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Therapeutic effects of anti-Delta1 mAb on Theiler's murine encephalomyelitis virus-induced demyelinating disease

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ABSTRACT

We examined the role of Notch ligand Delta-like 1 (Delta1) in the development of Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD). Blocking of Delta1 by anti-Delta1 monoclonal antibody (mAb) in the effector phase significantly suppressed the disease development of TMEV-IDD both clinically and histologically. The number of infiltrating inflammatory mononuclear cells in the spinal cords was also decreased in mice treated with anti-Delta1 mAb at the effector phase. Flow cytometric analysis of cytokine staining revealed that IFN-γ- or IL-4-producing $CD4^+$ splenocytes were significantly decreased in mice treated with anti-Delta1 mAb in the spleens, whereas IL-10-producing $CD4^+$ splenocytes were increased. Furthermore, IFN-γ-, TNF-α-, IL-4-, or IL-10-producing $CD4^+$ cells were decreased in spinal cords, and IL-17-producing $CD4^+$ cells were increased. These data suggest that Delta1 may play important roles in the development of TMEV-IDD and that antibodies to Delta1 could be used as a novel therapeutic treatment of demyelinating diseases such as human multiple sclerosis.

1. Introduction

Multiple sclerosis (MS) is an immune-mediated chronic inflammatory demyelinating disease of the human central nervous system (CNS). Histologically, MS is characterized by CNS lesions displaying inflammation, demyelination and axonal damage (Frohman et al., 2006). Although its etiology remains unclear, MS is generally considered to be an autoimmune T helper (Th) 1-mediated disease (Sospedra and Martin, 2005; Kebir et al., 2007). The morphology of the acute lesion and the susceptibility to MS apparently conferred by certain major histocompatibility complex (MHC) class II-restricted antigens suggest that autoreactive $CD4^+$ T cells have a crucial role in the disease process. $CD8^+$ T cells may also participate in the pathogenesis of MS and its animal model (Rodriguez et al., 2003; Tsunoda et al., 2006). Epidemiological evidence suggests that one or more infectious agents may be involved in the initial tissue damage leading to autoimmunity (Johnson, 1975; McFarlin and McFarland, 1982; Soldan et al., 1997).

Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD) serves as an infectious mouse model for MS because the disease displays histopathologic, genetic and clinical similarities to human MS. In this model, the development and progression of demyelinating disease correlate well with the level of Th1 responses specific for viral epitopes (Gerety et al., 1994; Yauch and Kim, 1994; Yauch et al., 1998; Kim et al., 2001). Administration of bacterial lipopolysaccharide (LPS) or interleukin (IL)-1β potentiating inflammatory Th1 responses into genetically resistant C57BL/6 mice infected with TMEV resulted in clinical symptoms (Pullen et al., 1995). Similarly, LPS treatment of susceptible SJL/J mice after infection with a non-pathogenic variant of TMEV causes the mice to develop clinical symptoms (Palma et al., 1996). Thus, the activation and polarization of $CD4^+$ T cells toward a Th1 lineage are considered to be essential steps in the pathogenesis of TMEV-IDD.

The Notch signaling pathway is highly conserved during evolution of vertebrates, and that is an intercellular signaling mechanism involved in embryonic patterning and controlling diverse aspects of development and tissue homeostasis (Rubio-Aliaga et al., 2009). In mammalian cells, four distinct Notch receptors (Notch1–4) and two families of Notch canonical ligands {Jagged 1, 2, Delta like (Delta) 1, 3, 4} have been identified (Zanotti and Canalis, 2010). Upon Notch ligand–receptor interactions, the intracellular domain of Notch (NICD) is cleaved by γ-secretase, released from the membrane, and translocated to the nucleus, where it binds a CSL/RBP-J/MAML/p300 to act as a transcriptional activator (Maillard et al., 2005).

Recent evidences have highlighted the role of Notch pathways in peripheral Th cell activation and differentiation (Yamaguchi et al.,

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Table 1
Experimental design.

	Group	Treatment	Number of mice	Date of treatment	MMS ± SD	MCS ± SD
Experiment 1	A	Non-specific Hamster IgG (Positive control)	8	Days 14, 17, 20, 23, 26, 29, 32, 35	2.7 ± 1.0	14.2 ± 9.2
	B	Anti-Delta1 mAb (Induction phase)	7	Days −3, 0, 3, 6, 9, 12, 15, 18	2.8 ± 0.6	19.5 ± 6.2
	C	Anti-Delta1 mAb (Effector phase)	8	Days 14, 17, 20, 23, 26, 29, 32, 35	0.9 ± 0.8	5.5 ± 7.0[a]
Experiment 2	D	Non-specific Hamster IgG (Positive control)	6	Days 20, 23, 26, 29, 32, 35, 38, 41	3.5 ± 0.5	33.0 ± 3.9
	E	Anti-Delta1 mAb (Effector phase)	6	Days 20, 23, 26, 29, 32, 35, 38, 41	1.6 ± 0.5[a]	12.1 ± 5.8[a]

MS: mean maximum clinical score.
MCS: mean cumulative clinical score.
[a] Significant difference between Groups A and C or D and E ($p<.05$).

2002; Amsen et al., 2004; Tsukumo and Yasutomo, 2004; Maillard et al., 2005). It has been proposed that differential expression of Notch ligands by dendritic cells (DCs) in response to exposure to different classes of pathogens underlies the ability of DCs to promote pathogen-appropriate Th responses. T-cell polarization and the profile of cytokine production may depend on the ligand interacting with the Notch receptors. It has been shown that Jagged1 and Jagged2 promote IL-4 expression and stimulate Th2-type responses, while Delta1 and Delta4 induce differentiation along a Th1-type pathway (Maekawa et al., 2003; Amsen et al., 2004; Rutz et al., 2005; Kostianovsky et al., 2007; Skokos and Nussenzweig, 2007).

Thus, it is possible that Notch ligands are actively involved in the development of immune-mediated diseases such as MS. The use of a γ-secretase inhibitor inhibited only Th1 cell differentiation and suppressed experimental autoimmune encephalomyelitis (EAE), a T cell-mediated disease that is used as a model for the study of MS (Minter et al., 2005). In another study, there was a significant decrease in the clinical score in the anti-Delta1 mAb-treated mice in EAE, while blockade of Jagged1 deteriorated the disease (Elyaman et al., 2007). Furthermore, we previously demonstrated that administration of anti-Delta4 mAb suppressed the development of TMEV-IDD (Takeichi et al., 2010). Here, we have described for the first time that blocking the Delta1-induced Notch signaling by anti-Delta1 mAb in the effector phase markedly suppresses the development of TMEV-IDD and decreases the number of infiltrating cells, suggesting that Delta1-induced Notch signaling may play a critical role in infiltrating inflammatory mononuclear cells (MNCs) in TMEV-IDD and Delta1 could be a novel therapeutic target in human multiple sclerosis.

2. Experimental procedure

2.1. Mice

Female SJL/J mice, 6 weeks old, were purchased from the Charles River Laboratories Japan, Inc. (Ibaraki, Japan), housed and cared for in an approved facility, in accordance with the Shinshu University Guide for Laboratory. The animals were kept in aluminum cages containing pine chips, and given food and water ad libitum. The protocol for animal experiments was approved by the Animal Care Committee of Shinshu University.

2.2. Virus

The BeAn strain of TMEV was expanded in baby hamster kidney (BHK) cell monolayers in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 7% donor calf serum. Cell lysates with known plaque forming unit (PFU) were used as viral stock for animal experiments. Partially purified virus was prepared following centrifugation through 30% sucrose as previously described and used for in vitro assays (Yauch et al., 1998). Viral titer was determined by the standard plaque assay on BHK cells.

2.3. Infection of mice with TMEV

We performed experiments in two patterns by changing concentration of TMEV or time of injection of mAb. Mice were intracerebrally (i.c.) infected with 30 μl (0.67×10^6 PFU or 1×10^6 PFU) of the BeAn strain of TMEV at day 0, and clinically observed and scored from day 0 to day 36 or 42 post i.c. infection. Mice were examined daily for clinical (neurologic) signs, which were recorded using the following grading scale: grade 0 = no clinical signs; grade 1 = mild waddling gait; grade 2 = moderate waddling gait and hindlimb paresis; grade 3 = severe hindlimb paralysis; grade 4 = severe hindlimb paralysis and loss of righting reflex; and grade 5 = moribund. A cumulative clinical score was calculated for each mouse in each group on animals in each experiment.

2.4. Treatment with mAbs

Six-week-old female SJL/J mice were separated into groups (Table 1). TMEV was infected into SJL/J mice i.c. at day 0. For neutralization of Delta1 in vivo, mice were intraperitoneally injected with 250 μg hamster anti-mouse Delta1 mAb (HMD1-5) (Moriyama et al., 2008) every third day from day −3 to day 18 in induction phase (group B) and from day 14 to day 35 or from day 20 to day 41 in effector phase (groups C or E) post viral infection. This dose was determined from our previous experiments (Moriyama et al., 2008). Control groups (groups A and D) were injected with 250 μg nonspecific hamster IgG (Jackson Immunoresearch Laboratories, Baltimore, USA) in the same time interval. Details of the experimental design are given in Table 1.

2.5. Isolation of infiltrating cells from spinal cords

Infiltrating cells into spinal cords were isolated by using the method of Michael A. Lyman (Lyman et al., 2004). Briefly, sterile Hanks balanced

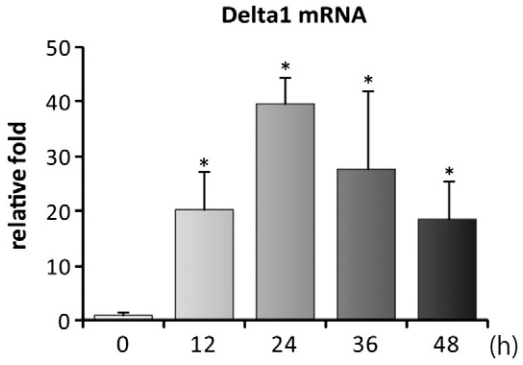

Fig. 1. TMEV induces the expression of Delta1 in bone marrow-derived dendritic cells (BMDCs). BMDCs from naïve SJL/J mice were infected with TMEV for the indicated time periods. Following stimulation, total RNA was harvested and the expression of Delta1–mRNA in BMDCs was measured by real-time RT-PCR. Relative fold increases in Delta1 expression were determined by comparing with unstimulated cultures. Gene expression levels of Delta1–mRNA in BMDCs significantly increased compared with unstimulated cultures (n = 4, $^*p<0.05$). Data are represented as the mean ± standard deviations (SD).