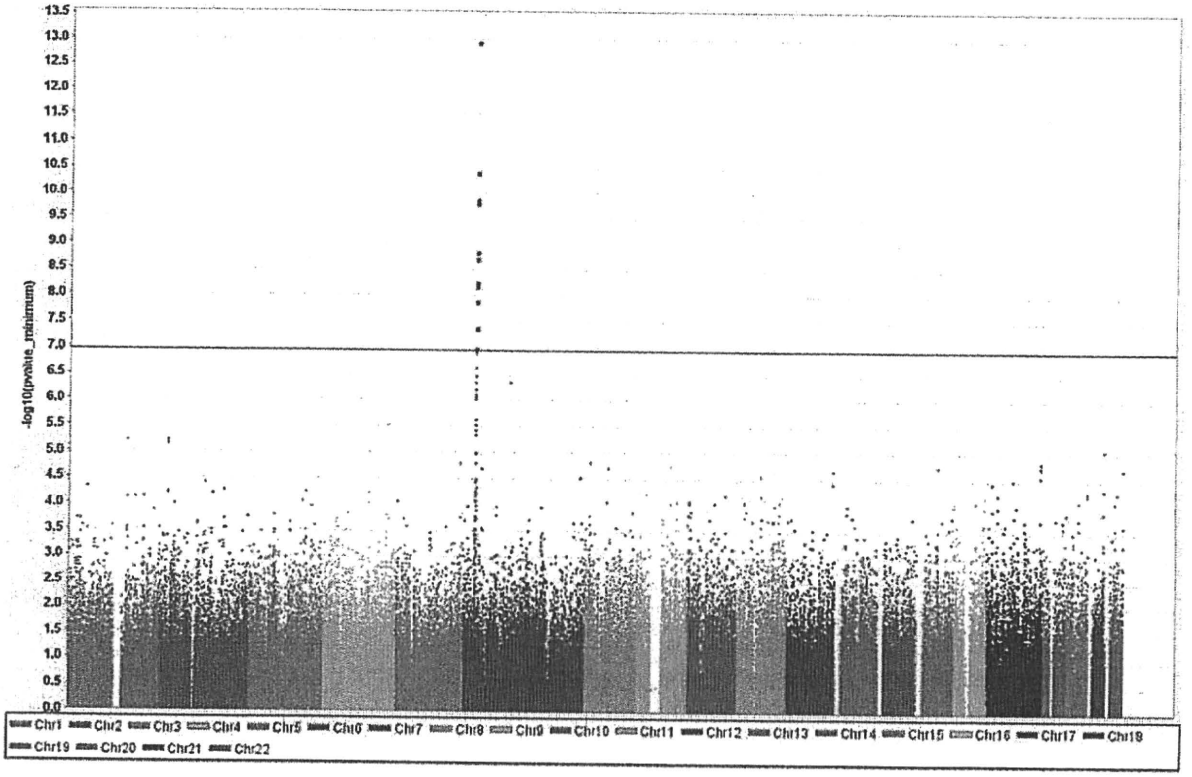

Figure 1. $-\text{Log}_{10}$ *P*-value plots at the GWAS. Each dot represents *P*-value obtained from GWAS using 53 patients with carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population in Japanese. The *Y*-axis represents the $-\log_{10}$ of the minimal *P*-values calculated by Fisher's exact tests for three models: dominant, recessive and allele frequency models in the case–control association study.

involvement [erythema multiforne (EM)] to progressive, fulminating, severe variant with extensive mucocutaneous epithelial necrosis [Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)]. Drug-induced hypersensitivity syndrome (DIHS) is also described as severe cADR, characterized by rash, fever and multiorgan systemic reactions such as lymphadenopathy, hepatitis and leukocytosis with eosinophilia (1,2). High fever, no mucocutaneous involvement and reactivation of human herpesvirus 6 (HHV-6) are important features of DIHS that can be distinguished from SJS and TEN (2,3).

Almost all drugs have been reported to have a risk to cause cADRs. Some drugs, such as anticonvulsants, antibiotics and non-steroidal anti-inflammatory drugs, are known to show higher incidences of cADRs, including SJS and TEN (2,3), while the culprit drugs for DIHS are limited to several drugs, including carbamazepine (CBZ), phenytoin (PHT), phenobarbital, dapsone, mexiletine, salazosulfapyridine, allopurinol and minocycline (4). Although several studies have indicated that T-cell-mediated allergic reactions might be related to pathogenesis of cADRs (5), the detailed mechanisms are not yet understood. Similarly, the underlying mechanism for DIHS also remains unknown, although the HHV-6 reactivation is suggested to associate with symptoms of DIHS, such as fever and hepatitis (6). Hence, there is no clinical test available to predict a risk of DIHS.

In case of CBZ, Taiwanese study demonstrated that *HLA-B*1502* was associated with SJS/TEN induced by CBZ (7). This strong genetic association could be applied for the prediction and prevention of cADRs. However, the allelic frequencies of the HLA loci differ significantly among different ethnic groups. For example, the *HLA-B*1502* allele is present at high frequency in south-eastern Asians (8.6%) (7), but it was only 0.1% in Japanese and Caucasian populations (http://www.allelefrequencies.net/). Thus, *HLA-B*1502* is not so useful as genetic predictors for the CBZ-induced cutaneous reactions in Japanese and Caucasian populations.

Single nucleotide polymorphisms (SNPs) are the most abundant DNA sequence variations, and a large body of SNP information was already constructed through the International HapMap project (8). In addition, the rapid technological development enabled us to perform genome-wide association study (GWAS) (9) routinely for identifying genetic risk factors for many complex diseases and traits (10). In the present study, we aimed to identify novel susceptibility loci associated with cADRs induced by CBZ in the Japanese population through case–control GWAS with the high-throughput SNP genotyping technology.

RESULTS

Genome-wide association study

We first genotyped 55 cases and 898 subjects of a general population in Japanese with Illumina HumanHap550v3 Genotyping

Table 1. Association of 12 SNPs showing *P*-value less than 1.12×10^{-7} in the GWAS with cutaneous adverse drug reactions induced by carbamazepine in Japanese population

SNP	Chr	Allele (1/2)	Case				General population				*P*-value		
			11	12	22	MAF	11	12	22	MAF	11 versus 12 + 22	11 + 12 versus 22	1 versus 2
rs1633021	6	A/G	19	32	2	0.340	736	142	3	0.084	1.18×10^{-13}	2.83×10^{-2}	1.58×10^{-12}
rs2571375	6	T/C	23	26	4	0.321	736	143	3	0.084	2.44×10^{-10}	2.85×10^{-4}	3.82×10^{-11}
rs1116221	6	T/C	4	26	23	0.321	4	149	729	0.089	5.46×10^{-4}	7.12×10^{-10}	1.35×10^{-10}
rs2844796	6	T/C	4	26	23	0.321	4	150	728	0.090	5.46×10^{-4}	8.26×10^{-10}	1.58×10^{-10}
rs1736971	6	A/C	6	35	12	0.443	27	282	573	0.190	8.43×10^{-3}	1.49×10^{-9}	1.46×10^{-8}
rs1611133	6	T/C	6	35	12	0.443	27	285	570	0.192	8.43×10^{-3}	1.90×10^{-9}	1.63×10^{-8}
rs2074475	6	A/G	28	21	4	0.274	750	129	3	0.077	9.99×10^{-8}	2.85×10^{-4}	5.60×10^{-9}
rs7760172	6	T/C	16	31	6	0.406	622	236	24	0.161	6.07×10^{-9}	5.16×10^{-3}	7.05×10^{-9}
rs2517673	6	T/C	6	31	16	0.406	24	237	621	0.162	5.16×10^{-3}	6.61×10^{-9}	7.67×10^{-9}
rs2524005	6	T/C	6	31	16	0.406	24	244	614	0.166	5.16×10^{-3}	1.28×10^{-8}	1.43×10^{-8}
rs12665039	6	T/C	17	30	6	0.396	621	236	25	0.162	4.96×10^{-8}	6.12×10^{-3}	4.19×10^{-8}
rs1362088	6	A/G	29	20	4	0.264	742	134	6	0.083	1.19×10^{-6}	1.50×10^{-3}	9.86×10^{-8}

53 cases of carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population.
Chr, chromosome; MAF, minor allele frequency.

BeadChip. After excluding one case and 16 subjects of the general population which were judged to be outliers in a principal component analysis (PCA), we applied SNP quality control [call rate of ≥ 0.99 in both cases and subjects of the general population, and *P*-value of the Hardy–Weinberg equilibrium test of $\geq 1.0 \times 10^{-6}$ in subjects of the general population] and excluded one case with the call rate of <0.98. Of 554 496 SNPs genotyped, 444 823 SNPs on autosomal chromosomes passed the quality control and were further analyzed.

Among the SNPs analyzed in the GWAS, 12 SNPs showed significant association with CBZ-induced cADRs after the correction of multiple testing, and rs1633021 revealed the lowest *P*-value for association ($P = 1.18 \times 10^{-13}$) (Fig. 1, Table 1). To validate the first genotyping data, we additionally performed genotyping by means of multiplex PCR-based Invader assays for the rs1633021 and confirmed the accuracy of the data obtained by the two platforms with the concordance rate of 100%.

Interestingly, all of these 12 SNPs were located within a 463 kb region on chromosome 6p21.33 (Table 1). Thus, we plotted linkage disequilibrium (LD) blocks using 882 subjects of the general population and found that 11 of these 12 SNPs were included in an LD block of 29.84–30.27 Mb and the remaining SNP rs1362088 was located closely to the particular LD block (Fig. 2). This region corresponded to the MHC I region containing the *HLA-A* locus.

HLA allele frequency

Since the most significant association was observed with the SNPs near the *HLA-A* locus, we further genotyped the individual *HLA-A* alleles for 61 cases including 7 additional cases and 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant subjects). As shown in Table 2, the frequency of *A*3101* was significantly higher in CBZ-induced cADR cases than CBZ-tolerant controls; *HLA-A*3101* was present in 37 (60.7%) of the 61 patients with cADRs induced by CBZ, while in only 47 (12.5%) of the 376 CBZ-tolerant controls [$P = 3.64 \times 10^{-15}$, odds ratio (OR) = 10.8, 95% confidence interval (CI) of 5.9–19.6]. In addition, the frequency of *HLA-A*2603* was nearly 3-fold higher (18.0 versus 5.9%) in the cADR cases with a suggestive *P*-value. However, this association should be validated by a larger number of cases. On the other hand, the allele frequency of *HLA-A*0206* was significantly lower in CBZ-induced cADR cases than CBZ-tolerant controls ($P = 2.74 \times 10^{-4}$, OR = 0.1, 95% CI 0.0–0.6).

To validate the significant associations of *HLA-A*3101* and *HLA-A*0206*, we performed a replication study using an independent Japanese case–control cohort which consisted of 16 CBZ-induced cADR cases and 44 CBZ-tolerant controls (Table 3). The association of *HLA-A*3101* was replicated in the second cohort ($P = 1.53 \times 10^{-2}$, OR = 5.3, 95% CI 1.5–24.5; combined-analysis $P = 1.09 \times 10^{-16}$, OR = 9.5, 95% CI 5.6–16.3), whereas that of *HLA-A*0206* was not replicated. In the case of *HLA-A*3101*, the *P*-value for the Breslow–Day test was not significant ($P = 0.32$), indicating the absence of significant heterogeneity in these two cohorts.

We further analyzed the association of *HLA-A*3101* according to the type of cADR using the combined cohorts (Table 4). *HLA-A*3101* showed significant associations with DIHS ($P = 2.06 \times 10^{-9}$, OR = 9.5, 95% CI 4.6–19.5) and SJS/TEN ($P = 2.35 \times 10^{-4}$, OR = 33.9, 95% CI 3.9–295.6) as well as other cADRs ($P = 4.74 \times 10^{-8}$, OR = 8.0, 95% CI 3.9– 16.6), respectively.

Although our GWAS analysis did not find any significant association with the *HLA-B* locus, *HLA-B*1502* has been reported its strong association with SJS/TEN induced by CBZ in Han-Chinese population (7). In order to confirm that the *HLA-B* locus was not associated with CBZ-induced cADRs, we carried out genotyping of the *HLA-B* alleles for 61 cases and 376 CBZ-tolerant subjects. The *HLA-B*1502* allele was absent in any of our cases. In addition, no *HLA-B* allele showed significant association with CBZ-induced cADRs (Supplementary Material, Table S1). Our LD analysis indicated that *HLA-G* was also located in the LD block including the landmark SNP, rs1633021 (Fig. 2). Thus, we also genotyped the *HLA-G* alleles for 61 cases and 376 CBZ-tolerant subjects and found that *HLA-G*010102* showed the most significant association with CBZ-induced cADRs ($P = 1.31 \times 10^{-7}$, OR = 4.8, 95% CI 2.6–8.9) (Supplementary Material,

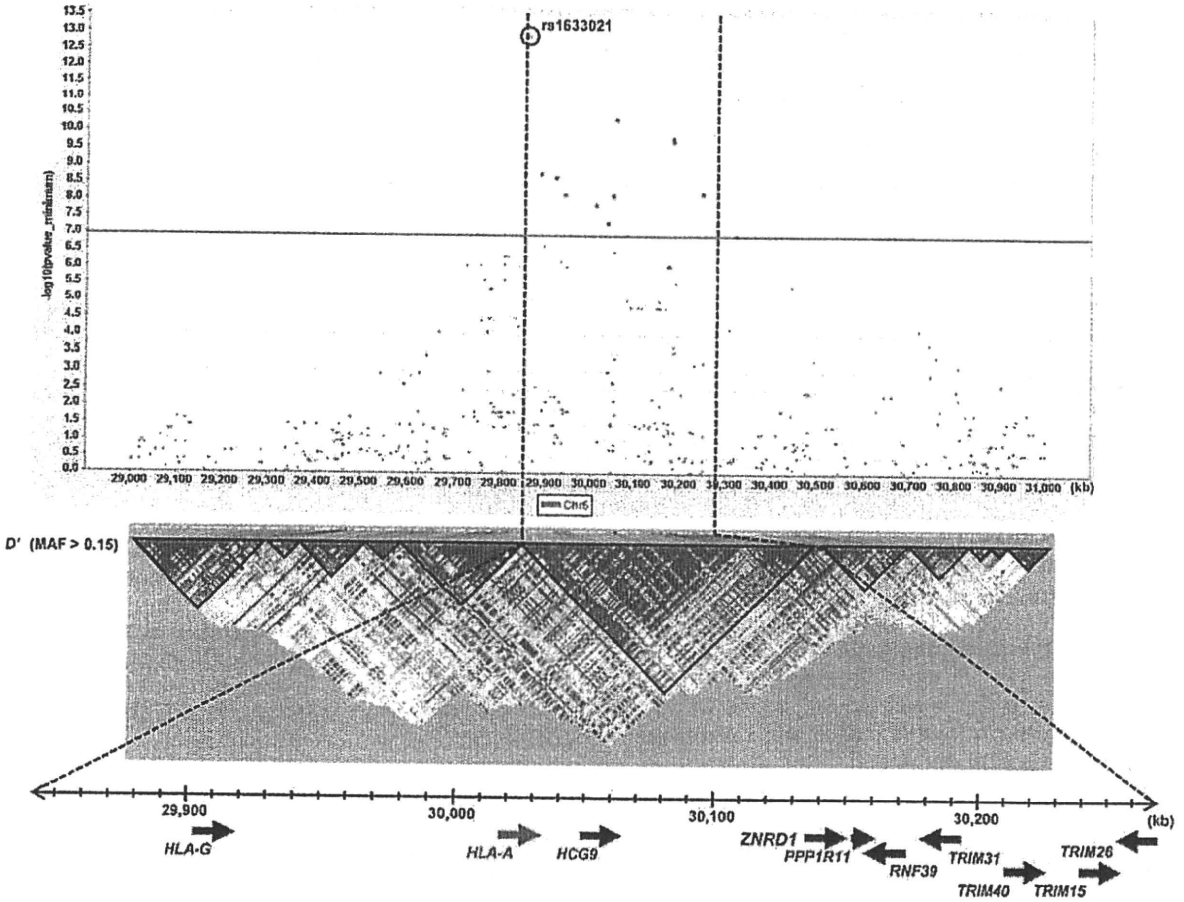

Figure 2. $-\text{Log}_{10}$ *P*-value plots, LD map and genomic structure of an *HLA-A* region. Each dot represents *P*-value obtained from GWAS using 53 patients with carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population in Japanese. The *Y*-axis represents the $-\log_{10}$ of the minimal *P*-values calculated by Fisher's exact tests for three models: dominant, recessive and allele frequency models in the case–control association study. Pairwise *D'* was based on the genotype data of the 882 subjects of the general population. MAF, minor allele frequency.

Table S2). However, multiple logistic regression analysis revealed that the *HLA-A*3101* genotype had the significant effect on risk of CBZ-induced cADRs (OR = 7.9, 95% CI 3.9–16.2, $P = 1.64 \times 10^{-8}$), but *HLA-G*010102* did not (OR = 1.7, 95% CI 0.8–3.7, $P = 0.16$) (Supplementary Material, Table S3).

DISCUSSION

This study is the first GWAS to investigate genetic factors associated with cADRs induced by CBZ. We demonstrated that the 11 SNPs showing the significant association were located within a 430 kb LD block, including the *HLA-A* locus. *HLA-A* belongs to the HLA class I heavy chain paralogues, which play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. We thus considered that the association of these SNPs with CBZ-induced cADRs should reflect variations in antigen-binding affinities of HLA-A that might affect the immune response in the pathogenesis of the cADRs. All of the HLA-A, B and G belong to the HLA class I molecules, while *HLA-B*1502* has been reported to be associated with SJS/TEN induced by CBZ in Han-Chinese population, and the *HLA-G* locus was located in the LD block including the landmark SNP, rs1633021, in our LD analysis. However, in our study, no *HLA-B* allele showed significant association with CBZ-induced cADRs in the Japanese population. Furthermore, multiple logistic regression analysis suggested that the association of *HLA-G*010102* was confounding of the association between HLA-A*3101 and CBZ-induced cADRs.

In the present study, the general population subjects were used for the case–control association study. The use of general population has a disadvantage, a reduced statistical power, because part of the general population had a potential to show CBZ-induced cADRs. However, the reduction of power is dependent on prevalence of ADRs and can be compensated by increasing the number of subjects. In the case of the CBZ-induced cADRs, the prevalence is generally low. For a prevalence of 2.9%, which was reported for Japanese population (http://www.info.pmda.go.jp/), statistical power estimates were 0.980 and 0.992 for the use of general

Table 2. Frequencies of HLA-A alleles in patients with carbamazepine-induced cutaneous adverse drug reactions and carbamazepine-tolerant controls

HLA allele	Number of carriers		*P*-value
	Case (%)	CBZ-tolerant control (%)	
*A*0101*	0 (0.0)	6 (1.6)	1.00
*A*0201*	5 (8.2)	93 (24.7)	2.74×10^{-3}
*A*0206*	1 (1.6)	68 (18.1)	[a]2.46×10^{-4}
*A*0207*	3 (4.9)	23 (6.1)	0.10
*A*0210*	2 (3.3)	2 (0.5)	0.03
*A*0301*	0 (0.0)	2 (0.5)	1.00
*A*1101*	7 (11.5)	68 (18.1)	0.27
*A*1110*	0 (0.0)	1 (0.3)	1.00
*A*2402*	37 (60.7)	211 (56.1)	0.58
*A*2405*	0 (0.0)	1 (0.3)	1.00
*A*2420*	0 (0.0)	4 (1.1)	1.00
*A*2601*	2 (3.3)	65 (17.3)	3.36×10^{-3}
*A*2602*	2 (3.3)	15 (4.0)	1.00
*A*2603*	11 (18.0)	22 (5.9)	2.61×10^{-3}
*A*2605*	1 (1.6)	1 (0.3)	0.26
*A*2901*	0 (0.0)	1 (0.3)	1.00
*A*3001*	0 (0.0)	2 (0.5)	1.00
*A*3101*	37 (60.7)	47 (12.5)	[a]3.64×10^{-15}
*A*3303*	5 (8.2)	59 (15.7)	0.17

61 cases and 376 CBZ-tolerant controls.
CBZ, carbamazepine.
[a]Significant after Bonferroni's correction.

population and CBZ-tolerant controls, respectively. These results indicate that the use of the general population, in place of the CBZ-tolerant controls, can yield sufficient power to permit the identification of strong genetic factors, such as our landmark SNP, rs1633021. Besides, since type I error rate will not be affected by using the general population, the possibility of false-positive results should be similar to that in the use of CBZ-tolerant controls. Consequently, we concluded that the use of the general population for our GWAS would be suitable.

We genotyped *HLA-A* alleles and identified a strong association of the *HLA-A*3101* allele with the risk of the CBZ-induced cADRs in Japanese population. Comparison of genotypes of the *HLA-A*3101* and the marker SNP rs1633021 in 376 CBZ-tolerant controls revealed that the G allele of rs1633021 was in strong LD with the *HLA-A*3101* ($r^2 = 0.79$, $D' = 0.95$). *HLA-A*3101* was present in 37 (60.7%) of the 61 subjects with cADRs induced by CBZ and was present in only 47 (12.5%) of the 376 CBZ-tolerant controls, implying that this allele has the 60.7% sensitivity and 87.5% specificity when we apply *HLA-A*3101* as a risk predictor for CBZ-induced cADRs in the Japanese population. If a prevalence of CBZ-induced cADRs was 2.9% (http://www.info.pmda.go.jp/), the positive and negative predictive values would be estimated to be 12.7 and 98.7%, respectively. That is, it might become possible to lower the frequency of CBZ-induced cADR from 2.9 to 1.1% by excluding the patient judged to be *HLA-A*3101* positive by the genetic diagnosis from the CBZ treatment. We are confident about the clinical benefit of the *HLA-A*3101* typing to predict the risk of CBZ-induced cADRs since there are several alternative drugs to CBZ for epilepsy and trigeminal neuralgia such as PHT and valproic acid, which induce cADRs with low prevalence. Although the efficacy of these alternative drugs might be inferior to CBZ for treating trigeminal neuralgia, a prevention of CBZ-induced cADRs, which are sometimes life-threatening, must be more important for patients. Our findings should provide useful information for making a decision of individualized medication for these diseases.

Recently, Kashiwagi *et al.* (11) performed HLA genotyping in Japanese subjects with CBZ-induced cADR and found an association with *HLA-A*3101* ($P = 4.0 \times 10^{-4}$ in the allele frequency, OR = 4.3, 95% CI 2.1–9.1). In the study, 22 cases (6 MPE/EM, 3 erythroderma, 4 DIHS, 2 SJS and 7 other drug eruptions) were included. Our results demonstrated that *HLA-A*3101* showed significant associations with CBZ-induced DIHS/SJS/TEN and other cADRs.

In the Han-Chinese population, the *HLA-A*3101* was reported to be associated with MPE induced by CBZ, but not with SJS/TEN (12). However, we demonstrated that *HLA-A*3101* was present in four (80.0%) of the five subjects with SJS/TEN induced by CBZ in the combined cohort. Thus, all of CBZ-induced cADRs including SJS/TEN and MPE are likely to be associated with the same *HLA-A* allele, *HLA-A*3101*, in Japanese population on the basis of the present study. The controversial results of the association of *HLA-A*3101* with SJS/TEN between Japanese and Chinese studies might be due to ethnic differences in allele frequencies of *HLA-B*1502* and *HLA-A*3101*. It has been found that the *HLA-B*1502* allele is extremely rare in Japanese (allele frequency: 0.1%) compared with Han-Chinese (allele frequency: 8.6%) (7). In contrast, the *HLA-A*3101* allele is present at a higher allelic frequency in Japanese (9.1%), but only 1.8% in Han-Chinese (http://www.allelefrequencies.net/). MHC-dependent presentation of drugs and/or the metabolites on HLA class II molecules to $CD4^+$ helper T cells and on class I molecules to $CD8^+$ cytotoxic T cells are considered to be critical for the severe cADRs (13,14). Both of *HLA-A* and *-B* belongs to MHC class I molecules. Thus, there might be common underlying mechanisms involved in the CBZ-induced cADRs, although *HLA-B*1502* seemed to be specifically involved in the CBZ-induced SJS/TEN.

To date, DIHS has been considered to be a different clinical entity from SJS and TEN, because of its delayed onset of symptoms from the beginning of the drug therapy as well as high fever, no mucocutaneous involvement and HHV-6 reactivation (2,3). However, the present study suggests that DIHS and other cADRs, including SJS/TEN, which were induced by CBZ, might share the common pathogenesis from the fact that they were associated with the same *HLA* allele, *HLA-A*3101*. Although further studies using a large sample size will be necessary to confirm this observation, the *HLA-A*3101* could be directly involved in the pathogenesis of all types of the CBZ-induced cADRs in the Japanese population, in view of the fact that *HLA-B*5801* has been reported to be a genetic factor associated with both SJS/TEN and DIHS induced by allopurinol in Taiwanese, Japanese and Europeans (15–17).

In conclusion, we have demonstrated that *HLA-A*3101* was significantly associated with susceptibility to cADRs induced by CBZ in Japanese population. Unfortunately, because of limitation of the subject information from BioBank Japan,

Table 3. Association of *HLA-A*0206* and *A*3101* alleles with carbamazepine-induced cutaneous adverse drug reactions

Population	*HLA-A*0206* Case (%)	CBZ-tolerant controls (%)	*P*-value	OR (95% CI)	*HLA-A*3101* Case (%)	CBZ-tolerant controls (%)	*P*-value	OR (95% CI)
First study	1/61 (1.6)	68/376 (18.1)	[a]2.46×10^{-4}	0.1 (0.0–0.6)	37/61 (60.7)	47/376 (12.5)	[a]3.64×10^{-15}	10.8 (5.9–19.6)
Replication study	2/16 (12.5)	8/44 (18.2)	0.72	0.6 (0.1–3.4)	8/16 (50.0)	7/44 (15.9)	[a]1.53×10^{-2}	5.3 (1.5–24.5)
Combined analysis	3/77 (3.9)	76/420 (18.1)	[a]1.02×10^{-3}	0.2 (0.1–0.6)	45/77 (58.4)	54/420 (12.9)	[a]1.09×10^{-16}	9.5 (5.6–16.3)

CBZ, carbamazepine; CI, confidence interval.
[a]Significant after Bonferroni's correction.

Table 4. Subgroup analysis of association of the *HLA-A*3101* allele with carbamazepine-induced cutaneous adverse drug reactions

Subgroup	Number of patients Positive for *HLA-A*3101*	Negative for *HLA-A*3101*	Total	*P*-value	OR (95% CI)
All CBZ-induced cADRs	45	32	77	[a]1.09×10^{-16}	9.5 (5.6–16.3)
DIHS	21	15	36	[a]2.06×10^{-9}	9.5 (4.6–19.5)
SJS/TEN	5	1	6	[a]2.35×10^{-4}	33.9 (3.9–295.6)
Others	19	16	35	[a]4.74×10^{-8}	8.0 (3.9–16.6)
CBZ-tolerant controls	54	366	420	–	–

cADRs, cutaneous adverse drug reactions; CBZ, carbamazepine; CI, confidence interval; DIHS, drug-induced hypersensitivity syndrome; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis.
[a]Significant after Bonferroni's correction.

the timings of the onset of reaction in relation to drug use, and CBZ doses were not available. Thus, although a prospective study of CBZ-induced cADRs with detailed clinical information and further investigations will be required to determine the clinical utility and to clarify the molecular mechanisms responsible for the risk of these cADRs, respectively, our findings should shed light on its pathogenesis and facilitate development of genetic test to identify individuals at risk for this potentially life-threatening condition caused by CBZ in the Japanese population.

MATERIALS AND METHODS

Participants

We obtained 62 patients with cADRs induced by CBZ (Supplementary Material, Table S4). The BioBank Japan Project started in 2003 with the aim of collecting genomic DNA and serum samples as well as clinical information from 300 000 patients diagnosed with any of 47 different diseases by collaboration with 66 hospitals in Japan (18). The biological materials and the clinical information were obtained with a written informed consent for participation in this project. From the registered samples in BioBank Japan from June 2003 to March 2008, we obtained 33 patients with non-DIHS cutaneous reactions induced by CBZ. The subjects included four patients with SJS/TEN, 16 EM, 4 MPE, 2 erythema, 1 erythroderma, 1 fixed dug eruption and 5 unclassified drug rashes. SJS and TEN were diagnosed as mucocutaneous disorders characterized by wide-spread erythema, blisters, detachment, erosions and fever. SJS was defined by identification of skin detachment of less than 10% of the body-surface area, whereas TEN was diagnosed by finding skin detachment of more than 10%, and excluding staphylococcal scalded skin syndromes (19). We obtained 29 patients with typical DIHS induced by CBZ who were recruited at Yokohama City University Hospital, Showa University Hospital, Kyorin University Hospital and Ehime University Hospital from October 2005 to October 2009. The diagnosis criteria of the typical DIHS were maculopapular rash (developing it more than 2 weeks after the beginning of the therapy with a limited number of drugs) as well as all of the following symptoms: fever (>38°C), hepatitis, hematological disorder [leukocytosis (>11 000 per mm^3), atypical lymphocytosis (>5%) or eosinophilia (>1500 per mm^3)], lymphadenopathy and HHV-6 reactivation. All the DIHS cases had all the manifestations listed.

Two control groups were used in this study. We used 898 volunteers recruited at the Midosuji and other related Rotary Clubs as the population of general Japanese individuals (general population) for GWAS (20). The 898 volunteers did not have any clinical histories of epilepsy, cranial nerve disorder, cancer and treatment of CBZ. As the second control group for the further detailed *HLA* genotyping, we selected 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant controls) from the BioBank Japan. The median ages of cases, the subjects of general population and CBZ-tolerant controls were 54 years (range 12–82), 55 years (18–93) and 52 years (1–88), respectively.

For a replication study, we enrolled 16 patients with CBZ-induced cADRs and 44 CBZ-tolerant controls, who were recruited at Yokohama City University Hospital, Showa University Hospital, Kyorin University Hospital and Ehime University Hospital, as the second cohort

(Supplementary Material, Table S5). The median ages of cases and CBZ-tolerant controls in the second cohort were 61 years (range 24–74) and 55 years (16–83), respectively.

Collection of blood samples and clinico-pathological information from patients and volunteers was undertaken with informed consent and was approved by the Ethical Committees at The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, and their use for this study was approved in The Institutes of Physical and Chemical Research (RIKEN), Yokohama, Japan.

Genome-wide association study

A genome-wide analysis for 55 cases and 898 subjects of the general population was conducted using Illumina HumanHap550v3 Genotyping BeadChip according to the manufacturer's protocols (San Diego, CA, USA). Of 62 cases mentioned above, 7 subjects were not included in the GWAS because these subjects were obtained after the GWAS. We did not include the SNPs of X-chromosome in our GWAS. In case of the X-chromosome, male and female subjects must be separately analyzed, leading to the decrease of sample size. Furthermore, in female, either one of the two X-chromosomes might be inactivated, which can impair genotype–phenotype correlations. A PCA was performed via an 'Eigen analysis' in the computer program smartpca, from the EIGENSOFT package (21). Genotype data for the cases and general population subjects and those for 89 East-Asian individuals (44 Japanese and 45 Han Chinese) from the International HapMap project (8) were analyzed for the PCA. PCA plots were obtained using the first two components (Eigenvectors 1 and 2). To validate the genotyping results, we performed genotyping by means of multiplex PCR-based Invader assays (Third Wave Technologies, Madison, WI, USA) (22) and compared the data obtained by the two platforms. To draw an LD map including SNPs which showed significant associations with CBZ-induced cADRs, we applied Haploview software (23).

HLA genotyping

HLA-A and *-B* genotyping was carried out using a WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan), which is based on PCR-sequence-specific oligonucleotide probes coupled with multiple analyte profiling (xMAP) technology (Luminex System; Luminex Corporation, Austin, TX, USA). The data analysis was performed using the WAKFLOW TYPING software (Wakunaga). *HLA-G* was genotyped by a sequence-based method reported previously (24).

Statistical analyses

A statistical significance of the association with each SNP or *HLA* allele was assessed using Fisher's exact test. For the GWAS, we carried out the statistical analysis for association by comparing the case and control groups using the allele-frequency model, dominant-inheritance model and recessive-inheritance model. SNPs were rank-ordered according to the lowest *P*-value in these models. Significance levels after Bonferroni's correction for multiple testing were 1.12×10^{-7} (0.05/444 823), 2.63×10^{-3} (0.05/19) and 2.50×10^{-2} (0.05/2) in the GWAS, *HLA-A* genotyping and the replication study, respectively. For power estimation, we used QUANTO (http://hydra.usc.edu/GxE/) (25), under the following conditions, assuming a dominant inheritance model: 53 cases, 881 controls; prevalence of CBZ-induced cADRs, 0.029; significance level, 1.0×10^{-7}; risk allele frequency, 0.08 for general population; ORs, 9.08 and 10.15 for general population and CBZ-tolerant controls, respectively. The Breslow–Day test (26) was used to evaluate the heterogeneity of the ORs between association studies of two cohorts.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Hashimoto, K., Yasukawa, M. and Tohyama, M. (2003) Human herpesvirus 6 and drug allergy. *Curr. Opin. Allergy Clin. Immunol.*, 3, 255–260.
2. Shiohara, T. and Kano, Y. (2007) A complex interaction between drug allergy and viral infection. *Clin. Rev. Allergy Immunol.*, 33, 124–133.
3. Shiohara, T., Inaoka, M. and Kano, Y. (2006) Drug-induced hypersensitivity syndrome (DIHS): a reaction induced by a complex interplay among herpesviruses and antiviral and antidrug immune responses. *Allergol. Int.*, 55, 1–8.
4. Sharma, V.K., Sethuraman, G. and Minz, A. (2008) Stevens Johnson syndrome, toxic epidermal necrolysis and SJS-TEN overlap: a retrospective study of causative drugs and clinical outcome. *Indian J. Dermatol. Venereol. Leprol.*, 74, 238–240.
5. Zanni, M.P., Schnyder, B., von Greyerz, S. and Pichler, W.J. (1998) Involvement of T cells in drug-induced allergies. *Trends Pharmacol. Sci.*, 19, 308–310.
6. Tohyama, M., Hashimoto, K., Yasukawa, M., Kimura, H., Horikawa, T., Nakajima, K., Urano, Y., Matsumoto, K., Iijima, M. and Shear, N.H. (2007) Association of human herpesvirus 6 reactivation with the flaring and severity of drug-induced hypersensitivity syndrome. *Br. J. Dermatol.*, 157, 934–940.
7. Chung, W.H., Hung, S.I., Hong, H.S., Hsih, M.S., Yang, L.C., Ho, H.C., Wu, J.Y. and Chen, Y.T. (2004) Medical genetics: a marker for Stevens-Johnson syndrome. *Nature*, 428, 486.
8. International HapMap Consortium. (2003) The International HapMap Project. *Nature*, 426, 789–796.

9. Ozaki, K., Ohnishi, Y., Iida, A., Sekine, A., Yamada, R., Tsunoda, T., Sato, H., Hori, M., Nakamura, Y. and Tanaka, T. (2002) Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. *Nat. Genet.*, **32**, 650–654.
10. Nakamura, Y. (2009) DNA variations in human and medical genetics: 25 years of my experience. *J. Hum. Genet.*, **54**, 1–8.
11. Kashiwagi, M., Aihara, M., Takahashi, Y., Yamazaki, E., Yamane, Y., Song, Y., Muramatsu, M. and Ikezawa, Z. (2008) Human leukocyte antigen genotypes in carbamazepine-induced severe cutaneous adverse drug response in Japanese patients. *J. Dermatol.*, **35**, 683–685.
12. Hung, S.I., Chung, W.H., Jee, S.H., Chen, W.C., Chang, Y.T., Lee, W.R., Hu, S.L., Wu, M.T., Chen, G.S., Wong, T.W. *et al.* (2006) Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet. Genomics*, **16**, 297–306.
13. Kehren, J., Desvignes, C., Krasteva, M., Ducluzeau, M.T., Assossou, O., Horand, F., Hahne, M., Kagi, D., Kaiserlian, D. and Nicolas, J.F. (1999) Cytotoxicity is mandatory for CD8(+) T cell-mediated contact hypersensitivity. *J. Exp. Med.*, **189**, 779–786.
14. Naisbitt, D.J., Farrell, J., Wong, G., Depta, J.P., Dodd, C.C., Hopkins, J.E., Gibney, C.A., Chadwick, D.W., Pichler, W.J., Pirmohamed, M. *et al.* (2003) Characterization of drug-specific T cells in lamotrigine hypersensitivity. *J. Allergy Clin. Immunol.*, **111**, 1393–1403.
15. Hung, S.I., Chung, W.H., Liou, L.B., Chu, C.C., Lin, M., Huang, H.P., Lin, Y.L., Lan, J.L., Yang, L.C., Hong, H.S. *et al.* (2005) HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc. Natl Acad. Sci. USA*, **102**, 4134–4139.
16. Kaniwa, N., Saito, Y., Aihara, M., Matsunaga, K., Tohkin, M., Kurose, K., Sawada, J., Furuya, H., Takahashi, Y., Muramatsu, M. *et al.* (2008) HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics*, **9**, 1617–1622.
17. Lonjou, C., Borot, N., Sekula, P., Ledger, N., Thomas, L., Halevy, S., Naldi, L., Bouwes-Bavinck, J.N., Sidoroff, A., de Toma, C. *et al.* (2008) A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet. Genomics*, **18**, 99–107.
18. Nakamura, Y. (2007) The BioBank Japan Project. *Clin. Adv. Hematol. Oncol.*, **5**, 696–697.
19. Bastuji-Garin, S., Rzany, B., Stern, R.S., Shear, N.H., Naldi, L. and Roujeau, J.C. (1993) Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch. Dermatol.*, **129**, 92–96.
20. Asano, K., Matsushita, T., Umeno, J., Hosono, N., Takahashi, A., Kawaguchi, T., Matsumoto, T., Matsui, T., Kakuta, Y., Kinouchi, Y. *et al.* (2009) A genome-wide association study identifies three new susceptibility loci for ulcerative colitis in the Japanese population. *Nat. Genet.*, **41**, 1325–1329.
21. Patterson, N., Price, A.L. and Reich, D. (2006) Population structure and eigenanalysis. *PLoS Genet.*, **2**, e190.
22. Ohnishi, Y., Tanaka, T., Ozaki, K., Yamada, R., Suzuki, H. and Nakamura, Y. (2001) A high-throughput SNP typing system for genome-wide association studies. *J. Hum. Genet.*, **46**, 471–477.
23. Barrett, J.C., Fry, B., Maller, J. and Daly, M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*, **21**, 263–265.
24. Turk, W.J., Mao, X.J., Bielawny, T., Brunham, R., Luo, M. and Plummer, F.A. (2008) Identification of novel human leukocyte antigen G alleles in an East African population by high-resolution sequence-based typing. *Hum. Immunol.*, **69**, 426–429.
25. Gauderman, W.J. (2002) Sample size requirements for association studies of gene-gene interaction. *Am. J. Epidemiol.*, **155**, 478–484.
26. Breslow, N.E. and Day, N.E. (1980) Classical methods of analysis of grouped data. In Davis, W. (ed.), *Statistical Methods in Cancer Research. Volume I—the Analysis of Case-Control Studies.* IARC Sci. Publ., Lyon, France, pp. 122–161.

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A Role of *Staphyococcus aureus*, Interleukin-18, Nerve Growth Factor and Semaphorin 3A, an Axon Guidance Molecule, in Pathogenesis and Treatment of Atopic Dermatitis

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Staphylococcus aureus (SA) is usually present not only in the skin lesions of atopic dermatitis (AD) but also in the atopic dry skin. SA discharges various toxins and enzymes that injure the skin, results in activation of epidermal keratinocytes, which produce and release IL-18. IL-18 that induces the super Th1 cells secreting IFN-γ and IL-13 is supposed to be involved in development of AD and its pathogenesis. Indeed, the number of SA colonies on the skin surface and the serum IL-18 levels in patients with AD significantly correlated with the skin scores of AD lesions. Also, there is strong positive correlation between the skin scores and serum IL-18 levels in DS-Nh mice ($P<0.0001$, $r=0.64$), which develop considerable AD-like legions when they are housed under conventional conditions, but develop skin legions with less severity and less frequency under specific pathogens free (SPF) conditions. Therefore, they are well-known as model mice of AD, in which SA is presumed to be critical factor for the development of AD lesions. Also, theses DS-Nh mice pretreated with Cy developed more remarkable AD-like lesions in comparison with non-treated ones. The levels of INF-r and IL-13 in the supernatants of the lymph node cell cultures stimulated with staphylococcal enterotoxin B (SEB) or ConA were increased in the Cy-treated mice, although the serum levels of total IgE were not. In this experiment, we revealed that Cy-treated mice, to which CD25 +CD4 + reguratory T cells taken from non-treated ones had been transferred, developed the AD-like legions with less severity and less number of SA colonies on the skin surface. Therefore, it is presumed that CD25 +CD4 + reguratory T cells might be involved in the suppression of super Th1 cells which are induced by IL-18 and are involved in the development of AD-like lesions rather than IgE production. The efficient induction of CD25 +CD4 + reguratory T cells is expected for the new type of treatment of AD. We also found that farnesol (F) and xylitol (X) synergistically inhibited biofilm formation by SA, and indeed the ratio of SA in total bacteria at sites to which the FX cream containing F and X had been applied was significantly decreased 1 week later, accompanied with improvement of AD, when compared with that before application and at placebo sites. Therefore, the FX cream is a useful skin-care agent for atopic dry skin colonized by SA. The nerve growth factor (NGF) in the horny layer (the horn NGF) of skin lesions on the cubital fossa was collected by tape stripping and measured using ELISA in AD patients before and after 2 and 4 weeks treatments. Simultaneously, the itch and eruptions on the whole body and on the lesions, in which the horn NGF was measured, were recorded, and also the peripheral blood eosinophil count, serum LDH level and serum total IgE level were examined. The level of NGF was significantly higher in AD patients than in healthy controls, correlated with the severity of itch, erythema, scale/xerosis, the eosinophil count and LDH level, and also significantly decreased after treatments with olopatadine and/or steroid ointment for 2 and 4 weeks. Therefore, the measurement of the NGF by this harmless method seems to be useful to assess the severity of AD and the therapeutic effects on AD. In AD patients, C-fiber in the epidermis increase and sprout, inducing hypersensitivity, which is considered to aggravate the disease. Semaphorin 3A (Sema3A), an axon guidance molecule, is a potent inhibitor of neurite outgrowth of sensory neurons. We administered recombinant Sema3A intracutaneously into the skin lesions of NC/Nga mice, an animal model of AD, and investigated the effect of Sema3A on the skin lesions and their itch. Sema3A dose-dependently improved skin lesions and attenuated the scratching behavior in NC/Nga mice. Histological examinations revealed a decrease in the epidermal thickness, the density of invasive nerve fibers in the epidermis, inflammatory infiltrate including mast cells and CD4 +T cells, and the production of IL-4 in the Sema3A-treated lesions. Because the interruption of the itch-scratch cycle likely contributes to the improvement of the AD-like lesions, Sema3A is expected to become a promising treatment of patients with refractory AD.

Key Words: Atopic dermatitis; *Staphylococcus aureus*; nerve growth factor; semaphorin 3A

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease characterized by highly pruritic, eczematous skin lesions. Various factors including the immunological and non-immunological abnormalities contribute to the pathogenesis and development of AD. Patients with AD generally have a familial predisposition of increasing in serum IgE antibodies to various allergens and development of eczematous skin lesions as the immunological abnormalities. On the other hand, physiological barrier dysfunctions of the skin, high detection of *Staphylococcus aureus* (SA) in the bacterial flora on their skin surface flora and an increased hypersensitivity to itch are known as the non-immunological abnormalities. In this paper, we would like to review the recent papers concerning the pathogenesis and biomarker of AD and its itch and to discuss a probable role of SA, interleukin-18 (IL-18), nerve growth factor (NGF) and semaphorin 3A (Sema3A) of an axon guidance molecule in pathogenesis and treatment of AD.

A PROBABLE ROLE OF *STAPHYLOCOCCUS AUREUS* (SA) AND INTERLEUKIN-18 (IL-18) IN PATHOGENESIS AND BIOMARKER OF AD

Recently, we measured the conductance and transepidermal water loss (TEWL) on the medical examination of infant. In order to examine their usefulness for early diagnosis of AD and reveal that the TEWL in the uninvolved skin of abdomen significantly increased in infants with AD already as early as 6 months and 1.5 years old, when compared with that in infants without AD. Therefore, the measurement of TEWL in the skin is useful for early diagnosis of infantile AD and the early care on the skin of these infants might be expected to effect protectively on development and aggravation of AD.[1] We have already reported that *Staphylococcus aureus* (SA) usually existed not only on the skin regions affected by AD but also on the atopic dry skin regions.[2] The number of SA detected on the skin surface of forearm and forehead in AD patients was remarkably more than that in healthy controls, as shown Fig. 1.[2] There was a significant correlation between eruption score and the number of SA detected on the same area of patients with AD (relationship coefficiency=0.54, $P<0.01$),[1] as shown in Fig. 2.[3] We have also reported that farnesol (F) of a perfume and xylitol (X) of sugar alcohol synergistically can inhibit the biofilm formation of SA and moreover can dissolve the formed biofilm of SA, as shown in Fig. 3-1 and Fig. 3-2.[3,4] Indeed, the treatment with FX cream improved significantly 4 out of 5 items in AD lesions at 2 weeks later, while the treatment with control cream improved only 1 item of dryness/desquamation as shown in Fig. 4.[3,4] Both of the number of SA and the ratio of SA to total bacteria at the sites, to which the FX cream containing F and X had been applied, significantly decreased in 2 weeks later, accompanied with clinical improvement of AD, as shown in Fig. 5.[3,4] The therapeutic effect of this FX cream is conceivable to be based on two functions of

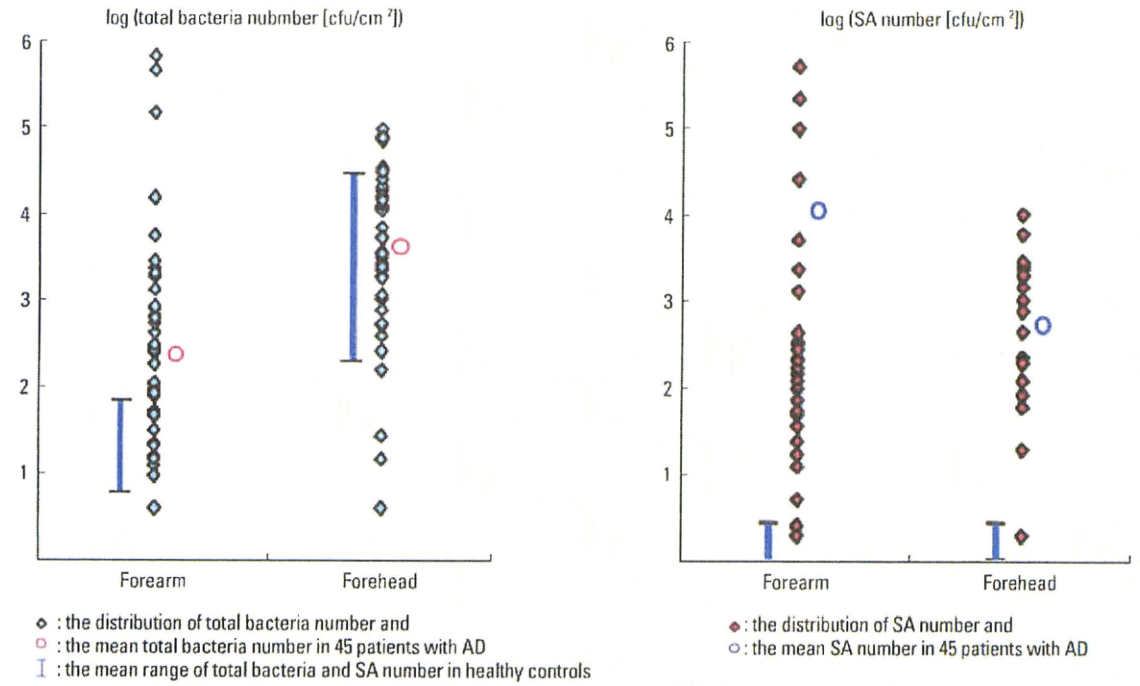


Fig. 1. The number of SA detected on the skin surface of forearm and forehead in patients with AD is remarkably more than in healthy controls.[2] cfu: colony forming unit.

it, namely the prevention of adhesion of SA to the skin and the removal of SA from the skin. Therefore, this FX cream is expected as a useful skin care agent for atopic dry skin colonized by SA.[3,4] Recently, we also found that AD-like lesions in DS-Nh of AD model mouse mice were significantly improved together with a decrease in number of SA detected from these lesions by treatment with the bamboo leaf extract cream having the antibacterial, viral and mycotic activity.[5] This improvement effect was also observed together with a decrease of the increased serum IgE levels and IFN-γ/IL-13 production in Con A-stimulated cultures of lymph node cells taken from these mice. From these results, this extract cream is suggested to have not only antibacterial effects but also immunological effects as the therapeutic agent.[5]

It is well-known that SA discharges various toxins and enzymes that injure the skin, results in activation and proliferation of epidermal keratinocytes, which produce and release IL-18. The IL-18 is supposed to be involved in pathogenesis of AD, because IL-18 induces the super Th1 cells producing and secreting IFN-γ and IL-13.[6] Indeed, the serum IL-18 levels in patients with AD

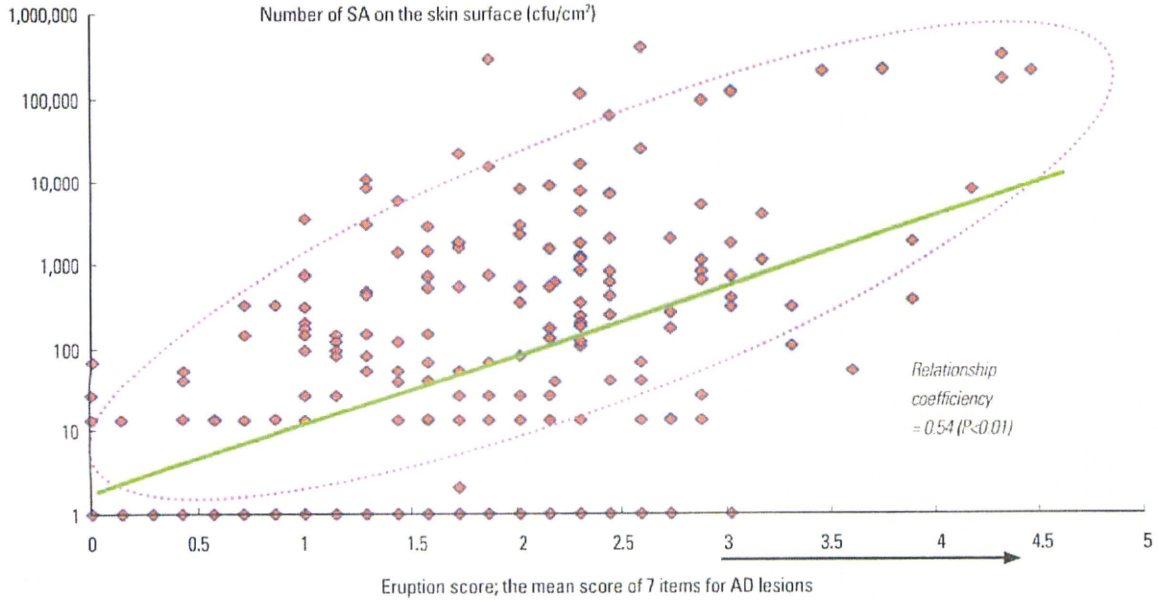


Fig. 2. The correlation between eruption score and the number of SA on the same area of patients with AD.[3] cfu: colony forming unit.

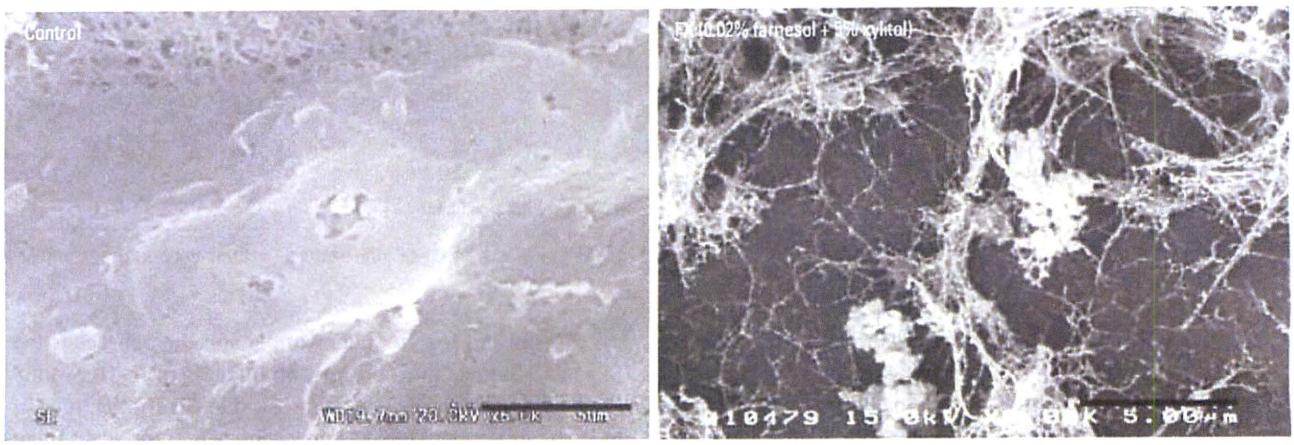


Fig. 3-1. Farnesol and xylitol (FX) have an inhibitory effect on biofilm formation of *SA*.[3] A biofilm of bacteria was formed by incubating a plastic coverslip coated with type IV collagen in each well of a 24-well tissue culture plate with the human plasma and TSB (1:1) medium, into which SA was inoculated. Cell suspensions of SA from an AD patient (1x10⁸ cfu/mL) were inoculated separately into 1 mL of the medium either alone (control) or supplemented with FX. After incubation at 37°C, each coverslip was observed visually and also under a scanning electron microscope (SEM) and number of SA colony adhered to the coverslip of was counted. TSB: tryptic soy broth.

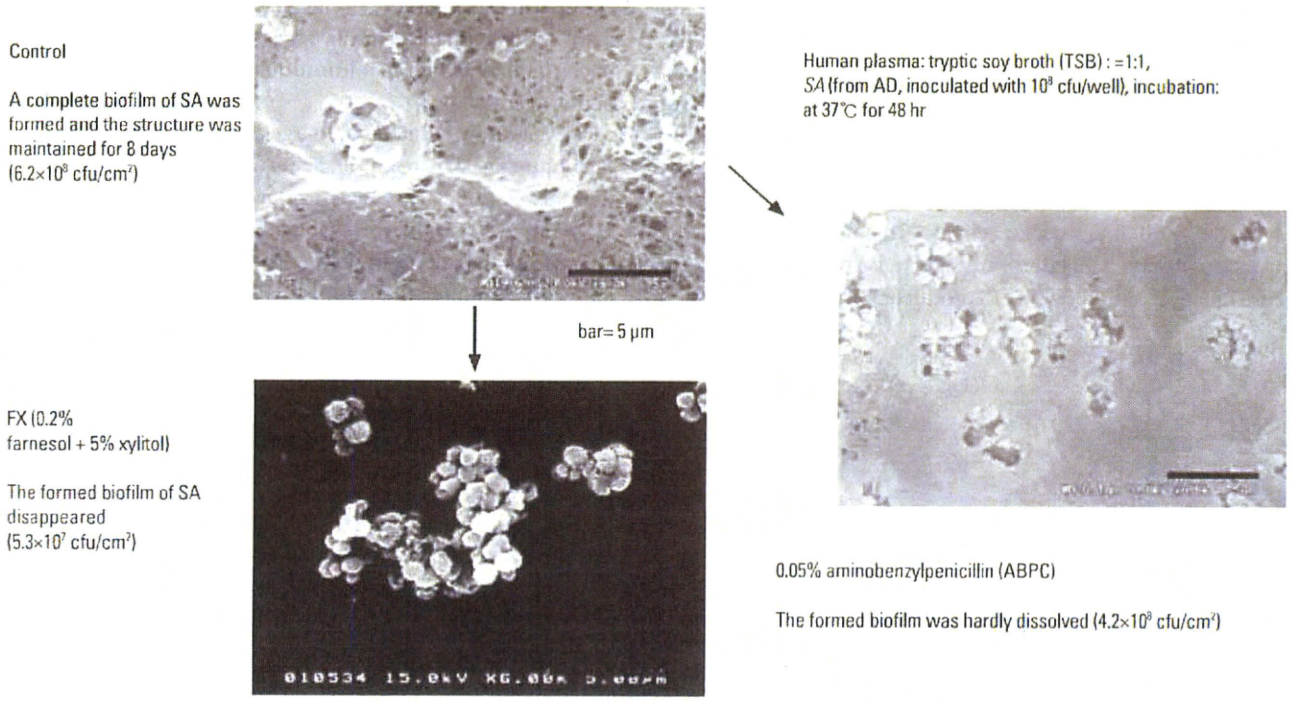
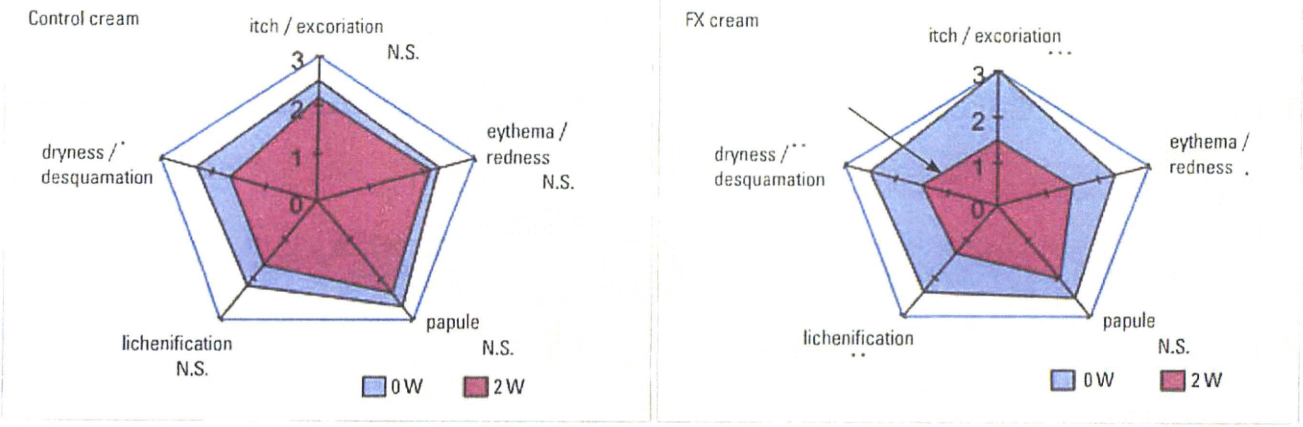


Fig. 3-2. FX dissolve the formed biofilm of *SA*.[3]



*** : $P<0.01$, ** : $P<0.05$, * : $P<0.1$, n.s. : not significant

Fig. 4. The treatment with FX cream improved significantly 4 items out of 5 items in AD lesions 2 weeks later, while the treatment with control cream improved only 1 item of dryness/desquamation.[3]

significantly correlated with skin scores of AD lesions, as shown Fig. 6-1.[7] From these lesions, SA is detected with high frequency and a correlationship is observed between eruption score and the number of SA detected, as above-mentioned. Also, there is a strong positive correlation between skin scores and serum IL-18 levels in DS-Nh mice ($P<0.0001$, $r=0.64$) with AD-like lesions, in which SA is supposed to be a critical factor for the development of AD lesions, as shown Fig. 6-2.[7,8]

DS-Nh mice develop AD-like legions when they are housed under conventional conditions, but develop skin legions with less severity and less frequency under specific pathogens free (SPF) conditions.[9] Furthermore, an increase of IL-18 production from epidermal cells was observed in AD model mice induced by subsequent topical application of SA products.[6] Therefore, in order to clarify the role of IL-18 in the pathogenesis of AD, we measured IL-18 levels in the horny layer (i.e. horn IL-18) by the method using tape stripping and ELISA, which has been already established for measurement of NGF level in the horny layer[10] and assessed the horn IL-18 level in relation to clinical severities and SA colonization on the involved skin of

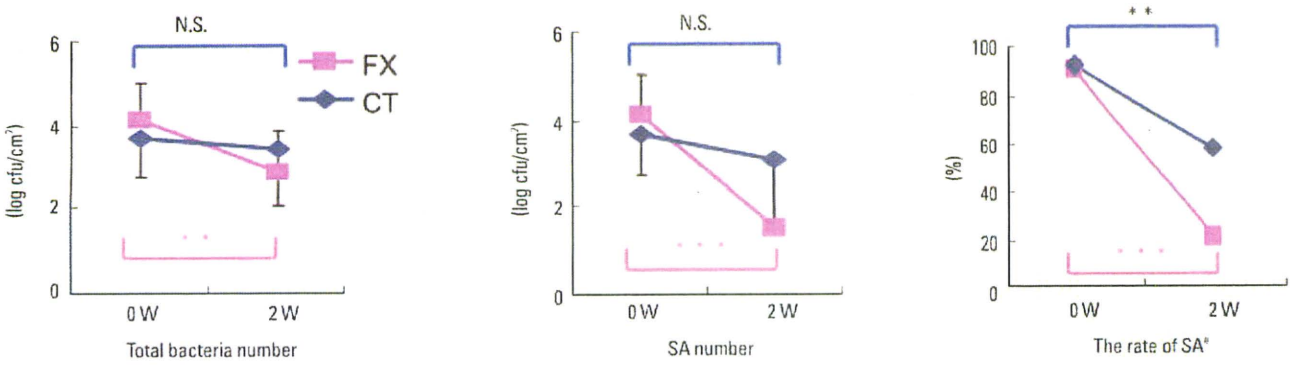


Fig. 5. Application of FX cream containing 0.2% farnesol and 5% xylitol to AD patients for 2 weeks decreased the number and rate of SA significantly when compared with the number at the start and with placebo control treatment.[3] FX: cream containing 0.2% farnesol and 5% xylitol, CT: control treatment, # the rate (%) of SA to total bacteria, N.S. : not significant, $^{**}P<0.05$, $^{***}P<0.01$.

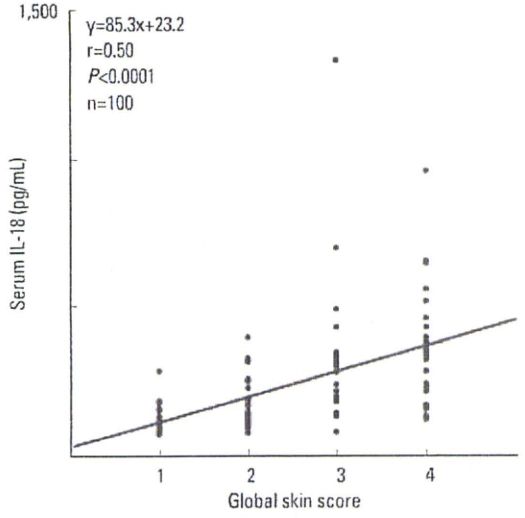


Fig. 6-1. Comparison between serum levels of IL-18 and global skin scores in AD patients divided into 4 groups based on their disease severities.[7]

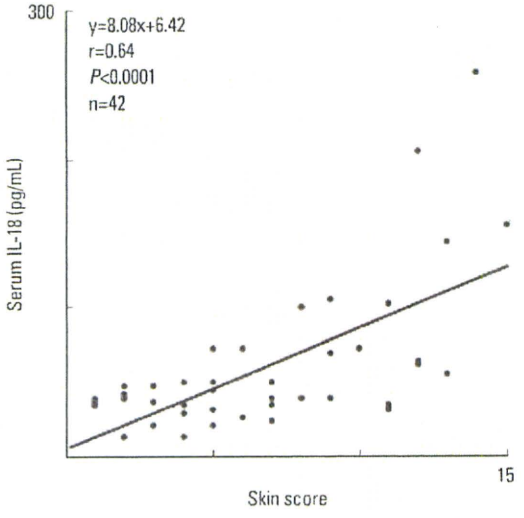


Fig. 6-2. Comparison between serum IL-18 levels and skin scores in DS-*Nh* mice (n=14 each group).[7]

AD patients. The horn proteins were collected via tape stripping from the horny layer of the skin in 61 AD patients and 40 healthy controls, and the horn IL-18 levels were measured using ELISA. Clinical severity, blood data and SA colonization of involved skin were also evaluated before and 4-8 weeks after treatment. The results showed that the horn IL-18 levels of skin lesions were significantly higher in AD patients than in healthy controls and correlated to SCORAD, serum IL-18, IgE, LDH, thymus and activation-regulated chemokine (TARC) and TEWL.[11] In the group of AD patients with IgE <1,500 IU/m, the horn IL-18 levels was significantly higher in patients whom SA was detected than in patients from whom SA was not.[11] From these results, SA colonization on the skin lesions is conceivable to contribute to the IL-18 production of epidermal keratinocytes especially in the group of AD patients with relatively low IgE production and the horn IL-18 levels seem to be associated with the severity of AD.

It is interesting that DS-Nh mice pretreated with cyclophosphamide (Cy) developed more remarkable AD-like lesions with more number of SA colonies on the skin surface, in comparison with untreated ones.[12] The levels of IFN-γ and IL-13 in the supernatants of the lymph node cell cultures stimulated with SEB or ConA were increased remarkably in the Cy-treated mice, although the serum levels of total IgE were not.[12] In this experiment, we also revealed that Cy-treated mice, to which CD25+ CD4+ regulatory T cells taken from non-treated ones had been transferred, developed AD-like legions with less severity and less number of SA colonies on the skin surface.[12] Table simply shows the summary of these results. Therefore, it is presumed that CD25+CD4+ regulatory T cells might be involved in suppression of super Th1 cells, which are induced by IL-18 and are involved in the development of AD-like lesions rather than IgE production. The efficient induction of CD25+CD4+ regulatory T cells may become new type of treatment for recalcitrant AD. Fig. 7 shows schematically the action mechanism of FoxP3+

Table. Summary of results of Cy-pretreated and transferred experiments in DS-Nh mice[12]

	Experiment 1			Experiment 2		
Treated/Sensitized recipient	Cy-untreated, TNCB-unsensitized	Cy-untreated, TNCB-sensitized	Cy-pretreated, TNCB-sensitized	Cy-pretreated, TNCB-sensitized	Cy-pretreated, TNCB-sensitized	Cy-pretreated, TNCB-sensitized
Transfer of T cells	-/+	+ ~ ++	+++ ~ ++++ Increased*	PBS	CD25- T (Te)	CD25+ T (Treg)
AD-like lesion score	-/+	+ ~ ++	+ ~ ++ Not increased	+++ ~ ++++	+++ ~ ++++	+ ~ ++ Decreased*
Serum IgE level						
Number of SA clonies	-/+	+ ~ ++	+++ ~ ++++ Increased**	+++ ~ ++++	+++ ~ ++++	+ ~ ++ Dcreased**

*: $P<0.05$, **: $P<0.01$.

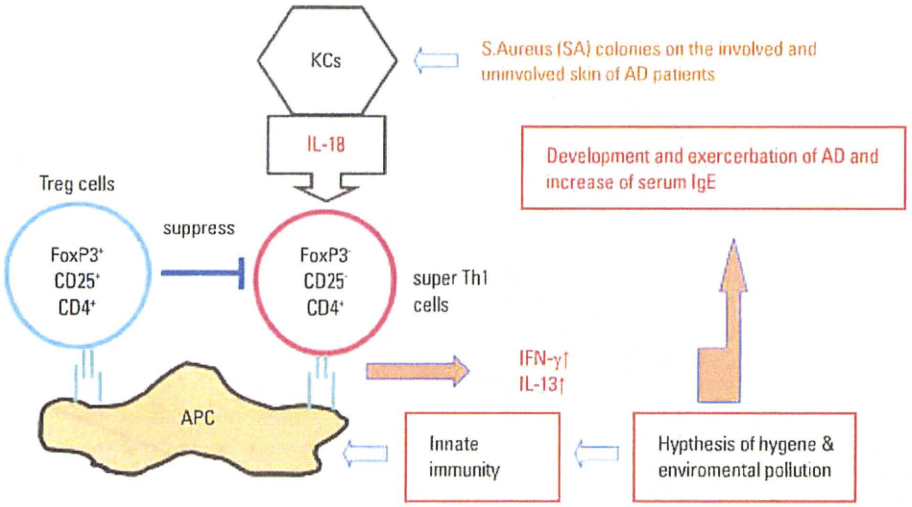


Fig. 7. FoxP3+CD25+CD4+ regulatory T cells are presumed to negatively regulate the induction and exercerbation of AD-like lesions through the suppression of IFNγ/L-13 production of by FoxP3-CD25-CD4+ super Th1 cells in DS-Nh of AD model mouse associated with SA colonization on the skin surface.[12,13]

CD25+CD4+ regulatory T cells, which are conceivable to suppress the induction and exacerbation of AD-like lesions through the suppression of IFN-γ/L-13 production of by FoxP3-CD25-CD4+ super Th1 cells in DS-Nh of AD model mouse associated with SA colonization on the skin surface.[12-14]

A ROLE OF NERVE GROWTH FACTOR (NGF) IN PATHOGENESIS AND BIOMARKER OF AD FROM THE QUANTITATIVE ANALYSIS OF NGF IN HORNY LAYER OF AD PATIENTS

Nerve growth factor (NGF), which belongs to the neurotrophin family, is mainly produced and released by the basal keratinocytes[15-19] and has diverse activity in the skin. Immunohistochemistry of human skin shows that NGF is expressed in suprabasal keratinocytes and in basal keratinocytes.[18] Recently, neurogenic inflammation, including effect of neuropeptides such as substance P (SP) and NGF, has provided a new perspective in understanding the pathogenesis of AD.[20] Increasing evidence suggests that neurotrophins are supposed to be involved in the neurogenic inflammation of AD and to be one of factors, which regulate development of AD.[21] It has been reported that NGF levels of AD patients are measured in plasma,[16] urine and saliva,[22] and the NGF level in plasma increases and correlates with the disease severity.[16] Furthermore, it has been also reported that expression of NGF increases in the AD lesions biopsied and relates to the aggravation of disease.[23] However, it is not so good method to measure the NGF level in the biopsied skin samples because it is invasive and cannot be done repeatedly. By the preliminary experiments, we established a method using tape stripping and ELISA for measurement of NGF in the horny layer (i.e. horn NGF), which is able to be accepted repeatedly in the same patient and then measured the horn NGF in AD patients by this method.[10] The results showed that the horn NGF level in the non-lesional skin of AD patients as well as in the lesional skin significantly increased, in comparison with the non-lesional skin of healthy subjects, as shown Fig. 8, being consistent with the previous histochemical findings that NGF expression in keratinocytes is increased in AD patients.[10] Then, we assessed a possible relationship be-

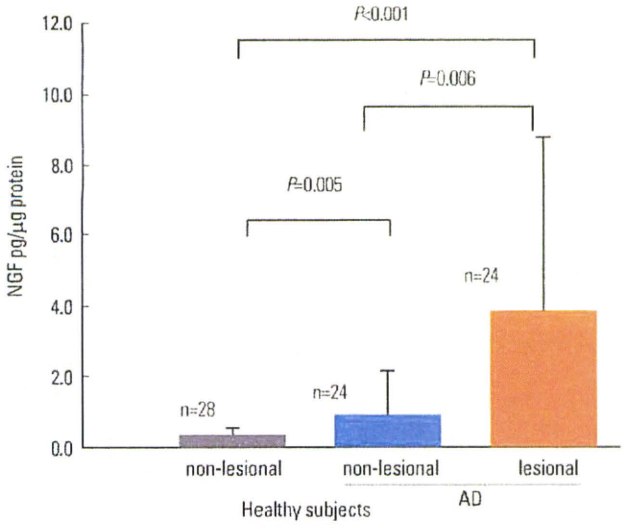


Fig. 8. NGF levels in the horny proteins.

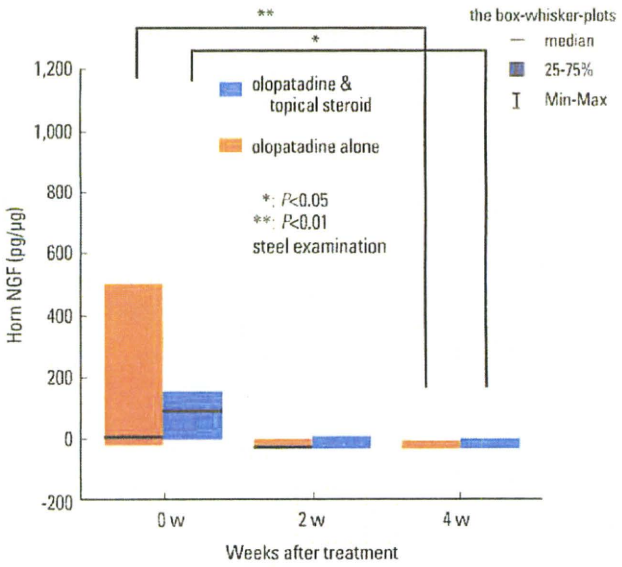


Fig. 9. Effect of treatment on horny NGF of AD patients.[10]

tween the horn NGF level and the severity of itch and AD, and also examined effect of treatment on the horn NGF level. The positive correlations were observed between horn NGF level and itch severity, eruption score such as erythema, scale/xerosis and total skin score on the skin lesions measured, eosinophil count and serum LDH level, but were not between horn NGF level and skin scores of papule, erosion/crust and lichenification on the skin lesions measured and serum IgE level.[10] The horn NGF level decreased significantly after treatment, which correlated with the decrease in itch severity, skin scores (erythema, papule, scale/xerosis, lichenification and total skin score) on the measured skin lesions, serum LDH level, and especially eosinophil count.[10] These findings indicate that the horn NGF level reflects to some extent the severity of itch, eruptions, and laboratory data. The level of horn NGF had decreased significantly not only at 4 weeks, but also at 2 weeks after beginning treatment.10 Considering the fact that the turnover of the horny layer is approximately 14 days, it is presumed that epidermal NGF production is suppressed immediately after beginning treatment. Also, a significant decrease in NGF level was observed not only in the group treated with olopatadine and topical steroid, but also in the group treated with olopatadine alone, as shown Fig. 9. How olopatadine suppresses the increase of horn NGF levels induced in AD patients? It has been reported that histamine enhances NGF production of human keratinocyte through the stimulation of H1 receptors.[24] Therefore, H1-antagtonist including olopatadine is conceivable to suppress the increase of horn NGF level by inhibiting the enhancement of NGF production by keratinocyte stimulated with histamine. Also, we have already reported that olopatadine suppresses the increase of SP levels in the skin lesions induced by the repeated application of 2,4,6-trinitrochlorbenzane in DS-Nh of atopic model mouse.[25] In AD patients, too, it has been reported that olopatadine significantly decreased the serum SP level because of down-modulatory effect on tachykinin release.[26] Other studies have also shown that whereas NGF stimulates the synthesis and releases of SP in the central and peripheral nervous system, SP induces NGF synthesis via cytokines, too.[27,28] These results suggest that the activations of NGF and SP are interdependent. Moreover, in studies of BALB/c mice with chronic contact hypersensitivity induced by oxazolone, it has been reported that oral administration of olopatadine suppressed the production of NGF and cytokines such as IL-4, IL-1β, IL-6 and GM-CSF in the affected ear tissues, unlike other histamine H1 receptor antagonists.[29-31] From these results, olopatadine is suggested to exerts its effect on AD not only by antihistamine effect but also by suppressing the production of NGF and SP. Taken all together, the horn NGF level is presumed to be able to serve as a marker to evaluate the clinical conditions of AD and the immediate effect of treatment because it can reflect the severity of itch and eruptions in AD. Therefore, quantification of NGF in the samples collected directly from the horny layer appears to be useful in assessing the severity of AD and the therapeutic effects on AD.

SEMAPHORIN 3A (SEMA3A) ALLEVIATES SKIN LESIONS AND SCRATCHING BEHAVIOR IN NC/NGA, MODEL MICE OF AD

Topical steroids and antihistamines are generally used for the treatment of AD. However, these treatments are not effective sufficiently for the recalcitrant AD with severe itching followed by uncontrollable scratching. In AD patients, the C-fiber in the epidermis increases and sprouts with the increase of horn NGF levels, inducing hypersensitivity to itching, which is considered

to aggravate the disease, as above-mentioned. Semaphorin 3A (Sema3A), an axon guidance molecule, is a potent inhibitor of neurite outgrowth of sensory neurons by exerting the plexin-A1-4 and neuropilin-1 (NRP-1) of its receptors.[32,33] Fig. 10 shows semaphorin family and its receptors. Sema3A may become effective drug against refractory itching skin diseases such as AD in future by suppressing the hypersensitivity to itching through inhibition of the extension and sprout of C-fiber in the epidermis. From this point of view, we administered the recombinant Sema3A intracutaneously into the AD-like skin lesions of NC/Nga mice, an animal model of AD, in order to investigate the effect of Sema3A on AD. Sema3A improved significantly AD-like lesions of NC/Nga, AD model mouse (Fig. 11-1), and the improvement effect was a dose-dependent manner (Fig. 11-2) and was presumed to be brought through the suppression of scratching accompanied with itching, because Sema3A suppressed significantly the scratching behavior of NC/Nga mice (Fig. 11-3). Histopathological examinations revealed an improvement of acanthosis, a remarkable reduction in the density of invasive nerve fibers, and a decrease of the inflammatory infiltrate including mast cells and CD4+ T cells and the interleukin-4 (IL-4) production in the Sema3A-injected area of AD-like lesions.[34] The improvement effect was associated with the suppression of scratching behavior in Sema3A-treated animals. These findings

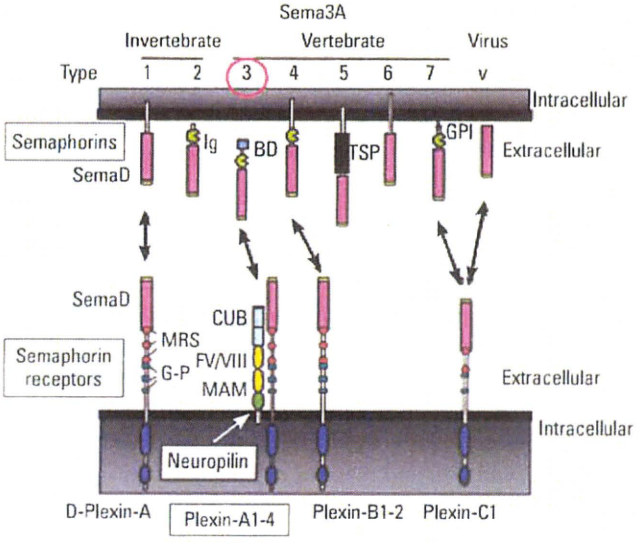


Fig. 10. Semaphorin family and its receptorsSema.[33,34] D: Semadomain, Ig: immunoglobulin, BD: Basic domain, TSP: Thrombospondin repeat, GPI: Glycerophosphatidyl-inositol anchor, MRS: Met-related sequence, G-P: glycine proline repeat, CUB: complement binding domains (a1 and a2 domains), FV/VIII: FactorV/VIII coagulation factor-like domains (b1 and b2 domains), MAM: Mepulin-A5-μ domain.

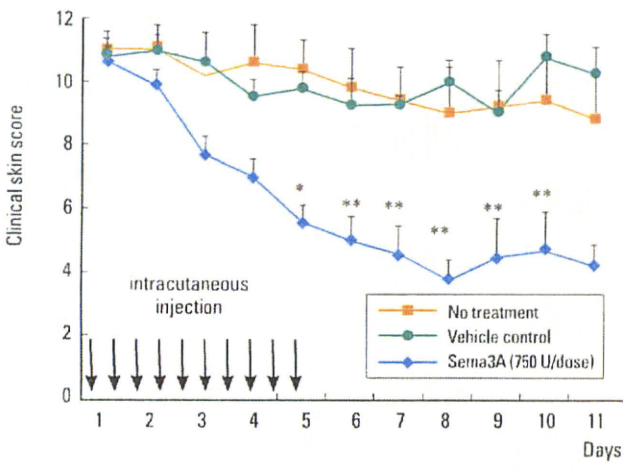


Fig. 11-1. Sema3A improves AD-like lesions of NC/Nga, AD model mouse.[34] The clinical skin score was defined as the sum of individual scores graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for the symptoms of erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness. $*P < 0.05$, $**P < 0.01$, compared to no treatment group and control group.

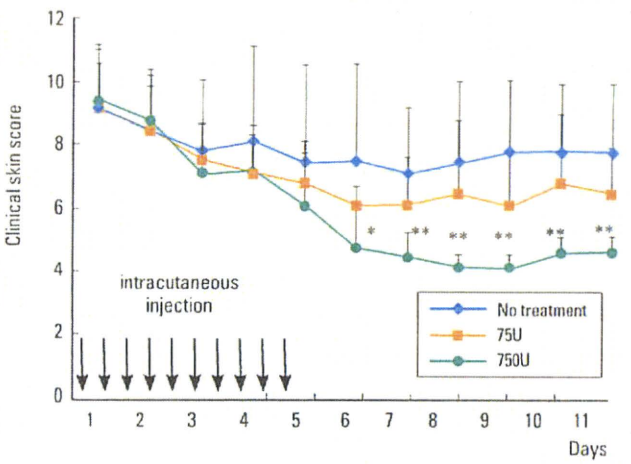


Fig. 11-2. Sema3A improves clinical skin score in a dose-dependent manner.[34] The clinical skin score was defined as the sum of individual scores graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for the symptoms of erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness. $*P < 0.05$, $**P < 0.01$, compared to no treatment group and control group.

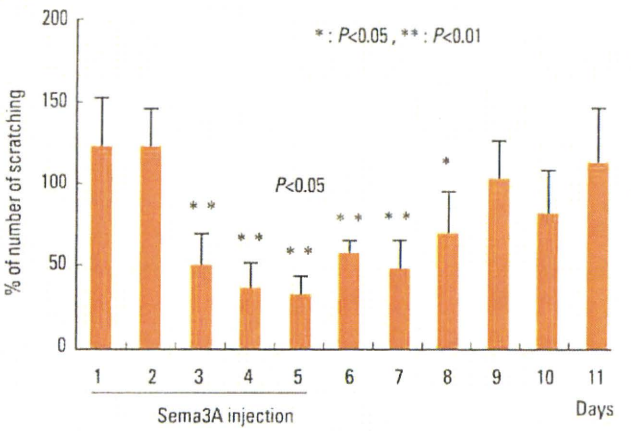


Fig. 11-3. Sema3A suppresses scratching behavior of NC/Nga mice.[34]

indicate for the first time that Sema3A in vivo remarkably improves AD-like lesions in NC/Nga mice, an animal model of AD. This is the first trial for a potential clinical application of semaphorins. Sema3A acts locally and exerts a continuous effect for a while even after discontinuation. But this effect of Sema3A on scratching was reversible, because the decrease in scratching behavior returned to the basal levels immediately after the discontinuation of Sema3A, followed by recurrence of lesions, as shown in Fig. 11-1, 2, 3. These results suggest that scratching behavior aggravates AD and Sema3A can ameliorate the symptoms of AD by interrupting the itch-scratch cycle, which is an important cause of aggravation and chronicity of AD. Recently, there has been an interesting report that epidermal Sema3A levels were decreased in patients with AD compared with healthy volunteers, concomitant with the increase of epidermal nerve densities.[35] This result shows that there is a good correlation between epidermal innervation and Sema3A levels, which may provide an important evidence for the therapeutic claim against itching. Many drugs have been developed for the treatment of AD. However there is no treatment other than Sema3A which targets the invasive nerve fibers in the epidermis. Therefore, Sema3A is expected to become an effective and useful drug for AD patients with severe recalcitrant itching, which is resistant to anti-histamine drugs of H1-blocker and anti-inflammatory steroid ointment.

Recently, transient receptor potential vanilloid receptor subtype 1 (TRPV1) and capsaicin are noticed as topics regarding treatment of itching. The generally accepted concept for the therapeutic application of capsaicin to mitigate itch is based on desensitizing effect of this vanilloid. However, the most notorious clinical limitation of capsaicin application is supposed to be acute excitation of the sensory C-afferents, which is induced by the TRPV1 and results in a remarkable "hot painful burning" sensation.[36] Does reduction in innervation density of intraepidermal nerve fibers by the local application of Sema3A cause such a sensation? In our experiments regarding the effects of Sema3A on AD-like lesions, no apparent abnormalities in antinociceptive response as well as in general symptoms were observed on local treatment with Sema3A. Answering above questions should be required before therapeutic attempts in human. Sema3A, when intracutaneously administered repeatedly, significantly improved AD lesions on day 2 or 3 after the start of the injections, as shown in Fig. 11-3. Recent studies indicate that semaphorins play diverse roles unrelated to axon guidance, including organogenesis, vascularization and angiogenesis.[37-39] In particular, attention has been given to the fact that several semaphorins play critical roles in the immune system.[40,41] It has been reported that Sema3A,[41,42] Sema3C,[43] Sema4A,[44] Sema4D[42,44-50] and Sema7A[51] function in various phases of immune system. From these recent knowledges and the above-mentioned histopathological findings in our experiments, the effects of Sema3A on AD-like lesions are conceivable to involve other mechanisms such as regulation of the immune and vascular systems, in addition to the suppression of the invasive sensory neurons in the epidermis. In addition, Sema3A and NRP-1 of its receptors expressed in human keratino-

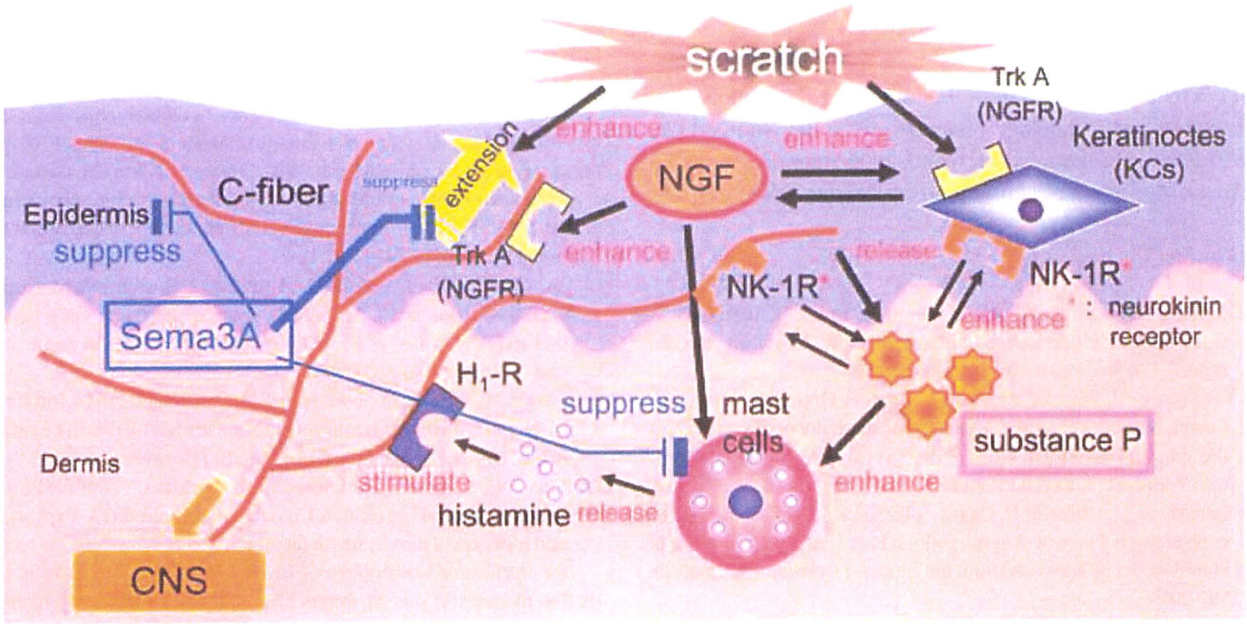


Fig. 12. Pathomechanism of itch and action mechanism of Sema3A on itching of AD lesions: Sema3A has not only the neurological activity, which inhibits the intraepidermal extension of peripheral nerve, but also the immunological anti-inflammatory activity, and is expected as a new type of drug effective for refractory AD patients who are resistant to existing drugs.

cytes[52] may be relevant to the above-mentioned suppressive effect of Sema3A on acanthosis observed in our present experiment. Also Sema3A and NRP-1 of its receptors expressed in dendritic cells and T cells[53,54] may be relevant to the immunological effect of Sema3A such as suppression of CD4+ T cells infiltration and IL-4 production observed in the AD-like lesions in NC/Nga mice treated with Sema3A, as above-mentioned.

In 2005, Takano et al., reported that anti-NGF antibodies inhibited the development of skin lesions and epidermal innervation in the NC/Nga of AD model mouse, accompanied with the reduction of scratching.[55,56] Thus Sema3A may function as well as anti-NGF antibodies may act on the both of immunological and neurological mechanisms. However, the combination therapy of Sema3A with anti-NGF antibody may not be justified for AD, because NGF is known to increase the expression levels of NRP-1[57] and to augment the effect of Sema3A to induce axon repulsion (or growth cone collapse) of dorsal root ganglia neurons.[58] Further studies should be required to elucidate the mechanism of action of Sema3A on AD. Fig. 12 shows schematically the pathomechanism of itch and action mechanism of Sema3A on itching of AD lesions. Sema3A has not only the neurological activity, which inhibits the intraepidermal extension of peripheral nerve, but also the immunological anti-inflammatory activity, and is expected as a new type of drug effective for refractory AD patients who are resistant to existing drugs. It is possible that Sema3A is also widely effective for severe itching skin diseases other than AD in which the itch-scratch cycle is involved.

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REFERENCES

1. Kanbara T, Nakamura K, Tanaka Y, Inoue Y, Tanaka K, Ikezawa Z. A study about the prevalence of infantile atopic dermatitis (the 5th report). Proceedings in Arerugi 2009;58:376.
2. Katsuyama M, Wachi Y, Kitamura K, Suga C, Onuma S, Ikezawa Z. Correlation between the population of staphylococcus aureus on the skin and severity of score of dry type atopic dermatitis condition. Nippon Hifuka Gakkai Zasshi 1997;107:1103-11.
3. Katsuyama M, Ichikawa H, Ogawa S, Ikezawa Z. A novel method to control the balance of skin microflora. Part 1. Attack on biofilm of Staphylococcus aureus without antibiotics. J Dermatol Sci 2005;38: 197-205.
4. Katsuyama M, Kobayashi Y, Ichikawa H, Mizuno A, Miyachi Y, Matsunaga K, Kawashima M. A novel method to control the balance of skin microflora Part 2. A study to assess the effect of a cream containing farnesol and xylitol on atopic dry skin. J Dermatol Sci 2005;38:207-13.
5. Fujita H, Takahashi H, Aihara M, Hirasawa T, Ikezawa Z. Effects of bamboo leaf extract on atopic dermatitis model mice. J Environ Dermatol 2006;13:87-94.
6. Terada M, Tsutsui H, Imai Y, Yasuda K, Mizutani H, Yamanishi K, Kubo M, Matsui K, Sano H, Nakanishi K. Contribution of IL-18 to atopic-dermatitis-like skin inflammation induced by Staphylococcus aureus product in mice. Proc Natl Acad Sci U S A 2006;103: 8816-21.
7. Kirino M, Kirino Y, Takeno M, Nagashima Y, Takahashi K, Kobayashi M, Murakami S, Hirasawa T, Ueda A, Aihara M, Ikezawa Z, Ishigatsubo Y. Heme oxygenase 1 attenuates the development of atopic dermatitis-like lesions in mice: implications for human disease. J Allergy Clin Immunol 2008;122:290-7, 7 e1-8.
8. Matsukura S, Aihara M, Hirasawa T, Ikezawa Z. Effects of TNCB sensitization in DS-Nh mice, serving as a model of atopic dermatitis, in comparison with NC/Nga mice. Int Arch Allergy Immunol 2005; 136:173-80.
9. Yoshioka T, Hikita I, Matsutani T, Yoshida R, Asakawa M, Toyosaki-Maeda T, Hirasawa T, Suzuki R, Arimura A, Horikawa T. DS-Nh as an experimental model of atopic dermatitis induced by Staphylococcus aureus producing staphylococcal enterotoxin C. Immunology 2003;108:562-9.
10. Yamaguchi J, Aihara M, Kobayashi Y, Kambara T, Ikezawa Z. Quantitative analysis of nerve growth factor (NGF) in the atopic dermatitis and psoriasis horny layer and effect of treatment on NGF in atopic dermatitis. J Dermatol Sci 2009;53:48-54.
11. Inoue Y, Aihara M, Harada I, Komori J, Kirino M, Ikezawa Z. Analysis of the pathogenesis by the quantitative measurement of interleukin-18 in the horny layer and Staphylococcus aureus colonization on the skin surface in patients with atopic dermatitis. Proceedings in Arerugi 2009;58:1262.
12. Ikezawa Y. Exacerbative effect of cyclophosphamide on symptom of atopic dermatitis in DS-Nh mouse. Yokohama Medical Journal 2004;55:437-43.
13. Ikezawa Y, Nakazawa M, Tamura C, Takahashi K, Minami M, Ikezawa Z. Cyclophosphamide decreases the number, percentage and the function of CD25+ CD4+ regulatory T cells, which suppress induction of contact hypersensitivity. J Dermatol Sci 2005;39:105-12.
14. Tatewaki S, Nakazawa M, Aihara M, Hongo N, Hotta Ch, Ikezawa Y, Takahashi K, Minami M, Ikezawa Z. Effects of cyclophosphamide on 2, 4-dinitrofluorobenzene-specific oral tolerance. J Environ Dermatol Cutan Allergol 2007;1:12-21.
15. Yoshioka T, Hikita I, Asakawa M, Hirasawa T, Deguchi M, Matsutani T, Oku H, Horikawa T, Arimura A. Spontaneous scratching behaviour in DS-Nh mice as a possible model for pruritus in atopic dermatitis. Immunology 2006;118:293-301.
16. Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. Br J Dermatol 2002;147:71-9.
17. Pincelli C, Sevignani C, Manfredini R, Grande A, Fantini F, Bracci-Laudiero L, Aloe L, Ferrari S, Cossarizza A, Giannetti A. Expression and function of nerve growth factor and nerve growth factor receptor on cultured keratinocytes. J Invest Dermatol 1994;103:13-8.
18. Botchkarev VA, Yaar M, Peters EM, Raychaudhuri SP, Botchkareva NV, Marconi A, Raychaudhuri SK, Paus R, Pincelli C. Neurotrophins in skin biology and pathology. J Invest Dermatol 2006;126: 1719-27.
19. Pincelli C, Marconi A. Autocrine nerve growth factor in human ke-

ratinocytes. J Dermatol Sci 2000;22:71-9.
20. Huang CH, Kuo IC, Xu H, Lee YS, Chua KY. Mite allergen induces allergic dermatitis with concomitant neurogenic inflammation in mouse. J Invest Dermatol 2003;121:289-93.
21. Peters EM, Raap U, Welker P, Tanaka A, Matsuda H, Pavlovic-Masnicosa S, Hendrix S, Pincelli C. Neurotrophins act as neuroendocrine regulators of skin homeostasis in health and disease. Horm Metab Res 2007;39:110-24.
22. Masahiko T. Useful therapeutic markers of atopic dermatitis: neurogenic factors. Skin Research 2005;4 (Suppl 5):87-93.
23. Dou YC, Hagstromer L, Emtestam L, Johansson O. Increased nerve growth factor and its receptors in atopic dermatitis: an immunohistochemical study. Arch Dermatol Res 2006;298:31-7.
24. Kanda N, Watanabe S. Histamine enhances the production of nerve growth factor in human keratinocytes. J Invest Dermatol 2003;121: 570-7.
25. Kojima M, Aihara M, Yamada M, Matsukura S, Hirasawa T, Ikezawa Z. Effects of neuropeptides in the development of the atopic dermatitis of mouse models. Allergol Int 2004;53:169-78.
26. Izu K, Tokura Y. The various effects of four H1-antagonists on serum substance P levels in patients with atopic dermatitis. J Dermatol 2005;32:776-81.
27. Lindsay RM, Harmar AJ. Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 1989;337: 362-4.
28. Braun A, Appel E, Baruch R, Herz U, Botchkarev V, Paus R, Brodie C, Renz H. Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. Eur J Immunol 1998;28:3240-51.
29. Tamura T, Matsubara M, Takada C, Hasegawa K, Suzuki K, Ohmori K, Karasawa A. Effects of olopatadine hydrochloride, an antihistamine drug, on skin inflammation induced by repeated topical application of oxazolone in mice. Br J Dermatol 2004;151:1133-42.
30. Tamura T, Matsubara M, Hasegawa K, Ohmori K, Karasawa A. Olopatadine hydrochloride suppresses the rebound phenomenon after discontinuation of treatment with a topical steroid in mice with chronic contact hypersensitivity. Clin Exp Allergy 2005;35:97-103.
31. Tamura T, Amano T, Ohmori K, Manabe H. The effects of olopatadine hydrochloride on the number of scratching induced by repeated application of oxazolone in mice. Eur J Pharmacol 2005; 524:149-54.
32. Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. Nat Rev Mol Cell Biol 2005;6:789-800.
33. Neufeld G, Kessler O. The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nat Rev Cancer 2008;8:632-45.
34. Yamaguchi J, Nakamura F, Aihara M, Yamashita N, Usui H, Hida T, Takei K, Nagashima Y, Ikezawa Z, Goshima Y. Semaphorin3A alleviates skin lesions and scratching behavior in NC/Nga mice, an atopic dermatitis model. J Invest Dermatol 2008;128:2842-9.
35. Tominaga M, Ogawa H, Takamori K. Decreased production of semaphorin 3A in the lesional skin of atopic dermatitis. Br J Dermatol 2008;158:842-4.
36. Biro T, Toth BI, Marincsak R, Dobrosi N, Geczy T, Paus R. TRP channels as novel players in the pathogenesis and therapy of itch. Biochim Biophys Acta 2007;1772:1004-21.
37. Kitsukawa T, Shimono A, Kawakami A, Kondoh H, Fujisawa H. Overexpression of a membrane protein, neuropilin, in chimeric mice causes anomalies in the cardiovascular system, nervous system and limbs. Development 1995;121:4309-18.
38. Behar O, Golden JA, Mashimo H, Schoen FJ, Fishman MC. Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. Nature 1996;383:525-8.
39. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998; 92:735-45.
40. Kikutani H, Kumanogoh A. Semaphorins in interactions between T cells and antigen-presenting cells. Nat Rev Immunol 2003;3:159-67.
41. Lepelletier Y, Smaniotto S, Hadj-Slimane R, Villa-Verde DM, Nogueira AC, Dardenne M, Hermine O, Savino W. Control of human thymocyte migration by Neuropilin-1/Semaphorin-3A-mediated interactions. Proc Natl Acad Sci U S A 2007;104:5545-50.
42. Delaire S, Billard C, Tordjman R, Chedotal A, Elhabazi A, Bensussan A, Boumsell L. Biological activity of soluble CD100. II. Soluble CD100, similarly to H-SemaIII, inhibits immune cell migration. J Immunol 2001;166:4348-54.
43. Mangasser-Stephan K, Dooley S, Welter C, Mutschler W, Hanselmann RG. Identification of human semaphorin E gene expression in rheumatoid synovial cells by mRNA differential display. Biochem Biophys Res Commun 1997;234:153-6.
44. Kumanogoh A, Marukawa S, Suzuki K, Takegahara N, Watanabe C, Ch'ng E, Ishida I, Fujimura H, Sakoda S, Yoshida K, Kikutani H. Class IV semaphorin Sema4A enhances T-cell activation and interacts with Tim-2. Nature 2002;419:629-33.
45. Bougeret C, Mansur IG, Dastot H, Schmid M, Mahouy G, Bensussan A, Boumsell L. Increased surface expression of a newly identified 150-kDa dimer early after human T lymphocyte activation. J Immunol 1992;148:318-23.
46. Herold C, Bismuth G, Bensussan A, Boumsell L. Activation signals are delivered through two distinct epitopes of CD100, a unique 150 kDa human lymphocyte surface structure previously defined by BB18 mAb. Int Immunol 1995;7:1-8.
47. Hall KT, Boumsell L, Schultze JL, Boussiotis VA, Dorfman DM, Cardoso AA, Bensussan A, Nadler LM, Freeman GJ. Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation. Proc Natl Acad Sci U S A 1996;93:11780-5.
48. Wu Y, Nadler MJ, Brennan LA, Gish GD, Timms JF, Fusaki N, Jongstra-Bilen J, Tada N, Pawson T, Wither J, Neel BG, Hozumi N. The B-cell transmembrane protein CD72 binds to and is an in vivo substrate of the protein tyrosine phosphatase SHP-1. Curr Biol 1998;8: 1009-17.
49. Kumanogoh A, Watanabe C, Lee I, Wang X, Shi W, Araki H, Hirata H, Iwahori K, Uchida J, Yasui T, Matsumoto M, Yoshida K, Yakura H, Pan C, Parnes JR, Kikutani H. Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity 2000;13:621-31.
50. Watanabe C, Kumanogoh A, Shi W, Suzuki K, Yamada S, Okabe M, Yoshida K, Kikutani H. Enhanced immune responses in transgenic mice expressing a truncated form of the lymphocyte semaphorin CD100. J Immunol 2001;167:4321-8.
51. Holmes S, Downs AM, Fosberry A, Hayes PD, Michalovich D, Murdoch P, Moores K, Fox J, Deen K, Pettman G, Wattam T, Lewis C. Sema7A is a potent monocyte stimulator. Scand J Immunol 2002; 56:270-5.
52. Kurschat P, Bielenberg D, Rossignol-Tallandier M, Stahl A, Klagsbrun M. Neuron restrictive silencer factor NRSF/REST is a transcriptional repressor of neuropilin-1 and diminishes the ability of semaphorin 3A to inhibit keratinocyte migration. J Biol Chem

2006;281:2721-9.
53. Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, Romeo PH. A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. Nat Immunol 2002;3:477-82.
54. Lepelletier Y, Moura IC, Hadj-Slimane R, Renand A, Fiorentino S, Baude C, Shirvan A, Barzilai A, Hermine O. Immunosuppressive role of semaphorin-3A on T cell proliferation is mediated by inhibition of actin cytoskeleton reorganization. Eur J Immunol 2006; 36:1782-93.
55. Takano N, Arai I, Kurachi M. Analysis of the spontaneous scratching behavior by NC/Nga mice: a possible approach to evaluate antipruritics for subjects with atopic dermatitis. Eur J Pharmacol 2003; 471:223-8.
56. Takano N, Sakurai T, Kurachi M. Effects of anti-nerve growth factor antibody on symptoms in the NC/Nga mouse, an atopic dermatitis model. J Pharmacol Sci 2005;99:277-86.
57. Pond A, Roche FK, Letourneau PC. Temporal regulation of neuropilin-1 expression and sensitivity to semaphorin 3A in NGF- and NT3-responsive chick sensory neurons. J Neurobiol 2002;51:43-53.
58. Messersmith EK, Leonardo ED, Shatz CJ, Tessier-Lavigne M, Goodman CS, Kolodkin AL. Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. Neuron 1995;14:949-59.

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症例

マイコプラズマ肺炎およびフェノバルビタール投与後に発症した小児 Stevens-Johnson 症候群の 1 例

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要　約

マイコプラズマ肺炎の 8 歳，男児。フェノバール投与後に Stevens-Johnson 症候群（SJS）を発症。ステロイドパルス療法とγグロブリン投与にて後遺症を残さず治癒。咽頭拭い液よりマイコプラズマ DNA-PCR 陽性，ペア血清でマイコプラズマ抗体価上昇あり。フェノバールのパッチテストと DLST が陽性。小児の SJS は感染症に合併するものが多く，マイコプラズマ感染の報告が多い。しかし，SJS 発症前に投与された薬剤の関与をも示唆する報告はまれである。小児 SJS においてマイコプラズマ肺炎などの感染症に合併した場合にも，パッチテストや DLST を施行し，薬剤の関与の可能性を調べる必要性がある。

キーワード：Stevens-Johnson 症候群，マイコプラズマ肺炎，薬剤アレルギー，フェノバール

Ⅰ．はじめに

Stevens-Johnson 症候群（以下 SJS）は口唇および口腔，眼，陰部などの皮膚粘膜移行部の水疱やびらんなどの粘膜疹が必発であり，中毒性表皮壊死症（toxic epidermal necrolysis；TEN）と並んで表皮および粘膜上皮の壊死性障害を本体とする重症の多形紅斑である。我々は，マイコプラズマ肺炎の合併とフェノバルビタールの薬剤の関与の両方が示唆された小児 SJS の 1 例を経験した。小児の SJS は感染症に合併するものが多く，特にマイコプラズマ感染が多いと報告されている[1)]。発症時の発熱，咳などの感染症症状に対し抗生剤，消炎剤がしばしば投与される。小児においてマイコプラズマ感染が SJS の契機と判断された症例のなかに，初期に投与された薬剤が SJS に関与しているかを調べた報告はまれである[2)3)]。マイコプラズマ肺炎などの感染症に合併した SJS の場合にも，薬剤が関与している可能性を考え，パッチテストや薬剤リンパ球刺激試験（drug lmgphocyte stimulation test，以下 DLST）などの確認をすることは，その後同じ薬剤の投与による再発を防ぐためにも重要であると考え，報告する。

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