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Viral gene expression potentiates reovirus-induced necrosis

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ABSTRACT

Infection of some cell types by reovirus evokes a caspase-independent form of cell death resembling necrosis. While reovirus strain T3D induces necrosis much more efficiently than strain T1L, which viral components contribute to this difference is not known. In this study, we identified that the sialic acid binding property of the reovirus σ1 protein affects necrosis efficiency. We found that in addition to sialic acid engagement by the virus particles, viral gene expression, in the form of viral RNA or protein synthesis, is also required for necrosis induction. Our studies reveal that sialic acid does not directly participate in necrosis induction by initiating a signaling pathway. Instead, sialic acid engagement augments necrosis induction indirectly, by increasing reovirus gene expression in each infected cell. Comparison of our results with previous studies suggests that reovirus-induced apoptosis and necrosis are initiated by distinct stages of viral infection.

Introduction

Programmed cell death is an antiviral mechanism (Flint et al., 2009; Upton and Chan, 2014). To overcome cell death mediated restriction of virus replication, viruses encode strategies to prevent or delay cell death (Hay and Kannourakis, 2002; Lamkanfi and Dixit, 2010; Mocarski et al., 2012). Regardless of whether viruses are hampered by the cell death response or have evolved mechanisms to overcome it, death of the infected cells contributes to viral pathogenesis (Clarke and Tyler, 2009; Mocarski et al., 2014).

Mammalian reovirus, henceforth referred to as reovirus, is used as an experimental system to understand how the interplay between viral and cellular factors controls the induction and execution of death responses (Danthi et al., 2013). One form of programmed cell death, apoptosis, contributes to encephalitis and myocarditis following reovirus infection of cells within the central nervous system and heart, respectively (Beckham et al., 2010; Berens and Tyler, 2011; Danthi et al., 2008a, 2008b, 2010; DeBiasi et al., 2004; O'Donnell et al., 2005; Richardson-Burns et al., 2002). Apoptosis induction following reovirus infection involves activation of host transcription factor NF-κB by the upstream IκB kinase (IKK) (Connolly et al., 2000; Hansberger et al., 2007). NF-κB activation is required for caspase-8-mediated cleavage of Bid, a BH3-only member of the Bcl-2 family of proteins (Danthi et al., 2010; Kominsky et al., 2002a, b). Cleaved Bid, tBid, activates the intrinsic apoptotic cascade leading to the activation of effector caspases (Danthi et al., 2010). A role for other proteins such as transcription factor IRF-3, the protein kinase c-Jun N-terminal kinase (JNK), and the protease calpain in reovirus-induced cell death has also been suggested (Clarke et al., 2004; Debiasi et al., 1999; Holm et al., 2007; Knowlton et al., 2012). Recent studies indicate that reovirus infection of some cell types results in an alternative, necrotic form of cell death (Berger and Danthi, 2013). Necrosis following reovirus infection occurs in absence of NF-κB function or caspase activity, but instead is diminished by blocking the kinase activity of RIP1 (Berger and Danthi, 2013).

Events in reovirus replication that result in apoptosis induction have been extensively studied. The efficiency of apoptosis induction following reovirus infection is affected by receptor engagement (Barton et al., 2001b; Connolly et al., 2001). In addition, events that occur after penetration of host cell membranes by reovirus but prior to synthesis of viral RNA and proteins also are implicated in apoptosis induction (Connolly and Dermody, 2002; Danthi et al., 2006). Consistent with the effect of early events in virus infection on apoptosis induction, the efficiency of apoptosis following reovirus infection maps to the viral S1 and M2 gene segments, which respectively encode the σ1 attachment protein and the μ1 membrane penetration protein (Connolly et al., 2001; Danthi et al., 2006; Rodgers et al., 1997; Tyler et al., 1996, 1995). The capacity of the σ1 protein to engage cell surface receptors affects NF-κB activation and caspase activation and therefore is a major determinant of apoptotic potential (Barton et al., 2001b; Connolly et al., 2001). Whether σ1-receptor interactions contribute to cell death directly by activating a signaling pathway, or

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indirectly through effects on other aspects of viral replication, remains unknown.

While it is not precisely known how necrosis is initiated following reovirus infection, there is a key difference in the viral requirement for apoptosis and necrosis induction. Reovirus-induced apoptosis can be triggered by genome-deficient reovirus particles or UV-inactivated virus particles (Connolly and Dermody, 2002; Danthi et al., 2006). In contrast, reovirus-induced necrosis requires the presence of transcriptionally competent viral RNA (Berger and Danthi, 2013). Here, we demonstrate that necrosis induction following reovirus infection requires viral gene expression and that viral gene expression is enhanced by the capacity of the viral attachment protein σ1 to bind sialic acid. Both, the requirement for viral gene expression for necrosis induction and the means by which sialic acid engagement affects necrosis induction indicate that reovirus-induced apoptosis and necrosis are initiated via distinct mechanisms.

Results

The reovirus S1 gene affects efficiency of necrosis induction

We previously showed that reovirus infection of L929 cells results in caspase-independent cell death (Berger and Danthi, 2013). Cell death in L929 cells is enhanced by RIP1 kinase activity and exhibits features of necrosis (Berger and Danthi, 2013). We also showed that among prototype reovirus strains, T3D induces necrosis much more efficiently than T1L (Berger and Danthi, 2013). Based on previous studies, which indicated that strain-specific differences in cell death induction are controlled by the viral S1 gene, we assessed the contribution of S1 to the necrosis-inducing capacity of reovirus (Connolly et al., 2001; Rodgers et al., 1997; Tyler et al., 1996, 1995). For these experiments we utilized recombinant monoreassortant viruses T1L/T3DS1 and T3D/T1LS1 (Boehme et al., 2009; Kobayashi et al., 2010). The capacity of these viruses to induce necrosis in L929 cells was compared to the parental strains T1L and T3D. Consistent with our previous work, T3D induced significantly higher amounts of cell death than T1L (Berger and Danthi, 2013) (Fig. 1). We observed that the presence of a T3D S1 gene in an otherwise T1L background (T1L/T3DS1) resulted in cell death equivalent to that observed following T3D infection. Conversely, the presence of a T1L S1 gene in an otherwise T3D background resulted in T1L-like levels of cell death. These data suggest that the viral S1 gene determines the efficiency of cell death induction following reovirus infection.

Sialic acid binding confers potency for necrosis

The viral S1 gene encodes the σ1 attachment protein and the σ1s non-structural protein (Dermody et al., 2013). In this study, we focused on the role of the σ1 protein. While T1L and T3D σ1 proteins both bind the JAM-A proteinaceous receptor, they bind different glycan receptors (Barton et al., 2001b; Campbell et al., 2005; Reiss et al., 2012; Reiter et al., 2011). Whereas T1L σ1 engages GM2 glycans, T3D σ1 engages GM3 glycans that terminate in sialic acid (Reiss et al., 2012; Reiter et al., 2011). To determine the contribution of sialic acid binding capacity of σ1 to the higher necrosis potential of T3D and T1L/T3DS1, we utilized reovirus strains T3SA+ and T3SA− (Barton et al., 2001a). While T3SA+ engages sialic acid, T3SA− contains a Leu-to-Pro change at residue 204 near the sialic acid binding site of σ1 and cannot bind sialic acid (Barton et al., 2001a; Reiter et al., 2011). All other gene segments in T3SA+ and T3SA− are derived from T1L and are identical. Comparison of cell death induction by T3SA+ and T3SA− indicates that T3SA+ induces significantly higher cell death than T3SA− at both 48 and 72 h following infection (Fig. 2A). These data suggest that sialic acid binding controls efficiency of cell death induction in L929 cells. As an alternate strategy to evaluate the importance of sialic acid binding to cell death induction, we pretreated the cells with neuraminidase to remove cell surface sialic acid. Neuraminidase treatment diminished the necrotic potential of T3SA+ but had no effect on cell death induction by T3SA− after 48 h of infection (Fig. 2B). Together our data indicate that sialic acid binding is required for efficient induction of cell death.

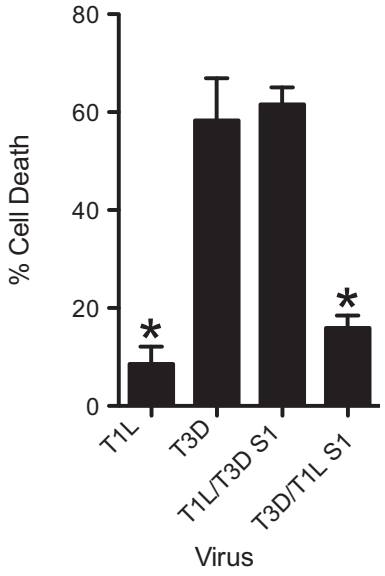

Fig. 1. The S1 gene determines efficiency with which reovirus induces necrosis. ATCC L929 cells were adsorbed with 10 PFU/cell of T1L, T3D, T1L/T3DS1, or T3D/T1LS1. Following incubation at 37 °C for 48 h, cells were stained with AOEB. The results are expressed as the mean percentages of cells undergoing cell death for three independent experiments. Error bars indicate SD. * $P < 0.05$ by student's t test in comparison to T3D.

Because both type 1 and type 3 reovirus strains kill L929 cells by necrosis (Berger and Danthi, 2013), we expected that T3SA+ would also induce necrotic cell death in this cell type. To confirm the mode of cell death induced by T3SA+, we measured the sensitivity of T3SA+ induced cell death to a pan-caspase inhibitor (Q-VD-OPh) and a RIP1 kinase inhibitor (Nec1)(Caserta et al., 2003; Degterev et al., 2008). Analogous to our previous observations with T1L and T3D (Berger and Danthi, 2013), cell death following infection was diminished by Nec1 but not Q-VD-OPh (Fig. 2C). Thus, cell death following infection of L929 cells with T3SA+ occurs in a caspase independent manner and is sensitive to blockade of RIP1 kinase function.

Sialic acid binding alone is not sufficient for necrosis induction

To determine if sialic acid binding is sufficient for necrosis induction, we assessed the capacity of UV-treated T3SA+ to induce necrosis. To ensure that UV treatment did not adversely damage the integrity of reovirus particles, we exposed the virus to UV for only 1 min. To confirm that UV treatment does not affect virus attachment and entry, we compared disassembly kinetics of untreated and UV-treated virus over the first 6 h of infection. Based on the evidence that equivalent amount of virus was found attached to cells at 0 h post-infection and that a comparable amount of δ was generated at 3 and 6 h following infection, we conclude that UV treatment did not influence virus attachment, internalization and disassembly (Fig. 3A). Due to the short duration of UV treatment, we observed that virus infectivity was not