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Efficacy of Low-Dose Oral Use of Type I Interferon in Cytomegalovirus Infections *In Vivo*

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ABSTRACT

Oral administration of type I interferons (IFNs; murine IFN-α and IFN-β) reduces early replication of murine cytomegalovirus (MCMV) in both the spleen and liver of MCMV-infected BALB/c mice. Examination of a range of doses of IFN (1 to 1000 IU) showed that 10 IU administered daily for 1 week prior to virus infection was optimal for inhibition of MCMV replication. Furthermore, low-dose orally administered IFN (10 IU/day) was effective in mice challenged with lethal and sublethal virus inocula. The antiviral efficacy of low-dose orally administered IFN was not restricted by either the route of virus inoculation or the mouse genotype. Analysis by immunohistochemistry of IFN-α receptor-bearing cells of the gastrointestinal tract revealed predominant staining of perivascular smooth muscle and the lamina propria of the anterior tongue, small intestine and rectum. These tissues, dense in IFN-α receptor-bearing cells, are likely to be the sites of interaction of the orally administered IFNs with the mucosal immune system. In conclusion, we propose that low-dose oral use of type I IFN therapy may have broad applications in the treatment of CMV infections.

INTRODUCTION

THE TYPE I INTERFERONS (IFN-α/β) are presently used in clinical treatment of several virus infections and diseases, including hepatitis C virus,[1] viral myocarditis,[2] condyloma acuminatum,[3] multiple sclerosis (MS),[4] Kaposi's sarcoma,[5] and hairy cell leukemia.[6] Conventional IFN therapy involves frequent administration of the highest dose of IFN tolerated by the patient, which often exceeds 5 million international units (IU) IFN-α/β injected either intramuscularly (i.m.), intravenously (i.v.), or subcutaneously (s.c.). Such a treatment schedule, associated with a number of dose-related side effects, commonly presents a range of clinical symptoms, including a flu-like illness, nausea, leukopenia, and injection site skin reactions.[5] It has also been reported that treatment of relapsing-remitting (RR) MS patients with high doses of human recombinant IFN alters endogenous cytokine levels, including an increase in interleukin-6 (IL-6) production.[7] More seriously, such high doses of IFN-α/β induce the production of neutralizing anti-IFN antibodies in approximately 40% of patients undergoing IFN treatment. The occurrence of these side effects highlights the need to improve the clinical efficacy of IFN therapy.

Low dose oral administration (LDOA) of IFN-α/β has provided a viable alternative to the current high-dose treatment regimes for several reasons. Low doses of IFN have not been associated with the development of severe side effects and the oral–mucosal route of administration presents a more acceptable and efficient mode of delivery with increased compliance by the patient. An early report of the antiviral efficacy of LDOA IFNs involved the protection of neonatal mice from lethal vesicular stomatitis virus (VSV) infection.[8] The efficacy of LDOA IFN therapy has now been demonstrated in experimental models[9,10] and clinical settings.[11,12] Several experimental studies including the nonobese diabetes (NOD) mouse model for diabetes,[13] murine B cell tolerance of ovalbumin,[14] vaccinia virus infections in the mouse,[15] and a guinea pig model of asthma[16] have shown the potential for the application of LDOA IFN therapy. In an animal model of MS, LDOA IFN treatment suppressed clinical relapse and adoptive transfer of chronic relapsing experimental autoimmune encephalomyelitis (CR-EAE).[7] It was found that treatment with 10 IU IFN-α/β delivered three times a week to the stomach and small intestine was optimal.

Previously, we have reported the antiviral efficacy of LDOA IFN therapy for a natural mouse pathogen, murine cytomegalovirus (MCMV),[17] a model used for human CMV infection and disease.[18] Daily treatment of BALB/c mice with 10 IU of IFN-α/β by the oral–mucosal route, starting 1 week prior to virus inoculation and continuing for the duration of the

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experiment, significantly reduced early virus replication in the spleen and liver. LDOA IFN was equally effective at reducing MCMV replication as a high dose (20,000 IU) of IFN injected intraperitoneally (i.p.).

In this study, we investigated the efficacy of LDOA IFNs in the context of the established MCMV model. Examination of the dose–response of the antiviral efficacy of IFN using a range of 1–1,000 IU IFN demonstrated that 10 IU was optimal. Efficacy was also investigated using a range of virus inoculum doses *in vivo* with intermediate doses being more responsive to IFN therapy. In addition, the efficacy of LDOA IFN was found to be unrestricted to the route of virus inoculation and the mouse genotype. Furthermore, we have characterized tissues of the gastrointestinal (GI) tract displaying dense type I IFN receptors to be located in both the anterior and posterior tongue, regions proximal to Peyer's patches of the small intestine, and the rectum. These sites correlate with specific binding of labeled IFN-α_1 identified in our previous study[17] and may represent the initial sites of interaction of the orally administered IFNs. These findings imply the potential for the wide application of LDOA IFN as antiviral therapy for CMV infections. Our results provide increased rationale for the delivery of low doses of IFN-α/β by the oral–mucosal route as an alternative mode of delivery in the treatment of patients with cytokine therapy.

MATERIALS AND METHODS

Mice

BALB/c, C57BL/6J, and CBA/CAH female mice were purchased from Animal Resources Centre (Murdoch, Western Australia) and used at 6–8 weeks of age.

Virus

MCMV (K181 strain) was prepared as a 20% salivary gland homogenate from MCMV-infected weanling female BALB/c mice and stored in the gas phase of liquid nitrogen. Virus titer was determined by plaque assay using mouse embryo fibroblasts as previously described.[19]

IFN administration

Murine IFN-α/β (Lee Biomolecular Inc, CA) was diluted to 10^3 IU/ml in pyrogen-free saline, and aliquots were stored at $-20°C$ until use. Unanaesthetised mice (5 per group) were administered 10 IU/10 μl by the oral–mucosal route daily for 7 days prior to virus infection and a further 2–3 days after virus infection as previously described.[17] Another group of mice received 10 μl of saline similarly via the oral–mucosal route and served as controls. In addition, a separate group of mice received 20,000 IU IFN-α/β by the i.p. route 6 h before virus inoculation.

Virus immunization

Virus inoculum was diluted in pyrogen-free phosphate-buffered saline. Mice (5 per group) were either injected i.p. with 100 μl of virus inoculum or injected with 50 μl of virus inoculum into the left footpad after 7 days of IFN treatment. Control groups of mice and mice injected with 20,000 IU IFN were similarly infected with virus by either the i.p. or footpad route. Approximately 1×10^3 plaque forming units (pfu) equates to 1 LD_{50} of MCMV when administered by the i.p. route in BALB/c mice.

Determination of virus titers in spleens and livers

Individual spleen and liver homogenates were prepared from virus-infected mice on day 2 and day 3 post infection (p.i.) and titrated in the plaque assay for quantitation of virus replication. Virus titers are expressed as mean pfu/gram of tissue $\pm$ SE from five individual samples. The limit of detection of virus was 50 pfu/gram.

Immunohistochemical examination of IFN receptors

GI tract samples of small intestine proximal to Peyer's patches, rectum, esophagus, masticatory muscle, posterior tongue, anterior tongue, posterior nasal cavity, anterior nasal cavity, and larynx and trachea were removed from a BALB/c mouse and frozen. Sections were prepared for immunohistochemical staining by incubation with 10% normal goat serum prior to incubation with rat anti-mouse IFN-α receptor immunoglobulin G (IgG) (Santa Cruz Biotechnologies, CA) and goat anti-rat IgG F(ab′) conjugated to horseradish peroxidase (Biosource, CA). The tissues were incubated with diaminobenzadine (Sigma, MO) substrate to give brown positive-reaction products and were counterstained with hematoxylin.

Statistical analysis

Levels of significance were determined by the unpaired *t*-test assuming unequal variance between the means.

RESULTS

Dose–response of orally administered IFN for reduction of MCMV replication

Previously, we have reported the antiviral properties of LDOA IFNs in the treatment of MCMV infection in the mouse.[17] In the present study we expand the investigations of the efficacy of LDOA IFN treatment for MCMV replication *in vivo*. BALB/c mice were treated with different concentrations of IFN-α/β ranging from 1 to 1,000 IU daily by the oral–mucosal route for 1 week prior to i.p. inoculation of MCMV (2.8×10^4 pfu). The virus titers produced in the spleen and liver following MCMV infection of LDOA IFN-treated animals were compared to those obtained from control animals given saline by the oral route. The administration of either 1 IU or 10 IU IFN orally to mice reduced virus replication in the liver and spleen at 2 days p.i. (Fig. 1). The lowest dose of IFN studied (1 IU) was shown to reduce the virus titer significantly in the spleen (Fig. 1A; two-fold reduction, $p = 0.008$) and in the liver (Fig. 1B). Also, a dose of 10 IU IFN clearly showed a decrease in MCMV titer in the spleen (Fig. 1A; 2.8-fold reduction, $p = 0.0003$) and liver (Fig. 1B). However, doses higher than 10 IU (50, 100, 500, 1,000 IU) did not reduce the early replication of virus in either the spleen or liver at day 2 p.i. (Fig. 1). Indeed, virus titers in the spleen of mice treated with doses of 50, 500, and 1,000 IU LDOA IFN were significantly increased ($p =$

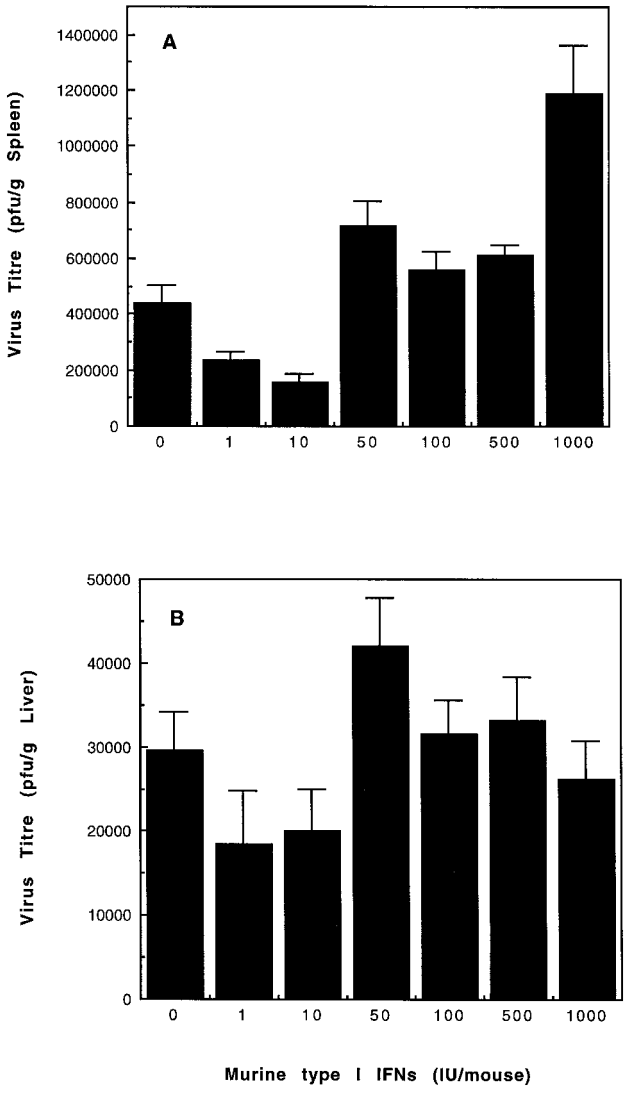

FIG. 1. Low doses of orally administered IFN are more effective at reducing MCMV replication in spleen and liver. Groups of 5 BALB/c mice were either given doses of MuIFN-α/β ranging from 1 to 1,000 IU or saline (0) by the oral–mucosal route daily for 7 days prior to i.p. inoculation with 2.8×10^4 pfu MCMV. Mean virus titers ± standard errors (pfu/gram of tissue) are shown for spleens **(A)** and livers **(B)** taken from mice at day 2 p.i.

0.0019, 0.0258, and 0.0014, respectively) compared to mice given saline orally. Although virus replication in the liver of mice treated with LDOA IFN, at doses greater than 50 IU, showed higher titers than mice given saline orally, these results were not significant. Overall, the dose–response resembled a U-shaped curve, with 10 IU being optimal for the antiviral effect. These results were reproduced over two separate experiments and confirm our earlier findings.[17]

LDO IFN treatment is effective for different doses of MCMV

The efficacy of LDOA IFN treatment using 10 IU IFN was assessed over a range of MCMV challenge doses. BALB/c mice were treated with LDOA IFN for 1 week before i.p. inoculation of MCMV (either 4.2×10^4 pfu, 2.8×10^4 pfu, or 6.7×10^3 pfu/mouse). Virus titers were again quantitated in the spleen and liver at day 2 p.i. and compared to titers obtained from control mice treated with saline prior to MCMV infection (Fig. 2). Virus replication in the spleens of mice given the highest and lethal virus load of MCMV (4.2×10^4 pfu) was not reduced by LDOA IFN (Fig. 2A), which may be associated with the markedly severe infection of the spleen at this time p.i. (1.25×10^6 pfu/g). However, the virus load in the liver, which was lower than that obtained in the spleen of saline-treated animals following infection with 4.2×10^4 pfu MCMV, was reduced with LDOA IFN treatment (Fig. 2B, 1.6-fold reduction). Groups of mice infected with the intermediate and sublethal dose of

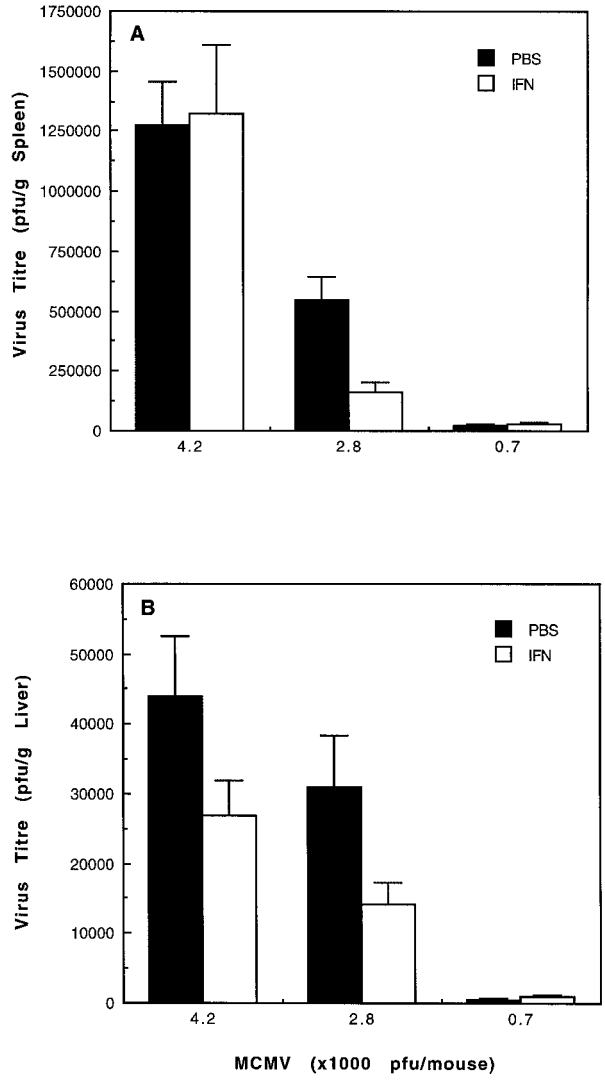

FIG. 2. Effect of LDOA IFN treatment with dose of MCMV. Groups of 5 BALB/c mice were either given a dose of 10 IU of MuIFN-α/β (IFN) or saline (PBS) by the oral–mucosal route daily for 7 days prior to i.p. inoculation with either 4.2×10^4, 2.8×10^4, or 6.7×10^3 pfu MCMV. Mean virus titers ± standard errors (pfu/gram of tissue) are shown for spleens **(A)** and livers **(B)** taken from mice at day 2 p.i.

MCMV (2.8×10^4 pfu), showed marked inhibition of virus replication in the spleen (Fig. 2A, 3.4-fold reduction, $p = 0.002$) and liver (Fig. 2B, 2.2-fold reduction, $p = 0.055$) associated with LDOA IFN treatment prior to infection. Mice infected with the lowest dose of virus studied (6.7×10^3 pfu) produced very low levels of detectable virus in both the spleen and liver which made a comparative analysis of LDOA IFN-treated groups and control groups of mice difficult (Fig. 2).

Efficacy of LDOA IFN treatment for mice infected with MCMV via different routes

We examined whether the phenomenon of virus reduction in titer by the LDOA IFN treatment was limited to the route of virus inoculation in our model of MCMV infection. In preliminary experiments, the kinetics of virus replication in the spleen and liver following dissemination from an initial footpad inoculation of MCMV (1×10^5 pfu/mouse) was determined in BALB/c mice (Fig. 3). Virus replication in both the spleen (Fig. 3A) and liver (Fig. 3B) was detectable at a low titer at day 2, with a peak titer at day 3, followed by a marked reduction in titer at days 4 to 7 p.i.

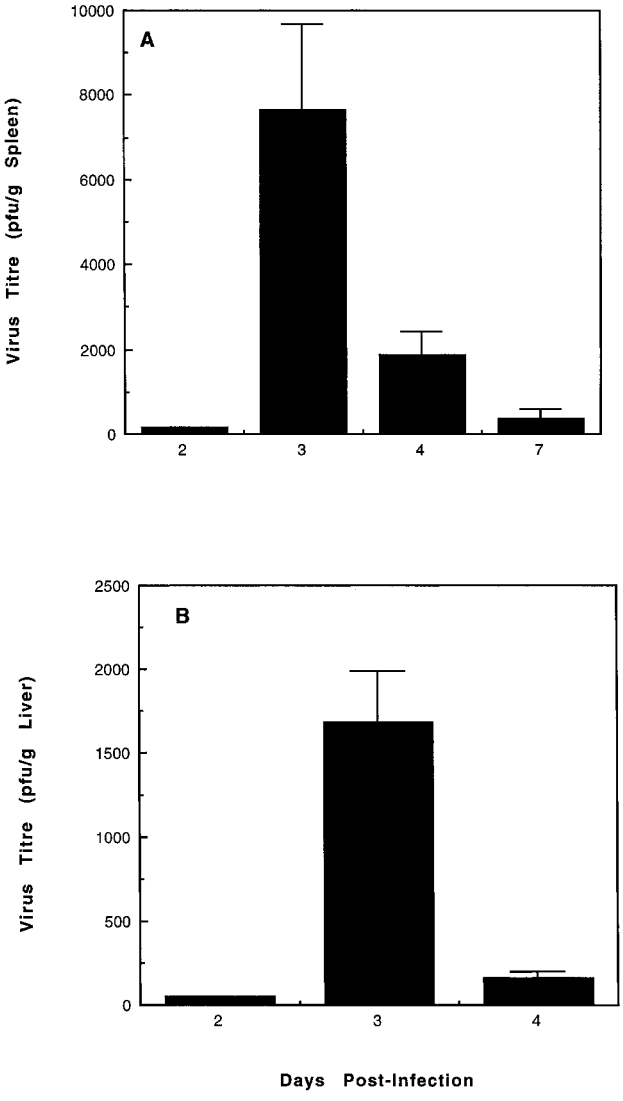

FIG. 3. Kinetics of MCMV replication in spleen and liver following footpad inoculation of virus. Groups of 5 BALB/c mice were infected with 1.0×10^5 pfu MCMV via the footpad. Mean virus titers ± standard errors (pfu/gram of tissue) are shown for spleens **(A)** and livers **(B)** taken from mice at various times p.i.

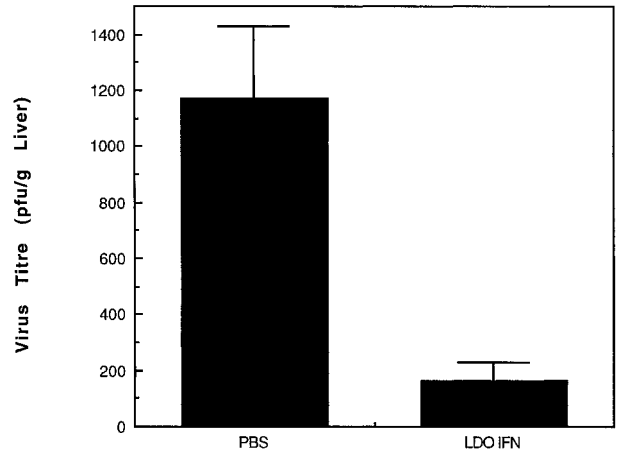

FIG. 4. LDOA IFN treatment reduces MCMV replication after footpad inoculation of virus. Groups of 5 BALB/c mice were either given a dose of 10 IU MuIFN-α/β (LDOA IFN) or saline (PBS) by the oral–mucosal route daily for 7 days prior to footpad inoculation with 1.0×10^4 pfu MCMV. Mean virus titers ± standard errors (pfu/gram of tissue) are shown for livers taken from mice at day 3 p.i.

We next investigated the efficacy of LDOA IFN treatment in the suppression of peak virus replication (day 3 p.i.) in mice inoculated with MCMV by the footpad. BALB/c mice were treated with LDOA IFN similarly to the previous experiments (10 IU IFN/mouse/day for 1 week) and were challenged with MCMV (1×10^4 pfu/mouse) via the footpad. There was no apparent reduction in virus titer in the spleens of mice treated with LDOA IFN (data not shown). Although the virus titers obtained at day 3 p.i. were low, mice treated with LDOA IFN and infected with MCMV via the footpad showed a marked reduction in virus titer in the liver (7-fold reduction, $p = 0.019$) compared with the titers obtained from liver of control mice treated with saline (Fig. 4).

A separate experiment was performed using an increased virus challenge dose of 3×10^5 pfu/mouse delivered by the footpad route in order to obtain higher virus titers at day 3 p.i. As a further control, mice were inoculated with 20,000 IU IFN by the i.p. route 6 h prior to virus infection. BALB/c mice treated with LDOA IFN showed significant reduction in the virus titer obtained from spleen (Fig. 5A, 3-fold reduction, $p = 0.004$) and liver (Fig. 5B, 1.5-fold reduction, $p = 0.049$) tissues compared to control mice treated with saline. Mice treated with a high dose of injected IFN were also partially protected from MCMV infection (Fig. 5). Indeed the LDOA treatment and the injected high dose of IFN were approximately equal at controlling virus replication, particularly in the spleen (Fig. 5A). Interestingly, the virus-induced splenomegaly was not affected by IFN treatment and increased spleen weights were a notable feature of footpad inoculation (210 ± 6 mg at day 5 p.i.) of the

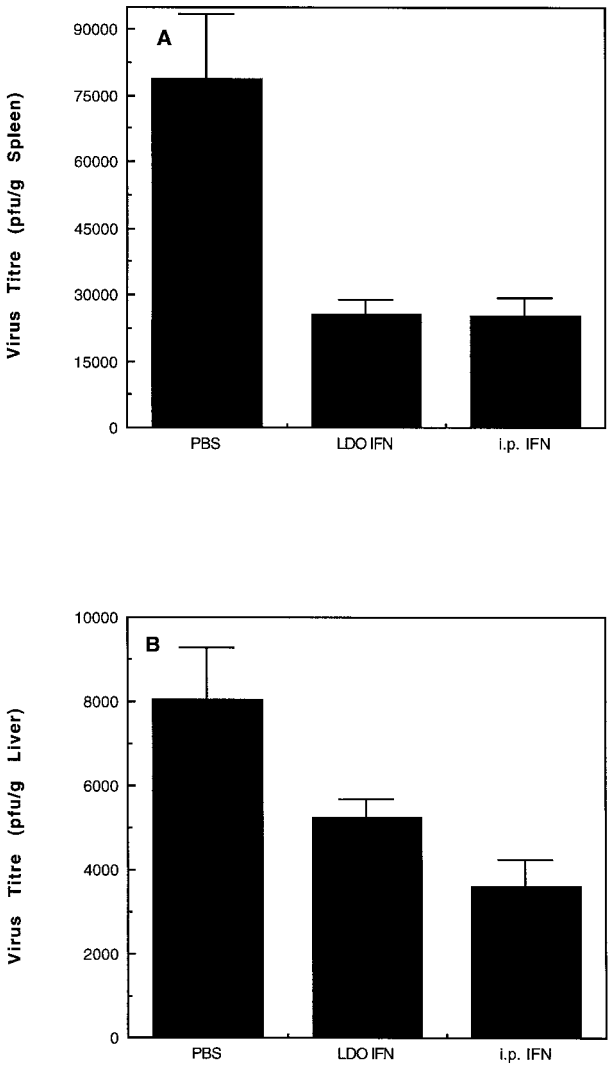

FIG. 5. LDOA IFN treatment is as effective as high-dose injected IFN at reducing MCMV replication after footpad inoculation of virus. Groups of 5 BALB/c mice were either given a dose of 10 IU MuIFN-α/β (LDO IFN) or saline (PBS) by the oral–mucosal route daily for 7 days prior to footpad inoculation with 3.0×10^5 pfu MCMV or injected with 20,000 IU MuIFN-α/β (i.p. IFN) 6 h before virus inoculation. Mean virus titers ± standard errors (pfu/g tissue) are shown for spleens **(A)** and livers **(B)** taken from mice at day 3 p.i.

virus compared to mice inoculated i.p. (110 ± 11.4 mg at day 5 p.i.). Furthermore, the variation in virus titers for spleen and liver samples from individual mice inoculated with MCMV in the footpad was decreased relative to the standard errors in individual titers obtained from groups of mice infected with MCMV by the i.p. route.

LDOA IFN efficacy is not restricted by mouse genotype

Our investigations into the antiviral efficacy of LDOAIFN treatment have used BALB/c animals, a mouse strain that is susceptible to MCMV infection. We next examined whether LDOA IFN treatment of MCMV infection was effective in other mouse strains with different genetic backgrounds to that of BALB/c (H-2^d). CBA (H-2^k) and C57B/6 (H-2^b) mice are more resistant to MCMV infection that BALB/c animals (20- to 30-fold, and 2- to 4-fold more resistant, respectively). CBA mice were treated with IFN and infected with MCMV (6×10^6 pfu via the footpad route or 5.4×10^5 pfu by the i.p. route) and analyzed for any reduction in virus titers obtained at day 3 p.i. in the spleen and liver (Fig. 6). CBA mice challenged with virus via the i.p. route showed a marked reduction in titer in the spleen (Fig. 6A, 4.5-fold reduction, $p = 0.013$) and liver (Fig. 6B, 4-fold reduction, $p > 0.05$). As expected, a single injection of

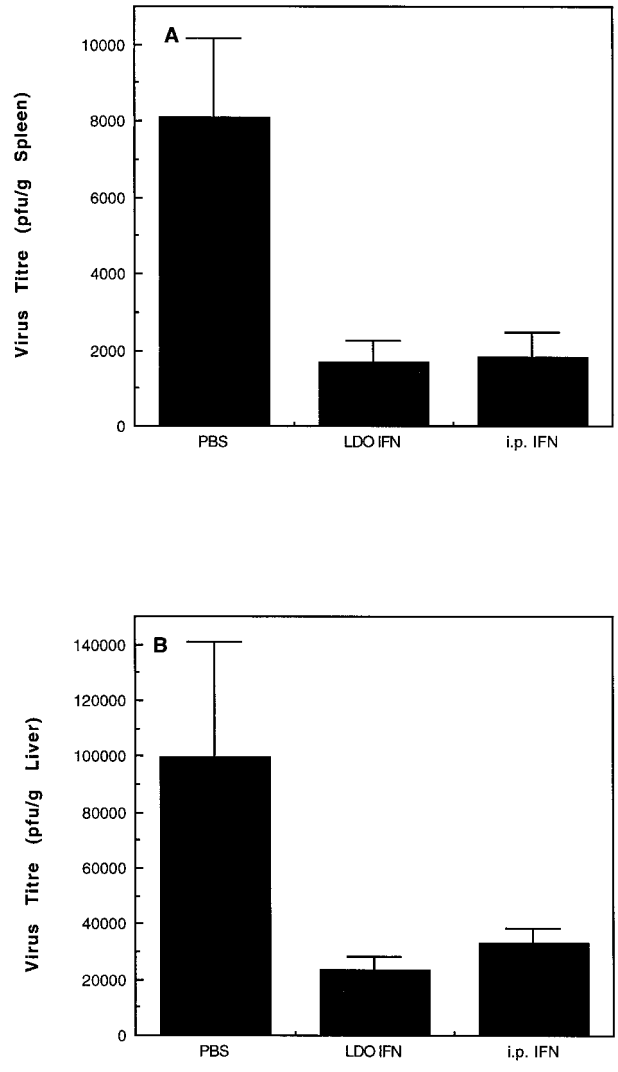

FIG. 6. LDOA IFN treatment is as effective as high-dose injected IFN at reducing MCMV replication in CBA mice. Groups of 5 CBA mice were either given a dose of 10 IU MuIFN-α/β (LDO IFN) or saline (PBS) by the oral–mucosal route daily for 7 days prior to i.p. inoculation with 5.4×10^5 pfu MCMV or injected with 20,000 IU MuIFN-α/β (i.p. IFN) 6 h before virus inoculation. Mean virus titers ± standard errors (pfu/g tissue) are shown for spleens **(A)** and livers **(B)** taken from mice at day 3 p.i.

20,000 IU IFN prior to virus inoculation also significantly decreased virus replication in the spleen and liver (Fig. 6). However, CBA mice challenged with virus via the footpad route showed only a modest reduction in virus titer in the liver at day 3 p.i. (Fig. 7A, 1.3-fold reduction, $p = 0.17$).

C57BL/6 mice given LDOA treatment and infected with MCMV (1.5×10^6 pfu) via the footpad showed a marked reduction in virus titer obtained from the liver at day 3 p.i. (Fig. 7B, 7.4-fold reduction, $p = 0.0007$). Very low titers of virus were detected in the spleen of these mice, making a comparative analysis of LDO treatment and saline-treated groups difficult.

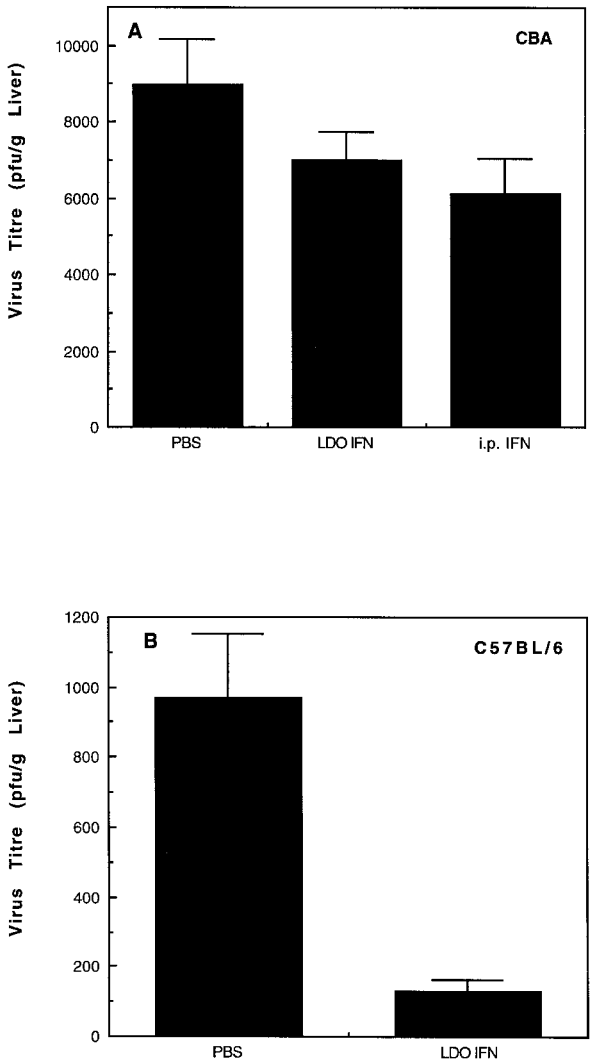

FIG. 7. LDO IFN treatment reduces MCMV replication in the livers of CBA and C57BL/6 mice inoculated with virus via the footpad. Groups of 5 mice, CBA **(A)** and C57BL/6 **(B),** were either given a dose of 10 IU MuIFN-α/β (LDO IFN) or saline (PBS) by the oral–mucosal route daily for 7 days prior to footpad inoculation with either 6.0×10^6 pfu MCMV (CBA) or 1.5×10^6 pfu MCMV (C57BL/6). CBA mice were also injected with 20,000 IU of MuIFN-α/β (i.p. IFN) 6 h before virus inoculation. Mean virus titers ± standard errors (pfu/gram of tissue) are shown for livers taken from mice at day 3 p.i.

IFN-α/β receptors are distributed throughout the GI tract

Previously, we reported the specific binding sites of IFN-α in the murine GI tract.[17] In the present study, we characterized the location of IFN receptor-bearing tissues of the GI tract in mice. Frozen tissue samples, posterior nasal cavity, anterior tongue, posterior tongue, masticatory muscle, esophagus, small intestine, and rectum, from a normal uninfected BALB/c mouse were analyzed by immunohistochemistry for IFN receptor-bearing cells. Tissue samples examined without the application of the rabbit anti-mouse IFN-α receptor antibody did not stain positive. Vascular and epithelial smooth muscle and skeletal muscle areas of the posterior and anterior tongue, small intestine, and rectum were positively stained for IFN-α receptors (data not shown). Tongue samples also showed positive staining, largely of the connective tissue of the lamina propria under the epithelium and on smooth muscle. In addition, the extracellular brush borders of the mucosal epithelium and the layers of mucous adjunct to brush borders of the small intestine and rectum demonstrated high IFN-receptor density. Modest staining was observed for surface epithelium and connective tissue of the esophagus (data not shown). The vascular smooth muscle and connective tissue of the larynx and masticatory muscle was lightly stained for IFN-receptor bearing cells (data not shown). Surface microvilli and cilia borders of the nasal epithelium were also positively stained (data not shown).

DISCUSSION

Efficacy of LDOA IFN therapy for CMV infection

Our earlier report of the antiviral properties of prophylactic LDOA IFN treatment[17] led us to investigate further the efficacy of LDOA IFN therapy for MCMV infection. Our results provide evidence for the role of the type I IFNs in a protective innate immune response for CMV infection. In the present study, a dose of 10 IU of IFN-α/β administered orally for 7 days prior to virus infection was shown to be optimal and significantly reduced the early replication of MCMV in both the liver and spleen of BALB/c mice. Furthermore, LDOA IFN treatment (10 IU/mouse per day) was not restricted to either the virus challenge dose, the route of virus inoculation, or the mouse genotype. Indeed, the s.c. route of inoculation into the footpad may more closely resemble a natural transmission pathway for MCMV than the i.p. route of virus inoculation. Although only a moderate reduction in virus titer (4- to 6-fold) was observed, this suppression of virus replication may result in a greater inhibition of virus load in host tissues that favors the development of a protective immune response. Our findings that larger doses of IFN (50, 100, 500, 10,000 IU/day per mouse) were not as effective as 10 IU of IFN suggests an immunoregulatory mechanism. The U-shaped dose–response curve is a characteristic feature of biological molecules operating through receptor/ligand interactions. It is hypothesized that high local concentrations of IFN induce downregulation of type I IFN receptors.[20] Our results, showing significantly higher spleen virus titers in mice given 50, 500, and 1,000 of IU LDOA IFN (Fig. 1A), may indicate such an event. This may result in an abrogation of the endogenous IFN response, allowing the virus

to replicate to a higher titer. Our results confirm other studies of experimental models where high doses of orally administered IFNs to mice were not protective against virus infection in contrast to the protection afforded by LDOA IFN.[21]

The ability of low doses of IFN, delivered via the oral–mucosal route, to stimulate a protective immune response is important for the control of virus infections. Our observations of IFN-α receptor dense tissues within the GI tract suggests the location of cells that may contact the ingested murine IFNs and transduce intracellular signals that ultimately are associated with the stimulation of the immune system. The GI-associated lymphoid tissue is composed of many immune cells, including macrophages, dendritic cells, B and T cells of the Peyer's patches, intraepithelial lymphocytes of villi, and lymphocytes within the lamina propria. These cell types may respond specifically to the ingested IFNs. We are currently investigating the stability of ingested type I IFNs in the lower GI tract.

The mechanisms of action of LDOA IFNs have not been fully elucidated. We are presently investigating possible mechanisms of action of the LDOA IFNs in the activation of immune cells that subsequently result in an antiviral state of the host. The orally administered IFNs may alter immune cell subset populations, with cytokine profiles leading to a switch in Th1/Th2 responses, enhanced NK cell cytotoxicity, increased antibody production, and the upregulation of cell surface molecules (MHC class I and class II, B7, B7.1, ICAM-1, VCAM-1, E-selectin). Such studies will lead to a better understanding of the link between the innate and acquired immune responses. Such a link was proposed by Tough *et al.*[22] in their study of bystander T cell activation by high-dose type I IFN. In this study, high-dose type I IFN, injected i.v., was shown to cause significant proliferation and long-term maintenance of the $CD8^{+}CD44^{hi}$ subset of memory T cells. The memory phenotype, a component of the acquired immune system, appears to be induced by type I IFN, a component of the innate system. This represents a direct link between the two components of the immune system.

Potential of LDOA IFN therapy for virus infections and other diseases

Our findings implicate the wide potential for LDOA IFN therapy in herpesvirus infections, although the effectiveness of other regimes with different dosing schedules and timing of the IFN treatment has not been examined. We are currently investigating the efficacy of LDOA treatment of mice following a virus infection and treatments that include multiple doses of IFN administered daily.

LDOA IFN therapy has been examined in various experimental models including vesicular stomatitis virus (VSV) infection of neonatal mice.[8] Protection from VSV infection (oral route of administration) was afforded to neonates after treatment with oral doses of IFN. In addition, suckling mice receiving an oral dose of IFN through the breast milk of Newcastle disease virus (NDV)-immunized lactating mothers, were also protected from lethal infection with VSV delivered via the oral route. Another virus model involved the treatment of C3H/HeN mice with LDOA IFN (1, 10, or 100 IU/mouse) 1 day before vaccinia virus infection and further daily treatment for 15 days p.i.[15] IFN-treated mice in this experimental model showed suppressed pock formation and, at doses greater than 1 IU/mouse, the mice showed enhanced virus-specific cytotoxic T lymphocyte (CTL) activity. Furthermore, antiviral treatment with LDOA human IFN has been reported to be effective between different species.[10] Induction of a transmissible gastroenteritis induced by corona virus infection of piglets (1–12 days old) was suppressed with LDOA IFN (1 to 20 IU/animal per day) treatment. A separate study showed the effectiveness of LDOA human IFN treatment in horses.[9] Standardbred racehorses with inflammatory airway disease showed reduced inflammation of the lower respiratory tract after treatment with LDOA IFN (50, 150, 450 IU/animal per day).

The effectiveness of LDOA IFN therapy for nonviral diseases, including autoimmune disease, has been investigated in several studies. NOD mice treated daily with LDOA murine IFN were partially protected from insulin-dependent diabetes and showed decreased islet cell inflammation.[13] In addition, ingested low-dose murine IFN suppressed acute attacks and clinical relapses in an EAE mouse model.[7] A model of ovalbumin-induced tolerance in mice has been investigated for the effects of orally administered IFN-α on breaking tolerance characterized by anti-ovalbumin antibody production.[14] Oral–mucosal use of IFN was shown to elevate levels of anti-ovalbumin antibodies in tolerized mice and augment 2-5 (A) synthetase activity, and mRNA levels for 2-5 (A) synthetase and interferon regulatory factor 1 (IRF-1) in splenocytes of normal mice.

An advantage of oral use of IFN over injected high doses of IFN in the clinical setting is that it cannot be blocked by anti-IFN antibodies because it is undetectable in the circulation. Therapy with LDOA IFN provides increased benefits for the patient, including prolonged treatment with less side effects. Furthermore, LDOA IFN therapy is less costly than treatments with high doses, and the low-dose IFN lozenges have room temperature stability.

Oral use of IFN in patients with a variety of diseases is showing great promise as a cytokine therapy. Therapy with orally administered IFN is also being developed as a treatment of Sjögren's syndrome, hepatitis B and hepatitis C virus infections, opportunistic infections in human immunodeficiency virus (HIV)-positive patients, and fibromyalgia (Joseph Cummins, Amarillo Biosciences, TX, personal communication). LDOA IFN treatment of patients with Sjögren's syndrome (150 IU $\times$ 3 times/day for 24 weeks) is currently undergoing phase III trials in the United States. With the growing evidence supporting the efficacy of low-dose oral IFN therapy for diseases of both viral and nonviral etiology, it seems feasible to propose that widespread application may be beneficial by reducing the side effects and cost of conventional IFN therapy. With added knowledge of the mechanism of action, these beneficial outcomes may be exploited to their full potential. This paper presents data depicting the ability of LDOA IFN therapy to cause a systemic immune response. The ability of such therapy to reduce the replication of MCMV in mice warrants further investigation to determine the mechanism of action of the LDOA type I IFN phenomenon.

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