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Orf virus inhibits interferon stimulated gene expression and modulates the JAK/STAT signalling pathway

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ABSTRACT

Interferons (IFNs) play a critical role as a first line of defence against viral infection. Activation of the Janus kinase/signal transducer and activation of transcription (JAK/STAT) pathway by IFNs leads to the production of IFN stimulated genes (ISGs) that block viral replication. The *Parapoxvirus, Orf virus* (ORFV) induces acute pustular skin lesions of sheep and goats and is transmissible to man. The virus replicates in keratinocytes that are the immune sentinels of skin. We investigated whether or not ORFV could block the expression of ISGs. The human gene *GBP1* is stimulated exclusively by type II IFN while *MxA* is stimulated exclusively in response to type I IFNs. We found that *GBP1* and *MxA* were strongly inhibited in ORFV infected HeLa cells stimulated with IFN-γ or IFN-α respectively. Furthermore we showed that ORFV inhibition of ISG expression was not affected by cells pretreated with adenosine N1-oxide (ANO), a molecule that inhibits poxvirus mRNA translation. This suggested that new viral gene synthesis was not required and that a virion structural protein was involved. We next investigated whether ORFV infection affected STAT1 phosphorylation in IFN-γ or IFN-α treated HeLa cells. We found that ORFV reduced the levels of phosphorylated STAT1 in a dose-dependent manner and was specific for Tyr701 but not Ser727. Treatment of cells with sodium vanadate suggested that a tyrosine phosphatase was responsible for dephosphorylating STAT1-p. ORFV encodes a factor, ORFV057, with homology to the vaccinia virus structural protein VH1 that impairs the JAK/STAT pathway by dephosphorylating STAT1. Our findings show that ORFV has the capability to block ISG expression and modulate the JAK/STAT signalling pathway.

1. Introduction

Interferons (IFNs) are a multifunctional family of cytokines that play a critical role as a first line of defence against viral infection. Type I IFNs α/β are induced in virus-infected cells by engagement of viral molecules, in particular nucleic acids, with pattern recognition receptors (Takeda and Akira, 2005; Kawai and Akira, 2007). Most human cell types can produce type I IFNs including keratinocytes that have evolved as immune sentinels within skin (Debenedictis et al., 2001; Liu et al., 2012; Nestle et al., 2009). These cytokines induce an antiviral state in virus infected and neighbouring cells through autocrine or paracrine mechanisms. IFN-γ is produced by T lymphocytes, natural killer cells and plasmacytoid DC that are recruited to skin by inflammatory mediators released from infected cells (Schroder et al., 2004; Debenedictis et al., 2001). IFN-γ has immunostimulatory as well as immunomodulatory roles in innate and adaptive immunity and like type 1 IFNs is able to inhibit viral replication directly.

The anti-viral state involves the upregulation of numerous interferon-stimulated genes (ISGs) that inhibit various stages of viral replication (MacMicking, 2012; Schoggins et al., 2011; Sadler and Williams, 2008; Liu et al., 2012). Type I IFNs induce ISGs through the interferon stimulated response element (ISRE) while IFN-γ induces ISGs through the interferon-gamma activated sequence (GAS) (Takaoka and Yanai, 2006). Both responses lead to the upregulation of hundreds of effector molecules, many of which inhibit and kill viruses and most of these effectors are stimulated by both responses (Schoggins et al., 2011; Liu et al., 2012). The receptors for IFN-α/β and IFN-γ signal through Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways.

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Type I IFN induced signalling initially results in the phosphorylation of the receptor-associated kinases JAK1 and tyrosine kinase 2 (Tyk2) that phosphorylate STAT1 and STAT2, respectively. Phosphorylated STAT1 and STAT2 form a heterodimer that translocates to the nucleus forming the heterotrimeric transcription factor complex IFN-stimulated genes factor 3 (ISGF3) with IFN regulatory factor (IRF)-9 that induces ISGs via the ISRE (Takaoka and Yanai, 2006; Audsley and Moseley, 2013). In comparison the JAK/STAT signalling pathway mediated by IFN-γ does not involve Tyk2 nor STAT2 but involves the phosphorylation of the transcriptional factor STAT1 that homodimerises and translocates to the nucleus where it binds to GAS, inducing the upregulation of ISGs (Takaoka and Yanai, 2006). In addition, Type I IFN signalling is also mediated through the p38 mitogen activated protein (MAP) kinase pathway (Halfmann et al., 2011).

The importance of the induction of ISGs as part of the host's anti-viral defences is highlighted by the fact that many viruses have evolved strategies to overcome the JAK/STAT signalling pathway so as to inhibit the production of the IFN effector responses. A number of viruses inhibit the JAK/STAT pathway induced by IFN-α/β however, many inhibit the JAK/STAT pathway induced by both type I and type II IFNs (Audsley and Moseley, 2013). Many viruses are known to target STAT1. The poxvirus *Vaccinia virus*, targets the activated form of STAT1 using a phosphatase that is part of the virion structure (Hakes et al., 1993; Najarro et al., 2001). *Hepatitis C virus* upregulates protein phosphatase 2A that impairs IFN-α induced anti-viral activity through inhibition of STAT1 tyrosine phosphorylation (Shanker et al., 2013). Measles virus V protein blocks JAK1 mediated phosphorylation of STAT1 (Caignard et al., 2007) and tick-borne flavivirus blocks phosphorylation of Tyk2 and JAK1 (Best et al., 2005). *Mumps virus* and *Newcastle disease virus* also target STAT1 by proteasomal degradation (Kubota et al., 2001; Huang et al., 2003). A number of other viruses target both STAT1 and STAT2 (Audsley and Moseley, 2013). HCV core protein blocks the JAK/STAT signalling pathway by upregulating cellular SOCS3 (Bode et al., 2003).

Orf virus (ORFV) is the prototype species of the *Parapoxvirus* genus of the *Poxviridae* family that induces acute pustular skin lesions in sheep and goats and is transmissible to man (Haig and McInnes, 2002; Fleming et al., 2015). The virus replicates in keratinocytes and epithelial cells of the oral mucosa. ORFV lesions normally persist for about 6–8 weeks. There is currently nothing known about the effects of ORFV on the induction of ISGs. We show here that ORFV has the capability to inhibit the transcriptional activation of guanylate binding protein (*GBP1*) by IFN-γ and the transcriptional activation of *MxA* by IFN-α. Furthermore we show that this could be as a consequence of ORFV reducing the levels of phosphorylated STAT1 in virus infected cells and our data suggests that this is related to a viral structural protein.

2. Materials and methods

2.1. Cells

HeLa cells were maintained in DMEM supplemented with fetal bovine serum (FBS) and penicillin/streptomycin/kanamycin (PSK). Primary lamb testis (LT) cells were maintained in minimum essential medium (MEM) (GIBCO, Invitrogen) and supplemented with FBS at 10% for growth and 5% for culture maintenance. LT cells were supplemented with PSK solution. HeLa cells and LT cells were incubated at 37 °C in a humidified 7% CO_2 atmosphere.

2.2. Virus

ORFV strain NZ2 (Robinson et al., 1982) was propagated in LT cells.

2.3. Inhibition of the IFN response: quantitative RT-PCR

HeLa cells, in 6-well plates were infected with ORFV at an MOI of 10 for 30 min on ice followed by 1 h at 37 °C. Cells were then treated with IFN-γ or IFN-α. Each assay was performed in duplicate and repeated 3 times. Cells were lysed for total mRNA extraction (RNeasy, Qiagen) and cDNA synthesis (SuperScript III, Invitrogen). Transcript analysis was carried out using Power-Mix SYBR Green (Applied Biosystems). Primers used: MxA (forward) 5′-TTC AGC ACC TGA TGG CCT ATC-3′, MxA (reverse) 5′-TGG ATG ATC AAA GGG ATG TGG-3′, hGBP1 (forward) 5′-CAC AGG CAA ATC CAT CCT GA-3′, hGBP1 (reverse) 5′-GCA CAC ACC ACA TCC AGA TT-3′, GAPDH (forward) 5′-CTC TGC TGA TGC CCC CAT GTT C-3′, GAPDH (reverse) 5′-GGT GGT GCA GGA GGC ATT GCT G-3′. MxA primer sequences are described in Jorns et al. (2006), GBP1 primer sequences were obtained from qPrimerDepot and GAPDH primer sequences are described in Wise et al. (2007). All primers were synthesised by Life Technologies.

2.4. Antibodies and cytokines

Donkey anti-goat IRDye 800CW, donkey anti-rabbit IRDye 800CW and donkey anti-mouse IRDye 680RD were obtained from Li-Cor, Biosciences. Mouse anti-STAT1 endogenous, mouse anti-STAT1 phosphorylated (tyrosine 701), mouse anti-STAT1 phosphorylated (serine 727) were obtained from BD Biosciences. Rabbit-anti-mouse-HRP was obtained from Dako Cytomation. Goat anti-actin was obtained from Santa Cruz. Rabbit-anti-goat-HRP and mouse-anti-FLAG-M2-HRP were obtained from Sigma–Aldrich. Rabbit anti-ORFV119 was manufactured by Mimotopes. Goat-anti-rabbit-HRP was obtained from Life Technologies. Monoclonal purified mouse antibody and rabbit-anti-GFP were obtained from Abcam. Polyclonal purified rabbit antibody and human IFN-α and human IFN-γ were obtained from GIBCO, Invitrogen.

2.5. Modulation of the IFN signalling pathway

HeLa cells were infected with ORFV in 6-well plates as described above. Each assay was performed in duplicate and repeated at least 3 times. After 30 min incubation at 4 °C, 1 ml of medium was added and cells incubated at 37 °C for 30 min. In all assays IFN (in 400 μl PBS) was added 60 min after virus was first added to cells. Cells were washed with PBS after 30 min of IFN treatment. Following this wash step, 1 ml of medium containing 10% FBS was added to the cells for the remainder of the assay. In all assays the addition of IFN to cells was taken as time-point 0. At the completion of the assay, medium was removed and cells washed twice with PBS. Cells were then removed from the wells using sterile rubber scrapers, washed with 1 ml of PBS before transfer to 2 ml microfuge tubes. Cells were centrifuged at 3000 × *g* for 3 min at 4 °C. Cell pellets were re-suspended in ice-cold PhosphoSafe (Novagen) extraction reagent by aspiration and incubated for 15 min on ice with vortexing 4 times for 30 s. Lysates were then centrifuged at 10,000 × *g* for 3 min at 4 °C and supernatants stored at −80 °C for Western blot analysis.

Where HeLa cells were pretreated with sodium orthovanadate (Na_3VO_4) (Sigma–Aldrich), cells were incubated with 100 mM (final concentration) Na_3VO_4 for 4 h prior to infection with ORFV. Where HeLa cells were treated with adenosine N1-oxide (ANO) (Sigma–Aldrich) cells were incubated with ANO 10 μg/ml (final concentration) for 6 h and then infected with ORFV.

2.6. Cloning

The ORFV gene *ORFV057* that encodes the polypeptide ORFV057 was amplified from plasmid pOV56 of ORFV strain NZ2 (Mercer et al., 1987) using the polymerase chain reaction