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Kawasaki Disease and the T-Cell Antigen Receptor

J. Jason, E. Montana, J. Fricks Donald, M. Seidman, K. L. Inge, and R. Campbell

ABSTRACT: We investigated the evidence for an infectious etiology of Kawasaki disease (KD), an acute vasculitis of unknown etiology, by assessing the effects of KD on the T cell antigen receptor variable beta region families (Vβ). Using 3-color flow cytometry, we studied KD patients pre- and post-intravenous gamma globulin (IVIG) therapy and at >40 days post therapy, additionally comparing them to matched pediatric control patients (PCC) and their own healthy parents (one parent/KD child). Of all the Vβ families examined, only $V_\beta 2$ exhibited statistically significant differences, between the pre- and post-IVIG samples and preIVIG and parent samples. No associations were found between $V_\beta 2$ findings and T cell memory, activation, or adhesion markers. For 2 KD patients, 4 parents, and 1 PCC participant, >15% of resting CD8+ lymphocytes and >15% of blastic CD8+ lymphocytes expressed a single Vβ family, which varied by individual, without similar expansions in the CD4+ cell populations. One of the participants with this abnormality was the only one with significant cardiac abnormalities. For all participants with the Vβ abnormality, other T-cell abnormalities were extensive and involved both CD4+ and CD8+ cells. We suggest that $V_\beta 2$ changes do occur in KD, as previously reported. However, these may not be involved in disease pathogenesis. Other Vβ changes also occur. Those occurring in parents may reflect asymptomatic reinfection with an infectious agent causing KD. Further, some KD patients may have restricted cytotoxic T-cell responses to that as yet unidentified agent; this restricted response may be associated with more severe cardiac involvement. *Human Immunology 59, 29–38 (1998).* Published by Elsevier Science Inc.

INTRODUCTION

Kawasaki Disease (KD) is an acute and transient vasculitis occurring in infants and children. It is an extremely common disorder, with the reported incidence in the United States being greatest between February and May [1]. Until the advent of intravenous gammaglobulin (IVIG) therapy, KD was associated with a 15%–30% incidence of inflammatory coronary arteritis [2–4] and a mortality rate of up to 5%, due to cardiac complications [5]. With IVIG therapy in the acute phase of disease, cardiac symptomatology is inhibited; fever and toxic symptomatology usually resolve in hours [6–9]. The mechanism of IVIG's action is unknown. However, because its effect is clinically significant, IVIG is now the treatment of choice for any case of suspected KD.

The etiology of KD is unknown despite intense investigation by numerous researchers. KD is generally assumed to be infectious in origin because of epidemiologic characteristics shared with other infectious diseases, i.e., a distinct age predilection (6 months to 5 years) [2, 10–12]; epidemic occurrences and seasonality [1, 10, 11]; and distinct clinical symptomatology [10]. This symptomatology includes fever, an erythematous rash, edematous hands and feet, subsequent digital desquamation, bilateral nonexudative conjunctivitis, inflammation of mucous membranes, and cervical adenopathy.

Because these symptoms are similar to those occurring with known superantigen-related infections, particularly staphylococcal and streptococcal, it has been proposed that KD involves superantigen (SAG) activity. It has recently been suggested that KD is associated with selective expansion of T lymphocytes expressing T-cell antigen receptor (TCR) variable beta region families (Vβ) 2 and 8 [13, 14]; selective Vβ expansion is consistent with SAG activity. The organisms suggested as

From the Immunology Branch, Division of HIV, Sexually Transmitted Diseases and Tuberculosis Laboratory Research, National Center for Infectious Diseases, Center for Disease Control and Prevention, Department of Health and Human Services, Public Health Service, Atlanta, GA (J.J., J.F.D., M.S., K.L.) and The Children's Heart Center, Egleston Children's Hospital at Emory University, Atlanta GA (R.C.).

Address reprint requests to: Janine Jason, Mailstop A-25, Immunology Branch, DASTLR, NCID, CDC, 1600 Clifton Road, N.E., Atlanta, GA 30333.

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causing KD have included streptococcus, retroviruses, parvovirus, herpesvirus, and most recently, a toxic shock syndrome toxin-secreting *Staphylococcus aureus* [15]. Research reports have supported [16, 17] or contradicted [18–21] the possibility of SAG activity and/or involvement of various suggested organisms [22–28]. One recent study presented evidence for TCR complementarity-determining region 3 (CDR3) size profiles suggestive of nominal antigen activity in KD, rather than SAG activity [21]. Most of these studies have included few participants and some, no, or poorly matched comparison groups. Some key studies [13, 14] of the TCR repertoire employed polymerase chain reaction techniques, which are unreliable for this purpose [29–31], and/or monoclonal reagents to only a few Vβ families [13, 14, 16–18].

To address the issues of pathogenesis, the role of SAGs, and the involvement of the TCR in KD, we studied patients during acute KD illness (within 10 days of fever onset), at 24–72 h post receipt of IVIG, and during convalescence (>40 days following fever onset). In addition, we studied one genetic parent residing with the child and a genetically unrelated, ethnically- and age-matched child without KD.

Participants and Methods

Participants were enrolled from hospitals throughout the Atlanta area in 1994–1996. Index participants were (a) 6 months to 8 years of age; (b) febrile for >5 to 10 days, with a documented temperature of >38.5°C; (c) without other discernible cause of illness; and (d) with at least 4 of the following findings, upon examination by a cardiologist: an erythematous rash, nonexudative bilateral conjunctivitis, a reddened inflamed tongue, fissured lips, cervical adenopathy, and arthralgia or arthritis. Only one participant had significant cardiac findings; he received 2 doses of IVIG over a 24-h period. Echocardiographic findings had not resolved completely by the time the convalescent sample was obtained. For all 30 participants with acute KD, we enrolled one genetic parent: 26 at the time of the patients's preIVIG sample was drawn; two, 1 day after the preIVIG sample; one, 4 days after the preIVIG sample; and one, 1 month after the preIVIG sample. Genetic siblings were not enrolled, to avoid clinically nonindicated venipuncture of a healthy child. For 20 KD participants, we enrolled an unrelated child being catheterized for a cardiac disorder not associated with KD, streptococcal infection, or immune deficiency. The pediatric "cardiac controls" (PCC) were matched to index cases by gender (19 male/11 female), ethnicity (15 white/15 black), and age ±3 years (mean 3.7 years, median 3.3 years, range 0.8–8.8 years), since these may be related to activation marker and T-cell subset profiles. This protocol was reviewed and approved by the Emory University and Centers for Disease Control and Prevention (CDC) human subjects review committees. Informed consent was obtained from the adult participants and parents of the pediatric participants.

Reagents. Fluorescein isothiocyanate-conjugated (FITC) murine monoclonal antibodies against the human TCR were purchased from three sources, with reactivity specified to date as follows: Immunotech* (Westbrook, ME; $V_\beta2$, $V_\beta3$, $V_\beta5S2$ $V_\beta8S1/8S2$, $V_\beta13S6$, $V_\beta17S1$, $V_\beta21S3$, $V_\beta22S1$); T Cell Diagnostics (Woburn MA; $V_\alpha2$, $V_\beta3S1$, $V_\beta5S1$ $V_\beta5S2/5S3$, $V_\beta5S3$, $V_\beta6S7$, $V_\beta8$ [referred to herein as "8A"], $V_\beta8$ [referred to herein as "8B"], $V_\beta12S1$, $V_\beta13S1/13S3$); and Becton Dickinson Immunochemistry (BD) (San Jose, CA) BD ($TCR_{\alpha,\beta}$ and TCR δ chain). Phycoerythrin-conjugated (PE) monoclonal anti-CD8 and Peridininin chlorophyll protein (PerCP) monoclonal anti-CD4 and anti-CD8 were obtained from BD, as were fluorochrome-conjugated monoclonal antibodies: CD3, CD19, CD16, CD56, CD45RA, CD45RO, HLA-DR, CD25, CD38, and CD71. Conjugated murine monoclonal antibody to CD29 was purchased from Coulter Corporation (Miami, FL) and to CD62L, from PharMingen (San Diego, CA). Natural killer cells (NK) are defined herein as those CD3− and CD16 or CD56+. Cells positive for CD3 and for CD16 or CD56 will be referred to as CD3+NK.

Flow cytofluorometry. Peripheral venous blood lymphocyte (PBL) samples were obtained on all participants. Three-color cytofluorometry was done using a whole-blood technique, a FACScan or FACSort (BD) flow cytometer, and Lysis II software. Negative control aliquots were stained with FITC mouse IgG1, PE-mouse IgG2a, and PerCP-mouse IgG1. From each sample, 20,000–30,000 ungated events were collected. For all parameters, results are reported for CD4+ and CD8+ cells in a lymphocyte scatter gate. In addition, for Vβ's and CD71, up to 3000 events were collected from each sample using the following gating: (a) forward and side scattering of $TCR_{\alpha\beta}$+ cells indicative of large, less dense (blastic) cells [as opposed to small, dense (resting) lymphocytes]; and (b) positivity for CD4 or CD8, but not both [32]. Vβ analyses are reported for the following four populations: resting CD4+ cells, resting CD8+ cells, blastic CD4+ cells, and blastic CD8+ cells. CD45RA, CD45RO, HLA-DR, CD25, CD38, and CD71 were assessed in resting CD4+ and resting CD8+ cells (6 antigens × 4 populations = 12 non-Vβ variables evaluated); CD71 was also assessed in blastic CD4+ and CD8+ cells (2 additional variables, for a total of 14 evaluated, non-Vβ variables). Detailed results for these variables, not in

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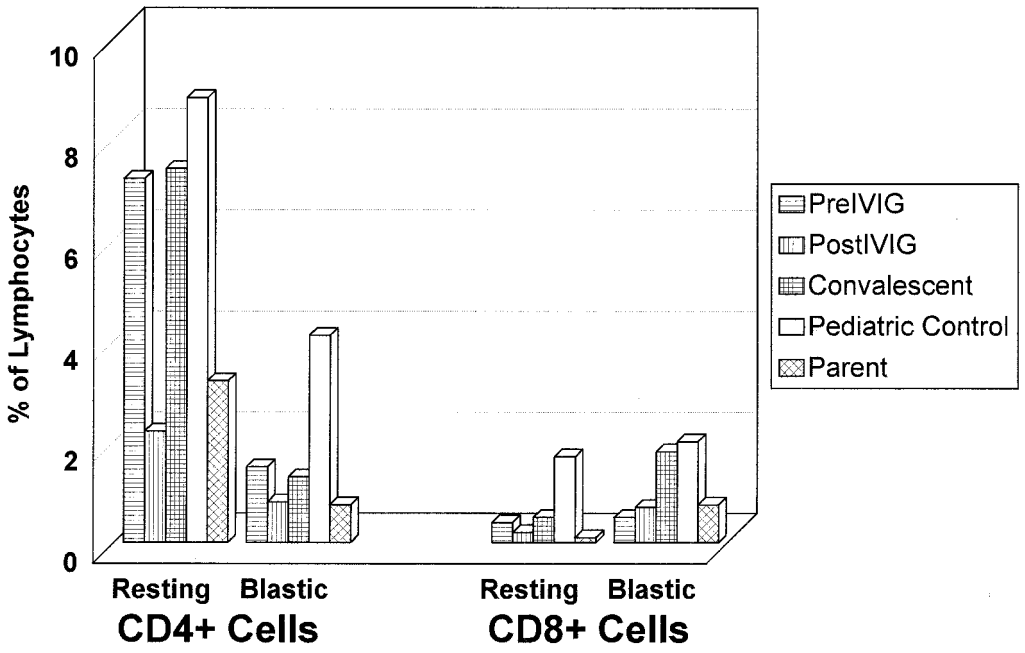

FIGURE 1 Median % of Lymphocytes Expressing TCR $V_\beta 2$, by Stimulation Status and Participant Characteristics, Matched Analysis 1. PreIVIG value shown is that for pre- to postIVIG comparison. Comparisons are to matched preIVIG values. Resting CD4+ cells: postIVIG, $p = .0039$; parent, $p = .0013$. Resting CD8+ cells: postIVIG, $p = .0015$; parent, $p = .0110$. Blastic CD8+ cells: pediatric control, $p = .0017$. 2. Numbers compared to PreIVIG: PostIVIG, resting CD4+ and CD8+ cells, $n = 29$; blastic CD4+ cells, $n = 28$; blastic CD8+ cells, $n = 26$. Convalescent, $n = 20$ for all. Pediatric Control, resting and blastic CD4+ cells, $n = 20$; resting CD8+ cells, $n = 19$; blastic CD8+ cells, $n = 20$. Parent, resting CD4+ and CD8+ cells, $n = 30$; blastic CD4+ cells, $- = 29$; blastic CD8+ cells, $n = 28$.

association with Vβ findings, are provided elsewhere [33].

Analytic techniques. Cellular markers were analyzed using Lysis II software. For all antigens except the Vβ's; all elements of an analytic cluster were analyzed at one time. All samples for a given cluster were collected on the same machine. PreIVIG, postIVIG, and parents' readings were done using identical settings, at the same time or within 72 h of one another. Convalescent and PCC samples were assessed at later times and not necessarily with the earlier settings. The same Vβ reagent panel was used for every sample in a given cluster, but the Vβ panel was enlarged as new reagents became available.

Statistical techniques. Values for the index case at presentation were compared with each of the following: those of the parent; the PCC; and his/her own findings at 24–72 h postIVIG therapy. For 20 patients, we obtained an additional blood sample at >40 days following acute illness (i.e., during convalescence, and compared these findings with those preIVIG). Because most of these parameters are not normally distributed, a wilcoxon signed rank test was used, rather than parametric-based analyses that assume normal distributions (e.g., means, standard deviations, etc.). The wilcoxon signed rank test provides a nonparametric matched comparison of the median value for the preIVIG sample to the median value of each of the other samples in that analytic cluster. A result will be referred to as significant if the two-sided p-value was $<.01$, rather than $<.05$, as a correction for multiple comparisons.

RESULTS

Of all the Vβ families examined, only $V_\beta 2$ exhibited statistically significant differences between the preIVIG samples and multiple other comparison groups (Fig. 1). For both resting CD4+ and resting CD8+ cells, a lower proportion of the postIVIG samples ($p = .0039$ for CD4+ cells and $p = .0015$ for CD8+ cells) and parent samples ($p = .0013$ for CD4+ cells and $p = .0110$ for CD8+ cells) expressed $V_\beta 2$. None of these parents had symptoms of KD. For 17 participant clusters, both the child's postIVIG and parent's percent of resting CD4+ cells expressing $V_\beta 2$ were lower than that child's preIVIG percent. For four, neither were lower; for four, the child's postIVIG value was lower but the parent's was not; for three, the parent's was lower but the child's postIVIG value was not (75% concordance between child and parent). For 16 participants, both the child's postIVIG and parent's percent of resting CD8+ cells express-

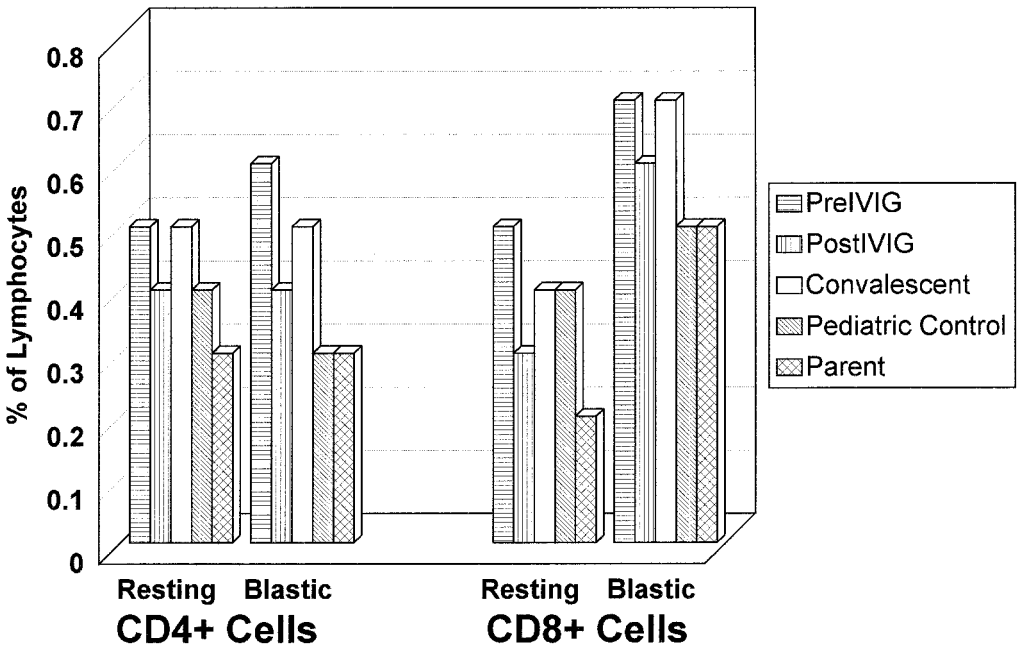

FIGURE 2 Median % of Lymphocytes Expressing TCR $V_\beta 5.2$, by Stimulation Status and Participant Characteristics, Matched Analysis 1. PreIVIG value shown ins that for pre- to postIVIG. Comparisons are to matched preIVIG values. Resting CD4+ cells: parent, $p = .0020$. 2. Numbers compared to PreIVIG: PostIVIG, resting CD4+ cells, $n = 18$; blastic CD4+ cells, $n = 18$; resting CD8+ cells, $n = 18$; blastic CD8+ cells, $n = 17$. Convalescent and pediatric control, $n = 11$ for all. Parent, resting CD4+ cells, $n = 19$; blastic CD4+ cells, $n = 18$; resting CD8+ cells, $n = 18$; blastic CD8+ cells, $n = 17$.

ing $V_\beta 2$ were lower than that child's preIVIG percent; for five, neither were lower; for five, the child's postIVIG value was lower but the parent's was not; for three, the parent's was lower but the child's was not (also 75% concordance).

For blastic cells, the proportion expressing $V_\beta 2$ was lower in the preIVIG samples than in the PCC samples ($p = .0017$). For $V_\beta 5S2$, the parents' proportions appear depressed relative to the patients' preIVIG levels ($p = .0020$, Fig. 2). No significant differences were found for $V_\beta 8$, using any reagent (reagent 8A shown in Fig. 3). PostIVIG and during convalescence, blastic cells expressed a higher proportion of TCRs expressing the δ chain ($p = .0150$ for CD4+ cells postIVIG, $p = .0028$ for CD4+ cells during convalescence, and $p = .0340$ for CD8+ cells during convalescence) (Fig. 4).

Of 29 patients with pre- and postIVIG values, the vast majority had some pre- to postIVIG decline in $V_\beta 2$: 24 had declines in the proportion of resting CD4+ lymphocytes expressing $V_\beta 2$; 15 had declines in the proportion of blastic CD4+ lymphocytes expressing the Vβ; 19, of resting CD8+ lymphocytes; and 13, of blastic CD8+ lymphocytes expressing $V_\beta 2$. Only 1 participant was without a decline in $V_\beta 2$ in any T lymphocyte subgroup.

There was no clear relationship between the extent of pre- to postIVIG declines in the proportion of cells expressing $V_\beta 2$ and any individual cellular markers of T cell activation, adhesion, or naive/memory status (i.e., HLA-DR, CD25, CD38, CD71, CD45RA, and CD45RO,) (data not shown). Further, no obvious relationship was found when these non-Vβ markers were analyzed as a group (methods section and [33]). Participants showed pre- to postIVIG changes of ⩾10% in from 0 to 9 of these non-Vβ evaluated variables (mean 4 variables, median 3 variables). The breadth of changes did not differ strikingly for the six participants with the greatest pre- to postIVIG $V_\beta 2$ changes, with "greatest" defined in four different ways: (1) the six participants with the greatest % change in $V_\beta 2$ for resting CD4+ cells, (2) the six with the greatest proportionate changes in $V_\beta 2$ (i.e., those with the highest preIVIG values - postIVIG value/preIVIG value) for resting CD4+ cells, (3) the six participants with the greatest % change in $V_\beta 2$ for resting CD8+ cells, and (4) the six with the greatest proportionate changes in $V_\beta 2$ for resting CD8+ cells (Fig. 5). Four participants had declines of $V_\beta 2$ in all 4 lymphocyte subgroups assessed (resting and blastic CD4+ and CD8+ cells); these four participants had 0, 2, 3, and 5 pre- to postIVIG changes of ⩾10%. The one participant with cardiac pathology, KD 05-I, had moderate $V_\beta 2$ decreases in both the resting (5.1–0.5%) and

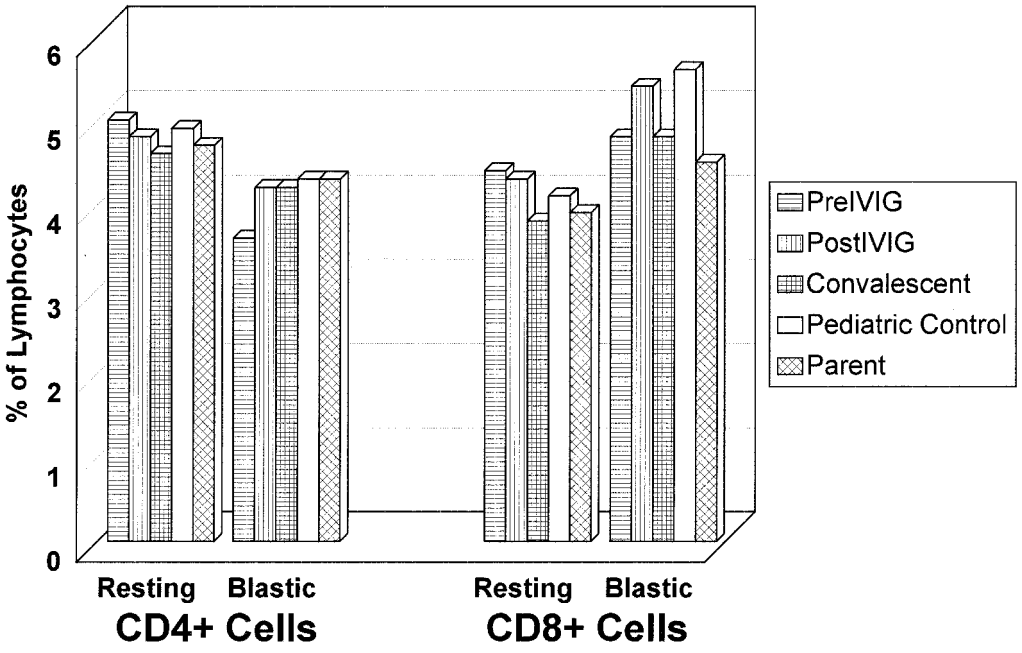

FIGURE 3 Median % of Lymphocytes Expressing TCR $V_\beta 8$, by Stimulation Status and Participant Characteristics, Matched Analysis 1. PreIVIG value shown is that for pre- to postIVIG comparison. 2. Numbers compared to PreIVIG: PostIVIG, resting CD4+ cells, $n = 28$; blastic CD4+ cells, $n = 28$; resting CD8+ cells, $n = 29$; blastic CD8+ cells, $n = 26$. Convalescent and pediatric control, $n = 20$ for all. Parent, resting CD4+ cells, $n = 30$; blastic CD4+ cells, $n = 30$; resting CD8+ cells, $n = 30$; blastic CD8+ cells, $n = 29$.

blastic CD4+ (0.5 to 0.1%) lymphocyte populations but not in CD8+ cells (0.1–0.1%; 0.1–2.6%).

For 2 KD patients, 4 parents, and 1 PCC participant, >15% of resting CD8+ lymphocytes and >15% of blastic CD8+ lymphocytes expressed a single Vβ family, without similar expansions in the CD4+ cell populations (Table 1). Both KD patients with this abnormality had extensive other abnormalities in immune markers (Fig. 6). One of the participants with this abnormality (KD 05-I) was the only one with significant cardiac abnormalities, including acute myocarditis with depressed myocardial contractility, a global pericardial effusion, and diffuse, long-segment dilation of multiple coronary arteries that persisted for >12 months post diagnosis. For a number of immunologic parameters, this participant's acute (preIVIG) values were remarkable. PreIVIG abnormalities included 61.4% of lymphocytes being negative for CD19, CD3, and NK markers, 33.1% of TCR+ lymphocytes being negative for both CD4 and CD8, and 11.6% of blastic CD8+ cells expressing the δ chain. All these, and most other immune abnormalities, had resolved by the postIVIG or convalescent evaluations. His Vβ finding in CD8+ cells may also have been resolving (Table 1). However, at the convalescent evaluation, 61.6% of his TCR+ cells were CD8+. KD 24-I also had extensive abnormalities. Of his lymphocytes, 17.9% expressed NK markers preIVIG and 28.9%, postIVIG. In his preIVIG sample, 57.9% of CD4+ cells expressed CD45RO (median for all preIVIG samples: 23.5%) and 22.6% of his CD8+ cells expressed this memory marker (median for all preIVIG samples: 8.7%). Most of these abnormalities had resolved by the time of the convalescent evaluation.

For all participants with this nonSAGlike Vβ abnormality, an extensive number of immune markers were abnormal, compared with values for other individuals in their participant groups. Immune abnormalities involved both CD4+ and CD8+ cells but included an increased proportion of TCR δ chain+ blastic CD8+ cells (2 KD, the PCC, 1 parent). For the KD and PCC patients, the % of cells expressing CD71 was elevated, especially in the blastic CD8+ population; for the parents, the % of CD8+ cells expressing CD38 was low (data not shown).

We were not able to obtain additional samples on any of the participants with CD8+ Vβ elevations, nor could we obtain additional clinical information on the PCC. However, we were able to assess additional memory markers on the convalescent PBL sample from KD 24-I (Table 2). These markers did not differ for $V_\beta 17S1+$ cells.

DISCUSSION

In this controlled study, we addressed the effects of KD on the TCR, to determine if there were evidence for or

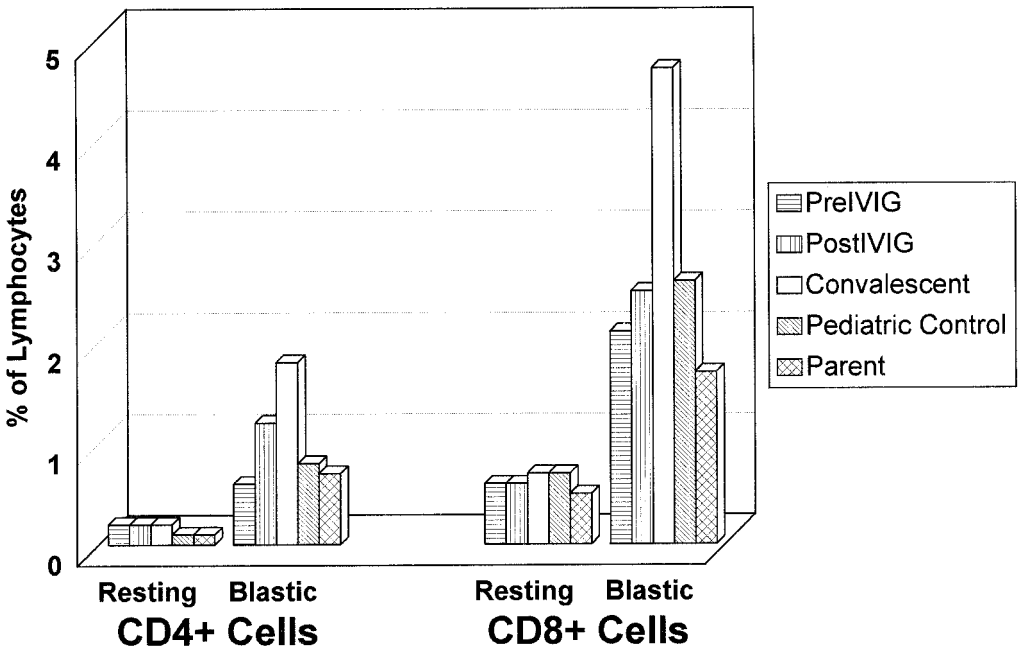

FIGURE 4 Median % of Lymphocytes Expressing TCR Delta, by Stimulation Status and Participant Characteristics, Matched Analysis 1. PreIVIG value shown is that for pre- to postIVIG comparison. Comparisons are to matched preIVIG values. Blastic CD4+ cells: postIVIG, $p = .0150$; convalescent, $p = .0028$. Resting CD8+ cells: convalescent, $p = .046$. Blastic CD8+ cells: convalescent, $p = .034$. 2. Numbers compared to PreIVIG: PostIVIG, resting CD4+ cells, $n = 28$; blastic CD4+ cells, $n = 29$; resting CD8+ cells, $n = 28$. Convalescent and pediatric control, $n = 20$ for all. Parent, resting CD4+ cells, $n = 29$; blastic CD4+ cells, $n = 30$; resting CD8+ cells, $n = 29$; blastic CD8+ cells, $n = 29$.

against SAG activity, cytotoxic T cell restriction, and an infectious cause of the disease. One laboratory has suggested that KD is associated with selective expansion of T lymphocytes expressing V_β's 2 and 8 [13, 14]. Our data, as well as those in one other study [17], support an effect of either KD or IVIG therapy on $V_\beta 2$, but not $V_\beta 8$. However, we found no association between pre- to postIVIG declines in $V_\beta 2$ and cellular markers of memory, activation, or endothelial adhesion. Further, the one patient with significant cardiac involvement did not have unusually large $V_\beta 2$ changes. This raises questions about the importance of SAG activity in KD disease pathogenesis. One author has suggested that anti-$V_\beta 2$ therapy may be appropriate in KD [34]. Our results imply that this therapy might not have as dramatic an effect upon the disease process as does IVIG.

Our finding of $V_\beta 2$ differences in the parents, as well as the KD patients, were unexpected and intriguing. Parents has been selected as a control group because of their genetic similarity to the index cases, since baseline Vβ patterns are genetically influenced, not because of their shared environment. As explanation for this unexpected finding, one might postulate that these parents, residing with the children, may have also been exposed to the as yet undetermined infectious agent causing KD. Their lack of clinical symptoms may have been due to previous exposure and subsequent immune protection against this putative organism.

FIGURE 5 Number of Large Pre- to Post-IVIG Activation Marker Changes, by Size of Pre- to Post-IVIG Change in $V_\beta 2$ 1) Size of $V_\beta 2$ change differentiated four ways: (a) the six participants with the greatest % change in $V_\beta 2$ for resting CD4+ cells; (b) the six with the greatest proportionate changes in $V_\beta 2$ (i.e., those with the highest preIVIG value-postIVIG value/preIVIG value) for resting CD4+ cells,; (c) the six participants with the greatest % change in $V_\beta 2$ for resting CD8+ cells, and (d) the six with the greatest proportionate changes in $V_\beta 2$ for resting CD8+ cells. 2) Triangles represent number of large pre- to post-IVIG activation marker changes for each of the patients defined in (a)-(d) above. Circles represent means for remaining patients; bars indicate standard deviations.

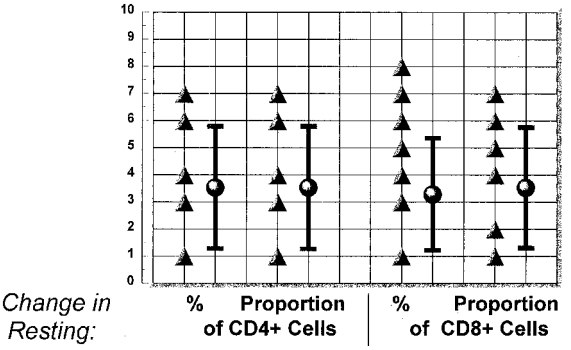

TABLE 1 Participants with >15% of both resting and blastic CD8+ lymphocytes being of one Vβ family, by cellular and participant characteristics

ID#	Vβ	% CD8 Cells Expressing This Vβ		% CD4 Cells Expressing This Vβ	
		Resting	Blastic	Resting	Blastic
Kawasaki Patients (n = 30):					
05-I	5S2/S3				
PreIVIG		20.3	30.6	8.9	0.7
PostIVIG		3.5	10.5	3.8	0.5
Convalescent		6.3	18.6	3.1	6.9
24-I	17S1				
PreIVIG		16.5	19.5	5.2	5.9
PostIVIG		19.4	26.0	4.8	5.7
Convalescent		16.2	18.4	4.8	4.8
Parents (*n* = 30)					
06-M	17S1	17.5	15.5	3.5	4.5
15-F	13S1/S3	13.7	53.5	3.8	3.8
18-F	22	50.9	68.3	5.7	3.8
25-M	3S1	11.7	20.2	7.2	7.7
Cardiac Controls (*n* = 20)					
20-C	13S1/S3	18.5	18.9	4.5	5.0

Our data concerning CD8+ cells provide further support to an infectious cause of KD. In addition to the $V_\beta 2$ effect seen in both CD4 and CD8+ cells, we noted an expansion of individual Vβs other than $V_\beta 2$ in 2 KD patients. Unlike the effects seen with SAGs or $V_\beta 2$ in our study, this finding was present only in CD8+ cell populations and did not involve a unique Vβ. Thus, this finding was more consistent with a restricted cytotoxic T-cell response to a nominal antigen than SAG activity. Further, this restriction did appear to be associated with extensive memory, activation, and adhesion marker abnormalities and possibly also with cardiac involvement in KD. A recent analysis of the TCR CDR3 regions of CD4+ and CD8+ cells from 26 KD patients also suggested the presence of conventional antigen, rather than SAG, in KD pathogenesis [21].

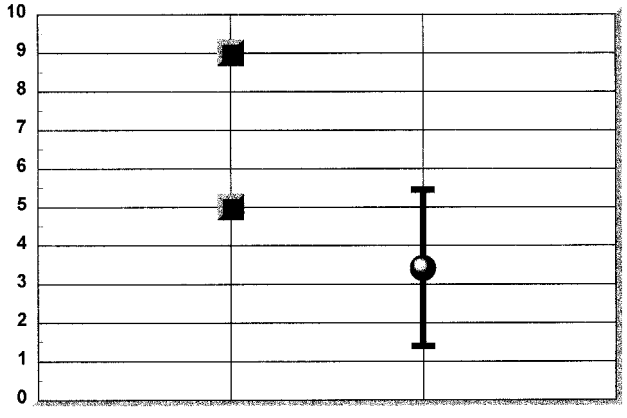

FIGURE 6 Number of Large Pre- to Post-IVIG Activation Marker Changes, for Those With and Without Dominant Vβ Families in CD8+ Cells 1. Squares represent number of large pre- to postIVIG activation marker changes for two patients with dominant Vβ families found in CD8+ cells. Circles represent means for remaining patients; bars indicate standard deviation.

Oligoclonal or monoclonal CD8+ expansions have been reported to occur in the acute response to human T-cell lymphotropic virus type I [35], influenza virus antigens [36], herpesvirus [37], and *toxoplasma gondii* [38]. They also have been noted in polymyositis [39], polyarteritis [34], and rheumatoid arthritis [40]. Their occurrence in these autoimmune disorders has been interpreted as due to a restricted response to an unknown antigen or as evidence of SAG involvement in these disorders, the latter because the clonality frequently includes $V_\beta 2$ [34] or $V_\beta 3$ [40] restriction. Chronic oligoclonal expansion occur in normal adults, at a rate that increases with age [41–44]. It has been postulated that these cells expanded in response to previous stimulation by gut flora or common viruses, such as cytomegalovirus [41]. They involve a broad spectrum of Vβs and reportedly involve CD8+CD28− and CD8+CD45RO+ cells [41, 44]. Associations with other cell markers, or with blastic, as well as resting cells, have not been reported.

We found 2 KD patients, 4 parents, and 2 PCCs had expansions of one Vβ in both resting and blastic CD8+ cells. We postulate that these may represent acute expansions in response to a recent infection or antigen contact. In the case of the KD patients, and possibly even the KD parents, this may have been to the agent causing

TABLE 2 % of lymphocytes[a] positive for memory markers, Kawasaki disease patient 24-I, convalescent sample

	Resting Lymphocytes		Blastic Lymphocytes
	V_β17S1 Positive	V_β17S1 Negative	V_β17S1 Negative
CD45RA+	70.0	72.1	57.3
CD45RO+	18.9	16.5	21.8
CD45RA+/RO+	0.8	0.5	8.4
CD62L+	56.5	64.9	58.9
CD62L+/CD45RO+	9.4	8.2	17.9
CD62L+/CD45RO−	47.1	56.7	40.8
CD62L−/CD45RO+	7.8	8.2	17.9

a) Number of V_β17S1-positive blastic cells inadequate for analysis.

KD. For the PCC, it could be in response to an acute undiagnosed viral infection. Unfortunately, we could not obtain information concerning, or additional blood samples from, either the parents or the PCCs, to examine this possibility further. However, consistent with our proposal, all patients with this Vβ finding also had other T-cell abnormalities. Of the KD patients with this finding, one appeared to have resolution, at least within the resting CD8+ cell population by the time of the post-IVIG sample and the convalescent sample, obtained at 41 days after his hospital admission. We were able to examine the other patients in greater depth, in part because this Vβ expansion had not resolved at the time his convalescent sample was drawn, 9 months following his acute infection. By flow assessment, resting lymphocytes V_β17+ did not express a disproportionately high % of memory cells.

In summary, we did find evidence of V_β2 changes in KD consistent with previous reports suggesting SAG activity. These changes were not associated with clinical or other immune defects. The presence of similar V_β2 changes in the parents of these patients supports that an infectious agent causes KD. We also report other Vβ abnormalities confined to the CD8+ cell population. We suggest that, in the case of the 2 patients with acute KD, these may represent restricted cytotoxic T-cell responses to the as yet unidentified agent causing KD. This restricted response may be associated with more severe cardiac involvement. These possibilities merit further evaluation.

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