研究成果の刊行に関する一覧表

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雑誌
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Variants of C-C Motif Chemokine 22 (*CCL22*) Are Associated with Susceptibility to Atopic Dermatitis: Case-Control Studies

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Abstract

Atopic dermatitis (AD) is a common inflammatory skin disease caused by multiple genetic and environmental factors. AD is characterized by the local infiltration of T helper type 2 (Th2) cells. Recent clinical studies have shown important roles of the Th2 chemokines, CCL22 and CCL17 in the pathogenesis of AD. To investigate whether polymorphisms of the *CCL22* gene affect the susceptibility to AD, we conducted association studies and functional studies of the related variants. We first resequenced the *CCL22* gene and found a total of 39 SNPs. We selected seven tag SNPs in the *CCL22* gene, and conducted association studies using two independent Japanese populations (1^{st} population, 916 cases and 1,032 controls; 2^{nd} population 1,034 cases and 1,004 controls). After the association results were combined by inverse variance method, we observed a significant association at rs4359426 (meta-analysis, combined $P=9.6\times10^{-6}$; OR, 0.74; 95% CI, 0.65–0.85). Functional analysis revealed that the risk allele of rs4359426 contributed to higher expression levels of *CCL22* mRNA. We further examined the allelic differences in the binding of nuclear proteins by electrophoretic mobility shift assay. The signal intensity of the DNA-protein complex derived from the G allele of rs223821, which was in absolute LD with rs4359426, was higher than that from the A allele. Although further functional analyses are needed, it is likely that related variants play a role in susceptibility to AD in a gain-of-function manner. Our findings provide a new insight into the etiology and pathogenesis of AD.

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Introduction

Atopic dermatitis (AD) is a pruritic and chronically relapsing inflammatory skin disease involving disturbed skin barrier functions, cutaneous inflammatory hypersensitivity and defects in the antimicrobial immune defense with a strong genetic background [1]. Predominant infiltration of Th2 cells is a hallmark of acute atopic AD skin lesions [2]. Most patients with AD have peripheral blood eosinophilia and increased serum IgE levels, which are reflected in an increased frequency of peripheral blood skin-homing Th2 cells producing IL-4, IL-5 and IL-13 [1]. C-C motif chemokine 22 (CCL22) and CCL17 are high-affinity ligands for CC-chemokine receptor 4 (CCR4) and induce selective migration of Th2 cells [3]. CCL22 plays a crucial role in controlling the trafficking of Th2 cells into sites of allergic inflammation and is considered to be involved in the pathology of

AD [4]. Keratinocytes from patients with AD highly express thymic stromal lymphopoietin (TSLP), and CCL22 is produced by TSLP-treated dendritic cells [5]. CCL22 is upregulated in lesional atopic dermatitis skin compared with healthy skin [6], and keratinocytes in the epidermal layer of AD skin express CCL17 and CCL22 [7]. Serum levels of CCL22 in AD patients are significantly higher than those found in normal controls [8], and the levels correlate positively with disease severity in AD patients [9]. Strong positive correlations between the levels of CCL17, CCL22, and total IgE in serum of patients with AD and SCORing Atopic Dermatitis (SCORAD) have also been reported [10]. Another study reported that overproduction of IgE induced CCL22 secretion from basophils, which are essential for IgE-mediated chronic allergic dermatitis [11]. These findings prompt-

Table 1. Frequencies of polymorphisms of the *CCL22* gene.

	SNP*	Location	Amino acid	MAF‡	NCBI†	
1	-3075G/A	5′-flanking region	-	0.125	rs223884	
2	-2938G/A	5′-flanking region	-	0.208	rs223885	
3	-2903T/A	5′-flanking region	-	0.333	rs223886	
4	-2668G/T	5′-flanking region	-	0.458	rs34569362	
5	-2550G/C	5′-flanking region	-	0.458	rs76295899	
6	-2511G/T	5′-flanking region	-	0.458	rs4784799	
7	-2191G/C	5′-flanking region	-	0.042	rs76720124	
8	-1795G/A	5′-flanking region	-	0.458	rs34885482	
9	-1775G/T	5′-flanking region	-	0.083	rs72784894	
10	-1618C/T	5′-flanking region	-	0.458	rs77239447	
11	-1515G/T	5′-flanking region	-	0.333	rs223887	
12	-1338A/G	5′-flanking region	-	0.208	rs182668	
13	-961G/A	5′-flanking region	-	0.208	rs223888	
14	-740A/G	5′-flanking region	-	0.083	rs3760071	
15	-488T/C	5′-flanking region	-	0.333	rs223889	§
16	-215WT/DelG	5′-flanking region	-	0.333	rs3214179	
17	5C/A	exon 1	Ala2Asp	0.125	rs4359426	§
18	88C/A	intron 1	-	0.458	rs2074543	§
19	493T/C	intron 1	-	0.458	rs72784897	
20	559G/A	intron 1	-	0.333	rs223816	
21	902C/T	intron 1	-	0.333	rs223817	
22	2030G/C	intron 2	-	0.208	rs223818	§
23	2134T/C	intron 2	-	0.208	rs223819	
24	2198T/C	intron 2	-	0.208	rs223820	
25	2314G/A	intron 2	-	0.292	rs598366	
26	2936A/G	intron 2	-	0.125	rs170359	
27	3062A/G	intron 2	-	0.458	rs73557194	
28	3766T/A	intron 2	-	0.042		
29	3970G/A	intron 2	-	0.125	rs223821	
30	4064WT/InsAAAAC	intron 2	-	0.125	rs72030112	
31	5222T/C	3′ UTR	-	0.125	rs170360	
32	5978WT/DelT	3′ UTR	-	0.125	rs57450696	
33	5979C/G	3′ UTR	-	0.375	rs57186204	
34	6089T/C	3′ UTR	-	0.125	rs223823	
35	6621A/G	3′ UTR	-	0.458	rs121565	§
36	6910G/A	3′ UTR	-	0.417	rs658559	§
37	7858C/T	3′-flanking region	-	0.458	rs3859048	§
38	7883G/A	3′-flanking region	-	0.458	rs72301	
39	8021G/A	3′-flanking region	-	0.042	rs11865093	

*Numbering according to the genomic sequence of *CCL22* (AC003665). Position 1 is the A of the initiation codon.
‡Minor allele frequencies (MAF) in the screening population (N = 12).
†NCBI, number from the dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).
§SNPs were genotyped in this study.
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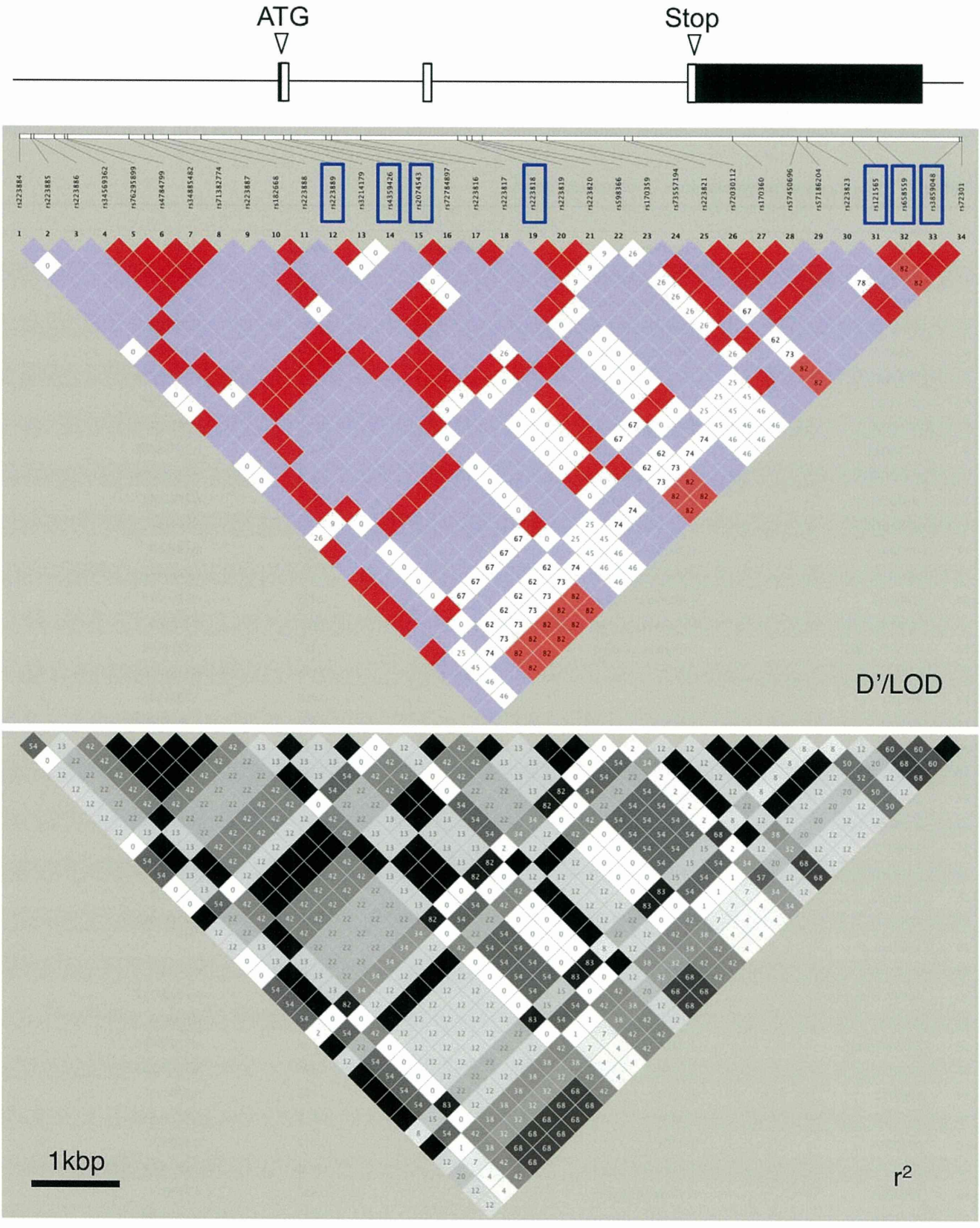

Figure 1. Pairwise linkage disequilibrium between 34 SNPs. LD was measured by D'/LOD (upper) and r^2 (lower) estimated using the Haploview 4.2 program (http://www.broad.mit.edu/mpg/haploview/). Boxed variants were genotyped in this study.
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Table 2. Clinical characteristics of the subjects.

	Case	Control
1st population		
Source	The University of Tokyo Keio University Kyushu University Takao Hospital	Control volunteers
Number of samples	916	1,032
Ethnicity	Japanese	Japanese
Female	43.6%	33.0%
Age (mean ± sd)	30.1±9.5	48.5±13.7
2nd population		
Source	BioBank Japan	University of Tsukuba
Number of samples	1,034	1,004
Ethnicity	Japanese	Japanese
Female	43.8%	54.4%
Age (mean ± sd)	30.8±12.7	50.0±9.2

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ed us to conduct an association and functional study to test whether genetic variations of *CCL22* contribute to AD susceptibility.

Several association studies using genetic variants of genes *CCL17* and *CCR4* in the CCR4 pathway have been conducted to discover genetic components in the pathogenesis of atopic dermatitis [12,13]. A promoter polymorphism of *CCL17*, -431C>T, increases the promoter activity and the 431T allele influences higher serum levels of CCL17 [12], but genetic variants in the *CCL17* gene are not associated with susceptibility to AD. A recent study also reported that C1014T polymorphism in the *CCR4* gene was not associated with AD [13]. However, those studies were performed with small sample sizes and without replication studies. Genetic study of the *CCL22* gene has not been conducted.

In this study, we focused on the *CCL22* gene, resequenced the gene regions including all exons and introns, and carried out linkage disequilibrium mapping. We performed an association study using two independent populations and functional analyses of the related variants.

Results

Polymorphisms of the *CCL22* gene and LD mapping

We identified a total of 39 polymorphisms (Table 1). We next performed linkage disequilibrium (LD) mapping and calculated

Table 3. Genotype counts and case-control association test results of seven tag SNPs.

	Allele		Case			Control				Frequency of allele 2			
db SNP ID	**1/2**	**1/1**	**1/2**	**2/2**	**N**	**1/1**	**1/2**	**2/2**	**N**	**Case**	**Control**	***P* value**	**OR (95%CI)**
1st population													
rs223889	T/C	321	435	151	907	360	502	161	1023	0.406	0.403	0.82	-
rs4359426	C/A	706	191	12	909	736	269	16	1021	0.118	0.147	0.0072	0.77(0.64–0.93)
rs2074543	G/C	386	404	113	903	447	469	110	1026	0.349	0.336	0.39	-
rs223818	A/G	563	311	39	913	596	369	56	1021	0.213	0.236	0.093	-
rs121565	A/G	294	439	173	906	325	509	195	1029	0.433	0.437	0.82	-
rs658559	G/A	333	434	134	901	374	491	162	1027	0.390	0.397	0.65	-
rs3859048	C/T	399	410	103	912	466	448	108	1022	0.338	0.325	0.40	-
2nd population													
rs223889	T/C	369	497	163	1029	364	485	150	999	0.400	0.393	0.65	-
rs4359426	C/A	815	202	12	1029	722	249	22	993	0.110	0.148	0.00037	0.71(0.59–0.86)
rs2074543	G/C	404	484	133	1021	418	459	120	997	0.367	0.351	0.26	-
rs223818	A/G	647	331	42	1020	585	351	57	993	0.203	0.234	0.019	0.84(0.72–0.97)
rs121565	A/G	317	530	179	1026	317	500	180	997	0.433	0.431	0.92	-
rs658559	G/A	389	486	154	1029	363	479	148	990	0.386	0.391	0.71	-
rs3859048	C/T	425	484	117	1026	441	446	113	1000	0.350	0.336	0.35	-
Combined													
rs223889	T/C	690	932	314	1936	724	987	311	2022	0.403	0.398	0.63	-
rs4359426	C/A	1521	393	24	1938	1458	518	38	2014	0.114	0.147	0.0000096	0.74(0.65–0.85)
rs2074543	G/C	790	888	246	1924	865	928	230	2023	0.359	0.343	0.16	-
rs223818	A/G	1210	642	81	1933	1181	720	113	2014	0.208	0.235	0.0044	0.86(0.77–0.95)
rs121565	A/G	611	969	352	1932	642	1009	375	2026	0.433	0.434	0.93	-
rs658559	G/A	722	920	288	1930	737	970	310	2017	0.388	0.394	0.56	-
rs3859048	C/T	824	894	220	1938	907	894	221	2022	0.344	0.330	0.21	-

P values of the two populations were calculated by logistic regression analysis under an additive model. The combined *P* values were calculated using the inverse variance method. OR, odds ratio; CI, confidence interval; -, not significant.

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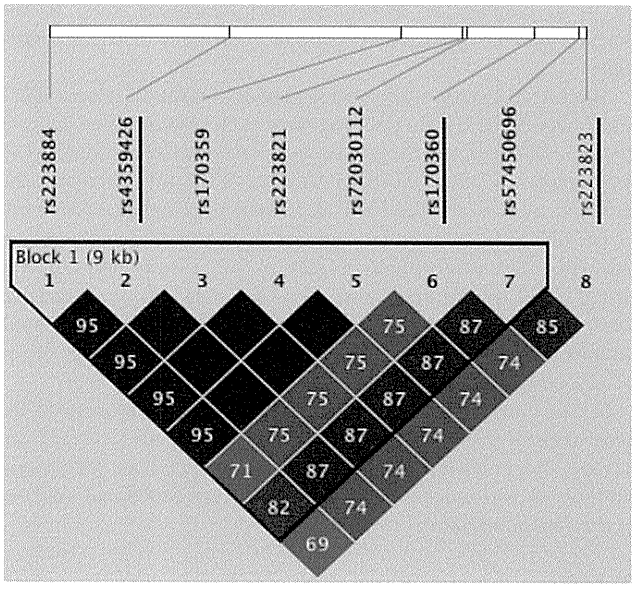

Figure 2. Pairwise linkage disequilibrium (r^2) among eight SNPs in strong LD with rs4359426 in 94 control subjects. Two tag SNPs, rs170360 and rs223823, were selected for further association study. Underlined SNPs were examined.
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pairwise LD coefficients D′ and r^2 among the 34 polymorphisms with MAF>10% using the Haploview 4.2 program (Figure 1). Seven tag SNPs were selected for association studies using tagger in Haploview 4.2, and these polymorphisms captured 34 of the 34 alleles with a mean r^2 of 0.990 (r^2>0.82). The HapMap JPT database contains genotype data for six SNPs with MAF>10% in the region (data not shown). The SNPs examined in this study covered all six SNPs shown in the HapMap JPT database.

Association of *CCL22* SNPs with susceptibility to atopic dermatitis

We recruited 916 cases and 1,032 control subjects for the 1st population and 1,034 cases and 1,004 control subjects for the 2nd population, respectively (Table 2). We genotyped seven tag SNPs and all genotype frequencies are shown in Table 3. The rs4359426 (A2D) SNP was associated with AD under an additive genotype model by logistic regression analysis in the first population (P=0.0072; OR, 0.77; 95% CI, 0.64–0.93) (Table 3). In a replication study, rs4359426 was also associated with AD in the second population (P=0.00037; OR, 0.71; 95% CI, 0.59–0.86) (Table 3). The direction of association of the SNP was similar in both of the populations. We combined the results using inverse variance method, and observed a significant association at rs4359426 (meta-analysis, P=0.0000096; OR, 0.74; 95% CI, 0.65–0.85) (Table 3). We next performed further mapping analyses using two genetic variants, rs170360 and rs223823. The two SNPs were selected from among SNPs that were in strong LD (r^2>0.87) with rs4359426 (Figure 2). Among the three variants, the strongest association was observed at rs4359426 (Table 4).

Contribution of 5′UTR rs4359426 SNP to mRNA expression levels of *CCL22*

Next, using allele-specific transcript quantification (ASTQ), we evaluated whether the related variants could affect the mRNA expression level in EBV-transformed lymphoblastoid cells. As rs4359426 was located at the 16th nucleotide from the 5′ end of the *CCL22* gene (NM_002990.3), we were not able to design primers of the SNP for ASTQ analysis. We therefore designed PCR primers to encompass a SNP in the 3′-UTR of *CCL22* (rs170360) that was in strong LD with rs4359426 (Figure 3A). We isolated total RNA from 24 cell lines that were heterozygous with rs170360, and genomic DNA was used as a control for equal biallelic representation. Predicted haplotype frequencies are shown in Figure 3B. The ratio of PCR products was approximately 1.6 for cDNAs and 1.0 for genomic DNA from 21 subjects who were heterozygous for rs4359426 (Figure 3C, left panel); however, such

Table 4. Genotype counts and case-control association test results for SNPs rs4359426, rs170360 and rs223823.

	Allele		Case			Control				Frequency of allele 2			
db SNP ID	1/2	1/1	1/2	2/2	N	1/1	1/2	2/2	N	Case	Control	*P* value	OR (95%CI)
1st population													
rs4359426	C/A	706	191	12	909	736	269	16	1021	0.118	0.147	0.0072	0.77(0.64–0.93)
rs170360	T/C	695	199	12	906	734	269	20	1023	0.123	0.151	0.011	0.78(0.65–0.95)
rs223823	T/C	728	170	11	909	765	252	10	1027	0.106	0.132	0.0093	0.77(0.63–0.94)
2nd population													
rs4359426	C/A	815	202	12	1029	722	249	22	993	0.110	0.148	0.00037	0.71(0.59–0.86)
rs170360	T/C	792	220	19	1031	728	238	26	992	0.125	0.146	0.055	0.84(0.70–1.00)
rs223823	T/C	823	189	8	1020	780	193	19	992	0.100	0.116	0.11	0.85(0.70–1.04)
Combined													
rs4359426	C/A	1521	393	24	1938	1458	518	38	2014	0.118	0.147	0.0000096	0.74(0.65–0.85)
rs170360	T/C	1487	419	31	1937	1462	507	46	2015	0.123	0.151	0.0017	0.81(0.72–0.93)
rs223823	T/C	1551	359	19	1929	1545	445	29	2019	0.106	0.132	0.0030	0.81(0.70–0.93)

P values of the two populations were calculated by logistic regression analysis under an additive model.
The combined *P* values were calculated using the inverse variance method. OR, odds ratio; CI, confidence interval.
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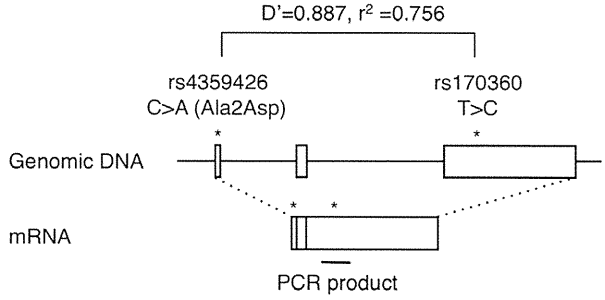
A



B

Haplotype	rs4359426 C>A	rs170360 T>C	Frequency
I	C	T	0.849
II	A	C	0.120
III	C	C	0.018
IV	A	T	0.013

C

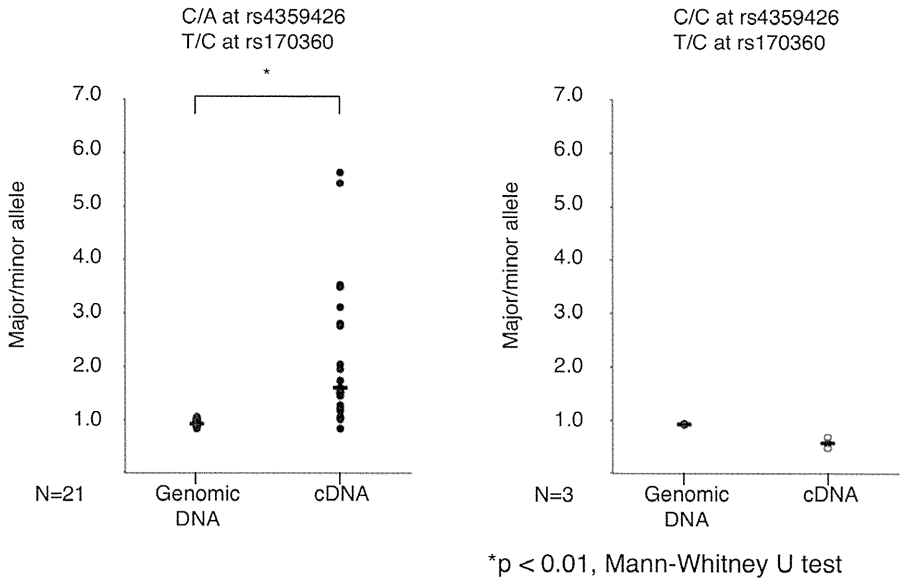


Figure 3. Allelic imbalance of gene expression of *CCL22* in EBV-transformed cells with heterozygous genotypes. (**A**) Genomic structures, locations and LD of the two SNPs. (**B**) Haplotypes for the two SNPs in the 1st population. (**C**) The allelic ratio of PCR products from individuals. Heterozygous (left) and homozygous (right) at rs4359426. *Two-tailed $P = 0.0000006$ by the Mann-Whitney U test.
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differences were not observed in cells from three subjects who were homozygous for the C allele at rs4359426 (Figure 3C, right panel). These results implied an effect of rs4359426 and/or variants in strong LD with rs4359426 on mRNA expression levels of *CCL22*. rs4359426 and rs170360 are in absolute LD in the HapMap Caucasian populations. We further examined the expression patterns of rs4359426 and rs170360 using Genevar 3.0.2 dataset, and confirmed that the expression patterns were similar to our findings (data not shown).

Transcription factor binding to the rs223821 SNP

As rs4359426 was in absolute LD with rs170359, rs223821 and rs72030112 (n = 94) (Figure 2), we further examined the allelic differences of these three SNPs in the binding of nuclear proteins by electrophoretic mobility shift assay (EMSA). We could not find any specific binding of nuclear factor(s) to oligonucleotides containing rs170359 and rs72030112. However, we observed that the signal intensity of the DNA-protein complex derived from the G allele of rs223821 was higher than that from the A allele in the presence of THP-1 nuclear extract stimulated with LPS (1 μg/ml) (Figure 4). We confirmed that the complex was diminished by an excess amount of a non-labeled allele-specific competitor probe (Figure 4). This result suggested that an unidentified nuclear factor(s) interacted with the genomic region at intron 2 of *CCL22* and the SNP might have an allele-specific effect on expression through varying affinity for a transcription factor.

Discussion

CCL22 plays an important role in the recruitment of Th2 cells into the inflammatory lesions of Th2-related diseases such as AD [14]. A recent study reported upregulation of *CCL17*, *CCL18* and *CCL22* expression in patients with AD, and suggested that the disease-specific chemokines might recruit specific memory T-cell subsets into the skin [15]. The plasma levels of CCL22 are

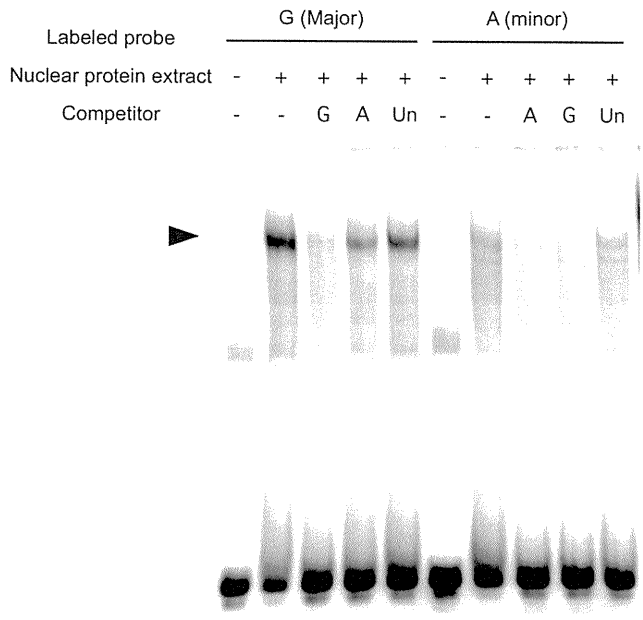

Figure 4. Electrophoretic mobility shift assays of rs223821. EMSA was performed using nuclear extracts from THP-1 cells stimulated with LPS (1.0 μg/ml) for 1 hour. DIG-labeled oligonucleotides corresponding to the G allele (lanes 1–5) and A allele (lanes 6–10) were used as probes. Three independent experiments were performed with similar results.
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significantly elevated in AD patients, and the values strongly correlate with disease severity [7,10]. We identified and replicated the rs4359426 (A2D) variant of *CCL22*, which was significantly associated with AD. rs4359426 is a non-synonymous SNP and causes an amino acid substitution in the signal peptide-encoding region. We examined the influence of the amino acid substitution on the structure using SIFT (Sorting Intolerant From Tolerant) software, and the substitution at position 2 from Ala to Asp was predicted to be tolerated. In addition, no possible impacts of the amino acid substitution on the structure and function of CCL22 were predicted by PolyPhen-2 (polymorphism phenotyping v2).

Functional analyses of the related variants of *CCL22* polymorphisms showed that the susceptible allele of rs4359426 might be involved in higher mRNA expression in ASTQ analysis. We confirmed that the expression patterns from Genevar 3.0.2 dataset were similar to our findings. We also demonstrated that the genomic fragment including the risk allele of rs223821 had much higher binding affinity to the nuclear factor(s). Although it is unclear whether higher mRNA expression is influenced by altering expression enhancer activity or mRNA stability, polymorphisms in the *CCL22* gene appear to be a genetic component of the pathologic mechanisms leading to atopic dermatitis, putatively via increased *CCL22* mRNA expression.

Genetic studies reveal underlying cellular pathways, and in some cases, point to new therapeutic approaches. A recent study using a humanized model of asthma showed a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation [16]. In the study, administration of a CCR4-blocking antibody abolished airway eosinophilia, goblet cell hyperplasia, IgE synthesis and bronchial hyperreactivity [16]. IL-13 is an important mediator of Th2 immune responses, and there many IL-13-positive cells in AD skin lesions [17]. A recent study has shown that IL-13 induces a significant increase in the expression of CCL22 in human keratinocytes, and blocking of CCL22 in IL-13-stimulated cells results in 70–90% inhibition in migration of CD4+CCR4+ T cells [18]. These findings suggest that targeting the CCL22/CCR4 pathway might be therapeutically efficacious as a new treatment for atopic dermatitis.

The involvement of CCL22 has been reported in several immune-mediated diseases. A recent study has shown by immunohistochemistry that CCL22 is not expressed in normal skin and is markedly expressed in the lesions of atopic dermatitis, allergic contact dermatitis, and psoriasis vulgaris [19]. Another report has shown that CCL22 is present within the synovial membrane in rheumatoid arthritis and osteoarthritis patients and in high amounts in the synovial fluid of patients with rheumatoid arthritis and psoriatic arthritis [20]. To examine whether the functional SNPs found in this study are associated with those diseases will be needed for understanding of the interconnectivity of the molecular mechanisms underlying distinct diseases.

In summary, we found a significant association between susceptibility to AD and polymorphisms affecting *CCL22* expression in Japanese populations. Our findings strongly support the important role of CCL22 in AD. Although the effect of the non-synonymous SNP on protein function remains unclear, it is likely that related variants play a role in susceptibility to AD in a gain-of-function manner. Further functional analyses and replication studies in other populations are needed; however, our findings provide insights into the pathophysiology of AD.

Materials and Methods

Subjects

A total of 1,950 case subjects with AD were recruited from several hospitals as described [21]. Case subjects in a second population were obtained from the BioBank Japan [22]. All case subjects were diagnosed according to the criteria of Hanifin and Rajka [23]. A total of 1,032 control volunteers in the first set who had no history of AD were recruited by detailed physicians' interviews. For the second set, a total of 1,004 controls who had never been diagnosed with AD were recruited during their annual health checkup in the University of Tsukuba (Table 2). All individuals were Japanese and gave written informed consent to participate in the study. This research project was approved by the ethics committees at the Institute of Medical Science, the University of Tokyo and the RIKEN Yokohama Institute.

Resequencing of the *CCL22* gene and genotyping

We first resequenced the *CCL22* region to identify genetic variations using DNA from 12 subjects with AD. We surveyed the gene from 3 kb of the 5′ flanking region to a 1 kb continuous 3′ flanking region of the last exon on the basis of genomic sequences from the NCBI database (NC_000016.9). The PCR product was reacted with BigDye Terminator v3.1 (Applied Biosystems), and sequences were assembled and polymorphisms identified using the SEQUENCHER program (Gene Codes Corporation, Ann Arbor, MI).

Genotyping of the seven SNPs in *CCL22* was performed by the TaqMan™ allele-specific amplification (TaqMan-ASA) method (Applied Biosystems) and multiplex-PCR based Invader assay (Third Wave Technologies).

Allele-specific transcript quantification (ASTQ)

We conducted allelic expression analyses by TaqMan assay using SNP genotyping probes as described [24]. EBV-transformed lymphoblastoid cells were obtained from the Health Science Research Resources Bank of Japan. Genomic DNA was used as a

control for equal biallelic representation. The allelic ratio for each cDNA and genomic DNA was measured.

Electrophoresis Mobility Shift Assay

EMSA was performed using nuclear extracts from THP-1 cells stimulated with LPS (1.0 μg/ml) for 1 hour. DIG-labeled oligonucleotides corresponding to the G allele (lanes 1–5) and A allele (lanes 6–10) were used as probes. The oligonucleotide sequences were 5′-ATCGCCTGAACCCGGGAGTTGGAG-GTT for the G allele and 5′-ATCGCCTGAACCCAG-GAGTTGGAGGTT for the A allele. For competition, a 100-fold excess of unlabeled G or A allele oligonucleotides or unrelated oligonucleotides (Un) (TFIID) was used.

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test. We then compared differences in the allele frequencies between case and control subjects by logistic regression analysis under an additive model and calculated odds ratios (ORs) with 95% confidence intervals (CIs). Results for the 1st and 2nd populations were combined by fixed effect inverse-variance method using Genome-Wide Association Meta Analysis (GWAMA, http://www.well.ox.ac.uk/gwama/tutorial.shtml) [25]. We applied Bonferroni corrections, the multiplication of P values by the number of variants investigated. Corrected P values of less than 0.05 were judged to be significant. The expression patterns of SNPs were obtained from Genevar (GENe Expression VARiation) 3.0.2 (Wellcome Trust Sanger Institute). We examined the influence of amino acid substitution on the structure using SIFT software (http://sift.jcvi.org/) and PolyPhen-2 (polymorphism phenotyping v2) (http://genetics.bwh.harvard.edu/pph2/).

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Author Contributions

Conceived and designed the experiments: TH MT. Performed the experiments: TH KT STanaka HM TSasaki STakeuchi. Analyzed the data: TH. Contributed reagents/materials/analysis tools: HS KE MS TY SF AM SD TE NH TSakamoto HM TSasaki TE MA HE STakeuchi MF EN YN MK. Wrote the paper: MT. Supervised the study: NK YN.

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Letter to the Editor

Significant correlation of serum IL-22 levels with *CCL17* levels in atopic dermatitis

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic eczematous skin disease, and Th1 and Th2 cytokines may differentially contribute to the pathogenesis of acute and/or chronic lesions of AD. The majority of allergen-specific T cells derived from skin lesions that had been provoked by the epicutaneous application of inhalant allergens were found to produce predominantly Th2 cytokines, which was initially considered to be a specific feature reflecting immune dysregulation in AD. Among various markers for AD including *CCL20* (macrophage inflammatory protein-3α) [1] and *CCL27* (cutaneous T cell-attracting chemokine) [2], serum CCL17 (thymus activation-regulated chemokine) level has been recognized to be a very sensitive and reliable parameter of disease severity of AD [2].

IL-22, a member of the IL-10 family, is known to be preferentially produced by Th17 cells [3,4]. IL-22 receptor is highly expressed on keratinocytes as well as other epithelial cells, inducing the production of antimicrobial proteins and keratinocyte proliferation [3]. Although IL-17 and IL-22 seem to be involved in the pathogenesis of psoriasis [3], attention has recently been drawn to a pathogenetic role of IL-22-producing T cells in AD [4,5]. There is a compelling evidence that IL-22-producing T cells, Th22 cells, distinct from Th17 and Th1 cells indeed exist and that the Th22 population is actually compartmentalized in lesional skin of AD with a reduction of Th17 cells [4,5]. It is intriguing that Th22 cells express the chemokine receptors CCR4, CCR6 and CCR10 targeted by *CCL17*, *CCL20* and *CCL27* [4]. In this study, we investigated the correlation of serum levels of these four chemo-cytokines in AD patients.

Sera were collected from 15 AD patients (mean ± SD age; 27.2 ± 6.1 years old) and 20 healthy volunteers (32.3 ± 6.5 years old) after written informed consent obtained. AD was diagnosed according to the Japanese Dermatological Association criteria [6]. The subjects received no systemic immunosuppressive drugs or corticosteroids. The experiment was approved by the Ethical Committee of Kyushu University. Quantification of the concentrations of IL-22, *CCL17*, *CCL20* and *CCL27* in sera was performed using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. Statistical analyses were performed using the 2-tailed Mann–Whitney *U* test for comparison between AD patients and normal controls and linear regression for ascertainment the correlation of serum levels of these markers. A *P*-value < 0.05 was considered significant.

The serum levels of IL-22 and *CCL17*, but not *CCL20*, were significantly higher in AD patients than those of normal controls (Fig. 1A). The serum *CCL27* levels tended to be elevated in AD, though they were not statistically significant compared with those in normal controls (Fig. 1A). As shown in correlation analysis, the serum levels of *CCL17* were significantly correlated with either those of IL-22 and *CCL27*, but not with *CCL20* (Fig. 1B). In addition, the serum IL-22 levels were significantly correlated with the serum *CCL27* levels ($r = 0.520$, $P = 0.039$). No significant association was observed either between the serum IL-22 and *CCL20* levels ($r = 0.446$, $P = 0.083$) or between the serum *CCL20* and *CCL27* levels ($r = 0.090$, $P = 0.748$).

In this study, serum IL-22 levels were elevated in AD patients compared with normal controls and were significantly associated with *CCL17* levels, suggesting that IL-22 could be considered as a activity marker for AD despite the narrow range of net values (10.2–53.4 pg/ml in this study) which was in sharp contrast to the wide range of net values (221.5–2144.0 pg/ml) of *CCL17* [2,5]. In the skin, IL-22 mediates keratinocyte proliferation and epidermal hyperplasia by downmodulating terminal differentiation genes, which highlighted a pathogenetic role of IL-22 in psoriasis [3]. Furthermore, patients with nickel contact dermatitis [7] and pityriasis rosea [8] showed elevated serum IL-22 levels. As for AD, the frequency of IL-22$^+$CD8$^+$ T cells in lesional skin of AD was correlated with the SCORing Atopic Dermatitis index [5], which supported our results. From the present and previous studies, IL-22 seems to play an important role in various inflammatory skin diseases including AD.

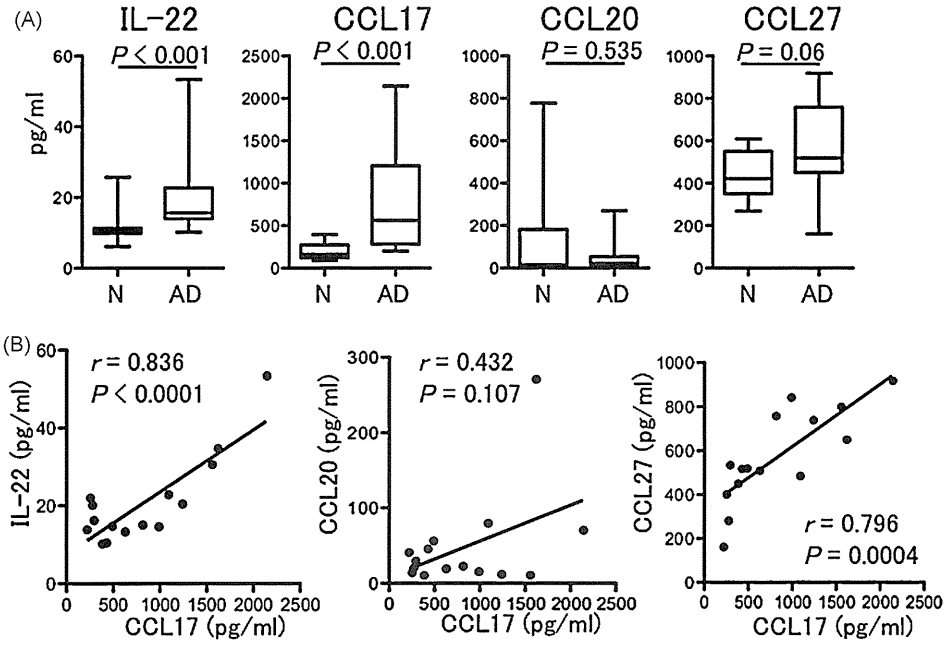

Fig. 1. (A) Serum concentrations of IL-22, *CCL17*, *CCL20* and *CCL27* in subjects using ELISA kits. The sera from normal controls (*N*; $n = 20$) and AD patients ($n = 15$) for measurement were collected. Boxes indicate 25–75% values, lines within boxes indicate medians, whiskers represent minimal to maximal of the data. *P*-values are determined by the Mann–Whitney *U* test. (B) Linear regression showing correlations of the serum levels of *CCL17* with those of IL-22, *CCL20* or *CCL27* in AD patients.

CCL27 is a skin-associated chemokine that attracts skin-homing memory T cells produced mainly by activated keratinocytes in various skin diseases [2]. Similar to *CCL17*, *CCL27* preferentially attracts cutaneous lymphocyte antigen-positive Th2 cells from peripheral blood and the serum levels of *CCL27* have been shown to correlate with severity of AD [2]. In our study, the levels of *CCL27* in AD also significantly correlated with *CCL17* levels, although the elevation of serum *CCL27* did not reach to a statistical significance, which may be attributable to small sample size in our study. The significant correlation of IL-22 with *CCL27* levels found in the present study further supports the mutual positive interaction among *CCL17*, IL-22 and *CCL27* in the development of AD.

CCL20 is a ligand for CCR6 that is constitutively expressed in normal skin and mucosa at low levels, but is strongly over-expressed in keratinocytes by proinflammatory cytokines and T cell-derived factors. Th17 cytokines including IL-17, IL-22 and TNF-α stimulate *CCL20* expression in keratinocytes in vivo and vitro [9]. Elevated serum levels and up-regulation of *CCL20* in lesional epidermis were shown in AD [1], however, Kim et al. demonstrated opposing evidence that AD skin was deficient in *CCL20* partly because of the overwhelming Th2-skewed milieu [10]. We also could not detect either the elevation of serum level of *CCL20* or its correlations with *CCL17*, IL-22 or *CCL27* level.

In conclusion, we have demonstrated for the first time that the elevated levels of IL-22 were significantly correlated with *CCL17* levels in AD. IL-22 could be a potential therapeutic target for the treatment of AD.

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Letter to the Editor

Histamine modulates the responsiveness of keratinocytes to IL-17 and TNF-α through the H1-receptor

Interleukin (IL)-17-producing $CD4^+$ helper T cells, Th17 cells, are involved in protection against bacterial pathogens and in the pathogenesis of various cutaneous inflammatory diseases, such as psoriasis and contact hypersensitivity [1]. In addition, a higher percentage of Th17 cells has been detected in the lesional skin and in the peripheral blood in the acute exacerbation phase of atopic dermatitis (AD), compared to normal controls [2,3]. It has been thought that IL-17 produced by Th17 cells infiltrating into the dermis acts on keratinocytes to produce inflammatory mediators, such as IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) to chemoattract neutrophils and T cells and to activate Langerhans cells and endothelial cells, respectively [2,4], which initiates and enhances cutaneous inflammations.

Initiation of cutaneous inflammation correlates with rapid upregulation of pro-inflammatory mediators such as IL-1α, IL-1β, and tumor necrosis factor (TNF)-α. Recently, TNF-α has been paid special attention, since neutralizing anti-TNF-α therapy is effective in the treatment of a wide variety of diseases, including psoriasis. Other mediators, such as histamine, are thought to play important roles in AD and even in psoriasis, since mast cells are activated early in the developing psoriatic lesion and later increase in number in the upper dermis with concomitant expression of cytokines, histamine, and TNF-α [5]. However, the impact of mediators such as TNF-α and histamine on IL-17-induced inflammatory mediator production remains unclear. In this study,

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T cell-specific overexpression of interleukin-27 receptor α subunit (WSX-1) prevents spontaneous skin inflammation in MRL/*lpr* mice

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Summary

Background Interleukin (IL)-27 and WSX-1, the receptor α-specific subunit, have been shown to play important roles in initiating Th1 responses and in inducing immune modulation, and the immunosuppressive effect of IL-27 appears to be exerted via suppression of IL-10 and IL-17, which may participate in the pathogenesis of human systemic lupus erythematosus (SLE).

Objectives To examine the significance of IL-27/WSX-1 signalling in spontaneous skin inflammation of MRL/lpr mice, a model for SLE.

Methods The severity and development of skin lesions, dermal inflammatory cells and epidermal–dermal depositions in the skin lesions of MRL/lpr mice with CD2-promoted *WSX-1* overexpression (WSX-1 Tg mice) and those with globally disrupted *WSX-1* (WSX-1 KO mice) were examined and compared with those of MRL/lpr mice.

Results By 4 months of age, both WSX-1 KO mice and control MRL/lpr mice developed predominantly similar skin inflammation, while WSX-1 Tg mice hardly did so, demonstrating that intensifying IL-27/WSX-1 signalling on T cells prevents the spontaneous skin inflammation. WSX-1 KO mice showed Th2-type skin inflammation as evidenced by the Th2-prone dermal infiltrating cells and an absence of cutaneous Th1-type IgG deposition. Interestingly, there were significant IL-17+ dermal infiltrating cells in both WSX-1 KO and control MRL/lpr mice, which might potentially contribute to the formation of skin inflammation in these mice.

Conclusions These data indicate that IL-27/WSX-1 signalling may play a protective role in the development of SLE-like skin inflammation, and modulating IL-27/WSX-1 signalling might be an interesting therapeutic strategy in the treatment of SLE.

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Conflicts of interest
None declared.

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that shows a wide range of manifestations, such as glomerulonephritis, arthritis and skin inflammation.[1] These types of tissue damage are presumably caused by immune complex deposition in the respective tissues and organs. Indeed, affected glomeruli in kidney as well as epidermal–dermal junctions in skin lesions[2] contain immunoglobulin depositions. The large majority of patients with SLE express some forms of cutaneous lupus erythematosus (LE) with a variety of clinical expressions during their disease courses. Among several murine models for SLE, MRL/lpr mice have the unique characteristic that skin inflammation occurs spontaneously at a high frequency as well as other characteristic symptoms of SLE, such as glomerulonephritis, arthritis and anti-DNA antibodies.[3] The skin lesion in these mice is the best characterized LE-like skin model and is thought to be a pertinent model of cutaneous LE, because the skin lesion is characterized by the liquefaction changes in basal keratinocytes, dermal T-cell infiltration and IgG depositions at the epidermal–dermal junction, which are similar to those characteristi-

cally seen in cutaneous lesions of human LE. Moreover, exposure to ultraviolet B radiation, which is an important exacerbating factor of human SLE, also accelerates the development of LE-like skin inflammation and cutaneous immune deposition in the SLE model mice.[4,5]

Interleukin (IL)-27 is a member of the IL-12 family, which shows both proinflammatory and anti-inflammatory properties.[6] WSX-1 is an IL-27 receptor subunit which confers the ligand specificity,[7] and the receptor is expressed on various leucocytes such as T cells, B cells, natural killer cells, mast cells, dendritic cells, macrophages and neutrophils.[8] Although IL-27/WSX-1 signalling was initially shown to be critical in inducing Th1 responses,[7,9] recent studies have also elucidated its immunosuppressive roles.[6,8] Indeed, our previous study has demonstrated that disruption of *WSX-1* gene induces augmentation of Th2-type autoimmune responses and changes the pathophysiology of autoimmune nephritis which develops in MRL/*lpr* mice,[10] and that CD2-promoted *WSX-1* overexpression in MRL/*lpr* mice renders the autoimmune-prone mice protected from the development of autoimmune diseases,[11] indicating that IL-27/WSX-1 signalling plays a protective role in the development of glomerulonephritis in MRL/*lpr* mice.

In this study, we investigated the significance of IL-27/WSX-1 signalling in the development of skin inflammation in MRL/*lpr* mice using the SLE model mice with globally disrupted or T-cell specifically overexpressed *WSX-1* gene.

Materials and methods

Mice

MRL/*lpr* mice with globally disrupted *WSX-1* were termed WSX-1 knockout (KO) mice and MRL/*lpr* mice with CD2-promoted *WSX-1* overexpression were termed WSX-1 transgenic (Tg) mice. *WSX-1* KO-heterozygous littermate mice were used as control MRL/*lpr* mice. The generation of WSX-1 KO and WSX-1 Tg MRL/*lpr* mice has been described previously.[10,11] Mice were maintained in the Laboratory of Animal Experiments of Kyushu University under specific pathogen-free conditions. All experiments conformed to the animal care guidelines of the American Physiologic Society and had been approved by the institutional animal research committee of Kyushu University.

Assessment of morbidity rate and degree of skin inflammation

In time course analysis, the rate of skin inflammation-free mice was plotted in a Kaplan–Meier curve to express the percentage of skin inflammation-free mice. Average involved skin area was determined monthly in each mouse as follows: 0, no lesion; 1, only facial lesion; 2, facial lesion + back skin lesion (< 1 cm in longitudinal diameter); 3, facial lesion + back skin lesion (> 1 cm in longitudinal diameter). The severity of skin inflammation was determined by the sum of the involved skin area score and the scorings for each eruption item: erythema/haemorrhage, erosion/excoriation, oedema and scaling/dryness. The degree of each eruption item was clinically assessed as follows: 0, no symptoms; 1, light; 2, mild; 3, severe, as previously described by others.[12]

Histopathological analysis

Skin samples from mice were fixed in 4% paraformaldehyde and were embedded in paraffin. Sections of 3 μm thickness were stained with conventional haematoxylin and eosin, or with toluidine blue for the detection of mast cells. Skin sections were also stained with anti-CD4, anti-CD8, anti-IL-17 (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), anti-IL-4 and anti-interferon (IFN)-γ (BioLegend, San Diego, CA, U.S.A.) antibodies using the peroxidase–diaminobenzidine method and were counterstained with haematoxylin. The mean number of total infiltrating cells, mast cells or other positively stained cells in inflamed dermis was counted in three randomly selected fields at a magnification of × 400.

Immunofluorescent analysis

Some skin samples were immediately frozen and stored at −80 °C. Frozen sections of 6 μm thickness were fixed in acetone. Direct detection of immunoglobulin deposition at the epidermal–dermal junction was conducted using fluorescein isothiocyanate-labelled anti-IgE (Bethyl Laboratories, Montgomery, TX, U.S.A.), anti-total IgG (Chemicon International, Inc., Temecula, CA, U.S.A.), anti-IgG1 or anti-IgG2a antibodies (Southern Biotechnology, Birmingham, AL, U.S.A.).

Statistical analysis

Results are shown as mean ± SD. Statistical analysis of the data was performed using Student's t-test, Bonferroni multiple comparison test or Kaplan–Meier method using MS EXCEL 2003. $P < 0.05$ was considered statistically significant.

Results

T cell-specific *WSX-1* overexpression prevents MRL/*lpr* mice from developing skin inflammation, while global disruption of *WSX-1* rather aggravates it

WSX-1 KO and control MRL/*lpr* mice developed clinically similar-appearing spontaneous skin inflammation characterized by erythema, crust formation, erosion, alopecia and lichenification on the dorsal, periocular, nasal and ear regions (Fig. 1a). Most WSX-1 KO and control MRL/*lpr* mice began to develop spontaneous skin inflammation at around 3 months of age, gradually expanding skin lesions from the face to the upper back, while only one of 10 WSX-1 Tg mice developed clinically apparent skin inflammation only on

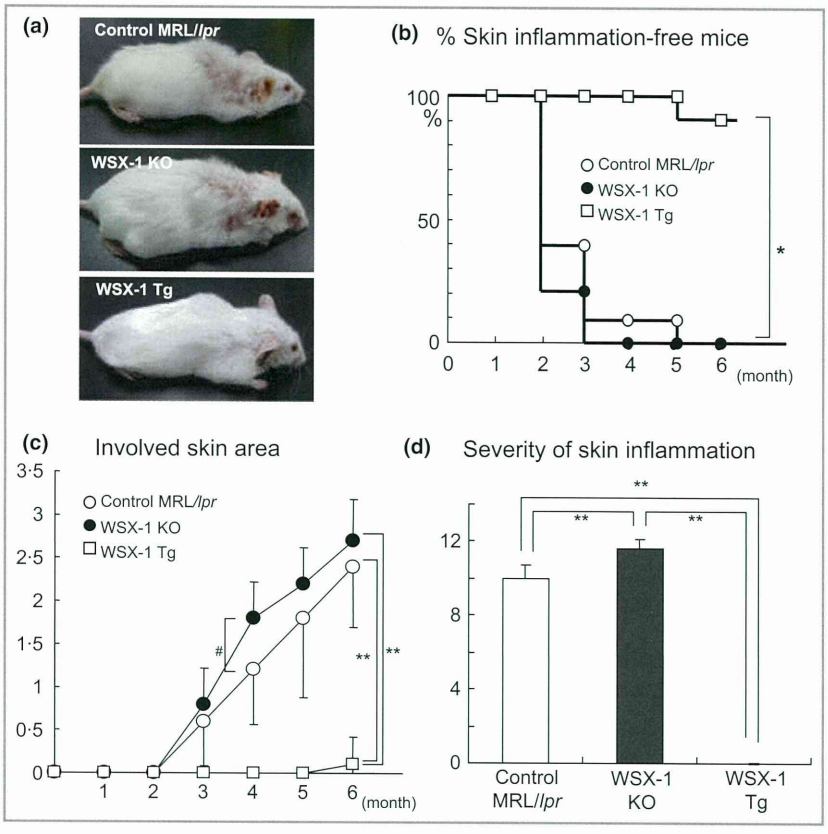

Fig 1. Prevention of skin inflammation in MRL/lpr mice by T cell-specific *WSX-1* overexpression. (a) Typical clinical manifestations of mice at 6 months of age. (b) Mice of the three genotypes were monitored for the development of skin inflammation. (c) Involved skin areas were assessed monthly. (d) The severity of skin inflammation was determined by sum of the involved skin area score and the scorings of each eruption item: erythema/haemorrhage, erosion/excoriation, oedema and scaling/dryness. Values are expressed as mean ± SD of 10 mice. *P < 0·05, **P < 0·01, #P < 0·05.

the face at 6 months of age (Fig. 1b). The involved skin area of both WSX-1 KO mice and control MRL/lpr mice was significantly higher than in WSX-1 Tg mice at 3–6 months of age, and the affected area of WSX-1 KO mice was significantly higher than that of control MRL/lpr mice at 4 months of age (Fig. 1c). The severity of skin inflammation in both WSX-1 KO and control MRL/lpr mice, as defined by the sum of involved skin area and scorings of each eruption item, was significantly higher than that in WSX-1 Tg mice, and the severity score in WSX-1 KO mice was also significantly higher than that in control MRL/lpr mice at 6 months of age (Fig. 1d).

There are significant inflammatory infiltrates and dermal mast cells in WSX-1 KO and control MRL/*lpr* mice, as compared with WSX-1 Tg mice

Histologically, epidermal hyperplasia and a considerable dermal infiltration of mononuclear cells were observed in both WSX-1 KO mice and control MRL/lpr mice, while there was little such infiltration in WSX-1 Tg mice (Fig. 2a, b). Toluidine blue staining revealed that dermal mast cells were significantly increased in WSX-1 KO mice and control MRL/lpr mice, as compared with WSX-1 Tg mice (Fig. 2c, d). The degree of infiltrating dermal eosinophils did not differ among the mouse strains (Figure S1; see Supporting Information). There were many IgE-positive cells in the dermis of WSX-1 KO mice, but not in control MRL/lpr mice (Fig. 2e).

There is significant dermal infiltration of interleukin-17+ cells in both WSX-1 KO mice and control MRL/*lpr* mice regardless of the Th1/Th2 balance

The number of dermal infiltrating CD4+ T cells in control MRL/lpr mice was significantly higher than in WSX-1 Tg mice (Fig. 3a, left). There were also some infiltrating CD4+ T cells in WSX-1 KO mice, but the number was not significantly higher than in WSX-1 Tg mice. The numbers of dermal CD8+ T cells in WSX-1 KO and in control MRL/lpr mice were equally and significantly higher than in WSX-1 Tg mice (Fig. 3a, right). The number of dermal IFN-γ+ cells in control MRL/lpr mice was significantly higher than in WSX-1 Tg mice. Meanwhile, the numbers of both dermal IL-4+ cells and IL-17+ cells in WSX-1 KO and in control MRL/lpr mice were significantly higher than in WSX-1 Tg mice (Fig. 3b). Representative images with corresponding isotype control staining are shown in Figure S2 (see Supporting Information). The Th1/Th2 balance (IFN-γ+ cells/IL-4+ cells ratio) of dermal infiltrating cells in control MRL/lpr mice was significantly higher than in WSX-1 KO mice (Fig. 3c).

Th1-type IgG deposition is predominant in control MRL/*lpr* mice, but is absent in WSX-1 KO mice

The lupus band test (LBT) was conducted to examine IgG deposition in the epidermal–dermal junction using back skin

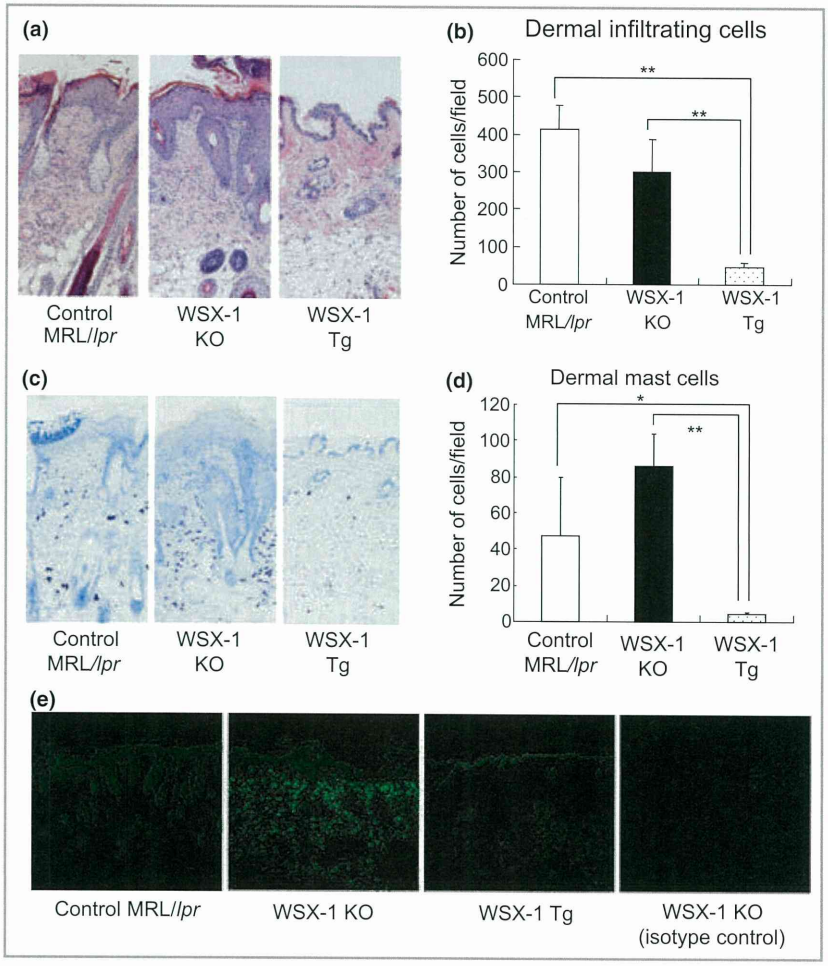

Fig 2. IgE-positive dermal infiltrates are observed in WSX-1 KO mice, but not in the control MRL/lpr mice. (a, b) The numbers of total infiltrating cells in the affected skin of mice were counted (haematoxylin and eosin). (c, d) Mast cells detected by toluidine blue in the affected skin of mice were counted. (e) Frozen skin sections were stained with fluorescein isothiocyanate-labelled anti-IgE (original magnification × 200). Values are expressed as mean ± SD of four or five mice. *P < 0·05, **P < 0·01.

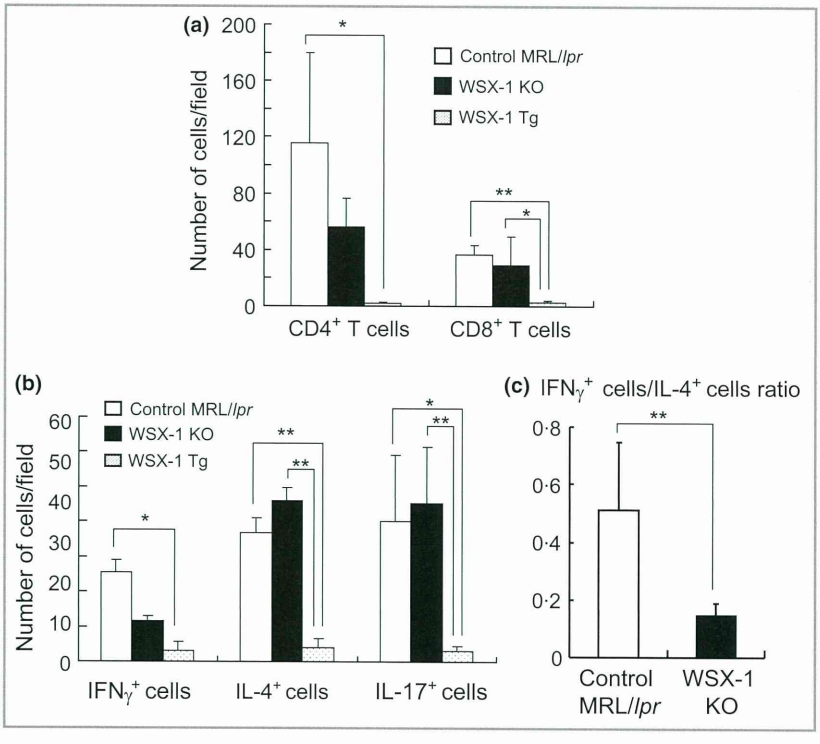

Fig 3. Disruption of *WSX-1* gene induces Th2-type infiltration in MRL/lpr mice. (a, b) Skin sections were stained with anti-CD4, anti-CD8, anti-interleukin (IL)-4, anti-interferon (IFN)-γ or anti-IL-17antibodies and the mean number of positively stained cells was computed. (c) The Th1/Th2 ratio (IFN-γ+ cells/IL-4+ cells) was calculated. Values are expressed as mean ± SD of four or five mice. *P < 0·05, **P < 0·01.

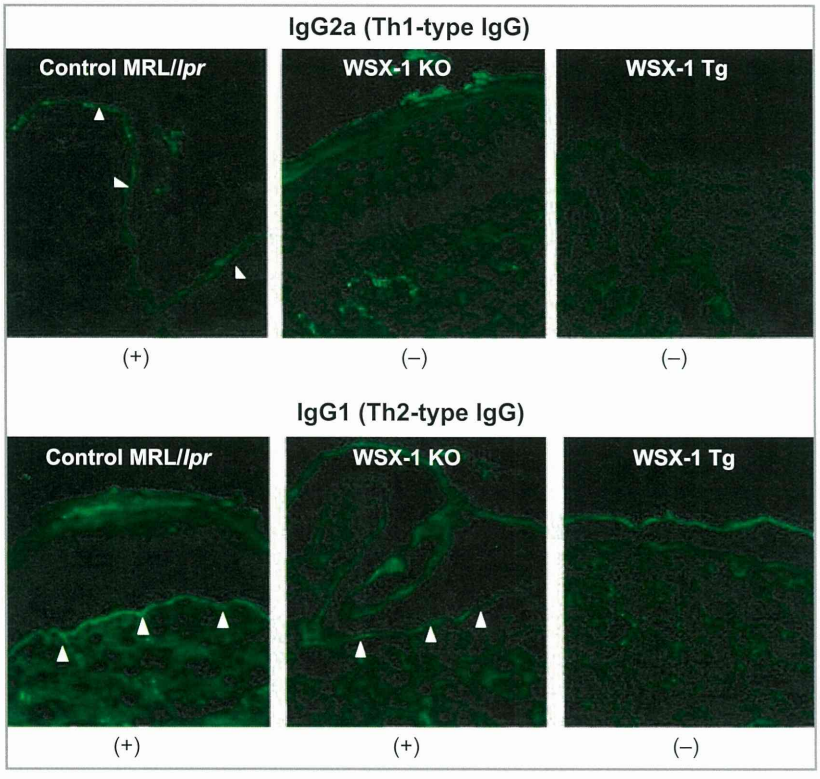

Fig 4. The differential deposition of various IgG subclasses. Representative data of lupus band test in each strain are shown. White arrowheads indicate immunoglobulin deposition.

(Fig. 4). LBT was positive in all the control MRL/lpr mice and in four of five WSX-1 KO mice, while it was negative in all the WSX-1 Tg mice examined (Table 1). Deposition of IgG2a (Th1-type IgG) was positive in four of five control MRL/lpr mice but was absent in WSX-1 KO mice, while deposition of IgG1 (Th2-type IgG) was positive in two of five control MRL/lpr mice and in three of five WSX-1 KO mice (Table 1). No deposition of either IgG2a or IgG1 was observed in WSX-1 Tg mice (Table 1).

Discussion

In this study, we assessed the influence of global cell disruption and T cell-specific overexpression of the IL-27 receptor (WSX-1) gene on spontaneous skin inflammation in MRL/lpr mice and clearly demonstrated a protective role of IL-27/WSX-1 signalling in the development of skin inflammation. T cell-specific overexpression of *WSX-1* by means of CD2 promoter resulted in protecting MRL/lpr mice from developing spontaneous skin inflammation, suppressing IL-17+, IL-4+ and IFN-γ+ cell infiltration and cutaneous IgG deposition. These results suggest that intensifying IL-27/WSX-1 signalling on T cells may be sufficient for preventing spontaneous skin inflammation in MRL/lpr mice. SLE has been classically considered as an autoantibody/immune complex-driven disease induced by a Th1/Th2 imbalance in cytokine production. However, recent reports also indicate that IL-17 may participate in the pathogenesis of SLE, augmenting both T-cell and B-cell activation,[13] and that IL-10 may contribute to B-lymphocyte hyperactivity in SLE because of its potential induction of B-cell differentiation.[14] IL-27/WSX-1 signalling might be associated with the onset and/or development of SLE because a relative decrease in serum IL-27 level has been shown in human SLE[15] and the immunosuppressive effect of IL-27 appears to be exerted via suppression of IL-17 and IL-10.[6]

We demonstrated that abrogation of IL-27/WSX-1 signalling by disrupting *WSX-1* skewed the spontaneous skin inflammation from Th1- to Th2-type. Imbalance of the cytokines produced by Th1 and Th2 cells is thought to play an important role in the pathogenesis of SLE, particularly in patterns of glomerulonephritis.[16,17] In the skin lesions of SLE it remains controversial whether Th1- or Th2-type cytokines contribute more to the pathogenesis,[18,19] but at least deposition of Th1-type IgG appears evident in human SLE.[20,21] Such Th1-type IgG deposition is even more evident in the skin lesions of patients with subacute cutaneous LE, a major cutaneous subtype of human LE.[20,22]

Table 1 Lupus band test in control MRL/lpr, WSX-1 KO and WSX-1 Tg mice

	Control MRL/lpr	WSX-1 KO	WSX-1 Tg
Total IgG	5/5	4/5	0/4
IgG2a (Th1-type IgG)	4/5	0/5	0/4
IgG1 (Th2-type IgG)	2/5	3/5	0/4

These data are expressed as the ratio of lupus band test-positive mice/investigated mice.

With IL-27/WSX-1 signalling globally disrupted, however, MRL/lpr mice showed even more severe skin inflammation than did control MRL/lpr mice. Note that the degree of total inflammatory infiltrates, IL-4+ cells and IL-17+ cells in the affected dermis was apparently unchanged between WSX-1 KO mice and control MRL/lpr mice, and the degree of IFN-γ+ cells and CD4+ T cells in WSX-1 KO mice tended to be even lower than in control MRL/lpr mice. Then what type of cells is responsible for the clinically more severe skin disease in the KO mice? A possible answer would be the combination of a relatively higher number of mast cells and their massive association with IgE in the dermis of WSX-1 KO mice (Fig. 2d, e) which was not observed in control MRL/lpr mice. It has been reported that WSX-1 KO mice show high serum IgE level.[10] By efficient linkage with such abundant IgE on the cell surface, mast cells must be highly activated and discharge numerous stored factors such as histamine, leucotrienes, prostanoids, proteases and cytokines, all of which could considerably aggravate skin inflammation.[23,24]

In conclusion, we found that enhanced IL-27/WSX-1 signalling on T cells could prevent MRL/lpr mice from developing spontaneous skin inflammation and that global cell disruption of IL-27/WSX-1 signalling resulted in skewing the skin inflammation towards a Th2 pattern, even more aggravating the affected skin. These findings indicate that IL-27/WSX-1 signalling may play a protective role in the development of SLE-like skin inflammation, and modulating IL-27/WSX-1 signalling may possibly be of interest in the treatment of SLE.

What's already known about this topic?

- Interleukin (IL)-27 has both proinflammatory and anti-inflammatory properties. WSX-1 is an IL-27 receptor subunit which confers the ligand specificity. The immunosuppressive effect of IL-27 may be exerted via suppression of IL-10 and IL-17, which may participate in the pathogenesis of systemic lupus erythematosus (SLE). IL-27/WSX-1 signalling was recently shown to play a protective role in the development of glomerulonephritis in MRL/lpr mice, a model for SLE.

What does this study add?

- We demonstrated that abrogation of IL-27/WSX-1 signalling by disrupting *WSX-1* skewed the spontaneous skin inflammation from Th1- to Th2-type and that overexpression of *WSX-1* on T cells resulted in protecting MRL/lpr mice from developing spontaneous skin inflammation. These data indicate that IL-27/WSX-1 signalling may play a protective role in the development of SLE-like skin inflammation, and modulating IL-27/WSX-1 signalling might be an interesting therapeutic strategy in the treatment of SLE.

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Supporting Information

Additional Supporting Information may be found in the online version of the article:

Fig S1. The degree of infiltrating dermal eosinophils was not different among mouse strains. Dermal eosinophils were detected with Sirius Red stain. (a) Infiltrating eosinophils (arrowhead) in the dermis of control MRL/lpr mice. (b) There is no significant difference in the numbers of eosinophils among mouse groups. Values are expressed as mean ± SD.

Fig S2. Staining with anti-CD4, anti-CD8, anti-interleukin (IL)-4, anti-interferon (IFN)-γ and anti-IL-17 antibodies in inflamed dermis. Representative images of each staining with corresponding isotype control staining are shown.

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Current status of atopic dermatitis in Japan

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Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic, eczematous skin disease. AD is the second most frequently observed skin disease in dermatology clinics in Japan. Prevalence of childhood AD is 12-13% in mainland Japan; however, it is only half that (about 6%) in children from Ishigaki Island, Okinawa. Topical steroids and tacrolimus are the mainstay of treatment. However, the adverse effects and emotional fear of long-term use of topical steroids have induced a "topical steroid phobia" in patients throughout the world. Undertreatment can exacerbate facial/periocular lesions and lead to the development of atopic cataract and retinal detachment due to repeated scratching/rubbing/patting. Overcoming topical steroid phobia is a key issue for the successful treatment of AD through education, understanding and cooperation of patients and their guardians.

Key words: Atopic dermatitis; History; Prevalence; Topical steroids; Dose

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic, eczematous skin disease. The incidence of AD is generally considered to be increasing worldwide [1, 2]. The percentage of adolescent- and adult-type AD has also been increasing [3, 4]. The etiology and pathogenesis of AD are not fully delineated. Recent studies demonstrate that it involves a complex interaction of skin barrier dysfunction, exposure to external allergens or microbes, Th2-prone response and psychosomatic reaction. Intense itching, skin inflammation, and other atopic symptoms impose a remarkable burden on the individual and society.

The history of AD is very old. Prior to the nomenclature of "atopic dermatitis" by Sulzberger et al. [5] in 1933, the disease was reported under various dermatological terms such as neurodermite diffusa (Brocq), lichen chronicus simplex disseminatus (Vidal), pruritus with lichenification, allergic eczema, hay-fever eczema and flexural eczema; each one highlighted important clinical aspects of this disease. Helmont (1607) and Trousseau (1850) pointed out a close association between asthma and certain types of pruritic skin disorder, and Vidal (1880) described a characteristic distribution of itchy lichenified plaques in this disease [5, 6]. Brocq and Jacquet (1891) referred to the disease as neurodermite diffusa, emphasizing its psychosomatic nature; later on, the disease was commonly reported in literature under the term neurodermatitis (neurodermitis) diffusa or disseminate, neurodermatitis constitutionalis, and disseminated neurodermatitis [7]. In 1892, Besnier named the disease "diathésique eczémato-lichénienne", mentioning that

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