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Research Paper

Variable transcription of pro- and anti-inflammatory cytokines in phocine lymphocytes following canine distemper virus infection

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ABSTRACT

Canine distemper virus (CDV) is a highly contagious viral pathogen. Domesticated dogs are the main reservoir of CDV. Although phocine distemper virus was responsible for the recent epidemics in seals in the North and Baltic Seas, most devastating epidemics in seals were also caused by CDV. To further study the pathogenesis of CDV infection in seals, it was the aim of the present study to investigate the mechanisms of CDV induced immunosuppression in seals by analyzing the gene transcription of different pro- and anti-inflammatory cytokines in Concanavalin A (Con A) stimulated and non-stimulated phocine lymphocytes in vitro following infection with the CDV Onderstepoort (CDV-OND) strain. Phocine lymphocytes were isolated via density gradient centrifugation. The addition of 1 μg/ml Con A and virus was either performed simultaneously or lymphocytes were stimulated for 48 h with Con A prior to virus infection. Gene transcription of interleukin (IL)-6, IL-12 and tumor necrosis factor alpha (TNFα) as pro-inflammatory cytokines and IL-4, IL-10 and transforming growth factor beta (TGFβ) as anti-inflammatory cytokines were determined by using RT-qPCR. CDV-OND infection caused an initial increase of pro-inflammatory phocine cytokines mRNA 24 h after infection, followed by a decrease in gene transcription after 48 h. A strong increase in the transcription of IL-4 and TGFβ was detected after 48 h when virus and mitogen were added simultaneously. An increased IL-10 production occurred only when stimulation and infection were performed simultaneously. Furthermore, an inhibition of IL-12 on IL-4 was noticed in phocine lymphocytes which were stimulated for 48 h prior to infection. In summary, the duration of the stimulation or the lymphocytes seem to have an important influence on the cytokine transcription and indicates that the outcome of CDV infection is dependent on various factors that might sensitize lymphocytes or make them more susceptible or reactive to CDV infection.

Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; CDV, canine distemper virus; Con A, Concanavalin A; e.V., German "**e**ingetragener **V**erein", incorporated society; IL, interleukin; MOI, multiplicity of infection; mRNA, messenger RNA; OND, Onderstepoort; PBL, peripheral blood lymphocytes; R^2, pseudo-R^2 (deviance explained), determined from 1-residual deviance/null; deviance, deviance explained, there is no real R^2 in geralized linear models (Zuur et al., 2009); RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation; $TCID_{50}$, tissue culture infectious dose 50%; TGF, transforming growth factor; TNF, tumor necrosis factor.

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1. Introduction

Canine distemper virus (CDV) belongs to the genus Morbillivirus, subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order Mononegavirales (International Committee on Taxonomy of Viruses, 2012). Members of this genus comprise other important contagious pathogens like measles virus, rinderpest virus (RPV), the peste des petites ruminants' virus (PPRV), the cetacean morbillivirus (CMV), the dolphin morbillivirus (DMV), the pilot whale morbillivirus, the porpoise morbillivirus (PMV) and the phocine distemper virus (PDV; International Committee on Taxonomy of Viruses, 2012; Lamb and Parks, 2007).

PDV was originally divided into PDV-1 and -2. PDV-1 is related but clearly distinct from CDV and caused the epidemics in the North and Baltic Seas in 1988 and 2002 when more than ten-thousand harbor seals died in the Wadden Sea (Dietz et al., 1989; Jensen et al., 2002; Müller et al., 2004). PDV-2 is most probably a strain of CDV (Visser et al., 1993). Evidence of a distemper virus infection, most likely PDV was also found in western Atlantic harbor seals, harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) in the western Atlantic (Duignan et al., 1993; Jauniaux et al., 2001; Lipscomb et al., 2001; Visser et al., 1993). Investigation of Baikal seals (*Phoca sibirica*) and Caspian seals (*Phoca caspica*) revealed also CDV as the responsible agent in seals (Grachev et al., 1989; Mamaev et al., 1995; Stanton et al., 2004). Furthermore antibodies to CDV were found in crabeater seals (*Lobodon carcinophaga*) and leopard seals (*Hydrurga leptonyx*; Bengtson et al., 1991).

CDV is a highly contagious viral pathogen which causes an abortive, subclinical or severe, systemic and often fatal infection in dogs and seals. The occurrence is depending on the host's immunity status, age of the animal at the time of infection and the virus strain (Baumgärtner et al., 2003; Beineke et al., 2009; Bengtson et al., 1991; Grachev et al., 1989; Krakowka et al., 1978; Siebert et al., 2007; Svansson et al., 1993; Visser et al., 1993). Following infection, the virus spreads via macrophages, lymphocytes and thrombocytes or cell independent within the blood followed by virus spread to epithelial, mesenchymal and neuroectodermal cells (Beineke et al., 2009; Krakowka et al., 1978). In dogs a virus associated immunosuppression caused by the lytic effect of CDV occurs at the beginning of the infection and may last for months (Blixenkrone-Møller et al., 1993; Gerber and Marron, 1976; Krakowka et al., 1978).

Worldwide CDV causes a major disease of domestic dogs that develops as a serious systemic infection in unvaccinated or improperly vaccinated dogs. It is well established that there are different strains of CDV which vary in their virulence for dogs and other susceptible species like wolves and foxes (Blixenkrone-Møller et al., 1992; Haas et al., 1999; Martella et al., 2008). CDV and PDV are genetically related with 75% match in the sequence of the amino acid of the hemagglutinin gene and show similar in vivo properties including systemic infection and immunosuppression (Jensen et al., 2002; Müller et al., 2008, 2004). The broad host range of CDV overlaps with PDV (Blixenkrone-Møller et al., 1992). CDV was the primary agent for several epizootics in arctic seals – starting from CDV-infected terrestrial mammals (Bengtson et al., 1991; Dietz et al., 1989; Grachev et al., 1989; Visser et al., 1993). Although PDV is the host specific morbillivirus species for harbor seals, they can also be infected by CDV (Svansson et al., 1993). Harbor seals are common marine mammal species in the German North and Baltic Seas. At present the population size in the Wadden Sea is estimated at 39,400 individuals (Tritaleral Seal Expert Group, 2013). Since the last epidemic in 2002 there is an average annual increase in population size of 9.6%, which is not far from the theoretical maximum growth rate (Tritaleral Seal Expert Group, 2012).

The origin of PDV that caused both epidemics remains unknown. Northwest Atlantic Harp seals and Ringed seals showed antibodies against CDV (Dietz et al., 1989; Kreuzter et al., 2008; Markussen and Have, 1992) and may serve and may serve as marine reservoirs like domesticated dogs as reservoirs of CDV (Kapil and Yeary, 2011).

In 1987/1988 a severe outbreak of CDV in northern Greenland and the Canadian Arctic killed up to 80% of the dogs in some settlements (Dickson, 1988; Dietz et al., 1989). It is discussed, whether infected foxes represent the origin of the outbreak or if dogs might have been infected by eating seal suffering from distemper. Vaccine-based prophylaxis has greatly helped to keep distemper under control; outbreaks of disease with significant mortality continue to occur amongst domestic dogs even in developed communities with high levels of vaccination (Bohm et al., 1989; Ek-Kommonen et al., 1997; Johnson et al., 1995).

Morbillivirus specific antibodies are currently not detectable in most harbor seals in the Wadden Sea (Bodewes et al., 2013). Moreover, *Phoca vitulina* is still exposed to various immunosuppressive factors like contaminants, noise or pathogens (Bodewes et al., 2013). Consequently, infection of seals by domestic dogs cannot totally be excluded (Gowtage-Sequeira et al., 2009; Svansson et al., 1993). However more likely, a similar outbreak as observed 1988 and 2002 may occur in the near future (Bodewes et al., 2013).

The aim of this study was to investigate the pathogenesis of a CDV induced immunosuppression in juvenile harbor seals by analyzing the gene transcription of different pro- and anti-inflammatory cytokines in Concanavalin A (Con A) stimulated and non-stimulated phocine lymphocytes following CDV-OND infection.

2. Materials and methods

2.1. Study design

Upon establishment of maximum phocine lymphocyte stimulation conditions using Con A as mitogen and confirmation of the infectivity of CDV-OND for phocine lymphocytes, various experiments were conducted. Gene transcription of different pro- and anti-inflammatory cytokines of Con A stimulated and CDV-OND infected phocine lymphocytes was compared to non-stimulated and non-infected lymphocytes. The addition of mitogen and virus was either performed simultaneously or lymphocytes were stimulated for 48 h with Con A prior to virus