warner T, Dohnalek M, Hilty M, Balish E. Probiotic effects of feeding heat-killed *Lactobacillus acidophilus* and *Lactobacillus casei* to *Candida albicans*-colonized immunodeficient mice. *J Food Prot* 2000; **63**: 638–644.
28 Chapat L, Chemin K, Dubois B, Bourdet-Sicard R, Kaiserlian D. *Lactobacillus casei* reduces $CD8^+$ T cell-mediated skin inflammation. *Eur J Immunol* 2004; **34**: 2520–2528.
29 Ukabam SO, Mann RJ, Cooper BT. Small intestinal permeability to sugars in patients with atopic eczema. *Br J Dermatol* 1984; **110**: 649–652.
30 Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996; **97**: 985–990.
31 Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998; **53**(Suppl 46): 20–25.

Letter to the Editor

Lipoxin A_4, a potential anti-inflammatory drug targeting the skin

Inflammatory response protects the body against injury and infection. However, such response can become dysregulated with deleterious consequences to the host. Present evidence shows that endogenous biochemical pathways such as lipoxin A_4 (LXA_4) activated during defense reactions can counter-regulate inflammation and promote resolution. This has shown promise in terms of the development of novel therapeutic approaches for inflammation-associated diseases based on endogenous agonists of resolution [1,2].

Lipoxins (LXs), a newly identified family, are short-lived endogenously produced nonclassic eicosanoids with potent anti-inflammatory bioactivities and are generated during the late phases of inflammation [3]. The original pathway for LX formation was via lipoxygenase–lipoxygenase (LO–LO) interactions; other pathways for LX synthesis involves the aspirin-triggered acetylation of cyclooxygenase-2 and the activation of 5-LO forming 15-epimer LX or aspirin-triggered LX (ATL) [3]. LXA_4 inhibits leukocyte-mediated injury, stimulates macrophage clearance of apoptotic neutrophils, and inhibits pro-inflammatory cytokine production and cell proliferation.

To circumvent rapid LX and ATL metabolism and inactivation, stable analogs bearing potent and long-lasting biological activity have been synthesized [4]. Some of these analogs have demonstrated therapeutic potential via their strong anti-inflammatory activity in several animal models of disease, including reperfusion injury, asthma, fibrosis, cancer and atherosclerosis [4].

The counter-regulatory signaling by LXA_4 and 15-epi-lipoxin A_4 is triggered by the activation of a seven-transmembrane domain receptor called ALXR, which belongs to G protein-coupled receptors and has been recognized as the main anti-inflammatory receptor. ALXR is expressed in synovial and lung fibroblasts (Fbs) [5–7]. However, to the best of our knowledge, there is as yet no report describing the anti-inflammatory effects of LXA_4 and ALXR expression in normal human skin keratinocytes (KCs) and Fbs.

A normal human dermal Fb cell line (ATCC CC-2509) was cultured as described in [5]. Normal human epidermal KCs (Clonetics-Bio Whittaker, San Diego, CA, USA) were cultured in serum-free keratinocyte growth medium (Lonza, Walkersville, MD, USA) supplemented with epinephrine, transferrin, hydrocortisone, insulin, human recombinant epidermal growth factor, and bovine pituitary extract in 5% CO_2 at 37 °C in a humidified atmosphere.

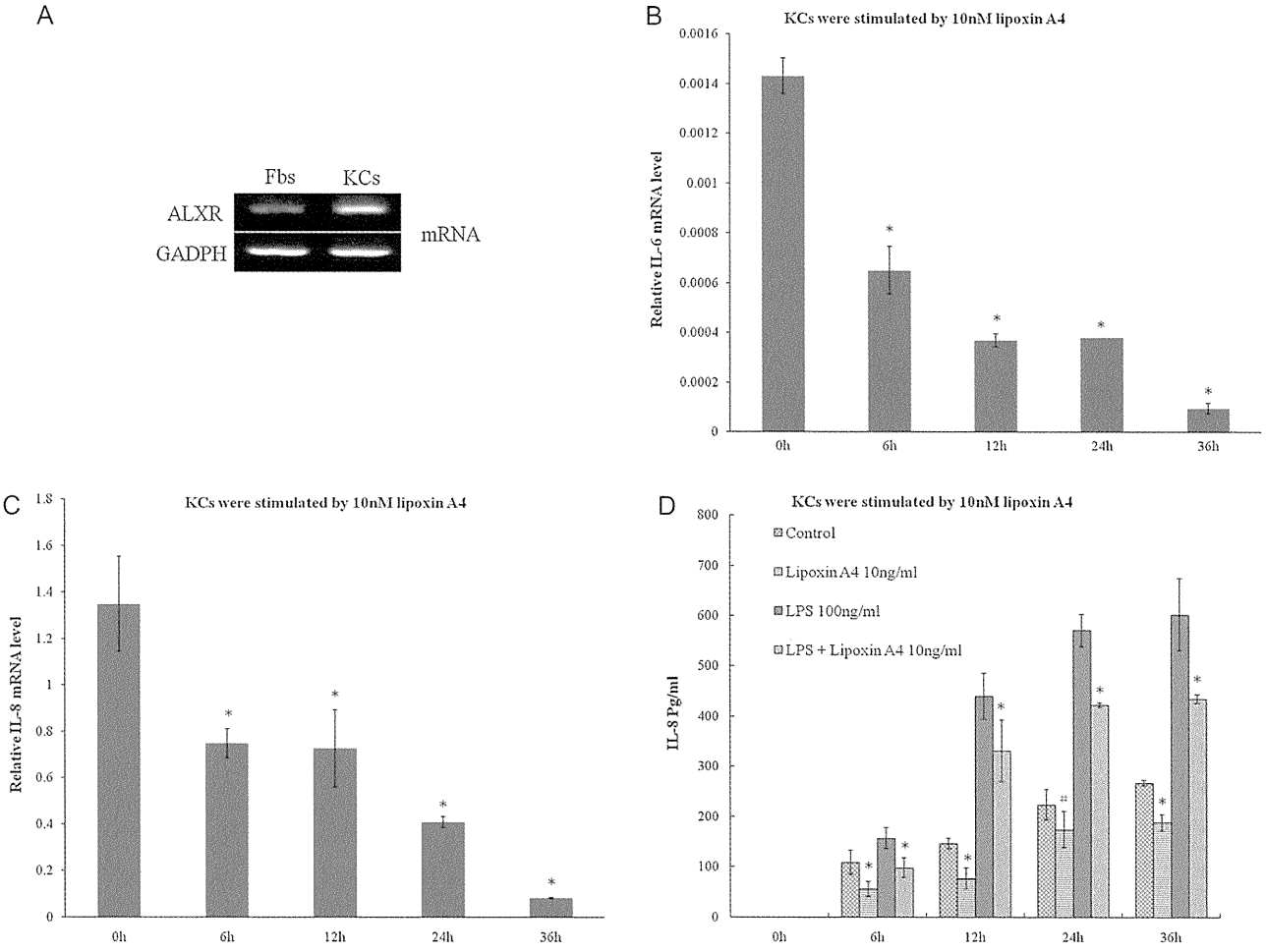

Fig. 1. Expression levels of mRNAs of ALXR, (interleukin) IL-6 and IL-8 and proteins of IL-6 and IL-8 in keratinocytes (KCs). (A) ALXR mRNA expression in KCs and Fbs; reaction products were separated on a 2% agarose gel, and band density was measured with Gel Dox TM XR (Bio-rad). Exogeneous lipoxin A_4 (LXA_4) decreased IL-6 mRNA (B) and IL-8 mRNA (C) expression levels of KCs in a time-dependent manner (0 h, 6 h, 12 h, 24 h, 36 h). (D) IL-8 concentrations were measured in KCsupernatants (0 h, 6 h, 12 h, 24 h, 36 h), both endogenous IL-8 (control) and pathological IL-8 (stimulated by LPS 100 ng/ml) levels were down-regulated by LXA4 stimulation (10 ng/ml), respectively. $^{\#}P < 0.05$; $^{*}P < 0.01$.

Synthetic LXA_4 was obtained from Cayman Chemical Company, USA. Lipopolysaccharide (LPS) was purchased from Sigma, USA. The mRNAs of normal skin KCs and Fbs were isolated using the Rneasy® Mini kit (Qiagen, Valencia, USA), and RNA (10 ng/μl) was reverse transcribed to cDNA with the Prime Script® RT reagent kit (Perfect Real Time, Takara, Japan) according to the manufacturer's protocol. Quantitative polymerase chain reaction was carried out with an Mx 3000 PTM (Strategene). Sequencing was performed with a Sequence Detection System using the Prime Script® RT reagent kit (Perfect Real Time, Takara) according to the manufacturer's protocol. The ALXR primer, the sequence of which was not provided, was designed by SABiosciences. Interleukin (IL)-6, IL-8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were designed by Takara, Japan. Levels of cytokines (IL-6 and IL-8) were measured by ELISA using commercially available BIOSOURCE IL-6, IL-8 EASIA kit (BioSource, USA).

Statistical analysis was performed using the SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL, USA). Student's *t*-test was performed to determine the presence of a significant difference. $P < 0.05$ was considered statistically significant.

KCs and Fbs constitutively expressed ALXR mRNA (Fig. 1A). As LXA_4 inhibits IL-6 and IL-8 synthesis in human synovial Fbs [5]. We next examined whether exogenous LXA_4 down-regulates endogenous IL-6 and IL-8 synthesis in KCs and Fbs. Also, IL-6 and IL-8 synthesis in pathological condition (stimulated by LPS 100 ng/ml) were detected in KCs and Fbs. The addition of 10 nM LXA_4 decreased the IL-6 and IL-8 mRNA expressions of KCs in a time-dependent manner (Fig. 1B and C). Both endogenous IL-8 (control) and pathological IL-8 (stimulated by LPS 100 ng/ml) levels in the KC supernatants were down-regulated by LXA_4 stimulation (10 ng/ml), respectively. Autocrine IL-6 production was not detected in the KC supernatants in the presence or absence of LXA_4. Similarly, both mRNA and protein synthesis of IL-6 and IL-8 were significantly and time-dependently reduced in Fbs in the presence of LXA_4 (Fig. 2A–D).

Effective host defense is regulated by inflammatory responses orchestrated through the transient and appropriate production of various pro- and anti-inflammatory mediators. LXs and their 15 epimers, ATLs, are eicosanoids derived from the sequential LO metabolism of arachidonic acid. The main routes of LX biosynthesis involve the cooperation between 15-LO and 5-LO, and between 12-LO and 5-LO [4,8].

The present results demonstrate for the first time that human normal skin KCs and Fbs stably express ALXR, and that anti-inflammatory actions are induced by the interaction of ALXR with its cognate endogenous ligand LXA_4. The inhibition of IL-6 and IL-8 release by LXA_4 is of interest because a significant elevation of IL-6 and IL-8 levels has been reported to be up-regulated in many skin inflammatory diseases such as psoriasis, suggesting that these cytokines are involved in the pathogenic processes [9]. IL-6 is a multifunctional cytokine that plays important roles in acute phase reactions, immune responses and hematopoiesis. The overexpression of IL-6 has been implicated in the pathology of numerous autoimmune and chronic inflammatory diseases, and IL-6 is

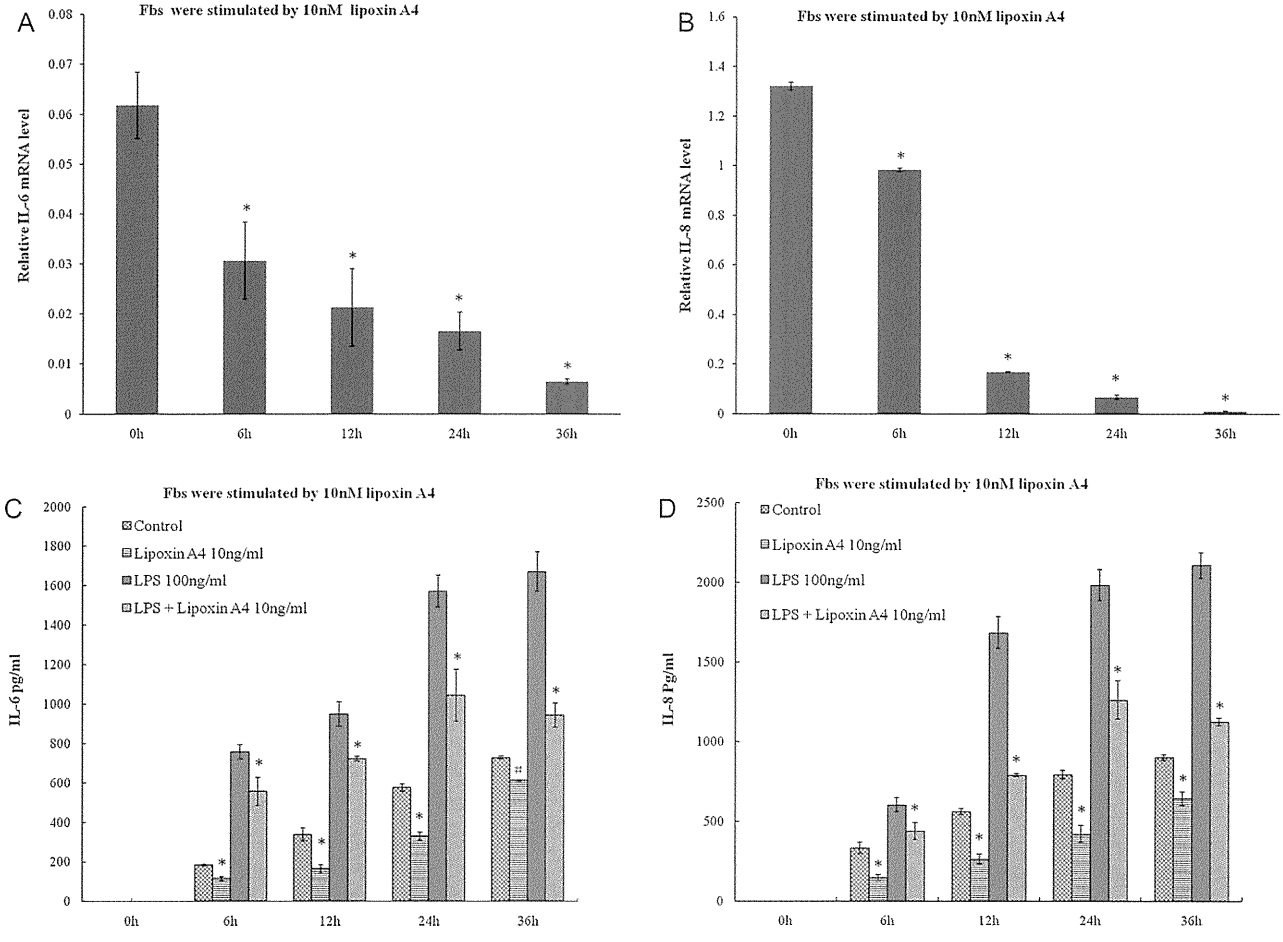

Fig. 2. Expression levels of mRNAs of IL-6 and IL-8 and proteins of IL-6 and IL-8 in fibroblasts (Fbs). (A) Exogeneous LXA_4 decreased IL-6 mRNA expression level of Fbs in a time-dependent manner (0 h, 6 h, 12 h, 24 h, 36 h). (B) Exogeneous LXA_4 decreased IL-8 mRNA expression level of Fbs in a time-dependent manner (0 h, 6 h, 12 h, 24 h, 36 h). (C) and (D). Concentrations of IL-6 and IL-8 were measured in Fb supernatants (0 h, 6 h, 12 h, 24 h, 36 h), both endogenous IL-8 (control) and pathological IL-8 (stimulated by LPS 100 ng/ml) levels were down-regulated by LXA_4 stimulation (10 ng/ml), respectively. $^{\#}P < 0.05$; $^{*}P < 0.01$.

expressed at high levels in psoriatic skin and stimulates the proliferation of cultured human KCs [9]. Recent findings have provided several new concepts that LX and 15 epi-LX play important roles in the resolution of acute inflammation and organ protection from leukocyte-mediated injury [8]. IL-8 is a member of the CXC chemokine family and was initially identified as a neutrophil chemotactic and activating factor, which is now recognized as having a wide range of functions, and plays an important role in neutrophil, basophil and T cell recruitment in the skin [10].

In conclusion, LXA_4 demonstrated inhibitory effects on the expression of pro-inflammatory cytokines IL-8 and IL-6 in KCs and Fbs. LXA_4 may be a novel therapeutic strategy for treating skin inflammatory diseases such as psoriasis.

Conflict of interest

None declared.

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References

[1] Serhan CN, Brain SD, Buckley CD, et al. Resolution of inflammation: state of the art, definitions and terms. FASEB J 2007;21:325–32.
[2] Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 2007;25:101–37.
[3] Devchand PR, Arita M, Hong S, et al. Human ALX receptor regulates neutrophil recruitment in transgenic mice: roles in inflammation and host defense. FASEB J 2003;17:652–9.
[4] Martins V, Valenca SS, Farias-Filho FA, et al. ATLa, an aspirin-triggered lipoxin A4 synthetic analog, prevents the inflammatory and fibrotic effects of bleomycin-induced pulmonary fibrosis. J Immunol 2009;182:5374–81.
[5] Sodin-Semrl S, Taddeo B, Tseng D, Varga J, Fiore S. Lipoxin A4 inhibits IL-1 beta-induced IL-6 IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metallo proteinases. J Immunol 2000;164:2660–6.
[6] VanCompernolle SE, Clark KL, Rummel KA, Todd SC. Expression and function of formyl peptide receptors on human fibroblast cells. J Immunol 2003;171:2050–6.
[7] Wu SH, Wu XH, Lu C, Dong L, Chen ZQ. Lipoxin A4 inhibits proliferation of human lung fibroblasts induced by connective tissue growth factor. Am J Respir Cell Mol Biol 2006;34:65–72.
[8] Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxin biosynthesis: an update and role in anti-inflammation and pro-resolution. Prostaglandins Other Lipid Mediat 2002;68–69:433–55.
[9] Zalewska A, Glowacka E, Wyczolkowska J, Tchorzewski H, Narbutt J, Sysa-Jedrzejowska A. Interleukin 6 and 8 levels in plasma and fibroblast cultures in psoriasis. Mediators Inflamm 2006;2006:81767.
[10] Skov L, Beurskens FJ, Zachariae CO, et al. IL-8 as antibody therapeutic target in inflammatory diseases: reduction of clinical activity in palmoplantar pustulosis. J Immunol 2008;181:669–79.

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