ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2011, p. 2067–2073 Vol. 55, No. 5
0066-4804/11/$12.00 doi:10.1128/AAC.01635-10

Treatment of Yellow Fever Virus with an Adenovirus-Vectored Interferon, DEF201, in a Hamster Model[▽]

Justin G. Julander,[1]* Jane Ennis,[2] Jeffrey Turner,[2] and John D. Morrey[1]

Institute for Antiviral Research, Utah State University, Logan, Utah,[1] and Defyrus Inc., 2 Bloor Street W, Suite 2602, Toronto, Ontario M4W 3E2, Canada[2]

Received 24 November 2010/Returned for modification 5 January 2011/Accepted 27 January 2011

Interferon (IFN) is an innate immune response protein that is involved in the antiviral response during viral infection. Treatment of acute viral infections with exogenous interferon may be effective but is generally not feasible for clinical use due to many factors, including cost, stability, and availability. To overcome these limitations, an adenovirus type 5-vectored consensus alpha IFN, termed DEF201, was constructed as a potential way to deliver sustained therapeutic levels of systemic IFN. To demonstrate the efficacy of DEF201 against acute flaviviral disease, various concentrations of the construct were administered as a single intranasal dose prior to virus infection, which resulted in a dose-responsive, protective effect in a hamster model of yellow fever virus (YFV) disease. A DEF201 dose of 5×10^7 PFU/animal administered intranasally just prior to YFV challenge protected 100% of the animals, while a 10-fold lower DEF201 dose exhibited lower, although significant, levels of protection. Virus titers in the liver and serum and levels of serum alanine aminotransferase were all significantly reduced as a result of DEF201 administration at all doses tested. No toxicity, as indicated by weight loss or gross morbidity, was observed in non-YFV-infected animals treated with DEF201. Protection of YFV-infected animals was observed when DEF201 was delivered as early as 7 days prior to virus challenge and as late as 2 days after virus challenge, demonstrating effective prophylaxis and therapy in a hamster model of disease. Overall, it appears that DEF201 is effective in the treatment of YFV in a hamster model.

Yellow fever virus (YFV) is a member of the flavivirus family endemic to tropical regions of Africa and South America, with reported imported cases outside this range (4). YFV causes a visceral disease, primarily targeting the liver and often involving hemorrhagic manifestations, which may result in lethality, with case fatality rates being up to 50% (33, 34). A highly effective vaccine is available, although it is underutilized in many countries with endemic YFV disease (25). No antivirals are approved for the treatment of YFV, and clinical management of disease involves only mitigation of symptoms.

YFV is susceptible to treatment with exogenous interferon (IFN) in cell culture (6, 7) and in animal models (3, 18, 24). Treatment with exogenous IFN has also been shown to be effective in other animal models of flaviviral disease, including disease caused by dengue virus (1), West Nile virus (26), St. Louis encephalitis virus (5), and Modoc virus (22), as well as in human disease cases (9, 14, 21). It has been shown that natural human alpha IFN (IFN-α) preparations are active in human and hamster cell cultures (13). There is also considerable evidence that consensus IFN-α (alfacon-1 IFN) is efficacious against herpesvirus infections in hamsters (12, 13).

IFN is a critical component of host antiviral response mechanisms. Some flaviviruses, such as YFV, evade the host innate immune response through various mechanisms, including activation of negative regulators, stimulation of suppressive cytokines (35), and blocking of interferon signal transduction (20, 23, 28). Therefore, timing and dose of IFN treatment are important. Generally, treatment with exogenous IFN is effective in animal models of flavivirus infection only when it is administered just prior to or shortly after virus challenge (18, 26, 27). In controlled trials with clinically diagnosed Japanese encephalitis virus infection, treatment with IFN alfa-2a was not effective in improving outcome (32). The majority of the patients in this study exhibited disease signs such as vomiting, convulsions, fever, and other symptoms at enrollment and prior to initiation of treatment. Efficacy of IFN treatment in animal models of viral disease is also associated with treatment initiation prior to the onset of disease signs.

IFN has a short half-life, which requires frequent (and expensive) treatment with bolus doses, which generally results in toxic side effects and often leads to patient-instigated cessation of treatment (11, 15). While IFN treatment has been shown to be effective against several flaviviruses *in vitro* and *in vivo*, the clinical use of IFN has been restricted during outbreaks of acute flavivirus infection because of high cost and need for repeated dosing. In contrast, an IFN drug which required only a single, inexpensive intranasal (i.n.) administration yet which produced a persistent IFN effect could have value as both a treatment and prophylactic in a yellow fever outbreak.

DEF201 is a replication-deficient adenovirus type 5 (Ad5) containing a gene for the production of human consensus IFN-α. After intranasal instillation of DEF201, the Ad5 vector infects (or enters) nasal epithelial cells, driving the expression of the IFN transgene. This transgene is then translated to produce IFN protein, which is secreted into the blood. The expression of mouse IFN using a similar construct resulted in

* Corresponding author. Mailing address: Institute for Antiviral Research, Utah State University, 5600 Old Main Hill, Logan, UT 84322-5600. Phone: (435) 797-7215. Fax: (435) 797-3959. E-mail: justin.julander@usu.edu.

▽ Published ahead of print on 7 February 2011.

systemic levels of protein several hours after infection (37). This article reports on the dose-dependent prophylactic and therapeutic efficacy of single-dose DEF201 in a hamster model of YFV disease.

MATERIALS AND METHODS

Animals. Female Syrian golden hamsters with an average weight of 100 g were used. After a 24-hour quarantine period and 7-day acclimation period, animals were randomly assigned to cages and individually marked with ear tags. All work with these animals was performed in the biosafety level 3 (BSL-3) area of the AAALAC-accredited Laboratory Animal Research Center (LARC) at Utah State University (USU). All animal study protocols were reviewed and approved by the Utah State University Institutional Animal Care and Use Committee.

Test articles. The adenovirus-vectored consensus interferon construct human DEF201 (hDEF201) was prepared at the Robert Fitzhenry Vector Laboratory (McMaster University, Hamilton, Ontario, Canada). The methods of preparation have been described previously (37). Briefly, the human consensus alpha interferon gene was cloned into a replication-deficient Ad5 vector (deletions of E1 and E3 genes), amplified in 293 cells, and purified by cesium chloride gradient centrifugation. Stock solutions of DEF201 were provided at 6×10^9 PFU/ml and stored at −80°C. Frozen stocks were thawed on ice and diluted in saline to the appropriate dose just prior to a single administration. Ribavirin was provided by ICN Pharmaceuticals, Inc. (Costa Mesa, CA) and was also prepared in saline. Ribavirin was prepared just prior to initial administration and was stored at 4°C.

Viruses. The hamster-adapted Jimenez strain of YFV was obtained as a generous gift from Robert B. Tesh (University of Texas Medical Branch, Galveston, TX) and prepared as previously described (18).

Experimental design for animal studies. Hamsters were randomly assigned to groups of 10 to 15 compound-treated and 10 to 20 placebo-treated animals. Toxicity controls, consisting of 3 animals per group, were included to measure any apparent toxicity associated with treatment. Vector control (sham-infected, vector-treated) and healthy control (uninfected, untreated) animals were also included. A concentration of 10^2 50% cell culture infectious doses ($CCID_{50}$s) of Jimenez YFV, which is an approximately 90% lethal dose (1 LD_{90}) of virus, was prepared in minimal essential medium. Hamsters were injected intraperitoneally (i.p.) with 0.1 ml of the YFV preparation. Animals were treated at various times prior to and after YFV challenge, depending on the study, with a single i.n. dose of DEF201 in a volume of 0.2 ml. Mortality was monitored twice daily, and weight was measured at 0, 3, 5, and 6 days postinoculation (dpi) with virus. Serum was taken at 6 dpi for quantification of serum alanine aminotransferase (ALT). Liver and serum virus titers were evaluated on 4 dpi. Ribavirin, prepared in saline at a dose of 50 mg/kg of body weight/day, was used as a positive-control compound, and the empty Ad5 vector was included as a negative control.

In the first experiment, a simple range-finding study was conducted to determine the effective prophylactic dose of DEF201 in hamsters infected with YFV. Mortality was monitored daily for 21 days, and weight was recorded on 0, 3, and 6 dpi. Liver tissue was taken at necropsy from 5 animals from each group for virus titration on 4 dpi. In follow-up studies, DEF201 and empty vector control were administered by i.n. instillation with a single dose of 3.6×10^7 PFU/animal at various times between −28 and 3 dpi. Disease parameters of mortality, weight change, and serum ALT level (on 6 dpi) were used to assess the efficacy of DEF201 in these experiments.

Serum alanine (ALT) assay. Serum was collected antemortem by ocular sinus collection from all of the animals in each group. ALT (serum glutamic pyruvic transaminase) reagent (Teco Diagnostics, Anaheim, CA) was used, and the protocol was altered for use in 96-well flat-bottomed microplates as previously described (17). The aminotransferase concentrations were determined according to the manufacturer's instructions.

Titration of yellow fever virus from livers. Vero cells were cultured in 96-well flat-bottomed microplates 1 day before use. Liver samples were homogenized in cell culture medium, and serial dilutions from 10^{-1} to 10^{-8} were added to microplates with semiconfluent Vero cells. Plates were incubated at 37°C for 9 days, after which the cells were observed microscopically for virus cytopathic effect (CPE). The observed titer in Vero cells, calculated by endpoint dilution (30), was adjusted on the basis of the weight of tissue prior to homogenization.

Statistical analysis. Survival data were analyzed using the Wilcoxon log-rank survival analysis, and all other statistical analyses were done using one-way analysis of variance using a Newman-Keuls multiple-comparison test (Prism, version 5; GraphPad Software, San Diego, CA).

RESULTS

DEF201 dose-ranging study. To determine the activity and effective dose of DEF201 in the hamster model of YFV, a single i.n. treatment of DEF201 was administered to hamsters 4 h prior to YFV challenge. Hamsters were treated with a single dose of DEF201 including 1×10^8, 5×10^7, 5×10^6, and 5×10^5 PFU/animal to titrate the effect of the DEF201 dose. The positive control, ribavirin, was included at a dose of 50 mg/kg/day given twice daily for 7 days.

Complete protection of hamsters was observed after a single i.n. treatment with the top two doses of 1×10^8 and 5×10^7 PFU of DEF201 (Fig. 1A). A dose response was seen, with some mortality occurring at lower doses. The lowest DEF201 doses of 5×10^6 and 5 $X10^5$ protected 90 and 70% of treated hamsters, respectively, which was a significant ($P < 0.001$) improvement compared with the results achieved with placebo. The mortality curve was also delayed in groups treated with these lower doses of DEF201 (Fig. 1A), with a mean day to death (MDD) of 9 ± 1.0 in the 5×10^5-PFU DEF201 group compared with a MDD of 7.5 ± 1.4 in placebo (empty vector)-treated animals. Ribavirin at a dose of 50 mg/kg/day also significantly ($P < 0.001$) improved survival of infected hamsters, with protection of 90% of treated animals, but required 14 injections over 7 days.

Weight gain was observed in animals treated with DEF201, which increased at between 0 and 5 dpi and had a slight decline or remained the same between 5 and 6 dpi, depending on the dose (Fig. 1B). A slightly significant increase ($P < 0.05$, compared with empty vector treatment) in weight was observed at DEF201 doses of 5×10^7 and 5×10^6, as well as in animals treated with ribavirin (Fig. 1B). The weight change at between 3 and 6 dpi was similar for all groups treated with DEF201 and did not appear to be dose responsive (data not shown).

ALT was measured in serum collected antemortem on 6 dpi from all animals surviving up to that time. The reduction in ALT levels appeared to be dose responsive in DEF201-treated animals, with the mean reduction in ALT level being highly significant ($P < 0.001$) compared with that achieved with empty vector treatment (Fig. 1C). ALT levels were uniformly reduced to the baseline in animals treated with the highest DEF201 dose and were similar to those in controls not infected with YFV (data not shown). Ribavirin treatment also significantly improved serum ALT levels ($P < 0.001$ compared with placebo).

Viremia and liver YFV titers were quantified by infectious cell culture assay of samples obtained at necropsy on 4 dpi. A significant reduction of YFV titers in both serum and liver was observed in animals treated with DEF201, as well as in ribavirin-treated animals (Fig. 1D). Treatment with the two highest doses of DEF201 reduced liver virus titers to below detectable limits of the assay, with increasing amounts of YFV in the liver being measured in samples obtained from animals treated with lower doses of DEF201 in a dose-dependent manner (Fig. 1D). Liver virus titers were similar in animals treated with the lowest dose of 5×10^5 of DEF201 and in ribavirin-treated animals, although a few animals in this DEF201 dose group had titers below the level of detection (Fig. 1D). Viremia showed a similar pattern, with reductions in the animals treated with the two top DEF201 doses to below the levels of detection and increas-

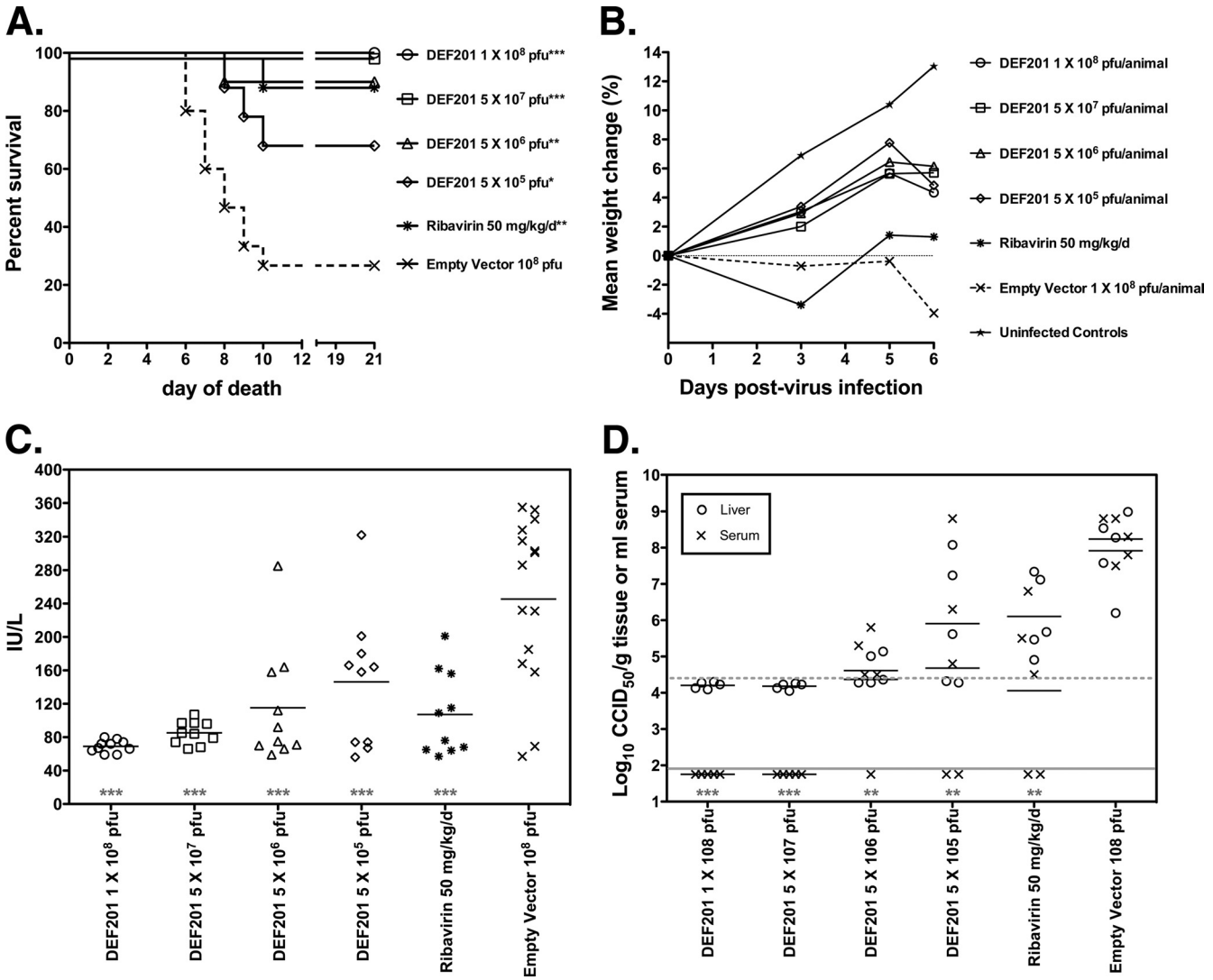

FIG. 1. Effect of a single i.n. treatment with various doses of DEF201 on disease of hamsters infected with YFV. Ribavirin was included as a positive control and was administered i.p. b.i.d. for 7 days beginning at −4 h dpi at a dose of 75 mg/kg/day. Disease parameters include survival (A), percent weight change (B), serum ALT level on 6 dpi (C), and virus titer (quantified as $CCID_{50}$s/g tissue or ml serum) in the liver and serum on 4 dpi (D). Dashed and solid gray lines in panel D, limits of detection of the liver and serum virus assays, respectively. ***, $P < 0.001$ compared with empty vector treatment; **, $P < 0.01$ compared with empty vector treatment; *, $P < 0.05$ compared with empty vector treatment.

ing numbers of animals with detectable viremia in the groups treated with lower doses of DEF201 and the ribavirin treatment group. Despite serum virus detection in some animals, all groups had a significant improvement in mean viremia compared with that for the placebo group (Fig. 1D).

Therapeutic treatment study. As YFV outbreaks occur randomly and unpredictably, it is important that effective antivirals have activity when they are administered after virus infection. To determine the therapeutic effect of DEF201, animals were treated at various times up to 4 dpi with a single i.n. DEF201 dose of 3.6×10^7 PFU/animal.

DEF201 treatment initiated at −4 h was effective, resulting in 100% protection (Fig. 2A), which confirmed the results of the initial study. Initiation of treatment on 1 or 2 dpi also resulted in protection (100% and 90%, respectively), while treatment on 3 dpi was not protective compared with empty vector treatment, despite a slightly higher overall survival rate and a slight delay in the mortality curve (Fig. 2A). Ribavirin, administered i.p. twice daily for 7 days at a dose of 50 mg/kg/day, also significantly ($P < 0.001$) improved survival of YFV-infected hamsters.

Despite an overall weight loss at between 5 and 6 dpi in all treatment groups, a significant overall improvement in weight change at between 3 and 6 dpi was observed in animals treated beginning at −4 h ($P < 0.05$) and 1 and 2 dpi ($P < 0.001$) (Fig. 2B). A trend toward improvement in mean weight change was observed in animals treated with DEF201 on 3 dpi compared to that for animals in the empty vector treatment group, although this improvement was not significant. Ribavirin also significantly improved weight change between 3 and 6 dpi ($P < 0.05$), although the overall percent weight change was similar to that of animals treated on 3 dpi with DEF201 (Fig. 2B). All

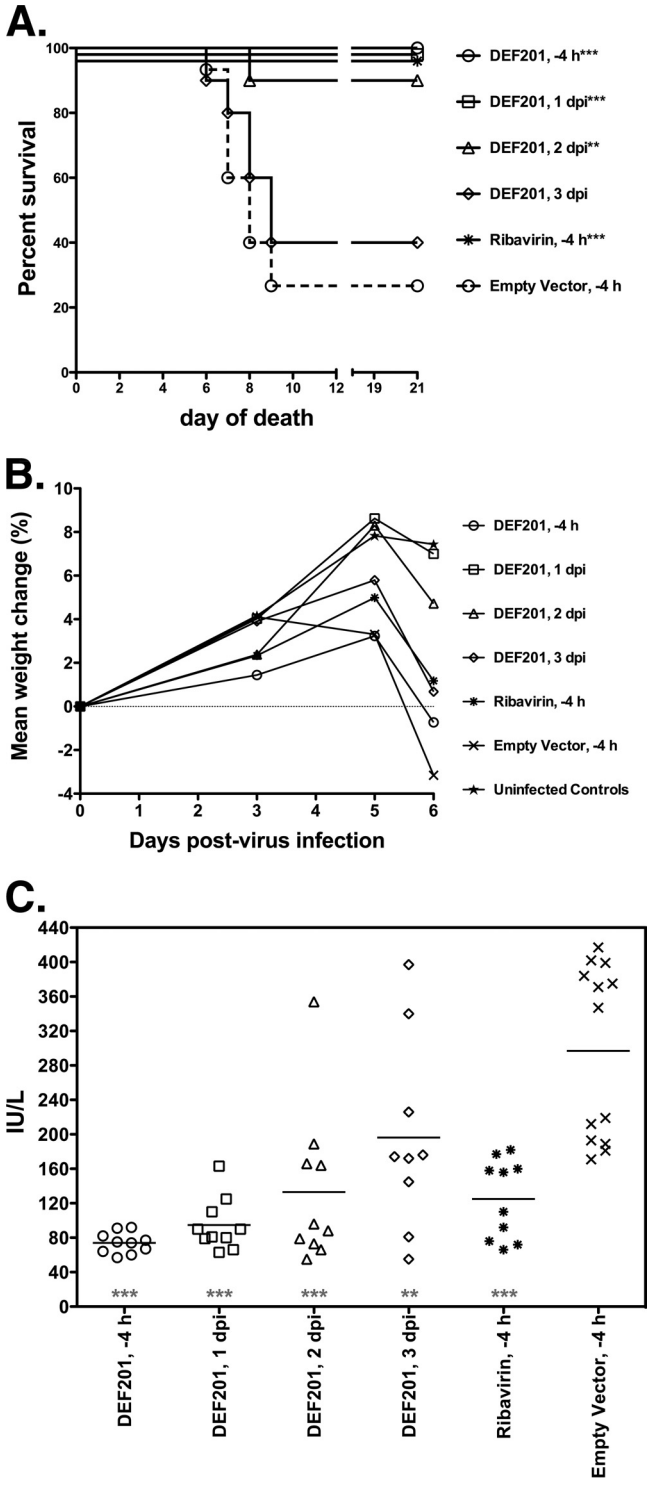

FIG. 2. Extended prophylactic efficacy of DEF201 at a dose of 3.6×10^7 PFU/animal administered at various times after virus challenge. Animals were treated with DEF201 at −4 h, 1, 2, or 3 dpi. Ribavirin was included as a positive control and was administered i.p. b.i.d. for 7 days beginning at −4 h dpi at a dose of 75 mg/kg/day. Disease parameters include survival (A), percent weight change (B), and serum ALT level on 6 dpi (C). ***, $P < 0.001$ compared with empty vector treatment; **, $P < 0.01$ compared with empty vector treatment.

treated animals gained weight at between 3 and 5 dpi, while empty vector control-treated animals lost weight during this time. All groups lost weight at between 5 and 6 days, regardless of treatment initiation time, although the degree was more severe in the group treated with empty vector (Fig. 2B).

Reduction of serum ALT levels occurred in a time-dependent manner, with earlier treatment time resulting in a reduction to baseline levels and an increasing delay resulting in higher levels of ALT (Fig. 2C). Interestingly, despite a high mortality rate and nonsignificance in improvements observed with other disease parameters, treatment with DEF201 on 3 dpi resulted in a significant ($P < 0.01$) reduction of the ALT level on 6 dpi (Fig. 2C). This indicates partial efficacy with treatment even at this later time point.

Prophylactic efficacy study. Prophylactic efficacy may also play a role in the prevention of yellow fever during an outbreak. To measure the effect of early administration, a dose of 3.6×10^7 PFU/ml of DEF201 was administered i.n. to hamsters at various times prior to i.p. challenge with YFV. Times of administration included −28, −21, −14, and −7 dpi, in addition to positive-control treatment with DEF201 at 4 h prior to virus challenge.

The effect of treatment with DEF201 administered at 4 h prior to virus challenge was similar to results obtained in the previous experiments in regard to survival (Fig. 3A). Single-dose administration of DEF201 on −7 dpi resulted in significant ($P < 0.001$) improvement of survival (Fig. 3A), with 90% survival of treated animals, which was similar to that achieved with treatment at −4 h or treatment with ribavirin for 7 days beginning at −4 h. Unfortunately, despite improvements in other disease parameters (described below), DEF201 did not significantly improve the survival of infected hamsters when it was administered at −14 dpi (Fig. 3A). Treatment at earlier time points of −21 or −28 dpi did not statistically improve any disease parameter, although there was a trend toward improvement of survival in animals treated at −28 dpi, which had a survival rate of 60%, compared with 20% survival for animals treated with the empty vector control (Fig. 3A). This is likely an artifact of model variability, as no other disease parameters were improved.

Treatment at −4 h showed a trend toward weight loss, which was not previously observed and which resulted in a nonsignificant improvement at between 3 and 6 dpi (Fig. 3B). Interestingly, despite the weight loss observed after treatment at −4 h, the weight change at between 3 and 6 dpi in animals treated on −7 dpi was significantly improved ($P < 0.001$) and similar to that seen in uninfected, toxicity controls. Trends in weight change also supported partial prophylactic protection after administration of DEF201 at −14 dpi, as weights increased at between 0 and 5 dpi, and despite a decline at between 5 and 6 dpi, a slightly significant ($P < 0.05$) improvement in weight change from 3 to 6 dpi compared with that with empty vector control treatment was observed (Fig. 3B).

Reduction of serum ALT levels to the baseline was observed in the −4 h as well as −7 dpi DEF201 treatment groups (Fig. 3C), which was consistent with survival data. Despite no protection against mortality, treatment administered on −14 dpi showed some indication of efficacy, as evidenced by a reduction in serum ALT levels on 6 dpi (Fig. 3C).

Ribavirin served as a suitable positive control. Treatment i.p.

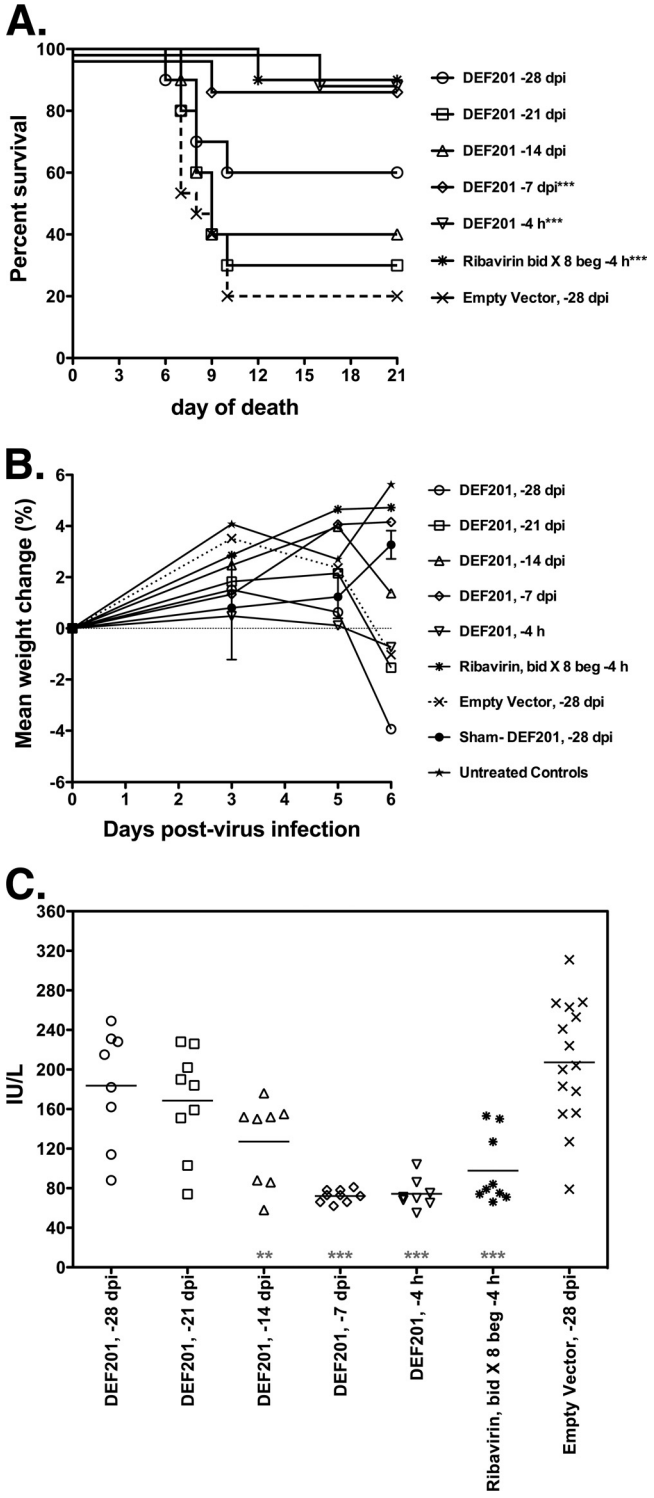

FIG. 3. Prophylactic efficacy of DEF201 at a dose of 3.6×10^7 PFU/animal administered at various times before virus challenge. Animals were treated with DEF201 at −4 h, −7, −14, −21, or −28 dpi. Ribavirin was included as a positive control and was administered i.p. b.i.d. for 7 days beginning (beg) at −4 h dpi at a dose of 75 mg/kg/day. Disease parameters include survival (A), percent weight change (B), and serum ALT level on 6 dpi (C). ***, $P < 0.001$ compared with empty vector treatment; **, $P < 0.01$, compared with empty vector treatment.

with ribavirin administered twice a day (b.i.d.) for 7 days beginning 4 h before virus challenge resulted in protection from mortality, with a 90% survival rate (Fig. 3A). Ribavirin treatment also significantly ($P < 0.001$) improved weight change (Fig. 3B) and serum levels of ALT (Fig. 3C), which were similar to results from previous studies.

DISCUSSION

When it was administered as a single intranasal treatment at −4 h, DEF201 offered protection against death at all tested doses, with efficacy being similar to or greater than that of the positive control, ribavirin, which was given 14 times over a 7-day period. Similar results were seen after daily treatment for 7 days with consensus alfacon-1 IFN (Infergen), which also displayed a dose response at doses between 0.5 and 5 μg/kg/day (18). The antiviral effect depended on the amount of DEF201 administered. The production of a steady-state level of IFN may bypass the need for the regular bolus dosing associated with traditional IFN treatment. The present study demonstrates that i.n.-delivered DEF201 protects animals against an i.p. YFV challenge both prophylactically and as a treatment. It is apparent that therapeutic doses of interferon can be produced by a transient gene therapy approach, which ameliorates the liver disease associated with YFV infection, as evidenced by reduction of virus titers in the serum and liver. Treatment with DEF201 resulted in positive weight gain at between 3 and 5 dpi, which is likely due to the animals feeling well enough to eat and drink normally or prevention of weight loss through another mode of action. This improvement, however, was not equivalent to the weight gain in uninfected controls, which gained weight steadily at between 0 and 6 dpi.

A similar study was conducted using an intramuscularly delivered Ad5-vectored mouse interferon for the protection of mice from i.n. challenge with western equine encephalitis virus (WEEV) at 24 h after DEF201 treatment (37). Similar results were also seen in a mouse model of Venezuelan equine encephalitis virus (VEEV) infection, with 24 h pretreatment protecting against a lethal challenge (29). In addition, it has been demonstrated that the murine form of DEF201 has efficacy in a mouse model of severe acute respiratory syndrome coronavirus (SARS-CoV), as DEF201 provided complete survival benefit up to 14 days prior to lethal challenge (19). Taken together, use of *in situ* production and secretion of IFN by an Ad5-vectored human IFN gene appears to be an effective way to treat a broad spectrum of viral infections.

One potential concern associated with adenovirus-vectored gene therapy is the potential for adverse reactions in people with preexisting immunity to adenovirus as a result of natural infection, which is estimated to be the case for 30 to 70% of the human population, depending on serotype (16). Indeed, a recent study with an investigational human immunodeficiency virus vaccine vectored by adenovirus failed, which was thought to be mainly due to preexisting adenovirus immunity in trial participants (31, 36). Intranasal administration of DEF201, which has been shown to bypass many of the negative effects associated with preexisting immunity to adenovirus, was utilized in the present study (8). Future studies will investigate the effect of i.n. treatment with DEF201 in the presence of neutralizing antibody to adenovirus type 5. Another potential con-

cern is the development of antibody to IFN in treated animals. Anti-interferon antibodies have been detected in patients undergoing prolonged treatment with IFN. The reported percentage of patients who develop antibodies varies widely (0 to 95%), depending on dosages, treatment schedules, and method of testing (2, 10). With short-term therapy with DEF201, such as treatment of infection with an acute flavivirus such as YFV, it can be expected that antibody development would be lower, but it will certainly be monitored in future animal experiments and eventual clinical work. Importantly, it has been determined that when antibodies to exogenous interferon do develop, they do not preclude the activity of native interferon (2).

Treatment with DEF201 initiated at 1 or 2 dpi offered significant protection compared with that offered by the empty vector control, while initiation on 3 dpi did not result in a significant increase in survival in this study, although the serum ALT level was significantly improved and a trend toward improved survival and weight change was observed. This treatment window is similar to that observed with the administration of exogenous recombinant interferon, which was given daily for 7 days beginning at 3 dpi and which was found to be effective in significantly improving survival (J. Julander, unpublished results). This is of particular interest, because although there is a YFV vaccine currently available, it is poorly utilized, resulting in a need for treatment options.

After infection of hamsters with YFV, virus is detectable in the serum during the course of disease, while virus is found in the liver at as early as 3 dpi, with peak titers occurring at 4 dpi (18). Some serum chemistry profiles are significantly affected at 2 to 3 dpi, but the majority of important changes in blood chemistry occur later during the course of infection (5 to 7 dpi), just prior to death. It appears that treatment with DEF201 on 3 dpi, while improving ALT levels, may be too late to impact mortality, while treatment on 2 dpi was highly effective in improving survival and affecting other symptoms of disease.

Other models of acute viral infection have been used to demonstrate the efficacy of DEF201. Treatment at 6 h after virus challenge in a mouse model of WEEV infection resulted in significantly improved survival (37). While efficacy after prophylactic treatment was demonstrated in a model of VEEV infection, therapeutic efficacy was not observed with Ad5-vectored mouse IFN, where delay of treatment to 6 h after virus challenge did not protect mice from death (29). Finally, it has been demonstrated that the murine form of DEF201 has significant treatment efficacy in a lethal mouse model of SARS-CoV at 6 h and 12 h postinfection (19).

Treatment on −7 dpi with DEF201 at 3.6×10^7 PFU/animal prevented any disease due to YFV infection, while treatments at longer time points prior to challenge (−14 and −21 dpi) were not protective. This is in contrast to effective protection in the WEEV mouse study, in which pretreatment at 13 weeks dpi was effective in protecting mice from virus challenge (37). Future studies will evaluate the immunological status of hamsters treated with DEF201 to determine the effect of this treatment on antibody production after YFV challenge.

Thus, DEF201 illustrates antiviral efficacy similar to that of IFN protein in the YFV model, with the added benefits of a single dose versus repeated daily dosing and the potential for self-administration intranasally. These benefits, if they are extrapolated to a clinical setting, allow rapid prophylaxis of people entering a suspected YFV-infected area and its use as a postexposure prophylactic or treatment in an outbreak scenario, as it may be quickly and easily distributed to an at-risk population, such as medical chain workers. More broadly, given the efficacy profile of DEF201 shown here and that in WEE (37), VEE (29), and SARS (19) models, DEF201 has significant potential as a broad-spectrum, host-directed antiviral.

ACKNOWLEDGMENTS

We thank Garret Child and Deanna Larson for their work in the animal and cell culture labs.

This work was supported by contract N01-AI-30063 from the Division of Microbiology and Infectious Diseases, NIAID, NIH.

REFERENCES

1. **Ajariyakhajorn, C., et al.** 2005. Randomized, placebo-controlled trial of nonpegylated and pegylated forms of recombinant human alpha interferon 2a for suppression of dengue virus viremia in rhesus monkeys. Antimicrob. Agents Chemother. **49:**4508–4514.
2. **Antonelli, G., and F. Dianzani.** 1999. Development of antibodies to interferon beta in patients: technical and biological aspects. Eur. Cytokine Netw. **10:**413–422.
3. **Arroyo, J. I., et al.** 1988. Effect of human gamma interferon on yellow fever virus infection. Am. J. Trop. Med. Hyg. **38:**647–650.
4. **Bae, H. G., et al.** 2005. Analysis of two imported cases of yellow fever infection from Ivory Coast and The Gambia to Germany and Belgium. J. Clin. Virol. **33:**274–280.
5. **Brooks, T. J., and R. J. Phillpotts.** 1999. Interferon-alpha protects mice against lethal infection with St. Louis encephalitis virus delivered by the aerosol and subcutaneous routes. Antiviral Res. **41:**57–64.
6. **Buckwold, V. E., J. Wei, M. Wenzel-Mathers, and J. Russell.** 2003. Synergistic in vitro interactions between alpha interferon and ribavirin against bovine viral diarrhea virus and yellow fever virus as surrogate models of hepatitis C virus replication. Antimicrob. Agents Chemother. **47:**2293–2298.
7. **Crance, J. M., N. Scaramozzino, A. Jouan, and D. Garin.** 2003. Interferon, ribavirin, 6-azauridine and glycyrrhizin: antiviral compounds active against pathogenic flaviviruses. Antiviral Res. **58:**73–79.
8. **Croyle, M. A., et al.** 2008. Nasal delivery of an adenovirus-based vaccine bypasses pre-existing immunity to the vaccine carrier and improves the immune response in mice. PLoS One **3:**e3548.
9. **De Clercq, E.** 2004. Antiviral drugs in current clinical use. J. Clin. Virol. **30:**115–133.
10. **Douglas, D. D., et al.** 1993. Randomized controlled trial of recombinant alpha-2a-interferon for chronic hepatitis C. Comparison of alanine aminotransferase normalization versus loss of HCV RNA and anti-HCV IgM. Dig. Dis. Sci. **38:**601–607.
11. **Du, Y., H. Tian, X. D. Gao, and W. B. Yao.** 2008. Pharmacokinetic properties of a 40 kDa branched polyethylene glycol-modified form of consensus interferon-alpha (PEG-CIFN) in rhesus monkeys. Biopharm. Drug Dispos. **29:** 481–484.
12. **Fish, E. N., K. Banerjee, H. L. Levine, and N. Stebbing.** 1986. Antiherpetic effects of a human alpha interferon analog, IFN-alpha Con1, in hamsters. Antimicrob. Agents Chemother. **30:**52–56.
13. **Fish, E. N., K. Banerjee, and N. Stebbing.** 1985. Efficacy of consensus interferon alpha against HSV-2 infections. Antiviral Res. Suppl. **1:**191–197.
14. **Heathcote, J.** 1998. Consensus interferon: a novel interferon for the treatment of hepatitis C. J. Viral Hepat. **5**(Suppl. 1)**:**13–18.
15. **Hutson, T. E., et al.** 2003. Phase I trial of consensus interferon in patients with metastatic renal cell carcinoma: toxicity and immunological effects. Clin. Cancer Res. **9:**1354–1360.
16. **Jiang, H., Z. Wang, D. Serra, M. M. Frank, and A. Amalfitano.** 2004. Recombinant adenovirus vectors activate the alternative complement pathway, leading to the binding of human complement protein C3 independent of anti-ad antibodies. Mol. Ther. **10:**1140–1142.
17. **Julander, J. G., Y. Furuta, K. Shafer, and R. W. Sidwell.** 2007. Activity of T-1106 in a hamster model of yellow fever virus infection. Antimicrob. Agents Chemother. **51:**1962–1966.
18. **Julander, J. G., J. D. Morrey, L. M. Blatt, K. Shafer, and R. W. Sidwell.** 2007. Comparison of the inhibitory effects of interferon alfacon-1 and ribavirin on yellow fever virus infection in a hamster model. Antiviral Res. **73:**140–146.
19. **Kumaki, Y., et al.** 2011. Single-dose intranasal administration with mDEF201 (adenovirus vectored mouse interferon-alpha) confers protection from mortality in a lethal SARS-CoV BALB/c mouse model. Antiviral Res. **89:**75–82.

20. **Laurent-Rolle, M., et al.** 2010. The NS5 protein of the virulent West Nile virus NY99 strain is a potent antagonist of type I interferon-mediated JAK-STAT signaling. J. Virol. **84:**3503–3515.
21. **Lewis, M., and J. R. Amsden.** 2007. Successful treatment of West Nile virus infection after approximately 3 weeks into the disease course. Pharmacotherapy **27:**455–458.
22. **Leyssen, P., et al.** 2003. Interferons, interferon inducers, and interferon-ribavirin in treatment of flavivirus-induced encephalitis in mice. Antimicrob. Agents Chemother. **47:**777–782.
23. **Mazzon, M., M. Jones, A. Davidson, B. Chain, and M. Jacobs.** 2009. Dengue virus NS5 inhibits interferon-alpha signaling by blocking signal transducer and activator of transcription 2 phosphorylation. J. Infect. Dis. **200:**1261–1270.
24. **Monath, T. P.** 2008. Treatment of yellow fever. Antiviral Res. **78:**116–124.
25. **Monath, T. P.** 2006. Yellow fever as an endemic/epidemic disease and priorities for vaccination. Bull. Soc Pathol. Exot. **99:**341–347.
26. **Morrey, J. D., et al.** 2004. Effect of interferon-alpha and interferon-inducers on West Nile virus in mouse and hamster animal models. Antivir. Chem. Chemother. **15:**101–109.
27. **Morrey, J. D., et al.** 2004. Modeling hamsters for evaluating West Nile virus therapies. Antiviral Res. **63:**41–50.
28. **Munoz-Jordan, J. L., et al.** 2005. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. J. Virol. **79:**8004–8013.
29. **O'Brien, L., et al.** 2009. Alpha interferon as an adenovirus-vectored vaccine adjuvant and antiviral in Venezuelan equine encephalitis virus infection. J. Gen. Virol. **90:**874–882.
30. **Reed, L. J., and C. H. Muench.** 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. **27:**493–497.
31. **Robb, M. L.** 2008. Failure of the Merck HIV vaccine: an uncertain step forward. Lancet **372:**1857–1858.
32. **Solomon, T., et al.** 2003. Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial. Lancet **361:**821–826.
33. **Tomori, O.** 2004. Yellow fever: the recurring plague. Crit. Rev. Clin. Lab. Sci. **41:**391–427.
34. **Tuboi, S. H., Z. G. Costa, P. F. da Costa Vasconcelos, and D. Hatch.** 2007. Clinical and epidemiological characteristics of yellow fever in Brazil: analysis of reported cases 1998-2002. Trans. R. Soc. Trop. Med. Hyg. **101:**169–175.
35. **Ubol, S., W. Phuklia, S. Kalayanarooj, and N. Modhiran.** Mechanisms of immune evasion induced by a complex of dengue virus and preexisting enhancing antibodies. J. Infect. Dis. **201:**923–935.
36. **White, A. M.** 2009. HIV-1 step study. Lancet **373:**805.
37. **Wu, J. Q., et al.** 2007. Pre- and post-exposure protection against Western equine encephalitis virus after single inoculation with adenovirus vector expressing interferon alpha. Virology **369:**206–213.