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Homeostatic interferon expression in neurons is sufficient for early control of viral infection ☆

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ABSTRACT

The mechanisms by which neurons respond to inflammatory mediators such as interferons (IFNs) remain largely undefined. We previously showed that the activation and nuclear localization of the core IFN signaling molecule, Stat1, are muted and delayed in primary mouse hippocampal neurons treated with IFN gamma as compared to control mouse embryonic fibroblasts (MEFs). Here, we show that the kinetics of Stat1 and Stat2 activation following type I IFN exposure are also unique in neurons, affecting gene expression and neuronal response. Specifically, despite lower basal expression of many IFN stimulated genes in neurons, basal expression of the type I IFN themselves is significantly higher in primary hippocampal neurons compared to MEF. Elevated homeostatic IFN in neurons is critical and sufficient for early control of viral infection. These data provide further evidence that neurons exploit unique signaling responses to IFNs, and define an important contribution of homeostatic IFN within the CNS. Such differences are likely critical for the ability of neurons to survive a viral challenge.

1. Introduction

The host immune response can efficiently resolve certain neurotropic infections, but unregulated or chronic immune responses in the central nervous system (CNS) can be pathogenic and often fatal. Immune dysregulation within the brain can result in encephalitis and meningitis, and contributes to many chronic neuroinflammatory diseases such as multiple sclerosis (Kundig et al., 1993; Binder and Griffin, 2001; Dorries, 2001; Griffin and Metcalf, 2011; Smith et al., 2012; Wilms et al., 2007). Therefore, a balance must be achieved in which pathogen control or clearance is achieved with minimal neuropathology. This is particularly relevant for infections of CNS neurons, which are a chiefly non-renewable cell population. Well-defined immune mechanisms that clear viral infections in the periphery, including perforins and granzymes, are underutilized in the brain, perhaps protecting the neuronal population from immune-mediated cytolysis. Instead, cytokines, including the interferons (IFNs), are fundamental contributors to CNS virus clearance. Thus, an overarching goal of our studies is to elucidate the unique interactions of IFNs with neuronal targets, and to define how the consequences of IFN signaling limit or clear neurotropic infections in the absence of CNS disease.

Viral reproduction in the CNS is a relatively rare, albeit serious, outcome of infection by a number of human viruses. While some viruses are well-known to be neurotropic (e.g., poliovirus, rabies virus, West Nile virus, and some herpesviruses), others that are primarily associated with peripheral infections, including influenza and measles, can also result in life-threatening CNS complications (Johnson, 1998). For example, influenza has been associated with encephalitis, Reye's syndrome, and acute necrotizing encephalopathy, particularly in children (Studahl, 2003). Moreover, measles virus (MV) infection of CNS neurons is associated with invariably fatal diseases such as subacute sclerosing panencephalitis, which can occur months to years after primary virus exposure (Dubois-Dalcq et al., 1974). While some of these viruses gain access to the brain parenchyma due to a weakened immune response (e.g., herpesviruses), most result in neuropathology via induction of the immune response (James et al., 2009).

An early and essential line of defense against viral infection is the induction of interferons (IFNs) that ultimately leads to antiviral gene expression (reviewed in Stark et al., 1998). Briefly, in most mammalian cells, viruses are detected by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and retinoic acid-inducible gene-I (Rig-I). Recognition of viral nucleic acid in infected cells by these receptors leads to downstream signaling, including activation of transcription factors NF-kB and IFN-regulatory factors 3 and 7 (Irf3, Irf7). Migration of these transcription factors into the nucleus induces expression of genes encoding type I IFNs (IFNα, IFNβ). Once released from the cell, IFNs then

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bind to their receptor (Ifnar, composed of Ifnar1 and Ifnar2 subunits) on the surface of neighboring cells, leading to activation of Janus-activated kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2). Signal transducers and activators of transcription 1 and 2 (Stat1, Stat2) are recruited to the activated receptor and are phosphorylated by Jak1 and Tyk2, resulting in a complex of Stat1, Stat2 and IFN regulatory factor 9 (Irf9). This ISGF3 complex then translocates to the nucleus and binds IFN-sensitive response elements (ISREs) in the promoters of IFN-stimulated genes (ISGs). Expression of ISGs leads to induction of an antiviral state (reviewed in Stark et al., 1998).

While IFNs are generally recognized as indispensable for an effective immune response, cell type-specific properties may diversify how cells respond to the same extracellular ligand. This is governed in part by the variety, level and distribution of Stat molecules in a given cell type, as type I IFNs are able to activate all seven known Stat molecules. Thus, following engagement of the IFN receptor, distinct Stat homo- and heterodimer complexes may form, and interactions between such complexes and promoter elements determine what genes will be up- or down-regulated (reviewed in von Boxel-Dezaire et al., 2006). For example, in immune cells, type I IFN aids in activation and Th1 skewing of naive T cells (Hibbert et al., 2003), promotes survival and cytolytic activity of $CD8^+$ T cells, and ensures efficient antibody response by B cells (Nishikomori et al., 2002; Curtsinger et al., 2005). Non-immune cells have varied responses to IFNs as well. For example, in primary human hepatocytes, IFNα triggers an antiviral and antitumor state (Radaeva et al., 2002). Cardiac myocytes, which, like neurons, are a non-renewable cell population, express higher basal levels of IFNβ than cardiac fibroblasts, likely as a "pre-arming" mechanism to protect against viral infection (Zurney et al., 2007). Within the CNS, distinct cellular responses to IFN could play a role in infection and immunity as well as neurodegenerative diseases and the response to injury or ischemia (Rose et al., 2007).

We utilize a mouse model of neuron-restricted measles virus (MV) infection, in which CNS neurons express a human MV vaccine strain receptor (CD46) under the transcriptional control of the neuron-specific enolase (NSE) promoter (NSE-CD46) (Rall et al., 1997). Using this model, we showed that adult mice clear MV infection from CNS neurons without neurological damage or neuronal loss in an IFNγ- and T cell-dependent manner (Patterson et al., 2002). Primary hippocampal neurons can be explanted from these mice and grown in culture; while all neurons of NSE-CD46 transgenic mice are CD46-positive, and therefore permissive, the hippocampus of day E15–16 embryos is very neuron-rich, enabling virtually pure cultures. When we assessed IFNγ signaling in these hippocampal neurons, we found that the response to IFNγ treatment was distinct from that observed in control MEF. Specifically, neurons responded to IFNγ with delayed and attenuated kinetics of Stat1 expression and activation, resulting in a concomitantly delayed and reduced expression of classic IFNγ-dependent genes (Rose et al., 2007). These foundational studies indicate that IFNγ activates a critical antiviral program in neurons, but that Stat1 plays a subordinated role in this response.

Because Stat1 is also a key mediator in type I IFN signaling, we wished to characterize the response of CNS neurons to type I IFN in the context of viral infection. Utilizing NSE-CD46 mice and primary neurons explanted from these mice, we show here that hippocampal neurons respond to type I IFN with a unique ISG signature. Moreover, exogenous type I IFN enhances resistance to viral infection in primary fibroblasts, but not in primary neurons. We provide evidence that neurons express increased basal levels of type I IFNs compared to fibroblasts, which we hypothesize is critical for controlling early infection in the absence of exogenous IFN. This suggests that within the CNS, neurons respond uniquely to crucial cytokines produced soon after infection, and we hypothesize that this may be a protective mechanism to ensure survival during infection and the subsequent adaptive immune response.

2. Materials and methods

2.1. Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was reviewed and approved by the Fox Chase Cancer Center Institutional Animal Care and Use Committee (Office of Laboratory Animal Welfare assurance number A3285-01).

2.2. Cells, viruses, mice, and infections

Primary hippocampal neurons were prepared from embryonic (E15–16) mice, as described (Rose et al., 2007; Rall et al., 1995; Banker and Goslin, 1991; Pasick et al., 1994). Neurons were plated on 15-mm glass coverslips or in 12-well plates coated with poly-L-lysine (Sigma-Aldrich) at a density of 2×10^5 cells/well, unless otherwise noted. Neuron cultures were quality-controlled, and were routinely >95% Map2-positive. Neurons were plated and incubated for 5 days (d) to allow for full differentiation prior to IFNβ treatment or infection. Primary mouse embryonic fibroblasts (MEFs) were isolated from the same embryos and maintained in complete DMEM medium (DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 ng/mL streptomycin). All cells were maintained at 37 °C, 5% CO_2 in a humidified incubator.

MV-Edmonston (vaccine strain) was purchased from the American Type Culture Collection and passaged and titered in Vero cells. Passages 2 or 3 of the MV stock were used for intracerebral (IC) injections and in vitro infection assays. LCMV Armstrong (LCMV-Arm; ATCC) was passaged in BHK-21 fibroblasts and plaque purified, and titers were determined on Vero fibroblasts.

Homozygous NSE-$CD46^+$ transgenic mice (line 18; H-2^b) (Rall et al., 1997) were maintained in the closed breeding colony of the Fox Chase Cancer Center. Homozygous NSE-$CD46^+$ and haplotype-matched homozygous immune knockout (KO) mice were intercrossed for three or more generations to obtain NSE-$CD46^+$ mice on the desired KO background. Ifnar-deficient mice (Muller et al., 1994) on 129S2/SvPas background were obtained from Luis Sigal (Fox Chase Cancer Center, Philadelphia, PA). Genotypes of all mice used in these experiments were confirmed by PCR analysis of tail biopsy DNA and/or flow cytometry on blood cells.

Isoflurane-anesthetized mice were infected with MV via IC inoculation (1×10^4 PFU in a volume of 30 μL, delivered along the midline using a 27 g needle). Mice were monitored daily post-infection for signs of illness, including weight loss, ruffled fur, ataxia, and seizures. Moribund mice were euthanized in accordance with IACUC guidelines.

2.3. Infection and interferon treatment of primary cells

Five days post-plating, primary hippocampal neurons or MEF from NSE-$CD46^+$ or CD46/Ifnar1 KO mice were infected with MV or LCMV (multiplicity of infection [MOI] = 1) for 1 h. Thereafter, the inoculum was removed and the cells were maintained in conditioned Neurobasal media. For cells treated with IFNβ, murine IFNβ (Millipore) was diluted in B27-free Neurobasal media, added to the cultures (100 U/mL final), and incubated for the indicated times prior to collection.

2.4. Immunoblots

Untreated and IFNβ-treated primary cells cultured on tissue culture plastic were lysed directly with protein solubilization buffer (106 mM Tris HCl, 141 mM Tris Base, 0.51 mM EDTA, 2% SDS). Where indicated, total cellular protein isolated from 2×10^5 cells per sample was separated on a NuPAGE 7% Tris–Acetate gel or 10% Bis–Tris gel (Invitrogen), and transferred (semi-dry) to PVDF-FL (Millipore). Within an experiment, corresponding samples from neurons and MEF were run on