Low-Dose Oral Use of Interferon Inhibits Virally Induced Myocarditis

CASSANDRA M. LAWSON and MANFRED W. BEILHARZ

ABSTRACT

Cytomegalovirus (CMV) infection has been associated with the development of myocarditis in humans. Our established mouse model for CMV myocarditis allows detailed investigation of the immunopathogenic mechanisms and therapies for cardiovascular disease. The type I interferons (IFN-α/β) are part of the innate immune response to CMV infections. Previously, we have reported that daily treatment with low doses of murine IFN-α/β administered by the oral–mucosal route significantly reduces early virus replication of murine CMV in the spleen and liver of infected mice. The oral–mucosal route provides an alternate delivery system to the current modes of IFN administration and is associated with fewer side effects. Since prophylactic treatment with type 1 IFNs may result in both antiviral and immunomodulatory effects that may lessen the development of disease, we wished to study the effect of IFN-α/β on the development of myocarditis. Low-dose oral use of type I IFN (10 IU/day for 7 days prior to virus infection) did not abrogate myocarditis but suppressed the inflammatory response in both the acute and chronic phase of the disease. Furthermore, low-dose oral use of IFN was as effective at inhibiting myocarditis as a single injection of a high dose of IFN (20,000 IU) on the day of virus infection. These findings indicate the need for evaluation of low-dose use of oral IFN in the development of improved clinical therapies for the treatment of cardiovascular disease.

THE CARDIOVASCULAR DISEASE, MYOCARDITIS, is characterized by inflammation and necrosis of cardiac muscle. The disease is associated with heart failure, arrhythmia, and sudden death. Myocarditis patients may have myocardial infarctions associated with severe chest pain, electrocardiographic changes, and elevated serum levels of creatine kinase.(1) The disease may be induced by a number of physical, chemical, and infectious agents, including viruses.(2) Viral myocarditis has been linked with the subsequent development of dilated cardiomyopathy, a disease that accounts for approximately 25% of all heart failures in North America.(3,4)

Viruses associated with myocarditis include, picornaviruses, orthomyxoviruses, and the herpesviruses. Human cytomegalovirus (CMV), a herpesvirus that is ubiquitous in human populations, is associated with seropositivity, often exceeding 50% in adults of developed countries. Overt disease is rare in immunocompetent individuals, but may follow blood transfusions resulting in a number of clinical manifestations, including myocarditis.(5) Active CMV infection is a common major complication in heart transplant recipients and patients with AIDS, resulting in allograft rejection and cardiac dysfunction, respectively.(6,7) Heart transplant recipients with a primary CMV infection have a 46% incidence of developing myocarditis.(8) Approximately 50–65% of patients with active myocarditis are positive for CMV DNA in heart tissues.(9) Importantly, myocardial damage post-CMV infection may persist in immunocompromised patients and lead to dilated cardiomyopathy.(10)

The strict host specificity of CMV has not allowed the development of a direct animal model for human CMV infection. However, murine cytomegalovirus (MCMV) infection of mice provides an excellent experimental model because it closely resembles human CMV in gene sequences, tissue tropism, and pathological sequelae. We have demonstrated myocarditis in various inbred strains of mice following sublethal infection with MCMV.(11,12) An inflammatory response is first observed in the heart at days 5–7 post infection (p.i.) and persists until at least day 100 p.i. The disease appears to be biphasic with an acute and chronic stage in susceptible strains of mice. Inflammation varies from focal lymphocytic infiltration to intense, dif-
fuse infiltration with degenerative changes ranging from loss of striations, vacuolation, to myofiber drop out. Host genetic factors affect the severity of MCMV-induced myocarditis, however susceptibility does not correlate with the presence of viral antigens in cardiac muscle.\(^{(12)}\) Indeed, viral DNA and viral transcripts for immediate early (ie1) genes, as detected by nested PCR, persists out to day 70 in the hearts of both susceptible and resistant mouse strains (Fairweather, Shellam, and Lawson, unpublished data).

While the pathogenesis of viral myocarditis in humans is not well understood, experimental infection models have increased our understanding of the pathogenic mechanisms involved in the disease.\(^{(2)}\) Our previous research has suggested that MCMV-induced myocarditis is immunopathological in nature. MCMV-induced myocarditis is associated with the production of autoantibodies to cardiac muscle and an inflammatory infiltrate. We have shown that T cells play a critical role in MCMV-induced myocarditis because athymic mice, in contrast to T cell-competent mice, do not develop myocarditis.\(^{(13)}\) Both CD4\(^+\) and CD8\(^+\) T cells are required for disease development (Fairweather, Lathbury, Allan, Scalzo, Shellam, and Lawson, unpublished data). CD4\(^+\) T cells are clearly important for immunoglobulin G (IgG) production, and CD8\(^+\) T cells may mediate cardiac tissue injury. However, the pathogenic T cell antigenic epitope(s) recognized in MCMV-induced myo-carditis remain to be elucidated. In contrast to T cells, we have found that NK1.1\(^+\) cells partially protect against acute myo-carditis (Fairweather, Lathbury, Allan, Scalzo, Shellam, and Lawson, unpublished data).

Our previous research has examined heart-reactive IgG antibodies, which are produced early during MCMV infection, peak at days 10–14 p.i., and persist into the late phase of myocarditis (days 28–100 p.i.).\(^{(14)}\) The early humoral immune response is directed against a number of heart proteins, including the contractile proteins troponin, tropomyosin, and myosin, whereas the late immune response is predominantly directed against the heavy chain of cardiac myosin.\(^{(14)}\) We have also found anti-myosin antibodies associated with myocarditis in wild mice that have been naturally infected with MCMV.\(^{(15)}\) In addition, MCMV-neutralizing monoclonal antibodies cross-react with cardiac myosin.\(^{(16)}\) Therefore, autoimmunity to myosin is a prominent feature of the immune response to MCMV infection.

Absorption experiments using myosin isoforms revealed that there were differences, both in titer and isoform specificity of anti-myosin antibodies, between mouse strains that are susceptible or resistant to disease. Only mice susceptible to MCMV-induced myocarditis (BALB/c) have a population of antibodies specific for the cardiac isoform of myosin. Although controversy exists as to the direct pathogenic role of antibodies reacting with internal antigens such as myosin,\(^{(17,18)}\) such autoantibodies appear to play an immunopathogenic role in MCMV-induced myocarditis.\(^{(19)}\) We have found that passive transfer of affinity-isolated anti-cardiac myosin antibodies, obtained from late immune sera of MCMV-infected BALB/c mice, induced cellular inflammation and myocardial necrosis in uninfected BALB/c mice. Our model suggests that some anti-CMV antibodies, while highly protective against CMV infection, may also be capable of inducing autoimmune disease.

These cardiac myosin-reactive antibodies (predominantly IgG\(_{2b}\)) cross-react with MCMV polypeptides (50, 100, 200 KDa) and the S2 region of myosin, suggesting that molecular mimicry between MCMV and cardiac myosin could contribute to the development of MCMV-induced myocarditis.\(^{(19)}\)

These findings taken together highlight the immunological nature of this disease and provide a basis for the development of immunomodulatory therapies. Our present study uses the well-established mouse model for CMV myocarditis to examine the antiviral and immunomodulatory roles of the type I IFNs (IFN-\(\alpha/\beta\)) in myocarditis. Our previous studies demonstrated the efficacy of low-dose orally administered IFN (LDOA IFN) treatment for reduction of early virus replication in the spleen and liver of MCMV-infected mice.\(^{(20,21)}\) The oral–mucosal route provides an alternate delivery system to the current modes (systemic delivery) of IFN administration which are limited, due to the production of neutralizing antibodies and may be associated with severe side effects. Treatment with type I IFNs may result in both antiviral and immunomodulatory effects that are less favourable for the development of myocarditis. In particular, we have studied the effects of LDOA IFN treatment of MCMV-infected mice for changes in both the severity and duration of heart disease.

The therapeutic efficacy of LDOA IFN in the development of myocarditis was examined in female adult BALB/c mice (6 weeks old) obtained from Animal Resources Centre (Murdoch, Western Australia). Mice (5/group) were treated with either saline (10 \(\mu\)l/mouse) or type I IFN-\(\alpha/\beta\) (Lee Biomedical Inc., CA; 10 IU/mouse per day in 10 \(\mu\)l of saline) orally for 7 days prior to intraperitoneal (i.p.) injection with MCMV (a sublethal dose of \(10^5\) pfu/mouse diluted in 100 \(\mu\)l of pyrogen-free phosphate-buffered saline). A separate group of mice were injected i.p. with 20,000 IU IFN-\(\alpha/\beta\) 6 h prior to MCMV infection. The MCMV stock (K181 strain) was prepared as a 20% salivary gland homogenate from virus-infected weaning female BALB/c mice and was titrated in mouse embryo fibroblasts by plaque assay and expressed as pfu/ml as described previously.\(^{(22)}\) Hearts from experimental mice were taken at day 6 and day 40 p.i., timepoints representing the acute and chronic stage of myocarditis, transected along the midline, and processed as paraffin embedded blocks. In previous experiments, the average number of foci from serial sections was consistent with the myocarditis score from a single level taken from the interior myocardium (data not shown). Heart sections (two sections cut per mouse with 5 mice/time point) were stained with hematoxylin and eosin (H&E) and were examined histologically for evidence of pathology (Table 1). Levels of significance were determined by the unpaired \(t\)-test assuming unequal variance between the means. Mice treated with LDOA IFN had reduced inflammation in the hearts taken at both timepoints (1.8-fold reduction, \(p \leq 0.03\)), indicating that LDOA IFN suppressed the development of myocarditis. The high dose of IFN, injected by the i.p. route, also reduced the severity of both acute (1.6-fold reduction, \(p \leq 0.06\)) and chronic (3.6-fold reduction, \(p \leq 0.00001\)) myocarditis.

To study the effect of LDOA IFN on myocarditis induced by a 10-fold higher dose of virus, BALB/c mice were again treated with either saline or IFN (LDOA and injected high dose) similar to the above experiment but were infected with \(10^4\) pfu...
MCMV by the i.p. route. Hearts were examined for myocarditis on days 7 and 35 p.i. Again, mice treated with IFN exhibited less severe myocarditis than untreated mice (Table 2). The inflammatory infiltrate of LDOA IFN-treated mice was often more perivascular. Mice receiving LDOA IFN treatment or 20,000 IU IFN (i.p.) showed 1.5-fold reductions ($p \leq 0.04$) in the number of inflammatory foci infiltrating the hearts at day 7 p.i. (Table 2). At day 35 p.i., mice treated with LDOA IFN displayed a two-fold reduction ($p \leq 0.0007$) and mice injected with 20,000 IU IFN showed a three-fold reduction ($p \leq 0.00003$) in myocarditis.

LDOA IFN treatment reduces MCMV titers in the spleen and liver early in infection.\(^{(20,21)}\) To assess further the effect of IFN treatment on virus replication in the infected mice, virus titers in the salivary gland, a site of persistence for MCMV, was quantitated at day 7 p.i. for mice receiving $10^4$ pfu MCMV and treated with or without IFN. This time point was chosen because virus does not reach the salivary gland until days 5–7 p.i., unlike virus replication of the spleen and liver, which is first observed at days 2–3 p.i. Individual salivary glands were prepared and titrated in the plaque assay as described previously.\(^{(22)}\) Virus titers are expressed as mean pfu/g tissue ± standard error from 5 mice/group (Fig. 1). The limit of detection of virus was 50 pfu/g tissue. No marked reduction in salivary gland virus titer was observed for mice treated with LDOA IFN. However, mice injected with a high dose of IFN on the day of virus infection showed significant ($p \leq 0.002$) reduction in virus replication in the salivary gland at day 7 p.i. These findings suggest that LDOA IFN treatment does not inhibit either the dissemination of virus to the salivary glands or its replication at this distal site, 7 days following i.p. challenge with virus. Interestingly, this result contrasts the suppression of early replication of disseminated MCMV in the liver and spleen observed in LDOA IFN-treated mice at days 2–3 p.i. Therefore, administration of IFN by the oral route appears to have different effects on virus replication depending on the tissue site and/or the kinetics of virus replication in organs. In contrast to the CD8$^+$ T cell-mediated control of MCMV replication in the spleen and liver,\(^{(23)}\) control of virus replication in the salivary glands is mediated by CD4$^+$ T cells.\(^{(24)}\) Thus, it is possible that LDOA IFN treatment may have different effects on the stimulation of the CD8$^+$ and CD4$^+$ T cell-mediated antiviral immune responses.

---

**Table 1. LDOA IFN-α/β Reduces the Severity of MCMV-Induced Myocarditis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 6$^b$</th>
<th>Day 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline$^c$</td>
<td>5.1 ± 0.9</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>LDOA IFN$^d$</td>
<td>2.8 ± 0.6</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>20,000 IU IFN$^e$</td>
<td>3.2 ± 0.7</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

$^a$Myocarditis was evaluated histologically as the average number of inflammatory foci per heart section ± standard error (two sections were examined from hearts transected through the midline for individual mice from groups of 5 animals).

$^b$Mice were infected with $1 \times 10^3$ pfu MCMV by the i.p. route and hearts examined histologically at the specified time points.

$^c$Mice were treated orally with $10 \mu l$ of saline/mouse per day for 1 week prior to virus infection.

$^d$Mice were treated orally with 10 IU MuIFN-α/β ($10 \mu l$/mouse per day) for 1 week prior to virus infection.

$^e$Mice were injected with 20,000 IU MuIFN-α/β by the i.p. route 6 h before virus infection.

---

**Table 2. LDOA IFN-α/β Reduces the Severity of Myocarditis in BALB/c Mice Infected with an Increased Dose of MCMV**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 7$^b$</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline$^c$</td>
<td>10.5 ± 1.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>LDOA IFN$^d$</td>
<td>7.6 ± 0.7</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>20,000 IU IFN$^e$</td>
<td>7.2 ± 1.1</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$Myocarditis was evaluated histologically as the average number of inflammatory foci per heart section ± standard error (two sections were examined from hearts transected through the midline for individual mice from groups of 5 animals).

$^b$Mice were infected with $1 \times 10^4$ pfu MCMV by the i.p. route, and hearts were examined histologically at the specified time points.

$^c$Mice were treated orally with $10 \mu l$ of saline/mouse per day for 1 week prior to virus infection.

$^d$Mice were treated orally with 10 IU MuIFN-α/β ($10 \mu l$/mouse per day) for 1 week prior to virus infection.

$^e$Mice were injected with 20,000 IU MuIFN-α/β by the i.p. route 6 h before virus infection.

---

**FIG. 1.** Low-dose oral type I interferon (LDOA IFN) treatment does not inhibit early virus dissemination and replication in the salivary gland. Groups of 5 BALB/c mice were either given doses of 10 IU of MuIFN-α/β (LDOA IFN) or saline (PBS) by the oral mucosal route daily for 7 days prior to i.p. inoculation with $10^4$ pfu MCMV or injected with 20,000 IU MuIFN-α/β (i.p. IFN) 6 h before virus infection. Mean virus titers ± standard errors (pfu/g tissue) are shown for mice at day 7 p.i.
It is unlikely that LDOA IFN treatment will prevent the establishment of persistence of MCMV in the host, especially the salivary gland, in which there is no evidence of reduction of infectious virus replication. However, our previous studies have shown that MCMV persistence in the salivary gland and low-level transcription of MCMV in the heart does not correlate with susceptibility to myocarditis (Fairweather, Shellam, and Lawson, unpublished data). Thus the antiviral effects of the type I IFNs are not entirely responsible for inhibition of the induction of myocarditis.

LDOA type I IFN (10 IU/day for 7 days prior to virus infection) did not completely abrogate myocarditis but suppressed the inflammatory response in both the acute and chronic phases. Furthermore, LDOA IFN was as effective as suppressing myocarditis as a single injection of a high dose of IFN (20,000 IU). We propose that predominant immunomodulatory effects of LDOA IFN treatment influence the development of myocarditis following MCMV infection. LDOA IFN therapy may provide a safe alternative in the development of improved clinical treatments of cardiovascular disease. This study provides support for further investigations into the efficacy of LDOA IFN as therapy for cardiovascular disease. The timing and dosage of IFN and the effects of IFN treatment on virus persistence in the heart warrant examination. Furthermore, we have recently shown that there are differential antiviral activities of individual IFN-α subtypes in vivo. It may be necessary to treat myocarditis patients with a particular subtype of IFN-α to achieve optimal protection from disease. These investigations will provide important information on prophylactic therapy for the benefit of people at risk of developing cardiovascular disease.

ACKNOWLEDGMENT

This work was supported by the Australian National Health and Medical Research Council (Grant No. 961302).

REFERENCES

phocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. J. Virol. 61, 3102–3108.


Address reprint requests to:
Dr. Cassandra M. Lawson
Division of Veterinary and Biomedical Sciences
Murdoch University
Perth, Western Australia, 6150
Tel: +61 8 9360 2267
Fax: +61 8 9310 4144
E-mail: cassiel@numbat.murdoch.edu.au

Received 2 June 1998/Accepted 2 September 1998