Virology 529 (2019) 23–28

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

The STING agonist 5,6-dimethylxanthenone-4-acetic acid (DMXAA) stimulates an antiviral state and protects mice against herpes simplex virus-induced neurological disease

Stacey Cerón[a,b], Brian J. North[b], Sean A. Taylor[b], David A. Leib[b,*]

[a] Guarini School of Graduate and Advanced Studies, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756, USA
[b] Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756, USA

ARTICLE INFO

Keywords:
Herpes simplex virus
STING
DMXAA
Immunotherapy antiviral therapy

ABSTRACT

Herpes simplex virus (HSV) − 1 is the most common cause of sporadic viral encephalitis and accounts for 5–10% of cases worldwide. A key factor in host control of viral infection is the initiation of the interferon (IFN) response, mediated in part by the stimulator of interferon genes (STING) pathway. In these studies, we examined the ability of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a STING agonist, to protect against HSV-1 infection. DMXAA reduced viral replication through increased production of type I IFN *in vitro*. Furthermore, administration of DMXAA to HSV-1 infected mice resulted in a reduction of viral burden in the peripheral and central nervous systems. This reduced viral burden also correlated with increased survival of DMXAA-treated infected mice. These results therefore demonstrate the potential of STING agonists for immunotherapy against HSV-1.

1. Introduction

Herpes simplex virus 1 (HSV-1) is a double stranded DNA virus with 80–90% seroprevalence worldwide (Xu et al., 2006). During primary infection, the virus replicates in a variety of mucosal surfaces before infecting innervating sensory neurons (Rowe et al., 2013). HSV then travels in a retrograde direction to establish lifelong latency in neuronal cell bodies often located in peripheral ganglia. Following reactivation, the virus may travel in an anterograde direction and return to the mucosal surface to cause herpetic disease and facilitate transmission (Roizman et al., 2007). In the majority of cases, HSV-1 is asymptomatic, with or without shedding. When symptoms arise, they can range in severity from orolabial and genital sores, to blinding stromal corneal keratitis or lethal encephalitis (Rowe et al., 2013; Fatahzadeh and Schwartz, 2007).

Herpes simplex encephalitis (HSE) accounts for 5–10% of encephalitis worldwide (Shives et al., 2017). The precise route of entry into the central nervous system (CNS) is not known in humans. Work in murine ocular models, however, suggests that HSE may result from uncontrolled viral spread from mucosal sites to the trigeminal ganglion (TG), and subsequently into the brain stem and brain (Shives et al., 2017; Slifer and Jennings, 2015). HSE is characterized by necrosis of neurons, astrocytes and oligodendrocytes as well as acute inflammation within the temporal lobes and/or the insular cortex of the cerebral hemispheres. This contributes to the clinical presentation in humans that includes fever, headaches, seizures, paralysis and psychiatric symptoms (Shives et al., 2017; Piret and Boivin, 2015; Gnann and Whitley, 2017). Although acyclovir reduces morbidity and mortality, there is still a 30% mortality rate associated with treatment. In addition, approximately 60% of survivors display neurological sequelae throughout their lives. There is, therefore, a clear need for improved treatments (Piret and Boivin, 2015).

Favorable clinical outcomes are determined by the ability of the immune response to control viral infection while mounting an appropriate, non-pathological, inflammatory response. This begins with innate immunity which is activated by components of HSV that stimulate specific pattern recognition receptors (PRRs) and the antiviral state (Paludan et al., 2011). The HSV genome itself is a potent stimulator of multiple PRRs including particular classes of toll-like receptors (TLRs), AIM2-like receptors (ALRs) and cytoplasmic DNA sensors (Paludan et al., 2011; Carty et al., 2014; Knipe, 2015). The majority of the cytoplasmic sensing pathways utilize stimulator of interferon genes (STING). This adaptor protein activates interferon regulatory factor 3 (IRF-3) and nuclear factor κB (NF-κB), which can in turn upregulate type I interferon (IFN) and IFN stimulated genes (ISGs) (Cai et al., 2014; Chen et al., 2016; Holm et al., 2012).

STING plays a key role in immune system activation and autophagy (Cai et al., 2014; Chen et al., 2016; Ishikawa and Barber, 2011). In

* Corresponding author.
E-mail address: david.a.leib@dartmouth.edu (D.A. Leib).

https://doi.org/10.1016/j.virol.2019.01.006
Received 13 July 2018; Received in revised form 4 January 2019; Accepted 4 January 2019
Available online 06 January 2019

addition, STING-dependent control of HSV-1 infection has been demonstrated (Ishikawa et al., 2009; Parker et al., 2015; Reinert et al., 2016). In a murine ocular model, however, a cell-type dependence on STING for control of HSV infection was shown (Parker et al., 2015). This is especially true in the CNS, where microglial cells prime the innate signaling pathways of resting astrocytes in a STING-dependent manner (Reinert et al., 2016). This results in the activation of astrocytes and production of type I interferon (IFN) therefore amplifying the antiviral response. Neurons in the CNS respond to type I IFN and subsequently repress viral replication (Reinert et al., 2016). STING is therefore a key contributor to the antiviral response within the CNS and represents a reasonable potential target for therapeutic agonists.

During infection, STING is activated upon the binding of 2′3′-cGAMP protein to its central crevice resulting in the initiation of the signaling cascade (Cai et al., 2014). There are other small molecules that are known to directly antagonize murine STING. These include cyclic dinucleotides, 10-(carboxymethyl) − 9(10 H) acridone (CMA) and 5,6 –dimethylxanthenone-4-acetic acid (DMXAA) (Conlon et al., 2013; Cavlar et al., 2013; McWhirter et al., 2009; Burdette et al., 2011; Woodward et al., 2010). Originally developed as a tumor vascular-disruption agent, DMXAA (Vadimezan or ASA404) is a potent inducer of various cytokines including IL-1α, IL-6, IL-12α, IL-21, tumor necrosis factor (TNF)-α and type I IFN (Conlon et al., 2013; Head and Jameson, 2010; Jassar et al., 2005). Furthermore, DMXAA induces effective antiviral responses against influenza, hepatitis B, chikungunya and HSV-2, hence showing promise for this approach (Shirey et al., 2011; Guo et al., 2015; Sali et al., 2015; Skouboe et al., 2018).

In this study, we have assessed DMXAA as a STING agonist for the treatment of HSV infection. DMXAA had demonstrable activity against HSV both *in vitro* and *in vivo*. Treatment of mice with DMXAA resulted in delayed and diminished neurological symptoms of infection and promoted survival. That said, DMXAA was only effective through treatment of mice prior to infection, a potential limitation for future clinical use. Our work therefore provides important information for the future development of STING agonists as antiviral therapeutics against HSV and other viral diseases.

2. Materials and methods

2.1. Mice

C57BL/6 J mice were bred in the barrier facility in the Center for Comparative Medicine and Research at The Geisel School of Medicine at Dartmouth College or purchased from Jackson Laboratories. There were no significant differences in outcomes between in-house bred and purchased mice. Goldenticket (Gt) STING-deficient mice in the C57BL/6 J background were purchased from Jackson Laboratories and bred in-house (Sauer et al., 2011). IRF-3$^{-/-}$ mice in the C57BL/6 J background were bred in-house (Sato et al., 1998; Murphy et al., 2013). This work was done in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health. The protocol was approved by the Dartmouth IACUC committee (protocol # 00002151).

2.2. Isolation of fibroblast cells and culture

Fibroblast cells were harvested from the pinnae of adult mice after being digested in 1000 U/ml collagenase type II (Invitrogen) and 0.05% trypsin (Cellgo). Triturated cell suspensions were plated in 6-well plates in Dulbecco's modified Eagle medium (DMEM) (HyClone) supplemented with 2% fetal bovine serum (HyClone), 1% nonessential amino acid (Lonza) and 1% penicillin-streptomycin (HyClone). Cells were passaged at least one time prior to experimental usage.

2.3. Virus and viral titers

HSV-1 strains KOS and McKrae were used as previously described (Wang et al., 2013; Summers and Leib, 2002). Vero cells were used to titer all viruses in these experiments as previously described (Rader et al., 1993).

2.4. In-vitro viral infection and DMXAA treatment

5,6-dimethylxanthenone-4-acetic acid (DMXAA) was purchased from Cayman Chemical and prepared freshly at 10 mg/ml in phosphate-buffered saline (PBS; HyClone). C57BL/6 J, IRF-3$^{-/-}$ and Sting$^{GT/GT}$ fibroblasts were cultured in 24 well plates. Based on data not shown, treated groups had media supplemented with 0.1 mg/ml of DMXAA 18 h prior to infection with KOS at a MOI of 1. Fibroblasts were washed three times with PBS before being replenished with fresh media containing DMXAA. Supernatants collected at 24 h postinfection were used to calculate viral titers as previously described (Rader et al., 1993).

2.5. IFN-β enzyme-linked immunosorbent assay (ELISA)

C57BL/6 J and Sting$^{GT/GT}$ fibroblast cells were cultured as previously described. DMXAA was supplemented in the media at 0.1 mg/ml. Polyinosinic: polycytidylic acid (poly(I:C); Invivogen) was transfected into cells using jetPRIME (Polyplus-transfection) at a final concentration of 500 ng/ml. Supernatants were harvested at 6 h post-infection and processed using a DuoSet ELISA Development Systems kit as per the manufacturer's protocol (R&D Systems).

2.6. In-vivo viral infection and DMXAA treatment

Male and female C57BL/6 J mice, aged 5–7 weeks were administered ketamine (87 mg/kg body weight) and xylazine (13 mg/kg body weight) via an intraperitoneal (i.p) injection. The corneas of the mice were scarified in a 10 × 10 crosshatching pattern using a 25 G syringe needle. Mice were inoculated with 2×10^5 PFU of virus in 5 µl of inoculation medium composed of DMEM and 2% fetal bovine serum. Upon waking from the anesthesia, mice were given bupranorphine (0.02727 mg/kg) via an i.p. injection. Mice were administered DMXAA (25 mg/kg) or 100 µl of phosphate-buffered saline through an i.p. injection on selected days (Shirey et al., 2011; Guo et al., 2015).

2.7. Disease scoring and viral titering

Mice were monitored daily for temperature, weight and disease. Clinical signs of disease were assessed by a masked observer: 0 = normal behavior; 1 = ruffled fur or hunched back; 2 = ruffled fur with a hunched back or jumpy with a hunched back; 3 = motor deficits on one side; 4 = endpoint criteria reached. Eye swabs were collected from mice on days 2, 4 and 6 postinfection (Leib et al., 1989). Mice were either sacrificed at specific days postinfection or at endpoint criteria as defined by our IACUC protocol. Survival was monitored daily for 20 days and brains, brain stems, trigeminal ganglion, livers and spleens were collected at endpoint and frozen in DMEM and 2% fetal bovine serum with glass beads at − 80 °C. Tissues were homogenized prior to sonication and plaque assays (Rader et al., 1993).

3. Results

3.1. DMXAA stimulates IFN-β production in a STING-dependent manner in murine fibroblasts

We assessed whether pre-treatment with DMXAA prior to HSV infection altered virus replication in Sting $^{GT/GT}$, IRF-3$^{-/-}$ and C57BL/6 J fibroblasts. As expected, C57BL/6 J fibroblast cells treated with DMXAA yielded significantly less virus in comparison to non-treated C57BL/6 J cells (Fig. 1A). There was no significant effect on virus replication in Sting$^{GT/GT}$ or IRF-3$^{-/-}$ cells treated with DMXAA, indicating that the antiviral state induced by DMXAA is STING- and IRF-3-dependent.

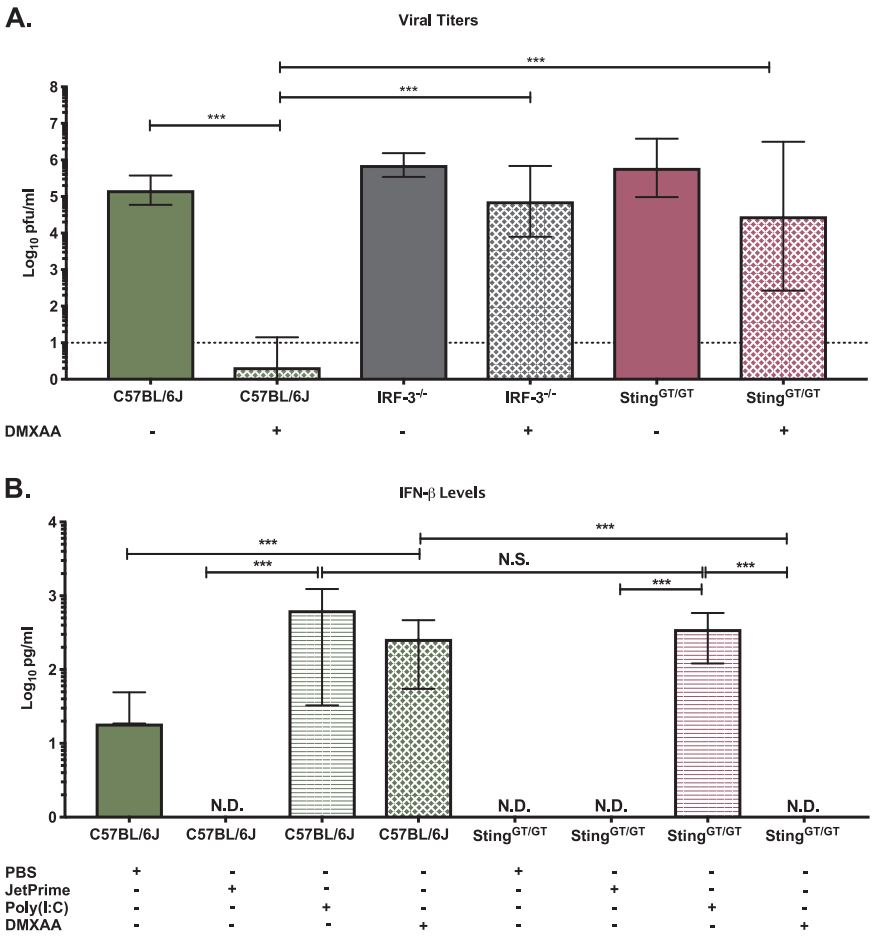

Fig. 1. DMXAA induction of the antiviral state and IFN-β in fibroblasts is STING-dependent. (A) Viral titers from C57BL/6J (green), IRF-3$^{-/-}$ (grey) and Sting$^{GT/GT}$ (magenta) fibroblasts treated with or without DMXAA 18h prior to infection with HSV-1 KOS (MOI = 1). Each bar represents the average of 3 wells in 2 independent experiments. The limit of detection is represented by the dotted line. (B) IFN-β levels evaluated by ELISA of extracts of C57BL/6J and Sting$^{GT/GT}$ fibroblasts treated with DMXAA or poly(I:C) for 6 h. N.D. = not detectable. N.S. = not significant. Error bars represent standard deviations (SD). Statistical significance was tested using a one-way ANOVA with Tukey posttests. * **, $p < 0.0005$.

Based on these data, we hypothesized that DMXAA is able to upregulate IFN-β in a STING-dependent manner. We therefore measured IFN-β levels in the supernatants of DMXAA-treated Sting$^{GT/GT}$ and C57BL/6 J fibroblast cells, using poly(I:C) as a positive control. Consistent with previous studies, IFN-β was significantly induced when cells were treated with DMXAA or poly(I:C) (Fig. 1B). Sting$^{GT/GT}$ cultures treated with poly(I:C) had comparable IFN-β levels to C57BL/6 J cultures. However, there was no IFN-β detected in Sting$^{GT/GT}$ cultures treated with DMXAA. These data confirm that DMXAA is a potent agonist of the STING pathway that upregulates IFN-β production in murine fibroblasts.

3.2. Viral replication and spread is reduced in the CNS of C57BL/6 J mice treated with DMXAA

Having demonstrated the potent antiviral response driven by DMXAA *in vitro* we wished to examine the effects of DMXAA in a mouse model of HSV-1. C57BL/6J and Sting$^{GT/GT}$ mice were pretreated with DMXAA or vehicle control, and treated again on days 1, 3 and 5 postinfection, and eye swabs were acquired. As expected, Sting$^{GT/GT}$ mice had a significantly higher viral load in corneas compared to the C57BL/6J mice, regardless of DMXAA treatment (Fig. 2A). Notably, 3 days postinfection C57BL/6J mice treated with DMXAA had significantly lower viral loads in corneas than mice treated with vehicle. An analogous trend was observed between the treated and non-treated C57BL/6J mice on days 5 and 7. This suggests that DMXAA was initially able to stimulate an antiviral response in the cornea.

Upon infecting the cornea, HSV-1 McKrae can spread to the TG and into the CNS, prompting the development of neurological disease (Slifer and Jennings, 2015). We therefore hypothesized that DMXAA would protect C57BL/6 J mice from HSV infection. In order to test this hypothesis, viral burden was measured in TGs and brainstems of C57BL/6 J and Sting$^{GT/GT}$ mice. On day 3 postinfection, C57BL/6 J mice treated with DMXAA had significantly reduced viral titers in TGs when compared to control-treated mice (Fig. 2B). By day 5 postinfection, however, DMXAA-treated and control-treated mice had comparable viral burdens in TGs. As expected, viral replication in Sting$^{GT/GT}$ mice was unaffected by DMXAA treatment throughout infection (Fig. 2B). Consistent with these data, we observed a trend of a lower viral burden in the brainstems of C57BL/6 J mice treated with DMXAA relative to control-treated C57BL/6 J mice, or relative to treated or untreated Sting$^{GT/GT}$ mice (Fig. 2C). These results support the hypothesis that DMXAA stimulates an anti-viral response to HSV-1 and can control viral dissemination from the PNS to the CNS.

3.3. Pre-treatment with DMXAA confers protection in C57BL/6 J mice against neurological disease and mortality

Given that STING plays a critical role in controlling the antiviral state in the CNS, we evaluated the ability of DMXAA to reduce HSV-induced CNS disease and mortality (Reinert et al., 2016). C57BL/6 J and Sting$^{GT/GT}$ mice were treated with DMXAA or vehicle control and challenged with McKrae and then temperature, weight, and neurological disease were monitored. Regardless of DMXAA treatment, Sting$^{GT/GT}$ mice lost 15% of their original weight by day 5 postinfection, whereas C57BL/6 J mice only lost approximately 10% of their original weight (Fig. 3A). By day 6 postinfection, all Sting$^{GT/GT}$ mice had met endpoint criteria. In contrast, the DMXAA-treated C57BL/6 J mice regained their original weight on day 9, while the PBS-treated group had all succumbed to infection. Consistent with the decrease of the core body temperature (data not shown), DMXAA-treated Sting$^{GT/GT}$ mice displayed significant neurological disease in comparison to C57BL/6 J

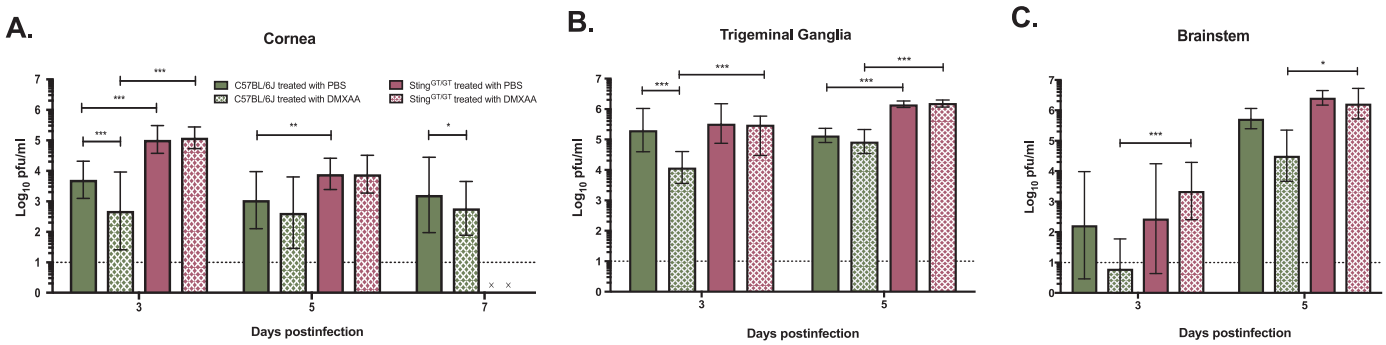

Fig. 2. DMXAA treatment lowers viral burden in tissues following corneal infection with HSV-1 McKrae. (A) C57BL/6J (green) and Sting$^{GT/GT}$ (magenta) mice were untreated (solid bars) or treated with DMXAA (stippled bars) one day prior to infection and subsequently on days 1, 3, and 5 postinfection. Eye swab samples were taken on days 3, 5 and 7, and viral titers were quantified, n = 12–13 mice per group. Statistical significance was tested by an unpaired Mann-Whitney t-test. (B-C) C57BL/6J and Sting$^{GT/GT}$ mice were treated with DMXAA or PBS one day prior to infection and subsequently on days 1 and 3 postinfection. Organs were harvested to quantify viral load in the TG (B) and brain stem (C). n = 7–8 mice per group. Graphs are the average of two independent experiments. X = no survivors, and dotted line represents the limit of detection. Error bars represent SD. Statistical significance was tested using a two-way ANOVA with Tukey posttests. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$.

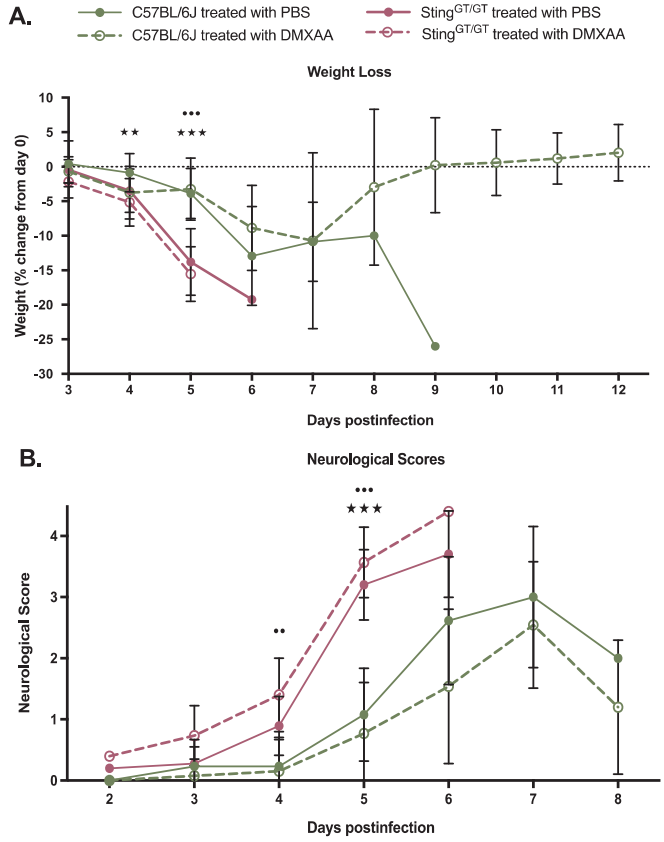

Fig. 3. DMXAA treatment results in reduced weight loss and neurological outcomes following infection with HSV-1 McKrae. C57BL/6J (green) and Sting$^{GT/GT}$ (magenta) mice were untreated (solid lines) or treated with DMXAA (dashed lines) one day prior to ocular challenge and then postinfection on days 1, 3 and 5. (A) Average percentage weight loss of mice relative to their weight on day 0. Statistical significance was tested using a two-way ANOVA with Tukey posttests. (B) Neurological score of mice over time as assessed by a masked observer: 0 = normal, 1 = hunched or ruffled fur, 2 = hunched and jumpy, 3 = loss of motor skills on one side, 4 = end point criteria. Statistical significance was tested using a two-way ANOVA with Tukey posttests. Each graph is the average of two independent experiments. Error bars represent SD. For both graphs, two symbols represents $p < 0.005$; three symbols represents $p < 0.0005$. • indicates significant differences between C57BL/6J treated with DMXAA and Sting$^{GT/GT}$ treated with DMXAA; and ★, between C57BL/6J treated with PBS and Sting$^{GT/GT}$ treated with PBS.

mice (Fig. 3B). Furthermore, C57BL/6 J mice treated with DMXAA displayed a trend toward lower neurological disease scores than control-treated mice. Notwithstanding, the DMXAA-treated mice displayed unilateral loss of motor function, although 4 out of 12 mice fully recovered from neurological disease by 21 days postinfection (Fig. 3B). This data contributed to the survival analysis (Fig. 4B). These data demonstrated that DMXAA had some efficacy in protecting against neurological disease in mice.

Given the aforementioned data, we wanted to address whether DMXAA treatment could prolong survival in infected mice, and confirm that this protection was IRF-3/IFN dependent. In order to test this, we administered DMXAA to C57BL6J and IRF-3$^{-/-}$ mice, infected them with HSV-1 via the cornea and measured survival (Fig. 4A). As expected, DMXAA treatment conferred a survival benefit ($p < 0.05$) on C57BL/6J but not IRF-3$^{-/-}$ mice, consistent with the hypothesis that DMXAA acts through the STING-IRF-3 pathway (Fig. 4A). Furthermore, untreated IRF-3$^{-/-}$ mice succumbed to infection more rapidly than untreated C57BL/6J mice therefore confirming an overall role for IRF-3 in control of HSV infections. Finally, we wished to determine if pretreatment of mice with DMXXA was necessary to confer a survival benefit, or whether postinfection treatment was sufficient. To assess this, C57BL/6J and Sting$^{GT/GT}$ mice were pretreated with DMXAA 1 day prior to challenge and further treated on days, 1, 3, and 5 postinfection. Pretreatment of C57BL/6J mice with DMXAA resulted in approximately 30% survival while untreated mice all reached end-point criteria within 9 days postinfection (Fig. 4B). In contrast, if mice were only treated postinfection, there was no survival benefit to administration of DMXAA (Fig. 4C). Together, these results demonstrated that DMXAA confers protection *in vivo* through the STING- IRF-3 pathway, but only if administered prior to HSV infection.

4. Discussion

Viral encephalitis is an important cause of morbidity and mortality in the United States and throughout the world with a disproportionate burden on individuals greater than 65 or less than 1 year of age (Shives et al., 2017). A survey of all hospitalizations for encephalitis in the USA from 2000 to 2010 identified more than a quarter of a million patients; the pathogen most frequently identified was HSV, which caused ~14% of all encephalitis cases (Shives et al., 2017; Gnann and Whitley, 2017; Spindler and Hsu, 2012). Despite antiviral therapy, mortality is still higher than 30%, and almost 60% of surviving individuals develop neurological sequelae (Piret and Boivin, 2015; Armangue et al., 2014). This underscores the need for improved treatments, and there has been significant interest in the development of agonists of innate immunity

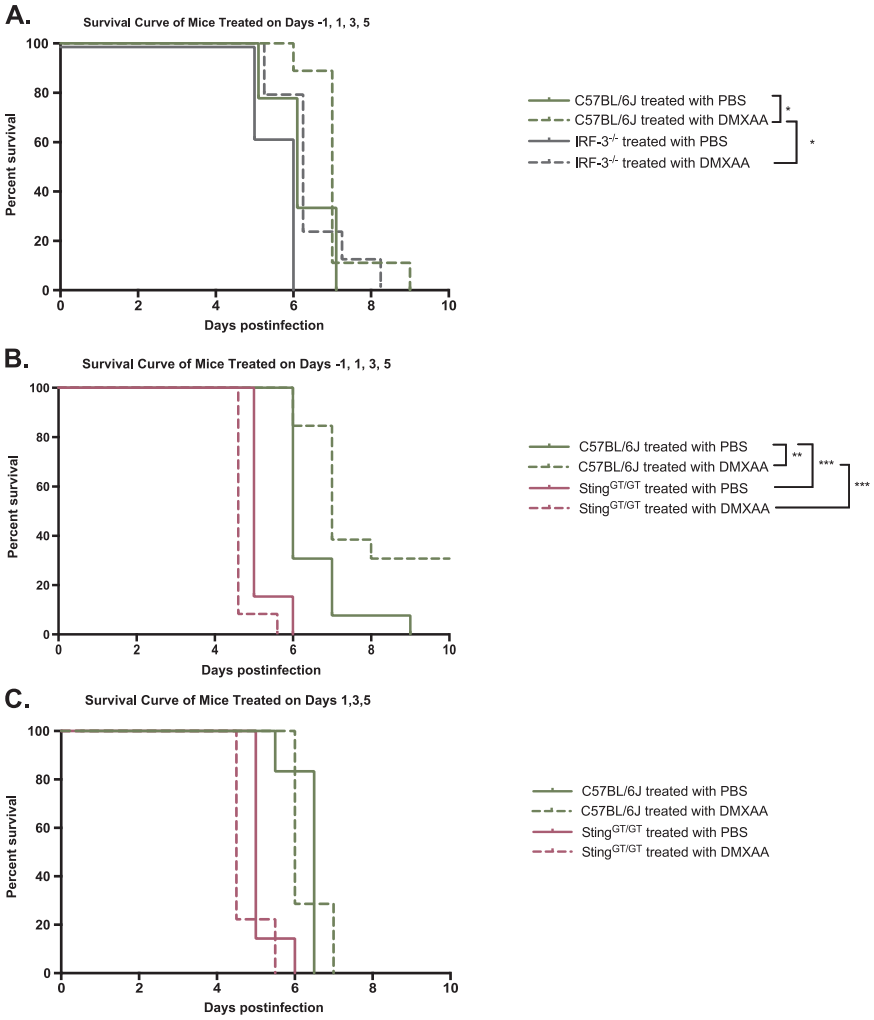

Fig. 4. DMXAA confers increased survival to HSV-1 McKrae-infected C57BL/6J mice in a STING-, IRF-3- and pretreatment-dependent manner. (A) Survival analysis of C57BL/6J (green) and IRF-3$^{-/-}$(grey) mice treated with DMXAA (dashed line) or PBS (solid line) prior to ocular challenge with 2.0×10^5 PFU/eye. Mice were treated postinfection on days 1, 3 and 5. n = 8–10 mice per group. (B) Survival analysis of C57BL/6J (green) and Sting$^{GT/GT}$ (magenta) mice challenged with HSV-1 after pre-treatment with DMXAA (dashed line) or PBS (solid line). Mice were also treated on days 1, 3 and 5 postinfection. Each graph is the average of two independent experiments n = 12–13 mice per group. (C) Survival analysis of C57BL/6J (green) and Sting$^{GT/GT}$ (magenta) mice challenged with 2.0×10^5 PFU/eye. Mice were treated with DMXAA (dashed line) or PBS (solid line) on days 1, 3 and 5 postinfection. n = 6–9 mice per group. Statistical significance was tested with Mantel-Cox tests. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$.

as alternatives to nucleoside analogue-based therapies (Cavlar et al., 2013; Skouboe et al., 2018). More specifically, the STING pathway has been highlighted as a key host defense pathway, and a possible nexus for targeting with agonists that could function as broad spectrum antivirals. As discussed further below, our studies suggest that while this approach has potential for success, significant caveats remain.

The STING pathway is clearly pivotal in the induction of an antiviral state within the CNS (Reinert et al., 2016). During CNS infection, activation of microglial cells induces the secretion of pro-inflammatory cytokines and chemokines that contribute to the recruitment of leukocytes across the blood brain barrier (Piret and Boivin, 2015). Microglial cells secrete IFN-β in a STING-dependent manner resulting in the induction of an antiviral state in astrocytes and neurons (Reinert et al., 2016). Consistent with these data, Sting$^{GT/GT}$ mice challenged with HSV-1 displayed neurological disease and rapidly reached end-point criteria. Our data showed no response to DMXAA in Gt mice and cells derived therefrom, suggesting that the antiviral effect of DMXAA is specific to STING. Moreover, given the robust production of IFN-β following DMXAA exposure, it seems likely that type 1 IFN is key to the antiviral state in this setting. To further support this idea, IRF-3$^{-/-}$ cells and mice were unresponsive to DMXAA, consistent with previous work (Shirey et al., 2011). Interestingly, ICP27 interacts with STING and TBK1 to prevent the phosphorylation of IRF-3 (Christensen et al., 2016). It is possible therefore that the high affinity binding of ICP27 to TBK-1 and STING limits the phosphorylation of IRF-3 caused by DMXAA, thereby limiting IFN-β upregulation.

In this study, we have explored DMXAA as a potential therapeutic against HSV-1 replication, disease, and mortality following corneal infection. Recent work has shown that DMXAA and other STING agonists are effective against HSV *in vitro* and *in vivo* following vaginal infection (Skouboe et al., 2018). Our findings are largely congruent, demonstrating that following DMXAA treatment there is a reduction in titers, disease and mortality in mice. Given the specificity of DMXAA for murine STING, any drug targeting human STING would have to be redesigned. That said, xanthenone-4-acetic acid analogues have been identified to induce robust cytokine in human leukocytes, suggesting potential translational applications (Tijono et al., 2013). Our data are consistent with the idea that agonists of STING may be therapeutic for HSV-1. DMXAA, however, appears effective only when used in a pre-treatment paradigm. In addition, we observed weight loss in C57BL/6J mice upon exposure to DMXAA, suggesting that DMXAA has some level of toxicity (data not shown). In a clinical setting, patients treated with IFN may experience mid to severe adverse events, although this is typically associated with the dose and duration of interferon (Vial and Descotes, 1994). These are important parameters for future analysis with DMXAA and future derivatives that are active against human STING.

It is unknown whether DMXAA directly stimulates innate immune cells that then enter the CNS, or whether DMXAA itself can cross the blood brain barrier to stimulate an innate response (Yung et al., 2014). Regardless, DMXAA is able to induce an antiviral response in the CNS and lessen the neurological disease and prevent mortality in 30% of mice. These surviving mice showed a notable absence of neurological symptoms. In addition, DMXAA did not prolong survival of STING- and

IRF-3 deficient mice, suggesting that DMXAA confers protection in a STING/IRF-3-dependent manner. Other studies have shown DMXAA is able to induce an antiviral response against influenza, hepatitis B and chikungunya (Shirey et al., 2011; Guo et al., 2015; Sali et al., 2015). Designing immunotherapies that can target such universal antiviral adaptor proteins promote the intriguing possibility of use of STING agonists as broad-spectrum treatments against a wide range of viruses.

Acknowledgments

This study was supported by a National Institutes of Health grant to D.A.L. (RO1 EY09083). We also acknowledge support from the Geisel School of Medicine Immunology Program Training Grant (T32 AI007363) and an NEI underrepresented minority supplement to S.C.

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