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Differential effects of virulent and avirulent equine infectious anemia virus on macrophage cytokine expression

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Abstract

Equine infectious anemia virus (EIAV) causes rapid development of acute disease followed by recurring episodes of fever, thrombocytopenia, and viremia. Most infected equid eventually bring the virus under immunological control. We recently reported the development of an equine-specific ribonuclease protection assay (RPA) to quantitate mRNA levels of 10 cytokines. Using this newly developed RPA, we now show significant differences in cytokine induction in equine monocyte-derived macrophages (EMDM) exposed to virulent and avirulent EIAV. Virulent $EIAV_{17}$ induced significant increases in interleukin (IL)-1α, IL-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α by 0.5–1 h postinfection (hpi). In contrast, the avirulent virus failed to induce any of the tested cytokines above that of control levels. These data show a direct correlation between cytokine dysregulation and EIAV pathogenesis.

Keywords: EIAV; Cytokines; Viral clones; Macrophage; RPA; Equine immunology

Introduction

Equine infectious anemia virus (EIAV) belongs to the family Retroviridae, genus lentivirus. The clinical course of EIAV infection is classically divided into acute, chronic, and inapparent carrier phases. The acute phase occurs within 5–30 days postinfection (dpi) and is characterized by viremia, fever, and thrombocytopenia (Issel and Coggins, 1979; Sellon et al., 1994). The acute symptoms vary from mild to severe to fatal depending on the strain of EIAV and the host immune status. Following the acute phase, the infected animal may develop chronic EIA, which manifests as recurring cycles of fever, severe anemia, weight loss, thrombocytopenia, hemorrhages, and anorexia (Issel and Coggins, 1979). About 90% of the infected horses survive the chronic phase and become clinically normal. Subsequent clinical episodes can be elicited in inapparent carriers by environmental stress or immunosuppressive drugs (Cheevers and McGuire, 1985). As in all retroviral infections, infected horses remain life-long carriers of EIAV (Issel and Coggins, 1979; Kono et al., 1976).

Tissue macrophages are the primary sites for EIAV replication and serve as cellular reservoirs during acute and persistent infection (Oaks et al., 1998; Sellon et al., 1992). EIAV can infect circulating monocytes, but virus expression is limited to differentiated tissue macrophages. Macrophages are the source of a myriad of cytokines, and perturbation of cytokine expression has been shown in other lentivirus-infected macrophages including human and simian immunodeficiency viruses (HIV and SIV, respectively), caprine arthritis encephalitis virus (CAEV), and maedi-visna virus (MVV) (Adeyemo et al., 1997; Bornemann et al., 1997; Ebrahimi et al., 2000; Horvath et al., 1991; Lechner et al., 1997; Legastelois et al., 1998; Sopper et al., 1996; Zink et al., 2001). Cytokines produced by infected macrophages have been shown to impact the outcome of lentiviral infections. For example, interleukin-

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1 (IL-1) and tumor necrosis factor-α (TNF-α) contribute to the neurological impairment associated with late-stage HIV and SIV infection by facilitating infiltration of macrophages into the CNS and stimulating production of neurotoxic substances by activated macrophages and microglia (Orandle et al., 2001; Sopper et al., 1996; Tan and Guiloff, 1998; Xiong et al., 2000). Macrophage-secreted cytokines and chemokines, including IL-1, IL-6, type I interferons (IFN type 1, TNF-α, RANTES, and macrophage inflammatory proteins-1α and 1β (MIP-1α and 1β), are known to regulate the expression and replication of HIV in macrophages and $CD4^+$ T-lymphocytes (Fauci, 1996; Vicenzi et al., 1997). Therefore, investigating the interaction of EIAV with equine monocytes and macrophages is important to the study of EIAV-induced disease.

In this study, viruses derived from two EIAV molecular clones, $EIAV_{19}$ and p19/wenv17, were examined for induction of cytokines in equine monocyte-derived macrophages (EMDM). Virus derived from clone $EIAV_{19}$ causes an asymptomatic infection in Shetland ponies (Payne et al., 1994). p19/wenv17 is a chimeric clone generated by replacing the 5′ and 3′ long terminal repeats (LTR) and the *env* sequences of $EIAV_{19}$ with those derived from the highly virulent Wyoming field strain of EIAV ($EIAV_{Wyo}$) (Payne et al., 1998). p19/wenv17 produces a virus named $EIAV_{17}$ that causes severe EIA in infected Shetland ponies (Payne et al., 1998). In vitro, however, both viruses replicate to high titer and induce cytopathic effects (CPE) in EMDM. Cytokine profiles of EMDM infected with $EIAV_{19}$ or $EIAV_{17}$ were determined to evaluate the correlation between cytokine dysregulation and EIAV pathogenesis. We utilized a newly developed set of antisense RNA probes to quantitate the mRNA expression of 10 equine cytokine genes by ribonuclease protection assay (RPA) (Lim et al., 2003).

Results

Characterization of $EIAV_{17}$ and $EIAV_{19}$

To verify the infectivity and compare the replication of the viral stocks, EMDMs were infected with 3000 cpm RT activity of $EIAV_{17}$ or $EIAV_{19}$ or were mock infected. Virus replication was monitored by measuring RT activity in culture supernatants. As shown in Fig. 1A, $EIAV_{17}$ and $EIAV_{19}$ replicated to the same levels in EMDM culture indicating that both virus stocks remained infectious following the process of purification and concentration. To further evaluate and compare the initial infectivity of the two virus stocks, provirus copy numbers were determined at 4 hpi of EMDM infected with equivalent RT units of $EIAV_{17}$ and $EIAV_{19}$. A quantitative real-time PCR assay using SYBR Green dye was performed as described in the methods. Using DNA from infected cells, specific product was detected before cycle 29 while uninfected controls and no template reactions showed no specific product through cycle 40. Provirus copy numbers were calculated per nanogram of input DNA, and as shown in Fig. 1B, there is about a 3-fold difference in the average provirus copy numbers. A set of control reactions was performed to determine if the virus stocks contained contaminating viral DNA that might have contributed to the amplification signals. Comparison of DNAse-treated to untreated virus stocks was performed, and it was verified that the treatment did not reduce the provirus copy numbers detected at 4 hpi of EMDM (data not shown). Therefore, the data presented in Figs. 1A and B suggest that equivalent RT units of input virus represent approximately equivalent levels of infectious, replication-competent virus.

The SU protein content of $EIAV_{17}$ and $EIAV_{19}$ stocks containing equal cpm of RT activity was estimated by Western blot analysis using a monoclonal antibody specific to a conserved epitope of EIAV SU (Hussain et al., 1987). A difference in electrophoretic mobility between the SU of the two viruses was evident (Fig. 1C), likely due to a difference in their glycosylation since $EIAV_{17}$ SU has five more potential N-linked glycosylation sites than $EIAV_{19}$ (Payne et al., 1998). Using Control Media (CM) as background, densitometry scans revealed $EIAV_{19}$ SU concentration is 2.2-fold higher than the SU derived from the virulent clone indicating elevated concentrations of $EIAV_{17}$ SU are not responsible for the differential cytokine induction. Further, Western blots were completed using equine immune sera. $EIAV_{19}$ showed 3-fold greater capsid protein (Fig. 1D), agreeing with the SU Western blot data.

$EIAV_{17}$-stimulated expression of IL-1α, IL-1β, IL-6, and TNF-α in EMDM

Initial experiments were performed to determine the effects of $EIAV_{19}$ and $EIAV_{17}$ on equine macrophage cytokine expression. EMDMs were cultured for 7 days and infected with $EIAV_{19}$ or $EIAV_{17}$ or mock-infected with CM. At 2, 4, 12, and 24 hpi, lysates of the infected cells were analyzed with the multiprobe RPA for IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12 p35, IL-12 p40, IFN-γ, TGF-β1, TNF-α, and β-actin mRNA expression.

These initial studies were performed beginning at 2 hpi. In EMDM exposed to $EIAV_{17}$, there was a readily apparent increase of IL-1α, IL-1β, IL-6, and TNF-α mRNA levels at 2 and 4 hpi (data not shown). Whereas, $EIAV_{19}$ infection induced a slight increase of IL-1β mRNA expression at 4 hpi and did not appear to affect the expression of other cytokines (data not shown). To confirm these initial observations, the experiment was performed in duplicate at 2, 4, 12, and 24 hpi using EMDM derived from four different horses (total of eight independent measurements). Cytokine mRNA levels were analyzed by phosphor imaging, normalized to the level of β-actin mRNA, and expressed as percentage of β-actin (relative mRNA levels) (Fig. 2). All four horses showed elevated IL-1α, IL-1β, and IL-6 at 2 hpi when EMDMs were treated with the virulent