Virology 396 (2010) 21–29

Contents lists available at ScienceDirect

Virology

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

Herpes simplex virus-1 infection causes the secretion of a type I interferon-antagonizing protein and inhibits signaling at or before Jak-1 activation

Karen E. Johnson, David M. Knipe *

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 19 June 2009
Returned to author for revision 28 July 2009
Accepted 16 September 2009
Available online 31 October 2009

Keywords:
HSV-1
Innate immunity
Immune evasion
ICP27
Secreted protein
Type I interferon

ABSTRACT

Host cells respond to viral infection by the production of type I interferons (IFNs), which induce the expression of antiviral genes. Herpes simplex virus I (HSV-1) encodes many mechanisms that inhibit the type I IFN response, including the ICP27-dependent inhibition of type I IFN signaling. Here we show inhibition of Stat-1 nuclear accumulation in cells that express ICP27. ICP27 expression also induces the secretion of a small, heat-stable type I IFN antagonizing protein that inhibits Stat-1 nuclear accumulation. We show that the inhibition of IFN-induced Stat-1 phosphorylation occurs at or upstream of Jak-1 phosphorylation. Finally, we show that ISG15 expression is induced after IFNα treatment in mock-infected cells, but not cells infected with WT HSV-1 or ICP27^{-} HSV-1. These data suggest that HSV-1 has evolved multiple mechanisms to inhibit IFN signaling not only in infected cells, but also in neighboring cells, thereby allowing for increased viral replication and spread.

Introduction

One of the first lines of defense that is activated upon infection of a host with a pathogen is the interferon (IFN) response. Type I IFNs (α, β, ω, τ) are a family of antiviral cytokines induced in most cell types by viral infection or the presence of double-stranded RNA and acts in an autocrine and paracrine manner to establish an antiviral state in host cells (Sato et al., 2000). Type II IFN (γ) is a pro-inflammatory cytokine induced in activated T cells and natural killer cells (Schiller et al., 2006). Though there are distinct similarities in the signaling pathways activated by each type of IFN, there are also some key differences. Each family of IFN binds to a distinct heterodimeric receptor (Kotenko et al., 2003; Platanias and Colamonici, 1992; Platanias, Uddin, and Colamonici, 1994; Sheppard and York, 1990), which causes the activation of Janus kinases (Jaks) by phosphorylation. The kinases Jak-1 and Tyk-2 are activated in the case of type I IFN, and Jak-1 and Jak-2 for type II IFN (Darnell, Kerr, and Stark, 1994; David et al., 1993; Platanias, Uddin, and Colamonici, 1994). The Jaks phosphorylate signal transducers and activators of transcription (Stats)-1 and-2, in type I IFN signaling, and only Stat-1 after exposure to IFNγ (Platanias, Uddin, and Colamonici, 1994; Schindler et al., 1992; Uddin, Chamdin, and Platanias, 1995). Once activated by phosphorylation, Stat-1 either homodimerizes (IFNγ) or forms a complex with Stat-2 and with interferon regulatory factor 9 (IFNα/β) (Bandyopadhyay et al., 1995; Kessler et al., 1990; Ramana et al., 2002). These complexes translocate into the nucleus and bind specific DNA elements, interferon stimulated response elements (ISREs, type I signaling) or gamma activated sequences (GASs, type II signaling), to activate transcription of interferon stimulated genes (ISGs). ISGs contribute to the pro-inflammatory or antiviral state and include RNase L, which degrades viral and cellular RNAs (Dong and Silverman, 1995; Kerr and Brown, 1978) and PKR, which inhibits protein synthesis by phosphorylating the translation initiation factor eIF2a (Der et al., 1998; Samuel, 1979a,b).

Viruses have evolved mechanisms to evade or counteract the effects of IFNα/β signaling. Several viral proteins, such as the influenza virus NS1 protein and the human papilloma virus (HPV) E6 oncoprotein inhibit expression of type I IFN by blocking the activation or activity of interferon regulatory factor 3 (IRF3), a transcription factor important for type I IFN production (Ronco et al., 1998; Talon et al., 2000). The vaccinia virus protein B18R is secreted from cells and binds IFN in the extracellular space to prevent its binding to cells (Alcamí and Smith, 1995; Colamonici et al., 1995). Other viral proteins, such as cytomegalovirus (CMV) IE1, measles V protein, and dengue virus NS4B, inhibit the signaling pathway itself (Gao et al., 1997; Muñoz-Jordan et al., 2003; Paulus, Krauss, and Nevels, 2006; Yokota et al., 2003).

Herpes simplex virus 1 (HSV-1) is a large, double-stranded DNA virus that productively infects epithelial cells and establishes a latent infection in sensory ganglia for the life of the host (Roizman, Knipe, and Whitley, 2007). In cells that have been exposed to IFNα prior to infection, HSV-1 replication is severely reduced compared with cells infected in the absence of IFN (Altinkilic and Brandner, 1988; Mittnacht et al., 1988; Oberman and Panet, 1988; Pierce et al., 2005). However, cells that are infected with HSV-1 and then treated with IFN show reduced IFN signaling and decreased ISRE reporter

* Corresponding author. Fax: +1 617 432 0223.
E-mail address: david_knipe@hms.harvard.edu (D.M. Knipe).

0042-6822/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2009.09.021

gene activity (Chee and Roizman, 2004; Johnson, Song, and Knipe, 2008; Yokota et al., 2001). One anti-IFN activity that has been characterized for HSV-1 is the ICP0-dependent inhibition of IRF-3 stimulated IFNβ expression (Melroe et al., 2007). Second, the HSV-1 late protein γ34.5 binds protein phosphatase 1 to counteract the activity of PKR, by causing the dephosphorylation and reactivation of eIF2a (Chou et al., 1995; He, Gross, and Roizman, 1997, 1998; Leib et al., 2000). We have also shown that HSV-1 ICP27 is necessary and sufficient to inhibit IFNα-induced Stat-1 phosphorylation and nuclear accumulation (Johnson, Song, and Knipe, 2008). The effect was observed by 2–4 hpi, so this is likely an early event in HSV infection.

ICP27 is a multifunctional immediate early protein with homologs in all herpesviruses (Roizman, Knipe, and Whitley, 2007) that is essential for transcription of some early and late viral proteins (Jean et al., 2001). Early in infection it is mostly nuclear but has been shown to shuttle between the nucleus and cytoplasm later in infection (Clements et al., 2004; Soliman, Sandri-Goldin, and Silverstein, 1997). It has roles in transcriptional regulation through association with RNA polymerase II (Zhou and Knipe, 2002) and translation through association with translation factors eIF3, eIF4g, and PABP (Ellison et al., 2005; Fontaine-Rodriguez and Knipe, 2008; Fontaine-Rodriguez et al., 2004). ICP27 also affects RNA processing through interactions with splicing machinery (Hardwicke and Sandri-Goldin, 1994; Hardy and Sandri-Goldin, 1994; Phelan et al., 1993; Sandri-Goldin and Hibbard, 1996) and regulation of differential polyadenylation (Hann et al., 1998; McGregor et al., 1996; McLauchlan et al., 1992; McLauchlan, Simpson, and Clements, 1989). ICP27 associates with RNA via its RGG box and CR1 regions to stabilize A/U-rich RNAs (Brown et al., 1995; Ingram et al., 1996). In some studies, it has also been implicated in nuclear export of some viral transcripts (Koffa et al., 2001; Mears and Rice, 1998; Pearson, Knipe, and Coen, 2004; Sandri-Goldin, 1998; Wadd et al., 1999). However, other groups have not seen a difference in RNA export during infection with ICP27 mutant viruses (Ellison et al., 2005; Fontaine-Rodriguez and Knipe, 2008; Pearson, Knipe, and Coen, 2004).

We performed immunofluorescence experiments to determine that ICP27 was necessary and sufficient for inhibition of Stat-1 phosphorylation and nuclear accumulation. In these experiments we also observed that even after IFNα-treatment, many cells that did not stain positive for ICP27 still did not show nuclear accumulation of Stat-1 (Johnson, Song, and Knipe, 2008). It appeared that ICP27 expression was causing a bystander effect in surrounding cells through an unknown mechanism.

There have been several hypotheses about the mechanism(s) by which IFN signaling is inhibited by HSV-1, with possible mechanisms being the HSV-1 virion host shut-off protein (vhs) or the cellular suppressor of cytokine signaling protein SOCS-3 (Chee and Roizman, 2004; Yokota et al., 2005; Yokota et al., 2004). However, the actual mechanism of inhibition is still unknown. In this study, we show that HSV-1 infection inhibits IFN signaling at or before the phosphorylation of Jak-1. In exploring the bystander effect of ICP27 on surrounding cells further, we have also found that HSV-1 infection and ICP27 transfection cause the secretion of a heat-stable, protease-sensitive soluble factor that inhibits IFNα-induced Stat-1 nuclear accumulation in trans.

Results

HSV-1 infection causes bystander cell inhibition of IFNα-induced Stat-1 nuclear accumulation

In our previous study, we observed that a number of cells that did not stain for ICP27 showed mostly cytoplasmic distribution of Stat-1 even after treatment with IFNα (Johnson, Song, and Knipe, 2008). To examine the relationship between ICP27 and Stat-1 distribution in HSV-1 infected cells, we mock-infected or infected Vero cells with WT HSV-1 for 10 h and treated with IFNα at 10^4 U/mL for 30 min prior to fixation. Cells were stained with antibodies to Stat-1 and ICP27 and 200–250 cells per cover slip were scored blindly for Stat-1 localization, as being nuclear, cytoplasmic, or both (Fig. 1A, black arrow–cytoplasmic, white arrow–nuclear, white arrow head–both).

In the absence of IFN, Stat-1 was mostly cytoplasmic in over 70% and both nuclear and cytoplasmic in over 25% of mock-infected cells, but after IFN treatment Stat-1 was redistributed to be approximately 75% nuclear and about 25% cytoplasmic and nuclear (Fig. 1B). After HSV-1 infection, when roughly 20% of cells appeared to be infected (as detected by ICP27 immunofluorescence), Stat-1 was cytoplasmic in nearly 70% of cells and both cytoplasmic and nuclear in about 30% of cells in the absence of IFN (Fig. 1B). After IFNα treatment however, Stat-1 accumulated in the nucleus of only approximately 45% of cells, which is significantly lower than in mock-infected cells ($p<0.01$).

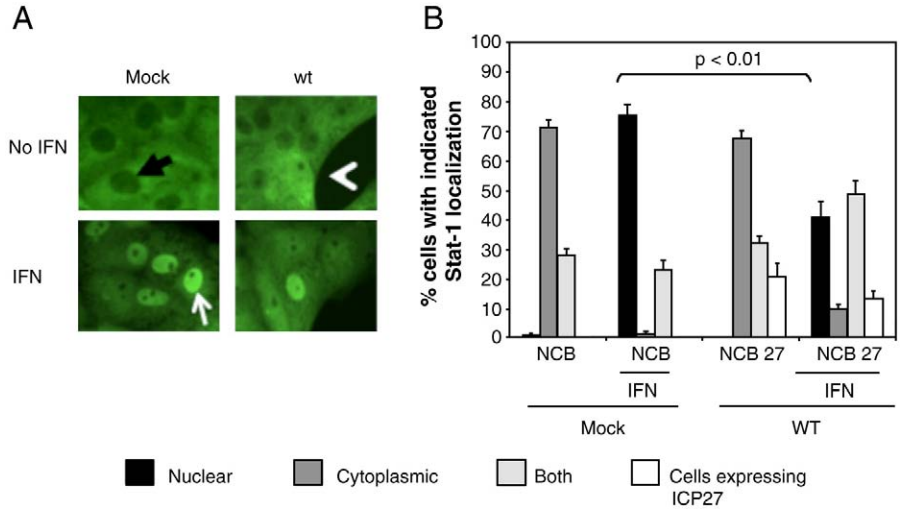

Fig. 1. HSV-1 infection inhibits IFN-induced nuclear accumulation of Stat-1 in surrounding cells. Vero cells were mock infected or infected (MOI = 3) with WT HSV-1 for 10 h and treated with IFN for 30 min before fixation, as indicated. Immunofluorescence was done with antibodies towards Stat-1 and ICP27. Stat-1 localization was scored as being predominantly nuclear (A: white arrow), predominantly cytoplasmic (A: black arrow), or both (A: white arrow head). The percent of cells infected was determined by counting cells that stained positive for ICP27 (B). Data shown are from cell counts from replicate cover slips, and statistical analysis was performed with the Student's *t*-test. The experiment shown is representative of multiple experiments.