Veterinary Microbiology 216 (2018) 132–141

Contents lists available at ScienceDirect

Veterinary Microbiology

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

# Modulation of type I interferon signaling by African swine fever virus (ASFV) of different virulence L60 and NHV in macrophage host cells

Raquel Portugal*,[1], Alexandre Leitão, Carlos Martins

*Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal*

ARTICLE INFO

*Keywords:*
African swine fever virus
Virulence
Type I interferon
Innate immunity
Signaling pathways

ABSTRACT

ASFV causes an important disease of domestic swine and wild boar. Currently no vaccine is available, highlighting the necessity to understand ASFV modulation of innate immune responses in natural host cells. With this aim, macrophage cultures enriched in SWC9 and CD163 differentiation markers were infected in parallel with high virulent ASFV/L60 and low virulent ASFV/NHV, the latter lacking MGF 360 and 505/530 genes associated with type I interferon (IFN I) control. IFN I production and signaling were studied after completion of the viral cycles. None of the viruses increased IFN I production in host cells, and accordingly, didn't cause activation of the central mediator of the pathway IRF3. However, upon stimulation by poly:IC treatment during infections, L60 and NHV similarly inhibited IFN I production. This didn't seem to depend on IRF3 modulation since its activation levels were not significantly decreased in L60 infection and were even increased in NHV's, in comparison to stimulated mock infections. The infections didn't evidently activate JAK-STAT pathway mediators STAT1 and STAT2, but did increase expression of interferon stimulated genes (ISGs), to higher levels in NHV than L60 infection. Interestingly, in presence of IFN-α, L60 but not NHV was able to decrease significantly the expression of some of the ISGs tested. Overall, both L60 and NHV were able to inhibit IFN I production in macrophages, through a mechanism not dependent on IRF3 modulation. The high virulent isolate showed however a more effective control of the downstream ISGs expression pathway.

## 1. Introduction

African swine fever virus (ASFV) is the causal agent of an important disease of domestic swine and wild boar, African swine fever (ASF), that constitutes a threat to worldwide pig production especially due to the lack of a vaccine or curative treatment. First described in 1921 in Kenya (Montgomery, 1921), ASFV was introduced into Europe in 1957 and 1960. With the exception of Sardinia where it is still endemic, ASF was eradicated from Europe in the ensuing decades. In 2007 however, it was introduced in Georgia at the Caucasus region, from where it continuously spread to neighbouring countries like the Ukraine and Russia, until in 2014 it reached the EU in Poland, Lithuania, Latvia and Estonia (Guinat et al., 2016). More recently also the Czech Republic and Romania suffered outbreaks of the disease (OIE, 2017).

ASFV is the only member of the family *Asfarviridae*, genus *Asfivirus*. It is a complex virus with a large dsDNA genome of 170–190 kbp, containing 150 to 167 ORFs depending on the isolate. The viral encoded proteins are involved not only in viral replication and morphogenesis but also in modulation of host cell functions and immune evasion (reviewed in Correia et al., 2013; Dixon et al., 2013; Reis et al., 2017). The main natural target cells of ASFV replication are macrophages, which are effectors of innate immune responses against pathogens and accordingly, are specialized in their phagocytosis and destruction, antigen presentation, or in producing signals triggering an inflammatory response and alerting the immune system of the threat (Wynn et al., 2013). To be able to replicate in macrophages, ASFV needs to circumvent all the above challenging processes, but on the other hand, infection of these cells constitutes an opportunity for the virus to immediately control the innate immune responses.

Viruses have since long evolved counteracting measures against their host cells innate immune defenses, where IFN I (IFN-α and IFN-β) produced by the generality of virally infected cells plays a fundamental role (reviewed in Hoffmann et al., 2015). The IFN I signaling pathway is triggered upon binding and entry of viruses, which cause perturbations at the cellular membrane, cytoskeleton and endocytosis pathways. The host cell response starts with the recognition of specific pathogen associated molecular patterns (PAMPs) by specialized cellular proteins, the pattern recognition receptors (PRRs), which are located in different

* Corresponding author.
*E-mail addresses:* raquel.portugal@pirbright.ac.uk (R. Portugal), alexandre@fmv.ulisboa.pt (A. Leitão), cmartins@fmv.ulisboa.pt (C. Martins).
[1] Current address: The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK.

https://doi.org/10.1016/j.vetmic.2018.02.008
Received 15 October 2017; Received in revised form 3 February 2018; Accepted 5 February 2018

cellular compartments (reviewed in Christensen and Paludan, 2017; Collins and Mossman, 2014). Once triggered, the PRRs initiate innate signal transduction pathways through a variety of adaptor proteins, which converge in the activation by phosphorylation of the transcription factor IFN regulatory factor 3 (IRF3), with some of the pathways also activating the transcription factor nuclear factor-kappa B (NF-κB). These translocate to the nucleus and activate the expression of IFN-β at a first stage (NF-κB also activates the transcription of pro-inflammatory cytokines), which then via an autocrine loop can induce IRF7 expression. IRF7 in its turn activates the expression of IFN-α subtype genes (Sato et al., 1998 and reviews by Gibbert et al., 2013; Ikushima et al., 2013; Hoffmann et al., 2015). The secreted IFN I (α/β) binds to its receptor on the infected and neighboring cells and activates the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway, leading to activation of central mediators STAT1 and STAT2 by phosphorylation. These form heterodimers with IRF9, assembling in the complex "interferon stimulated gene factor 3″ (ISGF3), which translocates to the nucleus and activates the transcription of genes that possess in their promoter areas an “interferon stimulated responsive element” (ISRE) (reviewed in Hoffmann et al., 2015). These interferon stimulated genes (ISGs) code for proteins targeting different viral components or virally induced processes, or activating host cell apoptosis (reviewed in Wang et al., 2017), creating an anti-viral state of the cells aimed at limiting further viral spread. For example, PKR recognizes double stranded RNA and phosphorylates the alpha subunit of the eukaryote translation initiation factor 2 (eIF2α) leading to a blockade of host and viral protein translation. Other roles for PKR in promoting IFN-β mRNA stability and NF-κB indirect activation have also been described (reviewed in Munir and Berg, 2013). ISG15 is strongly inducible by IFN I and related to ubiquitin. It conjugates with several cellular proteins, namely involved in innate immune responses such as IRF3, leading to their stabilization against degradation. ISG15 is also released from the cells, acting as a cytokine (reviewed in Schneider et al., 2014). Mx proteins constitute GTPases with anti-viral activity against a number of viruses. Their mechanism of action is not fully understood, but seems to involve recognition of nucleocapsid structures and restriction of their movement and localization in the cells (reviewed in Schneider et al., 2014). Bst-2 (Tetherin) is a trans-membrane protein that acts against enveloped viruses by retaining them at the cell membrane and hence preventing their release from infected cells, with a more recently described role also in NF-κB activation (reviewed in Sauter, 2014).

ASFV has several genes in its genome involved in evasion from the host defenses (reviewed in Reis et al., 2017). For instance, it possesses apoptosis inhibitor genes A179L, A224L, EP153R, homologous to Bcl-2, IAP or C-type lectins respectively (Galindo et al., 2008; Hernaez et al., 2013; Hurtado et al., 2004; Nogal et al., 2001), and ASFV of different virulence has been observed to differently modulate apoptosis in macrophages (Portugal et al., 2009a,b). Several ASFV genes were observed to modulate different steps of the interferon signaling pathways during transient expression in Vero cells using reporter assays (Correia et al., 2013). A276R prevented IFN-β induction through the inhibition specifically of the pathway leading to IRF3 activation but not NF-kB. A528R inhibited both of these pathways and additionally, was able to repress the impact of both IFN I and II, by inhibiting the JAK-STAT pathways leading to ISGs expression. The viral I329L has homology to TLR3 and acts as its antagonist, being suggested to block the TLR3 induced signaling route leading to NF-kB and IRF3/7 activation at the level of the adaptor molecule TRIF (Correia et al., 2013; de Oliveira et al., 2011). A238L is another viral gene that has the potential to intervene in a myriad of cellular pathways leading to the production of immunomodulatory proteins. It inhibits transcription factors NF-kB and NFAT, as well as CBP/p300 transcriptional coactivators, leading to inhibition of pro-inflammatory responses such as production of pro-inflammatory cytokines or expression of cyclooxygenase-2 and inducible nitric oxide synthase (Granja et al., 2006a; Granja et al., 2004; Granja et al., 2008; Granja et al., 2006b; Miskin et al., 2000; Miskin et al., 1998; Powell et al., 1996; Revilla et al., 1998).

Further determinants of ASFV pathogenesis, related with its immune evasion capacity, have been associated to the existence in the viral genome of a region containing several consecutive multigene family (MGF) 360 and 505/530 genes, a type of genes characteristic of ASFV that have no homology with known genes. This group of genes has been associated with virulence and related to the capacity of ASFV to fight IFN I anti-viral activity during infection *in vivo* and *in vitro* (Afonso et al., 2004; Golding et al., 2016; O’Donnell et al., 2015; Reis et al., 2016). It was observed that infection of swine macrophages *in vitro* with virulent isolates did not lead to IFN I expression, contrary to infection with low virulent ones possessing deletions of several of the MGF 360 and 505/530 members (Afonso et al., 2004; Gil et al., 2008; Golding et al., 2016; Reis et al., 2016). Interestingly, the opposite has been observed *in vivo*, with animals infected with virulent isolates showing IFN I in circulation (Golding et al., 2016; Karalyan et al., 2012), whereas infection with a low virulent isolate lacking several of the MGF 360 and 505/530 genes did not (Golding et al., 2016). Of note, the animals infected with the virulent virus also had high viraemia, indicating that virulent ASFV is able to replicate in spite of the IFN I induction in the host.

Overall, these different studies indicate that ASFV has the capacity to evade the innate immune response of the host with regard to IFN I production and its anti-viral effect, and suggest that virulent isolates have a greater capacity for evasion than low virulent or attenuated ones, where MGF 360 and 505/530 genes seem to have an important role. Indeed, natural isolates of ASFV that lack several of these genes have low virulence (Chapman et al., 2008; Golding et al., 2016; Leitao et al., 2001; Portugal et al., 2015), and their deletion from the genome of virulent isolates leads to attenuation for swine (Afonso et al., 2004; O’Donnell et al., 2015; Reis et al., 2016).

Using the virulent isolate L60 and the low virulent NHV, the latter also containing the deletion of several MGF 360 and 505/530 members (Portugal et al., 2015), our aim was to compare the impact of both infections on the intracellular signaling pathways leading to IFN I production and the downstream activation of the JAK-STAT pathway and antiviral ISGs transcription, simultaneously assessing whether the NHV isolate possesses a different capacity comparatively to L60 for modulating IFN I pathways.

## 2. Materials and methods

### 2.1. Primary macrophage cultures and virus

Swine blood from crossbred Large White x Landrace pigs (approximately 6-month old) was collected at the abattoir, at the moment of bleeding, into flaks containing heparin (20 IU/ml blood) and 10% v/v of a solution with 5% v/v Dextran T500 in Hank’s balanced saline solution. The blood samples were incubated at room temperature until clear sedimentation of erythrocytes was observed. The supernatant was then collected and overlaid on top of 15 ml Biocoll separating solution (L 6113, Biochrom) in 50 ml centrifuge tubes and centrifuged at 600xg for 30 min at room temperature, without break. The supernatant containing autologous plasma (AP) was recovered, centrifuged at 1500xg for 30 min and kept refrigerated until further use. Buffy coat layers with peripheral blood mononuclear cells (PBMC) were collected, washed twice in Hank’s balanced saline solution and resuspended in culture medium (RPMI 1640 with 100 IU/ml penicillin, 100 µg/ml streptomycin, and 20 mM HEPES) with 10% fetal calf serum (FCS). Viable cell numbers were determined through trypan blue dye exclusion. The collected PBMCs were screened for the presence of nucleic acids of Porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRS), porcine circovirus 2 (PCV2) and mycoplasma through PCR or real time PCR (qPCR) (see Supplementary data S1). Only PBMC samples that tested negative for these agents were further