Veterinary Immunology and Immunopathology 141 (2011) 162–167

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Expression of interferon gamma in the brain of cats with natural Borna disease virus infection

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ARTICLE INFO

Article history:
Received 7 January 2011
Received in revised form 10 February 2011
Accepted 18 February 2011

Keywords:
Type II interferon
Bornavirus
Staggering disease
Cat
Viral persistence

ABSTRACT

Borna disease virus (BDV) is a neurotropic, negative-stranded RNA virus, which causes a non-suppurative meningoencephalomyelitis in a wide range of animals. In cats, BDV infection leads to staggering disease. In spite of a vigorous immune response the virus persists in the central nervous system (CNS) in both experimentally and naturally infected animals. Since the CNS is vulnerable to cytotoxic effects mediated via NK-cells and cytotoxic T-cells, other non-cytolytic mechanisms such as the interferon (IFN) system is favourable for viral clearance. In this study, IFN-γ expression in the brain of cats with clinical signs of staggering disease ($N = 12$) was compared to the expression in cats with no signs of this disease ($N = 7$) by quantitative RT-PCR. The IFN-γ expression was normalised against the expression of three reference genes (HPRT, RPS7, YWHAZ). Cats with staggering disease had significantly higher expression of IFN-γ compared to the control cats (p-value ≤ 0.001). There was no significant difference of the IFN-γ expression in BDV-positive ($N = 7$) and −negative ($N = 5$) cats having clinical signs of staggering disease. However, as BDV-RNA still could be detected, despite an intense IFN-γ expression, BDV needs to have mechanisms to evade this antiviral immune response of the host, to be able to persist.

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1. Introduction

Borna disease virus (BDV) is a neurotropic, non-segmented, negative-stranded RNA virus that belongs to the family *Bornaviridae*, genus *Bornavirus* within the order *Mononegavirales* (Briese et al., 1994; Cubitt et al., 1994). The virus causes Borna disease, which was first described in horses in Germany over 200 years ago (Dürrwald and Ludwig, 1997). Today, natural infection has been confirmed worldwide in horses, sheep, cattle, cats, dogs and birds (Bode et al., 1994; Lundgren et al., 1995b; Rott and Becht, 1995; Weissenböck et al., 1998; Berg et al., 2001). Staggering disease has been observed in cats in Sweden since the early 1970s however at that time no etiological agent could be found (Kronevi et al., 1974). Later, BDV as the etiological cause of staggering disease was established through a series of studies, including virus isolation from and experimental infection in cats

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doi:10.1016/j.vetimm.2011.02.014

(Lundgren et al., 1995b, 1997). In most species BDV causes non-suppurative encephalomyelitis with predilection for the limbic system, the basal ganglia and the brain stem (Gosztonyi and Ludwig, 1995). In cats, immunohistochemical studies have shown the presence of a strong intracerebral immunological reaction. This reaction is T-cell dominated and associated with enhanced major histocompatibility complex (MHC) class II antigen expression in microglial cells (Lundgren et al., 1995a). The morphological features include presence of broad adventitial cuffs containing tightly packed lymphocytes, monocytes and plasma cells. These infiltrates are T-cell dominated immune reactions, associated with marked immune activation of macrophages and microglial cells in the neural parenchyma. In the brain, CD4+ cells predominate over CD8+ cells, while in the blood BDV-infection in cats is associated with an increase of peripheral CD8+ cells (Lundgren et al., 1995a; Berg et al., 1999). Studies on experimental BDV-infection in adult Lewis rats show that the immune response against BDV is primarily cellular in the acute phase and humoral in the chronic phase (Hatalski et al., 1998). Experimental infection in cats has been shown to induce high antibody titres against BDV, in contrast to naturally infected cats, which seem to develop a comparatively weak humoral immune response (Johansson et al., 2002).

Since many neurons are non-renewing, the cytolytic mechanisms mediated via NK cells, CD8+ cells and CD4+ cells are unfavourable in the CNS (Chesler and Reiss, 2002). Besides antibodies, the antiviral cytokine IFN-γ is an important factor in non-cytolytic virus clearance associated with the adaptive immune response (Rottenberg and Kristensson, 2002). IFN-γ has a potent antiviral activity; it has been shown to reduce viral titres by up to 100-fold in infected neuronal cells *in vitro* (Chesler and Reiss, 2002). IFN-γ is released during acute infections or immunological reactions but also for extended periods of time after clearance of infectious virus and during viral latency and could therefore also affect the brain when the acute inflammatory reaction has subsided (Rottenberg and Kristensson, 2002). BDV has been shown to be highly susceptible to the antiviral effects mediated via IFNs in certain cell lines, while other cell lines seem to lack undefined components of the IFN system, which mediate protection against BDV (Hallensleben and Staeheli, 1999; Sauder et al., 2004). The type I IFNs, IFN-α and -β have been shown to be powerful inhibitors of BDV replication in several cell lines, but have no effect on virus replication in the rat astrocyte cell line C6 (Hallensleben and Staeheli, 1999). IFN-γ has similar effects, where pre-incubation of IFN-γ can block *de novo* BDV infection of monkey kidney cells and human oligodendroglial cells, but is incapable of inhibiting infection in rat astrocyte and fibroblast cell lines (Sauder et al., 2004). Other studies have shown that IFN-γ can block BDV multiplication in mouse organotypic slice cultures (Friedl et al., 2004) and that the IFN-γ inducible protein IP-10 is strongly expressed in the brains of infected rats (Sauder et al., 2000; Jehle et al., 2003). Furthermore, CD8+ T-cells were shown to be dependent on IFN-γ for viral clearance in the CNS of experimentally infected mice (Hausmann et al., 2005).

Most immunological data concerning BDV infection come from experimental animal models such as mouse and rat. Much less is known about the exact immunological mechanisms against BDV in naturally infected animals such as the cat. In this study, we have investigated the expression of IFN-γ in the brain of cats showing clinical signs of staggering disease, with and without confirmed BDV infection, with the aim to better understand these mechanisms. This study shows that upon BDV infection and staggering disease, IFN-γ is highly expressed in the CNS. In spite of this antiviral cytokine expression, BDV was shown to persist in the CNS of infected cats.

2. Materials and methods

2.1. Animals

Twelve cats clinically diagnosed with staggering disease (cats 1–7 and I–V, Table 1) were euthanized due to animal welfare concerns and bad prognosis. Histopathological evaluation of the CNS revealed lesions consistent with BDV infection, i.e. non-suppurative meningoencephalomyelitis in the olfactory bulb, cerebral cortex, hippocampus, brainstem, and/or basal ganglia. Further confirmation of presence of BDV-RNA was performed (described below in *Detection of BDV*).

As controls, seven cats (cat A–G, Table 1) necropsied for other reasons than suspected BDV infection were used. No control had non-suppurative meningoencephalomyelitis. For detailed information about clinical history and pathology, see Table 1.

2.2. RNA extraction

Total RNA was extracted from fresh-frozen brain tissues using Qiagen Lipid Tissue RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To avoid genomic DNA contamination, a DNAse digestion step was included (RDD, Qiagen). RNA concentration was measured using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA).

2.3. cDNA synthesis

Five hundred ng of total RNA was used in the cDNA synthesis. For the investigation of BDV-RNA, gene-specific primers for the BDV phosphoprotein (P) gene (Berg et al., 2001), were used. For IFN-γ and reference gene expression analyses an Oligo(dT) primer (Invitrogen, Carlsbad, CA, USA) were used. Superscript III (Invitrogen) was used according to the manufacturer's recommendations. To investigate whether genomic DNA contamination could contribute to the achieved PCR-products, the RT enzyme was excluded from the cDNA synthesis.

2.4. Detection of BDV

To test for the presence of BDV, a previously described BDV P real-time RT-PCR was used (Wensman et al., 2007). The primers were modified by adding 5′ AT-rich overhangs to increase the sensitivity (Afonina et al., 2007). Total RNA was extracted as described above from different regions of the CNS (olfactory epithelium, cerebral cortex,

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