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Cerebral interleukin-15 shows upregulation and beneficial effects in experimental autoimmune encephalomyelitis

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Abstract

Interleukin (IL)-15 can cross the blood-brain barrier to act on its specific brain receptor (IL15R α) and co-receptors. The important roles of neuronal IL15 and IL15R α in experimental autoimmune encephalomyelitis (EAE) are suggested by the upregulation of IL15R α mRNA in different regions of the brain and spinal cord, and by double-labeling immunohistochemistry showing neuronal localization of IL15 and IL15R α in different neurons. Contrary to expectations, IL15 treatment lessened EAE severity. IL15 knockout mice showed heightened susceptibility to EAE with significantly higher scores that were decreased by treatment with IL15. Thus, IL15 improves this CNS autoimmune disorder as a potential therapeutic agent.

Keywords

IL15; cytokine; brain; immunity; autoimmune disease; blood-brain barrier

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) reflects the autoimmune aspect of the human disease multiple sclerosis (MS), a devastating demyelinating disorder of the central nervous system (CNS) (Hart *et al.* 1998; Hart and Amor 2003). Despite the use of disease modifying agents including β interferons, glatiramer, mitoxantrone, and newer immunomodulatory agents, the prognosis remains poor for the relapsing-remitting and primary progressive types of MS. This warrants better understanding of its neuropathology and the search for novel therapeutic agents. Although proinflammatory cytokines play important roles in the progression of MS (Merrill *et al.* 1984; Zozulya *et al.* 2009), very few studies have directly addressed the role of interleukin (IL)-15 in MS and EAE. Here, we tested the hypotheses that the cerebral IL15 system is regulated by EAE and that it in turn modifies the severity of EAE.

IL15 is a 14 kD polypeptide that belongs to the IL2 subfamily of the four-alpha-helix bundle family of cytokines. IL15 binds to its specific receptor IL15R α and two additional receptor subunits - IL2R β and IL2R γ . IL15 mRNA has been found to be constitutively expressed in parenchymal cells of many organs as well as immune cells. In the nervous system, IL15 is expressed by neurons, astrocytes, and microglia (Lee *et al.* 1996; Budagian *et al.* 2006).

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Elevated expression of IL15 at both transcriptional and translational levels is found in peripheral blood mononuclear cells from MS patients (Pashenkov *et al.* 1999; Blanco-Jerez *et al.* 2002). Furthermore, upregulated IL15 is observed in serum and cerebrospinal fluid of MS patients (Losy *et al.* 2002; Rentzos *et al.* 2006). We have recently shown that EAE affects the distribution and turnover of IL15 after intravenous delivery which also alters the in-vivo BBB permeability to IL15 (Hsueh *et al.* 2009b). This provides a basis to determine the functional roles of the IL15 system in EAE.

EAE is an ascending disease with a maximal increase in the permeability of the blood-brain and blood-spinal cord barrier (BBB) occurring in the lumbar and cervical spinal cord (Pan *et al.* 1996). Disruption of the BBB and demyelination are also pronounced in the brain. Although many EAE studies focused on neuropathology in the spinal cord, cerebral involvement probably has greater correlation with sickness behavior. In human MS, there is a higher incidence of supratentorial lesions than spinal cord demyelination. Therefore, the hippocampus was chosen as a region of interest for the study of EAE, since its susceptibility to neuroinflammation and corresponding behavioral changes have been well characterized (Pollak *et al.* 2002; Goshen *et al.* 2007; Dantzer *et al.* 2008). In this study, we determined both regulatory changes of the endogenous IL15 system and treatment effects of IL15. Further, the exciting finding that IL15 knockout (KO) mice show heightened susceptibility to EAE strongly supports an immunoprotective role of IL15 in EAE mice.

2. Materials and Methods

2.1. Induction of EAE and IL15 treatment

All studies were conducted following a protocol approved by the Institutional Animal Care and Use Committee. EAE was induced in female SJL/J (2 months old, Jackson Laboratory), IL15 KO (2 months old, C57BL/6NTac-*IL15^{tm1Imx}*, Taconic), and their matching control (C57BL/6NTac) mice. The SJL/J mice were injected subcutaneously (sc) with 80 µg of proteolipid protein fragment 139–151 (PLP_{139–151}, AnaSpec Inc., San Jose, CA), that was emulsified in either RIBI adjuvant (RIBI Immunochem Research Inc, Hamilton, MT), or complete Freund's adjuvant (CFA) containing 500 µg of heat-killed *Mycobacterium tuberculosis* H37RA. The injection was made in the lower flank (33 µl/spot × 3 injections). The control mice received adjuvant without PLP_{139–151}. The IL15 KO mice and their controls received 100 µg of myelin oligodendrocyte glycoprotein (MOG)_{35–55} peptide along with 100 µl of CFA containing 4 mg/ml of heat-inactivated *Mycobacterium tuberculosis* H37RA. In mice receiving CFA adjuvant, pertussis toxin (200 ng in 100 µl phosphate buffered saline, PBS) was given intraperitoneally (ip) on the day of immunization and again 48 h later.

EAE was scored daily as follows: 0, no detectable signs of weakness; 0.5, distal tail limpness, mild postural changes, or reduced locomotor activity; 1, completely limp tail; 1.5, limp tail and hindlimb weakness (unsteady gait and poor grip with hindlimbs); 2, unilateral partial hindlimb paralysis; 2.5, bilateral hindlimb paralysis; 3, complete bilateral hindlimb paralysis; 3.5, complete hindlimb paralysis and unilateral forelimb paralysis; 4, total paralysis of hindlimbs and forelimbs; 5, moribund or dead.

To determine the effect of IL15 treatment on EAE score, two groups of SJL/J mice were studied (n = 8 /group). The experimental group received daily injection of IL15 at 100 ng/day/mouse ip from day 7 to day 14 after PLP + CFA immunization on day 0 (n = 8 /group). The control received PBS ip daily from day 7 to day 14. To determine the effect of IL15 deletion on EAE score, two separate groups of mice on C57 background were studied (n = 8 /group). All mice received MOG + CFA immunization. After apparent changes in EAE score between the IL15KO and wildtype mice were observed, half of the IL15KO mice were treated with IL15 (100 ng/day/mouse ip) from day 40 to day 55.

2.2. Immunohistochemistry (IHC) to determine the expression of IL15 and IL15R α

SJL/J mice on day 12 after PLP + RIBI immunization were studied along with adjuvant controls. The mice were anesthetized with ketamine/xylazine and perfused intracardially with 4 % paraformaldehyde. Coronal sections of the hippocampus at 20 μ m thickness were obtained by use of a cryostat. IHC was performed as described previously (Pan *et al.* 2008a; Hsueh *et al.* 2009a). All primary antibodies and the corresponding blocking peptides were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The primary antibody for IL15 was L-20 (sc-1296, goat polyclonal against the C-terminus of IL15), and that for IL15R α was N-19 (sc-5526, goat polyclonal against the N-terminus of IL15R α). The secondary antibody was an Alexa488-conjugated anti-goat antibody (Invitrogen, Carlsbad, CA). The specificity of the IL15 and IL15R α immunostaining was confirmed by the lack of signal in sections where primary antibodies were preabsorbed with blocking peptide, and in sections with omission of primary antibodies. Double staining of the sections was conducted by inclusion of a primary mouse anti-NeuN antibody (MAB377B, Millipore, Temecula) with either IL15 or IL15R α antibodies, followed by incubation with respective Alexa-conjugated secondary antibodies. Fluorescent images of matching sections between the control and experimental groups were captured by use of an Olympus FV1000 confocal microscope. The co-localization of IL15 with NeuN, or IL15R α with NeuN, was determined by confocal microscopy.

2.3. ELISA of serum IL15

IL15 concentration in the serum of EAE mice after PLP + RIBI induction was measured with a mouse IL15 ELISA Ready-SET-Go kit (eBioscience, San Diego, CA). The linear range was 15.6 – 500 pg/ml with $R^2 = 0.99$. EAE mice on day 3, 7, 12, 16, and 24 after induction were studied along with an adjuvant control group studied on day 3 ($n = 3 - 4$ for each time point).

2.4. Real-time RT-PCR

Total RNA was extracted from different brain and spinal cord tissues in the control and EAE mice at different days after PLP + RIBI immunization by use of an RNeasy mini kit (Qiagen, Valencia, CA). The tissues analyzed included the hippocampus, striatum, hypothalamus, and cervical, thoracic, and lumbar spinal cord. After digestion with DNase I to eliminate trace amounts of DNA contamination, total RNA was purified with an RNA clean up kit (Zymo Research, Orange, CA) and the concentration was measured by absorbance at 260 nm with a Bio-Rad spectrometer (Hercules, CA). Reverse transcription of the total RNA was conducted with a High Capacity cDNA Transcription Kit (Applied Biosystems, Foster City, CA). The cDNA samples were incubated with Taqman $^{\circledR}$ universal PCR master Mix (Applied Biosystems). Amplification was detected by use of an ABI 7900 instrument. Standard curves for quantification were generated with template plasmids containing fragments of the respective target genes. The level of expression of the target gene was normalized to that of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) in the same sample. All primers and fluorescent probes used for amplification are listed in Table 1.

2.5. Statistical analysis

Statistical analyses were performed with GraphPad Prism 5 (GraphPad Software, La Jolla, CA). For EAE scoring and treatment effects between groups, repeated measures analysis of variance (ANOVA) was performed. When both genders were tested, two-way ANOVA was performed to determine the effects of treatment (KO vs wildtype). Otherwise, pairwise comparisons between control and treatment groups were conducted by one-way ANOVA followed by Tukey's post-hoc test. All graphs show mean $\pm$ standard error of the mean.

3. Results

3.1. EAE mice show behavioral deficits and elevated hippocampal IL15 and IL15R α immunoreactivity in neurons

Two groups of mice were studied ($n = 8$ /group): those inoculated with PLP_{139–151} along with RIBI adjuvant (EAE group), and those receiving RIBI adjuvant only (control group). Mice in the EAE group showed a monophasic pattern of locomotor deficits. The EAE score, reflecting the deficits in motor activity and overall health, peaked at day 12 with a maximal score of 4 in individual mice (total paralysis of hindlimbs and forelimbs) and a mean score of 3 at this time (complete bilateral hindlimb paralysis). Afterwards, the scores decreased gradually so that clinical symptoms were absent on day 24 (Fig. 1). At day 0, 3, 7, 12, 16, and 24 after induction by PLP and RIBI adjuvant in the SJL/J mice ($n = 4$ /time point), blood IL15 concentrations, hippocampal IL15 immunoreactivity, and hippocampal IL15 mRNA were determined in the EAE mice. The 0 time control received RIBI adjuvant only. In these mice, serum IL15 concentrations showed a significant ($p < 0.05$) increase on day 12, rising from the basal low level of 2.5 ± 2.31 pg/ml to 58.52 ± 2.75 pg/ml (Fig. 2A). At this time, hippocampal IL15 immunoreactivity was also increased (Fig. 2B). Double-labeling immunohistochemistry showed that IL15 was mainly expressed in neurons (Fig. 2C).

The increase of IL15R α immunoreactivity was most pronounced in the hilus and granular cell layer of the infrapyramidal blade of the dentate gyrus (Fig. 3A). IL15R α was also seen mainly in neurons, as shown by confocal microscopic analysis of tissue sections immunostained with both IL15R α and NeuN (Fig. 3B). The hippocampal area with the highest intensity for IL15R α was more caudal than that for IL15 seen in figure 2B. This suggests paracrine interactions.

3.2. Time course of increased mRNA expression of IL15R α , IL2R β , and IL2R γ in the hippocampus of the EAE mice

There was a robust increase of IL15R α mRNA in the hippocampus of mice 12 d after EAE induction ($p < 0.005$). The increase on days 7 and 16 was not significant. By day 24, the mRNA for IL15R α had returned to its low baseline (Fig. 4A). In comparison with the increase of IL15R α , the elevation of IL2R β mRNA occurred earlier and lasted longer (day 7 – 16) in the hippocampus, although that in the hypothalamus was also significant on day 12 (Fig. 4B). IL2R γ mRNA showed a significant increase on day 12 and day 16 (Fig. 4C).

IL15 mRNA in the hippocampus showed a significant overall change determined by one-way ANOVA. However, there was no significant increase in any of the EAE groups in comparison with the controls. The elevation of IL15 protein without a significant increase of its mRNA suggests that the increase occurred at a post-transcriptional level.

3.3. Spatial patterns of increased mRNA expression of IL15R α , IL2R β , and IL2R γ at the peak of EAE

Although we focused on the hippocampus in IHC analyses, IL15 and its receptors showed ubiquitous expression in the brain and spinal cord. High basal levels of IL15R α and its co-receptors were also seen in the striatum. At the peak of EAE (day 12 after induction by PLP_{139–152} with RIBI adjuvant), there was an elevation of mRNA for all three receptor subtypes in the striatum and hypothalamus, though other brain regions were not sampled. In the cervical, thoracic, and lumbar spinal cord, the mRNA for IL15R α , IL2R β , and IL2R γ also showed a significant increase on day 12 of EAE (Fig. 5A–C). This suggests a widespread activation of IL15 signaling within the CNS of the EAE mice.

3.3. IL15 treatment reduces the severity of EAE

We also used CFA instead of RIBI adjuvant in the induction of EAE by PLP peptide in the SJL/J mice. The mice challenged with PLP + CFA showed lower EAE scores and delayed occurrence of symptoms, but the weakness lasted longer. These mice were randomly divided into groups receiving IL15 (100 ng/mouse/d ip) and PBS (n = 8 /group). The mice were treated from day 7 to 14, and observed until day 50. The choice of time to initiate treatment was based on the observation of symptom onset in the SJL/J mice treated with PLP + RIBI shown in figure 1. Treatment endpoint was determined by the observation of weakness on day 15 in the mice treated with PLP + CFA. IL15 treatment induced a decrease of EAE score that was evident by day 15 and lasted until the end of the study. The IL15-treated group showed a maximal EAE score of 1.5 in the IL15-treated group, whereas the control group has a maximal score of 2.5 (Fig. 6A). The PBS treated mice showed an initial small increase of symptoms (day 15–18), followed by a brief plateau for 2 days and then a persistent increase of EAE score. The IL15 treated group showed a smaller, rather inconspicuous peak on day 21. This might reflect different stages of immune and inflammatory processes in these mice. It is probably not caused by technical problems such as injury of the sciatic nerve during the inoculation, which would induce an earlier onset of transient weakness. Although the overall effect of IL15 treatment was statistically significant when analyzed by repeated measures ANOVA, only 33 % of the treated mice showed EAE scores higher than 2.0. This is in contrast with an incidence rate of 88 % in the control group. This average reduction of EAE score by 55 % reflects an apparent alleviation of the symptoms. Fisher's exact test for an EAE score of 2 showed that the difference between the groups was significant ($p < 0.05$).

In comparison with the B6 wildtype mice receiving MOG + CFA, the IL15 KO mice showed severer weakness and significantly higher EAE scores, although the onset was as early as day 6 in both groups. The difference in the EAE score between the KO and wildtype mice persisted from day 15 to day 40 ($p < 0.01$). At this time, the KO mice were randomly divided into two groups that received either IL15 (100 ng/mouse/d ip) or PBS for 15 days (day 40–55, 2 wk duration). IL15 treatment tended to reduce the EAE score in the KO mice (Fig. 6B).

4. Discussion

In this study we show that EAE induction resulted in an increase of IL15 production and IL15 receptor expression in the brain, that IL15KO mice have a greater symptomatic deficit, and that IL15 treatment in both early and late stages of EAE progression can modify the disease course and reduce EAE severity. The results indicate that the cerebral IL15 system has immunomodulatory functions in restricting autoimmunity.

The blood concentration of IL15 was significantly higher at the peak of EAE. This is consistent with a previous report that IL15 is increased in serum and cerebrospinal fluid of MS patients (Rentzos *et al.* 2006). Elevated serum IL15 is also seen in inflammatory bowel disease, systemic lupus erythematosus, inflammatory synovitis, psoriasis, diabetes mellitus, bronchial asthma, and autoimmune vasculitis (Budagian *et al.* 2006). This suggests that IL15 is a weak biomarker for autoimmune diseases. In the hippocampus, both IL2R β /IL15R β (Petitto and Huang 2001) and the IL2R γ common chain (Winocur *et al.* 2005) have a high level of expression shown by in-situ hybridization. Consistent with these reports, qPCR showed that the upregulation of IL15 did not reside mainly at the mRNA level. Rather, IL15 protein expression, seen in neurons in the basal state, was increased at the peak of EAE. IL15 immunoreactivity showed a neuronal distribution in the rostral hippocampus. The differential regulation of IL15 mRNA and protein on day 12 indicates that the changes probably occurred at the level of post-transcriptional processing.

Whereas many studies have focused on disruption of the blood-spinal cord barrier in EAE mice with ascending paralysis, the brain shows specific changes in EAE mice that may represent the underlying mechanisms for the associated depressive-like and sickness behaviors. It is well known that neuroinflammation and autoimmune disorders impair memory and decrease feeding behavior, but specific involvement of the hippocampus in the EAE model or human MS has not been fully investigated. Interestingly, IL15 and IL2 play important roles in hippocampal function. IL2 is enriched in hippocampus and facilitates neurogenesis during development (Araujo *et al.* 1989; Hanisch and Quirion 1995; Petitto *et al.* 1998). IL2 KO mice show defective hippocampal structure (Petitto *et al.* 1999; Beck, Jr. *et al.* 2002; Beck, Jr. *et al.* 2005), have increased levels of IL15, IL12, and the chemokines IP-10 and MCP-1, and show greater hippocampal neurogenesis in male mice (Beck, Jr. *et al.* 2005). The IL2 KO also confers reduced susceptibility to EAE (Petitto *et al.* 2000). These reports provide a strong basis to focus on the role of IL15 in the hippocampus of EAE mice.

Besides the increase of IL15R α protein in different neurons in the hippocampus, the three receptor subtypes for IL15 all showed robust upregulation at the mRNA level. IL15R α mRNA was significantly increased only on day 12 (peak of EAE symptom), whereas IL2R β mRNA was increased from day 7 to 16, and IL2R γ mRNA was increased on day 12 and 16. The co-receptors IL2R β and IL2R γ for IL15 are shared by other cytokines in the same family of four-alpha-helix bundle cytokines. Their longer lasting changes may suggest that the cellular responses to IL2 and perhaps other cognate cytokines are also upregulated. The neuroprotective role of IL15R α is supported by the observation that IL15RKO mice have five times more motor neuron death after facial nerve axotomy (Huang *et al.* 2007), and that the serum concentration of IL15 is lower in Alzheimer's disease than in normal or vascular dementia patients (Rentzos *et al.* 2007).

To determine the functional role of IL15 and its receptors in the progression of EAE, we used two additional models of EAE that have a longer duration of symptoms. Whereas SJL/J mice after PLP + RIBI immunization showed monophasic EAE at day 6 – 22 with a higher score (behavioral deficits, Fig. 1), the model of SJL/J mice immunized with PLP + CFA showed a more protracted disease course and somewhat lower EAE score (Fig. 5A). B6 mice, known to be less susceptible to EAE, require induction by MOG + CFA, and showed an even lower score though the onset was earlier (Fig. 5B), making more apparent the striking increase in symptoms associated with the embryonic deletion of IL 15 after induction of EAE. IL15 treatment was effective in ameliorating the EAE symptoms in both SJL/J mice and IL15KO mice of a B6 genetic background. The treatment effect was evident both early in the disease course and after the symptoms were well established. These results strongly support a beneficial role of IL15 in restricting the autoimmune disease in EAE mice.

As a proinflammatory cytokine, IL15 has been postulated to mediate negative disease-promoting effects in MS (Kivisakk *et al.* 1998). The unexpected findings of a beneficial role of IL15 in EAE mice are in sharp contrast with that reported in peripheral autoimmune disease. IL15 blockade is considered a potential target for therapeutic intervention; anti-IL15 therapy has shown efficacy in rheumatoid arthritis and psoriasis. This includes the use of antibodies against IL15 in a phase II clinical trial of rheumatoid arthritis (Waldmann 2004) and suppression of psoriasis in mice by blockade of IL15 (Villadsen *et al.* 2003). The differing findings suggest differing roles or doses of IL15 in CNS and peripheral disorders and might even reflect differential functions of subtypes of immune cells in the disease processes. The CNS increase of IL15 in EAE and MS could represent a compensatory mechanism to ameliorate the disease process. Many substances such as insulin, TRH, and bombesin are known to exert opposite effects in the periphery and CNS (Banks and Kastin 1993).

We have shown that IL15 receptors in cerebral endothelial cells composing the BBB can be upregulated by tumor necrosis factor α (TNF) (Pan *et al.* 2009), and that the lipopolysaccharide model of altered innate immunity is associated with an increase of IL15 permeation across the BBB (Pan *et al.* 2008b). By comparison, EAE is associated with a reduction of IL15 permeation from blood to the CNS (Hsueh *et al.* 2009b). The immunoprotective effect of IL15 might involve multiple targets and cellular mechanisms, including promotion of T cell and natural killer (NK) cell proliferation (Grabstein *et al.* 1994; Carson *et al.* 1994), and CD4⁺ CD25⁺ regulatory T (Treg) cell activation or expansion. Treg has been recognized as a negative regulator of MS pathogenesis (Kohm *et al.* 2002; Viglietta *et al.* 2004). IL15 can stimulate Treg proliferation, reduce their apoptosis, and maintain their suppressive function in vitro (Wang *et al.* 2008; Ben *et al.* 2009). Thus, it is possible that the effect of exogenous IL15 treatment has an indirect effect on CNS disease processes, and that part of the exacerbation of EAE in the IL15KO mice is mediated by peripherally derived immune cytokines and other cytokines.

IL15 now can be added to the short list of protective cytokines in EAE (interferon α , leukemia inhibitory factor, IL10, and IL2). This assumes added importance because IL15 is usually considered a proinflammatory cytokine (Gomez-Nicola *et al.* 2006). Another unique feature is that the IL15 system is ubiquitously present in the CNS, suggestive of an important function in neuropathology. Even in the absence of inflammatory or autoimmune challenges, mice without IL15R α show distinctive changes in metabolism, locomotor control, and thermoregulation (He *et al.* 2009). These mice also show memory and emotional deficits (He *et al.* 2010). The absence of IL15 induces depressive-like behavior in these KO mice, and IL15 treatment reverses it (Wu X *et al.*, manuscript under review). Thus, the upregulation of the IL15 system in EAE might reflect its essential role in maintaining mood stability and reducing the depression commonly seen in MS patients.

In summary, EAE was associated with region- and time-specific upregulation of the IL15 system in mouse brain. Unexpectedly, IL15 treatment by peripheral injection reduced the severity of EAE, and IL15 KO mice showed lower EAE scores. The results indicate an important role of IL15 in restricting the progression of EAE.

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Fig 1. Progression of EAE score after induction with PLP₁₃₉₋₁₅₁. Signs of weakness occurred on day 7. The scores peaked on day 12 and then returned to 0 (asymptomatic) on day 24 (n = 8).

Fig 2.

Dynamic changes of IL15 expression in the EAE mice. (A) Hippocampal IL15 mRNA (left y-axis, squares) did not show a significant change from the control (time 0), though the levels from day 3 mice were significantly lower than those from day 16 and 24. Serum IL15 concentrations (right y-axis, closed circles) showed a significant increase on day 12 (58.51 ± 2.76 pg/ml) in comparison with controls (42.47 ± 2.31 pg/ml). *: $p < 0.05$. (B) IL15 immunoreactivity showed a neuronal pattern in the rostral hippocampus, and was higher in EAE (day 12) than control mice. Scale bar: 100 μ m. (C) Double-labeling IHC showed that IL15 (green) and NeuN (red) were present in the same cells, indicating a neuronal source of IL15. Mol, molecular layer of the dentate gyrus; GrDG, granular layer of the dentate gyrus; PoDG, polymorph layer of the dentate gyrus.

Fig. 3.

Immunofluorescence of IL15R α in the control and EAE mice. (A) On day 12 after EAE induction, there was a greater intensity of IL15R α staining in hippocampal neurons in comparison with the control. Scale bar: 100 μ m. (B) IL15R α and NeuN double-labeling showed neuronal localization of the receptor. Scale bar: 50 μ m. GrDG, granular layer of dentate gyrus; PoDG, polymorph layer dentate gyrus; Py, pyramidal cell layer of the hippocampus.

Fig 4.

EAE induction was associated with a time-dependent increase in the level of mRNA expression of IL15 receptors in hippocampus. (A) IL15R α ; (B) IL2R β ; and (C) IL2R γ . *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Fig. 5.
mRNA of IL15 receptors in striatum, hypothalamus, and cervical, thoracic and lumbar spinal cord on day 12 after EAE induction. (A) IL15R α ; (B) IL2R β ; and (C) IL2R γ . +: $p < 0.06$; ***: $p < 0.001$.

Fig. 6.

IL15 treatment of normal and IL15 KO mice after EAE induction. (A) In the SJL/J mice, IL15 treatment (100 ng/day/mouse) was administrated from day 7 to day 14 after EAE induction. The treated mice showed amelioration of their EAE severity. (B) IL15 KO mice displayed significantly deteriorated EAE symptoms compared with control mice ($p < 0.01$). Moreover, when given continuous IL15 treatment (100 ng/day/mice) from day 40, the IL15 KO mice showed improved EAE symptoms.

Table 1

Taqman primers and probes for real-time PCR

Gene	Forward primer (FP) and reverse primer (RP)	Probes
IL15	FP: ATGTTTCATCAACACGTCCTGACT RP: GCAGCAGGTGGAGGTACCTTAA	6-FAM-CATGCGAGCCTCTCCGTGTTTCT-TAMRA
IL15R α	FP: CCAGGCCATTCTGTGTTG RP: GATGTCAGCATGCTCAATAGATACG	6-FAM-CCGGGCACACGTGTCCACC-TAMRA
IL2R β	FP: CGACTTCCATCCCTTTGACAA RP: TGGGTATCAATGTGCAGAACTTG	6-FAM-CTTCGCCTGGTGGCCCCTCATT-TAMRA
IL2R γ	FP: GAAGCTGGACGGAATAAGTGAA RP: CTCCGAACCCGAAATGTGTAC	6-FAM-GAACCTAGATTCTCCCTGCCTAGTGTGGA-TAMRA
GAPDH	FP: TGTGTCCGTCGTGGATCTGA RP: CCTGCTTCACCACCTTCTTGA	6-FAM-CCGCCTGGAGAAACCTGCCAAGTATG-TAMRA