Veterinary Microbiology 162 (2013) 937–943

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

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

Short communication

Modulation of chemokine and chemokine receptor expression following infection of porcine macrophages with African swine fever virus

Emma Fishbourne, Charles C. Abrams, Haru-H. Takamatsu, Linda K. Dixon *

The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK

ARTICLE INFO

Article history:
Received 20 August 2012
Received in revised form 13 November 2012
Accepted 22 November 2012

Keywords:
African swine fever
Chemokines
Immune evasion
Transcription

ABSTRACT

African swine fever virus (ASFV) is the only member of the *Asfarviridae*, a large DNA virus family which replicates predominantly in the cytoplasm. Most isolates cause a fatal haemorrhagic disease in domestic pigs, although some low virulence isolates cause little or no mortality. The modulation of chemokine responses following infection of porcine macrophages with low and high virulence isolates was studied to indicate how this may be involved in the induction of pathogenesis and of effective immune responses. Infection with both low and high virulence isolates resulted in down-regulation of mRNA levels for chemokines CCL2, CCL3L, CXCL2 and chemokine receptors CCR1, CCR5, CXCR3, CXCR4 and up-regulation in expression of mRNAs for CCL4, CXCL10 and chemokine receptor CCR7. Levels of CCL4, CXCL8, CXCL10 mRNAs were higher in macrophages infected with low virulence isolate OURT88/3 compared to high virulence isolate Benin 97/1. Levels of CXCL8 and CCL2 protein were significantly reduced in supernatants from macrophages infected with Benin 97/1 isolate compared to OURT88/3 and mock-infected macrophages. There was also a decreased chemotactic response of donor cells exposed to supernatants from Benin 97/1 infected macrophages compared to those from OURT88/3 and mock-infected macrophages. The data show that infection of macrophages with the low virulence strain OURT88/3 induces higher expression of key inflammatory chemokines compared to infection with high virulence strain Benin 97/1. This may be important for the induction of effective protective immunity that has been observed in pigs immunised with the OURT88/3 isolate.

1. Introduction

African swine fever (ASF) is a fatal haemorrhagic disease of domestic pigs caused by a large, cytoplasmic DNA virus, African swine fever virus (ASFV). ASFV is currently the only member of the *Asfarviridae* family (Dixon et al., 2005). The natural reservoir hosts of ASFV are the warthog (*Phacochoerus africanus*) and bush pig (*Potamchoerus porcus*), which show few if any clinical signs of disease, although they can be persistently infected (Oura et al., 1998a). ASFV isolates differ in virulence and include, in addition to highly virulent isolates, isolates of moderate virulence from which up to 50% of pigs survive infection and low virulence isolates which cause few clinical signs. Low virulence isolates, such as OURT88/3 or NHVP68 (Boinas et al., 2004; Leitao et al., 2001), can induce protection against challenge with related virulent viruses.

ASF is characterised by apoptosis of bystander lymphocytes. Virulent isolates cause severe tissue destruction and cell depletion in lymphoid tissue (Carrasco et al., 1996; Oura et al., 1998b). ASFV primarily infects macrophages and soluble mediators produced by these cells have been implicated in ASFV pathogenesis. These mediators include tumour necrosis factor (TNF)α which has been shown to be elevated in the serum and tissues of pigs infected with virulent ASFV isolates (del Moral et al., 1999; Salguero et al., 2008).

* Corresponding author. Tel.: +44 1483 232441; fax: +44 1483 232448.
E-mail address: Linda.dixon@pirbrightac.uk (L.K. Dixon).

0378-1135
http://dx.doi.org/10.1016/j.vetmic.2012.11.027

ASFV encodes between 150 and 167 proteins, a number of which have been shown to modulate host immune responses (Dixon et al., 2004; Tulman et al., 2009). Proteins have been identified that inhibit interferon induction, host transcription factor activation, stress responses and apoptosis. The ASFV gene A238L codes for a protein that inhibits transcriptional activation of a range of host immunomodulatory genes, including pro-inflammatory cytokines and chemokines by inhibiting activation of NFκB, calcineurin phosphatase(Miskin et al., 1998; Powell et al., 1996) and the host transcriptional co-activator p300. This latter effect seems specific to transcription factors that act through the N-terminal but not C-terminal domain of p300 (Granja et al., 2009). An ASFV Toll like receptor (TLR)3 inhibitor, encoded by the ASFV gene I329L has been identified (de Oliveira et al., 2011) which could reduce transcription of genes through its effect on the pattern recognition receptors (PPRs) and resultant downstream signalling pathways. In addition deletion of 6 members of multigene family 360 (MGF360) and 2 of multigene family 530 (MGF530), from a virulent isolate Pr4, was shown to result in an increased type I IFN response and induction of IFN stimulated genes (ISGs). This suggests that these genes inhibit type I IFN induction and possibly IFN activated pathways (Afonso et al., 2004). Macrophages, the main target cells for ASFV replication, produce a range of inflammatory mediators including chemokines. Chemokines contribute to induction of pathology and protective immunity (Sallusto et al., 2000; Wack et al., 2011). Inflammatory chemokines promote the migration of leucocytes to an injured or infected cell, activate cells to raise an immune response and start wound healing (Baggiolini, 1998; Johnston and Butcher, 2002; Laing and Secombes, 2004; Moser and Loetscher, 2001). The importance of chemokines in the antiviral response is emphasised since other large DNA viruses have developed strategies to evade chemokine responses. These include virus-encoded proteins that mimic chemokines and their receptors or secreted proteins that are able to bind host chemokines. These proteins function to modulate the normal function of the host chemokine system (Alcami and Lira, 2010). Viral versions of chemokines may function as antagonists, agonists or facilitate dissemination and growth of the virus.

In the present study the effect of ASFV isolates, varying in virulence on the host chemokine system was compared in macrophages following infection *in vitro*, since these are the main cell type infected *in vivo*. The aim was to better understand the differences in response in the infected macrophages rather than in bystander cells and thus to identify how ASFV infection may initially modulate the host chemokine response. To achieve this, primary pig macrophages isolated from blood were infected at high multiplicity to obtain infection rates of greater than 90%. The mRNA levels were measured for a number of inflammatory chemokines and chemokine receptors known to be expressed in macrophages. The induction of chemotactic substances in supernatants from infected macrophages was also studied.

2. Materials and methods

2.1. Preparation and culture of porcine macrophages

Blood-derived macrophages were obtained from whole blood by incubating the blood with 6% Dextran for 30 min at 37 °C to sediment red blood cells with subsequent lysis of any contaminating red blood cells remaining using ammonium chloride. Remaining leucocytes were then washed and re-suspended in DMEM plus Hepes containing 30% porcine serum and plated in 12-well culture dishes. Cells were cultured at 37 °C and 5% CO_2 for 2 h then non-adherent cells were removed and cells were further cultured for 48 h before washing 3 times to remove non-adherent cells.

2.2. Virus isolates, titrations and infections

ASFV isolates used for infections were low virulence OURT88/3 isolate (Boinas et al., 2004) and the high virulence isolate Benin 97/1 (Chapman et al., 2008) which both belong to genotype I. These isolates were chosen because complete genome sequences are available and the proteins encoded are very closely related. Differences between the genomes include deletions of 5 members of MGF360 and 2 members of MGF530 from close to the left genome end and disruptions of genes EP402R and EP153R in the genome of the OURT88/3 isolate. These encode a CD2-like protein and a C-type lectin (Chapman et al., 2008).

These isolates were propagated on porcine bone marrow cells and purified (Zhang et al., 2006). Stocks were titrated by haemadsorption (Malmquist, 1960). Blood-derived macrophages were infected at a multiplicity of infection of 5 by incubating the cells for 2 h in serum free media. Mock-infected cells were treated in the same way using virus-free media. At 16 h post-infection, cell supernatants or lysates were recovered. Cell supernatants were centrifuged to remove any cellular debris and used in chemotaxis or ELISA assays. RNA was extracted from lysates as described below. Infection rates were measured by fixing cells in 4% paraformaldehyde, permeabilising with 0.2% Triton-X-100 and staining with anti-ASFV p30 and VP72 monoclonal antibodies C18 and 4H3 at a dilution of 1 in 10 (Cobbold and Wileman, 1998; Stefanovic et al., 2005), followed by Alexa-Fluor conjugated anti-mouse IgG1 antibodies (Invitrogen) at a concentration of 1 in 200 and visualised using a Leica TCSSP2 confocal microscope.

2.3. RNA preparation and quantitative reverse transcription PCR

RNA was isolated, from infected or mock-infected cells harvested at 16 h post-infection, using Qiagen RNAeasy Blood mini kits with Qiagen Shredders and eluted in a 50 μl volume. Contaminating DNA was removed using Turbo DNAse (Ambion) and RNA quality was assessed using a Bioanalyser 2100 (Agilent) and the quantity was measured using a nanodrop. Two hundred and fifty ng of total RNA was reverse transcribed into cDNA using Superscript III (Invitrogen) and anchored oligo dT primer.