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Loss of cholinergic phenotype in septohippocampal projection neurons: Relation to brain versus peripheral IL-2 deficiency

Danielle M. Meola[a,b], Zhi Huang[a], Michael King[b,c,d], John M. Petitto[a,b,c,*]

[a] Department of Psychiatry, McKnight Brain Institute, University of Florida, Gainesville, FL, USA
[b] Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL, USA
[c] Department of Pharmacology & Therapeutics, McKnight Brain Institute, University of Florida, Gainesville, FL, USA
[d] Veterans Administration Medical Center, Gainesville, FL, USA

HIGHLIGHTS

- Significant loss of choline acetyltransferase (ChAT) positive neurons in medial septum of IL-2KO mice.
- No difference in total cell number between IL-2KO mice and IL-2WT mice in medial septum.
- Decrease in ChAT staining due to loss of cholinergic phenotype in medial septum of IL-2KO mice rather than cell death.
- IL-2KO mice have higher levels of NGF in medial septum than IL-2WT.
- No difference in detectable cytokines and chemokines in medial septum of IL-2KO mice.

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ABSTRACT

In the peripheral immune system, IL-2 is essential for immune homeostasis, normal T regulatory cell function, and self-tolerance. IL-2 knockout (IL-2KO) mice develop spontaneous autoimmunity characterized by increased T cell trafficking to multiple organs. The IL-2 gene is also expressed in the brain, and in vitro studies have shown that IL-2 is a potent modulator of acetylcholine release from septohippocampal neurons and exerts trophic effects on septal neurons in culture. We previously described the apparent loss of cholinergic cell bodies in the medial septum of IL-2KO mice. Here we investigated if loss of brain-derived IL-2, or autoimmunity stemming from loss of peripheral IL-2, is responsible for the alteration in choline acetyltransferase (ChAT) expression in the medial septum of IL-2KO mice. To accomplish this objective, we compared ChAT-positive neurons between wild-type (WT) mice, IL-2KO mice, and congenic mice with a double gene deletion for the IL-2 gene and the recombinase activating gene-2 (RAG-2) which are referred to as IL-2KO/RAG-2KO mice (congenic mice which lack mature T and B cells as well as peripheral and brain-derived IL-2). We found that the loss of ChAT staining did not coincide with an overall loss of cells in the medial septum, suggesting that loss of brain IL-2 results in a change in cholinergic phenotype unrelated to cell death. No differences were noted in the endogenous expression of cytokines and chemokines tested in the medial septum. Evaluation of BDNF and NGF levels between WT and IL-2KO mice in medial septal homogenates revealed that IL-2KO mice have markedly higher levels of NGF in the medial septum compared to WT mice. Our findings suggest that brain-derived IL-2 plays an essential role in the maintainance of septohippocampal projection neurons in vivo.
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Interleukin-2 (IL-2) has been implicated in the pathogenesis of several major neurological and neuropsychiatric disorders including multiple sclerosis, Alzheimer's disease and schizophrenia [10,20]. The indispensable role of IL-2 for normal immune system functioning was discovered when IL-2 knockout (IL-2KO) mice demonstrated that IL-2 deficiency results in increased T cell trafficking and autoimmunity to multiple organ systems [12,17,24], and by research showing that IL-2 is essential for immune homeostasis, normal T regulatory cell function, and self-tolerance [21,27]. IL-2 is also expressed by brain cells. IL-2 receptors are enriched in the septohippocampal system where the cytokine has been shown to have trophic effects on fetal septal and hippocampal neurons, and have potent effects on acetylcholine release from septohippocampal cholinergic neurons [1,11,22,23,26]. In addition to these actions in the immune and central nervous systems, we have found that loss of brain IL-2 gene expression results in dysregulation of the brain's endogenous neuroimmunological milieu (e.g.,

* Corresponding author at: McKnight Brain Institute, 100 S. Newell Drive, Gainesville, FL 32610, USA. Tel.: +1 352 294 4952; fax: +1 352 294 0425.
E-mail address: jpetitto@ufl.edu (J.M. Petitto).

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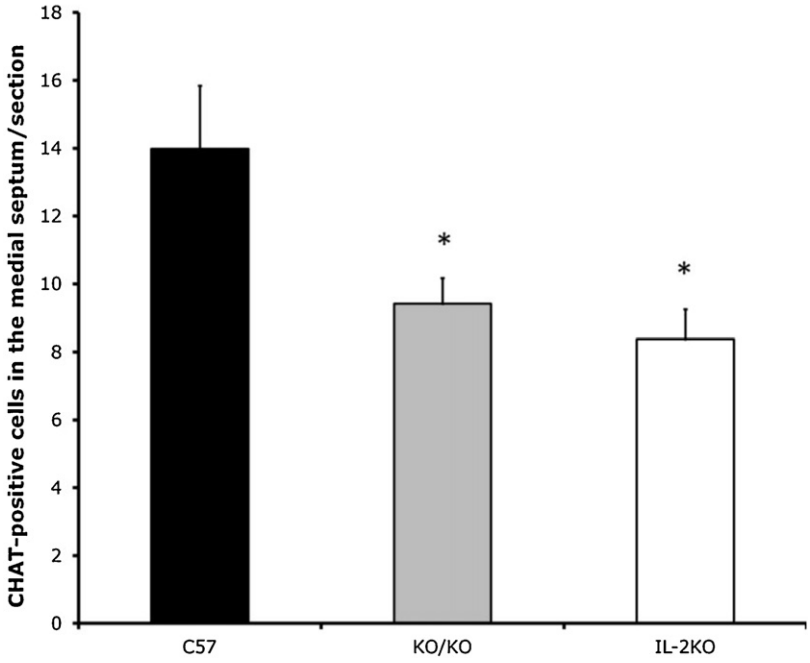

Fig. 1. Quantification of ChAT$^+$ cells/section (20 μm) in the mouse medial septum of subject groups at 8 weeks of age. Bars represent the mean ± S.E.M. for WT (C57), IL-2KO/RAG-2KO (KO/KO), and IL-2KO mice. $n = 5$ mice/group. $^*p < .05$.

alterations in the normal balance of cytokines and chemokines), and that such effects may be involved in initiating processes that lead to central nervous system (CNS) autoimmunity [4,13–15].

We found previously that compared to wild-type (WT) littermates, adult IL-2 deficient mice had a marked reduction of choline acetyltransferase (ChAT) positive medial septum/diagonal band of Broca (MS/vDB) cell bodies [3]. This loss of ChAT-positive neurons was selective for medial septum, as the cholinergic phenotype of WT and IL-2KO mice did not differ in the number of ChAT-positive neurons in the striatum, and GABAergic neurons in the MS/vDB did not differ between WT and IL-2KO mice [2]. Central versus peripheral immunological contributions on brain development and neuropathology are not well understood. Neuroimmunology studies revealed that T lymphocytes can have important effects on CNS neurons, and normal peripheral T cell function has been found to be essential for the preservation of the phenotype of injured motoneurons [7,16,25]. We previously found in IL-2KO mice that there is a marked infiltration of T cells to the brain that mirrors, in relative magnitude, the progression of autoimmunity in the periphery [13]. In the present study, we sought to test the hypothesis that the loss of quantifiable medial septal cholinergic neurons in IL-2KO mice is due to the loss of cholinergic phenotype rather than neuronal cell loss, and that the loss of phenotype is due to loss of brain-derived IL-2 rather than changes in neuroimmune status or T cell infiltration. In experiment 1, we sought to determine if the loss ChAT-positive neurons in the medial septum was due to loss of central (brain-derived) IL-2, peripheral IL-2 (autoimmunity), or a combination of both factors. To accomplish this objective, in experiment 1 we compared ChAT-positive neurons between WT mice, IL-2KO mice and congenic mice with a double gene deletion for the IL-2 and the recombinase activating gene-2 (RAG-2) – referred to as IL-2KO/RAG-2KO. These double knockout IL-2KO/RAG-2KO congenic mice have peripheral immunodeficiency resulting from the absence of mature T and B cells associated with the loss of both RAG-2 gene alleles, and also have both IL-2 gene alleles deleted. In experiment 2, we determined if the loss of the IL-2 gene resulted in changes in the endogenous expression of cytokines and chemokines in the medial septum. In experiment 3, we quantified total neurons in the medial septum to test our working hypothesis that the marked reduction of ChAT-positive neurons in the medial septum of IL-2KO mice is due to the loss of the cholinergic phenotype, rather than neuronal cell loss. Exploring a potential mechanism for downregulation of cholinergic phenotype [18,28,29], in experiment 3 we also quantified BDNF and NGF in the medial septum to assess how levels of these neurotrophic factors correlate with changes in ChAT-positive neurons in the medial septum of IL-2KO mice.

Mice used in these experiments were 8–12 weeks of age, and were matched for age and balanced for sex. IL-2KO mice were bred in our colony using IL-2 heterozygote by IL-2 heterozygote crosses as described previously [13]. IL-2KO/RAG-2KO mice were bred in our colony using recombinase activating gene 2 knockout (RAG-2KO) mice that were originally obtained from Taconic farms. The RAG-2 protein is necessary for the recombination of T cell receptors and immunoglobulins, therefore, RAG-2KO mice fail to develop a mature and functional T and B cells. The breeding of these congenic mice was performed as described previously by our lab, where IL-2 heterozygotic mice where bred with RAG-2KO mice, producing mice with both IL-2 and RAG-2 alleles deleted – referred to here as IL-2KO/RAG-2KO [14]. All mice used in study were on C57BL/6 background. Genotypes of mice were determined by PCR as described previously [14]. Statistical analyses for these studies were performed using analysis of variance (ANOVA), and post hoc comparisons were performed using Fisher's post hoc analysis.

Mice were anesthetized by a 0.5 mg/ml ketamine cocktail in a 3:3:1 ratio (ketamine/xylazine/acepromazine) and were perfused with 4% buffered paraformaldehyde. Brains were dissected, post-fixed for 2 h, and cryoprotected in 30% sucrose overnight. Tissue was snap frozen in isopentane and stored at −80 °C. Coronal sections were cut through the brain and brainstem at a thickness of 40 μm. Sections were collected in 0.1 M phosphate buffered saline (PBS) and immediately used in staining protocol. Tissue sections were incubated in normal goat serum (Vector; 1:30 in PBS) for 1 h at room temperature followed by overnight incubation at 4 °C with the primary antibodies rabbit anti-ChAT (Chemicon; 1:2000 in PBS with 0.3% Triton-X-100 and 1% normal goat serum (NGS)), or rabbit