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Delivery of Interferon- γ by an Adenovirus Vector Blocks Herpes Simplex Virus Type 1 Reactivation *in vitro* and *in vivo* Independent of RNase L and Double-Stranded RNA-Dependent Protein Kinase Pathways

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Abstract

HSV-1 is a significant human pathogen that can result in the loss of sight as a result of episodic reactivation of latent virus from sensory ganglion neurons. In this study the potential efficacy of anti-viral cytokine expression in preventing latent virus reactivation was investigated. Both type I (IFN- β) and type II (IFN- γ) IFN transgene expression following transduction of trigeminal ganglion explant cultures significantly reduced the incident of HSV-1 reactivation that in the case of IFN- β was dependent on the presence of double stranded RNA-dependent protein kinase and RNase L. *In vivo*, expression of the IFN- γ but not IFN- β transgene significantly delayed and reduced the frequency of reactivation of latent mice exposed to UV light without discernable inflammation. This result is the first report that demonstrates the ability to block reactivation using an ectopic cytokine expression system and warrants further exploration as a means to prevent HSV-1 reactivation.

Keywords

HSV-1; IFN- γ ; IFN- β ; CXCL10

1. Introduction

HSV-1 establishes a lifelong persistence in human and mouse trigeminal ganglion (TG) neurons where spontaneous molecular reactivation is thought to occur (Croen et al., 1987; Stevens et al., 1987; Feldman et al., 2002). Spontaneous or incomplete reactivation has been found to result in a persistent localized immune response in the corresponding ganglia (Shimeld

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et al., 1995; Cantin et al., 1995; Liu et al., 1996; Halford et al., 1996a; Theil et al., 2003). In vitro and in vivo studies have found CD8⁺ T lymphocytes are principally involved in maintenance of latent HSV-1 infection within the TG (Noisakran and Carr, 1999; Liu et al., 2000; Khanna et al., 2003; Sheridan et al., 2006). Although the mechanism involved in reactivation from latency is not understood, IL-6 has been tied to facilitating reactivation (Kriesel et al., 1997; Noisakran et al., 1998) whereas IFN- γ expressed by CD8⁺ T cells has been reported to antagonize reactivation associated with a reduction in ICP0 expression in the neurons undergoing reactivation (Cantin et al., 1999; Decman et al., 2005).

Type I (IFN- α s and IFN- β) and type II (IFN- γ) IFNs are potent anti-viral cytokines that block virus replication through the induction of the JAK/STAT pathway eliciting the production of Mx proteins, double-stranded RNA-dependent protein kinase (PKR), and the oligoadenylate synthetase effector molecule, RNase L (Stark et al., 1998; Torterella et al., 2000). The importance of these anti-viral pathways in the prevention of HSV-1 replication is underscored by the action of HSV-1-encoded proteins that specifically target IFN-induced pathways and effector molecules counteracting the anti-viral effect (Chou et al., 1995; Mossman and Smiley, 2002; Yokota et al., 2004; Halford et al., 2006). In addition to the anti-viral properties induced by IFNs, type I IFNs facilitate the development of the adaptive immune response through the activation of dendritic cells (Biron, 2001; Le Bon et al., 2003) which in terms of viral infection promote CD8⁺ T cell clonal expansion and memory cell formation (Kolumam et al., 2005).

Against this background, we sought to determine to what extent IFNs could antagonize latent HSV-1 reactivation. Since previous results reported success in delivery of the IFN transgenes by adenovirus (Ad) vector and hindering acute HSV-1 infection (Austin et al., 2005; 2007), we chose to compare IFN- γ to the most efficacious type I IFN transgene, IFN- β (Härle et al., 2002), in preventing HSV-1 reactivation in an in vitro and in vivo model. To the best of our knowledge, this is the first report demonstrating the ability of a lone cytokine to significantly block HSV-1 reactivation in vivo.

2. Material and Methods

2.1 Animals

Male and female C57BL/6 (The Jackson Laboratory), mice deficient in RNase L (RL^{-/-}) (Austin et al., 2005), mice deficient in PKR (PKR^{-/-}) (Austin et al., 2005), mice deficient in both RNase L and PKR (PKR/RL^{-/-}) (Austin et al., 2007), and NIH inbred mice (Keadle et al., 2002) were infected as previously described (Austin et al., 2005). The care and housing of mice was consistent 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 University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute Institutional Animal Care and Use Committees.

2.2 Virus and cells

Preparation and handling of HSV-1 (strain McKrae) and Vero cells used in plaque assays is previously described (Halford et al., 1996b).

2.3 UV-induced HSV-1 reactivation

NIH mice infected 30 days previously with HSV-1 underwent UV-B light exposure to induce reactivation as described (Keadle et al., 2002). Immediately following UV-B light exposure, the mice were transduced with Ad:Null (empty virus vector), Ad:IFN- β , or Ad:IFN- γ (Austin et al., 2005; 2007). Non-transduced, vehicle (PBS)-treated mice served as controls. At 3, 4, 5, or 7 days post UV-B light exposure, the mice were euthanized and the eyes removed, snap frozen, and stored at -80⁰ C until analysis of virus and cytokine/chemokine content could be

conducted. Frozen eyes were thawed in 4°C RPMI-1640 medium, homogenized using a tissue mizer (Fisher Scientific) and immediately assessed for viral content by plaque assay (Halford et al., 2006b) or cytokine/chemokine content by ELISA.

2.4 Detection of chemokines and cytokines by ELISA

CCL2, CXCL10, and IFN- γ were detected by commercially available ELISA kits (R&D Systems). IFN- β was detected by a commercially available ELISA kit as well (PBL Biomedical Lab).

Statistic analysis—Significant differences ($p \leq 0.05$) between groups were determined by ANOVA and Tukey's post hoc t -test. Differences comparing reactivated to non-reactivated groups was determined using Student's t -test with Bonferoni correction.

3. Results and Discussion

3.1 Transduction of latently infected TG explant cultures with either the IFN- β or IFN- γ transgene antagonizes reactivation

The removal and dissociation of latently infected TG into single cell suspensions results in lytic gene expression within 24 hr following culture of cells (Halford et al., 1996b) and detection of infectious virus 48-72 hr post explant. Therefore, we initially determined whether transduction of TG cell cultures from latently infected mice with Ad:IFN- β or Ad:IFN- γ suppresses HSV-1 reactivation. We initially chose to transduce with 5.0 transducing units (TU) since this dose has previously been found to significantly reduce HSV-1 replication in acute-infected primary TG cell cultures (Al-khatib et al, 2003; Austin et al., 2006). The results show Ad:IFN- β and Ad:IFN- γ delay and suppress HSV-1 reactivation (Fig. 1A). Transduction with the IFN- γ transgene appeared to be more effective compared to the IFN- β transgene although the differences were not significant. Transduction with a lower dose (1.0 TU) of Ad:IFN- β or Ad:IFN- γ had no significant impact on virus reactivation in terms of delay, frequency, or amount of virus reactivated (data not shown). To determine the specificity of the efficacy, neutralizing Ab directed against IFN- β or IFN- γ or isotypic control Ab was added to transduced TG explant cultures undergoing reactivation. Anti-IFN- β antibody completely blocked the action of the IFN- β transgene whereas anti-IFN- γ antibody partially (50% reduction) blocked the anti-viral effect mediated by the IFN- γ transgene (data not shown). The partial antagonism by the anti-IFN- γ antibody may be due to the incomplete neutralization of IFN- γ in the supernatant (data not shown).

3.2 Transduction with Ad:IFN- β but not Ad:IFN- γ requires the presence of functional PKR and RNase L pathways

We previously found the absence of RNase L or PKR modestly reduced the anti-viral effect induced by the expression of the IFN- β transgene but not IFN- γ transgene in primary TG cell cultures infected with HSV-1 (Al-khatib et al., 2003; Austin et al., 2006). The absence of functional oligoadenylate synthetases (Fig. 1B) or PKR (Fig. 1C) pathways had no effect on the capacity of the IFN transgenes to block HSV-1 reactivation following transduction of TG explant cultures undergoing reactivation. However, the absence of both PKR and RNase L blocked the anti-viral effect following Ad:IFN- β transduction of TG explant cultures undergoing reactivation (Fig. 1D). In contrast, the loss of these pathways had no such effect following transduction of TG explant cultures with Ad:IFN- γ in terms of frequency of reactivation (Fig. 1D). However, a comparison of the transduced cultures that reactivated found all cultures contained similar levels of virus (data not shown).

3.3 Transduction of mice with Ad:IFN- γ antagonizes HSV-1 reactivation

Within the human population, morbidity is typically associated with HSV-1 infection is due to the episodic reactivation of latent virus and ensuing immune response that impairs the visual axis often resulting in blindness (Corey and Spear, 1986; Liesegang, 2001). Previous studies have vaccinated latently infected mice using replication deficient virus or viral glycoproteins as a means to antagonize HSV-1 reactivation (Keadle et al., 2002; Richards et al., 2003). However, these studies induced reactivation post immunization. Our goal was to determine if the IFN transgenes could antagonize HSV-1 reactivation at the time of reactivation induction. Therefore, UV-induced reactivation of latent HSV-1 in mice subsequently transduced in the cornea with the Ad:IFN- β and Ad:IFN- γ transgenes was undertaken. Whereas mice transduced with the Ad:IFN- β transgene showed no appreciable impact on HSV-1 reactivation compared to Ad:Null transduced or vehicle-treated animals, mice transduced with Ad:IFN- γ showed a delay and reduction in HSV-1 reactivation in the eye (Fig. 2A). Similar to our *in vitro* results and what others have found *in vivo* (Keadle et al., 2002; Richards et al., 2003), we were not able to block 100% reactivation. Presently, we are unable to determine whether the block occurs at the site of transduction or within the ganglion as both sites express the transgene product (Al-khatib et al., 2004; Austin et al., 2005). Within the cornea, the transgene is not universally expressed by rather punctate pockets of expression are observed due to the scarification process (Al-khatib et al., 2004). Within the ganglion, we speculate not all neuron types latently infected with HSV-1 are capable of responding to IFN- γ via its receptor (Margolis et al., 2007).

3.4 Reactivation is associated with an increase in CXCL10

The occurrence of virus in the cornea of reactivated mice leads to infiltration of neutrophils and macrophages followed by CD4⁺ and CD8⁺ T cells (Shimeld et al., 1996). The recruitment of these cells may be due to expression of chemokines in response to virus including CCL2 and CXCL10 which are rapidly induced following acute infection (Carr and Tomanek, 2006). In fact, it is the inflammatory response to episodes of reactivated virus that leads to the decimation of the cornea requiring corneal transplants in the human population (Liesegang, 2001). Therefore, we investigated the expression of chemokines (CCL2 and CXCL10) along with IFN- γ comparing the transduced mice as well as groups that reactivated versus non-reactivated animals. Prior to reactivation, latently infected mice expressed either low or undetectable levels of CCL2, CXCL10, and IFN- γ (Fig. 2B-D). Following UV-exposure, there was no appreciable difference in soluble factor levels recovered in the eye comparing the Ad:Null, Ad:IFN- β , and Ad:IFN- γ transduced groups that did not reactivate (data not shown). The most striking difference noted was that for CXCL10 which was dependent upon the reactivation status. Corneas containing showed significantly elevated levels of CXCL10 in comparison to corneas that contained no virus (Fig. 2B). CXCL10 levels were sustained in the eyes over the course of evaluation (day 4 -day 7). In a similar fashion, IFN- γ was only detected in eyes from mice that reactivated and the expression was transient (Fig. 2C). By comparison, UV light exposure elicited a significant increase in CCL2 expression in the eye regardless of reactivation that diminished but remained post exposure (Fig. 2D). There was a modest but transient increase in CCL2 expression in the eyes of reactivated mice in comparison to non-reactivated animals (Fig. 2D).

Collectively, the study shows a specific anti-viral effect of the murine IFN- γ transgene in blocking HSV-1 reactivation *in vitro* and *in vivo* independent of the two prominent anti-viral pathways, PKR and oligoadenylate synthetases/RNase L. To our knowledge this is the first report which shows the ability to block reactivation when the reagent is delivered at the time of reactivation induction into the cornea without inducing a localized immune response.

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FIGURE 1.

Ad:IFN- β and Ad:IFN- γ antagonize HSV-1 reactivation from TG explant cultures. TG from C57BL/6 mice infected 30 days previously was removed, processed to single cell suspensions, and plated into 24 well plates at a concentration of $1-3 \times 10^5$ cells/well in 1.0 ml of media (9). At the initiation of culture, cells were transduced with 5.0 TU of Ad:Null (empty vector), Ad:IFN- β , or Ad:IFN- γ . Non-transduced cultures received only vehicle (PBS). Starting 24 hr post culture, 200 μ l of the supernatant was collected every 48 hr up to 11 days post culture condition and assayed for the presence and quantity of HSV-1 by plaque assay. (A) Summarizes the frequency of reactivation of latent infected wild type mouse TG explant cultures from 12-26 wells/condition from 3-4 experiments. ** $p < .01$ comparing Ad:IFN- γ to Ad:Null day 7-11 post infection. * $p < .05$ comparing Ad:IFN- β to Ad:Null day 7-11 post infection. (B) Summarizes the frequency of reactivation of latent infected RNase L deficient mouse TG explant cultures from 10-19 wells/condition from 3 experiments with the exception of Ad:IFN- γ which is taken from 6 wells. * $p < .05$ comparing Ad:IFN- β to Ad:Null day 9-11 post infection. (C) Summarizes the frequency of reactivation of latent infected PKR deficient mouse TG explant cultures from 12-19 wells/condition from 3 experiments with the exception of Ad:IFN- γ which is taken from 4 wells. ** $p < .01$ comparing Ad:IFN- β to Ad:Null day 7-11 post infection. (D) Summarizes the frequency of reactivation of latent infected PKR/RNase L deficient mouse TG explant cultures from 13-22 wells/condition from 3 experiments. * $p < .05$ comparing Ad:IFN- γ to Ad:Null day 9-11 post infection.

FIGURE 2.

Ad:IFN- γ antagonizes HSV-1 reactivation in vivo without inducing a localized immune response. Latently infected mice were exposed to UV light to induce reactivation as described (29). Immediately following UV light exposure, the cornea of mice were transduced with Ad:Null, Ad:IFN- β , or Ad:IFN- γ as described (26, 27). Mice were euthanized at the indicated time post UV light exposure and the eyes were harvested, homogenized, and the clarified supernatant was assessed for virus by plaque assay or the indicated cytokine/chemokine by ELISA. Each group consisted of 5-7 mice/group/time point for cumulative percent reactivation (A) and $n=6-12$ per group at each time point for CXCL10 (B), IFN- γ (C), and CCL2 (D) levels. * $p \leq .05$ comparing the Ad:IFN- γ to Ad:Null or vehicle-treated mice for percent cumulative reactivation at day 5-7 post reactivation. ** $p < .01$ comparing the reactivated to non-reactivated group for CXCL10 and CCL2 chemokines at 4-7 days post reactivation for CXCL10 and day 4 post reactivation for CCL2.