Vγ4+ T Cells Promote Autoimmune CD8+ Cytolytic T-Lymphocyte Activation in Coxsackievirus B3-Induced Myocarditis in Mice: Role for CD4+ Th1 Cells

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Myocarditis is an inflammation of the myocardium, and it often follows microbial infections (23). Enteroviruses are most frequently implicated in this disease (7). The virus may be directly pathogenic to cardiac myocytes, either by direct lysis of the cells during the virus replication cycle (29), disruption of cardiac myocyte function (30), or induction of locally produced proinflammatory cytokines which suppress myocyte contractility (4). Enteroviruses may also establish persistent infections in the heart, and these persistently infected cardiocytes may be dysfunctional (17). Nonetheless, presumably such injury would be largely restricted to infected cardiocytes. If the number of infected myocytes in the heart is small, then damage directly attributable to the virus would be minimal and may not produce clinical disease.

Either virus-specific or autoimmune responses also contribute to myocarditis. T-cell-deficient mice develop minimal cardiac injury despite high virus titers in the heart (31). Three distinct types of T-cell responses have been found in coxsackievirus B3 (CVB3)-induced myocarditis. These are lymphocytes expressing the Vγ4 T-cell receptor (TCR) (8), CD4+ Th1 (gamma interferon [IFN-γ] positive) (8, 9), and CD8+ αβ TCR+ autoimmune cytolytic T lymphocytes (CTL) (10, 11). Evidence to date strongly indicates that the autoimmune CD8+ αβ TCR+ CTL is the major mediator of cardiac damage. However, the activation of the CD8+ αβ TCR+ CTL is complex and poorly understood. A nonmyocarditic CVB3 variant (H310A1), which differs from the myocarditic variant (H3) by a single amino acid in the VP2 capsid protein, fails to activate any of the three T-cell types listed above (8, 12, 18). The key deficiency seems to be the lack of a Vγ4+ T-cell response, since adoptive transfer of activated Vγ4+ cells from H3 virus-infected donors into H310A1 virus-infected recipients completely restores myocarditis susceptibility (8) and, as shown here, a CD8+ αβ TCR+ CTL response. One key difference between H3 and H310A1 virus infections in the heart is that the myocarditic H3 virus induces expression of CD1d but the H310A1 virus does not (S. A. Huber, unpublished observations). CD1 is a nonpolymorphic cell-surface glycoprotein with structural homology to major histocompatibility complex (MHC) class I molecules (2, 24, 27). The studies presented here hypothesize that CD1d is important in myocarditis susceptibility in vivo because this antigen is necessary for Vγ4+ T-cell activation. In turn, the Vγ4+ T cells promote activation of CD4+ Th1 cells that are necessary for CD8+ αβ TCR+ effector cell responses. Although the CD8+ αβ TCR+ effector cell is the ultimate pathogenic agent, activation of these cells is a complex process involving both early innate (Vγ4+ T cell) and adaptive (CD4+ Th1 cell) immunity.

MATERIALS AND METHODS

Mice. Male mice, 5 to 6 weeks of age, were used in these experiments. BALB/cJ, BALB/c-Il4ra tm1Sz (interleukin-4 [IL-4] knockout), and C.129S7(B6)-Ifngtm1Ts (IFN-γ knockout) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Breeding pairs of BALB/c CD1 mice were originally obtained from Michael J. Grusby, Harvard School of Public Health, Boston, Mass.

Virus, virus infection, and virus titration. Animals were infected by intraperitoneal (i.p.) injection of 0.5 ml of phosphate-buffered saline (PBS) containing 106 PFU of either CVB3 H3 (myocarditic) or H310A1 (nonmyocarditic) variants as described previously (8).
Antibodies. Antibody class control (isotype control) and antigen-specific antibodies were obtained from Pharmingen (San Diego, Calif.). These included biotinylated and phycoerythrin (PE)-conjugated anti-CD3 (clone 17A2); PE-conjugated anti-TCRβ (clone H57-597); purified rat anti-mouse CD16/CD32 (Fc Block; clone 2.4G2); biotinylated, Cy-Chrome-, fluorescein isothiocyanate (FITC)-, and PE-conjugated rat immunoglobulin G1 (IgG1) (clone R3-34); FITC-conjugated anti-mouse IFN-γ (clone XMG 1.2); PE-conjugated rat anti-mouse IL-4 (clone BVD4-1D11); Cy-Chrome-conjugated rat anti-mouse CD4 (clone GK 1.5); purified anti-hamster IgG cocktail (clones G70-204/G94-56); FITC-conjugated rat anti-mouse CD8β (clone 53-5-8); PE–coconjugated hamster anti-mouse CD69 (clone H1.2F3); purified anti-mouse IAd (clone 39-10-8); purified anti-β TCR (clone GL3); and purified, FITC-conjugated anti-Vγ4 (clone U3C).

Isolation of splenic lymphocytes. Isolation of cell populations has been described previously (8). CD8+ or Vγ4+ cells were isolated by depleting populations of CD4+ cells, macrophages, and B lymphocytes by using anti-CD4 and anti-CD8 antibodies conjugated to species-specific fluorochromes and anti-mouse IgG-conjugated magnetic particles (PerSeptive Biosystems, Framingham, Mass.), followed by staining cells with either 1:50 dilutions of FITC-anti-CD8 and PE–anti-β TCR, or with FITC–anti-Vγ4 and PE–anti-CD3 for 20 min on ice, washing, resuspending the cells in RPMI 1640 containing 10% fetal bovine serum (Life Technologies), and sterile sorting the double-positive cell populations.

Isolation of neonatal myocytes. Isolation of neonatal mouse myocytes has been described previously (10). Briefly, hearts were obtained from mice within 72 h of birth, minced finely, and subjected to sequential digestion with 0.25% pancreatin (GIBCO) and 0.4% collagenase (Worthington Biochemical Co., Freehold, N.J.). The single-cell suspension was washed and depleted of endothelial cells and fibroblasts by two sequential 1-h adsortions to plastic. The nonadherent population was retrieved and plated into 1-mm tissue culture wells (Falcon) at 105 cells/well. After 48 h at 37°C in a humidified 5% CO2 incubator, the wells were used for cytotoxicity assays.

CMC assay. The cell-mediated cytotoxicity (CMC) assay has been described in detail previously (10). Targets were labeled with 100 μCi of 51Cr (Na2CrO4; ICN, Irvine, Calif.) for 2 h at 37°C, washed four times, and cultured for 12 h at 37°C in 105 lymphocytes (effector/target ratio of 10:1). Supernatants were removed and counted in a Packard gamma counter (Downers Grove, Ill.). Percent specific lysis was calculated as follows: [(CPM in supernatant) / (CPM in supernatant + CPM in pellet)] × 100. Each experiment was repeated at least twice.

Flow cytometry. The detailed methods used for the flow cytometry have been reported previously (8). For staining cell surface markers, cells were incubated for 20 min in PBS–1% bovine serum albumin (BSA) containing 1:100 dilutions of FcBlock and fluorescein-labeled antibodies, washed once, and resuspended in 2% paraformaldehyde. For intracellular cytokine staining, cells were cultured in medium containing 10 μg of Brefeldin A/ml, 50 ng of phorbol myristate acetate/ml, and 500 ng of ionomycin (Sigma Chemical Co., St. Louis, Mo.)/ml for 4 h at 37°C in 5% CO2, washed in PBS–1% BSA containing Brefeldin A, surface stained with Cy-Chrome-conjugated anti-CD4, washed again, fixed with 2% paraformaldehyde, resuspended in PBS–BSA buffer containing 0.5% saponin and 1:100 dilutions of FcBlock, FITC–anti-IFN-γ, and PE–anti-IL-4 for 30 min, washed once in PBS–BSA–saponin and once in saponin-free PBS–BSA, and then resuspended in 2% paraformaldehyde. Positive controls for cytokine staining were 2.5 × 105 of either Mice-1 (IFN-γ) or Mice-2 (IL-4)–fixed cells obtained from Pharmingen. Negative controls were fluorescein-conjugated species- and isotype-matched immunoglobulins. Stained cell populations were analyzed using a Coulter Epics Elite instrument with a single excitation wavelength (488 nm) and band filters for PE (575 nm), FITC (525 nm), and Cy-Chrome (670 nm). Each sample population was classified by cell size (forward scatter) and complexity (side scatter) and then gated on a population of interest. At least 10,000 cells were evaluated for each sample. Criteria for positive staining were established using isotype controls.

Adaptive transfer of purified cell populations. Vγ4+ and CD8+ TCR+ cell populations were isolated as described above, washed, and resuspended in PBS to a concentration of 5 × 106 (Vγ4+) or 5 × 107 (CD8+ TCR+ ) cells/ml. Mice were injected intravenously (i.v.) through the tail vein with 0.2 ml of the cell suspension 2 days after recipients were infected with virus.

Histology. Hearts were removed, fixed in 10% buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Stained sections were evaluated for myocarditis as described previously (8).

Statistics. Statistical evaluation was performed using either the Wilcoxon ranked score method or the Student t test. Each experiment was repeated at least two times.

RESULTS

Vγ4+ cell activity requires both IFN-γ and CD1. Previous studies (8) using Stat4−/− mice indicated that IFN-γ produced by Vγ4+ cells promotes myocarditis induction, since this transcription factor is involved in IFN-γ expression through its regulation of IL-12 (32). However, Stat4 might control expression of other genes besides IL-12, and some IFN-γ expression can be IL-12 independent. The following studies more directly investigate the role of IFN-γ in Vγ4+ cell modulation of myocarditis susceptibility. First, BALB/c, BALB/c IL-4−/−, and BALB/c IFN-γ−/− mice were infected with H3 virus and evaluated for myocarditis. CD4+ Th phenotype, and cytokine expression by Vγ4+ cells (Table 1). BALB/c and BALB/c IL-4−/− mice develop myocarditis and CD4+ Th1 (IFN-γ+) cell responses, whereas BALB/c IFN-γ−/− mice do not. Vγ4+ cells, isolated from H3 virus-infected BALB/c mice, express IFN-γ but little IL-4 (8). Vγ4+ cells from IFN-γ−/− mice showed neither cytokine (Table 1). Next, Vγ4+ T cells isolated by cell sorting from infected BALB/c, IL-4−/−, and IFN-γ−/− mice were adoptively transferred into either wild-type BALB/c recipient mice infected with the nonmyocarditic CVB3 variant (H310A1) (Table 1) or into BALB/c CD1−/− recipient mice infected with the myocarditic H3 variant (Table 1). Seven days after infection of the recipients, peripheral blood lymphocytes were retrieved and evaluated by cell surface and intracellular staining for CD4+ cells producing IFN-γ and IL-4. Hearts were evaluated for myocarditis and cardiac virus titers. CD8+ αβ TCR+ effectors were isolated with a fluorescence-activated cell sorter and assayed for cytotoxicity to uninfected BALB/c cardiac myocytes in a 51Cr release assay. Previously published studies have shown that adoptive transfer of γδ+ or Vγ4+ cells from myocarditic mice restores myocarditis susceptibility in syngeneic animals infected with the H310A1 virus (8). This experiment demonstrates that restoration of disease susceptibility requires both IFN-γ expression by the Vγ4+ cells and CD1+ expression in the recipients. Recipients not given Vγ4+ cells showed minimal cardiac inflammation, poor IFN-γ expression by peripheral blood CD4+ T cells, and poor CD8+ αβ TCR+ effector function. Giving Vγ4+ cells from BALB/c and BALB/c IL-4−/− donors restored myocarditis susceptibility, CD4+ IFN-γ expression, and CD8+ αβ TCR+ effector activity in wild-type BALB/c mice infected with the H310A1 virus but not in CD1−/− recipients. Vγ4+ cells from IFN-γ−/− donors were incapable of restoring myocarditis in either wild-type (CD1+) or CD1−/− recipients. These studies also show that CD8+ αβ TCR+ CTL responses correlate with myocarditis susceptibility and only occur when Vγ4+ cells promote CD4+ Th1 cell responses.

CD8+ αβ TCR+ CTL adoptively transfer myocarditis in both wild-type and CD1−/− recipients. Since 1984, we have theorized that the autoimmune CD8+ αβ TCR+ CTL is the dominant pathogenic factor in CVB3-induced myocarditis (10). CD8+ αβ TCR+ CTLs are activated only in CD1−/− mice.
To determine whether CD8+ αβ TCR+ CTL require CD1+ targets for induction of myocarditis, this population was sorted from H3 virus-infected BALB/c mice and adoptively transferred into infected and uninfected wild-type BALB/c and BALB/c CD1-/- recipients. The purity of the sorted CD8+ αβ TCR+ CTL was 98.1%. Table 2 shows the ability of these effectors to induce myocarditis in recipient animals. CD8+ αβ TCR+ CTL were equivalently pathogenic in both wild-type and CD1-/- recipients, indicating that these CTL do not require CD1 to mediate cardiac injury in vivo. CD8+ αβ TCR+ CTL were also capable of inducing myocarditis in uninfected recipients. This pathogenicity does not reflect transfer of virus with the T-cell population, since recipient mice showed no infectious virus in the heart (data not shown). Although adoptive transfer of CD8+ αβ TCR+ CTL induced myocarditis, no activation of Vγ4+ (data not shown) or CD4+ Th1 cells (Fig. 1) was observed in recipients, suggesting that once activated, CD8+ αβ TCR+ CTL pathogenicity is independent of both cell types. Figure 1 also shows that transferring Vγ4+ cells from H3 virus-infected BALB/c donor mice into H310A1 virus-infected BALB/c mice results in strong CD4+ Th1 cell responses, whereas giving Vγ4+ cells to uninfected BALB/c mice does not activate CD4+ T cells. These results indicate that virus infection in addition to Vγ4+ cell transfer is needed for CD4+ Th cell activation.

**Requirement of CD4+ cells for CD8+ αβ TCR+ CTL activation.** The above studies correlate CD8+ αβ TCR+ CTL activation with Vγ4+ cell responses in vivo. The next question is whether Vγ4+ cells activate CD8+ αβ TCR+ CTL directly or only through CD4+ Th1 cell responses. Vγ4+ cells were isolated from H3-infected BALB/c donor mice and adoptively transferred into BALB/c mice infected with H310A1 virus. Control mice were infected with H310A1 virus but did not receive Vγ4+ cells. Some mice also received i.p. injections of either monoclonal anti-CD4 (clone GK1.5) or anti-CD8 (clone 24.23) antibodies 2 days prior to infection (4 days prior to Vγ4+ cell transfer). Table 3 shows that anti-CD4 or anti-CD8 antibody treatment effectively eliminated the respective cell population. As shown above, adoptive transfer of Vγ4+ cells into H310A1 virus-infected recipients resulted in increased myocarditis, CD4+ IFN-γ, and CD8+ αβ TCR+ CTL responses. Depletion of CD4+ cells in recipients prior to adoptive trans-

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**TABLE 1. Vγ4+ cells promote myocarditis through IFN-γ expression**

<table>
<thead>
<tr>
<th>Strain</th>
<th>% Myocardium inflamed (10^5 PFU)</th>
<th>Cardiac virus titer</th>
<th>Flow staining of peripheral blood results</th>
<th>CD8+ αβ TCR+ cell (% lysis of uninfected myocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>10.3 ± 1.8</td>
<td>18.6 ± 7.5</td>
<td>10.7 ± 2.0</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>BALB/c IL-4 KO</td>
<td>18.7 ± 3.7*</td>
<td>15.9 ± 8.1</td>
<td>21.4 ± 4.5*</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>BALB/c CD1 KO</td>
<td>1.1 ± 0.7*</td>
<td>13.2 ± 5.9</td>
<td>0.3 ± 0.3*</td>
<td>7.9 ± 2.0*</td>
</tr>
<tr>
<td>H3-infected donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>2.7 ± 1.3</td>
<td>3.2 ± 0.9</td>
<td>5.7 ± 2.3</td>
</tr>
<tr>
<td>BALB/c</td>
<td>7.4 ± 1.6*</td>
<td>1.55 ± 0.32</td>
<td>10.5 ± 2.2*</td>
<td>0.8 ± 0.4*</td>
</tr>
<tr>
<td>BALB/c IL-4 KO</td>
<td>11.2 ± 3.1*</td>
<td>2.28 ± 1.25</td>
<td>19.7 ± 3.6*</td>
<td>0.8 ± 0.5*</td>
</tr>
<tr>
<td>BALB/c CD1 KO</td>
<td>1.0 ± 0.5</td>
<td>1.43 ± 1.01</td>
<td>1.7 ± 0.2</td>
<td>4.7 ± 0.5</td>
</tr>
</tbody>
</table>

**TABLE 2. Adoptive transfer CD8+ αβ TCR+ effectors into BALB/c and CD1-/- recipients**

<table>
<thead>
<tr>
<th>CD8+ αβ TCR+ cells</th>
<th>Recipients</th>
<th>Virus</th>
<th>% Myocardium inflamed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>BALB/c</td>
<td>None</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>+</td>
<td>BALB/c</td>
<td>H310A1</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>+</td>
<td>BALB/c</td>
<td>None</td>
<td>4.3 ± 0.8*</td>
</tr>
<tr>
<td>+</td>
<td>BALB/c</td>
<td>H310A1</td>
<td>12.7 ± 3.1*</td>
</tr>
<tr>
<td>−</td>
<td>CD1-/-</td>
<td>None</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>+</td>
<td>CD1-/-</td>
<td>H3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>+</td>
<td>CD1-/-</td>
<td>None</td>
<td>3.6 ± 1.2*</td>
</tr>
<tr>
<td>+</td>
<td>CD1-/-</td>
<td>H3</td>
<td>10.7 ± 2.6*</td>
</tr>
<tr>
<td>+</td>
<td>CD1-/-</td>
<td>H310A1</td>
<td>9.5 ± 1.7*</td>
</tr>
<tr>
<td>+</td>
<td>CD1-/-</td>
<td>None</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

a CD8+ αβ TCR+ cells were isolated from the spleens of H3 virus-infected BALB/c mice 7 days after infection. Recipients were either uninfected or were injected with 10^6 PFU of the indicated virus 1 day prior to injection of the CD8+ αβ TCR+ cells. Recipients received 10^6 CD8+ αβ TCR+ cells via iv. through the tail vein and were killed 6 days later.

b *, significantly different from the same mice not given CD8+ αβ TCR+ cells at a P value of < 0.05.
FIG. 1. Effect of Vγ4+ and CD8 αβ TCR+ cell transfer on CD4+ Th phenotype. Vγ4+ and CD8 αβ TCR+ cells were isolated as described in Materials and Methods from H3 virus-infected BALB/c donor mice 7 days after infection. These cell populations were transferred into either uninfected BALB/c (uBALB/c), H310A1 virus-infected BALB/c (vBALB/c), or H3 virus-infected CD1+/− (vCD1−/−) recipients. Recipients were infected i.p. with virus and injected 2 days later i.v. through the tail vein with either 10^4 Vγ4+ or 10^5 CD8 αβ TCR+ cells. Recipients were killed 7 days after infection. Peripheral blood lymphocytes were retrieved, stimulated with phorbol myristate acetate, ionomycin, and Brefeldin A for 4 h, then stained for CD4, fixed, permeabilized, and intracellularly stained for IFN-γ and IL-4. Numbers in the upper right corner indicate the percentage of peripheral blood cells in each quadrant of a single mouse in each group.
fer of Vγ4+ cells substantially reduced both myocarditis and CD8+αβ TCR+ CTL activity. Giving anti-CD8-treated mice Vγ4+ cells resulted in a strong CD4+ Th1 cell response, but no myocarditis.

**DISCUSSION**

This paper shows that innate immunity, in the form of Vγ4+ cells, is required for activation of autoimmune CD8+αβ TCR+ effectors. Vγ4+ cells require CD1 expression in the infected animal for activation and express high levels of IFN-γ but no detectable IL-4. This IFN-γ expression is necessary for CD8+αβ TCR+ cell activation, since CD8+ cells isolated from IFN-γ−/− mice are ineffective in either modulating CD4+ T-cell responses towards a Th1 phenotype or activating the CD8+αβ TCR+ cell population. Most likely, Vγ4+ cells do not directly interact with the CD8+αβ TCR+ cell population but promote autoimmune CTL responses through their ability to modulate CD4+ Th1 cell responses. BALB/c recipients depleted of CD4+ cells prior to transfer of Vγ4+ lymphocytes had substantially reduced CD8+αβ TCR+ CTL activity. Depletion of CD8+ cells in the recipients allowed Vγ4+ cell modulation of the CD4+ cell response toward a Th1 phenotype, but in the absence of the CD8+αβ TCR+ cells, minimal myocarditis was induced.

T cells expressing the γδ TCR form a part of the innate immune system and are most frequently distributed in mucosal membranes, intestinal epithelium, and skin (5, 26). γδ+ cell numbers often increase at inflammatory sites and may play an important role in modulating the adaptive (αβ TCR+ cell-dependent) immune response (5, 6, 19, 21, 22, 28). Previously, we have shown that γδ+ cells are important in determining susceptibility of mice to CVB3-induced myocarditis (8, 9, 12–15). Specifically, T cells expressing the Vγ4+ TCR promote myocarditis susceptibility, whereas T cells expressing the Vγ1 TCR cause myocarditis resistance (8, 15). Disease susceptibility always correlates with preferential activation of CD4+ Th1 (IFN-γ+) cells. The Vγ4+ cells express high levels of IFN-γ and localize to the infected myocardium within 1 to 2 days of virus inoculation (S. A. Huber, unpublished). This rapid Vγ4+ infiltration of the heart, and the ability of these cells to make IFN-γ, undoubtedly provides an environment conducive to CD4+ Th1 cell development. A major question is whether the Vγ4+ cell modulation of the CD4+ T-cell response is important in disease development or represents an epiphenomenon. CD8+ CTLs are the major pathogenic effectors in CVB3-induced myocarditis in BALB/c mice (10, 11). These are autoimmune CTL reacting to heart-specific antigens and killing cardiac myocytes but not other tissue-specific (noncardiac) targets. Furthermore, the CD8+ effectors can adoptively transfer myocarditis into uninfected syngeneic recipients. Preliminary data from this laboratory in collaboration with Kirk Knowlton (University of California, San Diego) and Madeleine Cunningham (University of Oklahoma) have recently identified an MHC class I Ld peptide in the 3D polymerase of the H3 variant which is recognized by both the CD8+αβ TCR+ effectors from H3 virus-infected BALB/c mice and by CD8+αβ TCR+ effectors isolated from BALB/c mice immunized with cardiac myosin (unpublished data). These results strongly indicate that autoimmunity results from antigenic mimicry between the virus and the target tissue. The nonpathogenic H310A1 variant has the identical Ld-restricted epitope, which means that the reason autoimmunity is not induced with this virus does not matter. The lack of CD1 expression in the heart, whereas H3 virus does not (S. A. Huber, unpublished observations). The crucial defect in H310A1 virus infections is that the lack of CD1 expression fails to activate Vγ4+ cells required for CD8+αβ TCR+ cell stimulation to the cross-reactive epitope in the virus.

The ability of either Vγ4+ or CD8+αβ TCR+ cells to transfer disease into uninfected recipient mice does not result from inadvertent transfer of virus, as recipients have no detectable virus and do not make anti-CVB3 antibodies and homogenates of the effector cells do not have infectious virus by the plaque forming assay. CD8+αβ TCR+ effectors kill both CD1+ and CD1− myocytes equivalently, showing that these effectors do not require CD1-presented antigen on target cells (16). This is also shown as CD8+αβ TCR+ cells equivalently transfer myocarditis into both CD1+ and wild-type mice, whereas Vγ4+ cells can only induce myocarditis and CD8+αβ TCR+ cell activation in CD1− mice. Clearly, the
activation of the CD8+ αβ TCR+ effectors is CD1 antigen dependent, but their effector function is independent of CD1. Vγ4+ cells do not directly activate the CD8− αβ TCR+ cell population, since adoptive transfer of Vγ4+ cells into mice made deficient of CD4+ cells promotes minimal myocarditis or CD8+ αβ TCR+ CTL activity. Adoptive transfer of Vγ4+ cells into recipients depleted of CD4+ cells allows generation of CD4+ Th1 cells, but without the CD8+ cell population no myocarditis is induced. This strongly implies that Vγ4+ cells act through the CD4+ Th1 cell response in promoting CD8− αβ TCR+ CTL generation. CD4+ cells are known to moderate CD8+ CTL responses in two ways. CD4+ Th1 cells directly promote CD8+ effector cell responses through cytokine release (20), whereas IL-4, produced by CD4+ Th2 cells, can suppress CD8− αβ TCR+ CTL generation (1, 3). Since up-regulation of CD4+ Th1 cell responses down-regulates CD4+ Th2 cell responses (25, 33), one might hypothesize that CD4+ Th1 cells promote CD8− αβ TCR+ CTL activation only by preventing the generation of regulatory CD4+ Th2 cells. It was somewhat surprising that CD8− αβ TCR+ cells did not promote CD4+ Th1 cell bias in vivo (3). Presumably, other environmental factors in the H310A1 virus-infected host prevent a CD4+ Th1 cell bias.

The mechanism by which Vγ4+ cells modulate the CD4+ Th cell response is still under investigation. We have shown that γδ T cells in the myocardium of H3 virus-infected mice express high levels of Fasl and are capable of specifically killing CD4− Th2 cells clones through Fas-dependent mechanisms (16). We have also observed that Th0-like CD4+ T cells (IFN-γ/IL-4+), in H3 virus-infected BALB/c mice, have murine CD1 (mCD1) on their cell surface as determined by flow analysis (S. A. Huber, unpublished observations). Although it is possible that a subpopulation of CD4+ T cells might inherently express CD1 protein, the more likely explanation is that these antigen-specific cells passively acquire the CD1 molecule during interactions with CD1+ antigen-presenting cells. However the CD1 molecule is obtained, CD1+ CD4+ T cells might directly interact with CD1-restricted Vγ4+ cells. IFN-γ produced by the Vγ4+ cells might then be highly effective in directing further CD4+ cell differentiation towards a Th1 phenotype.

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