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Reactive oxygen species activate NFκB (p65) and p53 and induce apoptosis in RVFV infected liver cells

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ABSTRACT

Rift Valley fever virus (RVFV) infection is often associated with pronounced liver damage. Previously, our studies revealed altered host phospho-signaling responses (NFκB, MAPK and DNA damage responses) in RVFV infected epithelial cells that correlated with a cellular stress response. Here, we report that RVFV infection of liver cells leads to an increase in reactive oxygen species (ROS). Our data suggests the presence of the viral protein NSs in the mitochondria of infected cells, hence contributing to early increase in ROS. Increased ROS levels correlated with activation of NFκB (p65) and p53 responses, which in conjunction with infection, was also reflected as macromolecular rearrangements observed using size fractionation of protein lysates. Additionally, we documented an increase in cytokine expression and pro-apoptotic gene expression with infection, which was reversed with antioxidant treatment. Collectively, we identified ROS and oxidative stress as critical contributors to apoptosis of liver cells during RVFV infection.

Introduction

Rift Valley fever virus (RVFV) is an arbovirus belonging to the genus *Phlebovirus*, family *Bunyaviridae* (Ikegami and Makino, 2011; Weaver and Reisen, 2010; Bouloy and Weber, 2010; Ikegami, 2012). The viral genome is a single stranded RNA, which exists as three segments namely, large (L), medium (M) and small (S) (Terasaki et al., 2011; Gauliard et al., 2006; Ikegami et al., 2005a, 2005b). The L segment codes for the viral RNA dependent RNA polymerase. The M segment codes for the two structural proteins Gn and Gc and two nonstructural proteins, 78 kDa protein and NSm. The S segment codes for the N protein and the nonstructural protein NSs. NSs plays an essential role in the viral life cycle by down regulating the host innate immune response early in infection (Kalveram et al., 2011, 2013; Benferhat et al., 2012; Mansuroglu et al., 2010; Ikegami et al., 2009a, 2009b; Habjan et al., 2009; Le May et al., 2008). NSm protein has been demonstrated to have an anti-apoptotic function in infected cells (Won et al., 2007).

RVFV is mainly transmitted by mosquitoes, although other arthropods including flies have also been demonstrated to be adequate hosts (Amraoui et al., 2012; Turell et al., 2008, 2010a, 2010b; Seufi and Galal, 2010). Viral infection of humans results in Rift Valley Fever (RVF) with flu-like symptoms (Ikegami and Makino, 2011). RVFV infections are often accompanied by liver damage. In a small percentage of cases, RVF can result in hemorrhage and neuronal phenotypes. Viral infection of livestock results in abortion rates that can reach 100% and high fatality among newborns (Ikegami and Makino, 2011; Bouloy and Weber, 2010). RVFV is classified as a category A select agent, an emerging infectious agent and an agricultural pathogen. The currently available vaccine candidates include mutant viruses (deleted for NSs, NSm segments), vaccines based on structural proteins, reconstructed virions, viral replicon particles or serially passaged attenuated virus (Bird et al., 2011; Kortekaas et al., 2012; Dodd et al., 2012; Kalveram et al., 2011; Jansen van Vuren et al., 2011; Morrill and Peters, 2011; Pichlmair et al., 2010; de Boer et al., 2010; Mandell et al., 2010; Ikegami and Makino, 2009). These candidates however, have not yet been approved for human use.

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Interactions between the virus and its host have been subject to intense investigation by multiple laboratories as this can provide critical information for the development of novel diagnostic and therapeutic avenues. Our previous approaches to understand host–pathogen interactions of RVFV and its human host using Reverse Phase Protein MicroArray (RPMA) have yielded significant information about host responses in infected cells (Popova et al., 2010). We have shown that RVFV infection of human cells induced multiple shifts in the anti- and pro-apoptotic pathways at early and late stages of infection. RVFV infection resulted in activation of stress responses and DNA damage responses (DDR) (Narayanan et al., 2011; Baer et al., 2012). Additionally, RVFV infection induced activation of critical transcription factors including NFκB (p65) and p53 (Narayanan et al., 2012; Austin et al., 2012). Our laboratory and those of our colleagues have demonstrated that inhibition of host responses during RVFV infection is a viable method to inhibit viral multiplication and afford protection to the host (Popova et al., 2010; Baer et al., 2012; Narayanan et al., 2012; Moser et al., 2012; Filone et al., 2010).

Studies with animal model systems have demonstrated that RVFV infection induces apoptotic responses in the liver of infected animals (Smith et al., 2010, 2012; Reed et al., 2012; Ding et al., 2005). Notably, strong inflammatory responses including cytokine secretion have been indicated to be involved in the apoptotic outcome in infected livers. Oxidative stress following infection is a key component that influences disease outcome in the host in the case of multiple viral infections including Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV), and Crimean Congo Hemorrhagic Fever virus (CCHF) (Duygu et al., 2012; Tawadrous et al., 2012; Lin et al., 2011; Rodrigues et al., 2012). Many viral proteins have been demonstrated to actively modulate the host oxidative stress responses to facilitate replication. For example, the viral core protein of HCV was shown to increase oxidative stress in HepG2 cells by interfering with the heme oxygenase mediated host response (Abdalla et al., 2012). HCV proteins including E1 and E2 were demonstrated to activate the antioxidant defense Nrf2/ARE pathway via several independent mechanisms (Ivanov et al., 2011). HCV nonstructural proteins contribute to pathogenesis by influencing mitochondrial redox potential ending in mitochondrial injury and apoptosis in liver cells (Abdalla et al., 2005; Gong et al., 2001). Use of antioxidants has had beneficial effects in HCV patients by attenuating multiple oxidative stress induced responses (Farias et al., 2012).

Reactive oxygen species (ROS) is an important host component that contributes to the innate immune response against multiple pathogens. ROS production may directly influence pathogen killing as seen in the case of infected macrophages (Jaeschke, 2011). ROS are also secondary signaling messengers that activate many host phospho-signaling responses including the nuclear factor kappa beta (NFkB), activating protein-1 (AP-1), mitogen-activating protein kinase (MAPK), phosphotidyl inositol-3 kinase (PI3K) and DNA damage response (DDR) pathways (Waris et al., 2001; Gwinn and Vallyathan 2006; Yang et al., 2010; Imai et al., 2008). Activation of transcription factors such as p65 and p53 due to increased intracellular ROS contributes to the inflammatory responses such as cytokine secretion.

In this manuscript, we have investigated the early onset of oxidative stress that leads to apoptotic responses in RVFV-infected liver cells. Our working hypothesis was that RVFV infection-induced oxidative stress activated both p65 and p53, which in turn modulated cytokine secretion and apoptotic gene expression profiles leading to cell death. Therefore, amelioration of oxidative stress in infected cells using antioxidants would decrease inflammatory cytokine expression, and apoptotic gene expression. Our data indicate that p65 activation occurred early in the temporal sequence of events after RVFV infection while p53 activation occurred at later time points. Antioxidants down regulated expression of inflammatory cytokines and apoptotic gene expression in liver cells. Therefore, antioxidants warrant further examination as a potential therapeutic strategy to achieve hepatoprotection in RVFV infections.

Results

HepG2 cells display increased reactive oxygen species following RVFV infection

We had demonstrated in our earlier studies that RVFV infection of epithelial cells resulted in increased expression of superoxide dismutase, an enzyme that is involved in oxidative stress response (Narayanan et al., 2011). The liver is one of the most prominently affected organs during RVFV infection. Oxidative stress has been demonstrated to play a critical role in liver pathologies both in the case of cancer and in virus infections such as HCV (Tawadrous et al., 2012). In many of these pathological responses in the liver, the outcome was associated with the host inflammatory responses including production of cytokines and resultant apoptosis (Duygu et al., 2012; Tawadrous et al., 2012; Lin et al., 2011). Therefore, we attempted to analyze oxidative stress and associated cytokine responses in RVFV infected liver cells. We first examined the generation of ROS in RVFV infected HepG2 cells following infection. HepG2 cells were infected with the MP-12 strain of RVFV, fixed and stained using MitoSox stain at 3 h post infection.

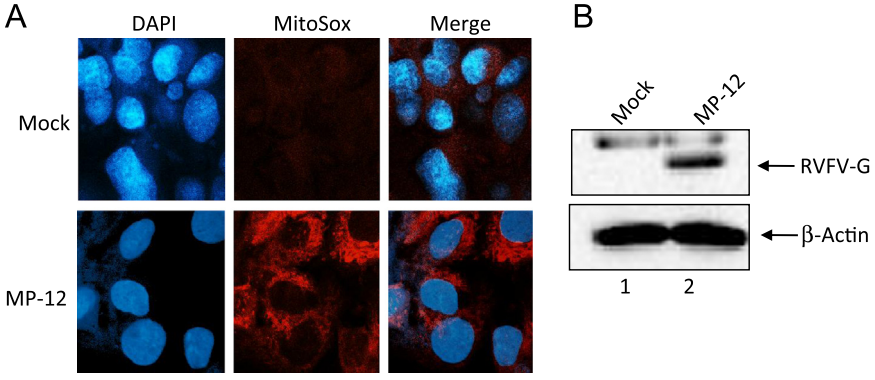

Fig. 1. Increased ROS in RVFV infected HepG2 cells. (A) HepG2 cells were infected with the MP-12 strain of RVFV (MOI: 3) and stained after 3 h using MitoSox stain and DAPI. The stained cells were visualized by confocal microscopy. (B) HepG2 cells infected with MP-12 (MOI: 3) were lysed at 24 h post infection and total protein lysate obtained. Lysates from uninfected cells were obtained as controls and both lysates were resolved by SDS-PAGE and western blots, carried out using anti-RVFV and anti-β-actin antibodies.