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Neuronal IFN signaling is dispensable for the establishment of HSV-1 latency

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ABSTRACT

IFN responses control acute HSV infection, but their role in regulating HSV latency is poorly understood. To address this we used mice lacking IFN signaling specifically in neural tissues. These mice supported a higher acute viral load in nervous tissue and delayed establishment of latency. While latent HSV-1 genome copies were equivalent, ganglia from neuronal IFN signaling-deficient mice unexpectedly supported reduced reactivation. IFNβ promoted survival of primary sensory neurons after infection with HSV-1, indicating a role for IFN signaling in sustaining neurons. We observed higher levels of latency associated transcripts (LATs) per HSV genome in mice lacking neuronal IFN signaling, consistent with a role for IFN in regulating LAT expression. These data show that neuronal IFN signaling modulates the expression of LAT and may conserve the pool of neurons available to harbor latent HSV-1 genome. The data also show that neuronal IFN signaling is dispensable for the establishment of latency.

Herpes simplex virus 1 (HSV-1) is a highly prevalent neurotropic virus, which cycles between lytic and latent phases of infection (Smith and Robinson, 2002; Xu et al., 2006). HSV-1 establishes latency in the neurons of sensory ganglia, and reactivation from latency can result in disease pathologies ranging from herpes labialis to herpes simplex encephalitis (HSE) (Rowe et al., 2013; Whitley and Gnann, 2002). The mechanisms governing the establishment of, maintenance of and reactivation from latency are unclear, particularly at the level of the sensory neuron. A global interferon (IFN)-driven antiviral response is critical for protection against HSV-1 infection in mice and humans (Andersen et al., 2015; Casrouge et al., 2006; Dupuis et al., 2003; Leib et al., 1999; Luker et al., 2003; Menachery et al., 2010; Zhang et al., 2007). Moreover, neuronal IFN signaling plays a pivotal role in controlling acute HSV-1 replication and pathogenesis (Rosato and Leib, 2015). Accordingly, patients with genetic defects in antiviral signaling suffer from recurrent HSE and neurons derived from these patients are more permissive to HSV infection (Lafaille et al., 2012). We therefore hypothesized that neuronal IFN signaling is important for the establishment and maintenance of HSV-1 latency.

Corneal HSV infection of mice lacking IFNαβγ receptors or STAT1, a critical transcription factor mediating IFN receptor signaling, results in 100% mortality (Pasieka et al., 2011). While these models demonstrate the importance of IFN signaling, the high mortality renders them unsuitable for studying latency. To address this, we used a conditional knock-out mouse, Stat1$^{N-/-}$, lacking STAT1 specifically in neural tissues (Rosato and Leib, 2015). We infected Stat1$^{N-/-}$ and Stat1$^{fl/fl}$ littermate control mice with WT HSV-1 strain KOS via the cornea (Smith, 1964). While infection of Stat1$^{N-/-}$ mice with 10^5 or 10^6 PFU HSV-1 led to near 100% lethality, approximately 75% of Stat1$^{N-/-}$ mice survived an inoculum of 10^3 PFU (Fig. 1A). Therefore, to maximize post-infection survival, we performed all following experiments using this inoculum. Disease scores and weight changes in the surviving Stat1$^{N-/-}$ mice were identical to those observed in Stat1$^{fl/fl}$ controls (Fig. 1B,C).

We previously observed increased viral titers in nervous system tissues of Stat1$^{N-/-}$ compared to control mice when infected with strain 17, a more neurovirulent strain of HSV-1 (Rosato and Leib, 2015). In the current model, at 8dpi, titers of strain KOS were low or undetectable in the trigeminal ganglia (TG), brain stem and brain of Stat1$^{fl/fl}$ mice (Fig. 2A). There were, however, significantly increased viral titers in the nervous system of Stat1$^{N-/-}$ mice, consistent with previous data (Rosato and Leib, 2015). This demonstrates that without STAT1 signaling there is prolonged acute replication and therefore delayed establishment of latency.

We next tested whether neuronal IFN signaling impacts the establishment or maintenance of HSV-1 latency. To assess establishment of latency, we quantified HSV-1 genomes at 28dpi by qPCR, measuring HSV TK copies normalized to the single-copy

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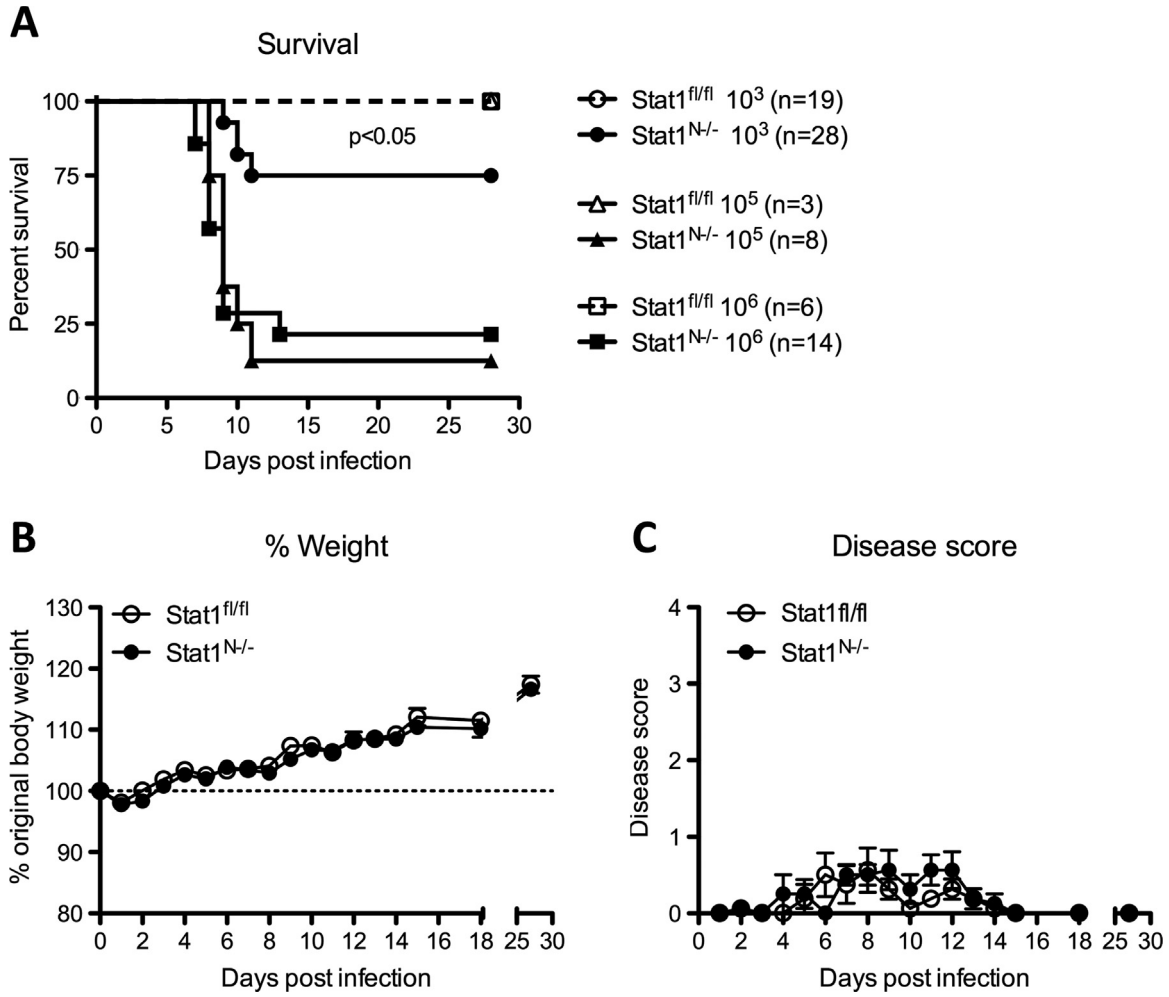

Fig. 1. Mouse model to study neuronal IFN signaling and HSV-1 latency. Mice expressing CRE recombinase under the neuron-specific nestin promoter were crossed with $Stat1^{fl/fl}$ mice to yield progeny with a neural-specific Stat1 deletion ($Stat1^{N-/-}$). (A) Survival of mice infected via the cornea with 10^3 (circles), 10^5 (triangles) or 10^6 (squares) HSV-1 PFU/eye. Mice were euthanized upon reaching endpoint criteria, here stated as survival. Significance was determined by Log-rank test. (B) The percentage of original body weight in infected $Stat1^{N-/-}$ or $Stat1^{fl/fl}$ mice over time. (C) Clinical score of HSV-1-infected $Stat1^{N-/-}$ or $Stat1^{fl/fl}$ mice over time. Mice were scored on a scale of 1–4 clinical severity of HSV-1 disease by a masked observer (Jiang et al., 2015). All data are collected from ≥ 2 independent experiments.

mouse adipsin gene (Kramer and Coen, 1995). We detected genomes in 20/23 $Stat1^{N-/-}$ TGs, and 29/30 $Stat1^{fl/fl}$ TGs. Additionally, infectious virus was undetectable in both $Stat1^{fl/fl}$ and $Stat1^{N-/-}$ mice, demonstrating that, by 28dpi, latency had been fully established in both groups of mice. To determine whether IFN signaling regulates the latent state, we measured the expression of the latency-associated transcript (LAT) by qRT-PCR (Pan et al., 2014). We found significantly more LAT transcripts per HSV genome in $Stat1^{N-/-}$ mice (Fig. 2B), consistent with the hypothesis that IFN signaling may downregulate the expression of LAT (Catez et al., 2012). Taken together, these data demonstrate that neuronal IFN signaling is dispensable for the establishment of HSV-1 latency, but may play a role in its regulation.

Given the crucial role that IFN signaling plays in controlling HSV lytic replication in neurons, we expected to find more latent genomes and greater reactivation in $Stat1^{N-/-}$ relative to $Stat1^{fl/fl}$ TGs. Unexpectedly, in the samples with detectable genomes, we found no significant difference in HSV genome levels between $Stat1^{N-/-}$ and $Stat1^{fl/fl}$ TGs (Fig. 2C). Moreover, using a TG explant model of reactivation (Leib et al., 1989), we detected significantly fewer reactivation events from $Stat1^{N-/-}$ TGs, compared to controls (Fig. 2D). This demonstrates that despite a 10–100 fold higher viral load during acute timepoints there is equivalent establishment of latency and reduced reactivation in TGs lacking IFN signaling.

IFNβ is required for long-term neuronal homeostasis even in the absence of infection (Ejlerskov et al., 2015), and can promote viability of neuron cultures after virus infection (Low-Calle et al., 2013; Samuel and Diamond, 2005). We therefore hypothesized that IFN signaling prevents neuronal loss during the initial infection, thereby sustaining the population of neurons that harbors latent HSV-1 genome and reactivation events. Due to the technical challenges of quantifying total numbers of neurons from latently infected TGs, we instead cultured TG neurons from 129SVEV (WT) or STAT1 null ($STAT1^{-/-}$) adult mice to test this hypothesis (Bertke et al., 2011; Durbin et al., 2002; Rosato and Leib, 2014). Since STAT1 is involved in IFNα/β (Type I), IFNγ (Type II) and IFNλ (Type III) signaling, we tested the ability of all three IFNs to promote neuronal survival after HSV infection. Adult TG neuron cultures were pre-treated with 100 U/mL IFNβ, or 100ng/mL IFNγ or IFNλ for 18 h and then infected with HSV-1 64-GFP KOS, a virus that expresses GFP under the CMV IE promoter (Van Heyningen and Leib, unpublished), to facilitate visualization and quantification of infected neurons. Since adult neurons are fully differentiated and do not undergo division, we were able to examine neuronal survival by quantifying the number of neurons remaining in the cultures, assessed by staining for the neuronal marker NeuN. At 24hpi, we found significantly fewer GFP-positive neurons in WT relative to $STAT1^{-/-}$ cultures in untreated and IFNβ treated groups