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Relation of Streptococcal Pyrogenic Exotoxin C as a Causative Superantigen for Kawasaki Disease

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ABSTRACT

We previously reported that the frequency of TCRBV2 and TCRBV6S5-bearing T-cells was high in patients in the acute phase of Kawasaki disease (KD) and that streptococcal pyrogenic exotoxin C (SPE-C) was a potent stimulator of these TCRBV-bearing T-cells. To further elucidate the pathogenesis of KD, we examined the T-cell receptor (TCR) repertoire, human leukocyte antigen (HLA)-DRB1 genotype, and antibody responses to recombinant(r) SPE-C in patients with KD. We also performed *in vitro* stimulation with rSPE-A and rSPE-C of peripheral blood mononuclear cells from healthy donors and characterized the reacting T-cells. The percentage of T-cells bearing TCRBV2 and TCRBV6S5 was high in patients in the acute stage of KD. rSPE-C stimulation of PBMC from healthy donors induced expansion of TCRBV2 and TCRBV6S5-bearing T-cells. Furthermore, serum levels of anti-SPEC antibodies, which did not display antimitogenic activity, were higher in patients with acute KD than in age-matched controls. The frequencies of the DRB1*04051, 0406, and 0901 were high, whereas that of the DRB1*1101 was low among patients with KD as compared with the healthy adults. **(*Pediatr Res* 53: 403–410, 2003)**

Abbreviations

HLA, human leukocyte antigen
IVIg, intravenous immunoglobulin
KD, Kawasaki disease
SPE-A, streptococcal pyrogenic exotoxin A
SPE-C, streptococcal pyrogenic exotoxin C
SSOPs, sequence-specific oligonucleotide probes
TCR, T-cell receptor
TSS, toxic shock syndrome
SA, superantigen

Kawasaki disease (KD) is an acute illness of early childhood. It is characterized by fever lasting >5 d, edema and redness of hands and feet, erythematous skin rash, bilateral conjunctival congestion, redness of oropharyngeal mucosa, strawberry tongue, red and fissuring lips, and nonsuppurative cervical lymphoadenopathy. The clinical features of the acute phase of KD were first described as a new mucocutaneous lymphocytotic syndrome by Kawasaki in 1967 (1). Although the symptoms of KD are usually self-limited, coronary artery abnormalities develop in 15% to 25% of patients. In Japan and the United States, KD has become one of the most common causes of acquired heart disease in children. It has been demonstrated that i.v. administration of gamma globulin during the acute phase of KD can significantly decrease the prevalence of coronary artery lesions.

In general, normal antigens to MHC class II and the complementarity-determining region 3 of TCR Vβ receptor recognize the specificity of antigens and lead to clonal expansion of T-cells. However, superantigens (SAs) produced by bacteria simultaneously bind to MHC class II and T-cell receptor (TCR) molecules via a certain TCR Vβ domain that is outside the normal antigen binding site; thus, a given SA can stimulate all T-cells that bear the appropriate TCR Vβ receptor in a polyclonal manner. These SAs also stimulate high systemic levels of proinflammatory cytokines that cause hypertension, fever, and shock.

Investigators have reported that there is marked activation of T-cells (2) and monocyte/macrophages (3–6) and increased production of cytokines (6) at the acute phase of KD. These immunopathological features are similar to those of diseases caused by bacterial toxins acting as SAs. Immunologic and clinical studies have revealed that there is a remarkable similarity among KD, toxin-mediated staphylococcal and streptococcal toxic shock syndrome (TSS) (7–9), and scarlet fever. The selective expansion of T-cells that bear TCRBV2 in patients with KD (10–13) or TSS (14) has been reported by

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several investigators. There are, however, several kinds of SAs, which stimulate TCRBV2-bearing T-cells (15), and the causative SAs have not been defined.

We previously reported selective expansion of TCRBV2 and TCRBV6S5 after *in vitro* stimulation of T-cells with recombinant streptococcal pyrogenic exotoxin-C (SPE-C) (16). We also reported polyclonal expansion of TCRBV2 and TCRBV6S5-bearing T-cells in patients in the acute phase of KD but not in those in the convalescent phase of KD or in age-matched controls (17). Furthermore, serum levels of anti-SPEC antibodies were significantly higher in patients with acute and convalescent KD than in age-matched controls, suggesting that these patients were infected with SPE-C–producing streptococcus (17). These findings suggest a possible role of SPE-C in the pathogenesis of KD.

In the present study, we confirm *1*) the expansion of TCRBV2- and TCRBV6S5-bearing T-cells in patients in the acute stage of KD and *2*) the expansion of these T-cells after *in vitro* stimulation with SPE-C of peripheral blood lymphocytes from healthy donors. We also present serological evidence of streptococcal infection in patients with KD.

METHODS

Samples. Peripheral blood specimens were obtained from 16 patients with KD (Table 1) at the acute stage (before treatment) and 206 age-matched controls. The patients received a diagnosis KD when they had more than five of the six major clinical signs—fever for 5 or more days, nonexudative conjunctival injection, mucosal changes such as dry fissured lips and strawberry tongue, cervical lymphadenopathy, polymorphous truncal rash, and extremity changes (erythema, edema, and desquama)—or if they had four of the six major signs and coronary arterial lesions. All of the patients and age-matched controls were hospitalized in the Wakayama Medical University Hospital. Peripheral blood specimens were also obtained from nine healthy donors for *in vitro* stimulation tests. Peripheral blood mononuclear cells (PBMC) and plasma were isolated from peripheral blood by Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. PBMC were washed in RPMI 1640 (BRL, Bethesda, MD, U.S.A.) and used in the experiments. The plasma samples were kept frozen until use. Informed consent was obtained before collection of blood samples, in accordance with the domestic guidelines of the hospital. The study was approved by the Wakayama University ethics committee.

Stimulation of PBMC with rSPE-A and rSPE-C. Human PBL were incubated at 1×10^6 cells in 100 μL of RPMI containing 10% FCS and 1 μg/mL of rSPE-A and rSPE-C in 24-well plates at 37°C for 4 d. The cells were harvested and used for analysis of the TCR repertoire.

Analysis of TCR repertoire. Crude cellular RNA were extracted from PBMC and stimulated cells by TRIzol LS Reagent (BRL), according to the manufacturer's instructions. Adaptor-ligation PCR and microplate hybridization assay were carried out as previously described (18–21). Briefly, 1 μg of total RNA was converted to double-stranded cDNA using the SuperScript cDNA synthesis kit (BRL) according to the manufacturer's instructions, except for priming with BSL-18e primer adaptor containing the Sph I site. The P20EA/10EA universal adaptors were ligated at the 5' end of BSL-18e primed cDNA. Three rounds of Cα- and Cβ-specific PCR were performed using CA and CB sequence-specific oligonucleotide probes (SSOP) to prepare amplified and biotinylated TCR cDNA pools. Hybridization was carried out between biotinylated PCR products, and VA or VB SSOP were immobilized on carboxylate-modified ELISA plate (Sumitomo Bakelite, Tokyo, Japan). The hybridization was visualized with *p*-nitrophenylphosphate (Nacalai Tesque, Osaka, Japan). The visualized signals were estimated at 405 nm using Immunoreader NJ-2000 (Nihon Intermed, Tokyo, Japan). The rela-

Table 1. *Demographic Data of Patients With KD*

			Clinical symptom						
	Sex	Age*	Fever	Nonexudative conjunctival injection	Dry fissured lips and strawberry tongue	Cervical lymphadenopathy	Polymorphous truncal rash	Erythema, edema, and desquama	Coronary arterial lesions
KD01	Female	4y10m	+	+	+	+	+	+	−
KD02	Male	1y9m	+	+	+	+	+	+	−
KD03	Female	1y7m	+	+	+	−	+	+	−
KD04	Male	4m	+	+	+	+	+	+	−
KD05	Male	6m	+	+	+	+	+	+	−
KD06	Female	10m	+	+	+	+	+	+	−
KD07	Female	7m	+	+	+	+	+	+	−
KD08	Male	2y9m	+	+	+	+	+	+	−
KD09	Female	1y9m	+	+	+	+	+	+	−
KD10	Female	3y7m	+	+	+	+	−	+	−
KD11	Female	1y3m	+	+	+	+	+	+	−
KD12	Male	7m	+	+	+	+	+	+	−
KD13	Male	7m	+	+	+	+	+	+	−
KD14	Male	2y6m	+	+	+	+	+	+	−
KD15	Male	1y5m	+	+	+	+	+	+	−
KD16	Male	2m	+	+	+	+	+	+	−

+, symptom present; −, symptom not present.
* Age at onset of fever.

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tive expansion of the TCRAV or TCRBV region repertoire was calculated by the following formula: relative expansion of subfamily (%) = corresponding SSOP signal × 100/sum of total TCR V SSOP signals.

CDR3 size analysis of TCRBV. Knowing the size of the CDR3 region in TCRBV would allow estimation of the polyclonal expansion of T-cells (22). We used this technique to determine the polyclonality of T-cells after *in vitro* stimulation with SA and of those from patients in the acute phase of KD. The second PCR products described above were labeled by 20-cycle PCR amplification with FAM-labeled Cβ-SSOP (22). The labeled PCR products were mixed with the size marker (GeneScan-500 TAMRA; Applied Biosystems, Warrington, UK) and loaded onto a 6% polyacrylamide sequencing gel to determine size and fluorescence intensity using an automated DNA sequencer (ABI 377; Perkin-Elmer Applied Biosystems, Foster City, CA, U.S.A.). Data were analyzed using GeneScan software (Perkin-Elmer Applied Biosystems).

DRB1 genotyping. Human leukocyte antigen (HLA)-DRB1 genotyping was carried out by the PCR-restriction fragment length polymorphism (RFLP) method as previously described (23), except that several endonucleases were included to detect new DRB1 alleles (24). Briefly, the DRB1 regions were amplified by PCR from the P20EA/10EA universal adaptor-ligated cDNA as described above. Each product was digested with the appropriate endonuclease, and the DRB1 genotype was determined by the RFLP band patterns on 10% polyacrylamide gel.

Detection of antibody to rSPE-C in serum samples. The rSPE-C was purified from fusion proteins expressed in *Escherichia coli* as previously described (16). Levels of immunoglobulin (Ig) antibodies against rSPE-C in the serum samples were assessed by ELISA methods using purified rSPE-C as antigens (25). Purified recombinant proteins were diluted to 2 μg/mL in 10 mM PBS (pH 7.4), and 100 μL was added to each well of 96-well microplates (Immuno Module F8 Maxisorp).

The plates were incubated overnight at 4°C to allow binding of the antigens to the wells. Unbound antigens were removed by aspiration, and the wells were washed four times with washing buffer (PBS containing 0.8 g/L Tween 20). PBS containing 10 g/L BSA was added to each well of the microplate and incubated at 30°C for 2 h for blocking. The wells were washed four times with washing buffer and filled with dilution buffer (PBS containing 1.0 g/L BSA). The SPE-C coated plates were stored at 4°C until use.

Serum samples from 38 patients with KD at the acute stage (2–14 d of illness; before treatment) and 206 age-matched controls were diluted to 1:200 with dilution buffer, and 100 μL of diluted serum was added to the rSPE-C coated wells. The plates were incubated at 4°C overnight, and the wells were then washed four times with washing buffer. Peroxidase-conjugated, F(ab')2 rabbit anti-human Ig was diluted to 1 000 ng/mL with dilution buffer, 100 μL of the reagent was added to each well, and the plates were incubated at 30°C for 2 h. The wells were again rinsed four times with washing buffer. The reaction was visualized by subsequent reaction with 100 μL of substrate solution (TMB Peroxidase EIA Substrate Kit; Bio-Rad, Hercules, CA, U.S.A.) for 5 min at room temperature. The reaction was terminated by addition of 50 μL of 1 M sulfonic acid, and the absorbance of each well was measured at 450 nm with an ImmunoReader NJ-2000.

Antimitogenic assay. A total of 10 μL of 20-fold dilution with RPMI1640 of human serum or 4 mg/mL intravenous immunoglobulin (IVIg; Teijin, Tokyo, Japan) was added to each well at the start of the human PBMC culture. Inhibition of the mitogenic activity was calculated by ^{3}H-Tdr (Amersham, Little Chalfont, UK) incorporation. Inhibition was assessed as positive when the difference of counts per minute between cultures containing both rSPE-C and pooled serum or diluted IVIg and those containing SPE-C alone were statistically significant according to the *t* test.

RESULTS

Stimulation of selected TCRBV-bearing T-cells by rSPE-A and rSPE-C. PBMC from nine healthy donors were stimulated *in vitro* with rSPE-A and rSPE-C for 4 d, and the changes of the TCRBV repertoire were analyzed. Expansion of these TCR-bearing T-cells was defined as significant when the percentage frequency of T-cells was greater than the mean percentage plus 2 SD of T-cells bearing the respective TCR in the PBMC from nine healthy donors without *in vitro* stimulation (Fig. 1). The percentage frequency of TCRBV2-bearing T-cells significantly increased in PBMC from eight of nine donors, and the percentage frequency of TCRBV6S5-bearing T-cells also increased in PBMC from four donors with rSPE-C. The percentage frequencies of TCRBV12-, TCRBV14-, and TCRBV15-bearing T-cells significantly increased in PBMC from all nine donors, and the percentage frequency of TCRBV13-bearing T-cells also increased in PBMC from six donors with rSPE-A. The increases of these TCRBV-bearing T-cells were due to polyclonal expansion based on the CDR3 size distribution analysis (Fig. 2).

We previously reported that PBMC of healthy donors was stimulated by mitogenic factors rSPE-A and rSPE-C (16) and that rSPE-C selectively stimulated TCRBV2- or TCRBV6S5-bearing T-cells when the donor had HLA-DRB1*0901 (16). In

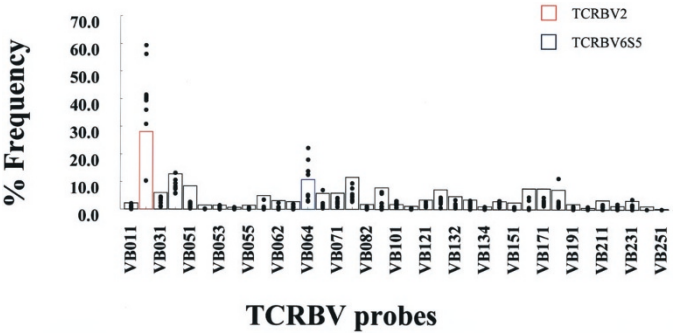

Figure 1. TCRBV repertoires in the PBMC from nine healthy donors who were stimulated *in vitro* with rSPE-A and rSPE-C. The bar indicates mean + 250 of the TCRBV repertoires in the PBMC from nine healthy donors without *in vitro* stimulation. Each dot indicates the percentage frequency of TCRBV-bearing T-cells in the PBMC from nine healthy donors who were stimulated *in vitro* with rSPE-C. The target families of VB02-1 and VB06-4 SSOP were TCRBV2 and TCRBV6S5. Red column indicates TCRBV2 and blue column indicates TCRBV6S5.

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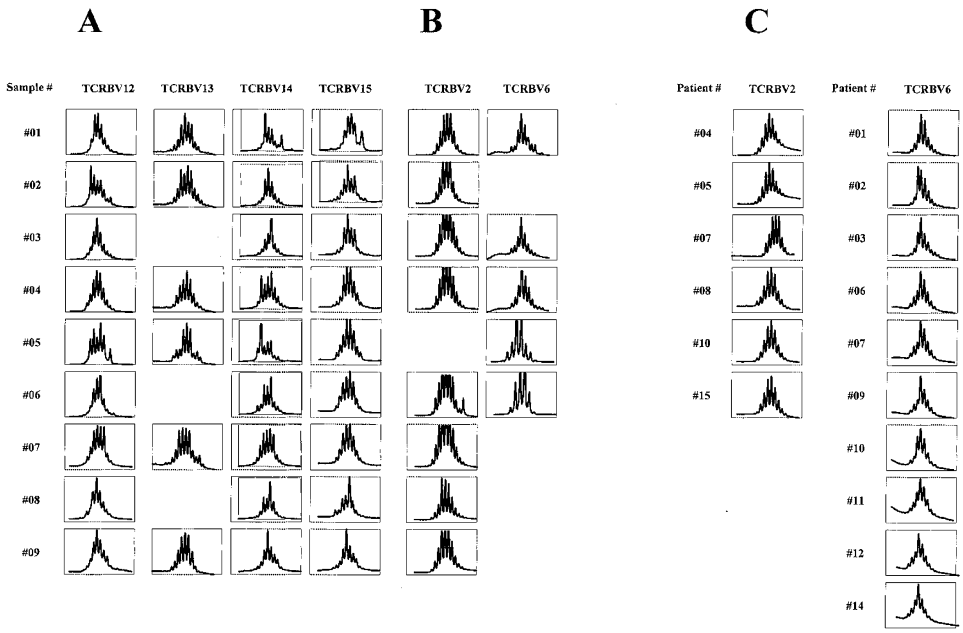

Figure 2. CDR3 size distribution pattern of TCRBV subfamilies in the T-cells after *in vitro* stimulation with SPE-A (*A*) and SPE-C (*B*) and in patients in the acute-phase of KD (*C*). The numbers of the peaks indicate the size variety of CDR3 of TCRBV. Several peaks in each square mean polyclonal expansion of T-cells, and one or two peaks mean oligoclonal expansion of T-cells.

the present study, the levels of TCRBV6S5-bearing T-cells were increased in PBMC from three (donors 1, 3, and 6) of four donors with HLA-DRB1*0901 after *in vitro* stimulation (Table 2).

Analysis of the expansion of TCRBV in patients with KD. We investigated TCRAV and TCRBV repertoires in PBMC from 16 children in the acute stage of KD and 14 children with other illnesses as age-matched controls, using adaptor-ligation PCR (18) and microplate hybridization assay (21). The expansion of TCR-bearing T-cells in patients with KD was defined as significant when the percentage frequency of T-cells was greater than the mean percentage plus 2 SD of T-cells bearing the respective TCR in age-matched controls (Fig. 3). There was no selective expansion of the TCRAV subfamily in patients with KD (data not presented). The expansion of TCRBV2 and TCRBV6S5 was markedly increased in six (38%) and ten (63%) patients with KD, respectively, compared with age-matched controls (Table 3). Thus, the levels of TCRBV2 and/or TCRBV6S5-bearing T-cells were significantly higher in

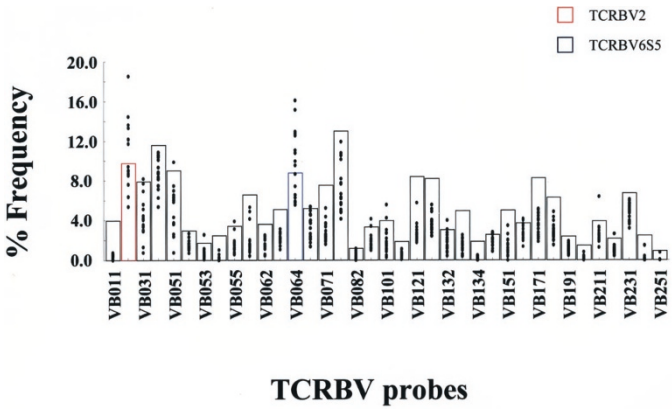

Figure 3. TCRBV repertoires in the PBMC from nine patients with KD. The bar indicates mean + 2 SD of TCRBV repertoires in the PBMC from 14 age-matched controls, and each dot indicates the percentage frequency of TCRBV-bearing T-cells in the PBMC from patients with KD.

Table 2. *HLA-DRB1 Genotypes of Healthy Donors and Increase of TCRBV2- and/or TCRBV6S5-Bearing T-Cells After In Vitro Stimulation With rSPE-C*

			Ratio (stimulated/ unstimulated)*	
Sample No.	HLA DR	Sex	BV2.1	BV6.5
1	0901/1502	M	3.7	2.2
2	1302/1501	M	4.3	0.3
3	0405/0901	M	2.2	1.9
4	0406/1502	M	1.9	1.4
5	1403/1502	F	0.4	1.7
6	0901/1406	F	3.7	1.5
7	1307/1502	M	4.0	0.6
8	0403/0901	F	3.8	0.8
9	1403/1502	F	3.4	0.7

* The ratio was calculated using the following formula: %TCRBV-bearing T-cells in SPE-C–stimulated PBMC/%TCRBV-bearing T-cells in unstimulated PBMC.

Table 3. *Comparison of Number of Acute Patients With KD and Expansion of TCRBV2- and/or TCRBV6S5-Bearing T-Cells in 1995–1996 and With That in 1999*

No. of patients with KD	Year	No. of positive (%)		
		TCRBV2	TCRBV6S5	BV2 and/or BV6S5
22	1995–1996	9 (41)	13 (59)	17 (77)
16	1999	6 (38)	10 (63)	13 (81)

Positive, Ratio > 1.0.

Ratio = % TCRBV-bearing T-cells in patients with KD/(mean + 2 SD) of % TCRBV-bearing T-cells in controls.

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13 (81%) of 16 patients in the acute phase of KD. Table 3 also allows comparisons of the number of patients with acute KD and expansion of TCRBV2 and/or TCRBV6S5-bearing T-cells in 1995–1996 (17) and the number with it in 1999. The number of patients with acute KD and expansion of these TCRBV-bearing T-cells in both periods paralleled each other. The expansion of these TCRBV2 and TCRBV6S5-bearing T-cells was polyclonal (Fig. 2).

The patients with KD, as individuals, did demonstrated expansion of TCRBV2 and TCRBV6S5-bearing T-cells compared with age-matched controls. We next compared the percentage frequency of theses TCRBV-bearing T-cells from 38 patients with KD who were hospitalized in 1995–1996 and 1999 at the early acute (ranging from 3 to 14 d), late acute (ranging from 19 to 22 d), and convalescent (ranging from 90 to 140 d) stages (Fig. 4). The expansion of these TCRBV2- and TCRBV6S5-bearing T-cells increased in the acute and late acute stages of KD, compared with the late convalescent stage of KD.

HLA-DRB1 genotyping. We previously reported that there was a relationship between selective expansion of T-cells that bear TCRBV2 and TCRBV6S5 subfamilies *in vivo* and HLA-DRB1*0901 in patients with KD (17) and after *in vitro* stimulation of PBMC with SPE-C (16). In the present study, we carried out HLA-DRB1 genotyping to determine the dominant DRB1 genotype among patients with KD. The frequencies of DRB1 in 56 patients with KD and healthy adults in the Kansai area are shown in Table 4. The frequencies of the DRB1*04051, 0406, and 0901 were higher, whereas that of 1101 was lower in patients with KD as compared with the healthy adults.

Antibody titers to rSPE-C in serum samples from patients with KD and age-matched controls. We examined serum antibody levels to rSPE-C in the serum from 38 patients in the acute phase of KD and from 205 age-matched controls to determine the potential pathogenic role of SPE-C in KD. We divided the patients and controls into eight age groups and compared the levels of antibodies against rSPE-C among the age groups (Fig. 5). Sera from older healthy children contained higher levels of antibodies against rSPE-C. Most patients with KD were in age groups younger than 2 years. Sera from most of these patients with KD at ages from 6 mo to 2 y contained higher levels of antibodies than the mean levels of the age-matched controls.

Antimitogenic antibodies in patients with KD. Figure 6 shows a representative experiment of antimitogenic antibodies of pooled sera from 16 patients with KD and nine healthy adults, and diluted IVIg. Pooled sera and diluted IVIg did not induce proliferation of PBMC. Weak inhibition of SPE-C mitogenicity was seen in the sera from healthy adults but strong inhibition in IVIg. Patients with KD had higher sera antibody titers against SPE-C than the age-matched controls, but these antibodies did not indicate antimitogenic activity.

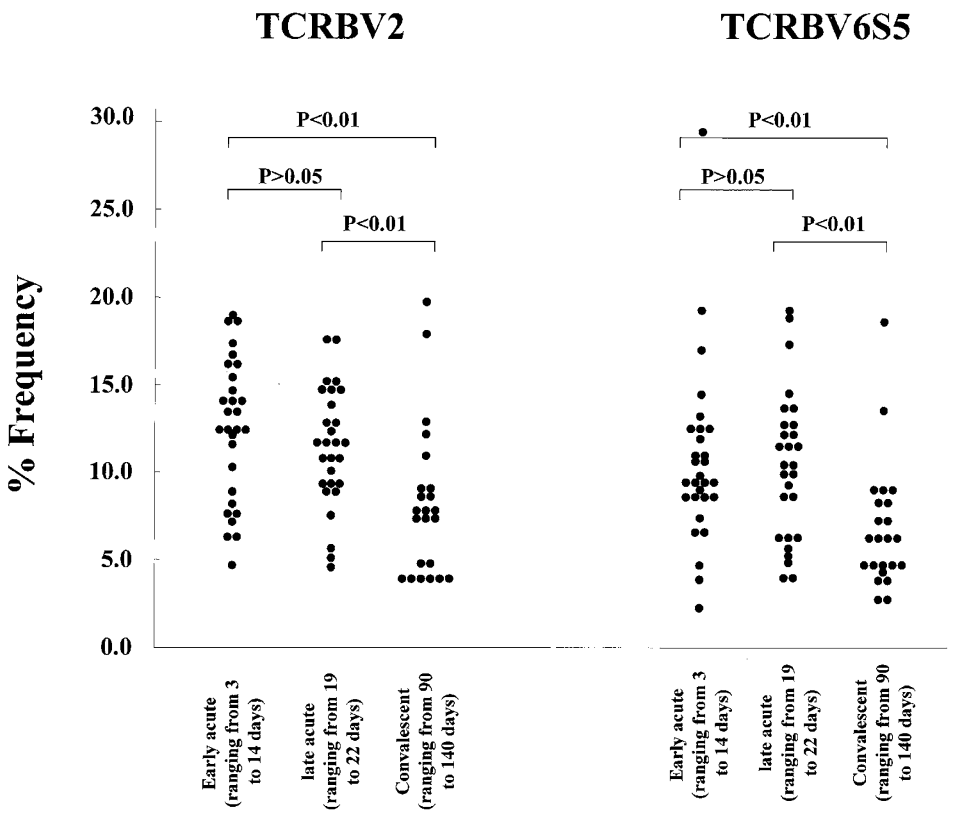

Figure 4. TCRBV2 and TCRBV6S5 expression of patients with KD at the acute, early convalescent, and late convalescent stages.

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Table 4. *Gene Frequencies of HLA-DRB1 Alleles*

DRB1	GF (%) KD (n = 56)	GF (%) Healthy adults (n = 498)	p
0101	5.4	7.4	
0401	1.8	0.5	
0403	1.8	2.8	
04051	*19.6*	*12.8*	0.0120
0406	*7.1*	*1.8*	0.0008
0407	1.8	0.8	
0410	0.9	2.0	
0802	3.6	6.6	
08032	8.0	6.1	
0901	*15.2*	*10.8*	0.0383
1001	1.8	0.5	
1101	*1.8*	*5.4*	0.0404
1201	2.7	3.3	
1302	4.5	7.4	
1401	1.8	3.3	
1405	1.8	2.8	
1406	1.8	2.3	
1501	6.3	7.9	
1502	10.7	7.4	
1503	1.8	–	

GF (%), genotype frequency.

DISCUSSION

Epidemiologic evidence strongly supports an infectious cause for KD (26). Some investigators reported selective and polyclonal expansion of T-cells that bear TCRBV2 in patients with KD (10–13), and SAs have been implicated in triggering this disease (12, 13). However, the causative SA for KD has not been defined, because there are several kinds of SAs that specifically stimulate TCRBV2 (15). We previously reported that T-cells with TCRBV2 and TCRBV6S5 subfamilies were expanded *in vivo* in approximately 80% of patients with KD (17) and that SPE-C specifically stimulates TCRBV2- and TCRBV6S5-bearing T-cells *in vitro* (16). Furthermore, there were similarities between the cytokines induced *in vitro* by stimulation of PBMC with SPE-C and those detected in patients with KD (16, 17). These results suggested a possible role of SPE-C in the pathogenesis of KD. In the present study, we confirmed the expansion of TCRBV2- and TCRBV6S5-bearing T-cells in patients in the acute phase of KD by analyzing different KD patients.

In Fig. 4, the percentage frequency did not fall to low levels until after day 20. This seems to be contrary to the kinetics of a superantigen-mediating response. It may be partially due to the patients' exposure to a low dose, not a high dose, of SA. However, patients with TSS may have been exposed to a higher dose of SA. The different of clinical features of these diseases (including kinetic T-cell response) may be due to different levels of SA exposure.

Our previous data demonstrated that there was some relationship between HLA-DRB1*0901 and expansion of T-cells that bear TCRBV2 and TCRBV6S5 after *in vitro* stimulation with rSPE-C (16). We carried out *in vitro* stimulation with rSPE-C using PBMC from nine healthy donors, four of whom had HLA-DRB1*0901. T-cells bearing TCRBV2 and TCRBV6S5 were expanded after stimulation in PBMC from three of four donors with DRB1*0901. It is not known whether high levels of expansion of some T-cells caused by SA are associated with certain HLA alleles, but this may in part be due to different binding affinities of the rSPE-C to specific HLA-DRB1 alleles of the donors. Yili *et al.* (27) reported the structure of SPE-C complexed with HLA bearing conventional antigen. Their data indicate that SA can bind to HLA with and without

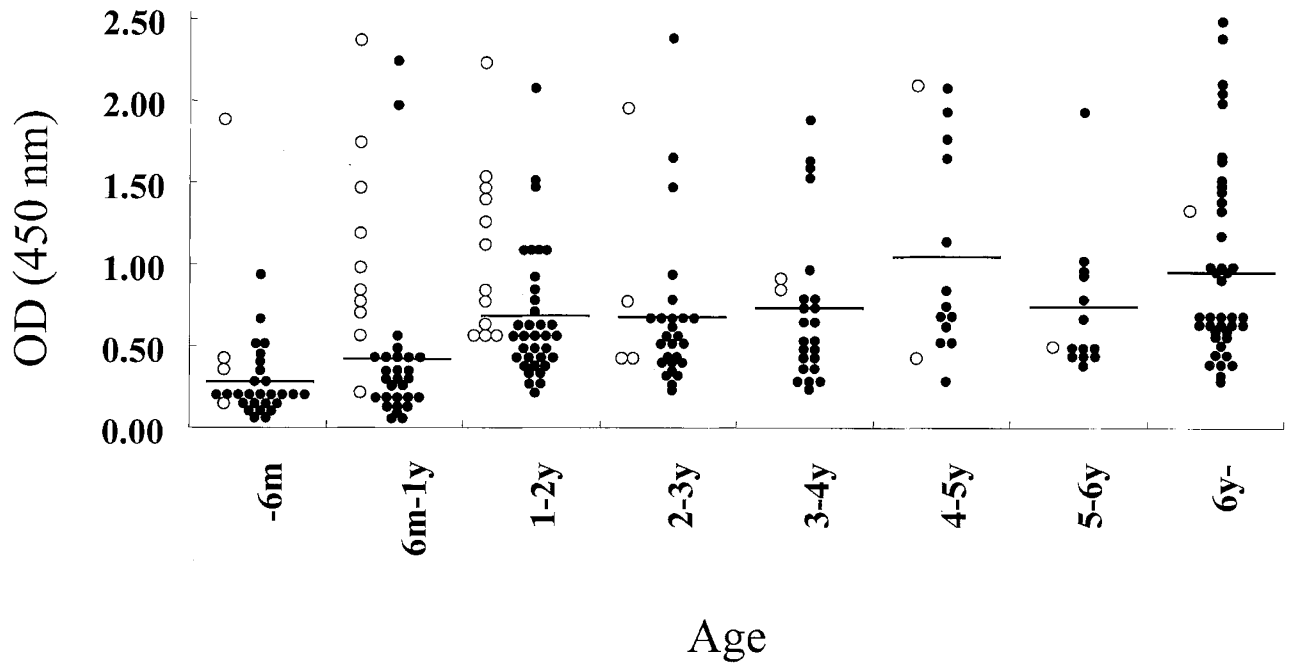

Figure 5. Antibody titers to rSPE-C in serum samples from patients with KD (n = 36) and age-matched controls. ○, antibody titers of patients with KD; ●, antibody titers of controls. Horizontal bars indicate the mean percentage of the controls.

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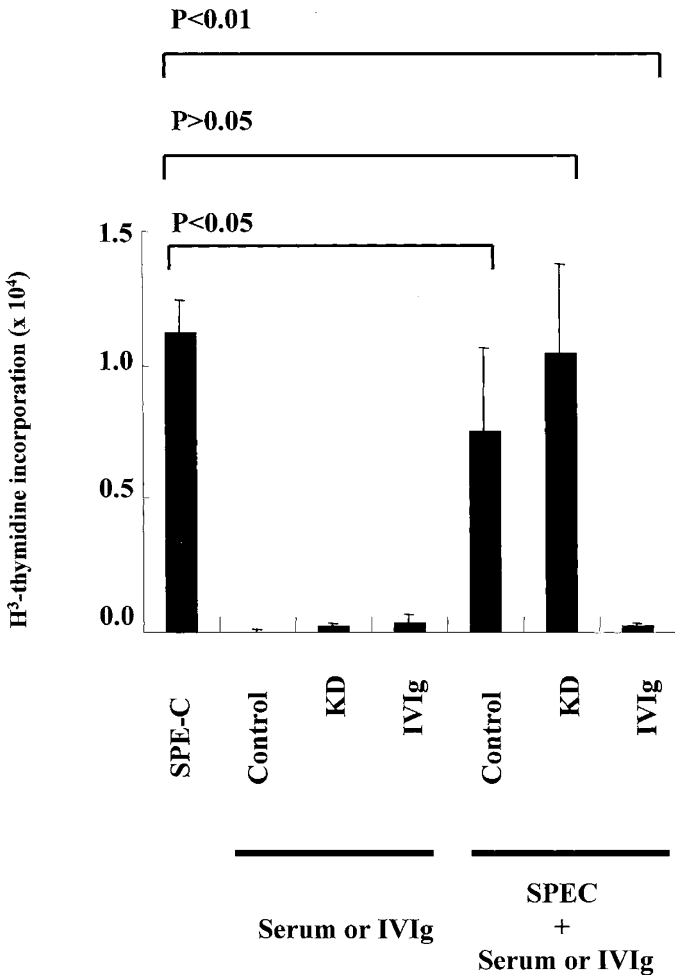

Figure 6. Inhibition of SPE-C mitogenicity by sera from patients with KD and healthy adults and IVIg. PBMC (1×10^6) were incubated in 96-well microtiter plates (Corning Costar Co., Cambridge, MA) with 5 ng/mL SPE-C, sera, IVIg, SPE-C + sera, and SPE-C + IVIg for 4 d. Cells were pulsed with 0.5 mCi/well ^{3}H-Tdr for 8 h and then harvested. The radioactivity was measured with a liquid scintillation counter.

peptide. They say that SA such as SPE-C are able to stimulate T-cells with an efficiency comparable to that achieved by specific peptide/HLA ligands.

The frequencies of HLA-DRB1*0405, 0406, and 0901 were higher and that of HLA-DRB1*1101 was lower in patients with KD compared with the data of healthy Japanese adults from the Kansai region of west Japan (1996). The high frequency of some HLA alleles in patients with KD may be due to high biding affinities of the causative SA to these HLA-DRB1 alleles. The other possibility is that patients with KD who have these alleles may be susceptible to infection by streptococcus and be prone to develop these diseases. Takata *et al.* (28) reported that subjects >90 years had extremely low HLA-DRw9 and high DR1 frequencies compared with healthy adults in various age brackets in Japan and concluded that DRw9 is highly associated with autoimmune or immune deficiency diseases. Furthermore, there is a significant linkage between HLA-DRB1*0901 and polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-α gene which regulates the levels of TNF-α production in immune responses to various stimuli (29). Thus, the higher frequency of some HLA alleles in patients with KD may be caused by multiple factors.

We previously reported that patients with KD had higher levels of sera antibodies to rSPE-C than age-matched controls (17). In the present study, we also examined the levels of antibody to rSPE-C in the sera samples from 38 patients with KD in the acute phase and from 205 age-matched controls. Young patients with KD at ages of 6 mo to 2 y possessed high levels of sera antibodies to rSPE-C compared with the mean levels of age-matched controls. However, some patients with KD had antibodies to rSPE-C at similar levels to the mean of age-matched controls. Interestingly, these patients had an incomplete type of KD. In severe group A streptococcal infection, patients with TSS showed an increase in T-cells of the TCRBV8, 12, and 14 families (30) but displayed a deficiency in antibodies against SAs (31). These findings of antibody titer in KD and other streptococcal infections are somewhat puzzling. Such differences may partly be due to the methods used to measure antibody titer. Mascini (31) measured antitoxin antibodies using ELISA in cases of severe group A streptococcal infection and did not measure the antibodies against SPE-A and SPE-B, using inhibition of lymphocyte mitogenicity by the toxins as an indicator. Abe *et al.* (32) described some discrepancy between neutralizing activity and ELISA results. In Fig. 6, we show that antibodies from healthy controls and IVIg displayed neutralizing activity against SPE-C but sera from patients with KD did not. Consideration of these findings led us to hypothesize that patients with group A streptococcal infection have neutralizing antibodies but that the amount of infected streptococcal toxins may exceed their neutralizing capacity. The sera from patients with KD had antibodies against SPE-C but did not show any defensive effect against this SA. Another hypothesis is that the deficiency of effective antibodies against SPE-C in patients with KD is the a characteristic of this disease. The therapeutic mechanism of IVIg has not been clear, but this is the first drug against KD. The data for IVIg assay is very interesting and may offer new insight into the therapeutic effects of IVIg treatments in patients with KD.

Our present data suggest that the SPE-C play an important role in the pathogenesis of KD; however, there are some questions about the relationship between the pathophysiology of KD and the biologic activities of SPE-C as SAs. A number of findings indicate that the fatal shock syndrome on systemic exposure to staphylococcal enterotoxins is caused mostly by their superantigenicity (33, 34). However, it is not clearly understood to what degree the superantigenicity is responsible for local, often unique, symptoms caused by the exotoxins. Yamaoka *et al.* (35) reported that there was a relationship between superantigenicity and skin erythema–forming activity of SPE-C, using single-residue mutant SPE-C. Our data along with these reports contribute to defining the role of T-cell stimulatory activity of streptococcal pyrogenic exotoxin C in the pathogenesis of KD.

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