Journal of Neuroimmunology 263 (2013) 43–54

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

IL-21 optimizes T cell and humoral responses in the central nervous system during viral encephalitis

Timothy W. Phares [a], Krista D. DiSano [a,c], David R. Hinton [d], Mihyun Hwang [b], Allan J. Zajac [e], Stephen A. Stohlman [a], Cornelia C. Bergmann [a,*]

[a] Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA
[b] Department of Immunology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA
[c] School of Biomedical Sciences, Kent State University, Kent, OH 44242, USA
[d] Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA
[e] Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form 24 July 2013
Accepted 26 July 2013

Keywords:
Central nervous system
Neuroimmunology
$CD4^+$ and $CD8^+$ T cells
Inflammation
Interleukin

ABSTRACT

Acute coronavirus encephalomyelitis is controlled by T cells while humoral responses suppress virus persistence. This study defines the contribution of interleukin (IL)-21, a regulator of T and B cell function, to central nervous system (CNS) immunity. IL-21 receptor deficiency did not affect peripheral T cell activation or trafficking, but dampened granzyme B, gamma interferon and IL-10 expression by CNS T cells and reduced serum and intrathecal humoral responses. Viral control was already lost prior to humoral CNS responses, but demyelination remained comparable. These data demonstrate a critical role of IL-21 in regulating CNS immunity, sustaining viral persistence and preventing mortality.

1. Introduction

Interleukin (IL)-21 is a pleiotropic type I cytokine secreted by multiple CD4 T cell subsets, including follicular helper T cells (T_{FH}), T helper 17 (T_H17) and natural killer (NK) T cells (Yi et al., 2010a). It acts as a co-mitogen to drive CD8 T cell activation, expansion, differentiation and/or survival (Zeng et al., 2005; Casey and Mescher, 2007; Ostiguy et al., 2007; Elsaesser et al., 2009; Frohlich et al., 2009; Yi et al., 2009; Barker et al., 2010; Novy et al., 2011) and is a critical regulator of humoral immunity (Ozaki et al., 2002; Linterman et al., 2010; Zotos et al., 2010; Rankin et al., 2011). The IL-21 receptor (IL-21R) is expressed on several cell types, including T, B, NK cells, and NK T lymphocytes, as well as dendritic cells (DC) and macrophages (Yi et al., 2010a) and consists of an IL-21 binding protein and the common cytokine receptor γ-chain, which is shared by the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 (Rochman et al., 2009). During peripheral viral infections IL-21 plays a beneficial role by supporting multiple CD8 T cell functions, survival and generation of long-lived memory (Barker et al., 2010; Novy et al., 2011; Pallikkuth et al., 2012). IL-21 deficiency during chronic infection induced by lymphocytic choriomeningitis virus (LCMV) results in impaired long-term CD8 T cell functionality, enhanced CD8 T cell exhaustion, and viral persistence (Elsaesser et al., 2009; Frohlich et al., 2009; Yi et al., 2009). IL-21 also influences long-lived humoral responses during peripheral viral infections including LCMV, vesicular stomatitis virus and influenza virus infection (Elsaesser et al., 2009; Yi et al., 2009; Rasheed et al., 2013).

The role of IL-21 during neuroinflammatory diseases is relatively unexplored. In multiple sclerosis (MS) and neuromyelitis optica elevated cerebrospinal fluid IL-21 levels and polymorphisms in the IL-21R gene implicate IL-21 in autoimmune pathogenesis (Nohra et al., 2010; Wu et al., 2012). In white matter MS lesions IL-21 expression is primarily restricted to CD4 T cells, while IL-21R is predominantly detected on T and B lymphocytes, and sparsely on cortical neurons (Tzartos et al., 2011). Administration of IL-21 before induction of experimental allergic encephalomyelitis (EAE) enhances encephalitogenic T cell and NK function in the periphery and increases disease severity supporting IL-21 as a factor promoting pathology (Vollmer et al., 2005). By contrast, severe neurological impairment during EAE in the absence of IL-21 signaling, associated with a defect in peripheral CD4 T regulatory cells, implicates a protective role for IL-21 (Liu et al., 2008; Piao et al., 2008). More recently, IL-21 was shown to be essential for maintenance of local T cell responses in the central nervous system (CNS) during chronic parasitic infection with *Toxoplasma gondii* (Stumhofer et al., 2013). However, the influence of IL-21 on CNS immunity during viral encephalitis has not been studied.

* Corresponding author at: Department of Neuroscience, Lerner Research Institute, The Cleveland Clinic, 9500 Euclid Avenue, NC30, Cleveland, OH 44195, USA. Tel.: +1 216 444 5922; fax: +1 216 444 7927.
E-mail address: bergmac@ccf.org (C.C. Bergmann).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.07.019

Infection with the sub-lethal glia-tropic JHM strain of mouse hepatitis virus (JHMV) induces an acute encephalomyelitis that resolves into a persistent infection restricted primarily to oligodendroglia (Parra et al., 1999; Bergmann et al., 2006). CD8 T cells are the primary effectors reducing virus replication using both perforin and gamma interferon (IFN-γ)-mediated mechanisms (Lin et al., 1997; Bergmann et al., 2004; Gonzalez et al., 2006). CD4 T cells play a vital supportive role by enhancing peripheral CD8 T cell priming/expansion and promoting local effector function within the CNS (Phares et al., 2012b). By contrast, humoral immunity is essential to control the persistent phase of infection (Lin et al., 1999; Tschen et al., 2002; Ramakrishna et al., 2003; Tschen et al., 2006). As CD4 T cells express IL-21 within the CNS during JHMV infection (Phares et al., 2011), we explored a potential role of IL-21 as a prominent factor providing local help for CD8 T cells as well as B cells. Infection of IL-21R$^{-/-}$ mice revealed that expansion and activity of antiviral CD8 T cells in draining cervical lymph nodes (CLN) as well as their accumulation within the CNS was independent of IL-21 signaling. However granzyme B, IFN-γ and most prominently IL-10 expression were diminished in CNS-derived IL-21R$^{-/-}$ CD8 T cells. IFN-γ and IL-10 expression was also reduced in CNS-derived IL-21R$^{-/-}$ CD4 T cells. The absence of IL-21R further delayed peripheral B cell activation and significantly impaired CNS humoral responses. While altered T cell activity in IL-21R$^{-/-}$ mice did not impede early viral control, infectious virus persisted prior to and subsequent to emergence of CNS humoral responses. Nevertheless, clinical scores and the extent of myelin loss were comparable throughout the early persisting phase. Overall, these data support IL-21 as a cytokine optimizing both CNS T cell antiviral activity and humoral responses, thus lowering the set point of viral persistence and ultimately preventing mortality.

2. Materials and methods

2.1. Mice and virus infection

C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD). IL-21R$^{-/-}$ mice on the C57BL/6 background were previously described (Yi et al., 2010b). All mice were housed under pathogen free conditions at an accredited facility at the Cleveland Clinic Lerner Research Institute. Mice were infected at 6–7 wks of age by intracranial injection with 1000 plaque forming units (PFU) of the J.2.2v-1 monoclonal antibody (mAb)-derived gliatropic JHMV variant (Fleming et al., 1986). Animals were scored for clinical signs of disease with: 0, healthy; 1, ruffled fur and hunched back; 2, hind limb paralysis or inability to turn to upright position; 3, complete hind limb paralysis and wasting; and 4, moribund or dead. All animal experiments were performed in compliance with guidelines approved by the Cleveland Clinic Lerner Research Institute Institutional Animal Care and Use Committee.

2.2. Virus titers and cytokine determination

Virus titers within the CNS were determined in clarified supernatants by plaque assay using the murine delayed brain tumor (DBT) astrocytoma as detailed (Fleming et al., 1986). Plaques were counted after 48 h incubation at 37 °C. Clarified supernatants were also used to measure IFN-γ by ELISA as described (Phares et al., 2009). Briefly, 96 well plates were coated overnight at 4 °C with 100 μl of 1 μg/ml of anti-IFN-γ (R4-6A2; BD Bioscience). Non-specific binding was blocked with 10% fetal calf serum in phosphate buffered saline (PBS) overnight before the addition of IFN-γ recombinant cytokine standard (BD Bioscience) and samples. After a 2 h incubation at room temperature bound IFN-γ was detected using biotinylated anti-IFN-γ (XMG1.2, BD Bioscience) and avidin peroxidase followed by 3,3′,5,5′ Tetramethylbenzidine (TMB Reagent Set; BD Bioscience) 1 h later. Optical densities were read at 450 nm in a Bio-Rad Model 680 microplate reader and analyzed using Microplate Manager 5.2 software (Bio-Rad Laboratories, Hercules, CA).

2.3. Mononuclear cell isolation and fluorescence activated cell sorting

CNS-derived cells were isolated as described (Bergmann et al., 1999). Briefly, brains from PBS-perfused mice ($n = 3$–8) were homogenized in ice-cold Tenbroeck grinders in Dulbecco's PBS. Homogenates were clarified by centrifugation at 450 ×*g* for 7 min, and supernatants stored at −80 °C for further analysis. Cell pellets were resuspended in RPMI medium, adjusted to 30% Percoll (Pharmacia, Piscataway, NJ), and underlaid with 1 ml of 70% Percoll. After centrifugation at 850 ×*g* for 30 min at 4 °C, cells were recovered from the 30%/70% interface, washed once, and resuspended in fluorescence-activated cell sorter (FACS) buffer (PBS with 0.5% bovine serum albumin). CNS-derived CD4 and CD8 T cells were purified from pooled brains ($n = 6$–8) using a BD FACS Aria (BD). The vast majority of CNS-derived T cells have an activated effector phenotype (CD44^{hi}CD62L^{lo}CD69^{+}PD-1^{+}) (Bergmann et al., 1999; Phares et al., 2009). CD44^{hi}CD62L^{lo} CD4 or CD8 (effector) and CD44^{lo}CD62L^{hi} CD4 or CD8 (naive) cells were also purified from pooled CLNs. A minimum of 1×10^5 cells were collected per pooled sample and frozen in 400 μl TRIzol (Invitrogen, Carlsbad, CA) at −80 °C for subsequent RNA extraction and PCR analysis as described (Ireland et al., 2009). Cell suspensions from CLN were prepared from identical animals as described (Bergmann et al., 1999).

2.4. Flow cytometric analysis

Cells were incubated with mixed serum (mouse, goat and horse) and rat anti-mouse FcγIII/II mAb (2.4G2; BD Bioscience, San Diego, CA) for 20 min on ice prior to staining. Expression of cell surface markers was determined by incubation of cells with mAb specific for CD45 (30-F11), CD4 (L3T4), CD8 (53-6.7), CD11b (M1/70), CD19 (1D3), CD25 (PC61), CD44 (IM7), CD62L (MEL-14), CD69 (H1.2F3), CD138 (281-2) (BD Bioscience, San Jose, CA), PD-1 (RMP1-30) (eBioScience) and F4/80 (CI:A3-1; Serotec, Raleigh, NC) for 30 min on ice. Virus-specific CD8 T cells were identified using D^{b}/S510 major histocompatibility complex (MHC) class I tetramers (Beckman Coulter Inc., Fullerton, CA) as described previously (Bergmann et al., 1999). Stained cells were washed with FACS buffer and fixed in 2% paraformaldehyde. For intracellular detection of granzyme B and Foxp3 cells were stained for cell surface markers prior to permeabilization with either Cytofix/Cytoperm Reagent (BD PharMingen) or Fixation/Permeabilization Reagent (eBioScience) and staining with allophycocyanin-labeled anti-granzyme B (GB12, Caltag Laboratories Burlingame, CA) or FITC-labeled anti-Foxp3 (FJK-16s; eBioScience San Diego, CA), respectively. For detection of Annexin-V, cells were stained with anti-CD4, anti-CD8 and anti-CD45 Ab, washed, resuspended in 1× Annexin-V binding buffer containing Annexin-V (BD Bioscience) and incubated for 15 min. Granzyme B, Foxp3 and Annexin-V were all measured directly ex vivo without stimulation. For detection of CXCR5, cells were stained with biotin rat anti-mouse CXCR5 Ab (BD Bioscience) and streptavidin phycoerythrin (BD Bioscience). A minimum of 2×10^5 viable cells were stained and analyzed on a FACSCalibur flow cytometer (BD, Mountain View, CA). Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Virus specific IFN-γ production by CLN-derived CD8 T cells was evaluated after peptide stimulation. Briefly, 2×10^6 CLN cells were cultured in the absence or presence of 1 μM S510 peptide encompassing the H-2D^{b} restricted CD8 T cell epitope in RPMI supplemented with 10% fetal calf serum for 5 h at 37 °C with 1 μl Golgi Stop (BD Bioscience)/ml. After stimulation, cells were stained for surface expression of CD8 and CD62L, fixed, and then permeabilized to detect intracellular IFN-γ as recommended by the supplier (BD Bioscience).

2.5. Gene expression analysis

Snap frozen brains, spinal cords or CLN from PBS-perfused individual mice ($n = 3$–7) were placed into Trizol (Invitrogen, Grand Island, NY) and homogenized using a TissueLyser with stainless steel beads (Qiagen,