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The pseudorabies virus US3 protein kinase possesses anti-apoptotic activity that protects cells from apoptosis during infection and after treatment with sorbitol or staurosporine

Kristin Geenen[a], Herman W. Favoreel[a,b,*], LeighAnne Olsen[c], Lynn W. Enquist[c], Hans J. Nauwynck[a]

[a]*Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*
[b]*Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*
[c]*Department of Molecular Biology, Princeton University, Princeton, NJ 08540, USA*

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Abstract

Most large DNA viruses, like herpesviruses, encode anti-apoptotic proteins to interfere with the apoptotic cellular response to infection. Previous studies have shown that the US3 protein kinase of herpes simplex virus, in contrast to US3 of bovine herpes virus 1, is very potent in protecting cells from apoptosis induced by the virus itself or by a broad range of exogenous apoptotic stimuli. Here, we demonstrate that US3 of the swine alphaherpesvirus pseudorabies virus (PRV) suppresses PRV-induced apoptosis in swine-testicle (ST) cells at late stages in infection, and that it protects ST cells from apoptosis induced by either sorbitol or staurosporine. Interestingly, PRV US3 encodes a short and a long isoform, the latter of which contains a functional mitochondrial localization sequence. Transient transfections showed that the PRV US3 long isoform is more efficient in protecting ST cells from PRV- or staurosporine-induced apoptosis, suggesting a potential advantage for the mitochondrial localization of PRV US3 in implementing its anti-apoptotic function.

Keywords: Pseudorabies virus, PRV; Apoptosis; US3; Mitochondrial localization

Introduction

During infection of a cell, most viruses generate pro-apoptotic signals that enable the host to limit virus replication and spread. Many viruses, especially large DNA viruses with a complicated and time-consuming replication cycle, have evolved various strategies to escape clearance by apoptosis of the infected cell. Indeed, poxviruses, adenoviruses and herpesviruses encode multiple anti-apoptotic genes to suppress apoptosis (reviewed by Koyama et al., 2000). Over the last few years, several anti-apoptotic genes have been identified in both herpes simplex virus serotypes (HSV-1 and HSV-2). The US3 protein kinase, which is conserved among all α-herpesviruses including the swine pseudorabies virus (PRV) and the bovine herpesvirus type 1 (BHV-1), appears to be one of the most potent anti-apoptotic HSV-encoded proteins. HSV US3 has been reported to counteract apoptosis triggered by virus infection, the overexpression of a variety of Bcl-2 family members, $CD8^+$ T cells or various exogenous inducers such as osmotic shock, UV irradiation and Fas ligand (Asano et al., 2000; Cartier et al., 2003a,b; Hata et al., 1999; Jerome et al., 1999; Leopardi et al., 1997; Murata et al., 2002; Ogg et al., 2004). In spite of the US3 conservation on a genetic level between all α-herpesviruses, no anti-apoptotic activity could be attributed to the US3 protein of BHV-1 (Takashima et al., 1999).

Interestingly, and unlike HSV and BHV-1, PRV encodes for two US3 isoforms, an abundant short isoform (>95% of the US3 protein in infected cells) of 41 kDa and a long

* Corresponding author. Laboratory of Virology and Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Salisbury-laan 133, 9820 Merelbeke, East Flanders, Belgium. Fax: +32 9 264 74 95.
E-mail address: Herman.Favoreel@UGent.be (H.W. Favoreel).

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isoform (<5% of US3 protein in infected cells) of 53 kDa (van Zijl et al., 1990). We recently found that both PRV US3 isoforms only differ by the presence of an operational 51 amino acid N-terminally located mitochondrial localization sequence in the US3 long isoform, which is absent in the US3 short isoform (Van Minnebruggen et al., 2003). Regarding the pivotal role of mitochondria in apoptosis (Desagher and Martinou, 2000), this mitochondrial localization of the long PRV US3 isoform may not be coincidental.

Although it has recently been shown that overexpression of the short PRV US3 isoform suppresses apoptosis induced by overexpression of the Bcl-2 family member Bax (Ogg et al., 2004), nothing is known about the anti-apoptotic properties of the long isoform of PRV-US3. Further, it is not known whether PRV US3 has a role in protecting cells from apoptosis during infection or exogenous apoptotic stimuli. The aims of the current study were therefore (i) to investigate whether PRV US3 protects cells from apoptosis during infection or induced by exogenous inducers like sorbitol and staurosporine, and, if so, (ii) whether there is a difference between the long and the short US3 isoform with this respect.

Results

PRV US3 expression suppresses virus-induced apoptosis

The induction of apoptosis is nowadays recognized as a general cellular response to virus infection, limiting virus replication and spread (Koyama et al., 2000). In this context, it has been shown that different members of the α-herpesvirus family including HSV-1, HSV-2, BHV-1, and PRV (Aleman et al., 2001; Koyama et al., 1998; Leopardi et al., 1997; Takashima et al., 1999) trigger apoptosis during infection. For both HSV serotypes, it was demonstrated that the US3 protein kinase is able to suppress this virus-induced apoptosis (Hata et al., 1999; Leopardi et al., 1997), whereas no such anti-apoptotic activity could be attributed to BHV-1 US3 (Takashima et al., 1999).

To determine whether the US3 orthologue of PRV protects cells from apoptosis during PRV infection, swine testicle (ST) cells were either mock-infected or infected with wild-type PRV (NiA3 WT), an isogenic US3 deletion mutant (NiA3 US3 null) or an isogenic US3 rescue strain (NiA3 US3 R) at an moi of 10. At 6, 12, 18, and 24 h postinoculation (hpi), cells were collected and processed for the detection of apoptotic-specific fragmented DNA using the TUNEL reaction or for the detection of active caspase-3, the latter of which has been suggested to be one of the most reliable markers for apoptosis (van Cruchten and van Den Broeck, 2002).

Fig. 1A shows a slight increase in caspase-3 activation in ST cells infected with NiA3 WT at 18 and 24 hpi compared to mock-infected ST cells (4.2 ± 1.6% and 4.5 ± 0.2% to 1.4 ± 0.2%, respectively), indicating that ST cells infected with wild-type PRV undergo apoptotic cell death to a limited extent at late stages in infection (≥18 hpi). Infection of ST cells with NiA3 US3 null, however, resulted in a slight increase in the percentage of active caspase-3-positive cells at 18 hpi and in a strong increase in active caspase-3-positive cells at 24 hpi compared to wild-type PRV-infected cells. At 24 hpi, 42.1 ± 2.0% of the NiA3 US3 null-infected ST cells were positive for active caspase-3 compared to 4.5 ± 0.2% and 4.6 ± 0.5% active caspase-3-positive cells after infection with NiA3 wild type or US3 rescue strain, respectively. Similar results were obtained using the TUNEL assay (Fig. 1B). Deletion of US3 from the wild-type NiA3 strain resulted in a slight increase in the number of TUNEL-positive cells at 12 and 18 hpi compared to the percentage of TUNEL-positive cells after infection with the wild type or rescue strain. However, this increase became very prominent at 24 hpi where deletion of US3 resulted in 39.1 ± 3.1% TUNEL-positive cells compared to 5.7 ±

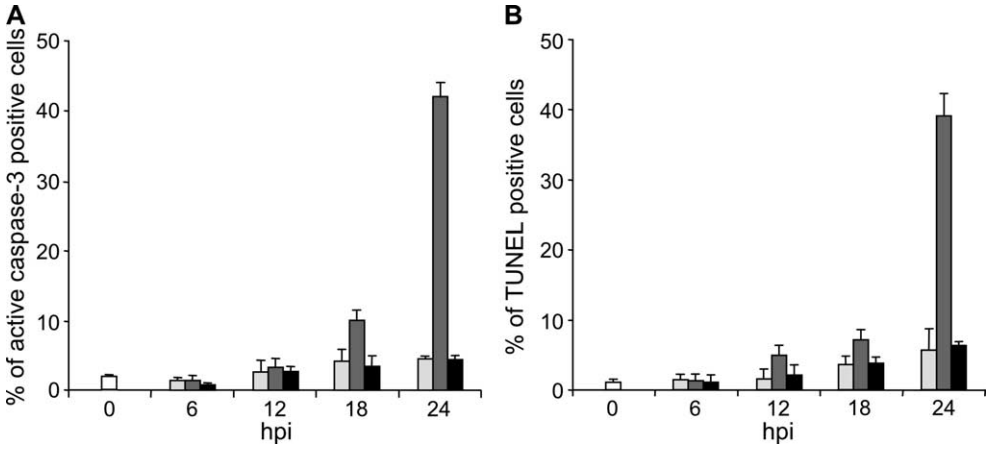

Fig. 1. PRV US3 protects ST cells from PRV-induced apoptosis at late stages in infection. ST cells were mock-infected (white column) or inoculated with NiA3 WT (light gray column), NiA3 US3 null (dark gray columns), or NiA3 US3 Rescue (black column) at an moi of 10. At 6, 12, 18, and 24 hpi, the percentage of apoptotic cells was determined either by visualizing active caspase-3 (A) or by TUNEL assay (B). Columns represent the mean, and error bars the standard deviation of triplicate assays.