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Altered levels of STAT1 and STAT3 influence the neuronal response to interferon gamma

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Abstract

As immune responses in the CNS are highly regulated, cell-specific differences in IFNγ signaling may be integral in dictating the outcome of host cell responses. In comparing the response of IFNγ-treated primary neurons to control MEF, we observed that neurons demonstrated lower basal expression of both STAT1 and STAT3, the primary signal transducers responsible for IFNγ signaling. Following IFNγ treatment of these cell populations, we noted muted and delayed STAT1 phosphorylation, no detectable STAT3 phosphorylation, and a 3–10-fold lower level of representative IFNγ-responsive gene transcripts. Moreover, in response to a brief pulse of IFNγ, a steady increase in STAT1 phosphorylation and IFNγ gene expression over 48 h was observed in neurons, as compared to rapid attenuation in MEF. These distinct response kinetics in IFNγ-stimulated neurons may reflect modifications in the IFNγ negative feedback loop, which may provide a mechanism for the cell-specific heterogeneity of responses to IFNγ.

Keywords: Cytokine; Signal transduction; Gene regulation; Neuron; Interferon gamma; STAT1; STAT3

1. Introduction

Interferon gamma (IFNγ), a pluripotent cytokine made primarily by T cells and NK cells, triggers the induction of genes that lead to antiviral and antibacterial responses, and modulates the expression of genes governing immune function, including components of the MHCI and MHCII antigen presentation pathways. IFNγ plays a crucial role in noncytolytic clearance of viruses in the "immune-privileged" environment of the central nervous system (CNS), including vesicular stomatitis virus (VSV) (Komatsu et al., 1996), measles virus (Patterson et al., 2002), Theiler's murine encephalomyelitis virus (Rodriguez et al., 2003), Sindbis virus (Burdeinick-Kerr and Griffin, 2005), and West Nile virus (Shrestha et al., 2006). IFNγ is also crucial for the resolution of some intracellular bacterial infections within the brain (Jin et al., 2004). However, IFNγ has also been implicated in the immunopathogenesis of demyelinating diseases such as multiple sclerosis (reviewed in Sanders and De Keyser, 2007), ischemia (Takagi et al., 2002), and other neurodegenerative disorders, such as Alzheimer's Disease (Bate et al., 2006). Moreover, IFNγ plays a key role in CNS homeostasis, development, and neurotransmitter receptor expression (Barish et al., 1991; Kraus et al., 2006; Wong et al., 2004).

Activation of IFNγ-stimulated gene expression occurs via a well-characterized signal transduction pathway (reviewed in Darnell, 1997; Stark et al., 1998). Briefly, IFNγ binding and subsequent assembly of its receptor complex (consisting of a heterotetramer of IFNγR1 and R2 subunits), stimulates the activation of receptor-associated JAK1 and JAK2 protein tyrosine kinases, resulting in the tyrosine phosphorylation of the cytoplasmic tail of the IFNγR1 subunits. Upon docking to the phosphorylated R1 subunit, signal transducer and activator of transcription (STAT)-1 is phosphorylated on tyrosine 701 (pY701), resulting in its homodimerization. The STAT1 (pY701) homodimer then translocates to the nucleus and binds to Gamma Activated Sequence (GAS) elements within

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the promoters of IFNγ-responsive genes, thus influencing their expression. In addition to STAT1, IFNγ stimulation also results (to a lesser degree) in phosphorylation of STAT3. Upon activation, STAT3 can homodimerize or form a heterodimer with phosphorylated STAT1, translocate to the nucleus and also bind to GAS elements.

In spite of the relatively straightforward nature of these well-characterized signal transduction pathways, the cellular response to IFNγ is complex and cell-specific. The genes that are induced in IFNγ-stimulated cells can result in a wide range of consequences, including cellular activation, proliferation, or the induction of apoptosis (reviewed in Stark et al., 1998). While it is clear that IFNγ can elicit varied outcomes, the mechanisms governing the way a given cell responds to IFNγ remain largely unclear. Previous studies have examined differences in GAS element binding and transcription factor specificity (e.g. Horvath et al., 1995; reviewed in Ramana et al., 2000; Schroder et al., 2004) to elucidate mechanisms of cell-specific responses to IFNγ. In addition, many of the studies characterizing the IFNγ response have focused on a single cell type (such as hepatocytes, fibroblasts, or transformed cell lines). This has resulted in the impression that the cytoplasmic signaling pathways triggered in response to IFNγ are somewhat generic, and potential cellular or developmental differences in upstream IFNγ signaling events have therefore been largely overlooked. Recently however, Qing and Stark demonstrated that in the absence of STAT1, IFNγ signals through STAT3 and induces overlapping but distinct gene products (Qing and Stark, 2004). These investigators proposed that differential use of signaling pathways could therefore explain some of the differences observed in IFNγ responses by diverse cell types. In addition, Costa-Pereira et al. demonstrated that cell lines expressing two IFNγ receptors differing in a single amino acid showed altered kinetics of STAT phosphorylation, which resulted in diverse profiles of downstream gene transcription (Costa-Pereira et al., 2005).

While a number of studies underscore the ability of neurons to make and respond to both type I and type II interferons (Chesler et al., 2004; Delhaye et al., 2006; Goody et al., 2007; Massa et al., 1999; Samuel et al., 2006; Trottier et al., 2005; Wang and Campbell, 2005; Yang et al., 2006), a direct comparison of IFNγ signaling in otherwise unmanipulated primary cells of varied tissue origin is lacking. Because immunity in the CNS is highly regulated, cell-specific differences in IFNγ signaling pathways may be particularly important in dictating the outcome of the host cell response in various pathogenic settings. We have therefore directly and quantitatively compared the responses of primary hippocampal neurons and matched primary fibroblasts to IFNγ. We have investigated expression and phosphorylation of STAT1 and STAT3, as well as the duration of the cellular response to IFNγ. We found that the neuronal response was remarkably distinct from that of control fibroblasts, providing support for the notion that differences at the level of signal transduction exist between cell types of distinct tissue origin. Furthermore, we demonstrated alterations in the expression of several IFNγ-responsive genes in treated neurons and fibroblasts, underscoring the importance of both the timing and magnitude of STAT signaling pathways in orchestrating the cell-specific response to exogenous IFNγ.

2. Materials and methods

2.1. Cells and culture conditions

Primary hippocampal neurons were prepared from embryonic (E14–15) inbred, c57Bl/6 mice (Rall et al., 1997) as previously described (Banker and Goslin, 1991; Pasick et al., 1994; Rall et al., 1995), with the exception that neurons were maintained in serum-free neurobasal medium (Life Technologies, Grand Island, NY) supplemented with B27 supplement (Life Technologies), glutamate (4 μg/ml), penicillin (100 U/ml), streptomycin (100 ng/ml), and glutamine (2 mM) in the absence of an astrocyte feeder layer. These cultures are routinely >95% pure, as assessed by MAP-2 immunostaining. Primary mouse embryonic fibroblasts (MEF) 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). Briefly, liver tissue was excised and discarded, and the remaining tissue was then dissociated in 0.4% trypsin, followed by trituration with a 10 ml pipette. The suspension was incubated for 10 min at 37 °C; 5 ml fresh trypsin was then added and the suspension was incubated for an additional 10 min at 37 °C. The suspension was transferred to a 15 ml conical tube, in which undigested tissue was allowed to settle for 2 min. The supernatant (containing MEF) was mixed with complete DMEM medium and centrifuged at 400 ×*g* for 5 min. The resulting pellet was resuspended in complete DMEM medium and plated into culture flasks. All cells were maintained at 37 °C, 5% CO_2 in a humidified incubator.

2.2. "Continual" IFNγ treatment

Neurons were plated on poly-L-lysine (Sigma, St. Louis, MO) coated glass coverslips or poly-L-lysine coated tissue culture plastic at a density of 560 cells/mm^2, and cultured for 5 days (unless specified otherwise). MEF were plated at a density of 280 cells/mm^2 one day prior to treatment. On the day of treatment, the culture medium was supplemented with either recombinant mouse IFNγ (BD Biosciences Pharmingen, San Jose, CA; 100 U/ml in Dulbecco's phosphate buffered saline (DPBS)), or with an equal volume of DPBS alone. Cells were incubated for the indicated times, and then lysed for protein or RNA isolation (described below).

2.3. "Pulsed" IFNγ treatment

Neurons and MEF were plated and treated as described above, with the exception that cells were incubated with or without IFNγ (100 U/ml) for only 30 min. After incubation, cells were washed 10 times with DPBS, to ensure removal of exogenously-added IFNγ. Unsupplemented conditioned culture medium was then added back to the cells, which were incubated