Immunobiology 221 (2016) 188–192

Contents lists available at ScienceDirect

Immunobiology

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

Short communication

The ribosomal S6 kinase inhibitor BI-D1870 ameliorated experimental autoimmune encephalomyelitis in mice

Ichiro Takada[a,b,*], Yoshiko Yogiashi[b], Makoto Makishima[a]

[a] Division of Biochemistry, Department of Biomedical Sciences, School of Medicine, Nihon University, Itabashi-ku, Tokyo 173-8610, Japan
[b] School of Medicine, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 2 September 2015
Accepted 4 September 2015
Available online 8 September 2015

Keywords:
Th17 cell
RORgt
RSK

ABSTRACT

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) caused by the infiltration of T_H1 and T_H17 cells into the CNS. Ribosomal S6 kinase 2 (RSK2; RPS6KA3) regulates T_H17 differentiation by attenuating RORγt transcriptional activities and IL-17A production. The pan-RSK inhibitor BI-D1870 also inhibits T_H17 differentiation, but the effect of BI-D1870 *in vivo* remains unclear. Here, we generated mice with experimental autoimmune encephalomyelitis (EAE) and treated them with BI-D1870. BI-D1870 administration protected mice from EAE by reducing the infiltration of T_H1 and T_H17 cells into the CNS and decreasing mRNA levels of *Ccr6* in T_H17 cells. These results suggest that RSK inhibition is a promising strategy for the treatment of MS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease in which T lymphocytes specific for myelin antigens initiate an inflammatory reaction in the central nervous system (CNS), leading to demyelination and subsequent axonal injury (McFarland and Martin, 2007). Although the cause of MS is still largely unknown, recent genome-wide association studies indicate that genetic factors play a critical role in the induction of MS (International Multiple Sclerosis Genetics et al., 2011).

Experimental autoimmune encephalomyelitis (EAE) is a $CD4^+$ T cell-mediated disease of the CNS, and it is commonly used as a model of MS (Fletcher et al., 2010). To induce EAE in mice, treatment with myelin oligodendroglial glycoprotein (MOG) leads to the development of a monophasic disease showing extensive demyelination and inflammation in the CNS. The generation of myelin protein-reactive T cells is an immunologic hallmark of EAE. The activated autoreactive T cells cross the blood-brain barrier to reach the CNS and it is likely that this process is common to the pathogenesis of EAE as well as MS.

IFN-γ-producing $CD4^+$ T cells (T_H1) and IL-17-producing $CD4^+$ T cells (T_H17) have been implicated in EAE induction (Rostami and Ciric, 2013). $CD4^+$ helper T cells differentiate into distinct subsets (such as T_H1, T_H2, iT_{reg} and T_H17) that express a number of cytokine signals (Zhou et al., 2009). In T_H17 cells, transcriptional factors such as RORγt and STAT3 regulate *Il17a* gene expression levels (Bettelli et al., 2008; Littman and Rudensky, 2010; McGeachy and Cua, 2008) and transcriptional co-factors modulate their activities. For example, RIP140 (Huang et al., 2012) and USP17 (Han et al., 2014) coactivate the transcriptional activity of RORγt. We previously identified the DGCR14/RSK2/BAZ1B complex as a transcriptional co-activator for RORγt in T_H17 cells (Takada, 2015).

RSK2 is a serine-threonine protein kinase belonging to the AGC kinase family and its mutation causes a genetic disease, Coffin–Lowry syndrome (Delaunoy et al., 2001). Recently, synthesized inhibitors of RSKs have been shown as good candidates for anti-cancer drugs (Nguyen, 2008). RSK2-deficient mice have reduced proliferation of $CD4^+$ T cells due to suppression of *Il2* mRNA expression (Lin et al., 2008), and the expression levels of IFN-γ in T_H1 and IL-4 in T_H2 are changed. RSK isoforms are downstream of ERK1/2 and control cell proliferation. Previously, we found that a pan-RSK inhibitor (BI-D1870 (Sapkota et al., 2007)) highly suppressed T_H17 cell differentiation but not that of T_H1, T_H2 or iTreg (Takada, 2015). However, the *in vivo* effect of RSK inhibitors on autoimmune diseases has not been studied.

In this study, we investigated the effect of BI-D1870 on EAE mice and found that BI-D1870 protected mice from EAE with reducing production of T_H17 cytokines, such as IL17A. These results provide an insight in the management of autoimmune diseases such as MS.

* Corresponding author.
E-mail address: takada.ichiro@nihon-u.ac.jp (I. Takada).

http://dx.doi.org/10.1016/j.imbio.2015.09.008
0171-2985/

2. Materials and methods

2.1. EAE

Myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (MEVGWYRSPFSRVVHLYRNGK) (BEX) was used to induce EAE in C57/BL6J mice purchased from CLEA Japan, Inc. (Matsumura et al., 2007). Mice were injected s.c. with 200 μg of MOG peptide in 100 μL of PBS emulsified in 100 μL complete Freund's adjuvant (CFA) that was further supplemented with five mg mL^{-1} *Mycobacterium tuberculosis* (H37Ra; Difco BD Biosciences). In addition, 500 ng pertussis toxin (Calbiochem) was injected i.p. on days zero and two. The RSK inhibitor (BI-D1870; 0.5 mg kg^{-1}) (Boehringer Ingelheim) was injected i.p. into mice two days after immunization with MOG peptide, and injection was repeated every other day for 11 days. Mice that received only dimethyl sulfoxide (DMSO) solution were used as controls. Paralysis was evaluated according to the following scale: zero, no disease; one, tail limpness; two, hind limb weakness; three, hind limb paralysis; four, fore limb weakness; five, quadriplegia; six, death. For histological analysis, CNS samples were fixed with 4% paraformaldehyde and sliced at 4 μm, and then hematoxylin & eosin (H & E) staining was performed.

2.2. ELISA

Supernatants were collected after the indicated periods of cell culture and were analyzed for IL-17A and IFN-γ with an ELISA kit (eBioscience) according to the manufacturer's instructions.

2.3. Primary T cell differentiation

$CD4^+CD25^-CD44^{low}CD62L^{high}$ naïve T cells from spleens and lymph nodes were enriched through negative selection using a magnetic cell sorting system (Miltenyi Biotec, Bergisch Gladbach, Germany) with biotin-conjugated anti-CD8.2 (53–6.7), anti-B220/CD44 (RA3–6B2), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD49b (Dx5, all from eBioscience, San Diego, CA) and anti-TER119 (BD Biosciences) antibodies as well as streptavidin-conjugated magnetic beads (Miltenyi Biotec). Cells were then flow cytometrically sorted using a BD FACSAria™ cell sorter (BD Biosciences). The purity of the sorted $CD4^+$ T cell populations was consistently >98%. T cells were maintained in a complete medium containing RPMI 1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 100 nM non-essential amino acids, two mM glutamine and 0.05 mM 2-mercaptoethanol. The culture conditions for different T_H 17 cell subsets were one μg mL^{-1} anti-CD-3ϵ, one μg mL^{-1} anti-CD-28, one μg mL^{-1} anti-IL-4, one μg mL^{-1} anti-IFN-γ, ten ng mL^{-1} IL-6 (Peprotech) and two ng mL^{-1} TGF-β with/without ten μM BI-D1870 or ten μM T0901317 (RORγ/RORα inverse agonist/liver X receptor agonist) for four days.

2.4. RNA analysis

Total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from total RNA using the SuperScript III synthesis system (Invitrogen) and random hexamer primers. The gene expression levels were analyzed by quantitative RT-PCR using a Thermal Cycler FCR96 System (BioRad) and SYBR Fast (BioRad). Primer sequences were as follows: m*Ccr6*, 5′-TCCTGGGTCTTTCGGACTTG-3′ (Fw) and 5′- GGGCAGTTCAACCACACTCTC-3′ (Rv); m*Il21*, 5′- CAGGAGGGGAGGAAAGAAACA-3′ (Fw) and 5′- CTTTCTAGGAATTCTTTGGGTGTCC-3′ (Rv); m*Il22*, 5′- GGTGACGACCAGAACATCCA-3′ (Fw) and 5′- CAATCGCCTTGATCTCTCCAC-3′ (Rv); mIl23r, 5′-AGAAACTGGCAGCCTTGGAG-3′ (Fw) and 5′- TGAGGTTCGTGGGATGATTTT-3′ (Rv); m*Gapdh*, 5′-TGTGTCCGTCGTGGATCTGA-3′ (Fw) and 5′-TTGCTGTTGAAGTCGCAGGAG-3′ (Rv). The mRNA levels of genes were normalized to that of *Gapdh* mRNA expression.

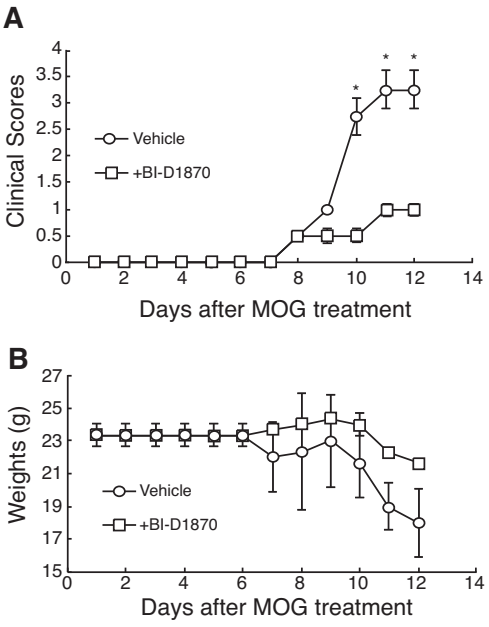

Fig. 1. BI-D1870 reduced development of EAE.
(A) Clinical scores of EAE-induced mice treated with/without BI-D1870. Mice were treated with BI-D1870 three times: before MOG injection, and one and three days after MOG injection ($n = 3$). $^*p < 0.05$.
(B) Weights of EAE-induced mice treated with/without BI-D1870 in A.

2.5. Statistical analysis

Data are presented as the means ± SD. Differences between groups were assessed by Student's paired two-tailed *t* test. Values of $p < 0.05$ were considered significant. All error bars shown in this article represent standard deviations.

3. Results and discussion

We evaluated the involvement of RSK activation in EAE mice immunized with MOG peptide. We used the pan-RSK inhibitor BI-D1870 (Sapkota et al., 2007) because we had previously found that overexpression of RSK1, 2 and 3 co-activated the transcriptional activity of RORγt (Takada, 2015). After myelin oligodendrocyte glycoprotein (MOG) immunization, mice developed severe paralytic symptoms from around day 11. Meanwhile, BI-D1870-injected EAE mice exhibited a delayed neural deficit (Fig. 1A). BI-D1870 treatment was moderately protective against weight loss (Fig. 1B). Histopathological analyses showed inflammatory cell infiltration and demyelination in the spinal cord in control mice, but not in BI-D1870-treated mice (Fig. 2). Then, we calculated the number of $CD4^+$ T cells in the spleen (SP), lymph node (LN) and CNS. In the SP and LN, the number of $CD4^+$ T cells did not differ between BI-D1870-treated EAE mice and control EAE mice (SP, $11.3 \pm 3.6 \times 10^7$ cells in control EAE mice and $14.0 \pm 6.0 \times 10^7$ cells in BI-D1870-treated EAE mice; LN, $4.9 \pm 0.69 \times 10^6$ cells in control EAE mice and $7.6 \pm 1.8 \times 10^6$ cells in BI-D1870-treated EAE mice. $n = 3$). However, the number of $CD4^+$ T cells that infiltrated into the CNS was lower in BI-D1870-treated EAE mice ($1.6 \pm 0.7 \times 10^5$ cells, $n = 3$) than control EAE mice (3.4×10^5 cells and 3.9×10^5 cells). These results showed that BI-D1870 protected against the infiltration of T_H1 or T_H17 cells into the CNS.