Published in final edited form as:

Neurosci Lett. 2011 July 25; 499(3): 213–218. doi:10.1016/j.neulet.2011.05.230.

Loss of CNS IL-2 gene expression modifies brain T lymphocyte trafficking: Response of normal versus autoreactive Treg-deficient T cells

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Abstract

Emerging data from our lab and others suggested that dysregulation of the brain's endogenous neuroimmunological milieu may occur with the loss of brain IL-2 gene expression and be involved in initiating processes that lead to CNS autoimmunity. We sought to test our working hypothesis that IL-2 deficiency induces endogenous changes in the CNS that play a key role in eliciting T cell homing into the brain. To accomplish this goal, we used an experimental approach that combined mouse congenic breeding and immune reconstitution. In congenic mice without brain IL-2 (two IL-2 KO alleles) that were reconstituted with a normal wild-type immune system, the loss of brain IL-2 doubled the number of T cells that trafficked into the brain in all regions quantified (hippocampus, septum, and cerebellum) compared to mice with two wild-type brain IL-2 alleles and a wild-type peripheral immune system. Congenic mice with normal brain IL-2 (two wild-type IL-2 alleles) that were immune reconstituted with autoreactive Treg-deficient T cells from IL-2 KO mice developed the expected peripheral autoimmunity (splenomegaly) and had a comparable doubling of T cell trafficking into the hippocampus and septum, whereas they exhibited an additional two-fold proclivity for the cerebellum over the septohippocampal regions. Unlike brain trafficking of wild-type T cells, the increased homing of IL-2 KO T cells to the cerebellum was independent of brain IL-2 gene expression. These findings demonstrate that brain IL-2 deficiency induces endogenous CNS changes that may lead to the development of brain autoimmunity, and that autoreactive Treg-deficient IL-2 KO T cells trafficking to the brain could have a proclivity to induce cerebellar neuropathology.

Keywords

IL-2; neuroimmunology; congenic mice; gene deletion; T cells; autoimmunity; brain

Immune surveillance of the central nervous system (CNS) occurs continuously under normal conditions by small numbers of circulating peripheral T lymphocytes [7, 14]. Increased T

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trafficking into the brain is a key feature of multiple sclerosis, and the animal model of the disease, experimental autoimmune encephalitis (EAE), where autoreactive T cells induce brain pathology [21, 40]. In the immune system, IL-2 is known to be indispensable for maintaining immunological homeostasis (e.g., self-tolerance, T regulatory cell development) and is essential for normal T regulatory cell function which is critical in self-tolerance [20, 35, 38]. IL-2 deficiency in the peripheral immune system of animals produces the spontaneous development of autoimmune disease affecting multiple organ systems characterized by T cell infiltration, and in some cases, autoantibody deposition [15, 18, 32]. IL-2 is also produced by brain cells [12, 19], however, the consequences of IL-2 deficiency in the CNS is less well understood. Emerging data from our lab and others suggests that dysregulation of the brain's endogenous neuroimmunological milieu may occur with the loss of brain IL-2 gene expression and be involved in initiating processes that lead to CNS autoimmunity [3, 6, 16]

Alterations in brain IL-2 and IL-2 receptors has been implicated in several brain diseases including developmental and neurodegenerative processes involving the septohippocampal system (e.g., schizophrenia, Alzheimer's disease) [4, 13, 28, 29, 45, 46], though less attention has been given to IL-2 related autoimmune effects on the brain [16, 25]. Receptors for IL-2 in the brain are enriched in the hippocampus and related limbic regions where brain-derived IL-2 has been ascribed neurotrophic and neuromodulatory effects [1, 23, 24, 27, 33]. Alterations in IL-2 can also modify neuroimmunological homeostasis in the CNS, changes that may be induced by IL-15, IL-2's partner/cognate cytokine which shares the same receptor subunits for signal transduction as IL-2 (IL-2/15 β and γ c) in the brain [5, 17, 23]. IL-15 and IL-15 receptors are upregulated, for example, in murine EAE and can modify disease outcome [11, 22, 42]. Our lab has shown previously that IL-2 deficient mice have increased hippocampal protein concentrations of endogenous IL-15, IP-10 (CXCL10) and MCP-1 (CCL2), potent T cell chemoattractant cytokines and chemokines [5, 17, 31, 41]. Coinciding with increased production of these endogenous brain T cell chemoattractants, we have shown that IL-2 KO mice have increased trafficking of autoimmune T cells into the brain [16]. In typical animal models of CNS autoimmunity like EAE, autoimmune T cells trafficking to CNS must be generated or induced by active immunization to components of myelin, whereas by contrast, brain autoimmunity in the context of IL-2 deficiency results spontaneously. It was unknown whether T cell entry into the CNS in IL-2 deficient mice is due to the propensity of autoreactive T cells to home to the brain, due to endogenous neuroimmunological alterations in the brain (i.e., increased endogenous brain expression of chemoattractant cytokines and chemokines) that leads to increased T cell homing to the brain, or a combination of these factors. Thus, in the present study we sought to test our working hypothesis that IL-2 deficiency induces endogenous changes in the CNS that play a key role eliciting T homing into the brain [16].

To accomplish this goal, we used the combination of congenic mouse breeding and immune reconstitution by adoptive transfer. We bred novel congenic mice of the C57BL/6 background with the RAG-2 gene deleted (RAG^{-/-}), that had either two wild-type IL-2 alleles (C57BL/6RAG2-IL-2^{+/+}, referred to here for clarity as brainIL2WT/RAG2KO) or both IL-2 alleles deleted (C57BL/6RAG2-IL-2^{-/-}, referred to here as brainIL2KO/RAG2KO). At weaning the peripheral immune system of these congenic immunodeficient RAG2-KO mice was reconstituted by adoptive transfer with either normal wild-type splenocytes or splenocytes from an autoimmune IL-2KO mouse. This resulted in 4 groups of mice that were compared to address the questions of interest in this study: 1) brainIL2WT/RAG2KO + IL2WTimmune (normal brain IL-2 gene expression with a wild-type immune system); 2) brainIL2WT/RAG2KO + IL2KOimmune (normal brain IL-2 gene expression with an IL-2 knockout immune system); 3) brainIL2KO/RAG2KO + IL2WTimmune (absence of brain IL-2 gene expression with a wild-type immune system), and; 4)

brainIL2KO/RAG2KO + IL2KOimmune (absence of brain IL-2 gene expression with an IL-2 knockout immune system). We then quantified CD3⁺ T cells in 3 different regions – the septum, the hippocampus, and the cerebellum – as described previously [16]. Spleen weights of the four subject groups were compared, as the substantial increase in T lymphocyte proliferation seen in the spleen of IL-2 knockout mice and other mouse models of autoimmunity results in increased spleen weight – a reliable measure to confirm if adoptive transfer resulted in normal splenic reconstitution (WT immune systems) or lymphoproliferative splenomegaly indicative of T autoimmunity [15, 16, 25].

A breeding strategy and that we have described previously was used to produce congenic severe combined immunodeficient (SCID) mutation mice on C57BL/6 background with either both IL-2 alleles deleted (IL2^{-/-}) or two normal IL-2 alleles (IL2^{+/+}) [26]. Briefly, in the present study C57BL/6-IL-2^{+/-} heterozygous and B6.129S6-RAG2^{-/-} (homozygous for the RAG2 mutation) were crossed, and mice heterozygous for both IL-2 and RAG2 were then crossed again. Mice heterozygous for IL-2 and homozygous for the RAG2 mutation were then used as breeders to generate 1) C57BL/6IL-2^{+/-}RAG2^{-/-} mice and 2) C57BL/6IL-2^{-/-}RAG2^{-/-} knockout mice. Genotyping by PCR was performed as described previously [16]. Mice were weaned, and received adoptive transfer at 21 days of age. One animal (recipient) to one animal (donor) adoptive transfer was performed; a total of 20 × 10⁶ splenocytes obtained from a young adult donor mice mouse (8 weeks of age) were adoptively transferred by intraperitoneal injection in a total volume of 0.2–0.5 ml of 1x PBS. This resulted in the 4 primary subject groups of interest described in the paragraph above: 1) brainIL2WT/RAG2KO + IL2WTimmune; 2) brainIL2WT/RAG2KO + IL2KOimmune; 3) brainIL2KO/RAG2KO + IL2WTimmune, and; 4) brainIL2KO/RAG2KO + IL2KOimmune. In addition, we also used standard 5) wild-type and 6) IL-KO mice as reference controls (littermates derived by breeding mice heterozygous for IL-2 and wild-type for the RAG2, to obtain C57BL/6IL-2^{+/-} RAG2^{+/+} and C57BL/6IL-2^{-/-} RAG2^{+/+} mice). The immunohistochemical identification and quantification of brain T cells was performed as described previously [16]. Assessments were made at a single time-point, 11 weeks of age (8 weeks after immune reconstitution by adoptive transfer), as this was found to represent an age at which the progression of T cells trafficking into the brain in this model is at stable plateau levels [16]. The sex ratio was 58% female/42% males, and all subject groups were balanced for sex and matched for age.

Examining the mean CD3⁺ T cells/mm² across the three regions, ANOVA showed that there was a significant main effect of subject group [F(5,23)=27.16, p < .0001]. Mean brain T cell counts did not differ between wild-type C57BL/6 mice and brainIL2WT/RAG2KO + IL2WTimmune mice, whereas both of these groups had significantly lower T cell counts than all of the other subject groups (Fisher's post-hoc, p < .05). There were no differences between brainIL2WT/RAG2KO + IL2KOimmune mice and brainIL2KO/RAG2KO + IL2WTimmune mice; although the former differed statistically from brainIL2KO/RAG2KO + IL2KOimmune mice (p < .01) the latter did not differ from brainIL2KO/RAG2KO + IL2KOimmune mice. Finally, for the analyses averaging across the three regions, the brainIL2KO/RAG2KO + IL2KOimmune mice did have significantly lower mean T cell levels across the three brain regions than standard (unmanipulated) IL-2 knockout mice (p < .01).

Figure 1 depicts the distribution of T cells for the subject groups by brain region. Figure 2 is a photomicrograph of representative sections from the four adoptive transfer groups in the cerebellum, hippocampus and septum. As can be seen in Figure 1, when comparisons between the subject groups were made by the specific brain regions, the results showed that a different pattern was present in the cerebellum versus the hippocampus and septum. Findings from the septohippocampal regions were similar, and those results are presented

first. In the hippocampus, ANOVA revealed that there was a significant main effect of subject group [$F(5,23)=23.61$, $p < .0001$]. Hippocampal T cell levels did not differ between wild-type C57BL/6 mice and brainIL2WT/RAG2KO + IL2WTimmune mice, whereas both of these groups had significantly lower T cell counts than all of the other subject groups ($p < .05$). There were no differences in hippocampal T cell levels between brainIL2WT/RAG2KO + IL2KOimmune mice and brainIL2KO/RAG2KO + IL2WTimmune mice, and the brainIL2KO/RAG2KO + IL2KOimmune mice were significantly higher than all other subject groups ($p < .01$). In the septum, ANOVA revealed that there was a significant main effect of subject group [$F(5,23)=23.61$, $p < .0001$]. Septal T cell levels did not differ between wild-type C57BL/6 mice and brainIL2WT/RAG2KO + IL2WTimmune mice, whereas both of these groups had significantly lower T cell counts than all of the other subject groups ($p < .05$). There were no differences in septal T cell levels between brainIL2WT/RAG2KO + IL2KOimmune mice and brainIL2KO/RAG2KO + IL2WTimmune mice, and neither group was statistically different from the brainIL2KO/RAG2KO + IL2KOimmune mice (although when the mean of the hippocampal and septum counts were analyzed, T cell counts in this group were significantly higher, $p < .05$). By contrast, the significant main effect of subject group in the cerebellum [$F(5,23)=11.66$, $p < .001$] resulted in a pattern of T cell counts that was notably different than in the other brain regions. As can be seen in Figure 1, cerebellar T cell levels did not differ between wild-type C57BL/6 mice and brainIL2WT/RAG2KO + IL2WTimmune mice. As in the other regions, brainIL2WT/RAG2KO + IL2WTimmune mice had significantly lower T cell counts than brainIL2KO/RAG2KO + IL2WTimmune mice. A striking difference, however, was seen in the cerebellum among the groups of mice that received adoptive transfer with splenocytes from IL-2 KO mice. The brainIL2WT/RAG2KO + IL2KOimmune mice and brainIL2KO/RAG2KO + IL2KOimmune mice had levels of T cells in the cerebellum similar to standard (unmanipulated) IL-2 KO mice; moreover, although these three groups did not differ from each other, they all exhibited significantly greater T cell levels than standard wild-type mice and the two groups that were immune reconstituted with wild-type splenocytes ($p < .05$). Finally, as seen in Figure 3, the 2 groups of congenic mice that were immune reconstituted with T cells from IL-2 KO mice had significantly larger spleens (spleen weights comparable to standard IL-2 KO mice) than the 2 groups immune reconstituted with cells from wild-type mice (who had spleen weights comparable to standard wild-type mice) [$F(5,23)=9.80$, $p < .001$].

These findings confirmed our hypothesis that brain IL-2 deficiency induces endogenous CNS changes that play a key role eliciting T homing into the brain [16]. As can be seen in *panel a* of Figure 1, in congenic animals without brain IL-2 (two IL-2 KO alleles) that were reconstituted with a normal wild-type immune system, the loss of brain IL-2 on average doubled the number of T cells that trafficked into the brain compared to mice with two wild-type brain IL-2 alleles and a wild-type peripheral immune system. This doubling of T cells was observed in all three brain regions tested; the number of T cells/cm² did not differ between the cerebellum, hippocampus and septum. As noted earlier, our lab showed previously that IL-2 KO mice have elevated levels of IL-2's partner (cognate) cytokine, the chemoattractive chemokine IL-15, as well as the chemokines MCP-1 (CCL-2) and IP-10 (CXCL10) in the brain [5]. IL-15 appears to be important for activation and release of brain chemokines by microglia [11], which likely occurs in response to a variety of physiological and pathophysiological states [22, 42]. Both IL-15 and MCP-1 (CCL-2) have been shown to play a role in selective T cell trafficking to injured neuronal cell bodies in the brain in response of facial nerve injury [10, 17], and MCP-1 (CCL-2) and IP-10 (CXCL10) upregulation has been shown to coincide with T cell trafficking into the denervated hippocampus in response to entorhinal dentate axotomy [2].

In contrast to the brain trafficking of normal (wild-type) T cells noted above, adoptively transferred IL-2 KO T cells - cells known to be autoreactive Treg-deficient T cells [20, 36, 38] - coincided with the development of peripheral autoimmunity assessed by splenomegaly (see Figure 3) [15, 25]. As depicted in *panel b* of Figure 1, when IL-2 KO T cells were adoptively transferred to congenic mice with two wild-type brain IL-2 alleles (normal brain IL-2), T cell counts in the septum and hippocampus were comparable to counts found in the mice with two brain IL-2 KO alleles (absence of brain IL-2) reconstituted with a normal wild-type immune system (Figure 1, *panel a*). That is, in mice with wild-type immune reconstitution, loss of CNS IL-2 gene expression changed the neuroimmunological status of the brain which resulted in a comparable effect size (i.e., increased CD3+ T cell trafficking) to that seen when peripheral immune reconstitution with autoreactive IL-2 KO Treg-deficient T cells was performed in mice with normal IL-2 gene expression.

A strikingly different response pattern was seen in the cerebellum compared to the septohippocampal regions. Although congenic mice with normal brain IL-2 that were immune reconstituted with T cells from IL-2 KO mice on averaged doubled the number of T cells that trafficked into septum and hippocampus, and they exhibited an additional two-fold proclivity for the cerebellum over the septohippocampal regions. Moreover, as illustrated in *panel b* of Figure 1, IL-2 KO T cell trafficking into the brain occurred at this higher level whether they were transferred into congenic mice with brain IL-2 (two brain IL-2 wild-type alleles) or without brain IL-2 (two brain IL-2 KO alleles). Neurodevelopmental and autoimmune processes involving the cerebellum have been speculated to be involved in the pathogenesis of cerebellar circuitry in autism spectrum disorder [37, 39]. Marked changes in GAD protein and mRNA levels have been found in cerebellar nuclei in autism spectrum disorder, and could underlie impairments in cerebellar communication to limbic and cortical areas [9, 43, 44]. GAD autoimmunity has been studied extensively in type 1 diabetes [8] as well as in clinical neurological syndromes including stiff-person syndrome and progressive cerebellar ataxia [30], and IL-2 allelic variants have been shown to be associated with disease susceptibility and Treg function in humans and in animal models of diabetes [34].

In summary, these data confirmed our hypothesis that IL-2 deficiency induces endogenous changes in the CNS that play a key role eliciting T cell homing into the brain [16]. A more in depth assessment of which T cell subsets and activation markers (e.g., CD69, CD44, double negative T cells) are involved under the conditions examined above and how they influence brain cytoarchitecture and function, will be important in future studies. Further analyses of the T cell subsets that comprise the lymphoproliferative autoimmune splenomegaly, and their relation to changes in the brain and potential interactions with the BBB [22], will also be informative. Unlike brain trafficking of wild-type T cells, the increased homing of IL-2 KO T cells to the cerebellum was independent of brain IL-2 gene expression. Our data demonstrate that brain IL-2 deficiency induces endogenous CNS changes that may lead to the development of brain autoimmunity, and suggest further that the proclivity of autoreactive Treg-deficient IL-2 KO T cells for the cerebellum could shed light on putative autoimmune disease processes associated with cerebellar pathology in certain neurological and neuropsychiatric disease (e.g., autism). These findings lend further support to emerging literature indicating that dysregulation of the brain's endogenous neuroimmunological milieu may occur with the loss of brain IL-2 gene expression and be involved in initiating processes that lead to CNS autoimmunity [3, 6, 16]. Further research using this approach may help to delineate further the complexity of IL-2 biology in the brain and immune system, as well as provide important new insights into how changes in immunological homeostasis may impact the development of brain autoimmunity.

Acknowledgments

Funding for this study was provided by NIH RO1 NS055018 (JP).

References

1. Araujo DM, Lapchak PA, Chabot JG, Nair NP, Quirion R. Characterization and possible role of growth factor and lymphokine receptors in the regulation of cholinergic function in the mammalian brain. *Prog Clin Biol Res.* 1989; 317:423–436. [PubMed: 2602427]
2. Babcock AA, Kuziel WA, Rivest S, Owens T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J Neurosci.* 2003; 23:7922–7930. [PubMed: 12944523]
3. Beck RD Jr, King MA, Ha GK, Cushman JD, Huang Z, Petitto JM. IL-2 deficiency results in altered septal and hippocampal cytoarchitecture: relation to development and neurotrophins. *J Neuroimmunol.* 2005; 160:146–153. [PubMed: 15710467]
4. Beck RD Jr, King MA, Huang Z, Petitto JM. Alterations in septohippocampal cholinergic neurons resulting from interleukin-2 gene knockout. *Brain Res.* 2002; 955:16–23. [PubMed: 12419517]
5. Beck RD Jr, Wasserfall C, Ha GK, Cushman JD, Huang Z, Atkinson MA, Petitto JM. Changes in hippocampal IL-15, related cytokines, and neurogenesis in IL-2 deficient mice. *Brain Res.* 2005; 1041:223–230. [PubMed: 15829231]
6. Cardona AE, Li M, Liu L, Savarin C, Ransohoff RM. Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation. *J Leukoc Biol.* 2008; 84:587–594. [PubMed: 18467654]
7. Cose S, Brammer C, Khanna KM, Masopust D, Lefrancois L. Evidence that a significant number of naive T cells enter non-lymphoid organs as part of a normal migratory pathway. *Eur J Immunol.* 2006; 36:1423–1433. [PubMed: 16708400]
8. Ellis TM, Atkinson MA. The clinical significance of an autoimmune response against glutamic acid decarboxylase. *Nature medicine.* 1996; 2:148–153.
9. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biological psychiatry.* 2002; 52:805–810. [PubMed: 12372652]
10. Flugel A, Hager G, Horvat A, Spitzer C, Singer GM, Graeber MB, Kreutzberg GW, Schwaiger FW. Neuronal MCP-1 expression in response to remote nerve injury. *J Cereb Blood Flow Metab.* 2001; 21:69–76. [PubMed: 11149670]
11. Gomez-Nicola D, Valle-Argos B, Nieto-Sampedro M. Blockade of IL-15 activity inhibits microglial activation through the NFkappaB, p38, and ERK1/2 pathways, reducing cytokine and chemokine release. *Glia.* 58:264–276. [PubMed: 19610094]
12. Hanisch UK, Quirion R. Interleukin-2 as a neuroregulatory cytokine. *Brain Res Brain Res Rev.* 1995; 21:246–284. [PubMed: 8806016]
13. Hanisch UK, Seto D, Quirion R. Modulation of hippocampal acetylcholine release: a potent central action of interleukin-2. *J Neurosci.* 1993; 13:3368–3374. [PubMed: 8340813]
14. Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. *J Neurosci Res.* 1991; 28:254–260. [PubMed: 2033653]
15. Horak I, Lohler J, Ma A, Smith KA. Interleukin-2 deficient mice: a new model to study autoimmunity and self-tolerance. *Immunol Rev.* 1995; 148:35–44. [PubMed: 8825281]
16. Huang Z, Dauer DJ, Ha GK, Lewis MH, Petitto JM. Interleukin-2 deficiency-induced T cell autoimmunity in the mouse brain. *Neurosci Lett.* 2009; 463:44–48. [PubMed: 19595743]
17. Huang Z, Ha GK, Petitto JM. IL-15 and IL-15R alpha gene deletion: effects on T lymphocyte trafficking and the microglial and neuronal responses to facial nerve axotomy. *Neurosci Lett.* 2007; 417:160–164. [PubMed: 17418948]
18. Kundig TM, Schorle H, Bachmann MF, Hengartner H, Zinkernagel RM, Horak I. Immune responses in interleukin-2-deficient mice. *Science.* 1993; 262:1059–1061. [PubMed: 8235625]

19. Lapchak PA, Araujo DM, Quirion R, Beaudet A. Immunoautoradiographic localization of interleukin 2-like immunoreactivity and interleukin 2 receptors (Tac antigen-like immunoreactivity) in the rat brain. *Neuroscience*. 1991; 44:173–184. [PubMed: 1770995]
20. Malek TR, Yu A, Zhu L, Matsutani T, Adeegbe D, Bayer AL. IL-2 family of cytokines in T regulatory cell development and homeostasis. *J Clin Immunol*. 2008; 28:635–639. [PubMed: 18726679]
21. Martino G, Hartung HP. Immunopathogenesis of multiple sclerosis: the role of T cells. *Curr Opin Neurol*. 1999; 12:309–321. [PubMed: 10499176]
22. Pan W, Yu C, Hsueh H, Khan RS, Kastin AJ. Cerebral microvascular IL-15 is a novel mediator of TNF action. *J Neurochem*. 2009; 111:819–827. [PubMed: 19719822]
23. Petitto JM, Huang Z. Cloning the full-length IL-2/15 receptor-beta cDNA sequence from mouse brain: evidence of enrichment in hippocampal formation neurons. *Regul Pept*. 2001; 98:77–87. [PubMed: 11179782]
24. Petitto JM, Huang Z. Molecular cloning of the coding sequence of an interleukin-2 receptor alpha subunit cDNA in murine brain. *J Neuroimmunol*. 1995; 59:135–141. [PubMed: 7797614]
25. Petitto JM, Huang Z, Lo J, Beck RD, Rinker C, Hartemink DA. Relationship between the development of autoimmunity and sensorimotor gating in MRL-lpr mice with reduced IL-2 production. *Neurosci Lett*. 2002; 328:304–308. [PubMed: 12147331]
26. Petitto JM, Huang Z, Lo J, Streit WJ. IL-2 gene knockout affects T lymphocyte trafficking and the microglial response to regenerating facial motor neurons. *J Neuroimmunol*. 2003; 134:95–103. [PubMed: 12507776]
27. Petitto JM, Huang Z, Raizada MK, Rinker CM, McCarthy DB. Molecular cloning of the cDNA coding sequence of IL-2 receptor-gamma (gamma_c) from human and murine forebrain: expression in the hippocampus in situ and by brain cells in vitro. *Brain Res Mol Brain Res*. 1998; 53:152–162. [PubMed: 9473647]
28. Petitto JM, McNamara RK, Gendreau PL, Huang Z, Jackson AJ. Impaired learning and memory and altered hippocampal neurodevelopment resulting from interleukin-2 gene deletion. *J Neurosci Res*. 1999; 56:441–446. [PubMed: 10340751]
29. Quirion R, Aubert I, Robitaille Y, Gauthier S, Araujo DM, Chabot JG. Neurochemical deficits in pathological brain aging: specificity and possible relevance for treatment strategies. *Clin Neuropharmacol*. 1990; 13(Suppl 3):S73–80. [PubMed: 2093419]
30. Ramirez-Montealegre D, Chattopadhyay S, Curran TM, Wasserfall C, Pritchard L, Schatz D, Petitto J, Hopkins D, She JX, Rothberg PG, Atkinson M, Pearce DA. Autoimmunity to glutamic acid decarboxylase in the neurodegenerative disorder Batten disease. *Neurology*. 2005; 64:743–745. [PubMed: 15728308]
31. Ransohoff RM. Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. *Immunity*. 2009; 31:711–721. [PubMed: 19836265]
32. Schorle H, Holtshke T, Hunig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature*. 1991; 352:621–624. [PubMed: 1830926]
33. Seto D, Kar S, Quirion R. Evidence for direct and indirect mechanisms in the potent modulatory action of interleukin-2 on the release of acetylcholine in rat hippocampal slices. *Br J Pharmacol*. 1997; 120:1151–1157. [PubMed: 9134229]
34. Sgouroudis E, Albanese A, Piccirillo CA. Impact of protective IL-2 allelic variants on CD4⁺ Foxp3⁺ regulatory T cell function in situ and resistance to autoimmune diabetes in NOD mice. *J Immunol*. 2008; 181:6283–6292. [PubMed: 18941219]
35. Sharma R, Fu SM, Ju ST. IL-2: a two-faced master regulator of autoimmunity. *J Autoimmun*. 2009; 36:91–97. [PubMed: 21282039]
36. Sharma R, Ju AC, Kung JT, Fu SM, Ju ST. Rapid and selective expansion of nonclonotypic T cells in regulatory T cell-deficient, foreign antigen-specific TCR-transgenic scurfy mice: antigen-dependent expansion and TCR analysis. *J Immunol*. 2008; 181:6934–6941. [PubMed: 18981113]
37. Shi L, Smith SE, Malkova N, Tse D, Su Y, Patterson PH. Activation of the maternal immune system alters cerebellar development in the offspring. *Brain, behavior, and immunity*. 2009; 23:116–123.

38. Turka LA, Walsh PT. IL-2 signaling and CD4+ CD25+ Foxp3+ regulatory T cells. *Front Biosci.* 2008; 13:1440–1446. [PubMed: 17981641]
39. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of neurology.* 2005; 57:67–81. [PubMed: 15546155]
40. Vogt J, Paul F, Aktas O, Muller-Wielsch K, Dorr J, Dorr S, Bharathi BS, Glumm R, Schmitz C, Steinbusch H, Raine CS, Tsokos M, Nitsch R, Zipp F. Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. *Annals of neurology.* 2009; 66:310–322. [PubMed: 19798635]
41. Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. *J Exp Med.* 1995; 181:1255–1259. [PubMed: 7869044]
42. Wu X, Pan W, He Y, Hsueh H, Kastin AJ. Cerebral interleukin-15 shows upregulation and beneficial effects in experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 223:65–72. [PubMed: 20430449]
43. Yip J, Soghomonian JJ, Blatt GJ. Decreased GAD65 mRNA levels in select subpopulations of neurons in the cerebellar dentate nuclei in autism: an in situ hybridization study. *Autism Res.* 2009; 2:50–59. [PubMed: 19358307]
44. Yip J, Soghomonian JJ, Blatt GJ. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta neuropathologica.* 2007; 113:559–568. [PubMed: 17235515]
45. Zalcman S, Green-Johnson JM, Murray L, Nance DM, Dyck D, Anisman H, Greenberg AH. Cytokine-specific central monoamine alterations induced by interleukin-1, -2 and -6. *Brain Res.* 1994; 643:40–49. [PubMed: 7518332]
46. Zalcman SS. Interleukin-2-induced increases in climbing behavior: inhibition by dopamine D-1 and D-2 receptor antagonists. *Brain Res.* 2002; 944:157–164. [PubMed: 12106675]

Highlights

1. IL-2 deficiency elicits T cell homing into the brain.
2. The loss of brain IL-2 with a normal wild-type immune system doubled the number of T cells that trafficked into the brain in all regions.
3. Normal brain IL-2 with autoreactive Treg- deficient T had a comparable doubling of T cell trafficking into the hippocampus and septum.
4. Unlike brain trafficking of wild-type T cells, the increased homing of IL-2 KO T cells to the cerebellum was independent of brain IL-2 gene expression.
5. Findings demonstrate that brain IL-2 deficiency induces endogenous CNS changes.

Figure 1.

Regional brain T cell counts in immune reconstituted congenic mice with and without brain IL-2 gene expression. The subject groups are 1) IL2WT/RAG2KO+WT (brainIL2WT/RAG2KO + IL2WTimmune); 2) IL2KO/RAG2KO+WT (brainIL2KO/RAG2KO + IL2WTimmune); 3) IL2WT/RAG2KO+KO (brainIL2WT/RAG2KO + IL2KOimmune), and; 4) IL2KO/RAG2KO + KO (brainIL2KO/RAG2KO + IL2KOimmune). Each bar represents mean $\pm$ S.E.M. of CD3⁺ T cells/mm² per region. Mice were immune reconstituted at weaning and brain T cell assessments were made at 11 weeks of age. Fig. 1 represents mean $\pm$ S.E.M. of 5 mice per group except for 4 animals/group for IL2WT/RAG2KO+KO mice.

Fig. 2.

Immunohistochemistry for CD3⁺ T cells in the cerebellum, hippocampus and septum for the subject groups. IL-2WT/RAG2KO+WT[A-C], IL-2KO/RAG2KO+WT[D-F], IL- 2WT/RAG2KO+KO[G-I] and IL-2KO/RAG2KO+KO[J-L]. High power magnification of T cells are shown in the insert of A-C. As viewed in the online version of the manuscript, CD3⁺ T cells were immunostained brown and are indicated by arrows, while neuronal and glial cell bodies were counterstained with cresyl violet and are shown in blue. Scale bar = 20µm.

Figure 3.

Assessment of lymphoproliferative autoimmunity using spleen weight. The subject groups are 1) IL2WT/RAG2KO+WT (brainIL2WT/RAG2KO + IL2WTimmune); 2) IL2KO/RAG2KO+WT (brainIL2KO/RAG2KO + IL2WTimmune); 3) IL2WT/RAG2KO+KO (brainIL2WT/RAG2KO + IL2KOimmune), and; 4) IL2KO/RAG2KO + KO (brainIL2KO/RAG2KO + IL2KOimmune). Each bar represents mean $\pm$ S.E.M of 5 mice per group except for 4 animals/group for IL2WT/RAG2KO+KO mice. Mice were immune reconstituted at weaning and brain T cell assessments were made at 11 weeks of age.