How does vaccinia virus interfere with interferon?

Geoffrey L Smith, Callum Talbot-Cooper and Yongxu Lu

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom

*communicating author: email: gls37@cam.ac.uk. tel: 00-44-1223-333692

For Advances in Virus Research

Key words: Vaccinia virus, vaccine, smallpox, innate immunity, interferon, interferon receptors, interferon inducible genes, signalling pathways, NF-κB, IRF-3, AP-1, JAK-STAT, ISRE, GAS, interferon alpha/beta, interferon gamma, interferon lambdas.

Contents

1. Introduction to vaccinia virus (VACV)

Vaccinia virus (VACV) is the only vaccine to have eradicated a human disease, smallpox, yet its origin and natural host are unknown (Baxby, 1981). VACV is the prototypic member of the *Orthopoxvirus* genus of the *Poxviridae*, a family of large and complex viruses with big double stranded (ds)DNA genomes that replicate in the cytoplasm of infected cells (Moss, 2007). Cytoplasmic replication is unusual for DNA viruses and poses challenges because the nuclear enzymes required for transcription and replication of DNA are not available and so poxviruses encode their own. Additionally, cytoplasmic replication of DNA creates an easily recognisable pathogen associated molecular pattern (PAMP) that can be detected by cytosolic pattern recognition receptors (PRRs) leading to activation of innate immunity. To combat this poxviruses encode many proteins that suppress activation of innate immune signalling pathways such as those leading to expression of interferon (IFN).

1.1 Origin of vaccinia virus

VACV is the vaccine used to eradicate smallpox but is distinct from cowpox virus (CPXV) the vaccine thought to have been used by Jenner in 1796 when he introduced vaccination against smallpox with a virus taken from the hand of a milkmaid. The distinction between CPXV and the 20th century smallpox vaccines was recognised in 1939 (Downie, 1939). Subsequently, it was suggested that the current smallpox vaccines might be derived from an orthopoxvirus that infects horses (horsepox virus, HSPV) (Baxby, 1981) and this is supported by: i) reports that early vaccinators, including Jenner, obtained smallpox vaccine from horses when cowpox was scare; ii) sequence comparisons of VACV with a virus isolated from a horse in Mongolia that show a closer relationship than with other orthopoxviruses (Tulman et al., 2006); iii) the observation that the IFN-γ receptor encoded by VACV can bind and inhibit equine IFN-γ (Symons et al., 2002); and iv) the recent finding that a strain of smallpox vaccine from 1902 is most closely related to HSPV (Schrick et al., 2017). VACV, CPXV and HSPV share considerable immunological cross-reactivity with each other and with other orthopoxviruses including variola virus (VARV), the cause of smallpox, monkeypox virus (MPXV), camelpox virus and ectromelia virus, the cause of mousepox. This immunological cross-reactivity is the basis for the protection that infection with members of this genus provides against subsequent infection by other members of this genus. Thus both VACV and CPXV were effective at preventing smallpox.

If Jenner used CPXV in 1796 and all the smallpox vaccines in use in 1939 were VACV, then sometime between 1796 and 1939 VACV replaced CPXV as the smallpox vaccine. It is uncertain when this happened but there are anecdotes suggesting this occurred early in the 19th century. First, Jenner and other early vaccinators were reported to use virus taken from horses (called the grease) when the supply of CPXV was limiting. Second, the smallpox vaccine taken from England to USA in the 1850s and which became the New York City Board of Health vaccine is VACV not CPXV. Third, pathologists who examined cells infected by the smallpox vaccine in the late 19th century described the eosinophilic cytoplasmic inclusion bodies made by both VACV and CPXV but failed to mention the more obvious A type inclusion bodies that are formed by CPXV but not VACV. Fourth, the genome sequence of a smallpox vaccine from 1902 shows this virus is very similar to the virus isolated from a horse in Mongolia (Schrick et al., 2017). Collectively, these observations are consistent with VACV being the smallpox vaccine by the first half of the 19th century.

1.2 Vaccinia virus replication cycle

VACV has a complicated replication cycle and each infected cell produces multiple forms of infectious progeny that differ in their surface proteins, the number of membranes surrounding the core, and their immunological and biological properties (Appleyard et al., 1971). There are two infectious forms of VACV called intracellular mature virus (IMV) and extracellular enveloped virus (EEV) that are surrounded by 1 or 2 membranes, respectively (Hollinshead et al., 1999). Entry into a new cell therefore presents these virions with differing challenges (Vanderplasschen et al., 1998). The IMV form with a single envelope can enter the cytosol by fusion of its envelope with either the plasma membrane or the membrane of an intracellular vesicle after the virion is taken up by endocytosis or macropinocytosis (Carter et al., 2005; Mercer and Helenius, 2008; Townsley et al., 2006), and there is evidence that different strains of VACV may use these different pathways preferentially (Bengali et al., 2009). Fusion is driven by a complex of 10 virus proteins on the IMV surface that are all essential for fusion (Moss, 2016; Senkevich et al., 2005). This is quite different from the fusion machinery of many other enveloped viruses such as influenza virus or human immunodeficiency virus that consists of a single protein that is cleaved into 2 subunits and is sufficient for fusion. Entry of EEV presents a topological challenge because fusion of the outer membrane of EEV with the plasma membrane or intracellular vesicle would just release an IMV surrounded by its single membrane into the cytosol, and so both virus membranes must be shed for replication to commence. To shed the EEV outer membrane VACV has evolved an unusual non-fusogenic mechanism that involves rupture of the outer membrane at the site of contact between the virus and cell, thereby exposing the

IMV membrane that can then fuse as described above (Law et al., 2006). The non-fusogenic dissolution requires negatively charged molecules, such as glycosaminoglycans, on the cell surface and the B5 protein on the EEV surface (Roberts et al., 2009).

After entry, the naked virus core is transported on microtubules deeper into the cell (Carter et al., 2003), where it is uncoated and replication starts forming a virus factory. Within this cytoplasmic replication site the three classes of virus genes (early, intermediate and late) are expressed in a temporal cascade (Broyles, 2003). DNA replication is a trigger for late gene expression and then virus proteins and genomes assemble to form new virions via distinct morphogenic stages (Morgan et al., 1954). IMV represent the majority of infectious progeny. Most IMV remain within the cell until cell lysis and spread only slowly from cell to cell. However, some IMV are transported away from factories on microtubules (Sanderson et al., 2000; Ward, 2005) to sites where they are wrapped by a double membrane deriving from early endosomes (Tooze et al., 1993) or the trans-Golgi network (Schmelz et al., 1994) to form a triple enveloped virus called intracellular enveloped virus (IEV), or wrapped virus. This virion is then transported by kinesin-1 on microtubules (Geada et al., 2001; Hollinshead et al., 2001; Rietdorf et al., 2001; Ward and Moss, 2001) to the cell periphery where the outer membrane fuses with the plasma membrane to expose a double enveloped virus on the cell surface. If this virion remains on the cell surface it is called cell associated enveloped virus (CEV) and if it is released it is called EEV. EEV mediate long range virus spread, whereas CEV induce the polymerisation of actin beneath the plasma membrane where the virion sits (Cudmore et al., 1995), and this growing actin tail propels the virus away to find new cells to infect. Actin tails are also induced upon super-infection of an infected cell by CEV/EEV that bind to the A33/A36 protein complex on the cell surface and repel super-infecting virions to find uninfected cells (Doceul et al., 2012; Doceul et al., 2010).

1.3 **Vaccinia virus genes**

VACV strain Copenhagen was the first poxvirus genome sequenced and is 191 kbp (Goebel et al., 1990). VACV genes are organised with those essential for virus replication in the central region of the genome and those non-essential and more variable present near each terminus (Gubser et al., 2004; Upton et al., 2003). It is estimated that approximately half the VACV genes are non-essential for virus replication in cell culture. Many of them encode proteins that affect host range, virulence or immune evasion. The latter group of genes and specifically those that encode proteins that interfere with IFN are the subject of this article.

2. Interferons

Interferons (IFNs) are a group of soluble glycoproteins that are produced and released from cells in response to virus infection (and other stimuli). They then bind to specific IFN receptors on cells to trigger signalling pathways that result in the expression of IFN stimulated genes (ISGs). The ISG products render the cell resistant to subsequent virus infection. Isaacs and Lindenmann are often cited as discovering IFN while working with influenza virus (Isaacs and Lindenmann, 1957; Isaacs et al., 1957), but a few years earlier Japanese scientists working with VACV reported that infection of rabbit skin with ultraviolet light-inactivated VACV induced the production of an “facteur inhibiteur” (inhibitory factor) that prevented subsequent virus infection (Nagano and Kojima, 1954; Nagano et al., 1954).

IFNs are categorised into three distinct classes known as type I, type II and type III IFNs (Pestka et al., 2004; Pestka et al., 1987; Platanias, 2005). In humans, the type I IFN family consists of IFNα, IFNβ, IFNε, IFNκ and IFNω, all of which share a high degree of structural similarity (Platanias, 2005). By contrast, IFNγ constitutes the sole member of the type II IFN family, and is structurally distinct from the type I IFNs (Farrar and Schreiber, 1993). The type III IFNs are the most recently discovered class of IFNs and include IFNλ1, IFNλ2, IFNλ3, and IFNλ4 (Kotenko et al., 2003; Prokunina-Olsson et al., 2013; Sheppard et al., 2003). It should be noted the whilst IFNλ1, IFNλ2 and IFNλ3 are also referred to as IL-29, IL-28A, and IL-28B respectively, the IFNλ nomenclature was favoured and is now accepted (Fox et al., 2009).

Deleted[gls37]: -

2.1 Induction of IFNβ expression

The expression of type I IFNs (mainly IFNβ) is induced rapidly after the sensing of virus infection by PRRs engaging their cognate PAMPs. This activates the IFN regulatory factor 3 (IRF3) pathway (Figure 1) leading to transcription from the IFNβ gene. PRRs that can trigger the activation of IRF3 include TLR3, TLR4, RIG-I, MDA5 and multiple DNA sensing proteins. These PRRs activate different signalling pathways that ultimately converge at the TANK-binding kinase (TBK1) and IκB kinase-ε (IKKε) (Fitzgerald et al., 2003). These kinases, in association with adapter proteins TANK (Pomerantz and Baltimore, 1999), nuclear factor kappa B (NF-κB)-activating kinase-associated protein 1 (NAP1) (Fujita et al., 2003), similar to NAP1 TBK1 adaptor SINTBAD

(Ryzhakov and Randow, 2007) and DEAD box protein 3 (DDX3) (Soulat et al., 2008), cause phosphorylation of IRF3.

In addition to IRF3 phosphorylation, IRF7 can also be phosphorylated by the same pathway. Unlike IRF3, which is expressed in a ubiquitous manner, expression of IRF7 is relatively low in unstimulated cells but activation of the pathway leads to an upregulation of IRF7 expression, thereby inducing a positive feedback loop (Honda et al., 2005; Sato et al., 2000). Phosphorylation of IRF3/7 leads to the formation of homo/heterodimers that are translocated into the nucleus.

In addition to the activation of IRF3/7, PRR-induced signalling activates the NF-κB pathway and the mitogen activated protein kinase (MAPK) pathway, and causes translocation of transcription factors NF-κB and AP-1 into the nucleus. Within the nucleus, these three transcription factors associate to form a complex known as the enhanceosome, driving the transcription of the IFNβ gene (Maniatis et al., 1998; Thanos and Maniatis, 1995).

2.2 IFN-induced signalling

After transcription of IFN genes, IFN mRNAs are translated in the cytoplasm and IFNs are secreted from the cell. To exert their effects, IFNs bind to transmembrane, cell surface IFN-receptors (IFNRs). The three classes of IFNs bind to distinct IFNRs known as the IFNα/β receptor (IFNAR), the IFNγ receptor (IFNGR) and the IFNλ receptor (IFNLR) (de Weerd and Nguyen, 2012). These receptors can be further divided into two distinct components: IFNAR1 and IFNAR2, IFNGR1 and IFNGR2, and IFNLR1 and IL-10R2 (Figure 2).

IFNAR and IFNGR are expressed on most cell types and therefore, both type I and type II IFNs exert their effects in a ubiquitous manner (de Weerd and Nguyen, 2012). In contrast, IFNLR has a far more limited distribution and is expressed predominantly on mucosal epithelial cells (Muir et al., 2010). Type III IFNs therefore promote an increased anti-viral state in epithelial tissues vulnerable to frequent and recurrent viral infection, while limiting the risk of systemic inflammation associated with other the IFNs (Wack et al., 2015).

Engagement of IFNs with their cognate receptors induces a signalling cascade known as the Janus associated kinase (JAK) and signal transducer and activator of transcription (STAT) pathway (Aaronson and Horvath, 2002). Although type I and type III IFNs act through distinct receptors, the JAK-STAT pathway is thought to be the same

(Crotta et al., 2013; Doyle et al., 2006; Zhou et al., 2007). In contrast, type II IFN activates a subtly different subset of JAKs and STATs (Platanias, 2005).

Upon receptor binding, the IFNR subunits undergo structural rearrangements and dimerise. This brings the receptor-associated JAKs within close proximity leading to their cross-activation (Leonard, 2001). Each of the subunits from the different IFNRs interacts with a specific member of the JAK family (Figure 2). The activated JAKs then phosphorylate the cytoplasmic region of the IFNRs, leading to the formation of docking sites to which inactive STAT proteins are recruited (Aaronson and Horvath, 2002; Li, 2008). Once recruited, the JAKs phosphorylate the STAT molecules at specific tyrosine residues near their C terminus, thereby inducing the formation of STAT dimers (Aaronson and Horvath, 2002; Li, 2008). In type I and type III IFN signalling, both STAT1 and STAT2 are recruited to form STAT1 and STAT2 heterodimers (Aaronson and Horvath, 2002). In turn, they further associate with IFN regulatory factor 9 (IRF9), to form a trimeric complex known as the IFN stimulated gene factor 3 (ISGF3) (Aaronson and Horvath, 2002; Schindler et al., 1992). Subsequently, ISGF3 translocates into the nucleus where it associates with the IFN-stimulated responsive element (ISRE) promoter to drive the transcription of ISGs (Aaronson and Horvath, 2002). In type II IFN signalling, only STAT1 is recruited to form STAT1 homodimers (Aaronson and Horvath, 2002; Shuai et al., 1992). These homodimers known as IFNγ associated factor (GAF) then translocate into the nucleus where they associate with the IFNγ associated sequence (GAS) promoter leading to the transcription of ISGs (Aaronson and Horvath, 2002).

2.3 IFN-stimulated genes (ISGs)

IFNs induce the transcription of hundreds of ISGs. While some are upregulated by all IFNs, others are upregulated selectively by distinct IFNs. For example, IRF1 is upregulated preferentially in response to IFNγ (Der et al., 1998). This type of specificity in IFN-induced responses is critical for a highly coordinated and fine-tuned innate immune response as well as a heightened anti-viral state within cells, thereby halting the spread of infection. Of the many hundreds of ISGs, well characterised examples include ISG15, protein kinase R (PKR) and 2'-5'-oligoadenylate synthase (OAS).

ISG15 is a 15-kDa protein that has sequence similarity to ubiquitin and is one of the most strongly induced genes upon viral infection (Blomstrom et al., 1986; Haas et al., 1987). ISG15 can become conjugated to both cytoplasmic and nuclear proteins via an isopeptidase bond in a process known as ISGylation. Conjugation of

ISG15 to target proteins such as IRF3, RIG-I, human MxA and PKR can increase their stability preventing their degradation (Villarroya-Beltri et al., 2017). Additionally, ISGylation through attachment of ISG15 can modulate JAK-STAT signalling (Malakhova et al., 2003).

PKR becomes activated by autophosphorylation upon binding dsRNA, a product often formed during infection by both RNA and DNA viruses. Subsequently, PKR phosphorylates the eukaryotic translational initiation factor 2 alpha (eIF2α) at serine 51, thereby preventing recycling of eIF2α and preventing further protein synthesis (Meurs et al., 1990).

2'-5'-OAS is also activated upon binding to dsRNA. Once activated, 2'-5'-OAS synthesises 2'-5'-oligoadenylates using ATP as a substrate. In turn, 2'-5'-oligoadenylates activate the ribonuclease RNAase L, which degrades both viral and cellular mRNAs as well as cellular tRNAs and rRNAs, leading to inhibition of translation (Silverman, 2007).

The importance of the IFN system for protection against viruses has been demonstrated in many ways, not least by the presence of many IFN antagonists in virus genomes. This is illustrated particularly well with VACV that has been known for decades to be relatively resistant to IFN and to confer resistance to IFN to other viruses such as vesicular stomatitis virus (Thacore and Youngner, 1973; Whitaker-Dowling and Youngner, 1983). The observation that large deletions in terminal regions of the VACV genome could arise spontaneously during passage of virus in tissue culture and that this was prevented in the presence of IFN, suggested that genes conferring resistance to IFN were present in these terminal regions (Paez and Esteban, 1985). We now know that VACV interferes with the production and action of IFNs in many ways.

3. Preventing production of IFN

3.1 Inhibition of protein synthesis in virus-infected cells

IFNs are cellular proteins whose production is blocked by inhibition of host protein synthesis induced by infection with VACV (Moss, 1968). In recent years some of the virus genes involved have been identified. These include the de-capping enzymes D9 and D10 that recognise and remove the cap structures from the 5' end of mRNAs (Parrish and Moss, 2007; Parrish et al., 2007). It might seem counter-intuitive for a virus that has capped mRNAs

to encode de-capping enzymes, but there are advantages to the virus in doing this. First, virus mRNAs may be more abundant than cellular mRNAs and therefore de-capping enzymes will cause a preferential inhibition of cellular rather than viral protein synthesis. Second, de-capping of virus mRNAs provides a mechanism to achieve an efficient shift from expression of early virus proteins to intermediate and then late proteins by removing the mRNAs of the earlier class. Another VACV protein, called 169, also inhibits protein synthesis and since this protein is excluded from virus factories, the site of virus protein synthesis, it may target cellular mRNAs preferentially. Loss of protein 169 causes an increased expression of cellular inflammatory proteins during infection in cultured cells and *in vivo* (Strnadova et al., 2015).

3.2 Limiting the formation or availability of virus nucleic acid PAMPs

Viral nucleic acids such as dsRNA, 5'-triphosphate RNA or cytoplasmic DNA are recognised by specific PRRs leading to activation of signalling pathways and the production of cytokines, chemokines and IFNs. A strategy employed by VACV to prevent IFN production is therefore to minimise the production or recognition of these nucleic acid PAMPs by PRRs.

3.2.1 Limiting dsRNA formation by gene arrangement

Production of dsRNAs is minimised early during VACV infection by gene arrangement (Smith et al., 1998). The genes at the left and right end of the genome are, with very few exceptions, transcribed outward from only one DNA strand (Goebel et al., 1990). Therefore, these transcripts lack complementary RNA to form dsRNA. In the central region of the genome, genes are encoded approximately equally on each DNA strand, but here genes are often arranged in blocks with several going left to right followed by an inversion with a block of genes transcribed right to left (Goebel et al., 1990). Furthermore, for pairs of adjacent genes that are transcribed towards each other early during infection, it is notable that the production of overlapping transcripts is minimised by the presence of transcriptional termination sequences (often multiple termination signals) on both DNA strands between these genes (Smith et al., 1998). This arrangement is unlikely to be accidental and may help reduce dsRNA formation and thereby IFN production.

3.2.2 Expression of a dsRNA binding protein E3

Late during VACV infection long transcripts are formed that run through several open reading frames due to a failure to terminate at specific sequences. Consequently, much dsRNA is formed late during infection. However, the availability of this PAMP is minimised by the VACV E3 protein that binds dsRNA and so restricts the activation of PRRs by dsRNA (Chang et al., 1992). Notably protein E3 is made early during infection and so is present before most virus dsRNA is formed. A mutant virus lacking the E3 protein is more sensitive to IFN and this sensitivity could be reversed by the expression of other dsRNA binding proteins (Beattie et al., 1995). Viruses lacking E3 are also attenuated *in vivo* and both the N-terminal domain, which has similarity to Z DNA binding proteins, and the C-terminal domain, which binds dsRNA, are required for virus virulence (Brandt and Jacobs, 2001). The E3 protein also serves to minimise RNA polymerase III-mediated dsDNA-sensing and the consequential activation of innate immunity (Marq et al., 2009; Valentine and Smith, 2010).

3.2.3 Targetting DNA-PK by protein C16

During replication VACV can produce thousands of copies of its ~ 200 kb dsDNA molecule in the cytoplasm. Given that cytoplasmic DNA is abnormal, DNA sensors such as cGAS (Gao et al., 2013; Li et al., 2013), DNA-PK (Ferguson et al., 2012; Morchikh et al., 2017) and several others (for review see Paludan, 2015), recognise this and activate an innate response via the STING-TBK1-IRF3 pathway (Figure 1). To counter the role of DNA-PK in DNA sensing, VACV encodes a protein called C16 that is made early during infection, contributes to virulence (Fahy et al., 2008) and binds to the Ku proteins that are part of the DNA-PK complex (Peters et al., 2013). By binding to Ku, C16 restricts the binding of DNA by Ku, resulting in reduced recruitment of DNA-PK and reduced activation of IRF3 via the STING-TBK1 pathway. Both in cell culture and *in vivo* C16 restricts the inflammatory response to transfected DNA or infection by DNA viruses (Fahy et al., 2008; Peters et al., 2013).

3.3 Inhibition of signalling pathways leading to expression of IFNβ

The IFNβ gene promoter contains binding sites for IRF3, NF-κB and AP-1 that form the enhanceosome for efficient transcription of the IFNβ gene (Maniatis et al., 1998; Thanos and Maniatis, 1995). VACV expresses a remarkable number of proteins that shut down IRF3 or NF-κB activation (Smith et al., 2013) and also expresses proteins that both positively and negatively influence activation of MAP kinases (Torres et al., 2016).

3.3.1 Inhibition of IRF3 activation

VACV expresses multiple proteins that inhibit IRF3 activation. These proteins and their mechanisms of action (where known) were described in a review of VACV immune evasion (Smith et al., 2013) and so are described only briefly here. They include A46, K7, C6 and N2 that are all known or predicted to be members of the VACV Bcl-2 family. A46 binds to TRIF, TRAM, MAL and Myd88 and therefore blocks activation of IRF3 early in the pathway downstream of TLRs (Stack et al., 2005) (Figure 1). K7 binds to the DEAD box helicase DDX3 and this interaction revealed that DDX3 is an adaptor protein for the TBK-1 / IKKε complex that promotes IRF3 phosphorylation (Schroder et al., 2008). Protein C6 also interferes with the production of IFNβ by binding to other TBK-1 adaptor proteins, TANK, SINTBAD and NAP1 (Unterholzner et al., 2011). Protein N2 acts further downstream in the pathway after IRF3 phosphorylation and translocation into the nucleus although its precise mechanism remains to be determined (Ferguson et al., 2013). Collectively, these proteins provide a co-ordinated assault on this pathway and their actions are non-redundant because deletion of any of these proteins causes a reduction in virus virulence *in vivo* (Benfield et al., 2013; Ferguson et al., 2013; Stack et al., 2005; Unterholzner et al., 2011).

3.3.2 Inhibition of NF-κB activation

VACV expresses a remarkable number of proteins that target NF-κB activation. Many of these were reviewed previously (Smith et al., 2013), but at least 10 inhibitors have been identified and, as for the IRF3 inhibitors, these proteins are non-redundant because deletion of A46 (Stack et al., 2005), A52 (Harte et al., 2003), N1 (Bartlett et al., 2002), K7 (Benfield et al., 2013), B14 (Chen et al., 2006), C4 (Ember et al., 2012), A49 (Mansur et al., 2013) and E3 (Brandt and Jacobs, 2001) individually each causes an *in vivo* phenotype despite the presence of the other inhibitors. These proteins work at different stages in the pathway. For instance, A46, K7 and A52 act early by binding to IRAKs and TRAFs (Bowie et al., 2000; Harte et al., 2003; Schroder et al., 2008; Stack et al., 2005), B14 binds to IKKβ part of the IKK complex where the IL-1- and TNF-activated pathways converge (Chen et al., 2008), and A49 binds to the E3 ubiquitin ligase β-TrCP to block p-IκBα ubiquitylation and degradation (Mansur et al., 2013). Binding of A49 to β-TrCP also prevents ubiquitylation and degradation of other β-TrCP substrates such as β-catenin and consequently results in activation of the wnt signalling pathway (Maluquer de Motes and Smith, 2017). Protein K1 acts further downstream within the nucleus to prevent the acetylation of RelA (Bravo Cruz and Shisler, 2016). Remarkably, a VACV deletion mutant lacking all known NF-κB inhibitors

was still able to inhibit NF-κB activation and did so after p65 translocation into the nucleus indicating a nuclear site of action (Sumner et al., 2014). Unpublished work from our laboratory has identified several other inhibitors of this pathway.

3.3.3 Modulation of AP-1 activation

VACV modulates MAPK pathways during infection (Andrade et al., 2004; de Magalhaes et al., 2001; Pereira et al., 2012; Silva et al., 2006). Recently some proteins involved have been identified and these are all Bcl-2 family members. VACV proteins A52, B14 and K7 promote activation of the pathway during infection and when expressed individually ectopically, and of these B14 gave the strongest activation. In contrast, protein A49 was inhibitory (Torres et al., 2016). The biological consequences of these effects remain incompletely understood.

4. Blocking binding of IFNs to IFN receptors

As illustrated above, VACV has many strategies to reduce the production of IFNs from infected cells, but unless these defences are deployed immediately after infection the inhibition of IFN production may be incomplete. In addition, these intracellular defences offer no protection against IFNs produced by activated uninfected cells that are recruited to the site of infection. To combat IFNs outside the cell VACV expresses 2 proteins called B8 and B18 that are secreted and bind and inhibit IFNs extracellularly (Figure 2).

Protein B8 was identified by virtue of its similarity to protein M-T7 from the leporipoxvirus myxoma virus that is the cause of myxomatosis in the European rabbit (Upton et al., 1992). M-T7 and VACV B8 share amino acid similarity to the extracellular ligand binding domain of the mouse and human IFNGR but lack the transmembrane anchor of the host protein and the intracellular domain that mediates signal transduction after ligand-receptor interaction (Upton et al., 1992). Direct analysis of the VACV protein confirmed it is secreted from cells and acts a soluble antagonist of IFNγ (Alcami and Smith, 1995; Mossman et al., 1995). B8 showed an unusually broad specificity for IFNγs from different species, unlike the cellular IFNGRs that are generally species specific. B8 bound human, bovine, rat and rabbit IFNγ well, but had much lower affinity for mouse IFNγ (Alcami and Smith, 1995; Mossman et al., 1995). Further studies showed that the B8 protein was a homodimer (Alcami and Smith, 2002) and also bound equine IFNγ (consistent with an equine origin of VACV) (Symons et al., 2002). *In vivo* loss

of the B8 protein did not affect VACV virulence in mice, consistent with the low affinity for mouse IFN$_\gamma$, however it did affect lesion formation in rabbit skin (Symons et al., 2002).

Protein B18 from VACV strain Western Reserve (WR) functions as a soluble inhibitor of type I IFNs. This protein was identified as a soluble inhibitor of type I IFNs in the culture medium of cells infected with VACV, and subsequently was mapped to the *B18R* gene (Symons et al., 1995). B18 is a member of the immunoglobulin superfamily and has a signal peptide and 3 Ig domains but no transmembrane anchor or cytosolic signal transduction domain (Smith and Chan, 1991). The B18 protein functions as a type I IFN inhibitor and prevents type I IFN-induced signalling (Colamonici et al., 1995; Symons et al., 1995). Like B8, B18 showed a broad species specificity and inhibited type I IFNs from human, rabbit, bovine, rat, and mouse and contributed to virulence in mouse models of infection (Symons et al., 1995). B18 has another important property not shared by B8 and can also bind to glycosaminoglycans on the surface of cells (Montanuy et al., 2011) and still bind and inhibit IFNs (Alcami et al., 2000).

Although B18 does not inhibit type III IFNs, a protein encoded by a yatapoxvirus, Yaba-like disease virus (YLDV), called Y136, shares amino acid similarity to B18 (Lee et al., 2001) and is able to bind and inhibit both type I and type III IFNs (Huang et al., 2007).

5. Blocking IFN-induced signalling pathways

Two VACV proteins are known to inhibit IFN signalling. VH1 is a tyrosine phosphatase and is packaged into the virus particle within the lateral bodies of the IMV particle that flank the biconcave virion core. Therefore, VH1 is delivered into cells immediately after infection. It dephosphorylates STAT1 and STAT2 that are phosphorylated upon type I, II or III IFNs binding to their receptors (Mann et al., 2008; Najarro et al., 2001). Thus VH1 provides a very rapid defence to IFN-induced signalling and can function within the infected cell immediately after the lateral bodies have been disassembled to release the proteins carried therein. Surprisingly, VH1 is essential for virus replication.

C6 also blocks IFN-induced signalling. It was characterised first as an inhibitor of IRF3 signalling (Unterholzner et al., 2011), but further work showed that it also inhibits JAK-STAT signalling in response to type I IFN (Stuart et al., 2016). Mapping its site of action showed that it did not prevent STAT phosphorylation or nuclear

translocation, nor binding of the ISGF3 complex to the ISRE promoter, but it prevented transcription from promoters bearing the ISRE. C6 interacts with STAT2 and this interaction requires the last 104 amino acids of STAT2 representing the transactivation domain (Stuart et al., 2016). Whether C6 can inhibit type II IFN signalling is unknown.

6. Blocking the anti-viral action of ISGs

VACV encodes several proteins that antagonise the anti-viral effects of ISGs. Well characterised examples are the E3 and K3 proteins. The dsRNA binding protein E3 (Chang et al., 1992), is able to sequester dsRNA produced during infection and thereby prevent activation of ISGs, such as PKR and 2'5'-OAS. Activation of PKR and 2'5'-OAS by binding dsRNA leads to an inhibition of protein synthesis (Figure 3). PKR phosphorylates eIF2α rendering it inactive to stop protein biosynthesis. The activation of 2'5'-OAS leads to the synthesis of oligoadenylates and these activate RNAaseL that then cleaves all forms of RNA and so also results in the inhibition of protein synthesis.

Protein K3 also prevents the inhibition of protein synthesis. This 88 amino acid protein shows sequence similarity to the N-terminal region of eIF2α including serine 51 that is phosphorylated by PKR. Consequently, K3 acts as a mimic and blocks the phosphorylation of eIF2α even if PKR has been activated (Beattie et al., 1991).

VACV proteins K1 and C7 were identified as factors that extended the range of host cells in which VACV could replicate (Perkus et al., 1990) and in their absence virus virulence was reduced (Liu et al., 2013). This host range restriction is related to the stimulation of ISG expression by type I IFN. VACV lacking K1 and C7 could replicate in Huh7 and MCF-7 cells but not if these cells were pre-treated with IFNβ. This blockage was reversed by the re-introduction of K1 or C7 (Meng et al., 2009). Screening of an ISG library identified IRF1 as the ISG targetted by K7 and C1-mediated suppression of anti-viral activity (Meng et al., 2012). Proteins K1 and C7 can also prevent the phosphorylation of eIF2α in VACV strain MVA infected cells (Backes et al., 2010).

7. Summary

VACV has acquired a remarkable arsenal of proteins that inhibit the production of IFNs, the binding of IFNs to their receptors, IFN-induced signalling and the anti-viral activity of ISGs. The existence of these VACV proteins

provides not only evidence of the importance of the IFN system for protection against viruses, but also illustrates how the IFN system works. It is likely that additional VACV proteins that target the IFN system remain to be discovered and, equally, it is possible that VACV proteins that target the IFN system will lead to the discovery of additional cellular proteins that function in the IFN system. Viruses are wonderful tools for learning about innate immunity and cell biology and so, although VACV was used as a vaccine against a disease that was eradicated nearly 40 years ago, there are compelling reasons to continue to study it.

8. Acknowledgements

Work in the senior author's laboratory has been supported by the Wellcome Trust, MRC, BBSRC and Lister Institute. GLS is a Wellcome Trust Principal Research Fellow.

9. Figure legends

Figure 1. Diagram showing a simplified version of the pathway leading to activation of the transcription factor IRF3. IRF3 together with the NF-κB and AP-1 form the enhanceosome that drives transcription of the IFNβ gene. Red circles depict individual VACV proteins that inhibit activation of this pathway.

Figure 2. Diagram showing the JAK-STAT signalling pathways induced by binding of type I, II or III IFNs to their cognate receptors. These pathways result in assembly of transcription factors in the nucleus that drive transcription from genes bearing the ISRE or GAS promoter elements. Red circles depict individual VACV proteins that inhibit activation of this pathway and the positions at which they function.

Figure 3. Diagram showing the activation of ISGs protein kinase R (PKR) and 2'-5'-oligoadenylate synthetase (2'-5'-OAS) by binding dsRNA and how this leads to inhibition of protein synthesis. Red circles depict VACV proteins E3 and K3 and the positions at which they inhibit these pathways to ensure that protein synthesis continues.

10. References

Aaronson, D. S., and Horvath, C. M. (2002). A road map for those who don't know JAK-STAT. Science 296, 1653.

Alcami, A., and Smith, G. L. (1995). Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity. J Virol 69, 4633.
Alcami, A., and Smith, G. L. (2002). The vaccinia virus soluble interferon-gamma receptor is a homodimer. J Gen Virol 83, 545.
Alcami, A., Symons, J. A., and Smith, G. L. (2000). The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. J Virol 74, 11230.
Andrade, A. A., Silva, P. N., Pereira, A. C., De Sousa, L. P., Ferreira, P. C., Gazzinelli, R. T., Kroon, E. G., Ropert, C., and Bonjardim, C. A. (2004). The vaccinia virus-stimulated mitogen-activated protein kinase (MAPK) pathway is required for virus multiplication. Biochem J 381, 437.
Appleyard, G., Hapel, A. J., and Boulter, E. A. (1971). An antigenic difference between intracellular and extracellular rabbitpox virus. J Gen Virol 13, 9.
Backes, S., Sperling, K. M., Zwilling, J., Gasteiger, G., Ludwig, H., Kremmer, E., Schwantes, A., Staib, C., and Sutter, G. (2010). Viral host-range factor C7 or K1 is essential for modified vaccinia virus Ankara late gene expression in human and murine cells, irrespective of their capacity to inhibit protein kinase R-mediated phosphorylation of eukaryotic translation initiation factor 2alpha. J Gen Virol 91, 470.
Bartlett, N., Symons, J. A., Tscharke, D. C., and Smith, G. L. (2002). The vaccinia virus N1L protein is an intracellular homodimer that promotes virulence. J Gen Virol 83, 1965.
Baxby, D. (1981). "Jenner's Smallpox Vaccine. The Riddle of the Origin of Vaccinia Virus." Heinemann, London.
Beattie, E., Denzler, K. L., Tartaglia, J., Perkus, M. E., Paoletti, E., and Jacobs, B. L. (1995). Reversal of the interferon-sensitive phenotype of a vaccinia virus lacking E3L by expression of the reovirus S4 gene. J Virol 69, 499.
Beattie, E., Tartaglia, J., and Paoletti, E. (1991). Vaccinia virus-encoded eIF-2 alpha homolog abrogates the antiviral effect of interferon. Virology 183, 419.
Benfield, C. T., Ren, H., Lucas, S. J., Bahsoun, B., and Smith, G. L. (2013). Vaccinia virus protein K7 is a virulence factor that alters the acute immune response to infection. J Gen Virol 94, 1647.
Bengali, Z., Townsley, A. C., and Moss, B. (2009). Vaccinia virus strain differences in cell attachment and entry. Virology 389, 132.
Blomstrom, D. C., Fahey, D., Kutny, R., Korant, B. D., and Knight, E., Jr. (1986). Molecular characterization of the interferon-induced 15-kDa protein. Molecular cloning and nucleotide and amino acid sequence. J Biol Chem 261, 8811.
Bowie, A., Kiss-Toth, E., Symons, J. A., Smith, G. L., Dower, S. K., and O'Neill, L. A. (2000). A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proc Natl Acad Sci U S A 97, 10162.
Brandt, T. A., and Jacobs, B. L. (2001). Both carboxy- and amino-terminal domains of the vaccinia virus interferon resistance gene, E3L, are required for pathogenesis in a mouse model. J Virol 75, 850.
Bravo Cruz, A. G., and Shisler, J. L. (2016). Vaccinia virus K1 ankyrin repeat protein inhibits NF-kappaB activation by preventing RelA acetylation. J Gen Virol 97, 2691.
Broyles, S. S. (2003). Vaccinia virus transcription. J Gen Virol 84, 2293.
Carter, G. C., Law, M., Hollinshead, M., and Smith, G. L. (2005). Entry of the vaccinia virus intracellular mature virion and its interactions with glycosaminoglycans. J Gen Virol 86, 1279.
Carter, G. C., Rodger, G., Murphy, B. J., Law, M., Krauss, O., Hollinshead, M., and Smith, G. L. (2003). Vaccinia virus cores are transported on microtubules. J Gen Virol 84, 2443.
Chang, H. W., Watson, J. C., and Jacobs, B. L. (1992). The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc Natl Acad Sci U S A 89, 4825.
Chen, R. A., Jacobs, N., and Smith, G. L. (2006). Vaccinia virus strain Western Reserve protein B14 is an intracellular virulence factor. J Gen Virol 87, 1451.
Chen, R. A., Ryzhakov, G., Cooray, S., Randow, F., and Smith, G. L. (2008). Inhibition of IkappaB kinase by vaccinia virus virulence factor B14. PLoS Pathog 4, e22.
Colamonici, O. R., Domanski, P., Sweitzer, S. M., Larner, A., and Buller, R. M. (1995). Vaccinia virus B18R gene encodes a type I interferon-binding protein that blocks interferon alpha transmembrane signaling. J Biol Chem 270, 15974.
Crotta, S., Davidson, S., Mahlakoiv, T., Desmet, C. J., Buckwalter, M. R., Albert, M. L., Staeheli, P., and Wack, A. (2013). Type I and type III interferons drive redundant amplification loops to induce a transcriptional signature in influenza-infected airway epithelia. PLoS Pathog 9, e1003773.
Cudmore, S., Cossart, P., Griffiths, G., and Way, M. (1995). Actin-based motility of vaccinia virus. Nature 378, 636.
de Magalhaes, J. C., Andrade, A. A., Silva, P. N., Sousa, L. P., Ropert, C., Ferreira, P. C., Kroon, E. G., Gazzinelli, R. T., and Bonjardim, C. A. (2001). A mitogenic signal triggered at an early stage of vaccinia virus infection: implication of MEK/ERK and protein kinase A in virus multiplication. J Biol Chem 276, 38353.

de Weerd, N. A., and Nguyen, T. (2012). The interferons and their receptors--distribution and regulation. Immunol Cell Biol 90, 483.
Der, S. D., Zhou, A., Williams, B. R., and Silverman, R. H. (1998). Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc Natl Acad Sci U S A 95, 15623.
Doceul, V., Hollinshead, M., Breiman, A., Laval, K., and Smith, G. L. (2012). Protein B5 is required on extracellular enveloped vaccinia virus for repulsion of superinfecting virions. J Gen Virol 93, 1876.
Doceul, V., Hollinshead, M., van der Linden, L., and Smith, G. L. (2010). Repulsion of superinfecting virions: a mechanism for rapid virus spread. Science 327, 873.
Downie, A. W. (1939). Immunological relationship of the virus of spontaneous cowpox to vaccinia virus. Br. J. Exp. Pathol. 20, 158.
Doyle, S. E., Schreckhise, H., Khuu-Duong, K., Henderson, K., Rosler, R., Storey, H., Yao, L., Liu, H., Barahmand-pour, F., Sivakumar, P., Chan, C., Birks, C., Foster, D., Clegg, C. H., Wietzke-Braun, P., Mihm, S., and Klucher, K. M. (2006). Interleukin-29 uses a type 1 interferon-like program to promote antiviral responses in human hepatocytes. Hepatology 44, 896.
Ember, S. W., Ren, H., Ferguson, B. J., and Smith, G. L. (2012). Vaccinia virus protein C4 inhibits NF-kappaB activation and promotes virus virulence. J Gen Virol 93, 2098.
Fahy, A. S., Clark, R. H., Glyde, E. F., and Smith, G. L. (2008). Vaccinia virus protein C16 acts intracellularly to modulate the host response and promote virulence. J Gen Virol 89, 2377.
Farrar, M. A., and Schreiber, R. D. (1993). The molecular cell biology of interferon-g and its receptor. Annu. Rev. Immunol. 11, 571.
Ferguson, B. J., Benfield, C. T., Ren, H., Lee, V. H., Frazer, G. L., Strnadova, P., Sumner, R. P., and Smith, G. L. (2013). Vaccinia virus protein N2 is a nuclear IRF3 inhibitor that promotes virulence. J Gen Virol 94, 2070.
Ferguson, B. J., Mansur, D. S., Peters, N. E., Ren, H., and Smith, G. L. (2012). DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. eLife 1, e00047.
Fitzgerald, K. A., McWhirter, S. M., Faia, K. L., Rowe, D. C., Latz, E., Golenbock, D. T., Coyle, A. J., Liao, S. M., and Maniatis, T. (2003). IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 4, 491.
Fox, B. A., Sheppard, P. O., and O'Hara, P. J. (2009). The role of genomic data in the discovery, annotation and evolutionary interpretation of the interferon-lambda family. PLoS One 4, e4933.
Fujita, F., Taniguchi, Y., Kato, T., Narita, Y., Furuya, A., Ogawa, T., Sakurai, H., Joh, T., Itoh, M., Delhase, M., Karin, M., and Nakanishi, M. (2003). Identification of NAP1, a regulatory subunit of IkappaB kinase-related kinases that potentiates NF-kappaB signaling. Mol Cell Biol 23, 7780.
Gao, D., Wu, J., Wu, Y. T., Du, F., Aroh, C., Yan, N., Sun, L., and Chen, Z. J. (2013). Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science 341, 903.
Geada, M. M., Galindo, I., Lorenzo, M. M., Perdiguero, B., and Blasco, R. (2001). Movements of vaccinia virus intracellular enveloped virions with GFP tagged to the F13L envelope protein. J Gen Virol 82, 2747.
Goebel, S. J., Johnson, G. P., Perkus, M. E., Davis, S. W., Winslow, J. P., and Paoletti, E. (1990). The complete DNA sequence of vaccinia virus. Virology 179, 247, 517.
Gubser, C., Hue, S., Kellam, P., and Smith, G. L. (2004). Poxvirus genomes: a phylogenetic analysis. J Gen Virol 85, 105.
Haas, A. L., Ahrens, P., Bright, P. M., and Ankel, H. (1987). Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. J Biol Chem 262, 11315.
Harte, M. T., Haga, I. R., Maloney, G., Gray, P., Reading, P. C., Bartlett, N. W., Smith, G. L., Bowie, A., and O'Neill, L. A. (2003). The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defense. J Exp Med 197, 343.
Hollinshead, M., Rodger, G., Van Eijl, H., Law, M., Hollinshead, R., Vaux, D. J., and Smith, G. L. (2001). Vaccinia virus utilizes microtubules for movement to the cell surface. J Cell Biol 154, 389.
Hollinshead, M., Vanderplasschen, A., Smith, G. L., and Vaux, D. J. (1999). Vaccinia virus intracellular mature virions contain only one lipid membrane. J Virol 73, 1503.
Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N., and Taniguchi, T. (2005). IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 434, 772.
Huang, J., Smirnov, S. V., Lewis-Antes, A., Balan, M., Li, W., Tang, S., Silke, G. V., Putz, M. M., Smith, G. L., and Kotenko, S. V. (2007). Inhibition of type I and type III interferons by a secreted glycoprotein from Yaba-like disease virus. Proc Natl Acad Sci U S A 104, 9822.
Isaacs, A., and Lindenmann, J. (1957). Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 147, 258.
Isaacs, A., Lindenmann, J., and Valentine, R. C. (1957). Virus interference. II. Some properties of interferon. Proc R Soc Lond B Biol Sci 147, 268.
Kotenko, S. V., Gallagher, G., Baurin, V. V., Lewis-Antes, A., Shen, M., Shah, N. K., Langer, J. A., Sheikh, F., Dickensheets, H., and Donnelly, R. P. (2003). IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat. Immunol. 4, 69.

Law, M., Carter, G. C., Roberts, K. L., Hollinshead, M., and Smith, G. L. (2006). Ligand-induced and nonfusogenic dissolution of a viral membrane. Proc Natl Acad Sci U S A 103, 5989.
Lee, H. J., Essani, K., and Smith, G. L. (2001). The genome sequence of Yaba-like disease virus, a yatapoxvirus. Virology 281, 170.
Leonard, W. J. (2001). Role of Jak kinases and STATs in cytokine signal transduction. Int J Hematol 73, 271.
Li, W. X. (2008). Canonical and non-canonical JAK-STAT signaling. Trends Cell Biol 18, 545.
Li, X. D., Wu, J., Gao, D., Wang, H., Sun, L., and Chen, Z. J. (2013). Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. Science 341, 1390.
Liu, Z., Wang, S., Zhang, Q., Tian, M., Hou, J., Wang, R., Liu, C., Ji, X., Liu, Y., and Shao, Y. (2013). Deletion of C7L and K1L genes leads to significantly decreased virulence of recombinant vaccinia virus TianTan. PLoS One 8, e68115.
Malakhova, O. A., Yan, M., Malakhov, M. P., Yuan, Y., Ritchie, K. J., Kim, K. I., Peterson, L. F., Shuai, K., and Zhang, D. E. (2003). Protein ISGylation modulates the JAK-STAT signaling pathway. Genes Dev 17, 455.
Maluquer de Motes, C., and Smith, G. L. (2017). Vaccinia virus protein A49 activates Wnt signalling by targetting the E3 ligase beta-TrCP. J Gen Virol.
Maniatis, T., Falvo, J. V., Kim, T. H., Kim, T. K., Lin, C. H., Parekh, B. S., and Wathelet, M. G. (1998). Structure and function of the interferon-beta enhanceosome. Cold Spring Harb Symp Quant Biol 63, 609.
Mann, B. A., Huang, J. H., Li, P., Chang, H. C., Slee, R. B., O'Sullivan, A., Anita, M., Yeh, N., Klemsz, M. J., Brutkiewicz, R. R., Blum, J. S., and Kaplan, M. H. (2008). Vaccinia virus blocks Stat1-dependent and Stat1-independent gene expression induced by type I and type II interferons. J Interferon Cytokine Res 28, 367.
Mansur, D. S., Maluquer de Motes, C., Unterholzner, L., Sumner, R. P., Ferguson, B. J., Ren, H., Strnadova, P., Bowie, A. G., and Smith, G. L. (2013). Poxvirus targeting of E3 ligase beta-TrCP by molecular mimicry: a mechanism to inhibit NF-kappaB activation and promote immune evasion and virulence. PLoS Pathog 9, e1003183.
Marq, J. B., Hausmann, S., Luban, J., Kolakofsky, D., and Garcin, D. (2009). The double-stranded RNA binding domain of the vaccinia virus E3L protein inhibits both RNA- and DNA-induced activation of interferon beta. J Biol Chem 284, 25471.
Meng, X., Jiang, C., Arsenio, J., Dick, K., Cao, J., and Xiang, Y. (2009). Vaccinia virus K1L and C7L inhibit antiviral activities induced by type I interferons. J Virol 83, 10627.
Meng, X., Schoggins, J., Rose, L., Cao, J., Ploss, A., Rice, C. M., and Xiang, Y. (2012). C7L family of poxvirus host range genes inhibits antiviral activities induced by type I interferons and interferon regulatory factor 1. J Virol 86, 4538.
Mercer, J., and Helenius, A. (2008). Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. Science 320, 531.
Meurs, E., Chong, K., Galabru, J., Thomas, N. S., Kerr, I. M., Williams, B. R., and Hovanessian, A. G. (1990). Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell 62, 379.
Montanuy, I., Alejo, A., and Alcami, A. (2011). Glycosaminoglycans mediate retention of the poxvirus type I interferon binding protein at the cell surface to locally block interferon antiviral responses. FASEB J 25, 1960.
Morchikh, M., Cribier, A., Raffel, R., Amraoui, S., Cau, J., Severac, D., Dubois, E., Schwartz, O., Bennasser, Y., and Benkirane, M. (2017). HEXIM1 and NEAT1 Long Non-coding RNA Form a Multi-subunit Complex that Regulates DNA-Mediated Innate Immune Response. Mol Cell 67, 387 e5.
Morgan, C., Ellison, S. A., Rose, H. M., and Moore, D. H. (1954). Structure and development of viruses observed in the electron microscope. II. Vaccinia and fowl pox viruses. J Exp Med 100, 301.
Moss, B. (1968). Inhibition of HeLa cell protein synthesis by the vaccinia virion. J Virol 2, 1028.
Moss, B. (2007). Poxviridae: the viruses and their replicaton. 5th ed. In "Fields Virology" (D. M. Knipe, ed.), Vol. 2, pp. 2905. Lippincott Williams & Wilkins, Philadelphia.
Moss, B. (2016). Membrane fusion during poxvirus entry. Semin Cell Dev Biol 60, 89.
Mossman, K., Upton, C., Buller, R. M., and McFadden, G. (1995). Species specificity of ectromelia virus and vaccinia virus interferon-gamma binding proteins. Virology 208, 762.
Muir, A. J., Shiffman, M. L., Zaman, A., Yoffe, B., de la Torre, A., Flamm, S., Gordon, S. C., Marotta, P., Vierling, J. M., Lopez-Talavera, J. C., Byrnes-Blake, K., Fontana, D., Freeman, J., Gray, T., Hausman, D., Hunder, N. N., and Lawitz, E. (2010). Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. Hepatology 52, 822.
Nagano, Y., and Kojima, Y. (1954). [Immunizing property of vaccinia virus inactivated by ultraviolets rays]. C R Seances Soc Biol Fil 148, 1700.
Nagano, Y., Kojima, Y., and Sawai, Y. (1954). [Immunity and interference in vaccinia; inhibition of skin infection by inactivated virus]. C R Seances Soc Biol Fil 148, 750.

Najarro, P., Traktman, P., and Lewis, J. A. (2001). Vaccinia virus blocks gamma interferon signal transduction: viral VH1 phosphatase reverses Stat1 activation. J Virol 75, 3185.
Paez, E., and Esteban, M. (1985). Interferon prevents the generation of spontaneous deletions at the left terminus of vaccinia virus DNA. J Virol 56, 75.
Paludan, S. R. (2015). Activation and regulation of DNA-driven immune responses. Microbiol Mol Biol Rev 79, 225.
Parrish, S., and Moss, B. (2007). Characterization of a second vaccinia virus mRNA-decapping enzyme conserved in poxviruses. J Virol 81, 12973.
Parrish, S., Resch, W., and Moss, B. (2007). Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci U S A 104, 2139.
Pereira, A. C., Leite, F. G., Brasil, B. S., Soares-Martins, J. A., Torres, A. A., Pimenta, P. F., Souto-Padron, T., Traktman, P., Ferreira, P. C., Kroon, E. G., and Bonjardim, C. A. (2012). A vaccinia virus-driven interplay between the MKK4/7-JNK1/2 pathway and cytoskeleton reorganization. J Virol 86, 172.
Perkus, M. E., Goebel, S. J., Davis, S. W., Johnson, G. P., Limbach, K., Norton, E. K., and Paoletti, E. (1990). Vaccinia virus host range genes. Virology 179, 276.
Pestka, S., Krause, C. D., and Walter, M. R. (2004). Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 202, 8
K. C., and Samuel, C. E. (1987). Interferons and their actions. Annu Rev Biochem 56, 727.
Peters, N. E., Ferguson, B. J., Mazzon, M., Fahy, A. S., Krysztofinska, E., Arribas-Bosacoma, R., Pearl, L. H., Ren, H., and Smith, G. L. (2013). A mechanism for the inhibition of DNA-PK-mediated DNA sensing by a virus. PLoS Pathog 9, e1003649.
Platanias, L. C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 5, 375.
Pomerantz, J. L., and Baltimore, D. (1999). NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. EMBO J 18, 6694.
Prokunina-Olsson, L., Muchmore, B., Tang, W., Pfeiffer, R. M., Park, H., Dickensheets, H., Hergott, D., Porter-Gill, P., Mumy, A., Kohaar, I., Chen, S., Brand, N., Tarway, M., Liu, L., Sheikh, F., Astemborski, J., Bonkovsky, H. L., Edlin, B. R., Howell, C. D., Morgan, T. R., Thomas, D. L., Rehermann, B., Donnelly, R. P., and O'Brien, T. R. (2013). A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet 45, 164.
Rietdorf, J., Ploubidou, A., Reckmann, I., Holmstrom, A., Frischknecht, F., Zettl, M., Zimmermann, T., and Way, M. (2001). Kinesin-dependent movement on microtubules precedes actin-based motility of vaccinia virus. Nat Cell Biol 3, 992.
Roberts, K. L., Breiman, A., Carter, G. C., Ewles, H. A., Hollinshead, M., Law, M., and Smith, G. L. (2009). Acidic residues in the membrane-proximal stalk region of vaccinia virus protein B5 are required for glycosaminoglycan-mediated disruption of the extracellular enveloped virus outer membrane. J Gen Virol 90, 1582.
Ryzhakov, G., and Randow, F. (2007). SINTBAD, a novel component of innate antiviral immunity, shares a TBK1-binding domain with NAP1 and TANK. EMBO J 26, 3180.
Sanderson, C. M., Hollinshead, M., and Smith, G. L. (2000). The vaccinia virus A27L protein is needed for the microtubule-dependent transport of intracellular mature virus particles. J Gen Virol 81, 47.
Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N., and Taniguchi, T. (2000). Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity 13, 539.
Schindler, C., Shuai, K., Prezioso, V. R., and Darnell, J. E., Jr. (1992). Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257, 809.
Schmelz, M., Sodeik, B., Ericsson, M., Wolffe, E. J., Shida, H., Hiller, G., and Griffiths, G. (1994). Assembly of vaccinia virus: the second wrapping cisterna is derived from the trans Golgi network. J Virol 68, 130.
Schrick, L., Tausch, S. H., Dabrowski, P. W., Damaso, C. R., Esparza, J., and Nitsche, A. (2017). An Early American Smallpox Vaccine Based on Horsepox. N Engl J Med 377, 1491.
Schroder, M., Baran, M., and Bowie, A. G. (2008). Viral targeting of DEAD box protein 3 reveals its role in TBK1/IKKepsilon-mediated IRF activation. EMBO J 27, 2147.
Senkevich, T. G., Ojeda, S., Townsley, A., Nelson, G. E., and Moss, B. (2005). Poxvirus multiprotein entry-fusion complex. Proc Natl Acad Sci U S A 102, 18572.
Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T. E., Kuestner, R., Garrigues, U., Birks, C., Roraback, J., Ostrander, C., Dong, D., Shin, J., Presnell, S., Fox, B., Haldeman, B., Cooper, E., Taft, D., Gilbert, T., Grant, F. J., Tackett, M., Krivan, W., McKnight, G., Clegg, C., Foster, D., and Klucher, K. M. (2003). IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat. Immunol. 4, 63.
Shuai, K., Schindler, C., Prezioso, V. R., and Darnell, J. E., Jr. (1992). Activation of transcription by IFN-gamma: tyrosine phosphorylation of a 91-kD DNA binding protein. Science 258, 1808.

Silva, P. N., Soares, J. A., Brasil, B. S., Nogueira, S. V., Andrade, A. A., de Magalhaes, J. C., Bonjardim, M. B., Ferreira, P. C., Kroon, E. G., Bruna-Romero, O., and Bonjardim, C. A. (2006). Differential role played by the MEK/ERK/EGR-1 pathway in orthopoxviruses vaccinia and cowpox biology. Biochem J 398, 83.
Silverman, R. H. (2007). Viral encounters with 2',5'-oligoadenylate synthetase and RNase L during the interferon antiviral response. J Virol 81, 12720.
Smith, G. L., Benfield, C. T., Maluquer de Motes, C., Mazzon, M., Ember, S. W., Ferguson, B. J., and Sumner, R. P. (2013). Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J Gen Virol 94, 2367.
Smith, G. L., and Chan, Y. S. (1991). Two vaccinia virus proteins structurally related to the interleukin-1 receptor and the immunoglobulin superfamily. J Gen Virol 72, 511.
Smith, G. L., Symons, J. A., and Alcamí, A. (1998). Poxviruses: interfering with interferon. Semin. Virol. 8, 409.
Soulat, D., Burckstummer, T., Westermayer, S., Goncalves, A., Bauch, A., Stefanovic, A., Hantschel, O., Bennett, K. L., Decker, T., and Superti-Furga, G. (2008). The DEAD-box helicase DDX3X is a critical component of the TANK-binding kinase 1-dependent innate immune response. EMBO J 27, 2135.
Stack, J., Haga, I. R., Schroder, M., Bartlett, N. W., Maloney, G., Reading, P. C., Fitzgerald, K. A., Smith, G. L., and Bowie, A. G. (2005). Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. J Exp Med 201, 1007.
Strnadova, P., Ren, H., Valentine, R., Mazzon, M., Sweeney, T. R., Brierley, I., and Smith, G. L. (2015). Inhibition of Translation Initiation by Protein 169: A Vaccinia Virus Strategy to Suppress Innate and Adaptive Immunity and Alter Virus Virulence. PLoS Pathog 11, e1005151.
Stuart, J. H., Sumner, R. P., Lu, Y., Snowden, J. S., and Smith, G. L. (2016). Vaccinia virus protein C6 inhibits type I IFN signalling in the nucleus and binds to the transactivation domain of STAT2. PLoS Pathog 12, e1005955.
Sumner, R. P., Maluquer de Motes, C., Veyer, D. L., and Smith, G. L. (2014). Vaccinia virus inhibits NF-kappaB-dependent gene expression downstream of p65 translocation. J Virol 88, 3092.
Symons, J. A., Alcami, A., and Smith, G. L. (1995). Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell 81, 551.
Symons, J. A., Tscharke, D. C., Price, N., and Smith, G. L. (2002). A study of the vaccinia virus interferon-gamma receptor and its contribution to virus virulence. J Gen Virol 83, 1953.
Thacore, H. R., and Youngner, J. S. (1973). Rescue of vesicular stomatitis virus from interferon-induced resistance by superinfection with vaccinia virus. I. Rescue in cell cultures from different species. Virology 56, 505.
Thanos, D., and Maniatis, T. (1995). Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. Cell 83, 1091.
Tooze, J., Hollinshead, M., Reis, B., Radsak, K., and Kern, H. (1993). Progeny vaccinia and human cytomegalovirus particles utilize early endosomal cisternae for their envelopes. Eur J Cell Biol 60, 163.
Torres, A. A., Albarnaz, J. D., Bonjardim, C. A., and Smith, G. L. (2016). Multiple Bcl-2 family immunomodulators from vaccinia virus regulate MAPK/AP-1 activation. J Gen Virol 97, 2346.
Townsley, A. C., Weisberg, A. S., Wagenaar, T. R., and Moss, B. (2006). Vaccinia virus entry into cells via a low-pH-dependent endosomal pathway. J Virol 80, 8899.
Tulman, E. R., Delhon, G., Afonso, C. L., Lu, Z., Zsak, L., Sandybaev, N. T., Kerembekova, U. Z., Zaitsev, V. L., Kutish, G. F., and Rock, D. L. (2006). Genome of horsepox virus. J Virol 80(18), 9244.
Unterholzner, L., Sumner, R. P., Baran, M., Ren, H., Mansur, D. S., Bourke, N. M., Randow, F., Smith, G. L., and Bowie, A. G. (2011). Vaccinia virus protein C6 is a virulence factor that binds TBK-1 adaptor proteins and inhibits activation of IRF3 and IRF7. PLoS Pathog 7, e1002247.
Upton, C., Mossman, K., and McFadden, G. (1992). Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science 258, 1369.
Upton, C., Slack, S., Hunter, A. L., Ehlers, A., and Roper, R. L. (2003). Poxvirus orthologous clusters: toward defining the minimum essential poxvirus genome. J Virol 77, 7590.
Valentine, R., and Smith, G. L. (2010). Inhibition of the RNA polymerase III-mediated dsDNA-sensing pathway of innate immunity by vaccinia virus protein E3. J Gen Virol 91, 2221.
Vanderplasschen, A., Hollinshead, M., and Smith, G. L. (1998). Intracellular and extracellular vaccinia virions enter cells by different mechanisms. J Gen Virol 79, 877.
Villarroya-Beltri, C., Guerra, S., and Sanchez-Madrid, F. (2017). ISGylation - a key to lock the cell gates for preventing the spread of threats. J Cell Sci 130, 2961.
Wack, A., Terczynska-Dyla, E., and Hartmann, R. (2015). Guarding the frontiers: the biology of type III interferons. Nat Immunol 16, 802.
Ward, B. M. (2005). Visualization and characterization of the intracellular movement of vaccinia virus intracellular mature virions. J Virol 79, 4755.
Ward, B. M., and Moss, B. (2001). Vaccinia virus intracellular movement is associated with microtubules and independent of actin tails. J Virol 75, 11651.

Whitaker-Dowling, P., and Youngner, J. S. (1983). Vaccinia rescue of VSV from interferon-induced resistance: reversal of translation block and inhibition of protein kinase activity. Virology 131, 128.
Zhou, Z., Hamming, O. J., Ank, N., Paludan, S. R., Nielsen, A. L., and Hartmann, R. (2007). Type III interferon (IFN) induces a type I IFN-like response in a restricted subset of cells through signaling pathways involving both the Jak-STAT pathway and the mitogen-activated protein kinases. J Virol 81, 7749.