CONCISE COMMUNICATION

CD8 T Lymphocytes and Macrophages Infiltrate Coronary Artery Aneurysms in Acute Kawasaki Disease

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The pathogenesis of coronary artery inflammation in acute Kawasaki disease (KD) is unclear. To test the hypothesis that the KD vascular lesion is an activated T lymphocyte-dependent process, immunohistochemical studies were done on coronary artery aneurysms from 8 fatal acute KD cases by using antibodies to CD45RO (activated or memory T lymphocyte), CD8 (cytotoxic T lymphocyte), CD4 (helper T lymphocyte), HAM56 (macrophage), and CD20 (B lymphocyte). Acute KD coronary arteritis was characterized by transmural infiltration of CD45RO T lymphocytes with CD8 T lymphocytes predominating over CD4 T lymphocytes. Macrophages were present primarily in the adventitial layer; B lymphocytes were notably absent. These data lend support to the hypotheses that KD results from infection with an intracellular pathogen, such as a virus, whose antigens are presented by major histocompatibility complex I molecules, and that CD8 T lymphocytes and macrophages are important in the pathogenesis of KD coronary aneurysms.

Kawasaki disease (KD) is an acute vasculitis occurring in young children that particularly affects the coronary arteries and can be fatal. Coronary arteritis in KD is characterized histologically by inflammatory cell infiltration and destruction of extracellular matrix, especially elastic tissue in vascular media, with resultant coronary artery aneurysm formation. Routine histologic staining of affected vessels demonstrates mononuclear cell infiltrates [1]; however, immunophenotypic characterization of these cells has been reported in only a single case [2]. Frozen sections of myocardium and coronary artery from the case demonstrated that infiltrating cells were predominantly T lymphocytes and macrophages. More cells stained with Leu-3a antibody (helper T lymphocytes) than with Leu-2a antibody (cytotoxic T lymphocytes). No staining was observed with B1 and Leu-12 antibodies (CD4 and CD20 on B lymphocytes). Plasma cells have been observed in KD vascular tissue [1], and we found that IgA plasma cells prominently infiltrate the vascular wall in all of 18 patients who died because of acute KD examined [3, 4].

We hypothesized that the KD vascular lesion is an activated T cell–dependent process. To determine the immunophenotype of the inflammatory cells in the primary target tissue in KD, we did immunohistochemical studies on coronary artery aneurysms from 8 patients who died of acute KD.

Patients and Methods

Patients. Formalin-fixed paraffin-embedded coronary artery aneurysms from 8 patients who died of acute fatal KD were studied (table 1). We also studied coronary artery aneurysm and myocardial tissue from a 6-month-old boy who underwent heart transplantation 3 months after the onset of KD [5]. Spleen tissue from patients 2 and 7 served as positive controls for CD4 and CD8 antibodies, and normal lymph node was used as a positive control for CD45RO, CD20, and HAM56 antibodies. Coronary artery sections from 5 children (ages 2 weeks, 2 months, 4 months, 8 months, and 16 years) who died of causes other than KD (pneumonia, congenital heart disease, sepsis, glioma, or Hodgkin’s disease) also were tested for the presence of CD4 and CD8 T lymphocytes.

Immunohistochemistry. Blocks were sectioned (5 μm), deparaffinized with xylene, and hydrated in graded alcohol. As recommended by Novocastra Laboratories, antigen retrieval for CD4 and CD8 antibodies was enhanced by heating sections in 0.001 M EDTA (pH 8.0) in a microwave oven. Endogenous peroxidase activity was quenched with 1% hydrogen peroxide in PBS for 30 min. Nonspecific binding was blocked with 3% normal horse serum in PBS for 30 min. Sections were incubated for 1 h at room temperature with mouse anti–human antibodies to CD20 (B lymphocyte), HAM56 (macrophage), and CD45RO (activated/memory T lymphocyte; all 3 antibodies from Dako) or overnight at 4°C with mouse anti–human antibodies to CD4 and CD8 (Novocastra Laboratories; NCL-CD4-1F6 and NCL-CD8-4B11). After a wash in PBS, staining was de-
Control spleen tissues from patients 2 and 7 showed the ex-
a 2-fold excess of CD4 T lymphocytes over CD8 T lymphocytes. Myocardium
showed patchy infiltration with greater numbers of CD8 (figure 1
pared with CD4 T lymphocytes (figure 1
G
T lymphocytes in the inflammatory infiltrates (figure 1
). Thus, there were
12–47) CD8 T lymphocytes per high-power field in the coronary
ary vessels (figure 1
A
). Determination of CD4:CD8 lymphocyte ratio in coronary aneu-
ysms. The number of CD4 and CD8 lymphocytes was identified separately in 15 high-power fields (×20 objective) from each case, and these values were used to determine a mean, SD, and range of CD4 and CD8 lymphocytes for KD coronary artery aneurysms. We used the Wilcoxon signed rank test to compare the means of CD4 and CD8 lymphocytes as matched random measurement samples.

### Results

All acute KD coronary artery aneurysms (n = 8) demonstrated marked transmural infiltration by CD45RO+ T lymphocytes (figure 1A). CD45RO+ T lymphocytes did not selectively infiltrate a particular layer of the vessel wall. Similar findings were observed in inflamed but nonaneurysmal coronary vessels (figure 1D) and in the myocardium, which often showed patchy infiltration. In an additional case in which heart transplantation was performed 3 months after the onset of KD, CD45RO+ T lymphocytes were prominent both in the aneurysm and in the myocardium.

CD4 T lymphocytes were present in the KD coronary artery aneurysm wall, but CD8 T lymphocytes were more prevalent in all 8 patients who died of acute KD. There was a mean of 6 ± 6 (range, 1–15) CD4 T lymphocytes and 27 ± 12 (range, 12–47) CD8 T lymphocytes per high-power field in the coronary artery aneurysms of the 8 patients who died of acute KD (P = .01). Thus, there were ≥4–5-fold greater numbers of CD8 T lymphocytes in the inflammatory infiltrates (figure 1H), compared with CD4 T lymphocytes (figure 1G). Myocardium showed patchy infiltration with greater numbers of CD8 (figure 1J) than of CD4 T lymphocytes (figure 1I) present. In the patient with KD requiring cardiac transplantation, there was a 2-fold excess of CD4 T lymphocytes over CD8 T lymphocytes. Control spleen tissues from patients 2 and 7 showed the expected marked predominance of CD4 T lymphocytes over CD8 T lymphocytes, with numerous CD4 T lymphocytes in and around germinal centers and fewer CD8 T lymphocytes in a perivascular distribution. CD4 and CD8 T lymphocytes were both absent in control coronary arteries, with the exception of rare CD4 and CD8 lymphocytes in the adventitial layer in 3 of the 5 cases.

Macrophages were present in the KD coronary artery aneurysm wall but were confined largely to the adventitial layer, with far fewer cells (often single cells) present in the neointimal layer and relative sparing of the media (figure 1C). Overall, macrophages were consistently only half as prevalent as CD45RO+ T lymphocytes. Similar findings were observed in inflamed but nonaneurysmal coronary arteries (figure 1F). Macrophages were particularly prominent adjacent to areas of neovascularization within the adventitia. In the myocardium, macrophages were half as prevalent as CD45RO+ T lymphocytes and were found in areas of adventitial fatty tissue surrounding the myocardium. In the patient with KD requiring transplantation 3 months after onset, the macrophage infiltrate was more diffuse. Numerous macrophages were present in all 3 layers of the coronary aneurysm wall, including the media.

CD20 B lymphocytes virtually were absent from coronary artery aneurysms (figure 1B), although they were occasionally found in lymphocyte clusters in the adventitial layer of the vessel. These cells also were absent in inflamed but nonaneurysmal coronary arteries (figure 1E), in myocardium, and in the coronary artery aneurysm of the child who had undergone a heart transplantation 3 months after onset. B lymphocytes in control lymph node sections stained appropriately with the CD20 antibody. These findings demonstrate that plasma cells are the only type of B lymphocyte present in the inflammatory infiltrate in the KD vascular wall; terminally differentiated plasma cells do not express CD20.

### Discussion

In our study, acute KD vasculitis was characterized by infiltration of the coronary artery wall primarily by CD8 T lymphocytes and macrophages and by a lack of undifferentiated B lymphocytes. We previously reported that IgA plasma cells infiltrate the vascular wall in acute KD [3, 4]. Terminally differentiated B lymphocytes, such as plasma cells, do not express CD19 and CD20, the markers common to undifferentiated B lymphocytes. The activated CD45RO+ T lymphocytes present in the vascular lesion may secrete cytokines or other factors that induce terminal differentiation of IgA B lymphocytes into plasma cells as they enter the vascular tissue. CD45RO+ T lymphocytes appear integral to the vascular lesion, since they are present transmurally. Macrophages are most prominent in adventitia and neointima, which suggests that they may serve as scavengers to “clean up” necrotic debris. They also may secrete factors to remodel vascular matrix, such as matrix me-

### Table 1. Characteristics of 9 children with Kawasaki disease (KD).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex/ethnicity</th>
<th>Time since KD onset</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 months</td>
<td>M/white</td>
<td>15 days</td>
<td>Ruptured CAA</td>
</tr>
<tr>
<td>2</td>
<td>10 months</td>
<td>F/black</td>
<td>5 weeks</td>
<td>MI</td>
</tr>
<tr>
<td>3</td>
<td>8 months</td>
<td>F/unknown</td>
<td>3 weeks</td>
<td>Ruptured CAA</td>
</tr>
<tr>
<td>4</td>
<td>4 months</td>
<td>M/Unknown</td>
<td>4 weeks</td>
<td>CHF</td>
</tr>
<tr>
<td>5</td>
<td>10 months</td>
<td>M/white</td>
<td>13 days</td>
<td>Ruptured CAA</td>
</tr>
<tr>
<td>6</td>
<td>2 years</td>
<td>M/white</td>
<td>5–6 weeks</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>7</td>
<td>4 months</td>
<td>M/white</td>
<td>3–4 weeks</td>
<td>Myocarditis/arrhythmia</td>
</tr>
<tr>
<td>8</td>
<td>3 months</td>
<td>F/white</td>
<td>2–3 weeks</td>
<td>MI</td>
</tr>
<tr>
<td>9</td>
<td>6 months</td>
<td>M/white</td>
<td>3 months</td>
<td>Survived; heart transplant done</td>
</tr>
</tbody>
</table>

NOTE: CAA, coronary artery aneurysm; CHF, congestive heart failure; MI, myocardial infarction.
Immunohistochemical stains for activated or memory T lymphocytes (CD45RO), B lymphocytes (CD20), macrophages (HAM 56), CD4 T lymphocytes (helper T lymphocytes), and CD8 T lymphocytes (cytotoxic/suppressor T lymphocytes) in coronary artery aneurysms (A–C, G, and H) and nonaneurysmal arteries (D–F, I, and J) from patients with Kawasaki disease (KD). A. All aneurysm wall layers infiltrated by memory T lymphocytes; B. lack of B lymphocytes within aneurysm wall; C. macrophages infiltrate adventitia and intima of aneurysm wall; D. inflamed nonaneurysmal coronary artery infiltrated by memory T lymphocytes; E. lack of B lymphocytes in same artery shown in panel D; F. macrophages infiltrate adventitial layer of same artery shown in panels D and E; G. lack of CD4 T lymphocytes in adventitial layer of coronary aneurysm; H. many CD8 T lymphocytes in region of aneurysm shown in panel G; I. few CD4 T lymphocytes in KD myocardium; and J. many CD8 T lymphocytes in KD myocardium. A–C and G–J, ×20 objective; D–F, ×10 objective. I, intima.

talloproteinases [6], as well as factors that stimulate neoangiogenesis and healing of the vascular lesion.

The finding that CD8 T lymphocytes predominate over CD4 T lymphocytes in the KD vascular lesion is intriguing. The CD4:CD8 ratio in the peripheral blood in acute KD has been reported to be elevated (≥2.3–2.7) and to return to a more normal ratio of ~2.0 in the convalescent phase of illness [7, 8]. It is particularly striking to have a ≥4–5-fold predominance of CD8 T lymphocytes, compared with that of CD4 T lymphocytes, in the KD vascular lesion, as elevations in CD8 T lymphocytes in the peripheral blood have not been reported during any phase of KD. We speculate that CD8 T lymphocytes are selectively removed from the circulation into the major target tissue of KD.

CD8 T lymphocyte populations may be significantly expanded during many virus infections [9, 10], and virus-infected tissues often are infiltrated by CD8 T lymphocytes [11]. Recent studies indicate that the majority of CD8 T lymphocytes responding to an acute virus infection are antigen specific [12]. An expansion of CD45RO⁺ expression in the CD8, but not in the CD4, population of peripheral blood T lymphocytes was demonstrated in acute KD, but no tissue localization was done [13]. Moreover, clonal expansion of CD8 T lymphocytes in peripheral blood of acute KD patients has been identified, as described elsewhere [14]. These studies complement our finding of CD8 T lymphocyte predominance in the KD vascular lesion and strongly suggest that an antigen-driven immune response involving CD8 T lymphocytes occurs in acute KD. The accumulating evidence that
CD8 T lymphocytes are important in the immune response to acute KD suggests antigen processing of an intracellular pathogen, such as a virus, by major histocompatibility complex class I molecules. An antigen-driven CD8 T lymphocyte response in acute KD also complements our recent finding of an oligoclonal antigen-driven IgA response in the acute KD vascular wall [15]. Clonal expansion of IgA B lymphocytes and CD8 T lymphocytes integrates well with the underlying hypothesis that KD results from an antigen-driven immune response to a mucosal, potentially viral, pathogen.

In summary, despite the reported CD4:CD8 ratio of 2.3–2.7 in the peripheral blood in acute KD, our data indicate a striking reversal of the CD4:CD8 ratio in the primary target tissue in KD, the coronary artery, suggesting that CD8 T lymphocytes are selectively recruited to this site. Our data indicate that activated/memory T lymphocytes, particularly CD8 T lymphocytes, and macrophages are important in the pathogenesis of coronary arteritis in KD.

References