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Kawasaki Disease: A Maturational Defect in Immune Responsiveness

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Kawasaki disease (KD), an acute febrile disease in children of unknown etiology, is characterized by a vasculitis that may result in coronary artery aneurysms (CAAs). In new patients with KD, a selective and prolonged T cell unresponsiveness to activation via the T cell antigen receptor CD3 was observed, whereas proliferation to other stimuli was intact. This "split T cell anergy" delineated KD from other pediatric infections and autoimmune diseases and correlated with CAA formation ($P < .001$). A transient immune dysfunction was also suggested by an incomplete responsiveness to measles-mumps-rubella (MMR) vaccination in patients with KD versus controls ($P < .0001$; odds ratio, 15.6; 95% confidence interval, 4.8–51.1), which was overcome by revaccination(s). The reduced responsiveness to MMR in patients with KD suggests a subtle and predetermining immune dysfunction. An inherent immaturity to clear certain antigens may be an important cause that precipitates KD and the immune dysregulation during acute disease.

Kawasaki disease (KD) is an acute illness of early childhood characterized by persistent fever, induration and erythema of hands and feet, polymorphous rash, inflammation of the mucous membranes, bilateral conjunctivitis, and (cervical) lymphadenopathy [1, 2]. A lack of response to antibiotics is a feature of the disease. In pathologic terms, KD is defined as an inflammatory disease affecting the heart by a small-to-medium-sized vasculitis of coronary arteries [3]. KD results in coronary artery aneurysms (CAAs) in ~25% of the affected children, as demonstrated by echocardiography. In Japan and the United States, KD is the leading cause of acquired heart disease in children, with an annual incidence in children aged <5 years of 90 per 100,000 in Japan and 5–10 per 100,000 in the United States and Europe. To date, therapy consists of intravenous immunoglobulin (IVIG) combined with aspirin [4], which has resulted in a decrease in the incidence of CAAs and in fatality rates [2, 4].

Formally, KD is a clinical diagnosis that cannot be explained by other causes [1]. The differential diagnosis includes bacterial (e.g., scarlet fever, Reiter syndrome, and rickettsiosis) and viral (e.g., measles, parvovirus, human herpesvirus [HHV]–6, and Epstein-Barr virus [EBV]) infections. Some of the bacterial and viral agents causing the above-mentioned illnesses have been directly associated with KD [5–8]. Microbial superantigens have also been incriminated as a cause of KD [9]. The immune system is generally implicated in the development of CAAs as a consequence of autoaggression against the coronary vasculature, caused by either cytokine-mediated toxicity, activated autoreactive T cells that have escaped from regulation, or autoantibody generation [10, 11]. In view of these data, we started a prospective study on the immunologic aspects of KD.

Methods and Materials

Study design. In this study we included new patients with KD, using the criteria for diagnosis and echocardiographic scoring of dilatation of coronary arteries of the revised and generally accepted 1984 guidelines of the Kawasaki Disease Research Committee in Japan [1]. Coronary artery dilatations with a diameter >3 mm or >1.5 times the adjacent vessel diameter are scored as aneurysmatic lesions. The main left and right coronary arteries, left anterior descending, and circumflexal arteries were scored for dilatations. Fractional shortening of the left ventricle, patency and function of the heart valves, and absence or presence of pericardial effusion were also scored. Standard therapy consisted of a single IVIG infusion (2 g/kg in 8–12 h) in combination with oral aspirin (80–100 mg/kg divided into 4 equal doses). A second IVIG infusion was administered when clinical symptoms (i.e., fever) persisted for 72 h or recurred or when echocardiographic findings were compatible with a progression or recurrence of CAAs or the presence of a giant CAA (diameter >8 mm) [12]. Echocardiographic findings were recorded before the IVIG infusion and 3–4 days thereafter; follow-up was at 1- to 3-week intervals during the first 2–3 months, with

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Informed consent was obtained from the parents of the children studied. The institutional medical ethics committee approved the study.

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an increase in frequency of visits depending on the presence, development, or persistence of CAAs.

Lymphocyte subsets and proliferation assays. Absolute numbers of B cell ($CD19^+$) and T cell ($CD2^+$, $CD3^+$, $CD4^+$, $CD8^+$) subsets were determined in the same longitudinal fashion by standard FACScan procedures with monoclonal antibodies (MAbs) produced by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam. Lymphocyte proliferation was simultaneously determined in a heparin-anticoagulated whole blood assay by addition of optimal concentrations of MAbs against T cell antigen receptor (TcR)/CD3 (CLB-CD3/4.E, IgE isotype), CD2 (CLB-CD2/1.1, CLB-CD2/2.1, and CLB-CD2/HIK27, all IgG1 isotype), and CD28 (CLB-CD28/15E8, IgG1 isotype) or the mitogen phytohemagglutinin (PHA) [13]. The advantage of CLB-CD3/4.E MAb is its IgE isotype (instead of IgG1 or IgG2a isotype). This unique and activating MAb makes the assay system independent of the patient's Fcγ-receptor profile (i.e., high vs. low response) [14]. Because of its extremely low avidity, CD23 expression on peripheral blood mononuclear cells (PBMC) is of no relevance in the system [15] (data not shown). The CD2 MAbs were characterized and described most thoroughly by Van Kemenade et al. [16]. The combinations of CD2/28 and CD3/28 MAbs were applied essentially as reported elsewhere [17, 18]. These MAbs were used at a final concentration of 2–5 μg/mL, except for CLB-CD2/HIK27 (0.5 μg/mL final concentration of purified MAb).

Blood from 2 healthy persons was included every day in the assay system as an internal control. The mean proliferation data of these daily controls did not differ from the reference values (historical controls). Under all culture conditions mentioned, proliferative responses were measured by means of [^{3}H] thymidine incorporation, added 24 h before harvest at day 5. Reactivity was calculated to counts/min in 10^3 $CD3^+$ T cells. The first blood samples were always obtained before IVIG infusion and aspirin use; subsequent samples were usually obtained during the visits for echocardiography.

Vaccine and serology tests. Standard measles-mumps-rubella (MMR) vaccine used for the national vaccination program was obtained from the National Institute for Hygiene and Environment. Specific IgG antibodies against measles and mumps were defined by ELISA (Virotech, Rüsselsheim, Germany). The analysis of seropositivity was calculated as follows: (extinction sample/extinction) × 10. Negative means were defined as <9 arbitrary units (AU), gray zone as 9–11 AU, and positive means as >11 AU. Antibodies against rubella virus were measured with IMx (Abbott Diagnostics, Amstelveen, The Netherlands) and were expressed in international units per milliliter (IU/mL) of serum. At least 2 control serum samples per plate were routinely included. Antibodies against tetanus toxoid (TT) were defined with a standard ELISA by use of human anti-TT IgG1 MAb (clone 151) as a reference antibody and expressed in IU/mL serum.

Statistics. Logarithmic values were used for statistical analysis. Analysis of variance was applied for normally distributed data, and the Wilcoxon 2-sample and Kruskal-Wallis tests were used for non–normally distributed data. χ^2 tests were used for statistical analysis of vaccination data.

Results

Split T cell anergy in KD. During the 20 months of this prospective study, 22 new patients admitted to our hospital for echocardiographic and immunologic evaluation were included (table 1). We routinely determined blood cell count, erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP), electrolytes, and aspartate aminotransferase, alanine aminotransferase, and creatinine levels and did microbiologic investigations (cultures from throat, blood, rectum/perineum, and urine for bacteria and serology for measles, EBV, cytomegalovirus [CMV], HHV-6, parvovirus, and streptococcal antistreptolysin O). Lymphocyte number and proliferation data were also determined in a longitudinal fashion. Although we started our study on lymphocyte cultures with purified PBMC, the data from these experiments, performed over 2 months, perfectly matched the findings in a whole blood assay ($n = 4$; data not shown). To minimize the amount of blood required for our follow-up studies, we continued the whole blood assay. Only in the presence of lymphopenia ($<1.0–1.5 \times 10^9$/L) is there a potential risk to underestimate the proliferative capacity of lymphocytes. This did not occur in our cohort. In contrast, we observed lymphocytosis (mainly $CD4^+$ T cells) during the acute and reconvalescent phases that lasted several weeks (figure 1) [19].

In contrast to the increased numbers of lymphocytes, we

Table 1. Clinical data of patients with Kawasaki disease (KD) in a prospective study on immunologic parameters.

Patient	Age	Sex	CAA	No. of IVIG doses: symptoms
Group 1				
1	5 m	M	+	1
2	5 m	M	+	1
3	10 m	M	+	3: progressive/giant CAAs
4	10 m	M	+	2: progressive CAAs
5	1 y, 6 m	M	+	3: progressive CAAs
6	1 y, 7 m	M	+	1
7	3 y, 2 m	M	+	2: recurrent CAAs
8	3 y, 3 m	F	+	1
9	4 y, 8 m	M	+	1
$n = 9$	21.3 ± 17.2 m	8 M/1 F	Yes	Total, 15; mean, 1.7/patient
Group 2				
10	2 m	F	−	1
11	7 m	F	−	1
12	8 m	M	−	1
13	9 m	F	−	1
14	1 y, 5 m	M	−	1
15	2 y, 6 m	F	−	2: persistent fever and rash
16	2 y, 7 m	M	−	1
17	3 y, 1 m	F	−	1
18	4 y, 1 m	F	−	1
19	5 y, 11 m	M	−	1
20	7 y, 10 m	M	−	2: recurrent KD
21	8 y, 6 m	F	−	1
22	9 y, 8 m	M	−	2: recurrent KD
$n = 13$	44.1 ± 39.3 m	6 M/7 F	No	Total, 16; mean, 1.2/patient

NOTE. CAA, coronary artery aneurysm; IVIG, intravenous immunoglobulin; F, female; M, male; m, month; y, year.

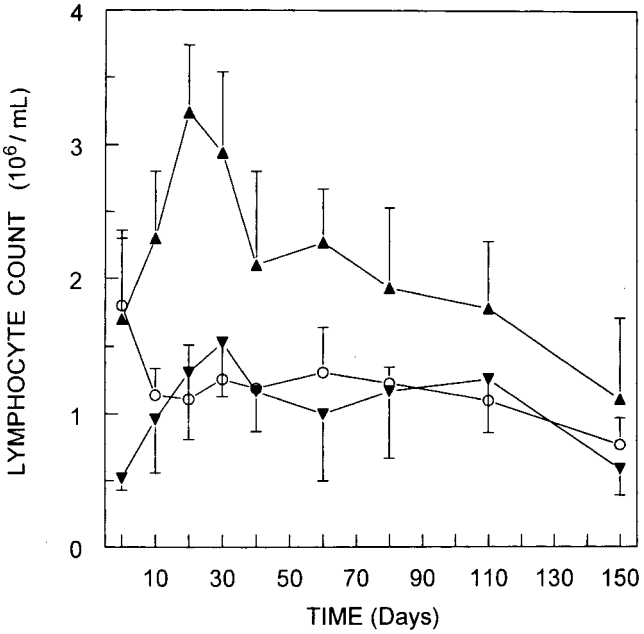

Figure 1. Absolute $CD4^+$ (▲) and $CD8^+$ (▼) T cells and $CD19^+$ B cells (○) in acute and convalescent Kawasaki disease stages. Numbers did not reach values below the lower range of normal age-matched controls for age group 1–5 years (not shown; see also [19]) and are expressed as mean ± SD (in 10^6/mL). Blood samples at $t = 0$ were obtained before intravenous immunoglobulin infusion and aspirin use.

observed a selective and prolonged decrease in T cell proliferation on activation via the TcR/CD3-complex. At the same time, proliferation induced by a mitogen or by the addition of combined CD2 and CD28 MAbs was essentially normal (figure 2). The intact T cell responsiveness toward the combination of CD2/CD28 MAbs and the prolonged nature of the observed split T cell unresponsiveness argue against artifactual factors as an explanation for these results.

When the patients with KD were subdivided into 2 groups, those with CAAs (group 1; $n = 9$) and those without CAAs (group 2; $n = 13$), some differences were noticed. Mean ages differed: 21.3 ± 17.2 months in group 1 and 44.1 ± 39.4 months in group 2; $P = .08$, not significant (NS). Moreover, girls were underrepresented in group 1 (table 1). In both groups, lymphocytosis, as shown in figure 1, was present. The numbers of absolute lymphocytes, $CD19^+$ B cells, and $CD4^+$ and $CD8^+$ T cells did not differ between the 2 groups (all serial samples, $P > .25$, NS). However, the selective TcR/CD3-dependent T cell unresponsiveness remained for a longer period in KD patients with CAA formation (11–90 days after onset of the disease) than in those without (figure 3*A;* $P < .001$, Kruskal-Wallis).

We analyzed several additional laboratory parameters, such as absolute leukocyte, neutrophil, and platelet counts and the inflammatory parameters ESR and CRP in relation to T cell unresponsiveness. However, a significant inverse relationship between these parameters was not seen (not shown).

Mechanisms involved in T cell anergy. We considered several mechanisms to explain the data for the so-called split anergy, such as the presence of suppressive factors in plasma or secreted by PBMC early during the whole blood lymphocyte culture [20]. First, experiments in which control cells were mixed with heparinized plasma (1 : 1) from patients with acute KD (≤1 week after treatment with IVIG and aspirin; $n = 3$, 6 plasma samples in total) did not indicate the presence of any suppressive effect of patient plasma on control lymphocyte cultures; however, the lymphocytes from these patients with KD showed a defective proliferation response. The fact that proliferation of isolated PBMC and simultaneously performed whole blood assays had previously shown identical patterns of T cell unresponsiveness also argued against a suppressive factor in plasma. Second, blockade of tumor necrosis factor–α, interleukin-10, or transforming growth factor–β by inhibitory polyclonal antisera also did not significantly influence proliferation of lymphocytes from patients with KD. Third, enhanced apoptosis (e.g., through activation-induced programmed cell death, as determined by staining with the AnnexinV–fluorescein isothiocyanate label to detect apoptotic cells in these cultures at

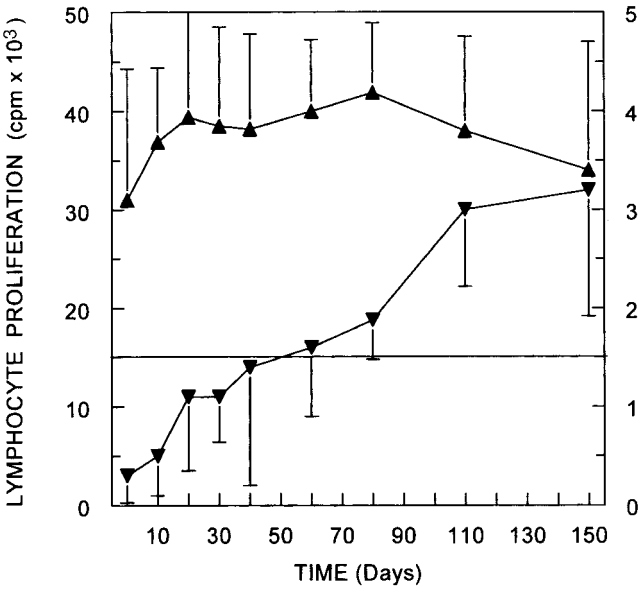

Figure 2. Lymphocyte proliferation in patients with Kawasaki disease (KD). CD2/CD28-induced T cell proliferation (▲, left axis) was intact in new patients with KD ($n = 22$) in contrast to T cell receptor/CD3–induced T cell proliferation (▼, right axis). Phytohemagglutinin-induced T cell proliferation was essentially unaffected (data not shown). Horizontal line separates normal (above) from abnormal lymphocyte proliferation (below), i.e., 2 SD below mean of 200 controls at 15,000 and 1500 cpm for CD2/CD28- and CD3-induced lymphocyte proliferation, respectively. Blood samples at $t = 0$ were obtained before therapy.

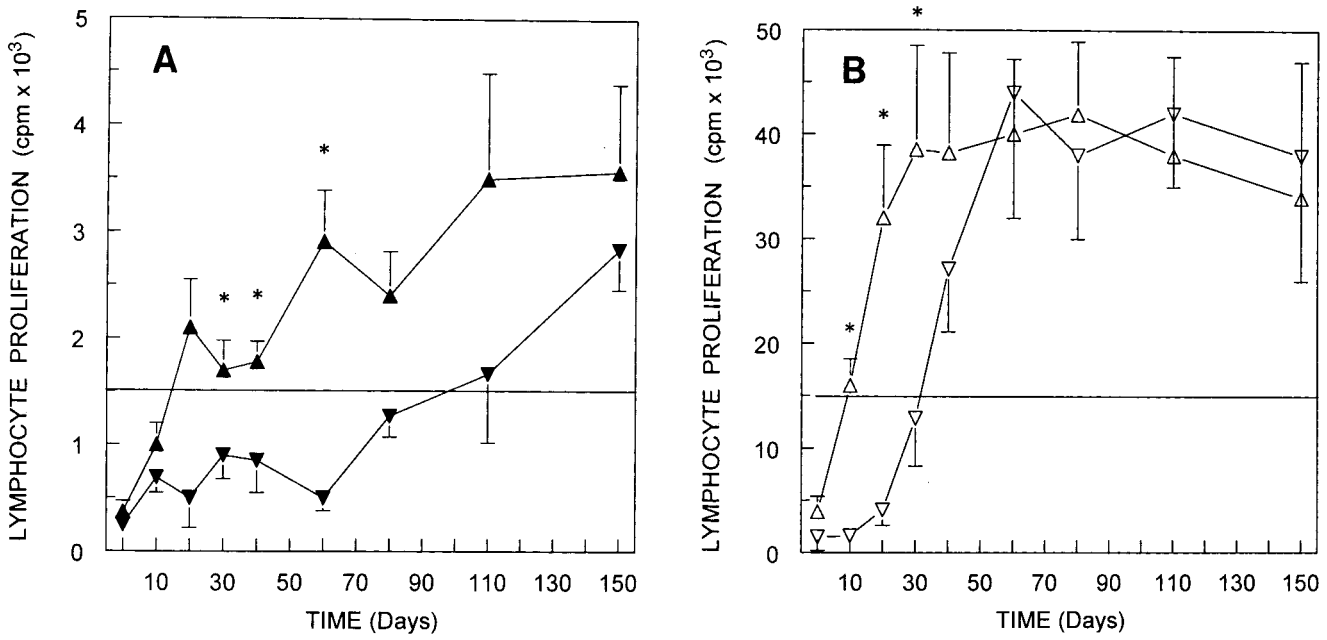

Figure 3. Split anergy reflects disease severity in Kawasaki disease. *A,* in vitro capacity of T cell receptor/CD3–induced T cell proliferation between patients with coronary artery aneurysms (CAAs; ▼; $n = 9$) and without CAAs (▲; $n = 13$). *B,* in vitro CD3/CD28-induced T cell proliferation between patients with CAAs (▽; $n = 9$) and without CAAs (△; $n = 13$). *Significant difference ($P < .05$) between 2 groups. *A,* 10–90 days after intravenous immunoglobulin (IVIG) infusion, $P < .001$; in *B,* significance was most apparent 10–40 days after IVIG infusion, $P < .01$. Horizontal line separates normal (above) from abnormal (below) lymphocyte proliferation. Blood samples at $t = 0$ were obtained before therapy (i.e., IVIG infusion and aspirin).

days 0 and 5) was not demonstrated in the lymphocyte cultures from patients with KD. Finally, the mean levels of CD3 expression on lymphocytes were not reduced, compared with those of age-matched control patients, and thus cannot explain the split T cell anergy (not shown).

An alternative mechanism to explain the data on split T cell anergy could be lack of costimulatory signals. However, even if the most important costimulatory molecules (CD80 and CD86) were expressed at very high levels, their functional capacity to trigger, via CD28, TcR/CD3-induced lymphocyte proliferation would have been strongly reduced. T cell anergy was also present when CD3 and CD28 MAbs were combined (figure 3*B;* $P < .01$, Kruskal-Wallis) for 11–40 days after the onset of the disease. For as yet unknown reasons, normalization of T cell responsiveness toward CD3/CD28 occurred earlier during follow-up than the effect of the CD3 MAb alone (figure 3). Together, these data are most compatible with a cell-related phenomenon.

Comparison with age-matched controls. To define the specificity of these results, we included the following pediatric patients as controls: 7 patients with sepsis (group A *Streptococcus pyogenes* [GAS], 3; *Streptococcus pneumoniae,* 2; and *Staphylococcus aureus,* 2); 3 patients with GAS-related toxic shock–like syndrome; 5 with scarlet fever; 6 with undefined pneumonia; 19 with viral diseases: measles, 7; EBV, 5; CMV, 4; and hepatitis B virus, 3); 5 with enterovirus-associated myocarditis; 3 with juvenile chronic arthritis; and 5 with idiopathic thrombocytopenic purpura within 7 days after IVIG infusion): infection associated, 3; herpes simplex virus type 1, 1; and CMV-EBV coinfection, 1. In these groups, lymphocyte proliferation did not show the form of split anergy, as found in the patients with KD. Proliferation was never as profoundly decreased as in patients with KD, either in severity or in duration. For clarity, the data for these separate patient cohorts are combined in figure 4.

Measles and scarlet fever, close clinical mimics, could be distinguished from KD. In contrast to earlier observations on measles-related immune suppression and mitogen-dependent defects in lymphocyte reactivity [21], the reduction in PHA-induced proliferation did not reach significance ($P = .14$, NS), whereas the CD3- and combined CD3/CD28-induced T cell proliferation responses were normal. In acute EBV infections, no defect in proliferation was observed (figure 4). On the other hand, longitudinal data from patients with organ transplants who were receiving immunosuppressive medication (prednisone, cyclosporin, and azathioprine) showed a selectively depressed CD3-induced lymphocyte proliferation observed only in the presence of EBV (or CMV) reactivation (unpublished data). Also, under these conditions, CD3/CD28-induced lymphocyte proliferation was always intact. In human immunodeficiency virus (HIV) infection, a progressive decline in T cell responses to CD3/CD28 stimulation has been observed (often

in the presence of low $CD4^+$ T cell counts) in seropositive persons who rapidly progress to clinical AIDS [18]. In KD, $CD4^+$ T cell counts were elevated yet transiently defective in proliferation reactivity (figures 1 and 3).

In vivo correlate for split T cell anergy? Measles virus infection and scarlet fever are the main infections to be excluded in patients suspected of having KD [22]. In the acute phase, cultures for bacterial and viral agents remained negative, and serologic tests for anti-streptolysin O, anti-DNAse B, and IgM antibodies against measles (among other viruses) were negative in all patients with KD but 1, who had chickenpox and IgM to varicella zoster virus [23].

Unexpectedly, a number of patients who ought to have had serologic proof of previous immunization with MMR vaccine were also negative for serum measles IgG antibodies. The incomplete seroconversion was further evaluated by determination of the complete serologic profile against all 3 mitigated viral strains in the MMR vaccine. Only 8 of 33 patients with KD showed a complete seroconversion (all were ⩾16 months old at KD diagnosis). The number of serologic responders against the complete vaccine or against the separate components differed from that of control patients matched for age, age at onset of disease, and disease-related symptoms (i.e., persistent fever and rash, stomatitis, vomiting, diarrhea, pulmonary infiltrates, or proven meningitis/encephalitis; table 2). There was a trend toward lower titers against all 3 MMR components in patients with KD, compatible with reduced T cell–dependent seroconversion responses (figure 5).

Table 2. Statistical analysis of the number of positive responders to measles-mumps-rubella (MMR) vaccination among patients with Kawasaki disease (KD) and control patients.

Condition	Control[a] (total)	KD (total)	χ^2	*P*	OR	95% CI
MMR[b]	30 (36)	8 (33)	24.3	<.0001	15.6	4.8–51.1
Measles	33 (36)	19 (33)	10.8	<.001	8.1	2.1–31.9
Mumps	30 (36)	14 (33)	12.5	<.001	6.8	2.2–20.7
Rubella	35 (36)	23 (33)	9.7	<.005	15.2	1.8–127.0

NOTE. OR, odds ratio; CI, confidence interval.

[a] Control patients were matched for age, age at onset of disease, and disease-related symptoms (i.e., all controls had persistent fever in combination with ⩾1 of the following symptoms: rash, stomatitis, vomiting, diarrhea, pulmonary infiltrates, or proved meningitis/encephalitis).

[b] Complete response to all 3 components of the MMR vaccine.

In vivo boosting responses in former KD patients. The in-

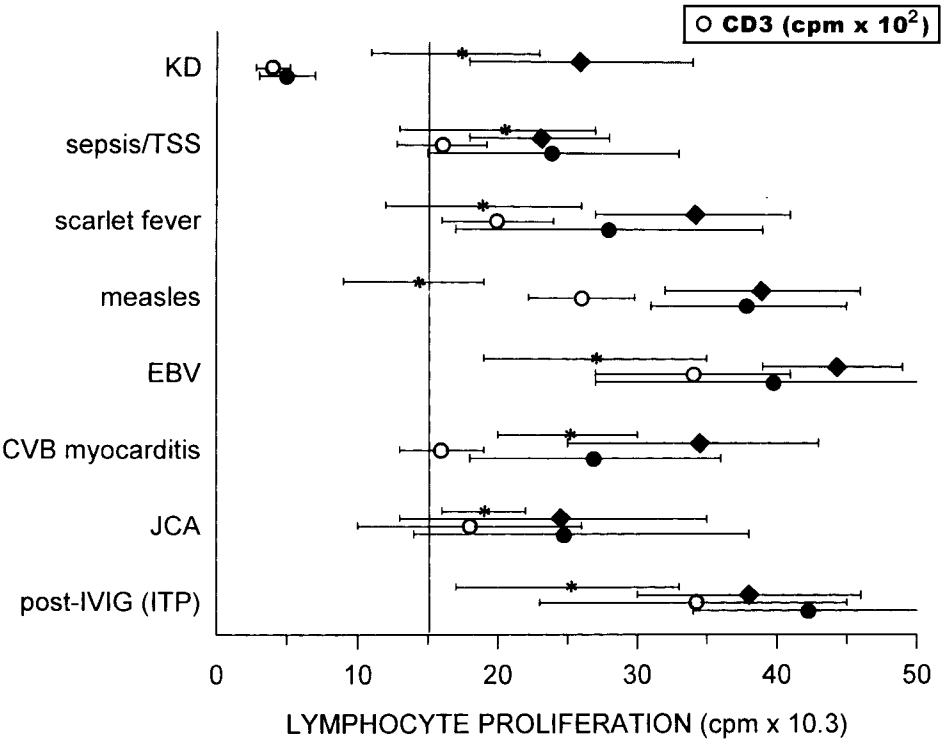

Figure 4. Lymphocyte proliferation cohorts of various pediatric diseases compared with Kawasaki disease (KD) cohort. Reactivity toward phytohemagglutinin (*), CD2/CD28 (♦), CD3 (○), and CD3/CD28 (●) are shown for children with acute KD ($n = 22$; mean age ± SD, 2.9 ± 2.7 years); sepsis and toxic shock syndrome (TSS; $n = 10$; age, 3.7 ± 3.19 years); scarlet fever ($n = 5$; age, 3.8 ± 0.64 years); measles ($n = 7$; age, 6.9 ± 4.47 years); Epstein-Barr virus (EBV; $n = 5$; age, 1.9 ± 0.81 years); Coxsackie B virus (CBV)–associated myocarditis ($n = 5$; age, 6.0 ± 3.46 years); juvenile chronic arthritis (JCA; $n = 3$; age, 4.5 ± 2.29 years); and idiopathic thrombocytopenic purpura (ITP; after intravenous immunoglobulin [IVIG]; $n = 5$; age, 2.7 ± 4.10 years). If serial measurements were done in 1 patient, proliferation results from first 2 weeks of clinical presentation were used for analysis. Data are expressed as mean proliferation ± SD (in cpm × 10^3) for all stimuli but CD3 response (expressed in cpm × 10^2).

herent unresponsiveness against MMR components was further evaluated by booster vaccinations in patients with KD who had an incomplete seroconversion ($n = 21$). Within this group, 9 former patients were included in whom KD had been diagnosed during infancy (age <1 year [$n = 10$]) and who had incomplete seroconversion on their first primary MMR vaccination. Normally, this national MMR vaccination program consists of an immunization at age 14 months followed by a booster vaccination at age 9 years. However, in these young patients with KD, the primary vaccination had been postponed in all but 1 until ⩾6 months (range, 4–12 months) had passed between the last IVIG and the primary MMR vaccination. Revaccination in these 9 patients was done ⩾6 months after the primary vaccination.

Also, in the remaining 12 former patients with KD selected for boosting, the vaccine was given at a time when IVIG can be assumed to no longer play a role. Similar to the situation with primary vaccination in nonimmune patients ⩽14 months old at the onset of KD, the 12 remaining "MMR-preimmune" patients who had KD after their primary vaccination were reimmunized ⩾6 months after the last IVIG administration during the acute phase of KD (post-KD range, 0.6–4.1 years [mean, 1.9 ± 0.98]). Thus, the exogenous antibody levels against specific antigens derived from the IVIG preparations are believed to have had no relevance because of the elapsed time after IVIG. Second, serologic findings were always checked in the patients prior to reimmunization to exclude subclinical infection with a wild-type strain of measles, mumps, or rubella. The MMR seroconversion pattern for these patients with KD was identical to the serologic findings of the incomplete primary response, either when they were immunized afterward (KD in infants) or when the pattern was determined in serum obtained prior to the IVIG infusion for acute KD (preimmune patients with KD). The humoral response to MMR reimmunization was defined by measuring specific antibody levels again 6–8 weeks after vaccination (most often followed by a second serum sample when the anti-MMR response was still not complete). Third, in vitro T cell proliferation had normalized in all patients before reimmunization. Of the 21 former patients with KD tested for boosting responses, 7 (only 2 with KD during infancy) had to be restimulated for a third time before complete seroconversion was reached (see table 3.)

Table 3. Result of measles-mumps-rubella (MMR) boosting in patients with Kawasaki disease who had incomplete seroconversion.

Condition	First response	Booster response	Repeated booster
MMR[a]	0/21	14/21 (66.7)	21/21 (100)
Measles	8/21 (38.1)	18/21 (85.7)	21/21 (100)
Mumps	5/21 (23.8)	15/21 (71.4)	21/21 (100)
Rubella	11/21 (52.4)	20/21 (95.2)	21/21 (100)

NOTE. Data are no. responding/no. inoculated (%).
[a] Complete response to all 3 components of the MMR vaccine.

Antibody catabolism in KD? The possibility of a general and rapid catabolism or sequestration of specific antibodies during KD was evaluated but was considered a less likely ex-

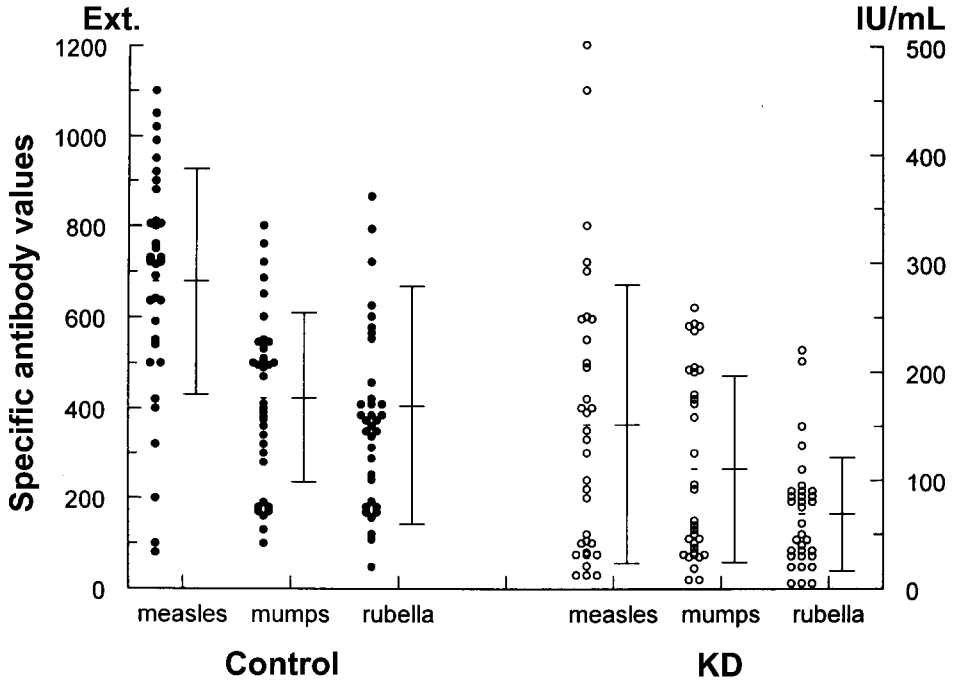

Figure 5. Antibody levels against measles-mumps-rubella vaccine components in patients with Kawasaki disease (KD) and in age- and disease-matched controls. Specific antibodies against measles and mumps were measured by ELISA; titers are expressed as mean extinction (Ext.) ± SD. Antibodies to rubella were defined by IMx (Abbott Diagnostics, Amstelveen, The Netherlands) and are expressed as mean ± SD (right axis).

planation, for the following reasons. First, anti–TT IgG antibodies were determined in a subset of patients with KD who had incomplete MMR seroconversion, for whom serial samples of the corresponding control patients (matched for age, age at onset of disease, and disease-related symptoms) were available during a similar period of follow-up (figure 6). Apart from being age and disease matched, the time range of follow-up for the serum samples of these 9 pairs of controls and patients with KD was almost identical (7.1 ± 1.2 vs. 7.7 ± 1.4 months for controls and patients with KD, respectively). As shown in figure 6, the anti-TT antibody levels in patients with KD (before IVIG) were slightly higher (NS). However, the anti-TT antibody ratio between the 2 samples, that is, the natural decline of antibody level during follow-up, was similar between matched control and patients with KD (0.73 ± 0.21 vs. 0.75 ± 0.15; NS). Second, a pattern of incomplete seroconversion is also often seen in patients with KD who have the disease during infancy. In these children, MMR vaccination took place after the acute stage of the disease. Among 10 of these young patients, 9 who formerly had KD showed an incomplete seroconversion at the first vaccination (whereas seroconversion was incomplete in only 1 of 10 age- and disease-matched controls tested). Among 23 subjects who had been vaccinated with MMR prior to KD, there were 16 incomplete responders. Third, all but 1 of the older patients with KD in whom seroconversion to MMR was incomplete before the acute episode of KD showed normal positive antibody responses to at least 1 and most often to 2 of the 3 different viral MMR components tested, excluding a general phenomenon. Moreover, revaccination (as expected) influenced the state of seroconversion (table 3). Finally, in experiments that mixed serum from patients with incomplete seroconversion with control sera, the presence of a nonspecific inhibitory factor in the serum of these patients was not indicated (not shown).

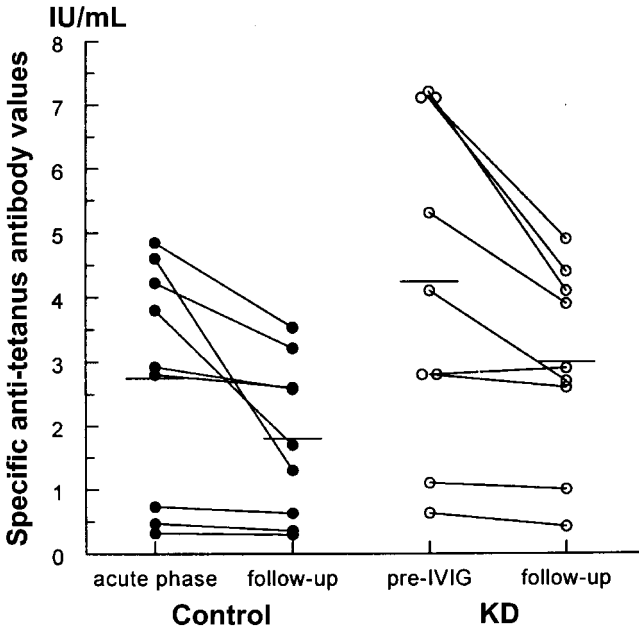

Figure 6. Presence and natural decline of antibodies against tetanus toxoid (TT) in patients with Kawasaki disease (KD) and in age- and disease-matched controls over time. Sera from patients with KD and from controls (respective mean ages, 2.9 ± 1.5 and 2.7 ± 1.5 years) obtained during acute phase of disease and after similar periods of follow-up (range, 6–9 months) were compared. Specific antibodies against TT were measured by ELISA and are expressed as mean ± SD.

Discussion

KD is a childhood vasculitis that especially affects the coronary arteries. Although some cases of KD have been precipitated by streptococcal, staphylococcal, or viral infections [2, 4–9, 23], the exact etiology and pathophysiology of KD has remained obscure. Numerous reports have noted the activated state of the immune system [2], but a clear immunologic hypothesis has never been formulated.

We became focused on MMR vaccination by serendipity. At the moment of acute KD, measles was routinely excluded by the lack of serum measles IgM antibodies. However, many of the children were also lacking measles IgG antibodies, despite prior vaccination. Incomplete seroconversion to ⩾1 components of the vaccine was subsequently found in 25 of 33 former patients with KD, in contrast to matched control patients (table 2; $P < .0001$). The National Institute for Hygiene and Environment has found a 93%–98% rate of seroconversion against each of the components 18 months after the first immunization [24]. In a wider age range, we found 6 (16.7%) of 36 control patients to have an incomplete serologic response. This number is in accordance with published data on complete seroconversion and persistence of specific antibodies of 85%–95% regardless of whether the children had a mild illness at the time of vaccination [25–29]. Repeated vaccination of the former KD patients who showed incomplete seroconversion overcame this lack of in vivo immune responsiveness, although >30% of the children needed a third MMR booster vaccination (table 3).

When individual antibody levels are considered, the lack of competent in vivo responses seems to be limited to some individually defined antigens and modulated in time and on restimulation. In groupwise comparison of controls and former KD patients (figure 5), patients with KD had lower antibody levels against MMR (NS); however, this was not a general phenomenon, as indicated by the presence of normal or even increased levels of TT antibodies in a group of patients with KD who had an incomplete seroconversion to MMR. Moreover, all showed the usual decline of antibody levels during follow-up when compared with age- and disease-matched controls (figure 6). Although it is unknown whether the increased TT antibody levels in patients with KD can be ascribed to the general increase of immunoglobulin during the acute stage of

the disease [2], the data contrast with the absence of specific antibodies against ⩾1 of the MMR components in many of these patients with KD. Thus, a general increased catabolism of specific antibodies cannot easily accommodate our serology findings on MMR and TT. We favor the idea of an as yet unknown predetermining factor in KD that defines a low primary response or failure to sustain an early response. To a certain extent, these two possibilities may be linked. In 3 patients with KD who had an incomplete seroconversion, we saw a brisk yet rapidly disappearing IgM response to 1 of the MMR components to which no antibodies were detected prior to reimmunization. This was not followed by a switch to specific IgG. When IgG antibodies had been generated, levels seemed to be normally sustained in patients with KD (figure 6; data not shown).

Our hypothesis of a subtle immune defect in KD is attractive for several reasons. First, it can explain why so many different antigens, mostly of infectious origin, have been reported to precipitate KD and why these children are clinically not immunocompromised sensu strictu. The so-called split T cell anergy in acute KD (figure 2) might be explained by the dysregulating effect of extensive antigenic stimulation on top of an imbalanced immune system, with the most extreme T cell unresponsiveness in patients who will develop CAAs (figure 3). This selective and prolonged form of split anergy was not seen in any of the cohorts of control patients tested (figure 4).

Lymphocytes obtained from patients with KD during the early stages of the disease also did not respond to the addition of an optimal concentration of costimulatory CD28 MAb. Of interest, the capacity to respond to costimulation normalized during follow-up slightly earlier than the CD3-induced lymphocyte proliferation (figure 3). Many suppressive mechanisms that depend on soluble factors or increased apoptosis have been excluded. However, T cell anergy may be related to a direct physical defect in the clustering or capping of the TcR with additional molecules or a temporary signaling defect downstream of the TcR/CD3 complex [30–32]. The resistance to CD28-dependent costimulation found in KD was previously observed in murine anergic T cells, which could still be activated by phorbol esters [33]. In KD, the combination of CD2/CD28 MAbs was found to circumvent the T cell unresponsiveness toward CD3 or CD3/CD28 MAbs (figure 2). The mechanism behind this split anergy in KD is unknown and clearly warrants further study. Collectively, these data remain most compatible with the hypothesis of a subtle cellular immune dysfunction.

A relevant study of the relationship of IVIG treatment to immune parameters in KD from an era in which patients were treated with aspirin or the combination of IVIG and aspirin showed a beneficial effect of IVIG on phenotypic changes of lymphocytes and in vitro immunoglobulin synthesis [34]. At present, all patients with KD are treated with IVIG and aspirin (table 1). As a further expansion on these findings, we can now add that IVIG does not always result in improved lymphocyte functions (e.g., proliferation) and that the duration of depressed T cell responsiveness significantly correlates with CAA formation.

The fact that this subtle immune defect in T cell responsiveness seems transient and maturationally defined can reconcile some other important issues of KD. First, age is an important risk factor for KD. Infants are at increased risk for the development of CAAs [35] (i.e., at an age when the immune system is still easily dysregulated). This is underscored by our data on split anergy (table 1; figure 2) and by the incomplete seroconversion in almost all young patients with KD. Second, KD is rarely diagnosed in adults but is more likely to occur if their immune system was imbalanced by HIV infection [36–38]. Finally, the MMR revaccination data reinforce the intrinsic capacity of patients with KD to mature and overcome the unresponsiveness (table 3).

In conclusion, we found a general phenomenon of incomplete seroconversion to routine MMR vaccination in former KD patients in combination with a dysregulated TcR/CD3-dependent T cell unresponsiveness during acute disease activity. A limited, antigen-specific, and perhaps maturationally defined immune defect is proposed to reconcile these and other published data on KD. For example, a reduced CD40L up-regulation on T cells may explain several deficient responses of the immune system, such as inadequate B cell help for seroconversion on infection or vaccination and a potentially decreased cytotoxicity against microorganisms during acute disease [39, 40]. Preliminary data showed a decreased up-regulation of early activation markers such as CD69 and CD40L on T cells in KD (data not shown). The beneficial effect of IVIG administered during the early phase of KD [4] can thus be explained by enhanced clearance or scavenging of infection-related antigens to which the immune system could not mount a sufficiently rapid humoral response. Although the precise mechanism involved in the T cell unresponsiveness in patients with KD remains to be unraveled, these findings open a new window on KD.

Holland/Flevoland KD Study Group Members

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