Veterinary Immunology and Immunopathology 156 (2013) 215–222

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

Dynamic changes of Foxp3$^+$ regulatory T cells in spleen and brain of canine distemper virus-infected dogs

V. Qeska[a,b,1], Y. Barthel[a,1], M. Iseringhausen[a,1], A. Tipold[b,c], V.M. Stein[c], M.A. Khan[a,b], W. Baumgärtner[a,b], A. Beineke[a,*]

[a] Department of Pathology, University of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany
[b] Center for Systems Neuroscience, Hannover, Germany
[c] Department of Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover, Bünteweg 9, D-30559 Hannover, Germany

ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form
25 September 2013
Accepted 8 October 2013

Keywords:
Canine distemper virus
Foxp3
Regulatory T cells
Cytokine expression
Demyelinating leukoencephalitis
Immunopathology

ABSTRACT

Canine distemper virus (CDV) infection causes immunosuppression and demyelinating leukoencephalitis in dogs. In viral diseases, an ambiguous function of regulatory T cells (Treg), with both beneficial effects by reducing immunopathology and detrimental effects by inhibiting antiviral immunity, has been described. However, the role of Treg in the pathogenesis of canine distemper remains unknown. In order to determine the effect of CDV upon immune homeostasis, the amount of Foxp3$^+$ Treg in spleen and brain of naturally infected dogs has been determined by immunohistochemistry. In addition, splenic cytokine expression has been quantified by reverse transcriptase polymerase chain reaction. Splenic depletion of Foxp3$^+$ Treg was associated with an increased mRNA-expression of tumor necrosis factor and decreased transcription of interleukin-2 in the acute disease phase, indicative of disturbed immunological counter regulation in peripheral lymphoid organs. In the brain, a lack of Foxp3$^+$ Treg in predemyelinating and early demyelinating lesions and significantly increased infiltrations of Foxp3$^+$ Treg in chronic demyelinating lesions were observed. In conclusion, disturbed peripheral and CNS immune regulation associated with a reduction of Treg represents a potential prerequisite for excessive neuroinflammation and early lesion development in canine distemper leukoencephalitis.

1. Introduction

Canine distemper virus (CDV) causes a systemic, often fatal disease in dogs, which is commonly associated with respiratory and/or gastrointestinal tract disorders and nervous system disturbances (Sips et al., 2007; von Messling et al., 2003; Wyss-Fluehmann et al., 2010). Similar to human measles, induced by a related morbillivirus, animals show profound immunosuppression and generalized depletion of lymphoid organs particularly during the acute phase of canine distemper (Beineke et al., 2009; Sellin et al., 2009; von Messling et al., 2006). Depending on CDV strain, host immune status and age, naturally infected dogs develop a demyelinating leukoencephalomyelitis, which shares similarities with human myelin disorders, such as multiple sclerosis (Confer et al., 1975; Ishizaki et al., 2010; Sips et al., 2007; Summers et al., 1984; Vandevelde and Zurbriggen, 2005; Young and Rall, 2009). Demyelination in canine distemper represents a biphasic process with directly virus induced neurodegeneration with a

* Corresponding author. Tel.: +49 0511 953 8640; fax: +49 0511 953 8675.
E-mail address: andreas.beineke@tiho-hannover.de (A. Beineke).
[1] Authors have contributed equally.

0165-2427/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2013.10.006

dominating pro-inflammatory cytokine environment in the brain during the early phase (Alldinger et al., 1996; Beineke et al., 2008; Markus et al., 2002; Tipold et al., 1999; Wünschmann et al., 1999) and CD4-mediated delayed type hypersensitivity with immune mediated tissue damage during the advanced phase (Alldinger et al., 1993; Beineke et al., 2009; Wünschmann et al., 2000).

Regulatory T cells (Treg), characterized by expression of the transcription factor forkhead box P3 (Foxp3), play a key role in the maintenance of immunological tolerance and prevent immunopathology (Feuerer et al., 2009, 2010; MacDonald et al., 2002; Sakaguchi, 2003; Sakaguchi et al., 2006; Vignali et al., 2008). However, in viral diseases Treg can exhibit both beneficial effects by reducing immune mediated tissue damage and detrimental effects due to their immunosuppressive properties, causing disease exacerbation or viral persistence, respectively (Gobel et al., 2012; Lund et al., 2008). For instance, Treg reduce antiviral immunity in experimental Theiler's murine encephalomyelitis (Herder et al., 2012; Richards et al., 2011), an infectious rodent model for demyelinating disorders. However, the significance of Treg in morbillivirus-induced diseases remains enigmatic (Aydin et al., 2010), since reports that Treg are increased in measles patients (Moss et al., 2002; Yu et al., 2008) have been contradicted by others (Li et al., 2008). Moreover, different rodent models for measles virus infection came to ambiguous conclusions regarding Treg-related effects upon immune responses, probably attributed to disease course-dependant effects or mouse strain-specific immune responses (Koga et al., 2010; Reuter et al., 2012; Sellin et al., 2009; Wu et al., 2012). Immunohistochemical and cytokine expression analyses revealed phenotypical changes of peripheral lymphoid organs which are supposed to foster migration of encephalitogenic $CD8^+$ T cells and immune derailment in the brain of CDV-infected dogs (Gröne et al., 1998; Markus et al., 2002; Tipold et al., 1999; Wünschmann et al., 1999, 2000). However, so far, the role of Treg in the pathogenesis of canine distemper has not yet been investigated.

Therefore, the aim of the present study was to quantify the amount of $Foxp3^+$ Treg in spleen and brain of naturally infected dogs to get further insights into the effect of CDV upon host immune homeostasis. Moreover, selected pro- and anti-inflammatory cytokines have been measured in the spleen to characterize peripheral immune alterations in canine distemper.

2. Materials and methods

2.1. Animals and tissue selection

A total of 23 dogs of different breeds and age with spontaneous CDV-infection were selected for this study. Affected animals were grouped (SI, SII, SIII, see below) according to the main pathological finding in the brain (supplemental table S1). Five non-infected healthy animals (beagles) were obtained from an animal experiment, which was approved and authorized by the local authorities (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Oldenburg, Germany, permission number 08A580) and used as controls (supplemental table S1). Formalin-fixed, paraffin-embedded cerebellum and spleen tissue were taken for histology and immunohistochemistry. In addition, snap frozen spleen tissue was used for molecular analyses.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetimm.2013.10.006.

2.2. Immunohistochemistry

CDV nucleocapsid protein (CDV-NP) in the CNS was visualized by immunohistochemistry using a mouse monoclonal antibody (clone 3991; kindly provided by Prof. Dr. C. Örvell, Stockholm, Sweden). For quantification of T cells, including Treg in brain and spleen samples a rabbit polyclonal CD3-specific antibody (A0452; Dako) and a rat monoclonal Foxp3-specific antibody (clone FJK-16s; eBioscience), respectively, were used. Antigen detection was performed by the avidin–biotin–peroxidase complex method as previously described (Alldinger et al., 1996; Gröters et al., 2005). In brief, paraffin-embedded tissues were deparaffinized in Roticlear (Carl Roth GmbH, Karlsruhe, Germany) and rehydrated through graded alcohols. Endogenous peroxidase activity was suppressed with 0.5% H_2O_2 in methanol, followed by incubation with primary antibodies (CDV: dilution 1:6000; CD3: dilution 1:1000; Foxp3: dilution 1:10) overnight at 4 °C. Antigen retrieval pretreatment was performed by incubation of slides with citrate buffer (pH 6) for 20 min in a microwave (800 W). Specificity controls included substitution of the respective primary antibody with ascitic fluid from non-immunized BALB/c mice (CDV), non-immunized rabbits (CD3) and an isotype-matched rat control antibody (Foxp3). Incubation with primary antibodies was followed by incubation with biotinylated secondary antibodies for 30 min at room temperature. Subsequently, the avidin–biotin–peroxidase complex (VECTASTAIN Elite ABC Kit; Vector Laboratories, PK 6100, Burlingame, CA) was added and incubated for 30 min at room temperature. Antigen–antibody reactions were visualized by incubation with 3,3′-diaminobenzidine-tetrahydrochloride with 0.03% H_2O_2 for 5 min, followed by counterstaining with Mayer's hemalaun.

2.3. Histological evaluation of white matter lesions in the cerebellum

Paraffin-embedded tissue slices (thickness 4 μm) from the cerebellum were stained with hematoxylin and eosin (HE) for morphological examination. The brain tissue was additionally stained with luxol fast blue (LFB) for detecting myelin loss and myelinophagia (active demyelination). Cerebellar white matter lesions were classified as described by Wünschmann et al. (1999) with slight modifications. Briefly, groups were classified as acute non-inflammatory encephalitis (SI), subacute non-inflammatory encephalitis (SII) and subacute to chronic inflammatory encephalitis (SIII). Acute white matter lesions (SI) were characterized by hypercellularity and vacuolization (predemyelinating lesions) whereas SII and SIII lesions showed active demyelination as demonstrated by decreased intralesional