Susceptibility to Myocarditis Is Dependent on the Response of $\alpha\beta$ T Lymphocytes to Coxsackieviral Infection

Mary Anne Opavsky, Josef Penninger, Karen Aitken, Wen-Hu Wen, Fayez Dawood, Tak Mak, Peter Liu

Abstract—Viral myocarditis is an important cause of heart failure and dilated cardiomyopathy. T lymphocytes are implicated in myocardial damage in murine models of coxsackievirus B3 (CVB3) myocarditis. We used knockout mice lacking CD4 (CD4$^{-/-}$), CD8 (CD8$^{-/-}$), both coreceptors (CD4$^{-/-}$CD8$^{-/-}$), or the T-cell receptor $\beta$ chain (TCR$\beta^{-/-}$) to address the contribution of T-cell subpopulations to host susceptibility to CVB3 myocarditis. Severity of disease was magnified in CD8$^{-/-}$ mice but attenuated in CD4$^{-/-}$ mice, consistent with a pathogenic role for CD4$^+$ lymphocytes. Elimination of both CD4 and CD8 molecules from T lymphocytes by genetic knockout better protected mice from myocarditis, demonstrating that both CD4$^+$ and CD8$^+$ T cells contribute to host susceptibility. The same benefit occurred in TCR$\beta^{-/-}$ mice, with prolonged survival and minimal myocardial disease observed after CVB3 infection. Elevated interferon-γ and decreased tumor necrosis factor-α expression are associated with attenuated myocardial damage in CD4$^{-/-}$CD8$^{-/-}$ mice. These results show that the presence of TCR$\alpha\beta^+$ T cells enhances host susceptibility to myocarditis. The severity of myocardial damage and associated mortality are dependent on the predominant T-cell type available to respond to CVB3 infection. One mechanism by which CD4$^+$ and CD8$^+$ T-cell subsets influence the pathogenesis of myocarditis may involve specific cytokine expression patterns. (Circ Res. 1999;85:551-558.)

Key Words: myocarditis ■ coxsackievirus ■ T lymphocyte ■ cytokine ■ transgenic mice

Viral myocarditis is an important cause of heart failure and dilated cardiomyopathy.1,2 The spectrum of disease spans from a fulminant course, including arrhythmias or sudden death to mild early symptoms, later progressing to chronic heart failure.1,2 Group B coxsackieviruses (CVB) have been associated commonly with viral myocarditis.1 Current evidence from CVB3 myocarditis in mice suggests that the acute phase of disease involves both direct, virus-mediated myocardocyte dysfunction and tissue injury mediated by the immune response.2 Whereas some elements of the immune response attenuate viral replication and protect the myocardium, others contribute to the development of myocardial inflammation and necrosis.2 In SCID mice, which lack both T and B cell functions, CVB3 infection induces severe disease.3 In contrast, depletion of T lymphocytes, through thymectomy, irradiation and bone marrow reconstitution or by injection of antithymocyte serum, attenuates myocarditis.4 Monoclonal antibody therapy directed against T-cell populations can modify myocarditis severity; however, depletion is not complete.5,6 A proportion of each T-cell subset remains available to participate in the immune response after antibody administration.5,6 The majority of circulating T lymphocytes express $\alpha$ and $\beta$ chains of the T-cell receptor (TCR) with either CD4 or CD8 coreceptor molecules, which augment TCR signaling pathways.7 Classically, after viral infection, the host immune system responds via activation of TCR$\alpha\beta^-CD4^+$ helper T cells and TCR$\alpha\beta^-CD8^+$ cytotoxic/suppressor T cells in a major histocompatibility complex (MHC) II– and MHC I–restricted manner, respectively.8 In addition, TCR$\alpha\beta^-CD4^-CD8^+$ (double negative; DN) T cells can participate in the immune response.8

Models using transgenic knockout technology provide a highly specific system to examine the contributions of specific subsets of T lymphocytes to pathology.9 In this study, we have used mice with targeted gene disruptions of CD4$^+$, CD8$^+$, both CD4$^+$ and CD8$^+$, or TCR$\beta$ genes to examine the role of $\alpha\beta$ T lymphocytes in the pathogenesis of viral myocarditis.

Materials and Methods

Virus

The cardiovirulent strain of CVB3 was adapted by Woodruff and Woodruff4 and passaged in the laboratory of Dr Charles Gauntt.10 Virus stock was prepared by passage through HeLa cell cultures and then titrated by plaque assay and stored at –70°C. Aliquots from the same stock were used for all animals.
Mice
Transgenic knockout mice, created by disruption of the CD4, CD8 (Ly-20), or TCRβ chain gene in embryonic stem cells through homologous recombination, have been described previously.11–13 Mice heterozygous for CD4 and CD8 genes were interbred to produce homozygotes for the CD4+/− or CD8+/− genotype. CD4+/−CD8+/− mice were subsequently bred with CD4+/−CD8+/− or CD4+/−CD8−/− to obtain CD4−/−CD8−/− offspring.14 After mating of double heterozygotes, CD4−/−CD8−/− offspring were produced.12 Mice of the CD4+CD8−/− or CD4−/−CD8−/− genotype will be referred to as CD4−/− and CD8−/− throughout the text. Mice with genotypes CD4+/−CD8+/− or CD4−/−CD8+/− were designated CD8−/−. No differences were found between the 2 genotypes within each group. CD4−/−CD8−/− (control) mice, with both CD4 and CD8 molecules intact, served as controls. All animals were backcrossed into an A/J strain (H2k/k) to generate mice (fifth generation) with uniform CVB3 susceptibility. An H2k/k haplotype was confirmed for the CD4b combination, have been described previously.11–13 Mice heterozygous for CD4 were examined qualitatively for evidence of inflammation and CD8 were censored from the mortality data.

Experimental Protocol
CD4+/− (n=47), CD8−/− (n=35), CD4−/−CD8+/− (n=56), TCRβ−/− (n=16), and control (n=64) mice ages 4 to 6 weeks were inoculated intraperitoneally with 105 plaque-forming units (PFUs) of CVB3. Animals were observed for spontaneous mortality, and a subgroup was randomly assigned to euthanization on 4, 7, 10, 14, or 28 days after infection. The animals who were randomized for euthanization were censored from the mortality data.

Histopathology
Transverse midsections of hearts were fixed in 4% paraformaldehyde and processed for hematoxylin and eosin staining. Histopathologic grading of cellular infiltrate and necrosis of the myocardium was on a scale of 0 to 4 as follows: 0, absence of infiltration or necrosis; 1, limited focal areas of infiltration or necrosis; 2, mild to moderate infiltration or necrosis; 3, moderate infiltration or necrosis; and 4, extensive areas of infiltration or necrosis involving the entire examined heart tissue.15 Paraffin-embedded pancreases and livers were examined qualitatively for evidence of inflammation and necrosis after CVB3 infection.

Viral Titters
After aseptic removal, hearts, pancreases, livers, and spleens were stored individually in RPMI (GIBCO-BRL) at −70°C for 24 hours to be used for detection. Organ samples were homogenized in 5 mL of RPMI. After 3 freeze-thaw cycles and centrifugation at 3000 rpm for 15 minutes, virus titters were determined in duplicate by standard plaque formation assay.

Neutralizing Antibody Titters
Neutralizing antibody titters were measured by inhibition of viral cytopathic effect (CPE). Sera were inactivated at 56°C for 30 minutes. Serial dilutions, in 2-fold increments in RPMI plus 10% FCS (GIBCO-BRL), were incubated for 1 hour at 37°C with 100 PFUs CVB3-CG. Sera were adsorbed onto HeLa cell monolayers in 96-well plates for 1 hour at room temperature and then replaced with RPMI plus 10% FCS and incubated for 48 hours at 37°C. The highest dilution of sera that inhibited CPE, determined after staining with 1% crystal violet in 10% formalin, was found to be the titer of neutralizing antibody against CVB3. The positive control was commercially produced anti-coxsackievirus antibody (Chemicon), and the negative control was uninfected mouse serum.

Immunohistochemistry
Sections of paraffin-embedded heart tissue were deparaffinized and rehydrated, and then endogenous peroxidase activity was blocked in 3% hydrogen peroxide in methanol for 10 minutes. Sections were permeabilized with 0.125% trypsin (Zymed Laboratories) at 37°C for 10 minutes and washed in PBS with 0.05% Tween 20 (PBST).

After blocking with 10% normal goat serum (Zymed Laboratories) in PBS for 30 minutes, samples were incubated overnight at 4°C with a rabbit polyclonal anti-CD3 antibody (Zymed Laboratories) to detect T cells or a monoclonal rat anti-mac-3 antibody (1:100; PharMingen; kindly provided by Dr Marlene Rabinovitch, The Hospital for Sick Children, Toronto, Ontario, Canada) to detect macrophages and then washed with PBST. Sections were incubated for 20 minutes with biotinylated secondary antibody (goat anti-rabbit, 1:250 [GIBCO-BRL]) or goat anti-rat, 1:100 (Jackson ImmunoResearch Laboratories) and then rinsed with PBST. Streptavidin–horseradish peroxidase conjugate (1:500; Jackson ImmunoResearch Laboratories) was then applied for 10 minutes. After washing, sections were developed for 5 minutes with aminoethyl carbazole (red staining; Zymed Laboratories).

Sections of OCT-embedded frozen myocardium were fixed in acetone at 4°C for 3 minutes and then incubated in 3% hydrogen peroxide in methanol for 30 minutes. After washing with PBS, samples were blocked with 10% goat serum (Sigma) and 3% BSA (BioShop) in PBS for 1 hour at room temperature. Samples were then incubated in rabbit anti-mouse asialo-GM1 polyclonal antibody (Cedarlane) for 1 hour at room temperature to detect natural killer (NK) cell infiltration. After washing in PBS, sections were incubated with biotinylated goat anti-rabbit secondary antibody (1:500), followed by streptavidin–horseradish peroxidase conjugate (1:500) and aminothyl carbazole detection, as described above.

Primary antibody was replaced with normal rat serum or rabbit serum, as negative control. Additionally, sections were processed in the absence of primary antibody or control serum. Sections of normal mouse spleen and thymus were used as positive controls. All samples were counterstained with hematoxylin.

To quantify differences in infiltrating cell populations at day 10, positive cells and total infiltrating cells were counted at high power in 5 randomly selected myocardial foci for each heart examined and then expressed as percentage CD3 (for T lymphocytes)– or mac-3 (for macrophages)–positive cells. Alternatively, at day 4 the total number of asialo-GM1–positive cells in each section was expressed as the number of positive cells per high-power field (HPF; ×250), with >10 HPF counted per section.

RNA Isolation and Reverse Transcriptase–Polymerase Chain Reaction (PCR)
Hearts were snap-frozen in liquid nitrogen at the time of euthanization. Total RNA was isolated using Trizol reagent (GIBCO-BRL) as directed. First-strand cDNA synthesis was performed by incubation of 1 μg of RNA with 200 ng/mL random hexamers (GIBCO-BRL) at room temperature for 10 minutes, followed by the addition of 200 units of M-MLV reverse transcriptase II (GIBCO-BRL), 10 mmol/L DTT, 0.5 mmol/L of each dNTP (Pharmacia), and first-strand buffer (GIBCO-BRL). The 20-μL reaction was incubated at 42°C for 45 minutes, and then reverse transcriptase was denatured at 70°C for 5 minutes.

Cytokine PCR primer sets for interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and β-actin were synthesized on the basis of published sequences.16 Each 25-μL PCR reaction contained 0.5 mmol/L of each primer pair, 100 μmol/L of each dNTP (Pharmacia), and 0.3 units of Taq polymerase (Pharmacia). Samples were analyzed in a thermal cycler (Perkin-Elmer Cetus) as follows: 94°C for 1 minute; 35 cycles of 95°C for 2 minutes, 60°C for 2 minutes, and 72°C for 2 minutes; and 95°C for 1 minute, 60°C for 1 minute, and 72°C for 7 minutes. PCR products were compared using ethidium bromide-stained agarose gel electrophoresis. Measurements of band intensity were made under ultraviolet light with a digital camera (GelDoc 1000, Bio-Rad Laboratories). Band intensities representing cytokine PCR products were compared in a semiquantitative fashion with Molecular Analyst software (version 2.1.2, Bio-Rad Laboratories) after expression as a ratio to β-actin for the same cDNA sample. Splenic cDNA and the plasmid pMCQ (courtesy of Dr Cornelia Platzer, Institut für Anatomie, Friedrich-Schiller Universität, Jena, Germany), which contains gene fragments of IFN-γ, TNF-α, and β-actin, served as positive controls.16
CD4+ T-cell subset intact, myocardial infiltration and necrosis were severe and comparable with that of control.

No differences were found in the extent of pancreatic or liver disease among knockout groups. In the pancreas, moderate to severe acinar destruction was observed in all groups, with islet cells remaining intact. Although mild to moderate cellular vacuolation and cytolysis of hepatocytes was evident in all groups, no frank necrosis was seen. Minimal inflammatory infiltrate was found in both organs, in striking comparison with the heart.

Knockout of T-Cell Subsets Influences the Participation of Immune Cells in CVB3 Myocarditis

Not only were differences in the extent of myocardial infiltration observed (Figure 3), but differences in the contribution of T cells and macrophages to the infiltrate were identified as well. On day 10 after infection, the proportion of T cells present in myocarditic foci of CD4+/−, CD8+/−, and CD4+/CD8+/− mice was decreased versus controls (Table 1 and Figure 4). Infiltration of macrophages was also less extensive in CD4+/− and CD8+/− mice as compared with control mice (Table 1 and Figure 4). The proportion of macrophages infiltrating the myocardium was lowest in CD4+/−CD8+/− mice.

Infiltration of the myocardium by NK cells was evident at day 4 after infection in our T-cell knockout models. NK cell infiltration tended to be more prominent in the myocardium of CD4+/− (2.5±0.8 cells/HPF) and CD4+/−CD8+/− mice (3.3±1.7 cells/HPF). Only 0.7±0.5 cells/HPF and 0.4±0.2 cells/HPF were found in the myocardium of CD8+/− and control mice, respectively.

The neutralizing antibody response, representative of B cell activation, was attenuated in some CD4+/− and CD4+/−CD8+/− mice (Figure 5). However, a rise in titer was observed in the majority of these mice, suggesting that in the absence of CD4+ T cells, neutralizing antibody can still be produced.

Myocardial Cytokine Expression Varies Among CD4 and CD8 Knockout Strains

To test the hypothesis that the T-cell repertoire of the host affects the cytokine response in myocarditis, cardiac cytokine gene expression was evaluated. On day 4, when cellular infiltration was minimal, cytokine expression differed among knockout groups (Figure 6). In CD4+/− and CD4+/−CD8+/− mice, myocardial IFN-γ gene expression was increased. This difference tended to persist until day 10. Day 4 TNF-α expression was lowest in the hearts of CD4+/−CD8+/− mice.

Viral Titers Do Not Correlate With Severity of Myocarditis and Mortality in CD4 and CD8 Knockout Mice

To test whether the differences in cardiac disease were due to differences in viral replication, cardiac CVB3 titers were analyzed. No significant differences among genotypes were observed, and no direct relationship between viral titers in the heart and outcome was apparent (Figure 7). The similarity in viral titers observed in the heart was apparent in other organs as well. No significant differences in splenic and hepatic viral...
titers were found among groups (Table 2). Pancreatic CVB3 titers were significantly lower in CD4−/−CD8−/− mice versus control, although this was not reflected in differences in tissue damage. Virus was cleared from spleens, livers, and pancreases in the majority of animals in all groups by day 7 (data not shown).

Discussion
T lymphocytes participate in the host immune response to viral infection. They can inhibit viral replication, but T-cell effector mechanisms can also promote host tissue damage via excess activation of cytokines and cellular cytotoxic responses.2 Gene-targeted removal of T-cell subsets alters the course of viral myocarditis, illustrating 2 key factors in the pathogenesis of myocarditis after viral infection. First, host susceptibility to myocarditis is dependent on activation of both CD4+ and CD8+ αβ T cells. Second, the predominant T-cell type available to respond to CVB3 infection determines the severity and course of myocarditis after CVB3 infection. Viral replication in the heart does not predict myocardial damage in αβ T-cell–deficient mice, underscoring the pivotal role of the T lymphocyte in CVB3 myocarditis.

Evidence from CD4−/− and CD8−/− knockout models demonstrates an important pathogenic role of CD4+ T cells in viral myocarditis. Knockout of CD4+ T cells alone provided protection from the myocardial infiltration and necrosis seen in control mice, but did not impact on survival. This is in contrast to anti-CD4 monoclonal antibody therapy experiments, which had no effect on myocarditis after CVB3 infection in A/J mice.3 The incomplete depletion of CD4+ T cells by monoclonal antibody therapy likely explains the differences observed. In CD4−/− mice, total T-cell numbers are normal, and the CD8+ cytotoxic T-cell response is intact.4 Approximately 10% of peripheral T cells are TCRαβ−DN, which can provide MHC II–restricted help for CD8+ T cells and B cells.9,10 Therefore, activation of the
intact CD8+ T-cell population in CD4−/− mice may proceed in response to CVB3 infection, albeit at a less intense level, resulting in less extensive myocardial damage. Survival was lowest in the CD8−/− mice, whereas the severity of myocardial disease was comparable with that observed in control mice. Animal survival is likely inversely related to the extent of myocardial infiltration and necrosis, such that more mice in the CD8−/− group had severe myocardial damage that is incompatible with life. Histopathologic examination of pancreases and livers did not reveal any differences in inflammation or tissue necrosis among knockout groups, which would offer an explanation other than death caused by fulminant heart dysfunction. This is consistent with the cardiovirulent nature of this strain of CVB3.10,18 CD8−/− mice are unable to produce any detectable CTL responses against viral antigens, but they do have normal total T-cell numbers and a normal CD4+ T-cell population that can mediate expected levels of T-cell help and immunoglobulin class switching.11 In conjunction with the less extensive myocarditis observed in CD4−/− mice, the severe disease in the CD8−/− model supports a deleterious role for CD4+ lymphocytes in myocarditis. Also, as suggested after monoclonal antibody depletion of CD8+ T cells in A/J mice, removal of suppressive CD8+ T cells may play a role.5

We found that CD4−/−CD8−/− mice had increased survival and decreased myocardial damage after CVB3 infection, without any impairment of viral clearance, indicating that both CD4−/− and CD8−/− T cells participate in cardiac tissue damage. Although CD4−/− and CD8−/− T cells do not develop in CD4−/−CD8−/− mice, other functional T-cell subsets are present. The majority of T cells (10% of normal numbers), are TCRαβ−DN, with TCRγδ+ T cells increased to 3% of the total leukocyte number.14 The CD3+ cells identified in the myocardial infiltrate of CD4−/−CD8−/− mice were most likely TCRαβ−DN or TCRγδ− T lymphocytes. MHC II−restricted TCRαβ−DN T cells provide T-cell help in CD4−/− mice in response to vesicular stomatitis virus.19 Human TCRαβ−DN T-cell clones can lyse target cells in a non-MHC−restricted manner.20 Cytokine regulation of TCRαβ−DN T-cell effector functions supports a functional diversity and physiological relevance of this T-cell population.21 TCRγδ− T cells can mediate both a cytotoxic response22,23 and B cell activation with neutralizing antibody production24 in viral infection and can influence effector cells via cytokine production.25 Thus, in CD4−/−/CD8−/− mice, TCRαβ−DN and TCRγδ− T cells may participate in the host response to CVB3 infection with cytolytic capacity and cytokine expression.

Susceptibility to myocarditis was decreased in TCRβ−/− mice, with the outcome comparable with that observed in CD4−/−CD8−/− mice. TCRβ−/− mice completely lack TCRαβ− T lymphocytes; however, development of TCRγδ− T cells is not impaired.13 The total thymocyte number in TCRβ−/− mice is 8% of controls, with peripheral T cells all γδTCR positive.13 In the absence of TCRαβ+ T cells, TCRγδ+ T cells may participate in viral clearance in conjunction with NK cells, macrophages, and B cells. We have shown that removal of all TCRαβ+ T cells is not required to optimally protect the host from myocarditis. Genetic knockout of CD4+ and CD8+ T-cell subsets is sufficient. Therefore, the exuberant response of this T-cell repertoire confers host susceptibility.

The elimination of specific T-cell subsets has the potential to alter other elements of the host response to CVB3 infection, including antibody production, cytokine expression, and NK cell response. The detection of neutralizing antibody in all knockout groups indicates that in the absence of CD4+ T cells, other lymphocytes, perhaps TCRαβ−DN and TCRγδ+ T cells, can participate in B cell activation and antibody production in CVB3-infected mice. Neutralizing antibody is likely not an important protective factor in these models with an A/J background, as titer does not correlate with outcome.

### TABLE 1. Infiltrating Cell Populations in Myocarditic Foci on Day 10 After CVB3 Infection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T Lymphocytes</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.6±5.2</td>
<td>40.6±8.4</td>
</tr>
<tr>
<td>CD4−/−</td>
<td>6.0±1.2*</td>
<td>18.8±4.0†</td>
</tr>
<tr>
<td>CD8−/−</td>
<td>10.6±3.8*</td>
<td>20.6±5.9†</td>
</tr>
<tr>
<td>CD4−/−CD8−/−</td>
<td>4.5±1.5*</td>
<td>6.1±3.6*</td>
</tr>
</tbody>
</table>

Sections of hearts 10 days after CVB3 infection were immunostained for T lymphocytes (CD3) and macrophages (mac-3). Data are reported as mean percentage CD3+ or mac-3+ cells±SEM per group (n=5 to 9).

*P<0.0005 and †P<0.05 within each column.
Cytokines are active participants in the induction of the immune response to viral infection and can influence both viral replication and immune-mediated tissue damage. Cardiac expression of IFN-γ and TNF-α may be significant factors in determining susceptibility to disease. The pattern of cytokine expression is dependent on the specific T-cell subset available to respond to CVB3 infection. IFN-γ and TNF-α expression in the myocardium on day 4 may reflect responses of cardiac myocytes, fibroblasts, endothelial cells, and dendritic cells. Myocardial cells may be influenced by differences in splenic cytokine production, determined by T-cell subsets in the knockout models.

Activation of NK cells occurs as early as day 3 after CVB3 infection and is compatible with the myocardial infiltration of NK cells observed on day 4. The increased NK cell infiltration of the myocardium in CD4−/− and CD4−/−CD8−/− mice in association with elevated IFN-γ expression may reflect a shift in the balance of the immune response, in favor of protective immune elements such as IFN-γ and NK cells. Similar day 4 cardiac viral titers among groups does not support widespread inhibition of viral replication by IFN-γ; however, perhaps myocyte-to-myocyte spread of virus is limited by local IFN-γ production, thus limiting the size and number of myocarditic foci.

In CD4−/−CD8+ mice, lower cardiac TNF-α expression, together with elevated IFN-γ, may contribute to decreased mortality by preserving heart function despite high viral titers. It has been shown previously that TNF-α administration to CVB3-resistant mice worsens myocarditis, whereas anti-TNF-α antibody treatment before encephalomyocarditis virus infection decreases the severity of myocarditis.
addition, the negative inotropic effects associated with TNF-α have been well documented. It has been suggested that the variable responses of murine strains to immunotherapy are the result of differences in the genetic repertoire of the host. This may explain observations, in different murine backgrounds, that CD4 knockout mice develop severe myocarditis, whereas β2-microglobulin knockout mice have minimal myocardial disease after CVB3 infection, in contrast to results shown here. Extrapolation of the present study suggests that it is in fact the complement of specific T-cell subpopulations in each host that determines susceptibility to myocarditis in mice and humans. The relevance of T-cell subset responses may have important diagnostic, prognostic, and therapeutic implications in the management of the disease.

In summary, the present study used a strategy of gene targeting to elucidate the role of the T-cell response in the pathogenesis of myocarditis after CVB3 infection. We have shown that the severity of myocarditis is dependent on the responding T-cell subset. Both CD4+ and CD8+ T lymphocytes can contribute to myocarditis and mortality after CVB3 infection. Distinct patterns of cytokine expression, associated with specific T-cell populations, may alter the intensity of the inflammatory response and severity of myocarditis. It appears that the balance of protective and destructive immune elements can be altered by the elimination of 1 or more T-cell subsets. Therefore, the role of the T cell in myocarditis is complex, and the contribution of mechanisms other than αβ T cells cannot be underestimated; consequently, elimination of the total αβ T-cell population may not always be advantageous to the host. Further investigation of the components of the immune response to CVB3 infection may identify other important factors of host susceptibility and lead to better understanding of the disease and more precise targeting of therapy.

Acknowledgments

This research was supported by grants from the Medical Research Council of Canada and the Heart and Stroke Foundation of Ontario. M.A.O. is a postdoctoral fellow of the Medical Research Council of Canada. P.L. is the Heart and Stroke/Polo Endowed Research Chair at the University of Toronto.

References


Susceptibility to Myocarditis Is Dependent on the Response of αβ T Lymphocytes to Coxsackieviral Infection
Mary Anne Opavsky, Josef Penninger, Karen Aitken, Wen-Hu Wen, Fayez Dawood, Tak Mak and Peter Liu

Circ Res. 1999;85:551-558
doi: 10.1161/01.RES.85.6.551

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/85/6/551

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/