Tocilizumab has been approved as a biological drug for the treatment of rheumatoid arthritis, systemic and polyarticular juvenile idiopathic arthritis, and Castleman's disease [12]. Recent case reports and pilot studies have reported the efficacy of tocilizumab for the treatment of various other autoimmune and inflammatory diseases [20]. The ameliorative effect of tocilizumab in our patient raises the possibility that BD could be another target disease for tocilizumab. The mechanism by which IL-6 blockade can lead to the suppression of disease activity in a patient with refractory BD remains unknown. However, it is known that tocilizumab inhibits the proinflammatory activities of IL-6 and also affects the function of effector T cells. One study found that the serum IL-17 level was higher in active BD than that in remission, which suggests a role for Th17 cells in the development of BD [7]. It has been reported that IL-6, together with transforming growth factor β (TGF-β), induces the differentiation of naïve T cells into Th17 cells, while IL-6 inhibits TGF-β-induced regulatory T cell (Treg) differentiation [21]. Dysregulation of IL-6 production thus causes imbalance in the Th17/Treg ratio. In fact, it was demonstrated that the blockade of IL-6 signaling in a murine model of autoimmune uveoretinitis suppressed the severity of uveoretinitis through Th17 and/or Th1 inhibition or Treg induction [22–24]. Whether tocilizumab can affect effector T cell function in BD patients remains to be determined. In conclusion, IL-6 blockade may constitute an optional treatment strategy for refractory BD with uveitis, although further clinical studies are required to elucidate the efficacy and safety of tocilizumab for BD.

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Conflict of interest Tadamitsu Kishimoto holds a patent for tocilizumab and receives royalties for Actemra. Atsushi Ogata has received a consulting fee as medical adviser and speaking fees from Chugai Pharmaceutical Co., Ltd. Toshio Tanaka has received speaking fees from Chugai Pharmaceutical Co., Ltd. The other authors declare no conflicts of interest.

References

1. Mendes D, Correia M, Barbedo M, Vaio T, Mota M. Behçet's disease—a contemporary review. J Autoimmun. 2009;32:178–88.
2. Fresko I, Yazici H. Treatment strategies for Behçet's disease. Expert Opin Pharmacother. 2008;9:3211–9.
3. Hatemi G, Silman A, Bang D, Bodaghi B, Chamberlain AM, Gul A, et al. EULAR recommendations for the management of Behçet disease. Ann Rheum Dis. 2008;67:1656–62.
4. Ohno S, Nakamura S, Hori S, Shimakawa M, Kawashima H, Mochizuki M, et al. Efficacy, safety, and pharmacokinetics of multiple administration of infliximab in Behçet's disease with refractory uveoretinitis. J Rheumatol. 2004;31:1362–8.
5. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R/IL12RB2 regions associated with Behçet's disease. Nat Genet. 2010;42:698–702.
6. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, et al. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. Nat Genet. 2010;42:703–6.
7. Hamzaoui K, Hamzaoui A, Guemira F, Bessioud M, Hamza M, Ayed K. Cytokine profile in Behçet's disease patients. Relationship with disease activity. Scand J Rheumatol. 2002; 31:205–10.
8. Hirohata S, Oka H, Mizushima Y. Streptococcal-related antigens stimulate production of IL6 and interferon-gamma by T cells from patients with Behçet's disease. Cell Immunol. 1992; 140:410–9.
9. Mege JL, Dilsen N, Sanguedolce V, Gul A, Bongrand P, Roux H, et al. Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. J Rheumatol. 1993;20:1544–9.
10. Wang CR, Chuang CY, Chen CY. Anticardiolipin antibodies and interleukin-6 in cerebrospinal fluid and blood of Chinese patients with neuro-Behçet's syndrome. Clin Exp Rheumatol. 1992; 10:599–602.
11. Hirohata S, Isshi K, Oguchi H, Ohse T, Haraoka H, Takeuchi A, et al. Cerebrospinal fluid interleukin-6 in progressive neuro-Behçet's syndrome. Clin Immunol Immunopathol. 1997;82:12–7.
12. Tanaka T, Ogata A, Narazaki M. Tocilizumab for the treatment of rheumatoid arthritis. Expert Rev Clin Immunol. 2010; 6:843–54.
13. The classification criteria for Behçet's disease. Japanese Ministry of Health and Welfare. 1987.
14. Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ. Behçet's disease: evaluation of a new instrument to measure clinical activity. Rheumatology. 1999;38:728–33.
15. Fukuhara S, Bito S, Green J, Hsiao A, Kurokawa K. Translation, adaptation, and validation of the SF-36 Health Survey for use in Japan. J Clin Epidemiol. 1998;51:1037–44.
16. Sfikakis N, Markomichelakis N, Alpsoy E, Assaad-Khalil S, Bodaghi B, Gul A, et al. Anti-TNF therapy in the management of Behçet's disease—review and basis for recommendations. Rheumatology. 2007;46:736–41.
17. Kotter I, Zierhut M, Eckstein AK, Vonthein R, Ness T, Gunaydin I, et al. Human recombinant interferon alfa-2a for the treatment of Behçet's disease with sight threatening posterior or panuveitis. Br J Ophthalmol. 2003;87:423–31.
18. Ohguro N, Yamanaka E, Otori Y, Saishin Y, Tano Y. Repeated intravitreal triamcinolone injections in Behçet's disease that is resistant to conventional therapy: one-year results. Am J Ophthalmol. 2006;141:218–20.
19. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood. 2008; 112:3959–64.
20. Tanaka T, Narazaki M, Kishimoto T. Anti-interleukin-6 receptor antibody, tocilizumab, for the treatment of autoimmune diseases. FEBS Lett (in press).
21. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. Nature. 2006; 441:235–8.

22. Yoshimura T, Sonoda K, Ohguro N, Ohsugi Y, Ishibashi T, Cua DJ, et al. Involvement of Th17 cells and the effect of anti-IL-6 therapy in autoimmune uveitis. Rheumatology. 2009;48:347–54.
23. Hohki S, Ohguro N, Haruta H, Nakai K, Terabe F, Serada S, et al. Blockade of interleukin-6 signaling suppresses experimental autoimmune uveoretinitis by the inhibition of inflammatory Th17 responses. Exp Eye Res. 2010;91:162–70.
24. Haruta H, Ohguro N, Fujimoto M, Hohki S, Terabe F, Serada S, et al. Blockade of interleukin-6 signaling suppresses not only Th17 but also interphotoreceptor retinoid binding protein-specific Th1 by promoting regulatory T cells in experimental autoimmune uveoretinitis. Invest Opthalmol Vis Sci. 2011;52:3264–71.

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Toll-like receptor 2 (*TLR2*) gene polymorphisms are not associated with sarcoidosis in the Japanese population

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Purpose: Sarcoidosis is a systemic inflammatory disease characterized by the formation of non-caseating granulomas, with varied clinical manifestations. The common etiology of sarcoidosis is uncertain, but it is thought to be triggered by an exogenous antigenic stimulus, such as some bacterial proteins. Toll-like receptors (TLRs) recognize microbial components and elicit innate as well as adaptive immune responses. It has been reported that polymorphisms in *TLR2* might be important in a small group of Caucasian sarcoidosis patients. The present study aimed to establish whether these findings are relevant to the Japanese population.

Methods: We genotyped 5 single-nucleotide polymorphisms (SNPs) in *TLR2* and assessed the allelic diversity between 257 Japanese sarcoidosis patients and 193 Japanese healthy controls.

Results: No significant differences in the frequency of *TLR2* alleles and haplotypes in the sarcoidosis cases were found in comparison with the controls. However, marginal associations were observed for *TLR2* at rs3804099 and rs3804100 in sarcoidosis patients with cutaneous manifestations.

Conclusions: Our results suggest that *TLR2* polymorphisms are not significantly related to the pathogenesis of sarcoidosis in the Japanese population.

Sarcoidosis is a systemic inflammatory disorder resulting in non-caseating granulomas in multiple organs, such as: lung, skin, eye, lymph nodes, central and peripheral nervous system, and heart [1-3]. Japanese patients have a higher likelihood of ocular involvement compared with other ethnic groups [4]. Ocular manifestation is one of the most common presentation in Japanese sarcoidosis patients [5]. Granulomatous inflammation can occur in any layer of the eyeball, and leads to wide variety of ocular pathology, including uveitis. A survey of almost 3,000 Japanese patients diagnosed with uveitis found that sarcoidosis was the most frequent (13.3%) cause of non-idiopathic uveitis [6].

The exact cause of sarcoidosis is unknown, but the fact that the frequency and course of the disease varies widely among racial groups suggests that genetic factors may be the basis of disease susceptibility. African Americans are more commonly and severely affected by sarcoidosis than Caucasian Americans. The annual sarcoidosis incidence for African Americans is threefold higher, compared with Caucasian Americans; at 35.5 versus 10.9 cases per 100,000, respectively [7]. In the Swedish, another ethnic group, the annual incidence of sarcoidosis is also high [8]. In Japan, the annual estimated prevalence is 1.01 per 100,000 [5]. In Korea, the reported incidence rate is similarly low [9].

Environmental factors are also thought to contribute to the disease progression. The DNA of *Mycobacterium tuberculosis* and *Propionibacterium acnes* has been detected

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in some sarcoid lesions by using polymerase-chain-reaction (PCR) methods [10-13]. Recent studies have also shown that the serum of some sarcoidosis patients contains antibodies against mycobacterial antigens [14]. These studies suggest that bacterial infections can affect the development of sarcoidosis.

Toll-like receptors (TLRs) recognize microbial components and elicit innate as well as adaptive immune responses. Stimulation with TLR ligands induces the production of proinflammatory cytokines and type I interferons in cells of the innate immune system through intracellular signaling cascades [15-17]. Accumulating data suggest that *TLR* polymorphisms are closely associated with many autoimmune diseases [18-20]. Among the TLR family members, TLR2 recognizes multiple components of several bacterial cell walls, including peptidoglycans and lipoproteins from the cell wall of several bacteria and mycoplasma, by forming a heterodimer with either TLR1 or TLR6, and plays a critical role in the activation of innate immunity [21,22]. Polymorphisms in *TLR2* are associated with impaired responses to bacterial infection in human [23-27]. Recently, Veltkamp et al. [28] reported that they found the single nucleotide polymorphisms (SNPs) located in the *TLR2* promoter lesion (rs4696480) was associated with sarcoidosis in a Dutch Caucasian population, but could not confirm this in their validation cohort. They inferred from these findings that a *TLR2* variant could play a role in a small percentage of patients. The association between *TLR2* polymorphisms and sarcoidosis needs to be confirmed by further replication studies, particularly in other ethnic groups. In the present study, we therefore evaluated the association of multiple SNPs in *TLR2* in Japanese patients.

METHODS

Subjects: Two hundred fifty-seven unrelated patients with a diagnosis of sarcoidosis and 193 healthy controls were recruited from Yokohama City University, Hokkaido University, Fujita Health University, Tokyo University, Keio University, and Kumamoto City hospital. All patients and control participants were of Japanese ethnicity. Sarcoidosis patients were diagnosed according to the diagnostic criteria developed by the Japanese Society of Sarcoidosis and Other Granulomatous Disorders (JSSOG) previously described [29]. Uveitis with sarcoidosis was assessed based on the "Guidelines for Diagnosis of Ocular Lesions in Sarcoidosis" prepared by the JSSOG. The ocular features of sarcoidosis were defined as granulomatous uveitis plus two or more of the following: infiltration of the anterior chamber (mutton-fat keratic precipitates/iris nodules), trabecular meshwork nodules and/or tent-shaped peripheral anterior synechia, masses of vitreous opacities (snowball-like or string of pearls-like appearance), periphlebitis with perivascular nodules; multiple candle-wax type chorioretinal exudates and nodules, and/or laser photocoagulation spot-like chorioretinal atrophy. All subjects had a similar social background and resided in the same urban area. The research methods were in compliance with the guidelines of the Declaration of Helsinki. Details of the study were explained to all patients and controls, and valid consent for genetic screening was obtained.

Analysis of TLR2 polymorphisms: Peripheral blood lymphocytes were collected, and genomic DNA was extracted from peripheral blood cells using the QIAamp DNA Blood Maxi Kit (Qiagen, Tokyo, Japan). We evaluated five single-nucleotide polymorphisms (SNPs): rs1898830, rs11938228, rs3804099, re3804100, and rs7656411 (Figure 1 and Table 1). These SNPs had minor allele frequencies (>5%) from the National Center for Biotechnology Information db SNP. Genotyping of all SNPs was performed using the TaqMan 5′ exonuclease assay using primers supplied by Applied Biosystems (Foster City, CA). Probe fluorescence signals were detected by TaqMan Assay for real-time PCR (7500 Real Time PCR System; Applied Biosystems) following the manufacturer's instructions.

Statistical analysis: Hardy–Weinberg equilibrium was tested for each SNP among the controls. Differences in allele and genotype frequencies between cases and controls were assessed by the χ^2 test. The Haploview 3.32 (Daly Lab at the Broad Institute, Cambridge, MA) program was used to compute pair-wise linkage disequilibrium (LD) statistics [30]. Standardized disequilibrium (D') value was plotted, and LD blocks were defined according to the criteria [31]. Haplotype frequencies were estimated using an accelerated expectation-maximization algorithm similar to the partition-ligation-expectation- maximization method [32]. P values <0.05 were considered statistically significant. The Bonferroni method was used to correct multiple comparisons.

RESULTS

We genotyped five common SNPs in the *TLR2* gene: rs1898830, rs11938228, rs3804099, re3804100, and rs7656411 (Figure 1 and Table 1). All five SNPs were in Hardy–Weinberg equilibrium in the controls (data not shown). The minor allele frequencies of all SNPs were over 5% in the control group (Table 1). In this study, we did not examine the polymorphism of rs4696480, because there were no data for this minor allele in the Japanese HapMap database.

Linkage disequilibrium (LD) blocks of five SNPs in *TLR2* were defined (Figure 1). The *TLR2* region was divided into two haplotype blocks, with substantial LD among the SNPs of both blocks (block 1: D'□1.00; block 2: D'□0.98). The allele frequencies of the five SNPs in both the cases and controls are listed in Table 1, and genotype frequencies are listed in Table 2. No statistically significant association was observed for any of the SNPs between the cases and controls ($p>0.05$). We analyzed clinical features according to five SNPs. In a stratified analysis according to lesion location, which included the eye, lungs, heart, and nerves, none of these

Table 1. Allele frequencies of SNPs of *TLR2* among sarcoidosis patients and controls.

dbSNP	Alleles (1/2)	Position (bp)	Gene location	Minor allele frequency, n (%) Cases (n=257)	Minor allele frequency, n (%) Controls (n=193)	OR	p value
rs1898830	A/G	154,827,903	Intron	232 (45.1)	185 (47.9)	0.89	0.41
rs11938228	C/A	154,841,396	Intron	228 (44.4)	180 (46.6)	0.91	0.50
rs3804099	T/C	154,844,106	Exon	165 (32.1)	108 (28.0)	1.22	0.18
rs3804100	T/C	154,844,859	Exon	155 (30.2)	97 (25.1)	1.29	0.097
rs7656411	G/T	154,847,105	3'UTR	218 (42.4)	168 (43.5)	0.96	0.74

In the "Alleles" column, 1 indicates the major allele and 2 indicates the minor allele. The position reflects the distance from short-arm telomere. p-values were caluculated by χ^2 test 2 · 2 contingency table. bp, base pairs. OR, odds ratio.

clinical features were found to be significantly associated with five SNPs (Table 3). Meanwhile, in 51 patients who had dermatitis, the minor allele frequencies of rs3804099 and rs3804100 were higher when compared with the frequency in 193 controls (p=0.021; p=0.013). However, these statistical differences disappeared after the Bonferroni correction was applied (p>0.05).

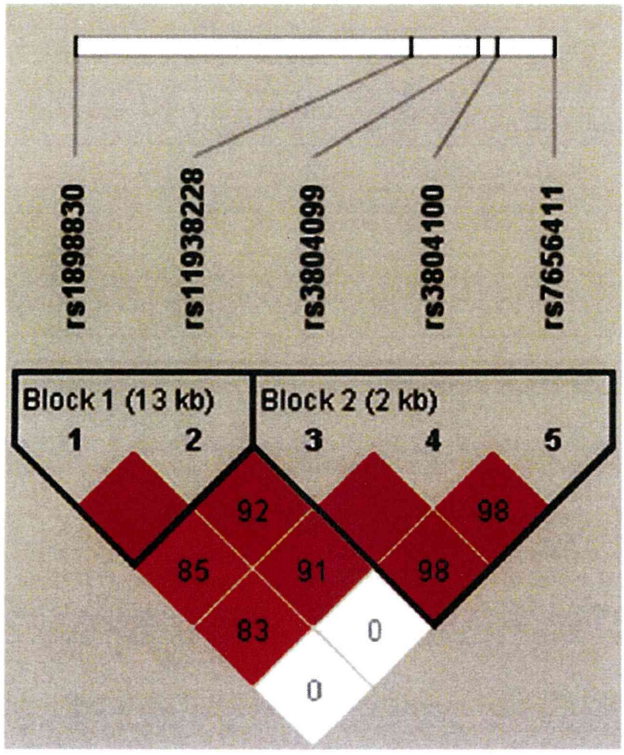

Figure 1. Linkage disequilibrium plot of five SNPs of *TLR2* in sarcoidosis patients and healthy controls. The schematic of the *TLR2* gene is shown as a black line, with boxes representing its three exons. The locations of the selected SNPs are indicated by the dotted lines. The Haplotype blocks were determined using the Haploview 4.2 software. Each box provides estimated statistics of the coefficient of determination, with brighter red representing a stronger Linkage disequilibrium. Values in squares represent pairwise D' values.

DISCUSSION

The current study was designed to determine whether *TLR2* polymorphisms affect the development of sarcoidosis in the Japanese population. Our results showed that all the *TLR2* polymorphisms so far examined were not significantly associated with any clinical subtype of sarcoidosis including ocular involvement in the Japanese population. However, a marginally significant p-value was observed for the SNPs rs3804099 and rs3804100 in patients with cutaneous manifestations, in comparison with the healthy control group. Healthy normal human skin contains two distinct major subsets of resident dendritic cells: Langerhans cells (LCs) and dermal DCs (DDCs). These DCs are located in the outer skin layers of the epidermis and play a critical role as the first line of defense against pathogens invading the skin. These DCs in the skin, especially DDCs, express TLR2 as well as TLR1 and TLR6. DDCs recognize bacteria and trigger the innate immune response [33]. Our results indicate that a possible connection may exist between *TLR2* polymorphisms and skin manifestations of sarcoidosis. The variants of the *TLR2* gene in DDCs may play a causative role in the development of cutaneous sarcoidosis in a site-specific manner.

Several reports have suggested that genetic variants of innate immune receptors might be associated with the risk of developing sarcoidosis [34]. Innate immune dysfunction caused by genetic factors may fail to eliminate pathogens. Consequently, it is postulated that frequent stimulation could lead to the chronic inflammation of sarcoidosis.

TLR4 is a major receptor for lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls. TLR4 and TLR2 signal transduction results in the activation of inflammatory pathways involving nuclear factor-kappa B (NF-kB). Conflicting reports about the association between

TABLE 2. GENOTYPE FREQUENCIES OF FIVE SNPS OF THE *TLR2* GENE IN SARCOIDOSIS PATIENTS AND CONTROLS.

SNP	Genotype	Cases, n (%)	Controls, n (%)	p value
rs1898830	AA	77 (30.0)	53 (27.5)	0.69
	AG	128 (49.8)	95 (49.2)	
	GG	52 (20.2)	45 (23.3)	
rs11938228	CC	79 (30.7)	56 (29.0)	0.76
	CA	128 (49.8)	94 (48.7)	
	AA	50 (19.5)	43 (22.3)	
rs3804099	TT	116 (45.1)	99 (51.3)	0.39
	TC	117 (45.5)	80 (41.5)	
	CC	24 (9.3)	14 (7.3)	
rs3804100	TT	123 (47.9)	107 (55.4)	0.24
	TC	113 (44.0)	75 (38.9)	
	CC	21 (8.2)	11 (5.7)	
rs7656411	GG	86 (33.5)	62 (32.1)	0.95
	GT	124 (48.2)	94 (48.7)	
	TT	47 (18.3)	37 (19.2)	

p values were calculated using the χ^2 test 3 × 2 contingency table.

TABLE 3. *TLR2* SNPS ALLELE FREQUENCIES AMONG SARCOIDOSIS PATIENTS INFLAMMATORY SITES AND CONTROLS.

		Minor allele frequency, n (%)						
			Patients					
SNP	Alleles (1/2)	Controls (n=193)	Cases (n=257)	Eye (n=211)	Lungs (n=138)	Skin (n=51)	Heart (n=49)	Nerve (n=12)
rs1898830	A/G	185 (47.9)	232 (45.1)	189 (44.8)	131 (47.5)	40 (39.2)	44 (44.9)	11 (45.8)
rs11938228	C/A	180 (46.6)	228 (44.4)	186 (44.1)	128 (46.4)	38 (37.3)	43 (43.9)	11 (45.8)
rs3804099	T/C	108 (28.0)	165 (32.1)	141 (33.4)	91 (33.0)	41 (40.2)*	30 (30.6)	8 (33.3)
rs3804100	T/C	97 (25.1)	155 (30.2)	134 (31.8)	84 (30.4)	39 (38.2)**	27 (27.6)	7 (29.2)
rs7656411	G/T	168 (43.5)	218 (42.4)	170 (40.3)	113 (40.9)	37 (36.3)	43 (43.9)	8 (33.3)

In the "Alleles" column, 1 indicates the major allele and 2 indicates the minor allele. *p=0.021, Pc>0.05 **p=0.013, Pc>0.06 These values are not significant after Bonferroni correction.

TLR4 and sarcoidosis have recently been published. It was shown that there is a significant association between patients with chronic sarcoidosis and *TLR4* polymorphisms in the Caucasian population [35]. Whereas, subsequent investigations by other groups found no significant association between polymorphisms and increased susceptibility to sarcoidosis [36,37]. Our group also could not find any association between *TLR4* polymorphisms and sarcoidosis in the Japanese population [29].

Nucleotide-binding oligomerization domain 2 (NOD2), a member of the NLR (Nod-like receptor) family, is an intracellular microbial sensor. This protein detects muramyl dipeptide (MDP), a component of bacterial peptidoglycans, and induces innate immune responses. Several reports have suggested there may be an association of polymorphisms in *NOD2* with early-onset sarcoidosis and Blau syndrome. However, no significant associations between the genetic polymorphisms in the *NOD2* gene and the risk of adult sarcoidosis were detected [36,38-41].

In the present study, genetic variations in TLR2 did not affect ocular sarcoidosis risk. However, some types of genetic predispositions underlying the pathogenesis of sarcoidosis can lead to ocular inflammation. Microbial pathogens have long been suspected as the cause of sarcoidosis. Therefore, further studies are needed to analyze other genes involved in the innate immune response against bacterial antigens.

In summary, the minor allele frequencies of *TLR2* do not appear to be significantly relevant to sarcoidosis in the Japanese population. However, in cutaneous sarcoidosis, rs3804099 and rs3804100 SNPs in *TLR2* are slightly associated with clinical disease. Further studies, especially in other ethnic populations, are required to elucidate what association there may be between sarcoidosis and *TLR2*.

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REFERENCES

1. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med 1997; 336:1224-34. [PMID: 9110911]
2. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet 2003; 361:1111-8. [PMID: 12672326]
3. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med 2007; 357:2153-65. [PMID: 18032765]
4. ACCESS Research Group. Design of a case control etiologic study of sarcoidosis (ACCESS). J Clin Epidemiol 1999; 52:1173-86. [PMID: 10580780]
5. Morimoto T, Azuma A, Abe S, Usuki J, Kudoh S, Sugisaki K, Oritsu M, Nukiwa T. Epidemiology of sarcoidosis in Japan. Eur Respir J 2008; 31:372-9. [PMID: 17959635]
6. Goto H, Mochizuki M, Yamaki K, Kotake S, Usui M, Ohno S. Epidemiological survey of intraocular inflammation in Japan. Jpn J Ophthalmol 2007; 51:41-4. [PMID: 17295139]
7. Rybicki BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. Am J Epidemiol 1997; 145:234-41. [PMID: 9012596]
8. Hillerdal G, Nou E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. Am Rev Respir Dis 1984; 130:29-32. [PMID: 6742607]
9. Kim DS. Sarcoidosis in Korea: report of the Second Nationwide Survey. Sarcoidosis Vasc Diffuse Lung Dis 2001; 18:176-80. [PMID: 11436538]
10. Abe C, Iwai K, Mikami R, Hosoda Y. Frequent isolation of Propionibacterium acnes from sarcoidosis lymph nodes. Zentralbl Bakteriol Mikrobiol Hyg [A] 1984; 256:541-7. [PMID: 6377763]
11. Saboor SA, Johnson NM, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 1992; 339:1012-5. [PMID: 1349051]
12. Ishige I, Usui Y, Takemura T, Eishi Y. Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. Lancet 1999; 354:120-3. [PMID: 10408488]
13. Eishi Y, Suga M, Ishige I, Kobayashi D, Yamada T, Takemura T, Takizawa T, Koike M, Kudoh S, Costabel U, Guzman J, Rizzato G, Gambacorta M, du Bois R, Nicholson AG, Sharma OP, Ando M. Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. J Clin Microbiol 2002; 40:198-204. [PMID: 11773116]
14. Dubaniewicz A, Kampfer S, Singh M. Serum anti-mycobacterial heat shock proteins antibodies in sarcoidosis and tuberculosis. Tuberculosis (Edinb) 2006; 86:60-7. [PMID: 16352470]
15. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007; 449:819-26. [PMID: 17943118]
16. Beutler BA. TLRs and innate immunity. Blood 2009; 113:1399-407. [PMID: 18757776]
17. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010; 140:805-20. [PMID: 20303872]
18. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004; 5:975-9. [PMID: 15454920]
19. Schwartz DA, Cook DN. Polymorphisms of the Toll-like receptors and human disease. Clin Infect Dis 2005; 41:S403-7. [PMID: 16237638]
20. Uematsu S, Akira S. The role of Toll-like receptors in immune disorders. Expert Opin Biol Ther 2006; 6:203-14. [PMID: 16503731]
21. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 1999; 11:443-51. [PMID: 10549626]
22. Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol 2000; 165:5392-6. [PMID: 11067888]
23. Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun 2000; 68:6398-401. [PMID: 11035751]
24. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunol Med Microbiol 2001; 31:53-8. [PMID: 11476982]
25. Bochud PY, Hawn TR, Aderem A. Cutting edge: a Toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. J Immunol 2003; 170:3451-4. [PMID: 12646604]
26. Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, Coskun M, Cilli A, Yegin O. The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. Eur Respir J 2004; 23:219-23. [PMID: 14979495]
27. Ben-Ali M, Barbouche MR, Bousnina S, Chabbou A, Dellagi K. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol 2004; 11:625-6. [PMID: 15138193]
28. Veltkamp M, Wijnen PA, van Moorsel CH, Rijkers GT, Ruven HJ, Heron M, Bekers O, Claessen AM, Drent M, van den Bosch JM, Grutters JC. Linkage between Toll-like receptor (TLR) 2 promotor and intron polymorphisms: functional effects and relevance to sarcoidosis. Clin Exp Immunol 2007; 149:453-62. [PMID: 17565608]
29. Asukata Y, Ota M, Meguro A, Katsuyama Y, Ishihara M, Namba K, Kitaichi N, Morimoto S, Kaburaki T, Ando Y, Takenaka S, Inoko H, Ohno S, Mizuki N. Lack of association between toll-like receptor 4 gene polymorphisms and sarcoidosis-related uveitis in Japan. Mol Vis 2009; 15:2673-82. [PMID: 20011079]
30. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263-5. [PMID: 15297300]
31. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward

R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science 2002; 296:2225-9. [PMID: 12029063]

32. Qin ZS, Niu T, Liu JS. Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. Am J Hum Genet 2002; 71:1242-7. [PMID: 12452179]
33. van der Aar AM, Sylva-Steenland RM, Bos JD, Kapsenberg ML, de Jong EC, Teunissen MB. Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. J Immunol 2007; 178:1986-90. [PMID: 17277101]
34. Schürmann M, Kwiatkowski R, Albrecht M, Fischer A, Hampe J, Muller-Quernheim J, Schwinger E, Schreiber S. Study of Toll-like receptor gene loci in sarcoidosis. Clin Exp Immunol 2008; 152:423-31. [PMID: 18422738]
35. Pabst S, Baumgarten G, Stremmel A, Lennarz M, Knufermann P, Gillissen A, Vetter H, Grohe C. Toll-like receptor (TLR) 4 polymorphisms are associated with a chronic course of sarcoidosis. Clin Exp Immunol 2006; 143:420-6. [PMID: 16487240]
36. Gazouli M, Koundourakis A, Ikonomopoulos J, Gialafos EJ, Rapti A, Gorgoulis VG, Kittas C. CARD15/NOD2, CD14, and toll-like receptor 4 gene polymorphisms in Greek patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2006; 23:23-9. [PMID: 16933467]
37. Veltkamp M, Grutters JC, van Moorsel CH, Ruven HJ, van den Bosch JM. Toll-like receptor (TLR) 4 polymorphism Asp299Gly is not associated with disease course in Dutch sarcoidosis patients. Clin Exp Immunol 2006; 145:215-8. [PMID: 16879239]
38. Schürmann M, Valentonyte R, Hampe J, Muller-Quernheim J, Schwinger E, Schreiber S. CARD15 gene mutations in sarcoidosis. Eur Respir J 2003; 22:748-54. [PMID: 14621080]
39. Ho LP, Merlin F, Gaber K, Davies RJ, McMichael AJ, Hugot JP. CARD 15 gene mutations in sarcoidosis. Thorax 2005; 60:354-5. [PMID: 15790995]
40. Milman N, Nielsen OH, Hviid TV, Fenger K. CARD15 single nucleotide polymorphisms 8, 12 and 13 are not increased in ethnic Danes with sarcoidosis. Respiration 2007; 74:76-9. [PMID: 16397396]
41. Akahoshi M, Ishihara M, Namba K, Kitaichi N, Ando Y, Takenaka S, Ishida T, Ohno S, Mizuki N, Nakashima H, Shirakawa T. Mutation screening of the CARD15 gene in sarcoidosis. Tissue Antigens 2008; 71:564-7. [PMID: 18384487]

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INFLAMMATORY DISORDERS

Correlation between elevation of serum antinuclear antibody titer and decreased therapeutic efficacy in the treatment of Behçet's disease with infliximab

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Abstract
Background Infliximab, an anti-TNF-α monoclonal antibody, administered to Behçet's disease (BD) patients in Japan with refractory intraocular inflammation, has shown excellent clinical results. However, some patients demonstrate a decreased response to infliximab during the course of the treatment. In the present study, we investigated the correlation between this reduced therapeutic effect and elevation of the serum antinuclear antibody (ANA) titers in patients with BD who were undergoing infliximab therapy.
Methods Seventeen patients (14 males and three females) with uveitis in BD who were undergoing treatment with infliximab for 2 years or longer were enrolled. Their blood test results and clinical histories were obtained from medical records.
Results One patient (5.9%) was ANA-positive prior to the initiation of infliximab, and 11 patients (64.7%) developed positive ANA during the therapy. The appearance of ANA was observed 6 months after the initiation of the infliximab therapy, and its titers gradually increased. None of the patients showed lupus symptoms. Five patients (29.4%) have suffered from ocular inflammatory attacks since the sixth month from the initiation of infliximab treatment and all of them were ANA-positive. In contrast, four patients (23.5%) who were ANA-negative experienced no ocular attacks during the follow-up period.
Conclusions Here we report the positive conversion and subsequent elevation of serum ANA titers in some patients with BD after the initiation of infliximab therapy. Since all recurrences of uveitis were shown only in the ANA-positive patients, serum ANA titer may be a helpful biomarker for predicting the recurrence of ocular attacks in BD patients treated with anti-TNF-α antibody therapies.

Keywords Behçet's disease · Retinal vasculitis · Uveitis · Antinuclear antibody · Infliximab · Biomarker · Anti-TNF-α monoclonal antibody

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Introduction

Behçet's disease (BD) is a chronic systemic inflammatory disease characterized by recurrent oral aphthous ulcers, genital ulcers, skin lesions, gastrointestinal involvement, vasculitis, neurological manifestations, and intraocular inflammation. BD is one of the major etiologies of endogenous uveitis in Japan [1], however, its prevalence and clinical features vary among countries and ethnic groups [2, 3]. Recurrent episodes of inflammatory ocular attacks can cause severe visual loss. To prevent the relapse of intraocular inflammation, colchicine and various immunosuppressive

agents are administered including cyclosporine A (CyA), which is a selective immunosuppressive agent of T-lymphocytes. However, some patients cannot use these drugs due to intolerable side-effects. Moreover, some patient's diseases are refractory to these agents and can progress to vision loss [4–6].

Infliximab (IFX) is a chimeric monoclonal antibody to TNF-α that can minimize the immunological response when used in humans [7]. It neutralizes both membrane-binding and soluble TNF-α, in addition to suppressing TNF-α production by macrophages. IFX is commonly administered to patients with rheumatoid arthritis [8, 9], Crohn's disease [10], psoriasis [11, 12], and in case of refractory uveitis with non-infectious etiologies including BD [13–20]. IFX is effective for preventing relapse of intraocular inflammations in BD and its efficacy has been well documented in previous studies [13–19]. In Japan, IFX was approved for use in BD patients with refractory uveoretinitis by the Ministry of Health, Labour and Welfare, Japan in January 2007 based on the excellent results from multicenter clinical trials [15, 21]. Though IFX is an excellent agent in the treatment of the BD with refractory uveoretinitis, it has been observed to have decreased efficacy in a subset of BD patients with uveoretinitis [19]. One report showed the development of autoantibodies including antinuclear antibody (ANA) during IFX treatment in BD [22], however, the mechanisms and the meanings of it remain unknown.

In the present study, we investigated ANA titers of the BD patients receiving IFX therapy and examined the correlation between the elevation of ANA and the therapeutic efficacy.

Materials and methods

BD patients with refractory uveoretinitis who had been administered IFX for 2 years or longer were enrolled at Hokkaido University Hospital. The results of their blood tests and clinical histories were obtained from medical records. BD was diagnosed based on the criteria set by the BD Research Committee of Japan, which is part of the Ministry of Health, Labour and Welfare, Japan [23]. The level of ocular inflammation was graded by means of the Standardization of Uveitis Nomenclature (SUN) grading criteria [24]. When a patient showed more than two steps of increase in level of inflammation or increase from grade 3+ to 4+, it was considered an inflammatory ocular attack. Ocular attacks of BD flare up repeatedly and usually disappear within a few weeks. Each ocular attack shows a varying degree of uveitis including only mild iridocyclitis or severe obstructive retinal vasculitis with retinal exudates. The number of ocular attacks was counted regardless of the severity and added both eyes.

Patients were administered 5 mg/kg of IFX intravenously at weeks 0, 2, and 6 as the initial series of infusions and thereafter every 8 weeks. Serum ANA and anti-double-stranded DNA (dsDNA) antibodies were examined prior to IFX infusion. ANA titers were quantified using an indirect immunofluorescence technique using human epithelial (Hep2) cells. Results were classified as positive (ANA≥80) or negative (ANA≤40) according to previous reports [25–31]. Anti-dsDNA antibodies were identified using enzyme-linked immunosorbent assay (ELISA).

Statistical analyses were performed using the Mann–Whitney U test; p values <0.05 were considered to be statistically significant. This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Hokkaido University Hospital.

Results

The demographics and clinical characteristics of the 17 Japanese patients, i.e., 14 (82.4%) males and three (17.6%) females ranging in age from 15 to 58 (mean age: 36.9) years, enrolled in the study are listed in Table 1. The rate of ocular inflammatory attacks during 6 months prior to the initiation of IFX was 3.8±2.1 (mean±SD). IFX therapy significantly reduced the rate of ocular attacks to 0.7±1.1 during the first 6 months after the initiation of IFX ($p<0.01$).

Table 1 Characteristics of Behçet's disease patients treated with IFX

Case	Age (years)	Sex	Treatment before IFX initiation	Concomitant treatment with IFX
1	39	M	CyA, Col, PSL	–
2	33	M	Col, PSL	PSL
3	58	M	Col	–
4	42	M	Col	–
5	40	F	PSL	PSL
6	31	M	Col	–
7	44	M	PSL	PSL
8	54	M	CyA, PSL	PSL
9	17	M	CyA, Col	–
10	52	M	Col	–
11	10	M	–	–
12	49	M	CyA	–
13	40	M	CyA	–
14	40	F	CyA	–
15	36	F	PSL	PSL
16	15	M	PSL	–
17	28	M	–	–

IFX – infliximab, CyA – cyclosporine A, Col – colchicine, PSL – prednisolone

Eight patients (47.1%) achieved no relapse of ocular inflammatory attacks between the first infusion and the 24-month visit. Five patients (29.4%) experienced only one ocular inflammatory attack and four patients (23.5%) experienced several ocular attacks during the follow-up period. It was not necessary to administer concomitant drugs with IFX for 12 patients. Three of five patients who were previously administered oral prednisolone (PSL) could decrease and gradually stop their therapy after IFX initiation. Two of these patients required continued PSL administration to control neurological symptoms.

Best-corrected visual acuities (BCVA) were reported 1 year after the initiation of IFX; IFX therapy had successfully maintained their vision acuity (Fig. 1).

ANA profiles and the frequency of ocular attacks in BD patients treated with IFX are shown in Table 2. One patient (5.9%) was ANA-positive prior to the initiation of IFX. Anti-dsDNA antibodies were never detected prior to IFX induction. The change in ANA-positive rates is shown in Fig. 2. The positive conversion of ANA became common 6 months after the initiation of IFX, and the positive titers continued to increase. At the end of the follow-up period, 13 patients (76.4%) were identified positive for ANA (Fig. 2). One patient (5.9%) developed anti-dsDNA antibodies (case #14). However, none of the patients showed lupus symptoms.

The correlation of ocular attacks with elevation of ANA titer is shown in Fig. 3. At the 6th month after the IFX induction, five patients (29.4%) were ANA-positive and 12 (70.6%) were negative. In the ANA-positive group, three patients (60%) had ocular inflammatory attacks during the first 6 months after IFX administrations, whereas in the ANA-negative group, four (33.3%) patients had these attacks. Ocular attacks were much milder than those before IFX therapy both in the ANA-negative group and ANA-positive group.

However, since the 6th month of IFX therapy, all of five patients (29.4%) suffering from a relapse of ocular inflammatory attacks were ANA-positive, and three of five patients had multiple ocular attacks. In two of these three patients, the administration interval was shortened from 8 to 7 weeks, and this successfully led to a lower rate of the ocular attacks. On the other hand, all of four patients (23.5%) with negative ANA had no ocular attacks.

Discussion

ANA appeared in the sera of BD patients 6 months after IFX induction, and its titer gradually increased. It was reported that the development of ANA and anti-dsDNA antibodies is seen during the course of anti-TNF-α therapy in patients with some autoimmune diseases such as rheumatoid arthritis [32–34], psoriasis [35], Crohn's disease [36], and BD [22]. In the present study, 75.0% of the patients converted to ANA-positive during the course of IFX therapy and positive ANA titers (1:80) had been detected in one patient on study enrolment. This patient experienced a twofold increase in

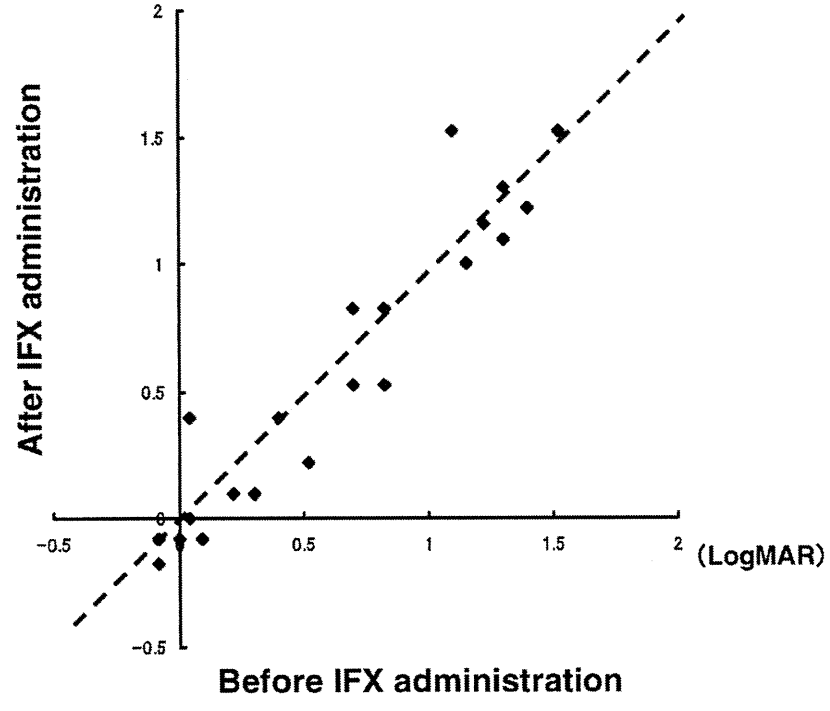

Fig. 1 Visual acuity before and after initiation of IFX. Best-corrected visual acuities (BCVA) 1 year after the initiation of IFX. IFX therapy successfully maintained the visual acuity in these patients

Table 2 ANA profile and the rate of ocular attacks of Behçet's disease patients treated with IFX

Case	Frequency of ocular attacks -6~0 month	ANA (titer) 0 month	Frequency of ocular attacks 0~6 month	ANA (titer) 6 month	Frequency of ocular attacks 6~12 month	ANA (titer) 12 month	Frequency of ocular attacks 12~18 month	ANA (titer) 18 month	Frequency of ocular attacks 18~24 month	ANA (titer) 24 month
1	0	0	0	80	0	40	0	80	0	80
2	2	0	0	0	0	0	0	0	0	40
3	8	0	0	0	0	0	0	0	0	40
4	4	0	0	40	0	80	0	80	0	160
5	4	0	0	40	0	80	0	80	0	160
6	5	0	0	40	0	160	0	320	0	640
7	3	0	0	40	0	80	0	160	0	640
8	1	0	0	40	0	40	0	40	0	40
9	4	0	1	0	0	40	0	40	0	80
10	2	0	1	0	0	80	0	80	0	80
11	4	0	1	0	0	0	0	0	0	0
12	4	40	1	80	0	80	0	80	0	80
13	4	0	0	160	1	80	0	160	0	80
14	7	0	1	160	5	160	4	320	3	640
15	2	0	0	40	1	80	1	80	0	40
16	6	80	2	160	1	160	1	160	0	160
17	4	0	4	40	1	80	0	40	0	40

ANA positive: ANA titer≥80, ANA – anti nuclear antibody

the titer (1:160). Only one patient (5.9%) converted to anti-dsDNA antibody-positive during the follow-up period. These findings are consistent with previous studies of other autoimmune rheumatoid diseases, which reported that 25–71% and 4–46% patients became positive for ANA and anti-dsDNA antibodies after IFX initiation in case of psoriasis and rheumatoid arthritis [32, 37, 38]. In these previous studies, a small number of patients had lupus-like symptoms [39–41]. Suhler EB et al. also reported the results of a prospective study in which 23 patients with non-infectious uveoretinitis including four of BD patients were enrolled [42]. In the report, ANA titers developed in 15 (75.0%) of the 20 patients and two patients with very high titer showed arthritis. Although none of the patients in our study have shown lupus symptoms, we have to observe the patients very carefully.

It is still unknown how ANA and anti-dsDNA antibodies develop during IFX therapy. One possible explanation is that TNF-α may up-regulate cellular expression of the adhesion molecule CD44, which plays a role in the

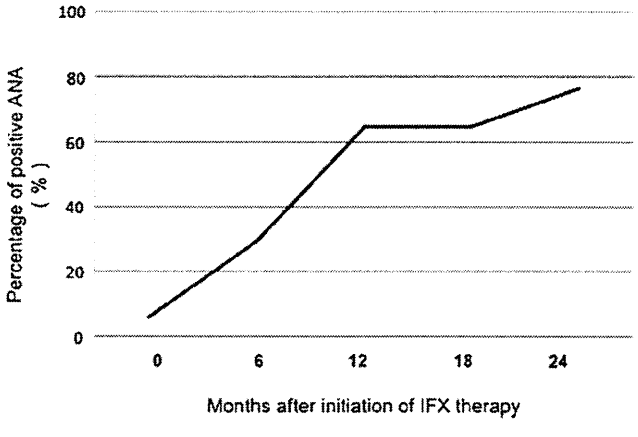

Fig. 2 Frequencies of ANA positivity in BD patients undergoing IFX therapy. The positive conversion of ANA became frequent 6 months after the initiation of IFX, and its positivity rate gradually increased

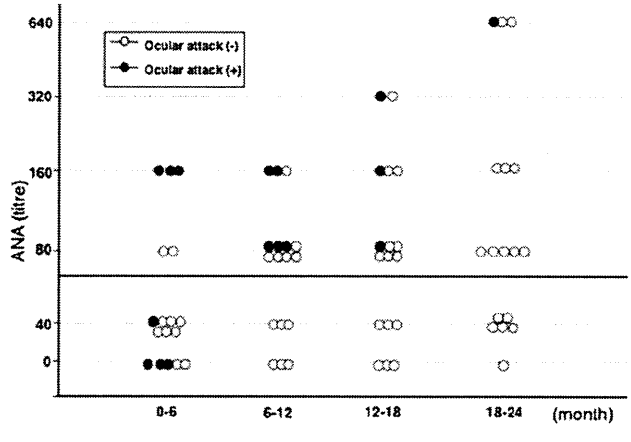

Fig. 3 Correlation of ocular attacks with elevation of ANA titer. Since the 6th month of IFX therapy, all five patients (23.5%) suffering from a relapse of ocular inflammatory attack were ANA-positive, and three of five patients had ocular attacks more than once throughout the observation period

clearance of apoptotic neutrophils by phagocytes [43, 44]. Impaired clearance of apoptotic cells and reduced CD44 expression on leukocytes has been reported in systemic lupus erythematosus (SLE) [45, 46]. IFX may down-regulate CD44 expression and induce an immune reaction toward their own nuclei by impairment of the clearance of apoptotic cells.

In the present study, we also demonstrated the association between the development of ANA and reduced effects of IFX therapy in BD patients. Only a few studies have reported the association of serum ANA development with the effects of IFX [35, 42]. Pink et al. reported that ANA titer was associated with the loss of response to anti-TNF-α therapy in psoriasis. In our study, during 6 months after the initiation of IFX therapy, several patients experienced mild ocular inflammatory attacks, both in the ANA-positive and ANA-negative groups. Presumably, it takes some time for IFX to exert an inhibitory effect on severe ocular inflammation in BD patients. However, after the 6th month IFX therapy, the cases suffering from the relapse of ocular inflammatory attacks were limited to ANA-positive patients. Similar to the study in psoriasis [35], these results suggest that the development of elevated levels of serum ANA may be associated with the reduction of IFX efficacy for BD patients. Suhler EB et al. mentioned no clear relation between the development of ANAs and ocular therapeutic response [42]. The subjects in the report included a variety of uveitis cases, in contrast to our study, which targeted only BD. The tight disease enrollment may be the reason why we could show the relation between recurrences of uveitis and high titer of ANA.

The exact association between ANA and IFX also remains unknown. It has also been found that repeated infusion of IFX leads to induction of antibodies to IFX (ATI) that reduce the efficacy of IFX. This phenomenon has been a serious issue in rheumatoid arthritis [47], Crohn's disease [48], psoriasis [49, 50] and BD [51] therapy. ANA and ATI, both of which appear during the course of IFX treatment, are likely to be involved in the reduced efficacy of IFX; however, the correlation between the two antibodies remains to be elucidated. We speculate that repeated administration of protein agents such as IFX may activate a systemic immune response, leading to the production of various autoantibodies including ANA and ATI. Therefore, detection of high titer of ANA indicates the development of ATI in the patients. According to this theory, the patients, such as cases #6 and #7, with high ANA titer (640×) may have already had ATI. These patients should be monitored closely for further symptoms. If the theory is confirmed that ATI is strongly correlated with the decreased therapeutic efficacy of IFX, we need to consider concomitant use of immune modulatory medicine in BD.

IFX has provided a new way to maintain good vision for a long time in many BD patients with severe uveitis. However, in certain cases IFX becomes less effective while long-term use of IFX. Serum ANA titers may be one of the helpful biomarker to predict IFX ineffectiveness.

Competing interests None.

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Ethics approval This study was approved by the institutional Ethics Committee of Hokkaido University

Patient consent Obtained.

References

1. Kitamei H, Kitaichi N, Namba K, Kotake S, Goda C, Kitamura M, Miyazaki A, Ohno S (2009) Clinical features of intraocular inflammation in Hokkaido, Japan. Acta Ophthalmol 87:424–428
2. Kitaichi N, Miyazaki A, Iwata D, Ohno S, Stanford MR, Chams H (2007) Ocular features of Behçet's disease: an international collaborative study. Br J Ophthalmol 91:1579–1582
3. Kitaichi N, Miyazaki A, Stanford MR, Iwata D, Chams H, Ohno S (2009) Low prevalence of juvenile-onset Behçet's disease with uveitis in East/South Asian people. Br J Ophthalmol 93:1428–1430
4. Kaklamani V, Vaiopoulos G, Kaklamanis P (1998) Behçet's disease. Semin Arthritis Rheum 27:197–217
5. Sakane T, Takeno M, Suzuki N, Inaba G (1999) Behçet's disease. N Engl J Med 341:1284–1291
6. Zouboulis C (1999) Epidemiology of Adamantiades-Behçet's disease. Ann Med Interne (Paris) 150:488–498
7. Knight D, Trinh H, Le J, Siegel S, Shealy D, McDonough M, Scallon B, Moore M, Vilcek J, Daddona P (1993) Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. Mol Immunol 30:1443–1453
8. Elliott M, Maini R, Feldmann M, Long-Fox A, Charles P, Bijl H, Woody J (1994) Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. Lancet 344:1125–1127
9. Maini R, St Clair E, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P (1999) Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 354:1932–1939
10. Hanauer S, Feagan B, Lichtenstein G, Mayer L, Schreiber S, Colombel J, Rachmilewitz D, Wolf D, Olson A, Bao W, Rutgeerts P, Group AIS (2002) Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 359:1541–1549
11. Zhang Z, Schmitt J, Wozel G, Kirch W (2009) Treatment of plaque psoriasis with biologics. A meta-analysis of randomized controlled trials. Med Klin (Munich) 104:125–136
12. Nestle F, Kaplan D, Barker J (2009) Psoriasis. N Engl J Med 361:496–509
13. Sfikakis P, Theodossiadis P, Katsiari C, Kaklamanis P, Markomichelakis N (2001) Effect of infliximab on sight-threatening panuveitis in Behçet's disease. Lancet 358:295–296

14. Muñoz-Fernández S, Hidalgo V, Fernández-Melón J, Schlincker A, Martín-Mola E (2001) Effect of infliximab on threatening panuveitis in Behçet's disease. Lancet 358:1644
15. Ohno S, Nakamura S, Hori S, Shimakawa M, Kawashima H, Mochizuki M, Sugita S, Ueno S, Yoshizaki K, Inaba G (2004) Efficacy, safety, and pharmacokinetics of multiple administration of infliximab in Behçet's disease with refractory uveoretinitis. J Rheumatol 31:1362–1368
16. Sfikakis P, Kaklamanis P, Elezoglou A, Katsilambros N, Theodossiadis P, Papaefthimiou S, Markomichelakis N (2004) Infliximab for recurrent, sight-threatening ocular inflammation in Adamantiades-Behçet disease. Ann Intern Med 140:404–406
17. Tugal-Tutkun I, Mudun A, Urgancioglu M, Kamali S, Kasapoglu E, Inanc M, Gül A (2005) Efficacy of infliximab in the treatment of uveitis that is resistant to treatment with the combination of azathioprine, cyclosporine, and corticosteroids in Behçet's disease: an open-label trial. Arthritis Rheum 52:2478–2484
18. Accorinti M, Pirraglia M, Paroli M, Priori R, Conti F, Pivetti-Pezzi P (2007) Infliximab treatment for ocular and extraocular manifestations of Behçet's disease. Jpn J Ophthalmol 51:191–196
19. Niccoli L, Nannini C, Benucci M, Chindamo D, Cassarà E, Salvarani C, Cimino L, Gini G, Lenzetti I, Cantini F (2007) Long-term efficacy of infliximab in refractory posterior uveitis of Behçet's disease: a 24-month follow-up study. Rheumatology (Oxford) 46:1161–1164
20. Al-Rayes H, Al-Swailem R, Al-Balawi M, Al-Dohayan N, Al-Zaidi S, Tariq M (2008) Safety and efficacy of infliximab therapy in active Behçet's uveitis: an open-label trial. Rheumatol Int 29:53–57
21. Yamada Y, Sugita S, Tanaka H, Kamoi K, Kawaguchi T, Mochizuki M (2010) Comparison of infliximab versus ciclosporin during the initial 6-month treatment period in Behçet disease. Br J Ophthalmol 94:284–288
22. Elezoglou A, Kafasi N, Kaklamanis PH, Theodossiadis PG, Kapsimali V, Choremi E, Vaiopoulos G, Sfikakis PP (2007) Infliximab treatment-induced formation of autoantibodies is common in Behçet's disease. Clin Exp Rheumatol 25:S65–S69
23. Mizushima Y (1988) Recent research into Behçet's disease in Japan. Int J Tissue React 10:59–65
24. Jabs DA, Nussenblatt RB, Rosenbaum JT, Group SoUNSW (2005) Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol 140:509–516
25. Fritzler MJ, Pauls JD, Kinsella TD, Bowen TJ (1985) Antinuclear, anticytoplasmic, and anti-Sjögren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. Clin Immunol Immunopathol 36:120–128
26. de Vlam K, De Keyser F, Verbruggen G, Vandenbossche M, Vanneuville B, D'Haese D, Veys EM (1993) Detection and identification of antinuclear autoantibodies in the serum of normal blood donors. Clin Exp Rheumatol 11:393–397
27. Forslid J, Heigl Z, Jonsson J, Scheynius A (1994) The prevalence of antinuclear antibodies in healthy young persons and adults, comparing rat liver tissue sections with HEp-2 cells as antigen substrate. Clin Exp Rheumatol 12:137–141
28. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, Gordon T, Hardin JA, Kalden JR, Lahita RG, Maini RN, McDougal JS, Rothfield NF, Smeenk RJ, Takasaki Y, Wiik A, Wilson MR, Koziol JA (1997) Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 40:1601–1611
29. Fernandez SA, Lobo AZ, Oliveira ZN, Fukumori LM, AM Prigo, Rivitti EA (2003) Prevalence of antinuclear autoantibodies in the serum of normal blood donors. Rev Hosp Clin Fac Med Sao Paulo 58:315–319
30. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, Tomita Y, Muro Y (2004) Anti-DFS70 antibodies in 597 healthy hospital workers. Arthritis Rheum 50:892–900
31. Al Jabri AA, Al Buloshi MS (2004) Anticardiolipin and antinuclear antibodies in the adult healthy Omani individuals. Saudi Med J 25:313–317
32. De Rycke L, Kruithof E, Van Damme N, Hoffman IE, Van den Bossche N, Van den Bosch F, Veys EM, De Keyser F (2003) Antinuclear antibodies following infliximab treatment in patients with rheumatoid arthritis or spondylarthropathy. Arthritis Rheum 48:1015–1023
33. Elkayam O, Burke M, Vardinon N, Zakut V, Yitzhak RB, Paran D, Levartovsky D, Litinsky I, Caspi D (2005) Autoantibodies profile of rheumatoid arthritis patients during treatment with infliximab. Autoimmunity 38:155–160
34. Eriksson C, Engstrand S, Sundqvist KG, Rantapää-Dahlqvist S (2005) Autoantibody formation in patients with rheumatoid arthritis treated with anti-TNF alpha. Ann Rheum Dis 64:403–407
35. Pink AE, Fonia A, Allen MH, Smith CH, Barker JN (2010) Antinuclear antibodies associate with loss of response to antitumour necrosis factor-alpha therapy in psoriasis: a retrospective, observational study. Br J Dermatol 162:780–785
36. Nancey S, Blanvillain E, Parmentier B, Flourié B, Bayet C, Bienvenu J, Fabien N (2005) Infliximab treatment does not induce organ-specific or nonorgan-specific autoantibodies other than antinuclear and anti-double-stranded DNA autoantibodies in Crohn's disease. Inflamm Bowel Dis 11:986–991
37. Smith CH, Jackson K, Bashir SJ, Perez A, Chew AL, Powell AM, Wain M, Barker JN (2006) Infliximab for severe, treatment-resistant psoriasis: a prospective, open-label study. Br J Dermatol 155:160–169
38. Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN (2000) Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum 43:2383–2390
39. Cairns AP, Duncan MK, Hinder AE, Taggart AJ (2002) New onset systemic lupus erythematosus in a patient receiving etanercept for rheumatoid arthritis. Ann Rheum Dis 61:1031–1032
40. Debandt M, Vittecoq O, Descamps V, Le Loët X, Meyer O (2003) Anti-TNF-alpha-induced systemic lupus syndrome. Clin Rheumatol 22:56–61
41. Favalli EG, Sinigaglia L, Varenna M, Arnoldi C (2002) Drug-induced lupus following treatment with infliximab in rheumatoid arthritis. Lupus 11:753–755
42. Suhler EB, Smith JR, Wertheim MS, Lauer AK, Kurz DE, Pickard TD, Rosenbaum JT (2005) A prospective trial of infliximab therapy for refractory uveitis: preliminary safety and efficacy outcomes. Arch Ophthalmol 123:903–912
43. Osada A, Nakashima H, Furue M, Tamaki K (1995) Up-regulation of CD44 expression by tumor necrosis factor-alpha is neutralized by interleukin-10 in Langerhans cells. J Invest Dermatol 105:124–127
44. Hart SP, Dougherty GJ, Haslett C, Dransfield I (1997) CD44 regulates phagocytosis of apoptotic neutrophil granulocytes, but not apoptotic lymphocytes, by human macrophages. J Immunol 159:919–925
45. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR (1998) Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum 41:1241–1250
46. Cairns AP, Crockard AD, McConnell JR, Courtney PA, Bell AL (2001) Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: relations with apoptotic neutrophils and disease activity. Ann Rheum Dis 60:950–955

47. Finckh A, Dudler J, Wermelinger F, Ciurea A, Kyburz D, Gabay C, Bas S, SCQM PO (2010) Influence of anti-infliximab antibodies and residual infliximab concentrations on the occurrence of acquired drug resistance to infliximab in rheumatoid arthritis patients. Joint Bone Spine 77: 313–318
48. Yamada A, Sono K, Hosoe N, Takada N, Suzuki Y (2010) Monitoring functional serum antitumor necrosis factor antibody level in Crohn's disease patients who maintained and those who lost response to anti-TNF. Inflamm Bowel Dis 16:1898–1904
49. Reich K, Nestle FO, Papp K, Ortonne JP, Evans R, Guzzo C, Li S, Dooley LT, Griffiths CE, investigators Es (2005) Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. Lancet 366: 1367–1374
50. Adişen E, Aral A, Aybay C, Gürer MA (2010) Anti-infliximab antibody status and its relation to clinical response in psoriatic patients: a pilot study. J Dermatol 37:708–713
51. Sugita S, Yamada Y, Mochizuki M (2010) Relationship between serum infliximab levels and acute uveitis attacks in patients with Behçet disease. Br J Ophthalmol 95:549–552

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REVIEW ARTICLE

Japanese Guideline for Allergic Conjunctival Diseases

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ABSTRACT

The definition, classification, pathogenesis, test methods, clinical findings, criteria for diagnosis, and therapies of allergic conjunctival disease are summarized based on the Guidelines for Clinical Management of Allergic Conjunctival Disease (Second Edition) revised in 2010. Allergic conjunctival disease is defined as "a conjunctival inflammatory disease associated with a Type I allergy accompanied by some subjective or objective symptoms." Allergic conjunctival disease is classified into allergic conjunctivitis, atopic keratoconjunctivitis, vernal keratoconjunctivitis, and giant papillary conjunctivitis. Representative subjective symptoms include ocular itching, hyperemia, and lacrimation, whereas objective symptoms include conjunctival hyperemia, swelling, follicular losis, and papillae. Patients with vernal keratoconjunctivitis, which is characterized by conjunctival proliferative changes called giant papilla accompanied by varying extents of corneal lesion, such as corneal erosion and shield ulcer, complain of foreign body sensation, ocular pain, and photophobia. In the diagnosis of allergic conjunctival diseases, it is required that type I allergic diathesis is present, along with subjective and objective symptoms accompanying allergic inflammation. The diagnosis is ensured by proving a type I allergic reaction in the conjunctiva. Given that the first-line drug for the treatment of allergic conjunctival disease is an antiallergic eye drop, a steroid eye drop will be selected in accordance with the severity. In the treatment of vernal keratoconjunctivitis, an immunosuppressive eye drop will be concomitantly used with the abovementioned drugs.

KEY WORDS

allergic conjunctivitis, antiallergic eye drop, atopic keratoconjunctivitis, giant papillary conjunctivitis, vernal keratoconjunctivitis

1. DEFINITION AND CLASSIFICATION OF ALLERGIC CONJUNCTIVAL DISEASE

1.1. DEFINITION

Allergic conjunctival disease (ACD) is defined as "a conjunctival inflammatory disease associated with a type I allergy accompanied by some subjective and objective symptoms." Conjunctivitis associated with type I allergic reactions is considered allergic conjunctival disease even if other types of inflammatory reactions are involved.[1]

1.2. CLASSIFICATION

ACD is classified into multiple disease types according to the presence or absence of proliferative changes, complicated atopic dermatitis, and mechanical irritation by foreign body (Fig. 1).

1.2.1. Allergic Conjunctivitis (AC) (Fig. 2)

Allergic conjunctival diseases without proliferative

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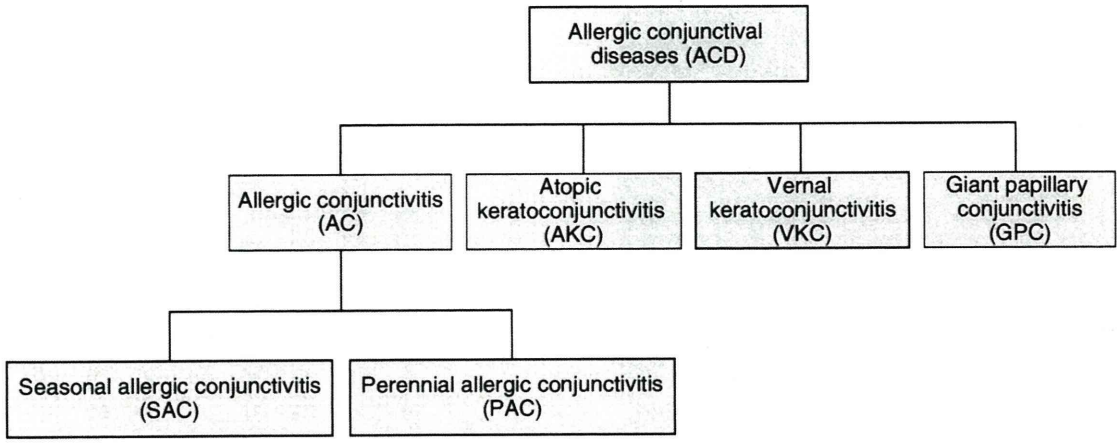

Fig. 1 Classification of ACD. ACD is classified as follows: (i) AC without proliferative change, (ii) AKC complicated with atopic dermatitis, (iii) VKC with proliferative changes, and (iv) GPC induced by irritation of a foreign body. Allergic conjunctivitis are subdivided into SAC and PAC according to the period of onset of the symptoms.

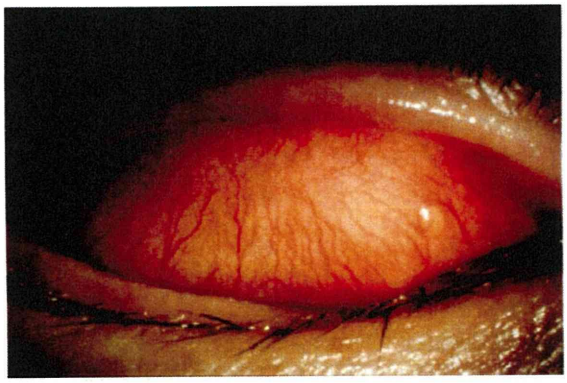

Fig. 2 Upper palpebral conjunctival findings in AC. Mild hyperemia and edema are present.

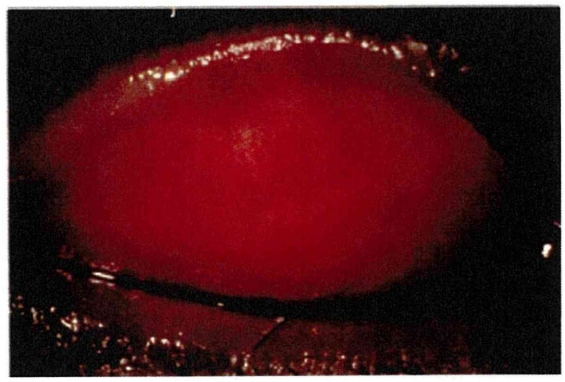

Fig. 3 Upper palpebral conjunctival findings in AKC. Hyperemia, opacity, and sub-conjunctival fibrosis are present.

changes in the conjunctiva include seasonal allergic conjunctivitis (SAC) where symptoms appear in a seasonal manner and perennial allergic conjunctivitis (PAC) where symptoms persist throughout the year.

1.2.2. Atopic Keratoconjunctivitis (AKC) (Fig. 3)

AKC is a chronic allergic conjunctival disease that may occur in patients with facial atopic dermatitis. Giant papillae may be present although many AKC cases have no proliferative changes.

1.2.3. Vernal Keratoconjunctivitis (VKC) (Fig. 4)

VKC is characterized by conjunctival proliferative changes such as papillary hyperplasia of the palpebral conjunctiva or its enlargement, and swelling or limbal gelatinous hyperplasia. Many VKC cases accompany atopic dermatitis. Corneal lesions with various severities including superficial punctate keratitis, corneal erosion, persistent corneal epithelial defect, corneal ulcers, or corneal plaque have been observed in VKC.

1.2.4. Giant Papillary Conjunctivitis (GPC) (Fig. 5)

GPC is conjunctivitis that accompanies proliferative changes in the upper palpebral conjunctiva induced by mechanical irritations such as contact lenses, ocular prosthesis, or surgical sutures. Clinically, GPC differs from VKC by the absence of a corneal lesion and by having a different papillary form.

2. EPIDEMIOLOGY OF ACD

In surveys of the entire population conducted by the Allergy Integrated Project Epidemiologic Investigation Group of the Ministry of Health and Welfare in 1993, the proportion of persons with bilateral ocular itching was 16.1% in children aged less than 15 and 21.1% in adults. The proportion of persons with allergic conjunctival diseases diagnosed by ophthalmologists was 12.2% in children and 14.8% in adults. From these results, the proportion of persons with allergic

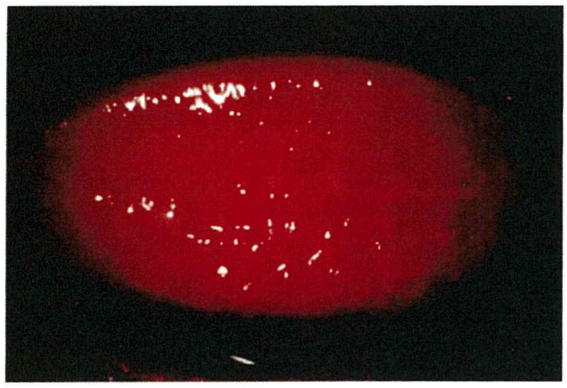

Fig. 4 Upper palpebral conjunctival findings in VKC. Conjunctival hyperemia, conjunctival edema, eye discharge, formation of giant papillae are present.

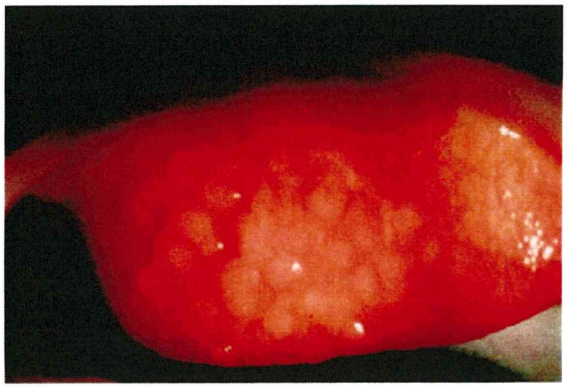

Fig. 5 Upper palpebral conjunctival findings in GPC. Hyperemia and dome-like giant papillae are present.

conjunctival diseases in the entire population is estimated to be about 15-20%.

A research group on allergic ocular disease of the Japan Ophthalmologists Association conducted epidemiologic surveys of all patients with allergic conjunctival diseases that were treated at 28 facilities (7 university attached hospitals, 5 general hospitals, and 16 ophthalmic hospitals and clinics) all over Japan during the period from January 1, 1993 to December 31, 1995. They found that female patients with SAC or PAC outnumbered male patients by 2 : 1, whereas male patients with VKC outnumbered female patients by 2 : 1. The number of patients with ACD was maximum at the age of 10 and the incidence decreased with aging (Fig. 6). The main subjective symptoms were an ocular itching, ocular hyperemia, eye discharge, and a foreign body sensation in each disease type. In SAC, symptoms of allergic rhinitis such as sneezing, rhinorrhea, nasal blockade were found in many cases.

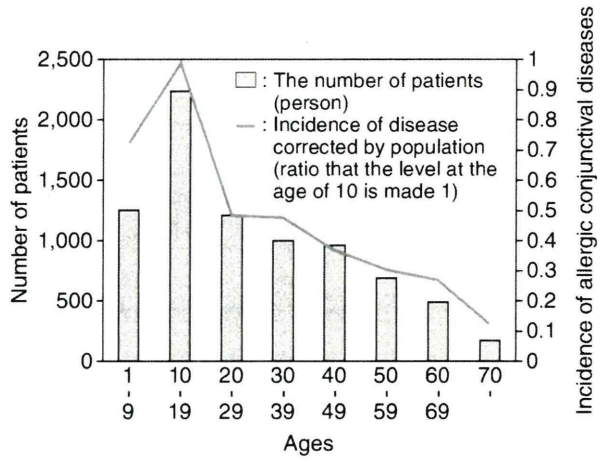

Fig. 6 Age distribution of patients with allergic conjunctival diseases. The number of patients with allergic conjunctival diseases who contacted the facilities was maximum at the age of 10 and the number decreased with aging. The peak incidence of each disease was also at the age of 10 and the incidence decreased with aging. (Incidence is calculated by dividing the number of treated patients in each age group by the entire population of the same age group in Japan. The incidence at the age of 10 is made 1 and the result is expressed as ratio.) Adapted from reference 1.

3. PATHOPHYSIOLOGY

3.1. NETWORK OF VARIOUS IMMUNE SYSTEM CELLS AND KERATOCONJUNCTIVAL RESIDENT CELLS

The pathological conditions of ACD with lesions in the conjunctiva is assumed to be caused by interactions between various immune system cells and resident cells, which are mediated by physiologically active substances (e.g. histamine and leukotriene), cytokines, and chemokines.

3.2. EOSINOPHILS AND CYTOTOXIC PROTEINS

Eosinophils are the main effector cells in ACD. Various cytotoxic proteins released from eosinophils infiltrating locally into the conjunctiva are thought to cause keratoconjunctival disorders such as severe AKC and VKC (Fig. 7).

3.3. HELPER T AND B CELLS AND CYTOKINES

An immunological feature of AKC and VKC is the infiltration of CD4+ helper T cells (Th) and IgE-producing B cells into the conjunctiva. In normal tunica propia conjunctivae, there are resident CD4+ T cells and CD8+ T cells.[2,3] Since T cell clones infiltrate into the conjunctiva in VKC patients and produce Th2 cytokines such as IL-4,[4] which is detected in high concentrations in the lacrimal fluid of VKC patients,[5]

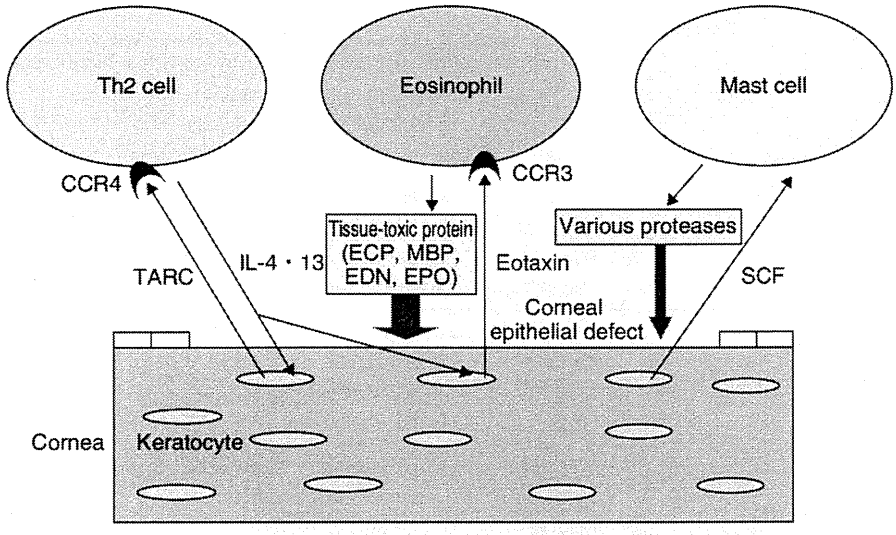

Fig. 7 Mechanisms of corneal disorder in severe allergic conjunctival diseases. Various cytotoxic proteins released from eosinophils and proteases released from mast cells induce corneal epithelial disorders. IL-4 and IL-13 infiltrate from the defective epithelium into the stromal layer to activate the keratocytes and produce chemokines such as TARC, eotaxin, and SCF, and cytokines. These molecules attract Th2 cells, eosinophils, and mast cells to the cornea. TARC, thymus-and activation-regulated chemokine; CCR4, Chemokine receptor 4; CCR3, Chemokine receptor 3; IL, Interleukin; SCF, stem cell factor; ECP, eosinophil cationic protein; MBP, major basic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase.

it appears that Th2 cells and Th2 cytokines predominate in VKC. On the other hand, it is reported that in the conjunctivae of AKC patients, Th1 and Th2 may be competitive.[6] It is also speculated that keratoconjunctival resident cells may be involved in the etiology of ACD by cytokine-stimulated production of chemokines such as eotaxin[7,8] and TARC,[9] which cause eosinophil and Th2 cell migrations from the circulation respectively (Fig. 7).

4. TEST METHODS

The objective of tests is to prove a type I allergic reaction in the conjunctiva and in the whole body. Clinical test methods for proving type I allergic reactions in the conjunctiva include the identification of eosinophils in the conjunctiva, instillation provocation test, and total IgE antibody measurements in lacrimal fluid. Systemic allergy tests detect antigen specific IgE antibodies in the skin and serum.

4.1. IDENTIFICATION OF EOSINOPHILS IN THE CONJUNCTIVA

Eye discharge or ocular secretions collected using spatulas and tweezers, are smeared onto glass slides, then stained by Hansel or Giemsa staining methods, and observed under an optical microscope.[10]

4.2. INSTILLATION PROVOCATION TEST

When an antigen can be presumed by skin test or serum antigen specific IgE antibody measurements, this test confirms the presence of conjunctivitis by instillation of a solution of the known antigen. If itching or hyperemia follows, the case is evaluated as being positive.

4.3. TOTAL IgE ANTIBODY MEASUREMENT IN LACRIMAL FLUID

A kit is commercially available to measure the level of total IgE antibody in lacrimal fluid using an immunochromatography.[11]

5. THE CLINICAL FEATURE AND EVALUATION CRITERIA

5.1. SUBJECTIVE SYMPTOMS

Representative subjective symptoms for ACD are itching, foreign body sensation, and eye discharge.

Itching is the most characteristic symptoms in ACD, but some patients complain of a foreign body sensation instead. The foreign body sensation is frequently present in ACD. Aside from cases where slight itching is felt as a foreign body sensation, it is very likely that when many conjunctival papillae sweep the cornea at the time of blinking, a foreign body sensation may occur. In ACD lymphocytes and eosinophils account for the majority of inflammatory

Table 1 Clinical evaluation criteria of allergic conjunctival diseases

Palpebral conjunctiva	Hyperemia	Severe	Impossible to distinguish individual blood vessels
		Moderate	Dilatation of many vessels
		Mild	Dilatation of several vessels
		None	No manifestations
	Swelling	Severe	Diffuse marked edema
		Moderate	Diffuse mild edema
		Mild	Localized edema
		None	No manifestations
	Follicle	Severe	20 or more follicles
		Moderate	10-19 follicles
		Mild	1-9 follicles
		None	No manifestations
	Papillae†	Severe	Diameter ≥0.6 mm
		Moderate	Diameter 0.3-0.5 mm
		Mild	Diameter 0.1-0.2 mm
		None	No manifestations
	Giant papillae	Severe	Elevated papillae in 1/2 or more of upper palpebral conjunctiva
		Moderate	Elevated papillae in less than 1/2 of upper palpebral conjunctiva
		Mild	Flat giant papillae
		None	No manifestations
Bulbar conjunctiva	Hyperemia	Severe	Vasodilatation of all vessels
		Moderate	Dilation of many vessels
		Mild	Dilation of several vessels
		None	No manifestations
	Chemosis	Severe	Cyst-like chemosis of entire conjunctiva
		Moderate	Diffuse thin chemosis
		Mild	Partial conjunctival swelling
		None	No manifestations
Limbus	Swelling	Severe	In ≥2/3 of circumference
		Moderate	In 1/3 to 2/3 of circumference
		Mild	In less than 1/3 of circumference
		None	No manifestations
	Horner-Trantas dots	Severe	≥9 dots
		Moderate	5-8 dots
		Mild	1-4 dots
		None	No manifestations
Cornea	Epithelial disorder	Severe	Shield ulcer or epithelial erosion
		Moderate	Superficial punctate keratitis with filamentary debris
		Mild	Superficial punctate keratitis
		None	No manifestations

† In cases having giant papillae, papillae and giant papillae should be graded simultaneously.

cells, while neutrophils are few, serous and mucous discharge is often present, and the nature of the discharge differs from the purulent discharge associated with bacterial conjunctivitis and viscous and serous discharges found in viral conjunctivitis.

5.2. OBJECTIVE SYMPTOMS

Conjunctival hyperemia with dilated conjunctival vessels is the most frequent conjunctival finding. Conjunctival swelling is a finding that is induced by circulatory failure of the palpebral conjunctival vessels and lymphatic vessels. And in many cases, conjunctival opacity is accompanied. A conjunctival follicle is a lymphoid follicle seen under the lower palpebral conjunctival epithelium. This finding can be discriminated from papillae by the condition of a smooth