Antiviral Activities of Individual Murine IFN-α Subtypes In Vivo: Intramuscular Injection of IFN Expression Constructs Reduces Cytomegalovirus Replication

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J Immunol 1998; 160:2932-2939; http://www.jimmunol.org/content/160/6/2932
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The IFN-α cytokines belong to a multigene family. However, the in vivo biological functions of each of the IFN-α subtypes is unknown. Recently, we developed an experimental model in which the tibialis anterior muscles of mice were transsected in situ with naked DNA plasmids encoding an IFN transgene. Here we use this model to investigate the in vivo effect of the expression of three murine IFN-α subtypes (A1, A4, and A9) on murine CMV replication in C57BL/6, BALB/c, and A/J mice. CMV was shown to replicate in the tibialis anterior muscles of mice for at least 6 days and induced an inflammatory infiltrate. However, mice expressing the IFN-α transgenes showed a marked reduction in the peak titers of virus replication, with less severe inflammation in the muscles compared with control mice that were inoculated with blank vectors. Moreover, mice expressing the IFN-α1 transgene had significantly lower CMV titers in the inoculated muscle than mice expressing either the IFN-α4 or the IFN-α9 transgenes. Furthermore, IFN-α/β receptor knockout mice had markedly higher levels of CMV replication in the tibialis anterior muscles than the wild-type parental strain (129/Sv/Ev) following IFN-α1 transgene inoculation, suggesting that the protection observed is due to host cell-mediated IFN signaling. These data provide the first evidence indicating that there are in vivo differences in the antiviral efficacy of the IFN-α subtypes. The Journal of Immunology, 1998, 160: 2932–2939.

The type 1 IFNs belong to a multigene family that includes multiple IFN-α subtypes, a single IFN-β, IFN-γ, and IFN-ω (1). Humans express >15 IFN-α (IFNA) subtypes, a single β (IFNB), and one ω (IFNW) located together on chromosome 9 (2). Similarly in the mouse, there are >10 IFN-α subtypes and one IFN-β subtype (3, 4), all of which are located on chromosome 4 (5). Collectively, these IFNs have diverse biologic functions, including antiviral (6, 7), antiproliferative (8), and immunomodulatory activities (9–11) mediated via signal transduction pathways (12). Although evidence from in vitro studies suggests differences in the biologic effects of the subtypes (13–16), the in vivo significance of these findings remains obscure. Some researchers have hypothesized that the multiple IFN-α subtypes are a series of evolutionary duplications of an original gene. It may therefore be the case that these subtypes have an equivalent function in vivo. Alternatively, it has been hypothesized that each subtype may have evolved specific functions (17). Increased knowledge of the activities of the individual subtypes in vivo will enhance utilization of such subtypes for improved clinical efficacy over the current usage of either a mixture of IFN subtypes or a single rIFN subtype.

One approach to study the functions of individual IFN subtypes in vivo was made using transgenic mice (18–21). Transgenic mice were created with either murine IFNA1 or IFNB under the control of the metallothionein I promoter and unexpectedly expressed the gene only in the testes (IFN-α and -β) and liver (IFN-β) upon induction with cadmium. IFN was only detected in the sera of the IFN-β transgenic animals. However, both transgenic lines showed inhibition of spermatogenesis, resulting in sterility of male mice. No further experimental work with these animals has been reported. We have also unsuccessfully attempted to produce transgenic mice expressing the murine IFN-α1 and IFN-α4 genes under the control of a mutated (non-leaky) metallothionein IIA promoter (W.-S. Yeow and M. W. Beilharz, Department of Microbiology, University of Western Australia, Perth, Australia, unpublished observations). It is likely that even low levels of expression of the IFN transgene in the developing embryo are conditionally lethal.

The advent of naked DNA transfer technology provided an alternative approach to the manipulation of gene expression in vivo (22, 23). The persistence of the plasmid allows ongoing expression of the transgene, and this finding has been pursued for gene therapy (24). Immunization with plasmids expressing viral genes has induced protective immune responses, and these plasmids are being developed for improved vaccines (25, 26). Recently, we have developed a mouse experimental model in which the tibialis anterior (TA)3 muscles were transfected in situ with naked DNA plasmids encoding an IFN transgene (27). In this model, a mammalian expression vector carrying MulIFNA9 was injected into the mouse skeletal TA muscle. Dot-blot analysis showed that the IFNA9 transcript was present in the DNA-inoculated muscle. Biologic IFN activities were found in both muscle homogenates and sera of inoculated animals for up to 2 mo post-DNA injection. This model allows for the first time investigations into the comparative

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Received for publication August 25, 1997. Accepted for publication November 25, 1997.

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1 This work was supported by the Australian National Health and Medical Research Council (Grant 961302).

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3 Abbreviations used in this paper: TA, tibialis anterior; MCMV, murine cytomegalovirus; IFNA1, the alpha chain of the interferon-ω receptor; B6, C57BL/6; pfu, plaque-forming unit; MORS, mouse osmolarity-buffered saline; IE, immediate early; p.i., postinfection; HCMV, human cytomegalovirus.
biologic efficacies of individual IFN-α subtypes. With this experimental model, we have chosen in the present study to compare the protective levels of a panel of three mouse IFN-α subtypes (namely, MuIFN-α1, -α4, and -α9) expressed in mice challenged with murine CMV (MCMV).

Type I IFNs play an important role in early defense mechanisms against MCMV infection. The administration of antiserum specific for type I IFN increased the susceptibility of adult mice to MCMV (28). The mouse genotype (H-2 and non-H-2 loci) determines the level of resistance to MCMV infection, with C57BL/6 (H-2b) mice being two- to fourfold more resistant to lethal challenge of virus, and A/J (H-2a) mice being twofold more susceptible to virus infection compared with BALB/c (H-2d) mice (29, 30).

In this paper, we report the in vivo effects of three individual subtypes of IFN-α on MCMV replication in skeletal muscle. Three murine IFN-α genes (A1, A4, and A9) were individually subcloned into a mammalian expression vector and were used to inoculate the TA muscles of C57BL/6, BALB/c, and A/J mice, which were subsequently challenged with MCMV i.m. (TA). Expression of MuIFNA1, -A4, and -A9 reduced MCMV replication in vivo to different extents in each of the mouse strains. The greatest antiviral effects were observed for MuIFNA1. The effects of IFN were presumably mediated by host cell signaling events, since IFN-α/β receptor (IFNAR1) knockout mice could not use the plasmid-expressed IFNs for protection against virus infection, in contrast to the parental wild-type mouse strain. Together, these findings provide supporting evidence for the hypothesis that individual IFN subtypes may have differential biologic functions in vivo.

Materials and Methods

Mice

Specific pathogen-free female C57BL/6 (B6), A/J, and BALB/c female mice were purchased from Animal Resources Centre (Murdock, Western Australia) and housed under minimal disease conditions. All mice used were between 6 and 8 wk of age. Specific pathogen-free male IFN-α/β receptor (IFNAR1) knockout mice (derived from M. Aguet, University of Zurich, Zurich, Switzerland) and the parental wild-type strain (129/Sv/Ev) were obtained from Drs. Alistair Ramsay and Malcolm Lawson (University of Western Australia).

Viruses

The K181 strain of MCMV was prepared as a salivary gland homogenate from infected 3-wk-old female BALB/c mice and stored in the gas phase under identical conditions. All mice used were between 6 and 8 wk of age. Specific pathogen-free female C57BL/6, BALB/c, and A/J mice, which were subsequently challenged with MCMV i.m. (TA). Expression of MuIFNA1, -A4, and -A9 reduced MCMV replication in vivo to different extents in each of the mouse strains. The greatest antiviral effects were observed for MuIFNA1. The effects of IFN were presumably mediated by host cell signaling events, since IFN-α/β receptor (IFNAR1) knockout mice could not use the plasmid-expressed IFNs for protection against virus infection, in contrast to the parental wild-type mouse strain. Together, these findings provide supporting evidence for the hypothesis that individual IFN subtypes may have differential biologic functions in vivo.

Plasmid constructs

The mammalian expression vector, pCMVInt.blank (Fig. 1A), was donated by Dr. James Mullins (31) (Stanford University School of Medicine, Stanford, CA). Large-scale plasmid preparations were obtained from terrific broth cultures of transformed Escherichia coli (XL1-Blue) using standard DNA extraction procedures with LiCl precipitation. DNA integrity was checked by agarose gel electrophoresis, and concentrations were determined by spectrophotometric analysis. Yields of 10 to 15 mg of plasmid DNA were routinely obtained. All DNA construct preparations were performed under identical conditions.

Muscle regeneration

To induce muscle regeneration by crush injury (27), mice were anesthetized with 2.5% avertin, fur was removed with depilatory cream from the hind legs, and TA muscles were exposed by surgical incision of the skin. The TA muscles were partially sheared away from the underlying bones with forceps and crushed in three equally spaced locations along the muscles for 3 s using artery forceps. The incisions were saturated, and the mice allowed to recover for 5 days before inoculation with the DNA constructs.

In separate experiments, muscle regeneration was induced by bupivacaine. Anesthetized mice were injected bilaterally in the TA muscles with 25 μl of 0.5% bupivacaine 5 days before inoculation of the DNA constructs.

Mouse injection of DNA constructs

Anesthetized (2.5% avertin) mice were inoculated with 200 μg of DNA plasmids in a 25-μl volume of saline bilaterally in the TA muscles by inserting the needle longitudinally into the muscle. Similarly, control groups of mice were given the blank expression vectors. The expression ability of these plasmids in vivo was confirmed using a similar vector that contained the lacZ gene cassette driven by the CMV IE-1 enhancer/promoter and intron A (pVR-1412, VICAL), and positive staining for β-galactosidase was found in the DNA-injected TA muscle (9 days post-DNA; data not shown).

Dot-blot analysis

Dot-blot analysis was performed to determine the presence of plasmid in the transfected muscles. The human CMV IE-1 enhancer/promoter probe (564 bp) was obtained as a Spe/I-SacII fragment from pCMVInt.blank (nucleotides 272–925), purified from agarose with Geneclean (BIO 101, Inc., La Jolla, CA), and labeled with [α-32P]CTP using random primers (Megaprime DNA labeling kit, Amersham International, Aylesbury, U.K.). The sequence of the probe has no homology with viral sequences of MCMV (GenBank U68299). DNA extracts were prepared from TA muscles of mice on day 7 post-DNA injection and dot-blotted onto Hybond-N+ membranes (Amersham International). Hybridization to the labeled probe was performed at 60°C for 5 h (6 × SSC/7% SDS and 100 μg/ml salmon sperm DNA).

IFN bioassay

The TA muscles were collected from mice at 7 days post-DNA inoculation and treated at pH 2 to remove acid-labile IFNs, and IFN content was titrated in an in vitro bioassay using encephalomyocarditis virus-infected L929 cells as previously described (27). The standard murine IFN-α/β (Lee Biomolecular, San Diego, CA; 1000 IU/ml) was also titrated in the bioassay as a control.

Histology

Muscles were removed, fixed in 10% formalin-buffered saline, and paraffin embedded. Sections (5 μm) were stained by hematoxylin and eosin and observed microscopically for evidence of pathology. Sections were also stained by the immunoperoxidase method using hyperimmune sera from MCMV-infected mice to reveal virus-infected cells (32). Whole TA muscles from mice injected with the plasmid containing the lacZ gene cassette were fixed with 4% paraformaldehyde and stained for the presence of β-galactosidase using X-gal, frozen in liquid nitrogen, and then sectioned and counterstained with eosin as previously described (33).

Results

MCMV replication in the TA muscles

To establish the kinetics of MCMV replication in a skeletal muscle compartment, we inoculated mice with MCMV bilaterally in the TA muscles. B6 mice were given a sublethal dose of MCMV (4 × 106 pfu/mouse), and the amount of virus present in the muscles at 4 h and 1, 2, 3, 4, and 6 days postinfection (p.i.) was titrated by
plaque assay (three mice per time point; Fig. 2). Within 24 h of MCMV inoculation, the virus titers in the muscles had decreased significantly \((p < 0.05)\) before increasing steadily with time. Virus replication peaked on day 3 and was almost cleared by day 6 p.i. The kinetics of MCMV replication in the muscles of a more susceptible mouse strain (A/J) were also investigated following inoculation of the same dose of MCMV as that used to infect B6 mice. There was also a significant decrease \((p < 0.005)\) in the MCMV titer in A/J muscles within 24 h of infection followed by an increase in the MCMV titer (Fig. 2), such that by day 3 the titer was approximately fourfold higher than that obtained from B6 muscles. Thus, MCMV replicated to markedly higher titers in the susceptible A/J mice compared with those in the resistant B6 mice, and no evidence of clearance was seen by day 4 p.i. Immunoperoxidase staining of TA muscle sections from the above MCMV-infected mice revealed further evidence of virus-infected cells (data not shown), providing confirmation of virus replication in the skeletal muscle tissue.

**MuIFNA9 expression reduces MCMV replication in vivo**

Previously, we have described the construction, delivery, and expression of IFN-\(\alpha\)9 in our mouse model (27). Preliminary experiments were conducted to assess the antiviral activity of **MuIFNA9** in B6 mice following MCMV infection in vivo. B6 mice (five mice per group) were injected with 200 \(\mu\)g of plasmid constructs carrying the full-length gene of **MuIFNA9** driven by the human cytomegalovirus (HCMV) IE enhancer/promoter (pkCMVint.IFNA9; Fig. 1B). Control groups of five mice received either no DNA or the blank expression...
We next wished to compare individual murine IFN-α subtypes for their relative antiviral activities in vivo using our mouse model of MCMV i.m. infection. Mammalian expression plasmids encoding the genes for murine IFN-α4 and IFN-α1 were constructed (see Materials and Methods; Fig. 1, C and D). Three mouse strains (B6, A/J, and BALB/c, eight mice per group) were injected with 200 μg of plasmid constructs carrying the full-length gene of MuIFNA1, MuIFNA4, or MuIFNA9 driven by the HCMV IE enhancer/promoter (pCMVint.IFNA1, pCMVint.IFNA4, and pCMVint.IFNA9, respectively). Control groups of mice received the blank expression vector, pCMVint.blank (Fig. 1A). The regenerating TA muscles were induced by crush injury 5 days before DNA injection. Seven days post-gene transfer, MCMV (4 × 10⁴ pfu/mouse) was injected bilaterally into the TA muscles. These muscles were sampled on both day 3 and day 4 p.i., around the time of peak virus replication in B6 mice (see Fig. 2), and the levels of MCMV present in the muscle homogenates were determined by plaque assay.

In control mice, in which no DNA was injected into the crush-injured muscles, MCMV titers obtained on both day 3 and day 4 p.i. were similar to the MCMV titers determined earlier for the kinetics experiment (Fig. 2). Since there was a small degree of protection observed in mice immunized with the blank vector compared with control mice that were not injected with plasmid DNA, all comparisons of plasmid constructs were performed with mice receiving the blank expression vector as the baseline. On day 3 p.i., the time when peak MCMV titers were found, the mean level of MCMV titer determined in muscles expressing the MuIFNA9 transgene (3.58 × 10³ pfu/ml of TA ± 8.78 × 10²) was significantly lower (p ≤ 0.04) than that determined in muscles injected with the blank vector (6.43 × 10³ pfu/ml of TA ± 1.00 × 10³). Furthermore, on day 4 p.i., virus titers were significantly lower in mice inoculated with pCMVint.IFNA9 (2.14 × 10³ pfu/ml of TA ± 6.24 × 10²; p ≤ 0.02) than in mice inoculated with blank vector (7.95 × 10³ pfu/ml of TA ± 1.95 × 10³).

Antiviral activities of MuIFNA1, MuIFNA4, and MuIFNA9 in vivo

We next wished to compare individual murine IFN-α subtypes for their relative antiviral activities in vivo using our mouse model of MCMV i.m. infection. Mammalian expression plasmids encoding the genes for murine IFN-α4 and IFN-α1 were constructed (see Materials and Methods; Fig. 1, C and D). Three mouse strains (B6, A/J, and BALB/c, eight mice per group) were injected with 200 μg of plasmid constructs carrying the full-length gene of MuIFNA1, MuIFNA4, or MuIFNA9 driven by the HCMV IE enhancer/promoter (pCMVint.IFNA1, pCMVint.IFNA4, and pCMVint.IFNA9, respectively). Control groups of mice received the blank expression vector, pCMVint.blank (Fig. 1A). The regenerating TA muscles were induced by bupivacaine inoculation 5 days before DNA injection. This alternative method to crush injury for the induction of muscle regeneration was used in all additional experiments. Seven days after DNA injection, half of the TA pools from three mice (of the eight) were collected and used for the determination of plasmid in the transfected muscle by dot-blot analysis and for the estimation of IFN protein titers by bioassay. All the plasmids (pCMVint.blank, pCMVint.IFNA1, pCMVint.IFNA4, and pCMVint.IFNA9) from muscle extracts of DNA-injected A/J mice showed similar hybridization signal intensities (the sensitivity limit was 50 pg detected in the 1/10 dilution of DNA samples; data not shown), suggesting that the plasmids were maintained at comparable levels in the muscles. DNA extracts were hybridized to the human CMV IE promoter/enhancer-labeled probe (657 bp) of the pCMVint vecto r. A further DNA sample of murine salivary gland-derived MCMV was included in the dot blot and resulted in no specific hybridization with the human CMV IE probe. Production of IFN proteins in the TA muscle from B6 mice injected with the different IFN constructs was evidenced by bioassay of muscle homogenates; results ranged from 24 to 150 IU/TA (24, IFNA9; 75, IFNA4; 150, IFNA1). Whereas B6 mice injected with the blank vector did not have detectable levels of IFN titer (≤1.7 IU/TA).

The remaining five mice (of the eight) were challenged with virus 7 days after DNA injection. MCMV was injected bilaterally into the TA muscles of the mice. B6 mice received 4 × 10⁴ pfu/mouse, while the more susceptible strains (A/J and BALB/c) received a lower virus dose (2 × 10⁴ pfu/mouse). The levels of

FIGURE 2. Kinetics of MCMV replication in the TA muscles of C57BL/6 and A/J mice. MCMV (4 × 10⁴ pfu/mouse) was injected bilaterally into the TA muscles of anesthetized mice. At specific times p.i., the muscles were collected and homogenized, and the amount of MCMV present in the individual mouse muscle homogenates (three mice per group) was titrated by plaque assay. The mean virus titer (plaque-forming units per milliliter of TA muscle ± SEM) is shown.

FIGURE 3. The antiviral activity of the MuIFNA1 subtype is greater than those of the MuIFNA4 and MuIFNA9 subtypes at reducing MCMV replication in the TA muscles of C57BL/6 and A/J mice. Plasmid DNA constructs (200 μg in MOBS) were injected bilaterally into regenerating TA muscles of adult mice 5 days after bupivacaine treatment. Seven days following DNA injections, the mice were challenged with MCMV. The challenge doses (4 × 10⁴ pfu/mouse in C57BL/6 mice and 2 × 10⁴ pfu/mouse in A/J mice) were injected bilaterally into the TA muscles (five mice per group). MCMV titers were determined on day 3 p.i. by plaque assay of the individual mouse muscle homogenates and are expressed as mean plaque-forming units per milliliter of TA muscle ± SEM. The mean reduction in virus titer in mice injected with plasmid constructs encoding IFN transgenes was compared with that in mice injected with the blank vector. The p values describe the significant reduction of MCMV titers by the IFN transgene.
MCMV present on day 3 p.i. in the muscle homogenates of all the mouse strains investigated were determined by plaque assay.

The bupivacaine-injured muscles of B6 mice that were transfected with an IFN transgene (A1, A4, or A9) had significantly reduced (p < 0.0001) MCMV titers compared with the muscles of control B6 mice given the blank vector (Fig. 3). Histologically, this is supported by reduced inflammation of the muscle from MuIFNA1. C, Perivascular infiltration is present in muscle from MCMV-infected B6 mice injected with pkCMVint. IFNA1. D, A mild perivascular infiltration is present in muscle from MCMV-infected B6 mice injected with pkCMVint IFNA4. E, Muscle from MCMV-infected BALB/c mice injected with pkCMVint. blank plasmid, showing marked necrosis. F, Muscle from MCMV-infected BALB/c mice injected with pkCMVint. blank plasmid, showing marked necrosis and evidence of calcified lesions associated with cellular infiltration. A, Severe and diffuse cellular infiltration associated with necrotic myofibers is present in muscle from MCMV-infected B6 mice injected with pCMVint. IFNA1. B, A mild focal infiltrate is present in muscle from MCMV-infected B6 mice injected with pCMVint. IFNA4. F, Muscle from MCMV-infected BALB/c mice injected with pkCMVint. blank plasmid, showing marked necrosis.

Our experimental mouse model of IFN transgene expression in vivo has allowed us to compare the antiviral activities of individual IFN-α subtypes (A1, A4, and A9). Each of the three IFN-α subtypes investigated could independently reduce MCMV replication in the TA muscle compartment, where both the transgene and virus were administered. The primary observation from this study suggests, for the first time, that there are differences in the biologic...
mice were challenged with MCMV (2 days after bupivacaine treatment. Fourteen days following DNA injections, the mice were injected bilaterally into regenerating TA muscles of adult mice 5 days after bupivacaine treatment. Fourteen days following DNA injections, the mice were challenged with MCMV (2 × 10^4 pfu/mouse) bilaterally into the TA muscles (five mice per group). MCMV titers were determined on day 3 p.i. by plaque assay of the individual mouse muscle homogenates. Data are expressed as mean pfu/ml TA muscle ± SEM. The mean reduction in virus titer in mice injected with plasmid constructs encoding IFN transgenes was compared with that in mice injected with the blank vector. The p values show the significant reduction of MCMV titers by the IFN transgenes.

The finding of this study, that the MuIFN-α1 subtype appeared to be more protective than the MuIFN-α4 or the MuIFN-α9 subtype in vivo, was unexpected. Bioassay of the muscle homogenates taken on day 7 after plasmid inoculation for type I IFN titers varied from 24 to 150 IU in C57Bl/6 mice. However, it must be emphasized that this read-out of titers reflects a wide array of in vivo antiviral activities have been determined from in vitro experiments using MuIFN-α1, -α4, and -α9 (15, 16) (S. J. Boyer and M. W. Beilharz, unpublished observations; B. Williams, unpublished observation). With the advent of naked DNA technology, we were able to successfully develop an in vivo model in which the expression of a specific IFN subtype predominates (27).

The inefficacies of the IFN-α subtypes in vivo. Indeed, the MCMV titers in TA muscles of B6, A1, and BALB/c mice transected with the MuIFNA1 transgene were consistently lower than the MCMV titers in TA muscles transected with the MuIFNA4 or MuIFNA9 transgene. Furthermore, MuIFNA1-transected mice had lower MCMV titers in liver and spleen, indicating partial protection from virus dissemination to these organs following i.m. virus challenge. Thus, mice allowed to express the MuIFNA1 transgene expressed intramuscularly are receptor-mediated.

In some cases partial protection was afforded by the blank vector via a non-IFN-specific mechanism. This has also been noted in other models of DNA vaccines including studies of influenza virus (C. M. Lawson and S. L. Epstein, National Institute of Allergy and Infectious Diseases and Food and Drug Administration, National Institutes of Health, unpublished observations). DNA sequences in the backbone of the plasmid containing cytosine guanine (CG) dinucleotides in a particular base context may have immunostimulatory effects on the immune system (34). Since we used the data obtained from the control group of mice injected with the blank vector as the baseline for comparison with other groups of mice injected with the vector constructs containing the IFN insert genes, we may be underestimating the degree of protection.

In considering the potential in vivo interactions of several genes and their effects on IFN transcription, we observed no hybridization of MCMV genes with the human CMV IE promoter/enhancer. Therefore, we believe that trans-activation of IFN expression through the virus would be unlikely and would be expected to occur equally with each of the individual plasmid constructs, since they have identical promoters. It is known that a rIFN (human/mouse hybrid) can turn off transcription of mouse CMV IE genes due to the negative regulation of IE enhancer sequences (35). However, since IFN does not appear to alter the mRNA levels of human CMV IE genes (36), putative IFN-α-responsive elements might be missing in human CMV IE genes. Our studies suggest that there is unlikely to be a strong negative regulation of murine IFN on the HCMV IE genes resulting in the termination of IFN transgene expression, since newly synthesized IFN from the plasmid does not turn off detectable plasmid IFN production in the TA, as assessed kinetically over a 2-mo period (27). Furthermore, the regulatory effect of murine IFN subtypes (endogenous and plasmid coded) on the human CMV IE promoter (if any) would be expected to occur equally with each of the individual plasmid constructs.

Many in vitro studies have shown differences in the biologic activities with either human IFN-α subtypes (37–41) or mouse IFN-α subtypes (15, 16). However, it has not been possible until now to conduct comparative studies of the different IFN-α subtypes in vivo. Early attempts to develop an in vivo model for such studies of the biologic activity of type 1 IFN subtypes have involved making transgenic mice. The failure of these mice to reproduce and/or to survive embryologic life makes this an inefficient approach (18–20) (W.-S. Yeow and M. W. Beilharz, unpublished observations; B. Williams, unpublished observation). With the advent of naked DNA technology, we were able to successfully develop an in vivo model in which the expression of a specific IFN subtype predominates (27).

Table I. Antiviral activities of the IFN-α transgene expressed intramuscularly are receptor-mediated

<table>
<thead>
<tr>
<th>Organ</th>
<th>IFNAR1 knockout</th>
<th>Wild-type (129/Sv/Ev)</th>
<th>Reduction</th>
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<tbody>
<tr>
<td>TA muscle</td>
<td>1.04 × 10^4 ± 1.07 × 10^4</td>
<td>5.38 × 10^3 ± 8.35 × 10^2</td>
<td>19.4 (p ≤ 0.0001)</td>
</tr>
<tr>
<td>Liver</td>
<td>2.04 × 10^3 ± 6.69 × 10^2</td>
<td>2.50 × 10^2 ± 0.00</td>
<td>8.2 (p ≤ 0.04)</td>
</tr>
<tr>
<td>Spleen</td>
<td>7.74 × 10^4 ± 1.01 × 10^4</td>
<td>2.92 × 10^2 ± 4.17 × 10^1</td>
<td>265.3 (p ≤ 0.0007)</td>
</tr>
</tbody>
</table>

*pCMVint.INS (200 μg in MOBS) was injected bilaterally into regenerating TA muscles of adult mice 5 days after bupivacaine treatment.

Seven days following DNA injections, mice were challenged with 2 × 10^4 pfu of MCMV injected bilaterally into TA muscles (six mice per group). MCMV titers were determined at day 3 p.i. by plaque assay of the individual mouse muscle homogenates. Data are expressed as mean pfu/ml organ ± SEM.
In our in vivo study, mice depleted of functional IFN-α/β receptors (knockout mice) were shown to be poor responders to the protective effects of MuIFN-α compared with mice with such receptors. We expected the parental wild-type strain (129/Sv/Ev), which has a similar susceptibility level to MCMV infection as A/J mice, to display virus titers in the same order of magnitude as A/J mice (9 × 10^4 ± 1 × 10^5 pfu/ml of TA). Thus, the titers obtained from 129/Sv/Ev mice injected with the IFN-α/α plasmid demonstrate a strong protective effect. On the other hand, the level of MCMV replication in the knockout mice was significantly higher than that in all other mouse strains tested with IFN-α/β receptors. These data clearly establish that the protective effect of IFN is receptor mediated.

Overall, our study demonstrates for the first time that there are in vivo differences in the relative biologic efficacies of the different MuIFN-α subtypes during a virus infection. Although many more questions have now been raised by these data, the mouse model we described should be capable of allowing experimental resolution of these issues. We are currently investigating whether the same MuIFN subtype (α1) can induce a better protective immune response in animals challenged with different viruses. Collectively, these studies will ultimately provide an improved rationale for effective clinical use of the IFNs.

Acknowledgments

We are grateful to VICAL for providing the pkCMVint expression vector, and to Dr. Alistair Ramsay for providing the pCMVIFNα/IFNα construct and the IFNAR1 knockout mice. We also thank the staff at PathCentre for histology services.

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