CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY
Vol. 84, No. 3, September, pp. 296–306, 1997
Article No. II974376

Immunoregulatory Changes in Kawasaki Disease

Janine Jason,* Lynda Gregg,† Alison Han,* Andy Hu,* K. Leigh Inge,*
Angelia Eick,* Irene Tham,* and Robert Campbell†

*Immunology Branch, Division of HIV, Sexually Transmitted Diseases, Tuberculosis Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (DHHS), Public Health Service (PHS), Atlanta, Georgia 30333; and †The Children's Heart Center, Egleston Children's Hospital at Emory University, Atlanta, Georgia 30322

Kawasaki disease (KD) is an acute vasculitis of unknown etiology, occurring in young children and treated with intravenous gamma globulin (IVIG) to prevent significant cardiac morbidity and mortality. We studied KD patients pre- and post-IVIG therapy and at >40 days posttherapy, additionally comparing them with matched pediatric control patients and parents. Using three-color flow cytometry, we examined immune changes in KD, especially previously unassessed markers of T-lymphocyte activation, memory, and adhesion. The percentage of cells positive for CD19, CD25, CD38, and CD71 was significantly lower during convalescence compared with pre-IVIG (medians: CD19, 18% vs 26%, $P = 0.0004$; CD25, 6% vs 9% for $CD3^+$ cells, $P = 0.0074$; CD38, 78% vs 89% for $CD8^+$ cells, $P = 0.0015$; CD71, 1% vs 6% for $CD4^+$ cells, $P = 0.0024$). The proportion of $CD3^+$ cells increased (medians: CD3, 66% vs 45%, $P < 0.0001$). Values for all parameters varied greatly pre- and post-IVIG, but not in a consistent direction. The sole patient with cardiac abnormalities had the greatest pre-/post-IVIG variability. These changes support the involvement of T-lymphocytes in the acute KD vasculitic process. They also suggest that T-lymphocytes involved in endothelial damage during acute KD may be subsequently removed or eliminated from the peripheral blood.

INTRODUCTION

Kawasaki disease (KD) is an acute and transient vasculitis occurring in infants and children and affecting small and medium blood vessels, including arterioles, venules, and capillaries (1). KD affects coronary arteries; untreated, it is associated with a 15–30% incidence of demonstrable inflammatory coronary arteritis (2–4) and a mortality rate of up to 5%, due to cardiac complications (5). The role of various components of the immune system in this vasculitic process is unresolved. With acute KD, the most striking peripheral blood finding is a marked granulocytosis, but it is felt that other immune components may have essential roles in the disease.

Monocytes and macrophages appear to be activated in KD (6, 7). Data concerning T- and B-lymphocyte involvement in KD are conflicting. Histologically, both neutrophils and $CD4^+$ T-lymphocytes infiltrate involved endothelium (1). Some studies using peripheral blood lymphocytes have shown evidence of B- and/or T-lymphocyte alterations (8–11) or activation (10–12), especially when there is clinically significant cardiac involvement (8). Others demonstrated no evidence of these (13, 14); still others had equivocal findings (15). Complicating the issue further, three laboratories reported alterations in the T-cell antigen receptor (TCR) variable β chain repertoire (16–19). These findings have been contradicted or not confirmed by others (20–22) but have been presented as evidence of superantigen involvement in KD (16, 17). There has been a recent report of similar findings in other vasculitides (23), suggesting that if and when these perturbations occur, they may be indicative of T-lymphocyte involvement in the vasculitic process, not necessarily suggestive of superantigen activity.

When given during the acute phase of KD, intravenous gamma globulin therapy (IVIG) rapidly reverses clinical symptomatology and prevents cardiac disease (24–27). The mechanism of IVIG's therapeutic and prophylactic activity in KD is unknown but is assumed to involve immune mechanisms. IVIG is now the treatment of choice for any case of suspected KD. Since the symptoms of KD overlap those of numerous infectious diseases to which young children are susceptible, this has led to extensive use of IVIG, an expensive therapeutic modality. It is of both practical and scientific value to elucidate the roles of various immune components in the KD process, to better refine therapy and perhaps to aid in more accurate diagnosis of KD. We therefore assessed immunologic markers on peripheral blood lymphocytes (PBLs) of 30 patients with acute KD and compared these findings with those of two matched comparison groups and to the patients' own findings post-IVIG therapy and during convalescence.

PARTICIPANTS AND METHODS

Participants were enrolled from hospitals throughout the Atlanta area in 1994–1996. Index participants

0090-1229/97 $25.00

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 another discernible cause of illness, and (d) with at least four of the following findings, on examination by a cardiologist: an erythematous rash, nonexudative bilateral conjunctivitis, a reddened inflamed tongue, fissured lips, cervical adenopathy, and arthralgia or arthritis. Only 1 participant had significant cardiac findings. He received two doses of IVIG over a 24-hr period. Echocardiographic findings had not resolved completely by the time the convalescent sample was obtained. For each of the 30 participants with acute KD, we enrolled one genetic parent; for 20, we enrolled an unrelated child being catheterized for a cardiac disorder not associated with KD, streptococcal infection, or immune deficiency. The "cardiac controls" 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 the 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. Fluorochrome-conjugated murine monoclonal antibodies to the following human antigens were purchased from Becton Dickinson Immunochemistry (BD; San Jose, CA)[1]: CD3, CD4, CD8, CD19, CD16, CD56, CD45RA, CD45RO, HLADR, CD25, CD38, CD71, TCR α/β chains, CD11a, and CD49d. Conjugated murine monoclonal antibody to CD29 was purchased from Coulter Corp. (Miami, FL). Natural killer (NK) cells 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 samples were obtained from 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 fluorescein (FITC) mouse IgG1, phycoerythrin (PE) mouse IgG2a, and PerCP mouse IgG1. A total of 20,000 to 30,000 ungated events were collected from each sample. For all parameters, results are reported for $CD4^+$ and $CD8^+$ cells in a wide lymphocyte scatter gate; for CD71 only, results are also provided for blastic $CD4^+$ and $CD8^+$ cells. Blastic cells were defined on the basis of high forward and side scatter within the lymphocyte scatter gate (28); findings were based on additional collection of up to 3000 cells, through a blastic, $CD4^+$ or $CD8^+$ collection gate.

Analytic techniques. Cellular markers were analyzed using Lysis II software; all elements of an analytic cluster were analyzed at one time. Data were analyzed in terms of percentage of cells positive for a given antigen; percentage of cells positive for a given antigen, using identical quadrant markers for all samples in that cluster; median fluorescence intensity of positive cells (MFI+); and overall fluorescence intensity. Results for these data were comparable with one another. Only percentage positivity will be presented here, except in the case of CD11a and CD49d, for which MFI+ findings reached significance while percentage positivity did not. Of note for interpretation of MFI+ findings, all samples for a given cluster were collected on the same machine. Pre-IVIG, post-IVIG, and parents' readings were done using identical settings, at the same time or within 72 hr of one another. Convalescent and cardiac control samples were assessed at later times and not necessarily with the earlier settings.

Statistical techniques. Values for the index case at presentation were compared with each of the following: those of the parent; the cardiac control; and his or her own findings at 24–72 hr following IVIG 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 pre-IVIG. Because most of these parameters are not normally distributed in any population, means and standard deviations are not used as summary statistics for these analyses. Instead, median values are provided and compared using a Wilcoxon signed-rank test. This test provides a nonparametric matched comparison of the pre-IVIG sample to each of the other samples in that analytic cluster (numbers provided in Table 1). A result will be referred to as significant if the two-sided P value was <0.05.

RESULTS

The proportion of lymphocytes expressing CD3 and the TCR was significantly lower for the pre-IVIG sample, compared with the post-IVIG, convalescent, and parent samples but not compared with matched pediatric controls (Fig. 1). The proportion increased from the pre-, to post-, to the convalescent samples; conversely, the proportion of $CD19^+$ cells declined (Fig. 1). Although the pre-/post-IVIG CD19 difference did not reach statistical significance, wide variations occurred. Three participants showed increases of ⩾10% of cells expressing CD19 between the pre- and post-IVIG samples (9 to 21%, 21 to 41%, and 5 to 31%), and 12 showed decreases of ⩾10% (41 to 27%, 33 to 19%, 46 to 23%,

[1] Use of any and all trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

TABLE 1

Samples Sizes for Matched Comparisons, by Parameter and Group Being Compared, with Pre-IVIG Values[a]

For all lymphocytes, CD19: 29, 20, 20, 30; CD3: 28, 19, 19, 29; TCR[b]: 28, 20, 19, 19; NK[b]: 27, 20, 20, 30; $CD3^+$ NK[b]: 27, 20, 20, 30; CD4: 18, 13, 11, 19; CD8: 19, 13, 12, 20.

For $CD4^+$ lymphocytes, CD25: 23, 16, 17, 26; CD29: 17, 7, 10, 16; HLADr: 28, 21, 20, 29; CD11a: 13, 7, 6, 14; CD49d: 12, 7, 6, 13; CD38: 29, 21, 20, 30; CD45RO: 29, 20, 20, 28; CD45RA: 29, 20, 20, 28; CD71: 28, 20, 20, 29.

For blastic $CD4^+$ lymphocytes, CD71: 20, 16, 14, 22. For $CD3^+$ lymphocytes, CD25: 29, 21, 20, 30.

For $CD8^+$ lymphocytes, CD29: 16, 7, 12, 16; HLADr: 27, 21, 20, 28; CD11a: 13, 7, 6, 14; CD49d: 11, 7, 6, 12; CD38: 28, 21, 20, 29; CD45RO: 29, 20, 19, 28; CD45RA: 29, 20, 19, 28; CD71: 28, 20, 20, 29.

For blastic $CD8^+$ lymphocytes, CD71: 20, 17, 15, 22.

[a] Provided in the order: post-IVIG, convalescent, pediatric (cardiac) control, parent.
[b] T-cell antigen receptor variable region α/β chain (TCR); $CD3^-$/CD16 or $CD56^+$ cells, natural killer cells (NK); $CD3^+$/CD16 or $CD56^+$ cells, $CD3^+$ natural killer cells ($CD3^+$NK).

36 to 20%, 45 to 26%, 24 to 10%, 50 to 30%, 19 to 8%, 14 to 4%, 51 to 41%, 33 to 17%, and 38 to 9%). Similar variations were not seen in the proportion of cells expressing NK markers (Fig. 1) or TCR+ lymphocytes expressing CD4 (pre-IVIG, 65%; post-IVIG, 66%; convalescent, 65%; parent, 63%; and pediatric cardiac control, 68%), CD8 (31, 31, 33, 31, and 28%, respectively), or both markers (0.4, 0.4, 0.5, 0.9, and 0.4%, respectively), nor were any paired comparisons for these variables significant.

Values for CD25, CD29, and HLADR did not differ significantly between pre- and post-IVIG samples (Fig. 2); however, individual participants showed great variation. Variations were greater for $CD8^+$ cells compared to $CD4^+$ cells and for $CD3^+CD4^-$ cells compared to $CD3^+CD4^+$ cells. For example, for participant 18, 21.6% of $CD3^+CD4^+$ cells were positive for CD25 before IVIG therapy and 10.7% were positive after therapy (Figs. 3A and 3B). However, the change for $CD3^+4^-$ cells was far greater: 1.1% expressed CD25 pre-IVIG and 66.0% expressed CD25 post-IVIG (Figs. 3C and 3D). For $CD4^+$ cells, 3 participants had increases of $\geqslant$10% in the proportion of cells expressing CD29 (54 to 64%, 19 to 30%, and 24 to 41%) and 2 had decreases of $\geqslant$10% (43 to 25% and 54 to 44%). For $CD8^+$ cells, 5 had increases of $\geqslant$10% (38 to 51%, 11 to 32%, 67 to 94%, 16 to 26%, and 37 to 58%) and 2 had decreases (47 to 37% and 39 to 11%). For HLADR, for $CD4^+$ cells, 4 participants showed similar, large increases (21 to 33%, 11 to 21%, 9 to 19%, and 29 to 47%) and 2 showed decreases (40 to 8% and 42 to 32%); for $CD8^+$ cells, 3 showed increases (21 to 32%, 15 to 30%, and 15 to 27%) and 3 showed decreases (27 to 8%, 45 to 13%, and 100 to 72%).

Pre-IVIG and post-IVIG samples also did not differ significantly for CD38, CD45RA, or CD45RO (Fig. 4), or for CD11a or CD49d. However, for these antigens also, individual participants showed variations of $\geqslant$10% over the 1–4 days between their initial blood sample and following receipt of IVIG. Variations of $\geqslant$10% for $CD4^+$ cells included the following: for CD38, two participants had decreases (85 to 59% and 89 to 77%); for CD45RA (CD45RA+RO−), five had increases (53 to 92%, 58 to 69%, 59 to 76%, 61 to 76%, and 68 to 78%) and four had decreases (86 to 75%, 34 to 23%, 82 to 67%, and 71 to 58%); for CD45RO (CD45RO+RA−), two had increases (12 to 22% and 14 to 28%) and two had decreases (30 to 17% and 38 to 23%); and for CD49d, two had decreases (95 to 41% and 24 to 9%). Variations of $\geqslant$10% for $CD8^+$ cells included the following: for CD38, two participants had increases (53 to 86% and 83 to 98%) and three had decreases (95 to 73%, 56 to 30%, and 30 to 20%); for CD45RA (CD45RA+RO−), three had increases (52 to 84%, 66 to 81%, 66 to 82%) and four had decreases (74 to 38%, 76 to 58%, 81 to 67%, and 86 to 69%); for CD45RO (CD45RO+RA−), three had increases (17 to 41%, 18 to 29%, and 12 to 25%) and none had decreases; and for CD49d, one had a decrease (28 to 14%).

For $CD4^+$ cells, 0 of 13 pre-/post-IVIG pairs had a $\geqslant$10% change in CD11a; for $CD8^+$ cells, 3 had increases in CD11a expression (24 to 34%, 25 to 72%, and 38 to 53%) and 4 had decreases (42 to 27%, 60 to 44%, 37 to 18%, and 33 to 22%). Similarly, for CD71, pre- and post-IVIG values did not change with any significant directionality (Fig. 5); however, large variations occurred in individual participants: for $CD4^+$ cells, 2 had increases (10 to 28% and 3 to 14%) and 6 had decreases (13 to 3%, 33 to 14%, 24 to 1%, 22 to 6%, 42 to 21%, and 20 to 3%); for $CD8^+$ cells, 1 increased (3 to 35%) and 3 decreased (30 to 2%, 16 to 3%, and 22 to 3%).

To summarize the overall pre-/post-IVIG variability described above, 15 participants had $\geqslant$10% differences between pre- and post-IVIG values for two to four of the above parameters, 5 participants had $\geqslant$10% differences between pre- and post-IVIG values for five or six parameters, and 4 participants had $\geqslant$10% differences between pre- and post-IVIG values for more than six

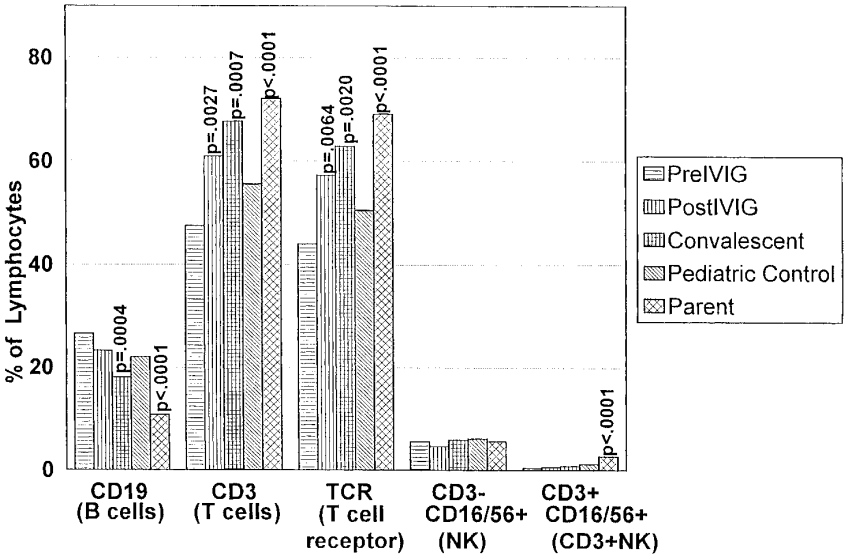

FIG. 1. Median percentage of lymphocytes expressing B, T, and NK markers, by participant characteristics, matched analysis. Pre-IVIG value shown is that for pre- to post-IVIG comparison; *P* values are for comparisons to matched pre-IVIG values.

parameters. Only 5 participants had variability in fewer than two parameters.

For CD25, CD29, and CD45RO, parents' values were significantly higher than pre-IVIG values (Figs. 2 and 4). CD25, CD45RO, and HLADR expression increase with age (29). For CD38 and CD45RA, parents' values were significantly lower than pre-IVIG values (Fig. 4); these normally decrease with age. CD45RA, CD45RO, and CD71 were the only parameters for which significant differences were found between the pre-IVIG KD patients and the matched pediatric controls.

For CD19, CD25, CD38, and CD71, the convalescent values for all lymphocyte groups assessed were significantly lower than the pre-IVIG values (Figs. 1, 2, 4, and 5). The median fluorescent intensities of cells positive for CD11a and CD49d in $CD8^+$ cells were lower in the convalescent samples (Fig. 6); however, convalescent samples were read at later dates and settings were not standardized.

We examined CD71 antigen in some depth; it was the only parameter specifically evaluated in blastic lymphocyte populations. Although the proportions of

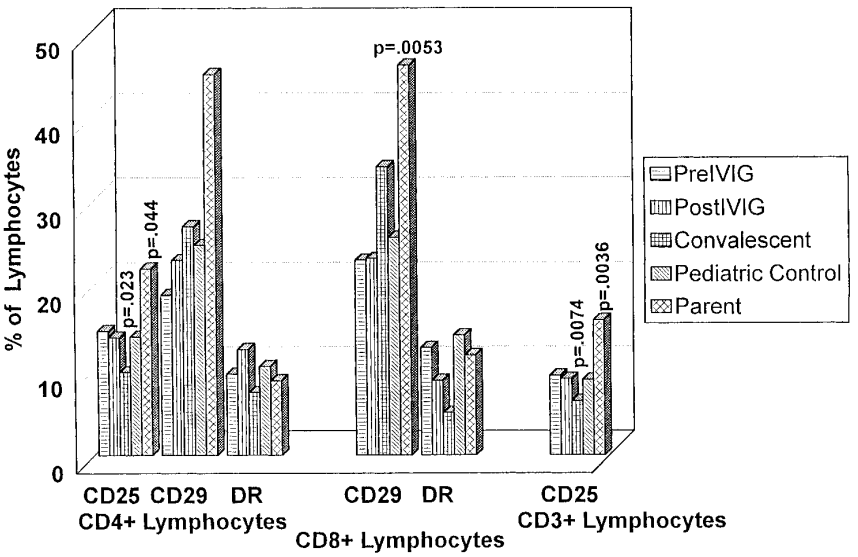

FIG. 2. Median percentage of lymphocytes positive for CD25, CD29, or HLA-DR, by participant characteristics, matched analysis. Pre-IVIG value shown is that for pre- to post-IVIG comparison; *P* values are for comparisons to matched pre-IVIG values.

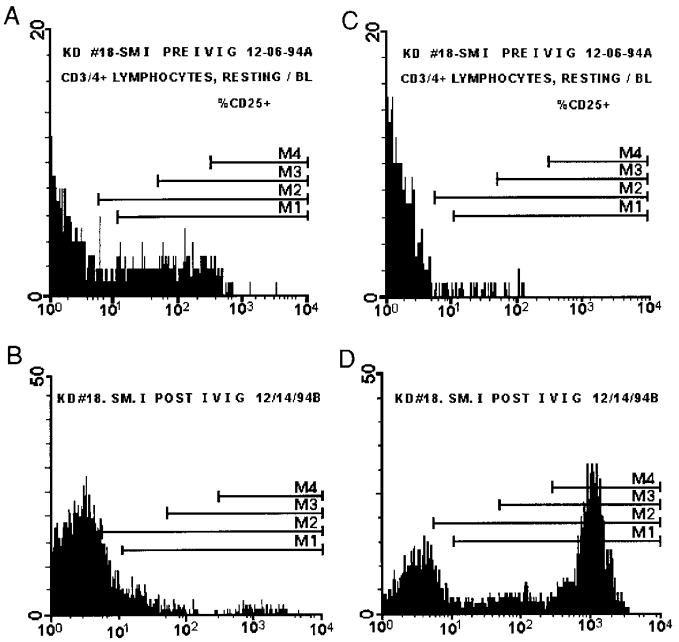

FIG. 3. Histogram of CD25 results for KD Patient 18, by nature of sample. (A) Pre-IVIG results for $CD3^+CD4^+$ lymphocytes; (B) post-IVIG results for $CD3^+CD4^+$ lymphocytes; (C) pre-IVIG results for $CD3^+CD4^-$ lymphocytes; (D) post-IVIG results for $CD3^+4^-$ results; (E) scatter gating used for wide lymphocyte scatter gate (R1) and blastic lymphocyte scatter gate (R5) (see Participants and Methods).

various lymphocyte subpopulations positive for CD71 were not sensitive parameters, alone or in combination with one another, they did differentiate acute KD cases from cardiac controls and parents (Table 2).

KD participant 5 was the only participant with significant cardiac abnormalities, including acute myocarditis with depressed myocardial contractility, a global pericardial effusion, and diffuse, long-segment dilatation of multiple coronary arteries that persisted for >12 months postdiagnosis. For a number of immunologic parameters, his acute (pre-IVIG) values were remarkable. Most immune abnormalities had resolved by the post-IVIG or convalescent evaluations (Table 3). Acute abnormalities included a high proportion of lymphocytes negative for both B- and T-cell antigens and a high proportion of TCR+ lymphocytes negative for both CD4 and CD8. By the time of the convalescent evaluation, these findings had resolved; however, at the convalescent evaluation, an unusually high proportion of his TCR+ cells were $CD8^+$. He had ⩾10% increases in nine of the parameters discussed above. This was the greatest breadth of variability found; the next highest numbers were variability in eight parameters (one patient) and seven parameters (two patients) each.

DISCUSSION

We used this extremely well-matched study population to examine immune changes in KD, especially markers of T-lymphocyte activation, memory, and adhesion that have not been previously evaluated in KD. In particular, we examined whether signs of T-cell activation were present in PBLs of children during the acute KD disease process, whether changes were reversed within 24–72 hr of IVIG therapy, and whether changes appeared to be related to cardiac disease. In a subset of participants, we also examined two adhesion markers purported to be associated with other forms of vasculitis (29, 30) as potential aids in laboratory discrimination of KD from other disease processes.

Shannon *et al.* compared 6 children with acute KD with age-matched controls; no differences were found in the proportion of cells positive for CD3, CD4, or CD8 (14). Leung *et al.* examined 15 acute KD patients before and after 4 days of IVIG therapy and noted significant declines in HLADR+ T-helper ($CD4^+$) lymphocytes and increases in $CD8^+$ cells (11). Furukawa *et al.* studied 17 KD patients prior to therapy and 21–64 days post-treatment and 12 healthy children (15). The number of PBLs positive for CD3, CD4, CD8, and HLADR did not differ significantly between the two KD groups and the control group; however, the number of $CD3^+$, $CD4^+$, and $CD8^+$ cells increased between the pretherapy and the convalescent samples. We found that the proportion of cells positive for CD3 increased post-IVIG therapy; this included both $CD4^+$ and $CD8^+$ cells. We did not find a change in HLADR expression in the $CD4^+$ lymphocyte population following single-dose IVIG therapy. In our study, other markers of activation showed

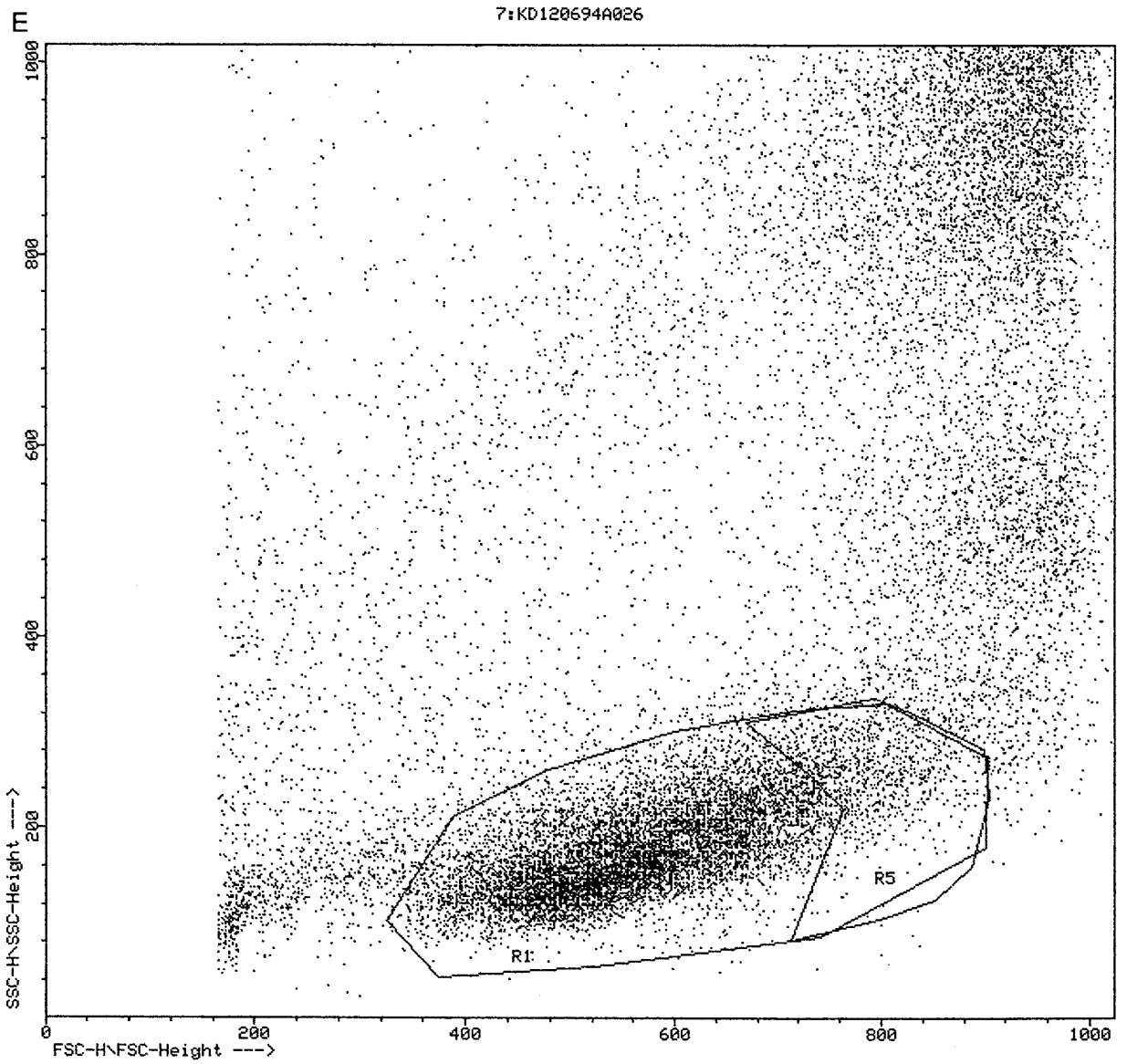

FIG. 3—*Continued*

more striking variation and $CD8^+$ cells showed as much variability as $CD4^+$ cells, if not more.

We found great variability for a variety of activation and adhesion markers during acute KD. This variability was far greater than that reported to occur in normal individuals over time (31). There was no obvious directionality to this variability nor any clear relation to IVIG therapy, suggesting either that the variability was due to the natural disease course or that our post-IVIG sample was taken before a definitive IVIG effect could be seen. Resolving the latter possibility would be difficult. The clinical effect of IVIG is so rapid that hospital discharge occurs within hours to days of therapy; we obtained our post-IVIG sample shortly prior to discharge. Even if later samples could be obtained, one could not assume that changes were due to IVIG therapy, especially given that the clinical response to IVIG occurs within hours. Resolving the role of IVIG in T-lymphocyte changes may be feasible only through the use of a non-IVIG-treated comparison group. This, of course, would now be unethical.

CD25 is a receptor for IL-2 and is expressed on activated lymphocytes. CD38 is also expressed on activated lymphocytes and is physically associated with the CD3–TCR complex (32). It is a weak coreceptor for antigen; proliferation via CD38 is mediated by IL-2, IL-2 receptors, and/or IL-6 (32, 33). CD38 is preferentially expressed by $CD4^+CD45RA^+$ ("naive") T-lymphocytes

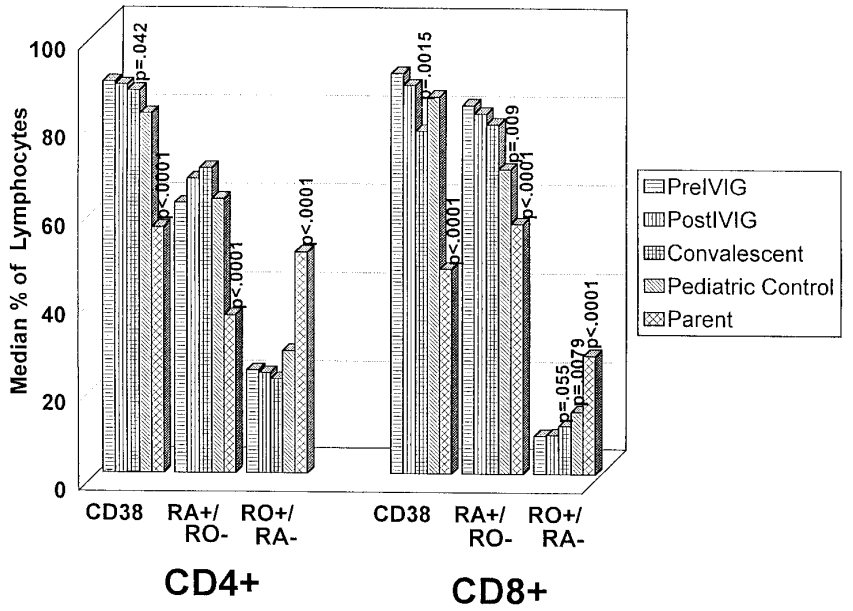

FIG. 4. CD38, CD45RA, and CD45RO. Age-related markers, matched analysis. Pre-IVIG value shown is that for pre- to post-IVIG comparison; no *P* values significant vs matched pre-IVIG values.

and mediates a selectin-like binding to endothelial cells (32, 34). CD71 is a receptor for transferrin, which is essential for DNA synthesis and metabolism (35). Interaction between transferrin and its receptor causes gene expression of both IL-2 and high-affinity IL-2R; it may also cause T-cell production of IFN-γ and B-cell growth factor (35). This interaction appears to play a role in at least some lymphocyte activation (36). CD11a, also known as lymphocyte function-related antigen 1 (LFA-1), is associated with endothelial adhesion and is expressed on activated lymphocytes—especially a subset of $CD8^+$ cells (37), as well as NK cells, monocytes, and granulocytes (38). Its engagement stimulates production of IFNγ, TNF-α, and TNF-β (39, 40). It may play a role in graft vasculitis (29). In one report, $CD3^+$LFA-1+ cells were proportionately decreased in

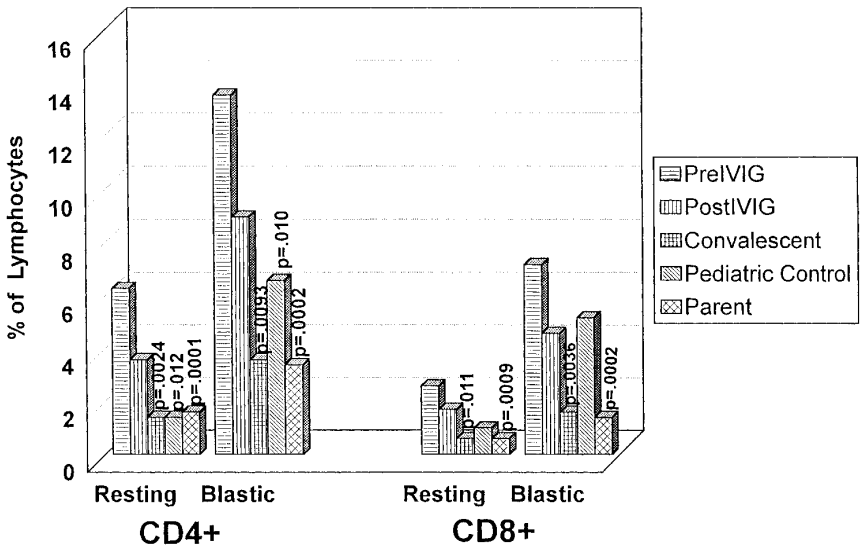

FIG. 5. Median percentage of $CD4^+$ or $CD8^+$ lymphocytes positive for CD71, by participant characteristics, matched analysis. Pre-IVIG value shown is that for pre- to post-IVIG comparison; *P* values are for comparisons to matched pre-IVIG values.

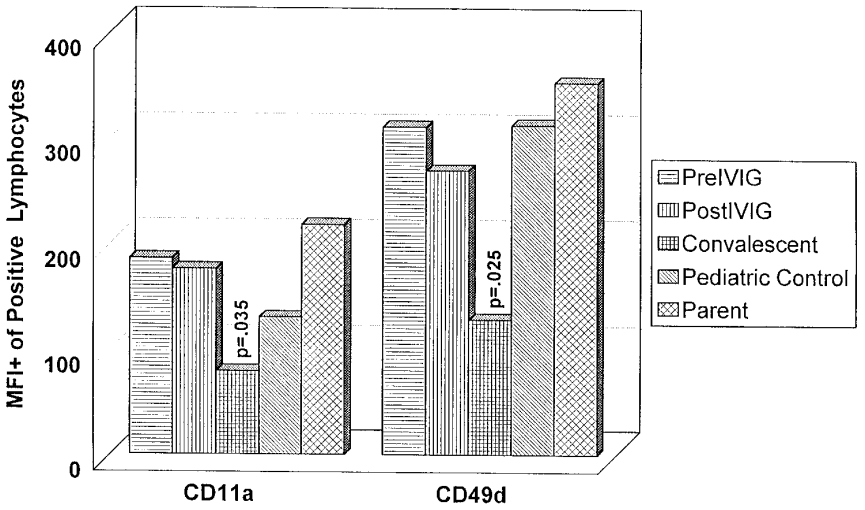

FIG. 6. Median fluorescence intensity of CD11a and CD49d, for $CD8^+$ lymphocytes, by participant characteristics, matched analysis. Pre-IVIG value shown is that for pre- to post-IVIG comparison; *P* values are for comparisons to matched pre-IVIG values. Pre-IVIG, post-IVIG, and parent samples read comparably. Others read at a later date.

acute KD, compared with convalescent disease (9); this is contrary to our findings. CD49d (very late antigen-4 or VLA-4) is a fibronectin receptor present on monocytes, thymocytes, B-lymphocytes, and some T-lymphocytes (38). It may play a role in the homing of $CD8^+$ cells to sites of viral infection (30) and mediates lymphocyte adhesion to cytokine-activated endothelium (41). It also has been implicated in graft vasculitis (29) and systemic lupus erythematosus-associated vasculitis (42, 43). We found declines in proportion or intensity of cells expressing these antigens during convalescent KD compared with acute KD. This was especially true of the one patient with cardiac abnormalities, which resolved during convalescence. These declines support the involvement of T-lymphocytes in the acute KD vasculitic process directly, as well as through the cytokine network. They also suggest that T-lymphocytes involved in endothelial damage during acute KD may be

TABLE 2

Proportions with Elevated Percentage of $CD71^+$ Lymphocytes, by Lymphocyte Subpopulation and Participant Category

	Patient	Pediatric (cardiac)	Parent
$CD4^+$ cells with			
>10% of cells $CD71^+$	34.5% (10/29)	10.5% (2/19)	0% (0/29)
>15% of cells $CD71^+$	27.6% (8/29)	0% (0/19)	0% (0/29)
Blastic $CD4^+$ cells with			
>15% of cells $CD71^+$	62.5% (15/24)	28.6% (4/14)	4.2% (1/24)
>30% of cells $CD71^+$	50.0% (12/24)	14.3% (2/14)	0% (0/29)
$CD8^+$ cells with			
>10% of cells $CD71^+$	13.8% (4/29)	0% (0/20)	0% (0/29)
Blastic $CD8^+$ cells with			
>10% of cells $CD71^+$	52.0% (13/25)	37.5% (6/16)	4.3% (1/23)
>30% of cells $CD71^+$	8.0% (2/25)	6.3% (1/16)	0% (0/23)
Proportion with at least 3 of the above increased[a]	29.2% (7/24)	0% (0/17)	0% (0/23)
Proportion with all 4 of the above increased[a]	21.7% (5/23)	0% (0/13)	0% (0/21)

[a] Defined as >15% of $CD4^+$ cells $CD71^+$, >15% of blastic $CD4^+$ cells $CD71^+$, >10% of $CD8^+$ cells $CD71^+$, and >10% of blastic $CD8^+$ cells $CD71^+$.

TABLE 3

Findings in Patient with Cardiac Lesions, by Nature of Sample

	Pre-IVIG		Post-IVIG		Convalescent	
	% Positive for					
CD3	13.3		53.6		81.1	
CD19	21.1		41.3		8.0	
TCR[a]	23.7		51.7		78.0	
NK[a]	4.5		1.2		3.1	
$CD3^+NK$[a]	1.4		0.1		1.7	
	Of TCR+					
CD4	34.7		58.1		37.2	
CD8	32.2		40.1		61.6	
	Of $CD4^+$	$CD3^+$	$CD4^+$	$CD3^+$	$CD4^+$	$CD3^+$
CD25	—	6.0	—	3.8	—	5.3
	Of $CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
CD29	43.4	38.9	25.0	11.1	34.9	37.5
HLADr	39.9	27.0	7.8	8.2	7.0	9.6
CD38	91.4	81.3	88.1	77.8	90.4	91.8
CD45RA	41.8	84.3	65.3	89.9	76.1	92.3
CD45RO	38.0	2.8	13.9	1.8	17.3	4.1
CD45RA+/RO+	1.3	0	0.2	0	0.3	0.2
CD71	24.2	15.5	1.4	3.2	2.4	3.2
Blastic CD71	36.7	10.6	—	—	2.1	1.6

[a] T-cell antigen receptor variable region α/β chain (TCR); $CD3^-$/CD16 or $CD56^+$ cells, natural killer cells (NK); $CD3^+$/CD16 or $CD56^+$ cells, $CD3^+$ natural killer cells ($CD3^+$ NK).

subsequently removed or eliminated from the peripheral blood.

Although the patients' own post-IVIG and convalescent samples provide the best matched comparisons for the pre-IVIG group, we had expected to find more differences between the pre-IVIG and the pediatric control groups. We hypothesize that, during acute infection, activated T-lymphocytes are eliminated or destroyed at a rate equivalent to the rate at which they are being supplied to the peripheral blood from the much larger lymphatic pool. Eventually, destruction is sufficient to be noted even in the peripheral blood, as reflected by the results found with the convalescent samples.

CD71 is normally expressed on $<2\%$ of PBMLs. The significant differences seen in CD71 expression between the pre-IVIG values and the pediatric controls suggest that CD71 represents a marker for cells involved in acute KD and possibly other forms of vasculitis. In addition, we found changes in $CD8^+$ cells, especially with regard to CD11a and CD49d, and an increase in $CD8^+$ seen in the convalescent sample of the sole patient with cardiac findings. Whether these adhesion markers and cytotoxic T-cells are affected by IVIG and/or play a role in KD merits further investigation.

ACKNOWLEDGMENTS

We are grateful for the cooperation of the participants in this study and for the assistance of numerous fellow, resident, and faculty physicians who identified potential participants. We are especially grateful to Eduardo Montana, M.D., who helped coordinate the clinical aspects of the study, and Sharon Collins, Ph.D., who obtained blood specimens from some participants.

REFERENCES

1. Leung, D. Y. M. Immunologic aspects of Kawasaki syndrome. *J. Rheumatol.* **17**(Suppl. 24), 15–18, 1990.
2. Kato, H., Koike, S., Yamamoto, M., Ito, Y., and Yano, E. Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. *J. Pediatr.* **86,** 892–898, 1975.
3. Suzuki, A., Tizard, E. J., Gooch, V., Dillon, M. J., and Haworth, S. G. Kawasaki disease: Echocardiographic features in 91 cases presenting in the United Kingdom. *Arch. Dis. Child.* **65,** 1142–1146, 1990.
4. Nakamura, Y., Fujita, Y., Nagai, M., Yanagawa, H., Imada, Y., Okawa, S., Kawasaki, T., and Kato, H. Cardiac sequelae of Kawasaki disease in Japan: Statistical analysis. *Pediatrics* **88,** 1144–1147, 1991.
5. Dhillon, R., Newton, L., Rudd, P. T., and Hall, S. M. Management of Kawasaki disease in the British Isles. *Arch. Dis. Child.* **69,** 631–638, 1993.
6. Furukawa, S., Matsubara, T., Jujoh, K., Yone, K., Sugawara, T.,

Sasai, K., Kato, H., and Yabuta, K. Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. *Clin. Immunol. Immunopathol.* **48,** 247–251, 1988.

7. Furukawa, S., Matsubara, T., Motohashi, T., Nakachi, S., Sasai, K., and Yabuta, K. Expression of FcεR2/CD23 on peripheral blood macrophages/monocytes in Kawasaki disease. *Clin. Immunol. Immunopathol.* **56,** 280–286, 1990.
8. Terai, M., Kohno, Y., Niwa, K., Toba, T., Sakurai, N., and Nakajima, H. Imbalance among T-cell subsets in patients with coronary arterial aneurysms in Kawasaki disease. *Am. J. Cardiol.* **60,** 555–559, 1987.
9. Furukawa, S., Matsubara, T., Tsuji, K., Okumura, K., and Yabuta, K. Transient depletion of T cells with bright CD11a/CD18 expression from peripheral circulation during acute Kawasaki disease. *Scand. J. Immunol.* **37,** 377–380, 1993.
10. Barron, K., DeCunto, C., Montalvo, J., Orson, F., and Lewis, D. Abnormalities of immunoregulation in Kawasaki syndrome. *J. Rheumatol.* **15,** 1243–1249, 1988.
11. Leung, D. Y. M., Burns, J. C., Newburger, J. W., and Geha, R. S. Reversal of lymphocyte activation *in vivo* in the Kawasaki syndrome by intravenous gamma globulin. *J. Clin. Invest.* **79,** 468–472, 1987.
12. Leung, D. Y. M., Siegel, R. L., Grady, S., Krensky, A., Meade, R., Reinherz, E. L., and Geha, R. S. Immunoregulatory abnormalities in mucocutaneous lymph node syndrome. *Clin. Immunol. Immunopathol.* **23,** 100–112, 1982.
13. Furukawa, S., Matsubara, T., Obara, T., Okumura, K., and Yabuta, K. Soluble CD2 levels in serum during acute Kawasaki disease and infectious mononucleosis. *J. Infect. Dis.* **167,** 778–779, 1993. [Letter].
14. Shannon, K., Fink, C. W., Buchanan, G. R., Punaro, M., and Stastny, P. Lymphocyte subpopulations in Kawasaki disease. *Clin. Exp. Rheumatol.* **1,** 59–62, 1983.
15. Furukawa, S., Matsubara, T., Tsuji, K., Motohashi, T., Okumura, K., and Yabuta, K. Serum soluble CD4 and CD8 levels in Kawasaki disease. *Clin. Exp. Immunol.* **86,** 134–139, 1991.
16. Abe, J., Kotzin, B. L., Jujo, K., Melish, M. E., Glode, M. P., Kohsaka, T., and Leung, D. Y. M. Selective expansion of T cells expressing T-cell receptor variable regions Vβ2 and Vβ8 in Kawasaki disease. *Proc. Natl. Acad. Sci. USA* **89,** 4066–4070, 1992.
17. Abe, J., Kotzin, B. L., Meissner, C., Melish, M. E., Takahashi, M., Fulton, D., Romagné, F., Malissen, B., and Leung, D. Y. M. Characterization of T cell repertoire changes in acute Kawasaki disease. *J. Exp. Med.* **177,** 791–796, 1993.
18. Curtis, N., Zheng, R., Lamb, J. R., and Levin, M. Evidence for a superantigen mediated process in Kawasaki disease. *Arch. Dis. Child.* **72,** 308–311, 1995.
19. Yamashiro, Y., Nagata, S., Oguchi, S., and Shimizu, T. Selective increase of $V_{\beta}2+$ T cells in the small intestinal mucosa in Kawasaki disease. *Pediatr. Res.* **39,** 264–266, 1996.
20. Peitra, B. A., DeInocencio, J., Giannini, E. H., and Hirsch, R. TCR Vβ family repertoire and T cell activation markers in Kawasaki disease. *J. Immunol.* **153,** 1881–1888, 1994.
21. Nishiyori, A., Sakaguchi, M., Kato, H., Igarashi, H., and Miwa, K. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* **343,** 299–300, 1994 [Letter].
22. Marchette, N. S., Kihara, S., Low, E., Cao, X., Martin, J., and Melish, M. Staphylococcal TSST-1 is not the cause of Kawasaki syndrome in Hawaii. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology, 1994. p. 44. Am. Soc. Microbiol., Washington, DC, 1994. [Abstract G-88].
23. Simpson, I. J., Skinner, M. A., Geursen, A., Peake, J. S., Abbott, W. G., Fraser, J. D., Lockwood, C. M., and Tan, P. L. J. Peripheral blood T lymphocytes in systemic vasculitis: Increased T cell receptor Vβ2 gene usage in microscopic polyarteritis. *Clin. Exp. Immunol.* **101,** 220–226, 1995.
24. Newburger, J. W., Takahashi, M., Burns, J. C., Beiser, A. S., Chung, K. J., Duffy, C. E., Glode, M. P., Mason, W. H., Reddy, V., Sander, S. P., Shulman, S. T., Wiggins, J. W., Hicks, R. V., Fulton, D. R., Lewis, A. B., Leung, D. Y. M., Colton, T., Rosen, F. S., and Melish, M. E. The treatment of Kawasaki syndrome with intravenous gamma globulin. *N. Engl. J. Med.* **315,** 341–347, 1986.
25. Newburger, J. W., Takahashi, M., Beiser, A. S., Burns, J. C., Bastian, J., Chung, K. J., Colan, S. D., Duffy, C. E., Fulton, D. R., Glode, M. P., Mason, W. H., Cody-Meissner, H., Rowley, A. H., Shulman, S. T., Reddy, V., Sundel, R. P., Wiggins, J. W., Colton, T., Melish, M. E., and Rosen, F. S. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N. Engl. J. Med.* **324,** 1633–1639, 1991.
26. Rowley, A. H., Duffy, C. E., and Shulman, S. T. Prevention of giant coronary artery aneurysms in Kawasaki disease by intravenous gamma globulin therapy. *J. Pediatr.* **113,** 290–294, 1988.
27. Nagashima, M., Matsushima, M., Matsuoka, H., Ogawa, A., and Okumura, N. High-dose gamma globulin therapy for Kawasaki disease. *J. Pediatr.* **110,** 710–712, 1987.
28. Jason, J., and Inge, K. L. The effects of mitogens, IL-2, and anti-CD3 antibody on the T-cell receptor Vβ repertoire. *Scand. J. Immunol.* **43,** 652–661, 1996.
29. Paul, L. C., Davidoff, A., Paul, D. W., Benediktsson, H., and Issekutz, T. B. Monoclonal antibodies against LFA-1 and VLA-4 inhibit graft vasculitis in rat cardiac allografts. *Transplant. Proc.* **25**(Pt 1), 813–814, 1993.
30. Christensen, J. P., Andersson, E. C., Scheynius, A., Marker, O., and Thomsen, A. R. Alpha 4 integrin directs virus-activated $CD8^+$ T cells to sites of infection. *J. Immunol.* **154,** 5293–5301, 1995.
31. Amlot, P. L., Tahami, F., Chinn, D., and Rawlings, E. Activation antigen expression on human T cells. I. Analysis by two-colour flow cytometry of umbilical cord blood, adult blood and lymphoid tissue. *Clin. Exp. Immunol.* **105,** 176–182, 1996.
32. Ausiello, C. M., Urbani, F., la Sala, A., Funaro, A., and Malavasi, F. CD38 ligation induces discrete cytokine mRNA expression in human cultured lymphocytes. *Eur. J. Immunol.* **25,** 1477–1480, 1995.
33. Malavasi, F., Funaro, A., Alessio, M., DeMonte, L. B., Ausiello, C. M., Dianzani, U., Lanza, F., Magrini, E., Momo, M., and Roggero, S. CD38: A multi-lineage cell activation molecule with a split personality. *Int. J. Clin. Lab. Res.* **22,** 73–80, 1992.
34. Dianzani, U., Funaro, A., DiFranco, D., Garbarino, G., Bragardo, M., Redoglia, V., Buonfiglio, D., DeMonte, L. B., Pileri, A., and Malavasi, F. Interaction between endothelium and $CD4^+CD45RA+$ lymphocytes. Role of the human CD38 molecule. *J. Immunol.* **153,** 952–959, 1994.
35. Suciu-Foca, N., Rosochacki, S. J., Cai, J., Reed, E., Rubinstein, P., and King, D. W. Immunological and biochemical characterization of an epitope of the transferrin receptor involved in the production of interferon-γ and B-cell growth factor. *Cell. Immunol.* **110,** 265–281, 1987.
36. Kemp, J. D., Thorson, J. A., Gomez, F., Smith, K. M., Cowdery, J. S., and Ballas, Z. K. Inhibition of lymphocyte activation with anti-transferrin receptor Mabs: A comparison of three reagents and further studies of their range of effects and mechanism of action. *Cell. Immunol.* **122,** 218–230, 1989.
37. Morimoto, C., Rudd, C. E., Letvin, N. L., and Schlossman, S. F. A novel epitope of the LFA-1 antigen which can distinguish killer effector and suppressor cells in human CD8 cells. *Nature* **330,** 479–482, 1987.

38. Sánchez-Madrid, F., and Corbi, A. L. Leukocyte integrins: Structure, function and regulation of their activity. *Semin. Cell Biol.* **3,** 199–210, 1992.

39. Fan, S. T., Brian, A. A., Lollo, B. A., Mackman, N., Shen, N. L., and Edgington, T. S. CD11a/CD18 (LFA-1) integrin engagement enhances biosynthesis of early cytokines by activated T cells. *Cell. Immunol.* **148,** 48–59, 1993.

40. Chong, A. S., Boussy, I. A., Graf, L. H., and Scuderi, P. Stimulation of IFN-gamma, TNF-alpha, and TNF-beta secretion in IL-2-activated T cells: Costimulatory roles for LFA-1, LFA-2, CD44, and CD45 molecules. *Cell. Immunol.* **144,** 69–79, 1992.

41. Issekutz, T. B. Dual inhibition of VLA-4 and LFA-1 maximally inhibits cutaneous delayed-type hypersensitivity-induced inflammation. *Am. J. Pathol.* **143,** 1286–1293, 1993.

42. Takeuchi, T., Amano, K., Sekine, H., Koide, J., and Abe, T. Upregulated expression and function of integrin adhesive receptors in systemic lupus erythematosus patients with vasculitis. *J. Clin. Invest.* **92,** 3008–3016, 1993.

43. Takeuchi, T., Aoki, K., Koide, J., Sekine, H., and Abe, T. Systemic lupus erythematosus with necrotizing vasculitis and upregulated expression of VLA-4 antigen. *Clin. Rheumatol.* **14,** 370–374, 1995.

Received February 7, 1997; accepted with revision April 11, 1997