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Pseudorabies virus induces a rapid up-regulation of nitric oxide synthases in the nervous system of swine

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Abstract

Nitric oxide (NO) is a free radical gas with important roles in the host's immune response against viral infections. In this study, we examined the kinetics and distribution of nitric oxide synthase (NOS) expression during the early steps of infection of the porcine nervous system by the alphaherpesvirus pseudorabies virus (PRV). To this end, we examined changes in the expression of the three major NOS isoforms, neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS), by immunohistochemistry in the trigeminal ganglia and brain of pigs inoculated intranasally with a virulent PRV strain. The results obtained show that infection of the porcine nervous system by PRV induced a rapid and progressive increment in NOS expression that coincided in timing, location, and magnitude with those of virus propagation in the nervous tissue. A major finding of this study was that PRV caused not only nNOS and iNOS induction in a variety of cell types, but also eNOS up-regulation in endothelial cells and neurons; therefore, all possible sources of NO are activated and probably contribute to the overproduction of NO during infection with the neurotropic alphaherpesvirus PRV in its natural host.

Keywords: Aujeszky's disease virus; Immune response; Nitric oxide synthase; Nervous system; Trigeminal ganglia; Immunohistochemistry

1. Introduction

A crucial feature shared by all members of the *Alphaherpesvirinae* subfamily is their ability to invade, propagate, and persist in a latent state in the nervous system of their hosts. It is well known that after primary replication in epithelial cells of mucosal surfaces, the alphaherpesviruses gain access to the termini of nerve fibers innervating the infected tissue, and through axonal transport invade the peripheral ganglia and often the central nervous system (CNS), usually resulting in lethal encephalitis. If the host immune response is able to restrain the primary

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infection, productive infection ceases but the alphaherpesviruses establish a latent infection for the lifetime of their hosts. When the latent infection reactivates, it appears that depending on the immune surveillance at the site of latency, infectious virus can be produced and transported to peripheral tissues (Enquist et al., 1999; Jones, 2003). Therefore, it is clear that the immune system has a key function in the interaction between alphaherpesviruses and their hosts in all the steps of the infectious process, that is during productive and latent infections, as well as in the transitional periods of establishment of and reactivation from latency.

The defensive molecules deployed by the host immune system during an alphaherpesvirus acute infection have been most thoroughly studied for the prototypical human alphaherpesvirus herpes simplex virus type 1 (HSV-1) using a mouse model of corneal infection. From these studies it was established that, from the mouse cornea, the virus is transmitted to the trigeminal ganglion (TG) within 2 days, reaching peak titers by 3–5 days, and then diminishing to undetectable levels by 7–10 days after infection. HSV-1 replication in the TG is associated with a marked infiltration of macrophages and T lymphocytes and the expression of various immunologically active molecules by these cells, such as the cytokines tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ), and the enzyme inducible nitric oxide synthase (iNOS) (Cantin et al., 1995; Kodukula et al., 1999; Liu et al., 1996; Shimeld et al., 1995, 1997). Evidence that the innate immunity controls primary virus replication in the mouse TG was provided by neutralization of TNF-α or IFN-γ or inhibition of NO production by iNOS, resulting in a dramatic increase in HSV-1 titers in the TG (Kodukula et al., 1999). Although both TNF-α and IFN-γ show direct antiviral activity against HSV-1, their main antiviral effect is to stimulate iNOS up-regulation in immune cells, and therefore, to induce the production of NO at sites of virus infection (Ellermann-Eriksen, 2005; Feduchi and Carrasco, 1991; Feduchi et al., 1989; Karupiah et al., 1993).

The role played by NO in the pathogenesis of virus infections has been, however, questioned during the past years, since both protective and cytotoxic effects of this molecule are frequently seen in parallel (Bogdan, 2001; Guix et al., 2005; Thippeswamy et al., 2006). The antiviral effect of NO seems to be provided by its ability to inhibit virus replication through suppression of viral mRNA and protein synthesis, probably by inhibition of the enzyme ribonucleotide reductase (Croen, 1993; Komatsu et al., 1999b). In line with this, it was shown that mice deficient in iNOS or treated with a NOS inhibitor were more susceptible to HSV-1 infection compared with similarly infected control animals (Adler et al., 1997; Gamba et al., 2004; MacLean et al., 1998). On the other hand, an increasing body of evidence indicates that NO is toxic, since it allows generation of a highly reactive nitrogen oxide species, peroxynitrite ($ONOO^-$), by reaction of NO with superoxide (O_2^-). Peroxynitrite causes cytotoxic effects and tissue damage through potent oxidation and nitration of various biomolecules, thus contributing to pathogenic processes (Akaike and Maeda, 2000; Zaki et al., 2005). This cytotoxic effect of NO has been pointed out following HSV-1 infection of the brain (Fujii et al., 1999; Meyding-Lamadé et al., 2002), and also in HSV-1-induced pneumonia (Adler et al., 1997).

In recent years, it has been demonstrated that not only iNOS, but also the other two major NOS subtypes neuronal NOS (nNOS) and endothelial NOS (eNOS) operate as players of the immune system (Bogdan, 2001). The three isoforms synthesize NO, but they mainly differ with respect to their regulation, amplitude and duration of the production of NO: nNOS and eNOS are Ca^{2+}-calmodulin-dependent and generate low amounts of NO lasting a few minutes, whereas iNOS is controlled by inflammatory mediators and cytokines and produces large quantities of NO for hours or days, thus being responsible for the overproduction of NO during an immune response (Guix et al., 2005). In the case of neurotropic viral infections, in addition to immune cells that infiltrate the nervous tissue and generate large quantities of iNOS-derived NO, it has been observed an increment in the number of neurons that express nNOS in virus-infected areas (Komatsu et al., 1999a; Serrano et al., 2002); the up-regulation of eNOS has also been reported in neurons and astrocytes, cell types that usually do not express this isoform in the uninfected nervous tissue (Barna et al., 1996; Shin et al., 2004). In this context, the present study set out to investigate the dynamics and sources of NO production during the initial stages of an alphaherpesvirus invasion of