Ectopic Expression of DNA Encoding IFN-α1 in the Cornea Protects Mice from Herpes Simplex Virus Type 1-Induced Encephalitis

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Ectopic Expression of DNA Encoding IFN-α1 in the Cornea Protects Mice from Herpes Simplex Virus Type 1-Induced Encephalitis

Sansanee Noisakran,* Iain L. Campbell,† and Daniel J. J. Carr‡*

A novel approach to combat acute herpes simplex virus type 1 (HSV-1) infection has recently been developed by administration with a plasmid DNA construct encoding cytokine genes. Cytokines, especially type I IFNs (IFN-α and IFN-β) play an important role in controlling acute HSV-1 infection. The purpose of the present study was to investigate the potential efficacy of ectopically expressed IFN-α1 against ocular HSV-1 infection following in situ transfection of mouse cornea with a naked IFN-α1-containing plasmid DNA. Topical administration of the IFN-α1 plasmid DNA exerted protective effect against ocular HSV-1 challenge in a time- and dose-dependent manner and antagonized HSV-1 reactivation. In addition, IFN-α1-transfected eyes expressed a fivefold increase in MHC class I mRNA over vector-treated controls. The protective efficacy of the IFN-α1 transgene antagonized viral replication, as evidenced by the reduction of the viral gene transcripts (infected cell polypeptide 27, thymidine kinase, and viral protein 16) and viral load in eyes and trigeminal ganglia during acute infection. The administration of neutralizing Ab to IFN-αβ antagonized the protective effect of the IFN-α1 transgene in mice. Collectively, these findings demonstrate the potential of using naked plasmid DNA transfection in the eye to achieve ectopic gene expression of therapeutically active agents. The Journal of Immunology, 1999, 162: 4184–4190.

Ocular infections caused by herpes simplex virus type 1 (HSV-1) are extremely common and can lead to encephalitis as well as to herpetic keratoconjunctivitis (1). Local immune responses to the corneal HSV-1 infection include the infiltration of mononuclear cells such as CD4+ and CD8+ T cells, macrophages, and neutrophils; the activation of NK cells; and the production of cytokines (2–4). Although there have been several studies assessing immunization strategies against HSV-1 infection, currently there is no successful vaccine commercially available. DNA immunization, a novel vaccination approach that induces both humoral and cellular immune responses, has been applied successfully to combat many infectious diseases (5–8). Studies evaluating DNA vaccination against HSV-1 infection using different types of viral vectors that carry HSV-1 Ag-encoding genes have had varying degrees of success (9–12). Although this approach is effective in generating an immune response, it is likely that viral vectors can reduce gene expression by killing transfected cells. In addition, the production of Ab can be induced, which modifies the immune response to the repeated administration of this type of vaccine (13).

Naked DNA vaccination against HSV-1 infection is another approach that has been developed using a plasmid construct consisting of a mammalian cell promoter, a polyadenylation site, a selective marker (e.g., drug-resistance gene), and an Ag-encoding gene as the immunogen. One in vitro study demonstrated that a plasmid construct encoding the immediate-early protein, infected cell polypeptide 27 (ICP27), of HSV-1 was capable of inducing HSV-1-specific CD8+ CTL generation (14). In a zosteriform model, i.m. immunization with glycoprotein B-encoded plasmid DNA construct has been shown to elicit CD4+ T cell activation and protect mice against acute HSV-1 infection (15). In another study, dendritic cells transfected with DNA as a delivery system were found to be more effective in comparison with the administration of naked DNA alone (16).

The concept of DNA immunization has been applied recently to gene therapy using vector constructs that encode cytokine genes. Presumably, following in vivo transfection, host cells take up plasmid DNA encoding the gene of interest; as a result, in situ expression of the transgene would either antagonize the microbial infection or alternatively, reduce the destructive inflammatory process associated with the infection. Recently, the possibility of using in vivo DNA transfection as a therapeutic option was supported by evidence showing that the topical administration of plasmid DNA encoding IL-10 reduced the incidence of herpetic stromal keratitis (17). To further explore this alternative approach, the present study was undertaken to characterize the efficacy of the topical administration of a plasmid encoding murine IFN-α1 in mice. IFN-α consists of multiple subtypes that are acid-stable, classically induced in cells by viruses or synthetic polyribo nucleotides, and show potent antiviral and immunoregulatory functions (18). The IFN-α subtype was investigated in the present study because it has been shown previously to have the greatest degree of protection against another herpesvirus, CMV, compared with other IFN-α subtypes (19).
Materials and Methods

**Virus and cells**

Vero and CV-1 African green monkey kidney cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 5% FBS (Life Technologies, Gaithersburg, MD) and an antibiotic/antimycotic solution (Life Technologies) in an atmosphere of 37°C/5% CO2/95% humidity. HSV-1 (McKrae strain) stock was prepared as described previously (20).

**Infection of mice**

Female ICR mice (weight 25–34 g, Harlan-Sprague-Dawley, Indianapolis, IN) were anesthetized by an i.p. injection of 0.1 ml of PBS containing xylazine (2 mg/ ml; 6.6 mg/kg) and ketamine (30 mg/ml; 100 mg/kg). Corneas were scarified with a 25-gauge needle, and tear film was blotted with tissue. Mice were inoculated with 450 plaque-forming units (PFU)/eye of HSV-1 (McKrae strain). Infection was verified by swabbing the eyes at 2–3 days postinfection (PI) and placing the swabs in CV-1 monolayer cultures to observe for a cytopathic effect.

Animals were handled in accordance with the National Institutes of Health guidelines on the Care and Use of Laboratory Animals (publication no. 85-23, revised 1996). All procedures were approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee.

**Plasmid DNA construct**

Plasmid pCMV-β (vector) was purchased from Clontech Laboratories (Palo Alto, CA). This eukaryotic expression vector (7.2-kb) contains *Escherichia coli* β-galactosidase (a reporter gene) expressed under the control of a human CMV immediate-early promoter/enhancer, an RNA splice donor and acceptor sequence, and an SV40 late polyadenylation signal. Plasmid pCMV-IFN-α1 was generated as follows: A 690-bp HindIII-EcoRI fragment of mouse IFN-α1 cdNA (a kind gift of Dr. E. Zwarthoff, Erasmus University, Rotterdam, The Netherlands) was excised from pGEM-4. After the addition of NotI linkers, this fragment was cloned in the NotI site of pCMV-β, thus the generated construct was called pCMV-IFN-α1. The plasmid DNA constructs were transformed into shortINV (antisense). Primers specific for the IFN-α1 transgene are: 5'-GGACCTGATTTCCCAGTTCAAC-3' (sense) and 5'-TTTATTCACCAAGGATGTCATCCG-3' (antisense). Plasmid DNA constructs were transformed into shortINV (antisense). Primers specific for the IFN-α1 transgene are: 5'-GGACCTGATTTCCCAGTTCAAC-3' (sense) and 5'-TTTATTCACCAAGGATGTCATCCG-3' (antisense). Plasmid DNA constructs were transformed into shortINV (antisense).

**Administration of plasmid DNA construct**

After mice were anesthetized, corneas were scarified with a 25-gauge needle and blotted with tissue before 100 μg/eye of either pCMV-β (vector) or pCMV-IFN-α1 was administered at 24 h, 72 h, or 2 wk before HSV-1 injection. In addition, to test the prophylactic efficacy of the IFN-α1 construct on acute HSV-1 infection, we anesthetized HSV-1-infected mice and topically treated their eyes with 100 μg/eye of either pCMV-β (vector) or pCMV-IFN-α1 at 24 h PI. For the dose-response study, mice were treated with either PBS or pCMV-IFN-α1 (5, 25, 50, or 100 μg/eye) and infected with 450 PFU/eye of HSV-1 24 h later.

**RT-PCR**

RT-PCR was performed as described previously (20). Briefly, RNA was extracted from excised tissue in Ultraspec RNA isolation reagent (Biotecx, Houston, TX). First-strand cdNA was synthesized using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). PCR was performed in a thermal cycler (Ercopim D cycler, Ercopim, San Diego, CA) with 30–35 cycles of 94°C for 75 s, 57–65°C for 75 s, and 72°C for 30–45 s. PCR primers for glycereraldehyde-3-phosphate dehydrogenase (GAPDH), latency-associated transcript (LAT) RNAs, IC27, IFN-γ, IL-6, and IL-10 were as described previously (20, 22). IFN-α1 (consensus sequence for IFN-α1, α2, and 7), CD4, and CD8 primer sequences were obtained from Clontech Laboratories. Primers for viral protein 16 (VP16) (VP16) were 5'-GGACCTGATTTCCCAGTTCAAC-3' (sense) and 5'-CGTCTTGGCGCGTGGGATC-3' (antisense). Primers for thymidine kinase (TK) were 5'-ATGTTCCGTAGCCAGTCCG-3' (sense) and 5'-GGTATGTCCGGCGGCGCGGTA-3' (antisense). Primers specific for the IFN-α1 transgene were 5'-ATTCCCCAGGGAAGATGTTGAGTTCGGCCCA-3' (sense) and 5'-GAGTATGTACATAGAATGATTACA-3' (antisense) based on the published sequence for the murine IFN-α1 cDNA sequence upstream primer starting at nucleotide 550 (GenBank accession no. X01974) and the downstream primer sequence starting at nucleotide 20 of the SV40 late region polyadenylation sequence, which was used as a 3' untranslated region in the pCMV-IFN-α1 fusion gene construct (sequence according to Clontech Laboratories). Primers for JE/monocyte chemoattractant protein-1 (MCP-1) and the setting for the amplification of the specific products were as described previously (23). The primers for cytokine response gene-2 (CRG-2) were 5'-CAGCCACCATGAAACCTAATGC-3' (sense) and 5'-GTGGTCACCTTCTAGAAGACC-3' (antisense).

**In vivo neutralization assay**

In indicated experiments, mice received rabbit anti-mouse anti-IFN-αβ (Access Biomedical, San Diego, CA; 1000 neutralizing units) or normal rabbit Ig at the time of infection and at 3 and 6 days PI as described previously (22).

**Statistics**

The Mann-Whitney U test was used to determine significant (p < 0.05) differences between the IFN-α1 plasmid construct- and vector construct-treated groups relative to cumulative survival using the GBSTAT program (Dynamic Microsystems, Silver Springs, MD). All other statistical analyses comparing vehicle- or vector-treated mice with IFN-α1 construct-treated animals involved ANOVA and Tukey's post hoc test.

**Results**

**Transient expression of plasmid DNA encoding IFN-α1 in eyes**

To verify the exogenous expression of IFN-α1 in eyes, mice were topically administered with 100 μg/eye of either pCMV-β (vector control) or pCMV-IFN-α1 at 24 h, 72 h, or 2 wk before sacrifice. The transgene was expressed in uninfected eyes in a time-dependent manner by RT-PCR (Fig. 1) and ribonuclease protection assay (data not shown). Likewise, IFN-α1 transgene expression was detected in the eyes of IFN-α1 construct-treated mice at 3 days PI but was absent in the vector-treated mice (Fig. 1). However, expression during infection was transient, disappearing at 6 days PI (Fig. 1). A possible explanation for this result is that the host-transfected corneal epithelial cells are compromised or destroyed as a result of the infection, leading to a loss in transgene expression during the acute phase of the infection.

**In vivo transfection with plasmid DNA encoding IFN-α1 protects mice against ocular HSV-1 challenge**

To determine the in vivo efficacy of a plasmid IFN-α1 construct against ocular HSV-1 infection, either pCMV-β (vector) or pCMV-IFN-α1 (100 μg/eye) were applied topically to the corneas of mice at 24 h, 72 h, or 14 days before ocular HSV-1 infection, or at 24 h PI. Topical administration of pCMV-IFN-α1 at 24 h before infection significantly (p < 0.05) enhanced the survival of mice compared with vector-treated mice (Fig. 2). Treating mice with the IFN-α1 construct at 72 before infection or at 24 h following HSV-1 infection had no significant effect against acute HSV-1 infection (Fig. 2). In addition, none of the mice receiving the IFN-α1 construct at 2 wk before the infection survived in the period surveyed (Fig. 2). The data illustrate the transient nature of the protective effect, which is consistent with the expression of the transgene within the cornea. To determine the dose-dependent efficacy of the IFN-α1 construct against HSV-1, mice were topically given either vehicle (PBS) or pCMV-IFN-α1 (5, 25, 50, or 100 μg/eye) and subsequently challenged 24 h later with HSV-1. The results show that compared with mice receiving vehicle (PBS) or pCMV-IFN-α1 (5 μg/eye), mice receiving higher concentrations...
Transient expression of IFN-α1 construct in eyes. A, Mice (n = 2/experiment, repeated three times) were topically treated with 100 μg/eye of pCMV-IFN-α1 and then sacrificed at 24 h, 72 h, or 2 wk post-treatment. B, Mice (n = 3/group, repeated three times) were treated with 100 μg/eye of either pCMV-β (V) or pCMV-IFN-α1 (α1), infected with HSV-1 24 h later, and then sacrificed on days 3 and 6 PI to determine the expression of the IFN-α1 transgene using RT-PCR. This figure shows a representative RT-PCR analysis. C, A summary of the RT-PCR results is shown as a pixel ratio of IFN-α1 to GAPDH.

Effect of IFN-α1 construct on expression of MHC, viral transcripts, immune cell transcripts, and cytokine as well as chemokine transcripts

Type I IFNs possess many antiviral characteristics, including the induction or up-regulation of MHC class I molecules, which facilitates CTL recognition of virally infected cells. To determine whether application of the IFN-α1 transgene onto the cornea elicited such an effect, mouse eyes were transfected with the pCMV-β vector or with pCMV-IFN-α1 and assessed for MHC class I mRNA expression. The results show a fivefold elevation in the expression of MHC class I RNA in eyes that were transfected with the IFN-α1 transgene compared with the vector-treated group (Fig. 3, A and B). Equivalent amounts of RNA for each sample were analyzed based on the hybridization intensities for β-actin (Fig. 3A). To further characterize the protective mechanism elicited by the IFN-α1 construct, viral loads were assessed in the eyes and trigeminal ganglia (TG) during acute infection. There was a significant reduction in the amount of infectious virus recovered from the eyes of mice topicaly treated with pCMV-IFN-α1 compared with vector-treated controls at 3 and 6 days PI (Fig. 4). Likewise, 7 of 12 mice treated with the vector had detectable virus in the cerebellum (100 PFU/cerebellum) compared with 3 of 12 mice treated with the pCMV-IFN-α1 construct (10 PFU/cerebellum). Consistent with these results, viral transcript expression was also modified. Specifically, treatment with the IFN-α1 construct reduced the expression of HSV-1 ICP27 and HSV-1 VP16 in the TG at 3 days PI, whereas the expression of TK was not detected (Fig. 5). In the eye, HSV-1 TK was not detected in the pCMV-IFN-α1-treated cornea (Fig. 5). At a later timepoint during acute infection (i.e., day 6 PI), topical administration with the IFN-α1 construct reduced the expression of all viral transcripts tested in both the eyes and TG (ICP27, TK, and VP16) (Fig. 5). Because cytokines and chemokines are detected in the eyes and TG during an acute, ocular HSV-1 infection (2–4, 20, 23) and the antigenic stimulus

Table I. IFN-α1 transgene protects mice from ocular HSV-1 infection in a dose-dependent fashion

<table>
<thead>
<tr>
<th>Days Post infection</th>
<th>Vehicle (PBS)</th>
<th>Treatment (IFN-α1)</th>
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<tr>
<td></td>
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<td>5.0 μg/eye</td>
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* Mice (n = 3/group/experiment) were administered 3.0 μl of PBS or PBS containing the indicated amount of the IFN-α1 construct at 24 h prior to infection with HSV-1 (450 PFU/eye). Mice were monitored for survival.

** Numbers indicate surviving animals per total number tested. This table is a summary of three separate experiments. *, p < 0.05 comparing the experimental group with the vehicle-treated controls as determined by the Mann-Whitney U test.
IFN-α acute infection (i.e., 6 days PI), topical administration of the pCMV-IFN-α construct reduced viral load in the eyes and TG at 6 days PI. Mice were topically treated with 100 μg/eye of either pCMV-β or pCMV-IFN-α-1 and infected with HSV-1 after 24 h. The viral load in the eyes and TG was determined at 3 and 6 days PI as described previously (35). Results are reported as the mean ± SEM from five experiments; n = 3 mice/experiment (pCMV-β) and n = 3 mice/ experiment (pCMV-IFN-α-1). *p < 0.05, comparing the pCMV-IFN-α-1-treated group with the vector-treated control group.

To further ensure that the protective effect elicited by the transgene was due to IFN-α rather than to the induction of an immune response to the plasmid DNA (25–28), neutralizing Ab to IFN-α/β or control rabbit Ig was administered to mice undergoing transgene administration. Whereas the pCMV-IFN-α-1-treated mice administered control Ab showed an increased cumulative survival compared with the vehicle-treated group, pCMV-IFN-α-1-treated mice administered anti-IFN-α/β succumbed to infection at the same rate as the untreated group of mice (Fig. 6).

**Topical administration of the IFN-α construct suppresses HSV-1 reactivation**

To determine the effect of a topical administration of naked plasmid DNA on the establishment of HSV-1 latency and reactivation, TG explant cocultures were established using HSV-1-infected mice that had survived into viral latency (i.e., day 30 PI). The results show that the TG from mice that had been administered the IFN-α construct either at 72 h before infection or at 24 h PI reactivated to a level similar to the vector control (Fig. 7). However, none of the TG from mice treated with the IFN-α construct at 24 h before infection reactivated (Fig. 7). LAT expression was weakly detected in the TG from nonreactivated explant cultures obtained from the mice pretreated with pCMV-IFN-α-1 at 24 h before infection as determined by RT-PCR compared with samples from mice treated with the transgene PI or vector-treated mice (Fig. 7). These results suggest that the presence of the transgene may reduce the establishment of latent HSV-1 in the sensory ganglia.

**Discussion**

Type I IFNs (IFN-α and IFN-β) are known to be potent antiviral agents, blocking viral replication, transcription, and translation as well as augmenting antiviral immune components (29, 30). IFN-α has been used against established tumors and chronic viral infections (e.g., hepatitis B and C virus and AIDS-associated Kaposi’s sarcoma) (31, 32). Recent work has focused on a cost-effective, low-dose oral application of IFN-α to control melanoma (33) and viral infections (34–36). Similar to other viruses, HSV-1 has been found to be sensitive to the antiviral effects of IFN-α (37, 38), making this cytokine a likely candidate to pursue using additional therapeutic applications including gene therapy.

Plasmid DNA delivery is an important subclass of gene therapy that shows promise, as it is thought to be qualitatively safer than virus vectors due to the potential for less adverse effects (39). Because previous studies have identified murine IFN-α as a key component controlling the HSV-1 replication that is generated in
vivo following active immunization (40, 41), and because transgenic mice expressing IFN-α1 under the control of a glial fibrillary acidic protein (GFAP) (expressed primarily in astrocytes) promoter (GFAP-IFN-α1) are resistant to HSV-1 infection (42), a study was undertaken to investigate the potential efficacy of the IFN-α1 transgene in ocular HSV-1 infection using a pCMV delivery system. In the present study, the expression of the IFN-α1 transgene in the cornea is transient and short-lived in infected animals. However, the pCMV-IFN-α1 plasmid construct but not the vector plasmid protected mice from HSV-1-induced encephalitis in a dose- and time-dependent manner when applied to the cornea before infection. The protective effect is mediated through the expression of the transgene rather than through exposure to plasmid DNA, as indicated in experiments using neutralizing Ab to IFN-αβ that showed that IFN-α1-treated mice that were administered the neutralizing Ab but not control Ab succumbed to the infection similar to controls.

Ectopic expression of IFN-α1 in the eye was found to reduce viral replication, as evidenced by a reduction in the viral load and expression of viral genes during the course of acute infection. Consistent with these findings, in vitro studies have found that type I IFNs block HSV-1 immediate-early gene expression (43, 44) or elicit the production of defective, noninfectious HSV-1 particles from infected cells (45). Consequently, the data suggest that placement of the IFN-α1 transgene in the eye before infection prevented the replication and spread of the virus from the origin of infection (cornea) to the sensory ganglia and possibly the central nervous system. The measurement of viral loads in the cerebellum showed...
a reduction in HSV-1 (6 days PI), which supports this point. Because the immune response to acute infection results in significant pathology (46), a reduction in viral replication and spread would reduce the inflammatory response to the infection. In fact, the findings in this study showed a reduction in some chemokines (MCP-1 and CRG-2), cytokines (IL-6 and IL-10), and immune cell (CD8) transcripts in the eye and TG of mice treated with pCMV-IFN-α as evidenced on day 6 PI. Although there was no apparent difference in the expression of IFN-γ in the TG when vector-treated mice and pCMV-IFN-α-treated mice were compared at 6 days PI, IL-10 levels were reduced in the pCMV-IFN-α-treated mice. Recently, IL-10 has been reported to reduce HSV-1 elicited IFN-α class I in the eye and presumably with increased Ag presentation.

The application of cytokine gene therapy in controlling viral replication is illustrated in the present report. The present study showed that preexposing mice to the IFN-α1 transgene construct become resistant to HSV-1 infection may reside with the enhanced expression of MHC class I in the eye and presumably with increased Ag presentation. In addition to protection against acute HSV-1 infection, topical administration of the IFN-α1 construct at 24 h before infection reduced the establishment of latency as evidenced by LAT expression and the lack of viral reactivation in TG explant cultures. The weak detection of LAT in TG from the mice treated with the pCMV-IFN-α1 at 24 h before infection compared with TG from mice treated with the pCMV vector or treated with pCMV at 72 h before infection suggests that either fewer neurons/ganglia were infected in the TG of mice pretreated (24 h) with the transgene or, alternatively, fewer HSV-1 genome copy numbers/neuron were infected in the TG of mice treated with the pCMV vector before infection. Administration of the transgene before infection followed scarification of the cornea to facilitate the growth of new tissue and uptake of plasmid DNA. Although this approach has little practical value in treating patients presenting with herpetic stromal keratitis, it is possible that a greater degree of protection in HSV-1-infected mice may be realized by increasing the frequency of administration of the IFN-α1 transgene after infection. The present study only treated mice with a single application before or after infection. Furthermore, facilitating the uptake of plasmid DNA by alternative means may also increase the efficacy of the transgene through a greater degree of incorporation into the target tissue.

Collectively, the present study serves to illustrate that naked DNA plasmid construct technology may provide an alternative therapeutic option in treating localized infections in an attempt to influence the immune response and promote a favorable outcome in the host. Moreover, the transient nature of transgene expression suggests that the introduction of foreign DNA into selective target tissue may be short-lived depending upon the infectious disease and the time of application. One advantage to decreased expression of the transgene is that an immune response to the product is potentially reduced. Recent findings suggest that multiple exposures to plasmid DNA containing transgenes encoding chemokines generate a humoral immune response to the transgene product (54). Finally, the application of cytokine gene therapy may prove to be equally or better suited for other microbial pathogens, sites of infection, or the control of an unwarranted inflammatory response (55).

References


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