Brn-3a suppresses pseudorabies virus-induced cell death in sensory neurons

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Sensory neurons of the trigeminal ganglion (TG) are of crucial importance in the pathogenesis of many alphaherpesviruses, constituting major target cells for latency and reactivation events. We showed earlier that a subpopulation of porcine TG neurons, in contrast to other porcine cell types, is highly resistant to cell death induced by infection with the porcine alphaherpesvirus pseudorabies virus (PRV). Here, we report that expression of Brn-3a, a neuron-specific transcription factor implicated in cell survival of sensory neurons, correlates with the increased resistance of TG neurons towards PRV-induced cell death. In addition, overexpression of Brn-3a in the sensory neuronal cell line ND7 markedly increased resistance of these cells to PRV-induced cell death. Hence, Brn-3a may play a hitherto uncharacterized role in protection of sensory neurons from alphaherpesvirus-induced cell death, which may have implications for different aspects of the alphaherpesvirus life cycle, including latency/reactivation events.

Pseudorabies virus (PRV) is an alphaherpesvirus causing devastating disease and economic losses worldwide in the swine population. Besides its importance as an animal pathogen, PRV has been shown to be useful as a model to study aspects of alphaherpesviruses in general (Enquist, 1999; Pomeranz et al., 2005), including the closely related human alphaherpesviruses herpes simplex virus 1 (HSV-1) and varicella-zoster virus (VZV). An important characteristic of all these viruses is their ability to establish lifelong latent infections in sensory neurons of the trigeminal ganglia (TG) (for PRV and HSV-1) or dorsal root ganglia (DRG) (for VZV), from which they may reactivate sporadically and spread to naive hosts (Efstathiou & Preston, 2005; Enquist et al., 1998; Pomeranz et al., 2005; Roizman & Pellet, 2001; Thompson & Sawtell, 2001).

We reported earlier that a subpopulation of TG neurons is remarkably more resistant towards cell death resulting from productive infection with PRV compared to a broad range of other porcine cell types, including porcine primary sympathetic neurons from the superior cervical ganglion (SCG) (Geenen et al., 2005). Remarkable resistance of TG neurons against PRV-induced and immune cell-induced apoptotic cell death has also been demonstrated in vivo (Aleman et al., 2001). Such postponed cell death may be important for alphaherpesvirus pathogenesis, since it may give the virus enough time to travel long distances via axons to reach mucosal surfaces during primary infection and reactivations. In addition, it may supply the time needed for the immune system to divert virus replication to viral latency (Jones et al., 2006). Different elegant studies have shown that viral factors, especially latency-associated transcripts, are able to suppress (apoptotic) cell death during alphaherpesvirus infection of neurons (Gupta et al., 2006; Perng et al., 2000; Thompson & Sawtell, 2001). Besides these viral factors, cell-type-specific cellular anti-apoptotic factors may also be involved in increasing resistance of sensory neurons to alphaherpesvirus-induced cell death. In support of this, we have demonstrated recently that TG neurons display an unusual resistance to different non-viral apoptotic assaults, including treatment with different apoptosis-inducing reagents (staurosporine, camptothecin and genistein) (Geenen et al., 2006). We therefore hypothesized that, in addition to viral factors, a sensory neuron-specific cellular factor may also be of importance in postponing alphaherpesvirus-induced cell death in TG neurons.

A potential cellular candidate that may increase resistance of TG neurons to PRV-induced cell death is Brn-3a, a member of the IV-POU family of transcription factors. Brn-3a has been shown to be expressed in sensory TG and DRG neurons and was found to protect these neurons, but not sympathetic SCG neurons, from apoptotic cell death induced by growth...
factor deprivation (Ensor et al., 2001; Smith et al., 1998a, b, 2001).

The aim of the current study was therefore to investigate whether the sensory neuron-specific cellular anti-apoptotic factor Brn-3a may be involved in the high resistance of a subpopulation of sensory TG neurons towards PRV-mediated cell death.

First, expression of Brn-3a was analysed by confocal microscopy in primary in vitro cultures of porcine TG neurons and compared with expression in in vitro cultures of porcine SCG neurons [which show no increased resistance towards PRV-induced cell death (Geenen et al., 2005)]. Primary porcine TG and SCG cells were isolated, cultured and inoculated with PRV strain 89V87 as described before (Geenen et al., 2005). Briefly, ganglia were dissociated using 0.2% collagenase and the single cell suspension obtained was seeded on poly-D/L-ornithine and laminin-coated glass coverslips. At 7 days after seedings, cultures were used in experiments. Viability staining, fixation, permeabilization, indirect immunofluorescence and analysis by fluorescence microscopy in the cultures were performed essentially as described before (Geenen et al., 2005). Expression of Brn-3a was analysed using rabbit polyclonal antibodies directed against mouse Brn-3a (Huang et al., 2001). Staining of tissue sections of mouse and porcine TG showed that the mouse-specific Brn-3a antibody displays cross-reactivity with porcine Brn-3a, resulting in nuclear staining of TG neurons in tissue sections of both species (data not shown). Detection of Brn-3a in in vitro cultures of primary porcine TG and SCG neuronal cultures revealed a nuclear expression pattern typical of Brn-3a in 50–60% of the TG neurons, whereas no or only a very low level of Brn-3a expression could be observed in the remaining TG neurons and in the SCG neurons (Fig. 1). Lack of Brn-3a expression in SCG neurons is in agreement with earlier findings in murine neurons (Wyatt et al., 1998). These results indicate that Brn-3a is expressed to a high level in a subpopulation of primary porcine TG neurons, but not in SCG neurons.

Next, we investigated whether Brn-3a expression in a subpopulation of TG neurons correlates with the high resistance of a subpopulation of TG neurons towards PRV-induced cell death. Primary porcine TG neurons were inoculated with PRV 89V87 and at different time points post-inoculation (p.i.) simultaneously assessed for viability and expression of Brn-3a. During the 48 h time period of infection, the total percentage of dead TG neurons increased from 18.4 to 47.0%, which is similar to what we observed before (Geenen et al., 2005) (Fig. 2a). However, Fig. 2(b) shows that the percentage of dead TG neurons in the subpopulation that lacks detectable expression of Brn-3a increases significantly within this time period, whereas there is no such increase in the percentage of dead TG neurons that show Brn-3a expression (Fig. 2b). Results are the means ± SD of three independent experiments and were compared with an ANOVA and a least significant difference post-hoc test for a multiple comparison of means with α = 0.05. As a control, no differences in cell death were observed in Brn-3a-positive vs -negative cells in mock-infected cultures over a similar time period and no differences in the percentage of Brn-3a-positive cells could be observed during the 48 h course of the PRV infection (data not shown). Together, these results indicate that primary porcine TG neurons that express high levels of Brn-3a have a higher resistance towards PRV-induced cell death and that the subpopulation of TG neurons that expresses high levels of Brn-3a corresponds to the subpopulation of TG neurons that shows increased resistance to PRV-induced cell death. The differences observed in neuronal survival in Brn-3a-positive vs -negative neurons could not be explained by a difference in susceptibility to infection between both subpopulations since (i) we showed earlier that virtually all (>95%) of the TG neurons in our in vitro culture show homogeneous, comparable infection kinetics (Geenen et al., 2005), and (ii) we performed a triple fluorescent staining on Brn-3a-positive and -negative neurons at 24 h p.i. with PRV, showing the typical granular Golgi staining of PRV glycoproteins, indicative for late stages of PRV infection as we observed before (Geenen et al., 2005), in >90% of both subpopulations of TG neurons (Fig. 2c). A negative effect of Brn-3a on PRV replication would have been surprising, since, on the contrary, Brn-3a has been shown before to activate the immediate-early promoters of the closely related alphaherpesvirus HSV (Lillycrop et al., 1995).

These data provide correlative evidence that the sensory neuron-specific cell survival factor Brn-3a is of importance
for the postponement of cell death of a subpopulation of sensory TG neurons during infection with the alphaherpesvirus PRV that we observed before (Geenen et al., 2005).

The sensory neuronal cell line ND7 has been shown to express little or no detectable Brn-3a protein (Hudson et al., 2005). Overexpression of Brn-3a in ND7 has been reported to increase resistance of these cells to apoptotic stimuli (Ensor et al., 2001). To investigate directly the involvement of Brn-3a in resistance of sensory neuronal cells towards PRV-induced cell death, the sensitivity of ND7 cells to PRV-induced cell death was assessed in Brn-3a-transfected ND7 cells and compared to PRV-induced cell death in ND7 cells that were not transfected or transfected with a GFP-encoding control plasmid. The ND7 cell line, which was obtained by immortalization of primary sensory neurons from rat dorsal root ganglia (Wood et al., 1992), was cultured as described before, and transfection using Lipofectamine reagent (Invitrogen), inoculation with PRV 89V87, viability staining, fixation, permeabilization, indirect immunofluorescence and microscopic analysis were performed using established protocols as described before (Favoreel et al., 2005; Wood et al., 1992; Geenen et al., 2005).

Fig. 3(a) shows that ND7 cells that were not transfected or transfected with a control plasmid showed no detectable expression of Brn-3a, which is in agreement with earlier findings (Hudson et al., 2005). Fig. 3(b) shows that virtually all non-transfected and mock-transfected ND7 cells succumb to a PRV infection within 24 h. However, over 50 % of Brn-3a-transfected ND7 cells survive PRV infection at 24 h.p.i. No difference in PRV antigen expression could be observed between transfected and non-transfected cells, with over 90 % of ND7 cells expressing PRV antigens at 24 h.p.i. irrespective of transfection (illustrated in Fig. 3d).

Together, the data of the current report show that Brn-3a lowers PRV-induced cell death in sensory neurons.
PRV-induced cell death. This indicates that Brn-3a may play a potentially important role during different aspects of the virus life cycle. Indeed, extended survival of TG neurons during primary infection may supply the time needed for immune responses to suppress virus replication, leading to establishment of latency (Cantin et al., 1995, 1999a, b; Halford et al., 1996; Jones et al., 2006; Oakes & Lausch, 1984; Pierce et al., 2005). In addition, extended survival of TG neurons after infection may enhance virus spread since it may allow newly produced progeny virus to travel long, time-consuming distances (often >0.1 m) towards the axon termini where spread to mucosal or epidermal epithelium and finally to neighbouring hosts may occur. Therefore, the prolonged survival may aid in a more efficient spread of alphaherpesviruses to naive hosts.

Different reports have clearly demonstrated a role for viral anti-apoptotic factors, especially latency-associated transcripts, in protecting sensory neurons from cell death during alphaherpesvirus infection (Perng et al., 2000; Thompson & Sawtell, 2001). Our data show that, in addition to these viral factors, a cell type-dependent cellular anti-apoptotic factor like Brn-3a may also play an as yet unappreciated role in this process.

Further unravelling the protective effect of Brn-3a on sensory neurons against PRV-induced cell death may also enhance knowledge on the mechanism of the anti-apoptotic activity of Brn-3a. Although Brn-3a has been suggested to protect neuronal cells from apoptosis through the activation of the anti-apoptotic Bcl-2 (Latchman, 1998; Smith et al., 1998a), it remains unclear whether Brn-3a expression consistently leads to Bcl-2 upregulation (Eng et al., 2003, 2004).

In conclusion, the data presented here provide a first clue that the cellular anti-apoptotic factor Brn-3a may be of importance in the survival of TG neurons during PRV infection, which may possibly have important consequences for alphaherpesvirus pathogenesis.

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promoters by neuronally expressed POU family transcription factors. 


