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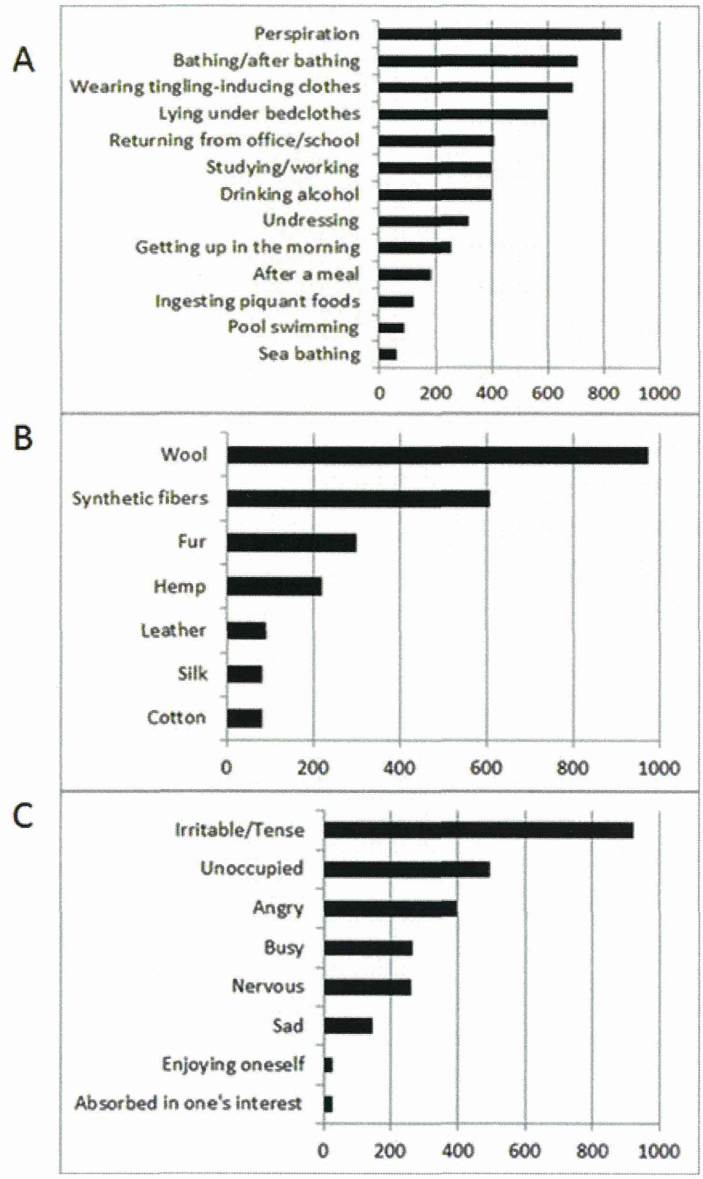
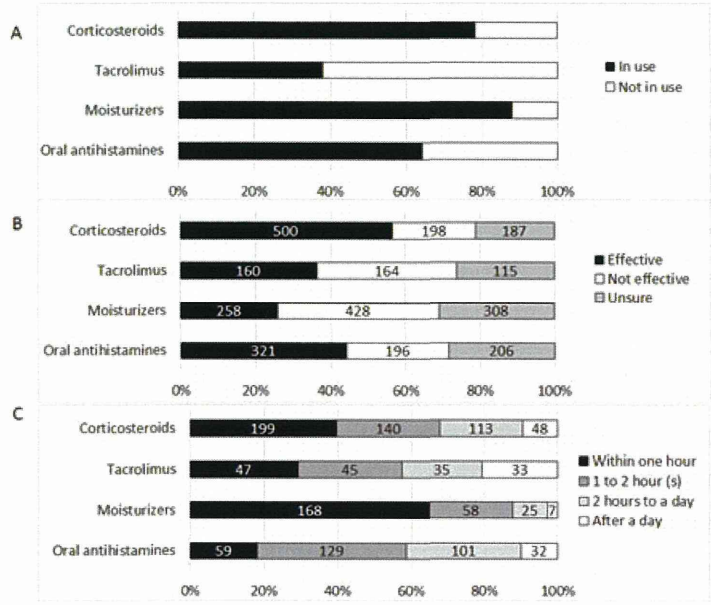
	Severity of pruritus	Efficacy	p-value	
Corticosteroids	Mild vs Moderate	51.3% vs 47.0%	1.000	n.s.
	Mild vs Severe	51.3% vs 37.0%	0.061	n.s.
	Moderate vs Severe	47.0% vs 37.0%	0.006	**
Tacrolimus	Mild vs Moderate	23.7% vs 14.4%	0.100	n.s.
	Mild vs Severe	23.7% vs 11.5%	0.015	*
	Moderate vs Severe	14.4% vs 11.5%	0.552	n.s.
Moisturizers	Mild vs Moderate	34.2% vs 23.9%	0.145	n.s.
	Mild vs Severe	34.2% vs 18.1%	0.005	**
	Moderate vs Severe	23.9% vs 18.1%	0.092	n.s.
Oral antihistamines	Mild vs Moderate	30.3% vs 31.1%	1.000	n.s.
	Mild vs Severe	30.3% vs 22.3%	0.426	n.s.
	Moderate vs Severe	31.1% vs 22.3%	0.009	**

*: $p<0.05$, **: $p<0.01$, n.s.: $p\geqq0.05$





CLINICAL REPORT

Incidence, Serum IgE and TARC/CCL17 Levels in Atopic Dermatitis Associated with Other Allergic Diseases: An Update from the Ishigaki Cohort

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Population cohort studies are important for understanding the current status of the target disease and its relation to comorbidity, gender, age, or environmental factors. To better understand atopic dermatitis (AD) and its related diseases, we initiated in 2001 a population cohort study of nursery school children from Ishigaki Island, Okinawa, Japan. The cohort study comprised a dermatologist-based physical examination, questionnaire administration, and blood sample analysis. The mean prevalence of AD was 6.3%. Questionnaire-based bronchial asthma and egg allergy in the children and paternal and sibling AD were statistically significant risk factors for AD. Boys with AD had a high incidence of asthma that was coexistent with a high serum total immunoglobulin E level. Also a high incidence of egg allergy was associated with greater AD severity as assessed by TARC/CCL17. ***Key words: atopic dermatitis; prevalence; incidence; cohort; epidemiology.***

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Atopic dermatitis (AD) is a common, chronically relapsing, severely pruritic, eczematous skin disease. The incidence of AD, including that of adolescent- and adult-type AD, is increasing worldwide (1–4). The aetiology and pathogenesis of AD are not fully understood; however, recent studies suggest that AD involves a complex interaction of skin barrier dysfunction, exposure to external allergens or microbes, a Th2-prone response, and psychosomatic reactions. Intense itching, sleep disturbance, appearance of severely affected skin, and other atopic symptoms impose a remarkable burden on affected individuals, their families, and society (5).

In 2001, we initiated a population cohort study that included nursery school children aged ≤6 years from Ishigaki Island, Okinawa, Japan; this study was named the Kyushu University Ishigaki Atopic Dermatitis Study (KIDS). Through the cohort study, we reported AD prevalence, serum total immunoglobulin E (IgE) and thymus and activation-regulated chemokine (TARC) levels, the spontaneous regression ratio, risk factors for AD, and the relationship between skin infections and childhood AD (6–9). In the present study, we report an update from the KIDS cohort on the dermatologist-examined AD prevalence and questionnaire-based incidence of AD and other related allergic diseases, risk factors for AD, serum total IgE and TARC levels, and factors other than AD that affect disease severity.

METHODS

Study design

The study design has been previously described (7). Briefly, the KIDS cohort comprises a dermatologist-based physical examination, questionnaire survey of the children's parents or guardians, and analysis of collected blood samples. The study was approved by the Ethics Committee of Kyushu University and the directors and classroom teachers of the nursery schools. The parents or guardians provided written informed consent for participation of the children.

Ishigaki Island is located approximately 410 km southwest from the main island of Okinawa and has a subtropical oceanic climate. The mean annual temperature and humidity are 24.4°C and 73.1%, respectively, which are higher than those of Tokyo, Japan (16.5°C and 60%, respectively).

Physical examination and questionnaire

Dermatologists from the Department of Dermatology, Kyushu University Hospital, conducted medical skin examinations of the children since 2001. AD was diagnosed according to the Japanese Dermatological Association's diagnostic criteria for AD (10). The parents or guardians of the children completed a structured questionnaire that included the children's personal history of allergic diseases such as AD, allergic rhinitis (AR), bronchial asthma (BA), and food allergy. The current form of the questionnaire has been used since 2006.

Measurement of serum TARC and total IgE levels

Serum TARC levels were measured using enzyme-linked immunosorbent assay (ELISA, Shionogi & Co., Ltd., Osaka, Japan), according to the manufacturer's protocols. Serum total

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IgE levels were determined using radioimmunoassay (Shionogi & Co., Ltd., Osaka, Japan), as previously described in detail (8).

Statistical analysis

The Mann-Whitney *U* test was used to compare variables between the 2 groups. Gender-based difference in the incidence of allergic diseases and the association between AD and other allergic diseases were examined using the χ^2-test or Fisher's exact test, depending on the variable distribution. Univariate and multivariate logistic regression analyses were used to assess risk factors for AD incidence. A p-value <0.05 was considered statistically significant.

RESULTS

Study population

In total, 7,856 nursery school children aged ≤6 years from approximately 13 nursery schools participated in the KIDS cohort from 2001 to 2011. The data of AD prevalence from 2001 to 2004 were taken from our previous report (7). The data on serum total IgE levels were obtained from a total of 2,841 blood samples (1,507 boys and 1,334 girls) collected from 2001 to 2009, after excluding data of individuals duplicated in later years, if any, to avoid the possibility of children with any allergic diseases participating more often than healthy children, which would potentially affect mean total IgE values. For assessing disease risk factor, we examined questionnaires from 2006 to 2008, excluding data of individuals duplicated in later years, if any, to collect as much information on the examinees' incidence of each disease. We used the data obtained from blood samples of 743 nursery school children in 2008 in order to assess the relation between disease severity and AD-related comorbidities.

Incidence of AD-related allergic diseases and risk factors for AD

The mean annual prevalence of AD through the 11 years in the KIDS cohort was 6.3%. The mean prevalence of AD in boys was 5.7%, and that of girls was 7.0%; the prevalence rates did not significantly differ between the 2 genders (Table SI[1]).

From 2006 to 2008, 1,195 answers to the questionnaires were obtained from 658 boys and 537 girls. The results of the questionnaire regarding incidental AD and related allergic diseases such as AR, BA, and food allergy (mostly egg allergy [EA]) based on gender are shown in Table I. The AD incidence (during infancy) in boys and girls was 13.2% and 14.4%, respectively, with no gender-based difference. The AR incidence in boys and girls was 3.1% and 2.5%, respectively, with no gender-based difference. The incidence of BA in boys and girls was 17.4% and 11.9%, respectively, with a statistically significant difference ($p=0.008$). Among food allergies, EA was the most prevalent (67.1%) and has sufficient numbers for subsequent analysis; therefore, EA incidence was used to represent food allergy. In the questionnaire, a total of 54 children were identified as having been diagnosed as EA. Excluding 7 children identified in 2007 whose questionnaire lacked the free field regarding detailed symptoms of food allergy due to a printing problem, 30 out of the remaining 47 patients (63.8%) referred to their detailed symptoms as having eczema/urticaria (86.7%), itching only (6.6%), diarrhoea (3.3%) and a positive skin patch test (3.3%). The EA incidence in boys and girls was 5.3% and 3.8%, respectively. When nursery school children were divided into incidental AD and non-AD groups regardless of gender, the AR incidence in AD and non-AD groups was 5.5% and 2.4%, respectively. The BA incidence was 28.1% and 12.9%, and the EA incidence was 18.4% and 2.3%, respectively. All allergic diseases known as AD-related diseases examined in the questionnaire showed significantly higher disease incidence rates in the AD group than in the non-AD group (Table II). Specifically, the difference in EA incidence between the AD and non-AD groups was remarkable. Univariate analysis of familial history of allergic diseases revealed that incidences of paternal AD, AR, and BA; maternal AD and food allergy; and sibling AD and AR were significantly associated with the subjects' AD incidences. Further, multivariate logistic regression analysis, including age, gender, and incidence of other allergic diseases of the subject's and family history of allergy, revealed that the incidences of subject's BA and EA and paternal and sibling AD were statistically significant risk factors for AD as determined by an odds ratio >2 (Table SII[1]).

Table I. *Incidences of allergic diseases based on gender of nursery school children from Ishigaki Island*

	Boys		Girls		
	No *n*	Yes *n* (%[a])	No *n*	Yes *n* (%)	*p*-values χ^2-test
Atopic dermatitis	571	87 (13.2)	460	77 (14.3)	0.524
Allergic rhinitis	632	20 (3.1)	517	13 (2.5)	0.524
Bronchial asthma	536	113 (17.4)	466	63 (11.9)	0.008
Egg allergy	614	34 (5.3)	508	20 (3.8)	0.234

[a]% of total number of children.

Table II. *Atopic dermatitis (AD)-associated allergic diseases in nursery school children from Ishigaki Island*

	AD		Non-AD		
	No *n*	Yes *n* (%[a])	No *n*	Yes *n* (%)	*p*-values χ^2-test
Allergic rhinitis	154	9 (5.5)	995	24 (2.4)	0.036[b]
Bronchial asthma	115	45 (28.1)	887	131 (12.9)	0.00000
Egg allergy	122	30 (18.4)	1,000	24 (2.3)	0.00000

[a]% of total number of children. [b]Fischer's exact method.

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1989

Serum total IgE levels in children from Ishigaki Island

The analysis of blood samples of children obtained from 2001 to 2009 revealed an increase in serum total IgE levels from the age of 2 years, and the levels in boys from the age of 2–4 years were significantly higher than those in girls of the same age (Fig. 1). When children were divided into groups based on whether they had allergic disease or not, according to the data from the questionnaire 2006–2008, the mean total IgE levels in incidental AD and non-AD children were 425.3 ± 678.3 IU/ml and 141.9 ± 315.4 IU/ml, respectively; the levels for those with incidental AR and non-AR were 454.8 ± 592.4 IU/ml and 172.7 ± 385.0 IU/ml, respectively; the levels for those with incidental BA and non-BA were 328.3 ± 505.6 IU/ml and 157.3 ± 376.6 IU/ml, respectively; and the levels for those with incidental EA and non-EA were 496.7 ± 784.6 IU/ml and 164.1 ± 358.0 IU/ml, respectively (graphs not shown). These mean total IgE levels in the incidental disease groups of each allergic disease were statistically higher than those in the non-incident groups ($p < 0.01$). In incidental AD children with coexistent BA, but without AR or EA, the serum total IgE levels increased significantly compared to incidental AD children without BA (Fig. 2).

Disease severity in children with AD and EA obtained from answers to the questionnaire

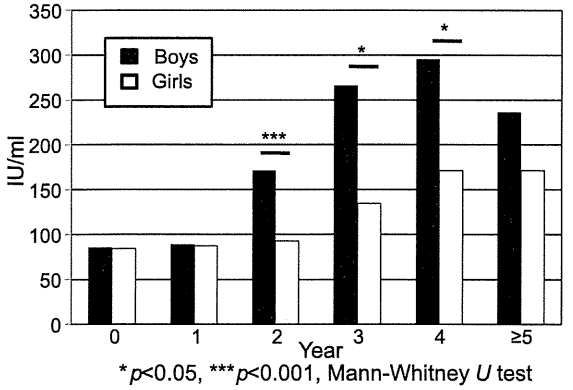
Among the 743 children examined in 2008, blood samples were successfully obtained from 696 children, from whom we received 520 valid answers to the questionnaire. The mean TARC levels of non-allergic children decreased with age, while those of the AD group did not change with age (Fig. 3).

Among the 520 valid answers on EA history, TARC and total IgE levels were measured for 520 and 519, respectively. The mean TARC and total IgE levels of the children with EA history were 733.5 ± 461.1 pg/ml and 385.7 ± 580.4 IU/ml, respectively, while those in children without EA history were 554.0 ± 371.1 pg/ml and 181.0 ± 324.0 IU/ml, respectively. The TARC and total IgE levels in children with EA history were significantly higher than those in children without EA history (graphs not shown). We obtained 31 answers for EA history in the group with AD, and serum TARC levels were measured for 30 out of the 31. The mean TARC levels of the AD children with EA history was 965.0 ± 487.8 pg/ml, while that of AD children without EA history was 703.5 ± 440.0 pg/ml. The serum TARC level in incidental AD children with EA history was significantly higher than in those without EA history (Fig. 4).

DISCUSSION

The AD incidence assessed by questionnaire (13.7% in total) was considerably higher than the AD prevalence assessed by physical examination (6.3% in annual average) because those with incidental AD included a population who had AD but had remitted naturally at the time of examination in the present study. Most infantile AD cures or remission occurred naturally. Additionally, in the follow-up of the KIDS study, we found that AD in 71.6% of the children who were diagnosed by dermatologists alleviated naturally within 3 years (7), explaining the gap between the AD incidence rate via the answers to the questionnaire and the mean annual AD prevalence.

The AD prevalence on Ishigaki Island was apparently lower than that reported by Yamamoto S where from 2000 to 2002, a research team of the Japanese Ministry of Health, Labour, and Welfare examined 48,072 children living in Asahikawa, Iwate, Tokyo, Gifu, Osaka, Hiroshima, Kochi, and Fukuoka (11). They reported that the national mean prevalence of AD was 12.8% in individuals aged 4 months, 9.8% in those aged 18 months, 13.2% in those aged 3 years, 11.8% in those aged 6–7 years, 10.6% in those aged 12–13 years, and 8.2% in those aged 18 years (11). The AD prevalence on Ishigaki Island (6.3%) was approximately half that of correspondingly aged children in mainland Japan. There are many possible explanations for this, such as different pathogens, dietary habits, or flora. However, we believe that the high temperature (24.4°C) and humidity (73.1%) of Ishigaki Island might reduces the



Age	Boys	Girls
0	52	35
1	391	376
2	443	354
3	316	263
4	208	215
5	97	91

Fig. 1. Total serum IgE levels based on gender and age differences in nursery school children from Ishigaki Island. The data on total IgE levels were obtained from 2,841 blood samples (1,507 boys and 1,334 girls) taken from 2001 to 2009, without individual duplication. *$p < 0.05$ and ***$p < 0.001$ using the Mann-Whitney U test.

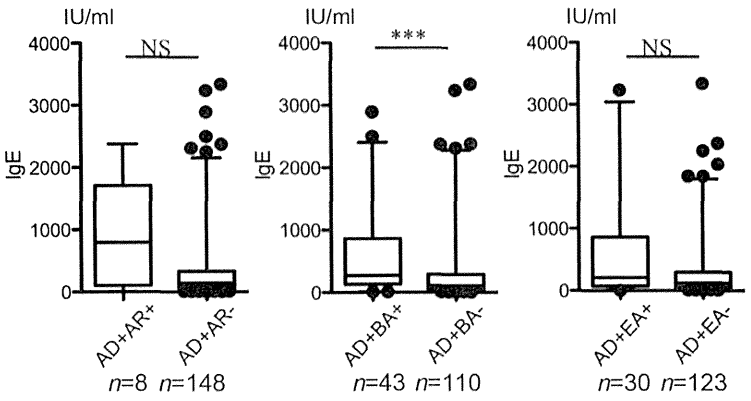


Fig. 2. Total serum IgE levels in nursery school children from Ishigaki Island who have atopic dermatitis (AD) coexistent with other allergic diseases. These data were obtained from questionnaires and blood samples obtained from 2006 to 2008, excluding individual duplication. ***$p<0.001$ using the Mann-Whitney U test. AR: allergic rhinitis; BA: bronchial asthma; EA: egg allergy. NS: non-significant.

AD incidence, presumably reducing AD onset in those who have filaggrin mutation (12) or a defect of filaggrin-digesting enzyme (13). Indeed, a genetic analysis in the KIDS cohort in collaboration with the Department of Dermatology, Keio University School of Medicine, did not find a positive association between filaggrin mutations and AD incidence in Ishigaki Island children (14).

There was a gender difference in total IgE levels (significantly higher in boys aged 2–4 years than girls of the same age group) (see Fig. 1). Serum total IgE levels are increased in children with any allergic disease (either AD, AR, BA, or EA), as shown elsewhere; however, in our study, only BA showed a significantly higher disease incidence in boys (see Table I). In addition, BA coexistence alone further increased serum total IgE levels in children with AD. Therefore, we believe that the gender difference in total IgE levels may be because of the high BA incidence in the boys in our study. We further examined whether there is a gender difference of TARC levels since severity of AD is reported to be associated with prevalence of asthma (15) and serum IgE levels (16). However, there was no significant gender difference between boys and girls by age in the present study (Fig. S1[1]).

Another interesting finding was a high coexistence of EA in children in the incidental AD group. The coexistence of EA in children with AD was 7.9 times higher than that in children without AD. This rate was considerably higher than the rates in other diseases (AR or BA), which were approximately 2 times (Table II). Incidental EA was also a significant risk factor for AD (Table SII[1]). These data indicate an intimate relation between AD and EA. Another interesting finding was that the serum TARC levels, a disease severity marker, in children with AD and EA were significantly higher than those in children without EA (see Fig. 4). The serum TARC levels reflect disease severity of infantile AD (17, 18), and AD severity appears to be correlated with the degree of EA (19). The significant increase in TARC levels in children with AD and EA in the present study may indicate that EA comorbidity might be an important exacerbation factor in children with AD, although we currently do not know whether it is really causative or a bystander. Alternatively, children with more severe AD may be prone to EA, presumably by repeated percutaneous sensitisation through the impaired barrier in the lesional skin.

In conclusion, we obtained the current status of AD and other associated allergic diseases in children from Ishigaki Island. Subjects' BA and EA and paternal and sibling AD were statistically significant risk factors for AD. We found that a high BA incidence in boys was coincident with high total IgE levels, and that EA was associated with AD severity. Further studies focusing on these findings are of interest.

ACKNOWLEDGEMENTS

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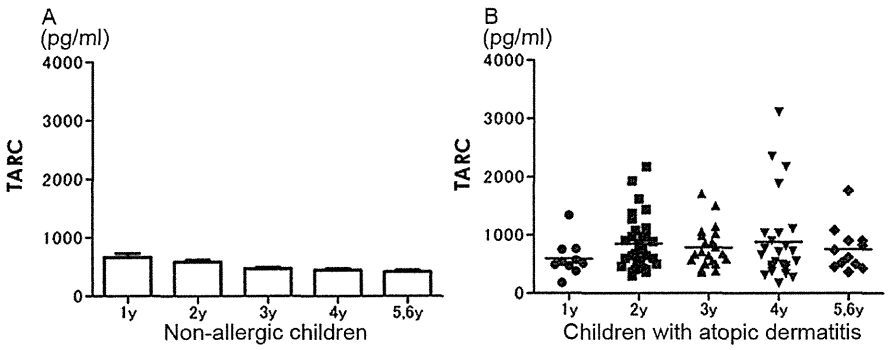

Fig. 3. Changes in serum TARC levels according to the age of nursery school children from Ishigaki Island who have no allergies or have atopic dermatitis. The data were obtained from blood samples taken in 2008.

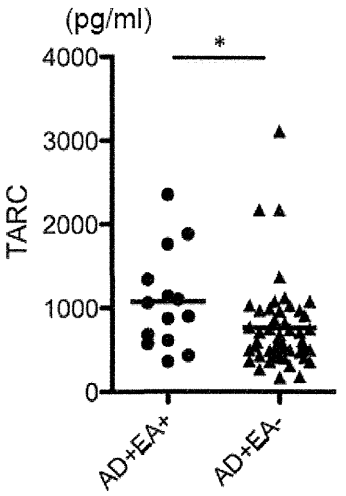

Fig. 4. Mean serum TARC levels of nursery school children with incidental atopic dermatitis (AD) and coexistent incidenatal egg allergy (EA) are significantly higher than those of children without EA. The data were obtained from questionnaires and blood samples in 2008. $*p<0.05$ by Mann-Whitney *U* test.

The authors declare no conflict of interest.

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Reduction of serum TARC levels in atopic dermatitis by topical anti-inflammatory treatments

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Summary

Background: **Serum thymus and activation-regulated chemokine (TARC) levels are associated with the disease activity of patients with atopic dermatitis (AD) and sensitively reflect short-term changes in skin conditions. The main treatment for AD is topical agent application.**

Objective: **This study investigated the relationship between serum TARC levels and the dosage of topical agents, including corticosteroids and/or tacrolimus, in patients with AD.**

Methods: **The serum TARC levels of 56 AD patients and the amounts of topical agents prescribed to them were investigated retrospectively. The weekly reduction in serum TARC levels and weekly dosage of topical agents among AD patients were compared and their associations were evaluated.**

Results: **The dosage of topical agents was closely related to serum TARC levels. One gram of strong rank steroid or the equivalent amount of steroid/tacrolimus is required to reduce serum TARC levels by 9.94 pg/mL weekly in moderate to severe AD patients. Higher initial TARC levels require more topical agent, which results in a more rapid decrease in TARC levels. The serum TARC levels and eosinophil numbers in peripheral blood are significantly correlated.**

Conclusion: **Serum TARC level improvement and topical agent dosage are strongly correlated. TARC and eosinophil numbers are significantly correlated, but the wider range of TARC levels seems to be clinically more useful for monitoring AD severity. The serum TARC level is a very sensitive biomarker for monitoring the severity and treatment response in AD.** ***(Asian Pac J Allergy Immunol 2014;32:240-5)***

Keywords: *atopic dermatitis, serum thymus and activation-regulated chemokine (TARC) levels, topical corticosteroids, topical tacrolimus, total equivalent amounts (TEA)*

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Introduction

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic, eczematous skin disease that manifests not only in humans but also in other mammals, such as dogs.[1,4] The waxing and waning clinical course of AD results in deterioration in patients' quality of life because of spontaneous or seasonal flare-up.[2,3] The most important clinical symptom is intolerable itch. By scratching, patients easily fall into a vicious circle called the "itch-scratch cycle", resulting in chronic sleep disturbance.[6] The main treatment of AD is skin moisturization with emollients and topical anti-inflammatory agents, such as corticosteroids and tacrolimus.[2,3]

Thymus and activation-regulated chemokine (TARC), a chemokine involved in Th2 cell migration, was recently found to be closely associated with AD.[7,8] The measurement of TARC was recently covered by medical insurance in Japan. Serum TARC levels are significantly elevated in patients with AD, particularly in those severely affected by the disease, compared with patients with other inflammatory skin diseases and healthy controls.[7-12] The TARC levels are significantly correlated with the clinical severity scores of AD. Therefore, serum TARC level is now considered a specific and objective indicator of AD disease activity.[7-12] Another feature of serum TARC level that makes it

a useful and reliable biomarker is its wide range of values - 100 to 50000 or more pg/mL - allowing it to sensitively correspond to the waxing and waning of AD severity.[11,12] Tamaki et al. reported that it is now feasible to quantify a patient's AD severity according to the serum TARC level (mild state: ≤700 pg/mL, moderate/severe state: >700pg/mL).[12] This is very advantageous because both dermatologists and their patients can evaluate the severity state of AD by using the same measures. Dermatologists can confidently ask the patients to change treatment strategy to reduce his/her TARC level to ≤700 pg/mL or 450 pg/mL (the normal control level); this greatly increases patients' adherence to treatment in routine clinical practice.

The concept of finger-tip units is a useful application method and is recommended in therapeutic guidelines.[2,13] In general, doses of topical steroids and tacrolimus are closely related to the severity of AD.[14,15] However, how topical dosage affects serum TARC levels is not well understood. This study statistically assessed the influence of topical agent dosage on the reduction of serum TARC levels.

Methods

Patients

Patients were diagnosed as AD according to the diagnostic criteria of the Japanese Dermatological Association.[2] A total of 349 patients with AD (184 men and 165 women), whose serum TARC levels were measured between April 2008 and October 2012, were initially enrolled. The following patients were excluded: patients whose TARC levels were measured only once (n = 188), those taking oral immunosuppressants such as cyclosporine and prednisolone and/or receiving ultraviolet therapies (n = 54), children aging 14 years and under (n = 13), those whose TARC levels were checked at an interval exceeding 3 months (n = 36), and mild patients with TARC levels ≤700 pg/mL (n = 2). Finally 56 moderate/severe patients out of 349 patients (16.05%, 31 men and 25 women, mean age 34.75±12.76, range 15-73 years) whose TARC levels were examined before and after treatment within 3 months were included. If a patient had multiple TARC measurements, only the first pair was used. All 56 patients were treated with topical anti-inflammatory treatments continuously during those periods and all of them received oral anti-histamines and emollients in addition.

Methods

We divided the patients into 2 groups; those with TARC levels ≥3001 pg/mL (severe AD group) and 701 to 3000 pg/mL (moderate AD group). To calculate the weekly reduction in TARC levels, the difference between the pre- and post-treatment TARC levels was divided by the number of weeks in the intermediate periods. Meanwhile, the amounts of topical steroids and/or topical tacrolimus prescribed for each patient were checked during these periods by asking how much topical agents the patient used or by checking the number of used tubes and the weekly dosages of topical agents were calculated. The amounts of topical agents per week are expressed as the total equivalent amount (TEA) and were calculated by multiplying by potency equivalent factors as follows[16]: strong rank steroids, x1; mild rank steroids, x0.5; very strong rank steroids, x2, and strongest rank steroids, x4. For example, 1g of the strongest rank steroid represented 4 TEA. Tacrolimus ointments (0.1% and 0.03%) were classified as strong rank (x1) and mild rank (x0.5) steroids, respectively. Among the 56 patients, 31 (55.4%) used topical steroids only and 25 patients (44.6%) used both topical steroids and tacrolimus but there were no patients treated with tacrolimus only. The study was approved by the ethical committee of Kyushu University Hospital.

Statistical analysis

Statistical analysis was performed using the Microsoft Excel software under the Windows 7 operating system, and the SPSS statistical software package for Windows (Version 11.0, SPSS Inc., Chicago, IL, USA). Data are expressed as means±standard error (SE). The weekly TARC reduction, weekly TEA and the rate of change in TARC levels between the moderate and severe AD groups were analyzed using unpaired *t*-tests. The factors affecting the serum TARC levels were analyzed using analysis of covariance (ANCOVA). A *P*-value of <0.05 was considered to indicate statistical significance.

Results

Serum TARC levels of patients with moderate/ severe AD

The overall pre-treatment serum TARC levels of the 56 moderate/severely affected patients with AD ranged from 829 to 52000 pg/mL (mean±SE; 7076.48±1336.73 pg/mL). Post-treatment TARC levels within 3 months after topical treatment ranged

Table 1. Weekly TARC reduction and weekly TEA in all the patients and those patients with TARC levels 701-3000 and ≥3001 pg/mL

	TARC (701-3000 pg/mL) mean ± SE/frequency(%)	TARC (≥3001 pg/mL) mean ± SE/frequency(%)	TARC (≥701 pg/mL) mean ± SE/frequency(%)	*p*-value
Age(years)	35.46 ± 2.39	34.03 ± 2.47	34.75 ± 1.71	0.679306
Gender(M)	15(53.6%)	16(57.1%)	31(55.3%)	0.788077
Pre-treatment TARC(pg/mL)	1847.89 ± 137.60	12305.07 ± 2246.94	7076.48 ± 1336.73	0.000097
Post-treatment TARC(pg/mL)	971.11 ± 113.88	2138.32 ± 342.48	1554.71 ± 198.07	0.003205
TARC reduction per week(pg/mL)	143.67 ± 24.34	2089.67 ± 411.25	1116.68 ± 245.78	0.000079
TEA per week	61.85 ± 7.96	104.57 ± 14.45	83.21±8.77	0.013490

from 159 to 6740 pg/mL (1554.71±198.07 pg/mL) (Table 1, Figure 1A). The pre-treatment TARC levels in the moderate AD group (1847.89±137.60 pg/mL) decreased significantly to 971.11±113.88 pg/mL post-treatment (Figure 1B). The pre-treatment TARC levels of the severe AD group (12305.07±2246.94 pg/mL) decreased rapidly to 2138.32±342.48 pg/mL within 3 months (Figure 1C). The rate of change in the severe AD group was significantly faster than in the moderate TARC group ($p < 0.001$).

Weekly dosage of topical agents and reduction in serum TARC levels

The weekly TEA of the overall moderate/severely affected patients ranged from 10 to 340 (83.21±8.77) (Table 1). The weekly TARC reduction of the study group as a whole ranged from 3.1 to 7765 (1116.68±245.78 pg/mL). The weekly TARC reduction was significantly correlated with weekly TEA (Figure 2). The weekly TARC reduction (2089.67±411.25 pg/mL) of the severe AD group was significantly larger than that of the moderate AD group (143.67±24.34 pg/mL) ($p < 0.001$). Accordingly, the weekly TEA (104.57± 14.45) of the severe AD group was significantly larger than that of the moderate AD group (61.85±7.96) ($p < 0.001$) (Table 1).

Factors affecting the reduction of serum TARC levels

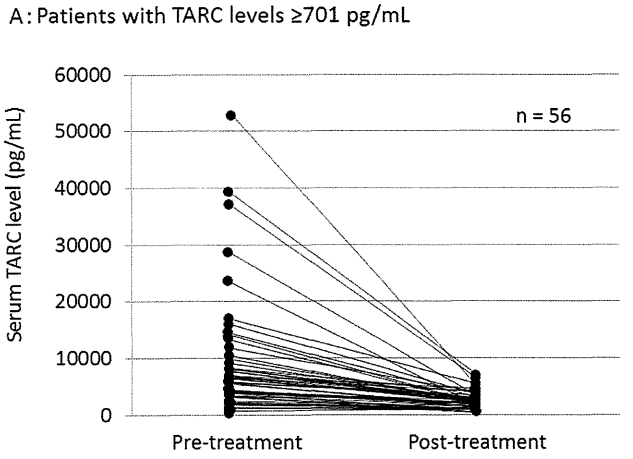
Next we analyzed whether the following factors affected the serum TARC levels using regression analysis: age, gender, treatment duration (weeks), TEA values, and initial severity (TARC ≤3000 or ≥3001 pg/mL). The TEA values ($p < 0.01$) and initial severity ($p < 0.001$) were significantly related to the reduction of TARC levels (Table 2). The results of ANCOVA demonstrated that 1 TEA per week reduced serum TARC levels by 9.94 pg/mL.

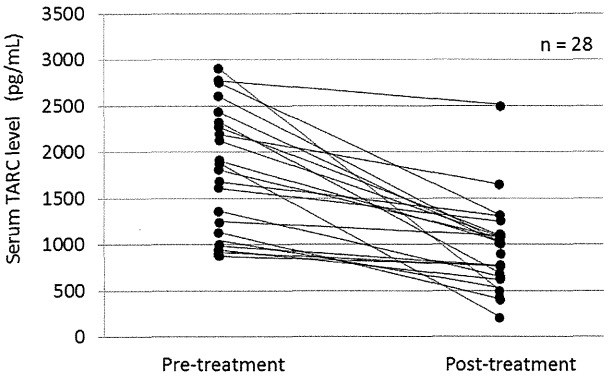
Correlation between TARC and eosinophil number

There was a moderate and significant correlation between pre-treatment TARC levels and eosinophil numbers ($R^2 = 0.2822$, $P = 0.000003585$), as has been reported by Kakinuma et al.[7] A significant correlation was also observed between the pre- and post-treatment reduction of TARC levels and the reduction of eosinophil numbers ($R^2 = 0.236$, $P = 0.0002978$). The pre-treatment values of TARC and eosinophil numbers ranged from 829-52000 and 162-7634, respectively, and the pre- and post-treatment reduction of TARC levels and eosinophil numbers ranged from 28-48140 and 1571-7295, respectively, confirming that the wider range of TARC levels seemed to be clinically more useful for evaluating AD activity than eosinophil numbers.

Discussion

Although the topical application of steroids and tacrolimus is the mainstay of the treatment of AD, dosages of topical agents prescribed in daily clinical practice were actually small, possibly reflecting patients' aversion to steroid use which is spreading worldwide.[14,15,17] As documented previously, up to 75% of adolescent/adult patients with AD are prescribed a total of less than 180g topical steroids per 6 months (7.5g/week) and less than 59g topical





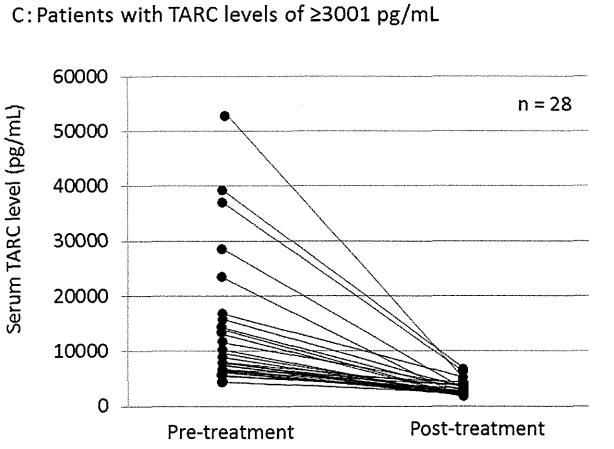


Figure 1. Serum TARC levels pre- and post-treatment. A: Patients with TARC levels ≥ 701 pg/mL. B: Patients with TARC levels 701 to 3000 pg/mL. C: Patients with TARC levels ≥3001pg/mL.

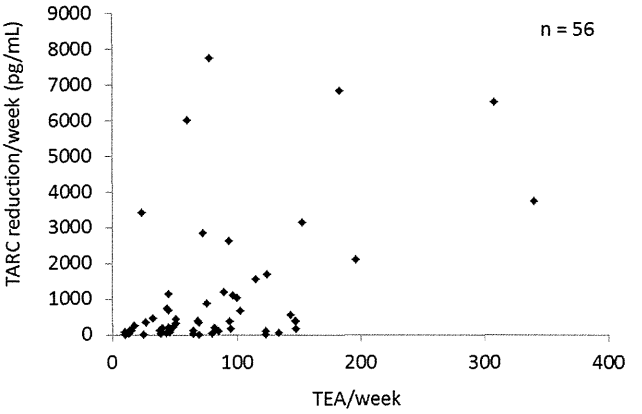


Figure 2. Correlation between weekly TARC reduction and weekly TEA

tacrolimus per 6 months (2.5g/week).[14,15] Treatment outcomes were unsatisfactory in this situation because 19% of adolescent and adult AD patients remained in a very severe or severe state or experience exacerbation.[14] Since Japanese medical insurance began to cover the monthly measurement of serum TARC levels in AD patients, monitoring TARC levels has been recognized as a very useful tool for setting treatment goals through mutual discussion between a patient and a dermatologist. Kimura et al.[18] stresses the importance of bringing TARC levels down and keeping them under 500 pg/mL. However, considering patients' aversion to steroids, it is difficult to persuade or negotiate with a patient to use a suitable amount of topical agents.

The present study examined the dose impact of topical agents on the reduction of TARC levels. The TEA was calculated by summing up the amounts of different topical agents multiplied by their respective potency equivalent factor. As expected, a greater increase in the TEA resulted in a greater decrease in TARC levels. One TEA contributed to a roughly 10 pg/mL reduction in TARC levels per week. Patients in the severe AD group exhibited a more rapid decrease in TARC levels than those in the moderate AD group. Although the exact reason for this remains unknown, we assume that more severely affected patients with more damaged skin may absorb topical agents to a greater extent, consequently inducing a dramatic reduction of TARC levels.

Intrinsic and extrinsic AD have recently received attention.[19-21] In this study, 4 female patients with normal IgE levels (104 to 138 IU/mL) were identified; their pre- and post-treatment TARC

Table 2. Regression analysis of factors affecting serum TARC level reduction

Factors affecting TARC reduction	*B*	SE	*p*-value	
Age	-19.57	15.62	0.2159	
Gender	467.00	394.87	0.2425	
Weekly TEA	9.94	3.44	0.0056	<0.01
Weeks	48.53	79.35	0.5436	
Initial TARC3000 (ref.:<3000 pg/mL)	-1626.71	436.44	0.0005	<0.001

levels changed from 839 to 394, 2778 to 1040, 4980 to 748, and 12300 to 847 pg/mL, respectively. These findings indicate TARC is likely to be a reliable biomarker of AD irrespective of IgE level.

In addition, the TARC levels significantly correlated with the number of eosinophils, as has been documented previously.[7] However, due to wider range of the values, the TARC levels seemed to be more advantageous than the number of eosinophils in evaluating the disease activity.

Oral anti-histamines are effective therapeutic adjuncts in AD.[2,3] Interestingly, Shoji et al.[22] demonstrated that antihistamines inhibit TARC production by human CD14+ monocytes/macrophages *in vitro*. Concordant with this evidence, Kimura et al.[18] found that the addition of oral antihistamines to topical steroids decreases TARC levels to a significantly greater extent than topical therapy alone. Since all patients in the present study received antihistamines simultaneously, we were unable to investigate the effect of antihistamines on the reduction of TARC levels.

This study has the following limitations; (1) the TEA may not represent the actual consumption of topical agents, (2) 3 months of post-treatment duration may be too long to adequately investigate the dose-effect relationship, and (3) we cannot exclude the beneficial effects of antihistamines and emollients, etc.

In conclusion, the results of the present study suggest that the weekly application of 1 TEA reduces TARC levels by 10 pg/mL in AD patients with serum TARC≥701 pg/mL.

Acknowledgement

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ORIGINAL ARTICLE

Safety and efficacy of topical E6005, a phosphodiesterase 4 inhibitor, in Japanese adult patients with atopic dermatitis: Results of a randomized, vehicle-controlled, multicenter clinical trial

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ABSTRACT

The safety and efficacy of topical E6005, a novel phosphodiesterase 4 inhibitor, in Japanese adults with atopic dermatitis were evaluated. A total of 78 patients were randomized to receive either the 0.2% E6005 ointment or vehicle control (without E6005) at an allocation ratio of 2:1. The randomization phase of 4 weeks was followed by an extension phase of 8 weeks. In the extension phase, all 67 subjects who completed the randomization phase were treated with 0.2% E6005 ointment. The 4-week application of topical E6005 twice daily was safe and well tolerated. The safety profile for up to 12 weeks was similar to that for the first 4 weeks. No deaths or other serious adverse effects were observed during the entire study period of 12 weeks. Plasma E6005 was undetectable in all subjects at all sampling points while very low plasma concentrations of an E6005 metabolite were detected in 47% of subjects receiving E6005 treatment. At the end of week 4, Eczema Area and Severity Index (EASI), Severity Scoring Atopic Dermatitis (SCORAD)-objective, SCORAD-C (visual analog scales for pruritus and sleep loss), itch Behavioral Rating Scale, and the severity of the targeted eczematous lesions in the topical E6005 group showed trends toward improvement compared with those in the vehicle group (not statistically significant). However, the group receiving topical E6005 for 12 weeks showed significant score reductions from baselines for EASI ($P = 0.030$), SCORAD-objective ($P < 0.001$) and SCORAD-C ($P = 0.038$). These results further support the development of topical E6005 for the treatment of atopic dermatitis.

Key words: atopic dermatitis, E6005, inflammation, phosphodiesterase 4 inhibitor, topical drug.

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic, relapsing, eczematous skin disease characterized by pruritus and inflammation, and is associated with cutaneous barrier dysfunction.[1,2] Both genetic and environmental factors contribute to the development of AD but the precise interplay between genetic predisposition and environmental triggers remains to be clarified. Immune cells in AD patients have elevated phosphodiesterase 4 (PDE4) activity.[3] In fact, PDE4 inhibitors reduce inflammatory parameters in AD patients.[4,5] *In vitro* and animal studies have shown that PDE4 inhibitors cause activation of effector proteins such as protein kinase A, resulting in inhibition of the production of inflammatory cytokines, degranulation and migration of neutrophils, and accumulation of T-cell chemoattractants.[5–10]

Roflumilast is an oral PDE4 inhibitor which is available in the USA and EU for the maintenance treatment of patients with severe chronic obstructive pulmonary disease (COPD) associated with chronic bronchitis. However, roflumilast may be associated with systemic adverse effects (AEs) such as diarrhea, emesis, weight loss, headache, insomnia, and symptoms of depression.[11]

In general, topical agents might have less activity than oral and injectable drugs but they have significantly less systemic side effects. Treatment of AD using PDE4 inhibitors as a topical ointment is currently being trialed, including AN2728, AN2898,[12] and E6005, which has the following structure: methyl 4-[({3-[6,7-dimethoxy-2-(methylamino)quinazolin-4-yl] phenyl} amino)carbonyl] benzoate. E6005 suppressed the production of various cytokines from human lymphocytes and

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*See Appendix 1 for the JAPANESE E6005 study investigators
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monocytes, and topical application in mice models induced an immediate antipruritic effect as well as an anti-inflammatory effect with reduced expression of cytokines and adhesion molecules.[13] A single center, randomized, open-label, vehicle-controlled, 10-day study of topical E6005 in the treatment of hospitalized adult AD subjects had been conducted. This multiple ascending-dose study of E6005 from 0.03% to 0.2% ointment indicated that topical E6005 had a good safety profile and significant efficacy was observed using the 0.2% ointment (http://clinicaltrials.gov with an identifier of NCT01179880, F. Ohba, S. Matsuki, S. Imayama, M. Doi, S. Hojo and H. Akama 2014, unpublished data). In the present multi-center study, 0.2% E6005 ointment was applied twice daily in AD outpatients. The primary endpoint was long-term safety and tolerance, and the secondary endpoint was efficacy. The study consisted of an initial 4-week randomization phase and an 8-week extension phase in which all subjects were treated with topical E6005. The safety and efficacy of topical E6005 application for up to 12 weeks were evaluated.

METHODS

This study was conducted in 11 medical institutions in Japan as a randomized, parallel group study using a vehicle control. The study protocol was approved by the institutional review board of each study institution, and the study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guideline. Investigators obtained written informed consent from each subject before conducting the screening procedures. This study was registered at http://clinicaltrials.gov with an identifier of NCT01461941.

Subjects

Eligible outpatients were those who were aged 20–64 years with a definite diagnosis of AD based on the Japanese Dermatological Association's "Guidelines for the management of AD",[2] and had eczema covering 5–30% of the body surface area at the time of enrollment.

The following subjects were excluded: those with a history of biological therapy in the 6 months prior to study drug application; those who received systemic therapy such as corticosteroids (oral, injection, suppository and inhalant preparations), immunosuppressants, antihistamines/antiallergic agents, Chinese herbal medicines for AD, or phototherapy within 28 days before study drug application; those who had used topical corticosteroids that are classified as very strong or strongest[2] within 28 days of study commencement; those who had used topical corticosteroids that are classified as weak, medium, or strong,[2] tacrolimus ointment, and topical analgesic, antipruritic or anti-inflammatory preparations such as nonsteroidal anti-inflammatory drugs within 7 days before application of the study drug. The following subjects were also excluded: those suffering from an active infection; and those suffering from Kaposi's varicelliform eruption, scabies, molluscum contagiosum, impetigo contagiosum, psoriasis, Netherton's syndrome or other autoimmune disorders, which may affect the pathological evaluation of AD.

Study design

This study consisted of three phases: pre-randomization (screening period and baseline assessment), randomization (4 weeks) and extension (8 weeks) phases (Fig. 1). After the screening period, the subjects were randomized to receive either 0.2% E6005 ointment or vehicle at an allocation ratio of 2:1 (the planned number of subjects was 75). Subjects who completed the randomization phase entered the extension phase in which all subjects were treated with 0.2% E6005 ointment. Subjects who completed the extension phase and those who discontinued the study underwent a follow-up examination 30 days after the final application of the study drug.

An appropriate amount of 0.2% E6005 ointment or vehicle control was applied according to the size of the lesion (0.5 g of ointment for each area covering 2% of the body surface area). The study was not conducted in a double-blind manner because the colors of the E6005 ointment and the vehicle control are slightly different. The study was conducted in a manner similar to that in a double-blind study to reduce evaluation bias by: (i) placing the study drugs in unidentifiable tubes and packing the tubes in unidentifiable containers; and (ii) not informing subjects or investigators about the color of the study drugs. The concurrent use of the following treatments was prohibited during the entire study period: biological agents such as TNF-α inhibitors, anti-IgE antibodies, and anti-CD20 antibodies; corticosteroid preparations except eye drops and nasal sprays; immunosuppressants; antihistamines/antiallergic agents; Chinese herbal medicines for AD; topical analgesic or antipruritic preparations; and phototherapy. Concomitant use of moisturizers was permitted.

Assessments

Prior medications used for AD were recorded and topical corticosteroids were classified into five categories (strongest, very strong, strong, medium, and weak) according to the "Guidelines for management of atopic dermatitis".[2]

Safety was evaluated according to occurrence of AEs, laboratory test results (hematology, blood chemistry, and urinalysis), body weight, and physical findings. AEs were defined according to "Common Terminology Criteria for Adverse Events (CTCAE, version 4.0). Descriptions of AEs were based on standardized medical terminology using the Medical Dictionary for Regulatory Activities (MedDRA, version 15.0). The severity of AE was graded from 1 to 5 according to CTCAE.

Blood samples were collected on day 0 (baseline) and 1, 4, 8, 12 and 16 weeks after the start of the study drug application. Plasma concentrations of E6005 and its metabolite M11 (a hydrolyzed form at methyl ester bond of E6005) were determined using the validated liquid chromatography tandem mass spectrometry method (lowest detectable concentration was 1 ng/mL for both E6005 and its metabolite). The pharmacokinetic analysis of the drug and its metabolite were based on data of plasma drug concentrations.

Assessments of efficacy during the study period were based on scores derived from Eczema Area and Severity Index (EASI, 0–72 points),[14] severity Scoring Atopic Dermatitis (SCORAD),[15] and itch Behavioral Rating Scale (0–8 points).[16] SCORAD-A

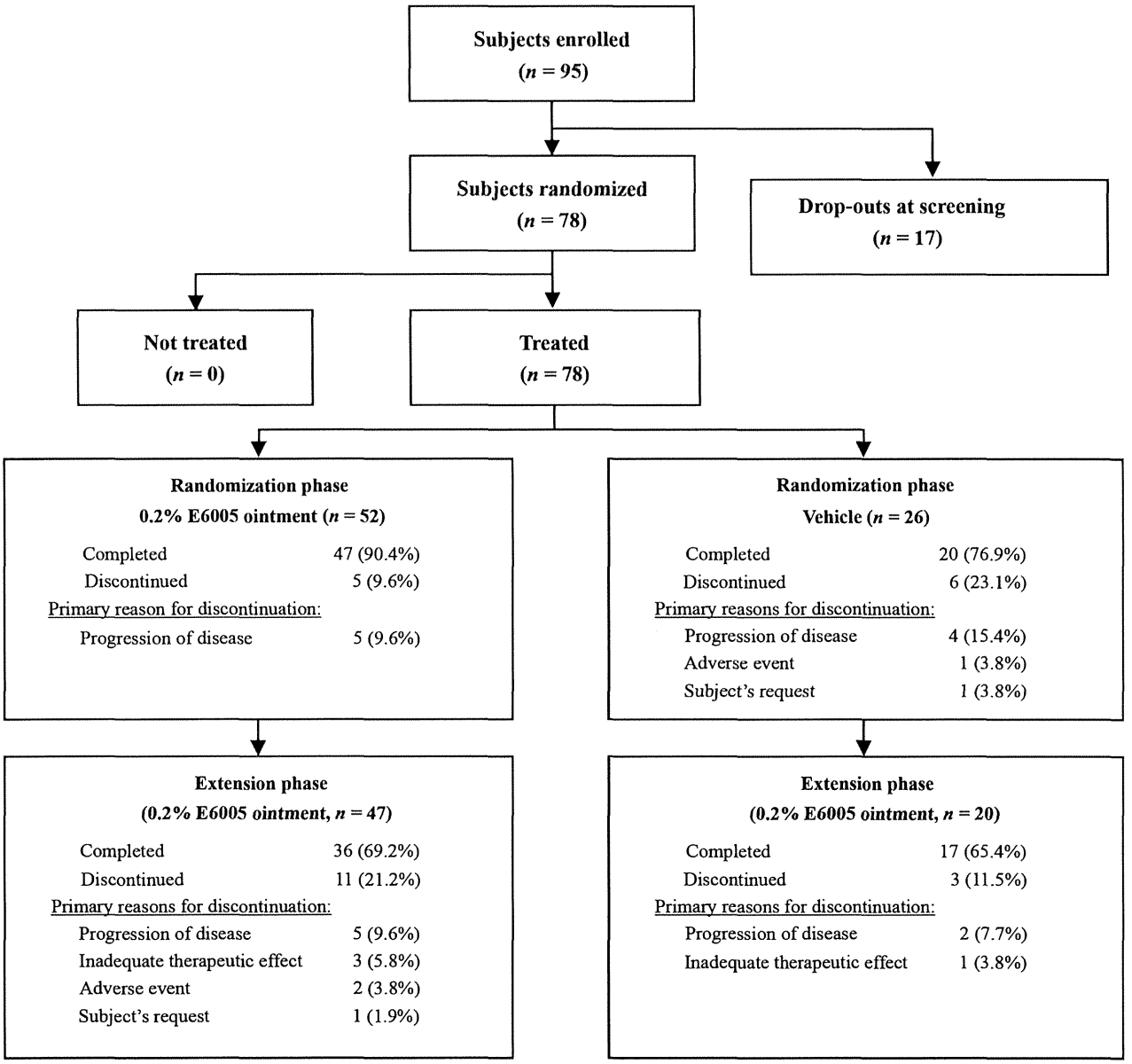

Figure 1. Study flow, subject disposition, and primary reasons for discontinuation of treatment.

(affected area%, score 0–100); SCORAD-B (intensity score, 0–18 points); SCORAD-objective ([SCORAD-A]/5 + 7[SCORAD-B]/2, 0–83 points); SCORAD-C (sum of two 10-cm visual analog scale scores for pruritus and sleep loss, 0–20 points); and SCORAD-total (SCORAD-objective + SCORAD-C, 0–103 points) were also assessed.

Investigators took digital photographs of the targeted lesion and their severity scores during the randomization phase. The location of the targeted lesions was predetermined before the start of the study drug application. Targeted lesions were described according to the following five features: erythema, edema/papulation, oozing/crust, excoriation, and lichenification. Severity was graded on a scale of 0 to 3 (0, none; 1, mild; 2, moderate; and 3, severe) and the sum of the severity scores of each of the five features represented the total severity score of the targeted lesion (0–15 points). The severity evaluation was validated by an independent dermatologist using digital photographs obtained from individual investigators.

Statistical analysis

Safety and efficacy analyses were performed for the randomization phase (4 weeks) and for the entire study period (12 weeks). Subjects who were treated with study drug at least once and had at least one safety or pharmacokinetics assessment were subjected to safety or pharmacokinetics analyses.

Analysis of efficacy in the randomization phase was based on full analysis set (FAS) and per protocol set (PPS) at the end of week 4 (last observation carried forward, LOCF). Analysis of efficacy in the entire period was based on FAS. Efficacy in the randomization phase was assessed by examining the mean percent change from baseline at week 4 in the E6005 and vehicle control groups. The least square mean difference of the percent change in efficacy between the E6005 and vehicle groups was evaluated using the ANCOVA model. Changes in the parameters of efficacy from baseline for the entire period were examined at week 12, and the paired *t*-test was used to assess dependent samples for statistical significance level of 0.05.

RESULTS

Study population, baseline demographics and clinical characteristics

Of the 95 subjects screened, 78 randomized subjects were treated with either vehicle (n = 26) or E6005 (n = 52) (Fig. 1). Subjects who completed the randomization phase entered the extension phase (n = 67). For the randomization phase, FAS and safety analysis set consisted of all 78 subjects who entered the randomization phase. The PPS consisted of 68 subjects (E6005 group, n = 45; vehicle group, n = 23) excluding 10 subjects who had hardly applied the study drugs. For the entire period, the FAS and safety analysis set consisted of 72 subjects, including five subjects who discontinued E6005 treatment in the randomization phase (E6005 group, n = 52; vehicle group, n = 20). The pharmacokinetic analysis set consisted of 78 randomized subjects (E6005 group, n = 52; vehicle group, n = 26).

Table 1 shows the subject demographics. In the FAS analysis (n = 78), the mean age of subjects was 31.2 years, 56.4% were men, mean weight was 61.7 kg, and the mean AD duration was 23.6 years. At baseline, the mean EASI score was 9.2 ± 4.8 and SCORAD-objective was 30.4 ± 7.3. Prior medications used for the treatment of AD are listed in Table 1. The percentage of subjects who had been treated with topical corticosteroids in the E6005 group (78.8%) was slightly higher than that in the vehicle group (69.2%). In contrast, the use of antiseptics and disinfectants was higher in the vehicle group

Table 1. Baseline demographics and clinical characteristics

	E6005 (n = 52)	Vehicle (n = 26)	Total (n = 78)
Demographics			
Age, years, mean (range)	31.4 (20–53)	30.7 (20–49)	31.2 (20–53)
Sex, *n* (%)			
Male	29 (55.8)	15 (57.7)	44 (56.4)
Female	23 (44.2)	11 (42.3)	34 (43.6)
Weight, kg, mean (range)	61.8 (41.4–91.9)	61.5 (41.7–91.1)	61.7 (41.4–91.9)
Race, *n* (%)			
Japanese	52 (100.0)	26 (100.0)	78 (100.0)
Clinical characteristics			
AD duration, year, mean (range)	22.7 (0–49)	25.5 (7–45)	23.6 (0–49)
EASI score, mean ± SD	9.2 ± 4.6	9.4 ± 5.3	9.2 ± 4.8
SCORAD			
Total, mean ± SD	38.9 ± 10.0	37.9 ± 9.7	38.6 ± 9.8
SCORAD-A, mean ± SD	19.2 ± 8.3	18.8 ± 8.5	19.1 ± 8.3
SCORAD-B, mean ± SD	7.8 ± 2.0	7.2 ± 1.8	7.6 ± 1.9
SCORAD-C, mean ± SD	7.8 ± 4.3	8.8 ± 5.4	8.1 ± 4.7
Behavioral rating scale, mean ± SD	4.2 ± 1.4	4.4 ± 1.4	4.2 ± 1.4
Severity of targeted lesion, mean ± SD	6.1 ± 1.7	5.7 ± 2.2	6.0 ± 1.9
Prior medications for AD, *n* (%)			
Systemic treatment	23 (44.2)	14 (53.8)	37 (47.4)
Topical treatment			
Emollients and protectants	41 (78.8)	21 (80.8)	62 (79.5)
Corticosteroids (all)	41 (78.8)	18 (69.2)	59 (75.6)
Strongest*	5 (9.6)	0 (0.0)	5 (6.4)
Very strong*	28 (53.8)	12 (46.2)	40 (51.3)
Strong*	21 (40.4)	7 (26.9)	28 (35.9)
Medium*	35 (67.3)	10 (38.5)	45 (57.7)
Weak*	2 (3.8)	1 (3.8)	3 (3.8)
Calcineurin inhibitor	12 (23.1)	8 (30.8)	20 (25.6)
Antiseptics and disinfectants	4 (7.7)	6 (23.1)	10 (12.8)

AD, atopic dermatitis; EASI, Eczema Area and Severity Index; SCORAD, Scoring Atopic Dermatitis; SD, standard deviation.
*The potency of topical corticosteroids was classified into categories (strongest, very strong, strong, medium, and weak) according to the "Guidelines for management of atopic dermatitis".[2]