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Ablation of CD11c^{hi} dendritic cells exacerbates Japanese encephalitis by regulating blood-brain barrier permeability and altering tight junction/adhesion molecules

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ABSTRACT

Japanese encephalitis (JE), characterized by extensive neuroinflammation following infection with neurotropic JE virus (JEV), is becoming a leading cause of viral encephalitis due to rapid changes in climate and demography. The blood-brain barrier (BBB) plays an important role in restricting neuroinvasion of peripheral leukocytes and virus, thereby regulating the progression of viral encephalitis. In this study, we explored the role of CD11c^{hi} dendritic cells (DCs) in regulating BBB integrity and JE progression using a conditional depletion model of CD11c^{hi} DCs. Transient ablation of CD11c^{hi} DCs resulted in markedly increased susceptibility to JE progression along with highly increased neuro-invasion of JEV. In addition, exacerbated JE progression in CD11c^{hi} DC-ablated hosts was closely associated with increased expression of proinflammatory cytokines (IFN-β, IL-6, and TNF-α) and CC chemokines (CCL2, CCL3, CXCL2) in the brain. Moreover, our results revealed that the exacerbation of JE progression in CD11c^{hi} DC-ablated hosts was correlated with enhanced BBB permeability and reduced expression of tight junction and adhesion molecules (claudin-5, ZO-1, occluding, JAMs). Ultimately, our data conclude that the ablation of CD11c^{hi} DCs provided a subsidiary impact on BBB integrity and the expression of tight junction/adhesion molecules, thereby leading to exacerbated JE progression. These findings provide insight into the secondary role of CD11c^{hi} DCs in JE progression through regulation of BBB integrity and the expression of tight junction/adhesion molecules.

1. Introduction

Among all neurotropic causes of viral encephalitis, Japanese encephalitis virus (JEV) is considered to be the most serious, due to its neuropathological impacts, higher fatality rate, and permanent neuropsychiatric sequelae [1]. Currently, JEV is spreading to previously unaffected regions such as Indonesia, Pakistan, and northern Australia due to rapid changes in climate and demography [2,3]. Considerable progress has been made in our understanding of JE pathogenesis, an acute neuroinflammation caused by JEV infection, in both infected patients and murine models [4–9]. JEV replicates within monocytes/macrophages and dendritic cells (DCs) as primary target cells [10], through which the virus is routed from the periphery to the CNS, leading to neurological disorders [11].

Like acute viral encephalitis caused by other flaviviruses, JE is characterized by CNS infiltration of peripheral leukocytes in the perivascular space and parenchyma [11]. Furthermore, the blood-brain barrier (BBB) plays a critical role in regulating neuroinvasion of peripheral leukocytes, although it seems infrangible until the late phase of encephalitis [12,13]. Junctional proteins on infiltrated monocytes and macrophages contribute to contemporaneous interactions with the BBB as they drift across the barrier, and are responsible for virus influx into the brain and subsequent outcomes of neuro-inflammation [4,12]. The core structural and anatomical basis of BBB integrity is covalently related to the appearance of tight junction transcripts and adhesion molecules

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regulating the microenvironment. Physiologically, tight junction proteins (TJPs) like claudins and occludin are connected to the cytoskeleton by cytoplasmic proteins zonula occludens (ZOs), and BBB integrity is correlated with the expression of TJPs and adhesion molecules [14]. The BBB and TJP transcripts contribute to maintaining a distinct internal atmosphere and controlling the diffusion of solutes and polarized molecules through the intercellular space [15]. Indeed, the BBB alters and can become highly compromised as encephalitis progresses, and simultaneous loss of TJ integrity is associated with brain migration of monocyte in human immunodeficiency virus (HIV) and West Nile virus (WNV) encephalitis [16,17]. Likewise, upon JEV infection, altered patterns of cellular tight junctions in non-neuronal cells results in alteration of BBB integrity supervening a critical role in viral dissemination, and exacerbation of encephalitis [18]. Thus, alterations in the BBB and adhesion molecules may be highly related to the severity of neurotropic disease pathogenesis.

Based on various immunological functions like the ability to express MHC class II, naïve T cell-stimulating capacity and potency both in vivo and in vitro, DCs act as APCs [19,20]. Furthermore, DCs play critical roles in the regulation of innate immune responses such as crosstalk with NK cells as well as links between innate and adaptive immune responses against viral infections [21], resulting in specific types of $CD4^+$ Th responses and establishing a memory T-cell pool using costimulatory signals from DCs [22]. However, the study of DCs in the progression of neuroinflammation induced by neurotropic viral infections relies on complex phenomena of multi-cellular interactions, immune homeostasis, and tolerization, due to their relationship with other innate immune cells, including macrophages and monocytes, at the level of differentiation [23,24]. $CD11b^+Ly\text{-}6C^{hi}$ monocytes tend to significantly dampen the immune-privileged CNS [25] and exacerbate the pathogenesis of viral encephalitis [26], and their CNS infiltration is highly related to increased production of inflammatory cytokines and chemokines [27], alteration of BBB integrity [28], and a reduction in transcripts of tight junctions and adhesion molecules [29]. Recently, a detailed map of the close relationship between $Ly\text{-}6C^{hi}$ monocytes and DCs at the level of differentiation sheds light on the role of DCs in JE progression through regulation of monocyte differentiation [30]. In addition, elevated levels of systemic pro-inflammatory cytokines in the sera of DC-ablated mice may be critically related to CNS infiltration of $CD11b^+Ly\text{-}6C^{hi}$ monocytes and other cellular components, along with alteration in the BBB [31,32]. However, very little is known regarding the role of DCs in regulating BBB integrity during JE progression.

CD11c-DTR transgenic (Tg) mice, which express the diphtheria toxin receptor (DTR) gene under control of a cloned *Itgax* promoter and thus allow conditional DC depletion upon DT injection, are a critical tool in the study of DC immunology in neurotropic viral infections [33]. Here, we explored the role of DCs in the regulation of BBB integrity and JE progression using a conditional depletion model of DCs. We found that higher expression of proinflammatory cytokines and dampening of tight junction molecules were closely associated with BBB alterations, along with concurrent exacerbation of JE progression. Therefore, this fact provides insight into the secondary role of DCs in JE progression via regulation of BBB integrity, providing a mechanism that allows peripheral leukocytes to gain access into the CNS.

2. Materials and methods

2.1. Ethics statement

All animal experiments described in the present study were conducted at Chonbuk National University according to the guidelines set by the institutional Animal Care and Use Committees (IACUC) of Chonbuk Nation University (http://lac.honamlife.com/research_05.php) and were pre-approved by the Ethical Committee for Animal Experiments of Chonbuk National University (permission code 2013-0028), which has been fully accredited by the Korean Association for Laboratory Animal Sciences (KALAS), adopted by Council of the Korean Government for Animal Care. All experimental protocols requiring biosafety were approved by Institutional Biosafety Committees (IBC) of Chonbuk National University.

2.2. Animals, cells, and viruses

C57BL/6 ($H\text{-}2^b$) mice (4–6 weeks old) were purchased from Samtako (O-San, Korea), and CD11c-DTR transgenic (Tg) mice (B6.FVB-Tg Itgax-DTR/EGFP 57Lan/J [DTR]) were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were genotyped and bred in the animal facilities of Chonbuk National University. JEV Beijing-1 strain was propagated in the mosquito cell line (C6/36) using DMEM supplemented with 2% FBS, penicillin (100 U/ml), and streptomycin (100 U/ml), as described elsewhere [30]. The virus stocks were titrated by conventional plaque assay using BHK-21 cells (CCL-10; American Type Culture Collection), and stored in aliquots at −80 °C until use. Primers specific for JEV and cytokines (Table 1) were synthesized at Bioneer Corp. (Daejeon, Korea) and used for PCR amplification of target genes.

2.3. Quantification of viral burden and cytokine expression

Viral burden and the expression of cytokines and chemokines in inflammatory and lymphoid tissues were determined by quantitative SYBR Green-based real-time RT-PCR (real-time qRT-PCR). Mice were infected intraperitoneally (i.p.) with JEV (1.0 LD_{50}) and tissues including brain, spinal cord, and spleen were harvested at 2, 4, and 6 dpi. Total RNAs extracted from tissues using easyBLUE (iNtRON, INC., Daejeon, Korea) were employed in real-time qRT-PCR using a CFX96™ Real-Time PCR Detection system (Bio-Rad Laboratories, Hercules, CA). Following reverse transcription of total RNAs with High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster, CA), each reaction mixture contained 2 μl of template cDNA, 10 μl of 2 × SYBR Primix Ex Taq, and 200 nM primers to a final volume of 20 μl. The reactions were denatured at 95 °C for 30 s and then subjected to 45 cycles of 95 °C for 5 s and 60 °C for 20 s. After the reaction cycle was completed the temperature was increased from 65 °C to 95 °C at a rate of 0.2 °C/15 s, and the fluorescence was measured every 5 s to construct a melting curve. A control sample that contained no template DNA was run with each assay, and all determinations were performed at least in duplicate to ensure reproducibility. The authenticity of the amplified product was determined by melting curve analysis. Viral RNA burden in the infected samples was expressed as viral RNA copies per microgram of RNA, and the relative ratio of cytokines and chemokines in infected samples to uninfected samples was determined. All data were analyzed using Bio-Rad CFX Manager, version 2.1 analysis software (Bio-Rad Laboratories).

2.4. Histological examination of the brain

For histological examination of the brain, brain tissues derived from mock and JEV-infected mice were embedded in paraffin and 10-μm sections were prepared and stained with H&E by the Pathology Lab (College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea). Sections were analyzed using a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan). Photomicrographs were taken from coronal sections of the septo-striatal regions of the brain.