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Loss of Neuronal Phenotype and Neurodegeneration: Effects of T Lymphocytes and Brain Interleukin-2

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Abstract

Loss of neuronal phenotype and reversal of neuronal atrophy have been demonstrated in different models of central nervous system (CNS) injury. These processes may be generalizable to different types of brain neurons and circuitry. The idea that some injured neurons may lose their phenotype and/or atrophy with the potential to rejuvenate is a remarkable and potentially promising form of neuronal plasticity that is not well understood. In this paper, we present some of our laboratory's basic neuroimmunology research showing that peripheral T cells entering the CNS, and brain-derived interleukin-2 (IL-2), play significant roles in these intriguing processes. Our findings suggest, for example, that T cell immunosenesence could be involved in related processes of brain aging and contribute to neurodegenerative disease. Neuroimmunological approaches may provide new insights into yet undiscovered factors and brain mechanisms that regulate changes in neuronal integrity associated with aging and disease. Such findings could have important implications for discovering more effective strategies for treating patients with neurotrauma and neurodegenerative diseases (e.g., Alzheimer's disease).

Keywords

Cytokines; Neuroimmunology; Interleukin-2; Congenic mice; Knockout mice; Cholinergic; Immunodeficient mice; T cells; Autoimmunity; Neuronal atrophy; Neurodegeneration

Introduction

The idea that damaged neurons may lose their phenotype and/or atrophy rather than die, has intrigued neuroscientists for more than two decades in the field of neurodegeneration research. This remarkable and potentially promising form of neuronal plasticity has been demonstrated in different models of central nervous system (CNS) injury [1-4], and may be generalizable to different types of neurons and neuropathology in the CNS. Understanding the mechanisms by which neurons survive such insults, and how interactions between complex systems (i.e., the nervous and immune systems) promote survival, are essential to devise novel and more effective treatments for human neurodegenerative diseases. In this paper, we present some of our lab's neuroimmunology research that suggests that both peripheral T cells entering the CNS and brain-derived interleukin-2 (IL-2) play significant roles in these intriguing processes by which neurons appear to survive with the potential to rejuvenate their normal phenotype and regain function. The first half of the review discusses the role that normal T cells play in neuronal preservation and recovery in models of

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motoneuron injury, and relate that to how immunosenescence, the gradual deterioration of the immune system due to advanced age, may contribute to loss of function and vulnerability in the aging brain. In the second half, we review our research that has identified important actions of brain-derived IL-2 in the maintenance of septohippocampal cholinergic projection neurons, circuitry involved in cognition that has been found to be altered in some forms of neurodegenerative disease in humans.

T lymphocytes, Neuronal Atrophy And Regeneration T cells and CNS function

The question of whether the immune system is actively involved in the maintenance and protection of the CNS has been explored over the last decade and the term “protective autoimmunity” has emerged. It has been demonstrated that the peripheral immune system contributes significantly to the outcome of neuronal trauma during toxic, ischemic, hemorrhagic, infective, degenerative, metabolic and immune-mediated insults and also assists in the process of repair after injury [5].

The role of T cells in the CNS is complicated as evidenced by conflicting data emerging from different models; however multiple studies have made it clear that T lymphocytes have important effects on neuronal integrity and function in the CNS. Under normal conditions, continuous immune surveillance of the CNS occurs by small numbers of circulating peripheral T lymphocytes [6,7]. During pathogenic states such as multiple sclerosis and infection, it is well established that the presence of T cells in the brain can have detrimental effects, however, in other contexts T cells act in concert with glial cells to promote neuroprotection and survival. Perhaps one of the best examples of such a neuroprotective role of peripheral immunity is in the facial nerve axotomy model where T cells slow the rate of neurodegeneration and neuronal loss [8,9]. Following facial nerve axotomy in mice, T cells cross the blood–brain-barrier (BBB) and home to the neuronal cell bodies in the facial motor nucleus (FMN) [10]. Severe combined immunodeficient (SCID) and recombination activating gene-2 knockout (RAG-2 KO) mice, which both lack functionally mature T and B lymphocytes, exhibit a faster rate of neuronal death than wild-type mice [9,11] which can be prevented by adoptive transfer of wild-type T cells prior to injury [8,9]. Surprisingly, the effects of old age on neuronal outcomes using this model have not been well studied. Several lines of evidence suggest that T cell aging could play a significant role in contributing to impairments of functional recovery in this model.

A connection between immunosenescence and brain aging?

Though innate immunity seems to be better preserved, more severe and often detrimental age-dependent changes occur in the adaptive immune system [12]. As aging is associated with decreased T cell function [13-15], older T cells may be less effective at protecting injured or aging neurons. Immunosenescence that develops in the elderly may similarly be linked to the cognitive behavioral deficits associated with normal aging. The elderly are more vulnerable to various forms of systemic infection that lead to emotional and cognitive complications as well as life threatening delirium. The animal model of LPS-induced sickness behavior has been widely studied to advance this field. In animals, LPS administration elicits an exaggerated neuroinflammatory response characterized by increased activation of microglia and elevated expression of proinflammatory cytokines that is accompanied sickness/ depressive-like behaviors as well as cognitive changes in aged mice [16,17]. As mice age, increased baseline levels of T cells are found in the CNS of normal mice [18,19], and aging mice exhibit higher expression of certain immune response genes following immune challenge with LPS [20]. Surprisingly, the role of T cells in this

neurobehavioral/ neuroimmunological model of LPS sickness related behavior is largely unknown.

Injured neurons under the radar: T cells and neuronal atrophy

Increasing evidence suggests that in some forms of neuronal injury, neurons may not actually die following nerve injury but reside in an atrophic state, characterized by extreme cell shrinkage and a decreased ability to take up stains such as Nissl stain [21]. McPhail et al. showed that a large population (i.e., up to 40%) of mouse facial motoneurons undergo a protracted period of degeneration or atrophy following peripheral resection of the facial nerve. Re-injuring the facial nerve stimulated a reversal in the atrophic status of the injured neurons, causing an increase in both their size and number. Those atrophied motoneurons appear to be gone and are thus not accounted for using conventional neuronal counting methods to assess survival. The atrophied motoneurons in the injured FMN that reside undetected, surprisingly regain their normal phenotype – they increase to normal size and uptake Nissl stain when that same injured facial nerve is stimulated by re-injury [21]. Moreover, adult facial motoneurons that atrophy following axotomy in mice have been rescued by GDNF delivered by a lentiviral based vector [22].

We examined the role of T cells in mediating the reversal of neuronal atrophy using the facial nerve re-injury model [21,23]. We used the nerve resection re-injury paradigm to test the hypothesis that the re-injury induced regeneration seen in wild-type (WT) mice would be impaired in RAG2-KO mice [21,23]. Briefly, WT and RAG2-KO mice were subdivided into two treatment groups, referred to here as “chronic resection+sham” and “chronic resection +re-injury.” In both treatment groups, the main branch of the right facial nerve was exposed and resected, where a portion of the nerve was removed to prevent nerve reconnection. Ten weeks after the initial injury, the right facial nerve was re-exposed in the “chronic resection +re-injury” group and the re-injury was performed by removing the neuroma that had formed at the proximal nerve stump. In the “chronic resection+sham” group the nerve was exposed but the neuroma was left intact. We later compared neuronal survival and average neuronal cell size in the groups at day 14 following the second surgery (mice were euthanized and brains were assessed at week 12). Motoneurons throughout the rostro-caudal extent of the FMN were quantified and neuronal sizes were measured. There was a significant increase in motoneuron survival from 38.0 ± 2.7% in chronically resected WT mice that received sham re-injury compared to 49.1 ± 3.6% survival in those that received nerve re-injury. By contrast, the level of motoneuron survival did not differ between chronically resected RAG2-KO mice that received re-injury compared to RAG2-KO mice that received sham re-injury. Average neuronal cell size was significantly increased, from 74.6 ± 3.4% in chronically resected WT mice that received sham re-injury, to 101.8 ± 8.7% in those that received re-injury. By contrast, average cell size did not differ between chronically resected RAG2-KO mice that received re-injury versus sham re-injury. Moreover, comparing neurons by cell size revealed a noticeable shift from smaller to larger neuronal cell sizes following re-injury in WT mice, where the size distribution of neurons following re-injury returned to the normal distribution seen on the uninjured side; by contrast, this shift in cell size following nerve re-injury was not apparent in the RAG2-KO mice. These data provide clear evidence that in WT mice with a normal immune system, resection results in atrophy of injured facial motoneuron that is reversed by re-injury. By contrast, immunodeficient RAG2-KO mice do not show this re-injury induced regeneration response across the neuronal size distribution [24].

The hypothesis that we are currently testing in our lab is that T cells prevent neuronal death by enabling axotomized motoneurons to enter a long-term atrophic state where they are viable and can be stimulated (i.e., by re-injury) to regenerate the size and phenotype. It is

likely that T cells trafficking to injured motoneurons work in concert with glial cells to provide trophic support to those neurons. It should be noted here that B cells are not found in the FMN following axotomy, and do not appear to influence neuronal recovery like T cells do following the adoptive transfer of splenocytes to reconstitute the immune system of immunodeficient mice such as RAG-2KO mice [8,24].

Interleukin-2 plays a Role in the Maintenance of Septohippocampal Cholinergic Neurons

One of the earliest and most widely studied phenomena showing that neurons may lose their phenotype and atrophy rather than die, came from studies investigating fimbria-fornix axotomy of septohippocampal cholinergic neurons, where it was found that these alterations could be reversed with nerve growth factor (NGF) [1,2]. One major focus of research from our lab has been on IL-2's actions on septohippocampal system neurobiology and behavior using knockout animals for the cytokine and its receptor(s) to disentangle the complex actions of peripheral and central IL-2 on the brain. Some of the pathophysiology and neuropathology observed in this model is reminiscent of abnormalities seen in human neurodegeneration, and to our knowledge, some of our most recent data described below is among the first evidence in a non-injury model (e.g., axotomy) showing that loss of cholinergic projection neurons in the medial septum can be associated with IL-2 deficiency in the brain.

IL-2, the septohippocampal system, and cognition

Among the earliest evidence of IL-2's ability to act in the brain came from cancer patients. In the peripheral immune system IL-2 is responsible for the activation of immune cells and exogenously administered IL-2 can be used therapeutically to combat the disease. Treatment with IL-2 can induce cognitive dysfunction and other untoward neuropsychiatric side effects at doses significantly above what would be considered physiological [25,26]. Though many subsequent studies have documented the diverse effects of IL-2 in the brain, of particular relevance to cognition and neurodegeneration are the observations from studies investigating the effects of IL-2 in the septohippocampal system. Depending on the methodology and conditions, there is data showing that neurons and glial cells can produce IL-2 [27]. In culture, IL-2 provides trophic support to neurons from the hippocampus and medial septum, enhances neurite branching [28-30], is one of the most potent modulators of acetylcholine (ACh) release from rat hippocampal slices[31,32], and can also increase the activity of its precursor enzyme, choline acetyltransferase (ChAT) [33]. Exogenously applied IL-2 has also been shown to modify long-term potentiation by interaction with NMDA receptors in the hippocampus [34]. Preclinical studies in animals have substantiated the in vitro effects of IL-2 on septohippocampal circuitry where IL-2 alters memory processing via interactions with septohippocampal cholinergic nerve terminals in the hippocampus, and alters cognitive performance in rodents [34-36]. It appears that the predominant effects of IL-2 in the brain occur in the hippocampal formation where receptors for this cytokine are enriched [37,38]. It is also noteworthy that in post-mortem hippocampi of Alzheimer's disease patients IL-2 levels were found to be elevated compared to controls [39].

Virtually all of these studies have used the strategy of administering exogenous IL-2. Thus, one of the goals of our research has been to study IL-2 knockout (IL-2KO) mice to better understand the role of endogenous IL-2 on brain function. We have found that IL-2KO mice have altered learning and memory performance, sensorimotor gating, fewer infrapyramidal granule cells, and reductions in hippocampal infrapyramidal mossy fiber length [40,41]. Infrapyramidal mossy fiber length correlates positively with spatial learning ability in rodents, thus alterations in these regions may be functional significant [42].

We had previously found that choline acetyltransferase (ChAT)-positive neurons in the medial septum/vertical diagonal band of Broca (MS/vDB) of IL-2KO and IL-2WT littermates on the C57BL/6 background differed as a function of age [40,43]. At 8-12 weeks of age IL-2KO mice show considerable evidence of peripheral autoimmunity (e.g., marked splenomegaly), whereas 3-week-old IL-2KO mice did not yet develop autoimmunity. We postulated that the selective loss of septal cholinergic neurons in IL-2KO mice (no differences were found in ChAT-positive neurons of the striatum or in GABAergic neurons of the MS/vDB) was due to autoimmune mediated neurodegeneration that occurs postnatally between weaning and early adulthood (medial septal development is essentially complete by embryonic day 17)[44]. Thus, we quantified $CD3^+$ T cells in the septum, hippocampus, and cerebellum of IL-2KO and IL-2 WT mice at ages ranging from 2-14 weeks. Although brain T lymphocyte levels in IL-2KO mice positively correlated with the degree of peripheral autoimmunity, contrary to our earlier hypothesis, they were not selective for the septum and we did not detect $CD19^+$ B lymphocytes, IgG-positive lymphocytes or IgG deposition indicative of autoantibodies in the brains of IL-2 KO mice [45].

Deficiency of brain-derived IL-2: loss of cholinergic neuronal phenotype in the medial septum

It is well established that IL-2 is essential for immune homeostasis, normal T regulatory cell function, and self-tolerance. Loss of IL-2 in IL-2KO mice leads to the development of spontaneous autoimmunity characterized by increased T cell trafficking to multiple organs including the brain [45]. Our research and others suggested that dysregulation of the brain's endogenous neuroimmunological milieu may occur with the loss of brain IL-2 gene expression and may be involved in initiating processes that lead to CNS autoimmunity [40,45,46]. We found that IL-2 deficiency induces endogenous changes in the CNS that play a key role in eliciting T cell homing into the brain [47]. We used an experimental approach that combined mouse congenic breeding and immune reconstitution to test this hypothesis. In congenic mice without brain IL-2 (two IL-2KO alleles) that were reconstituted with a normal WT immune system, the loss of brain IL-2 doubled the number of T cells that trafficked into the septum and hippocampus compared to mice with two WT brain IL-2 alleles and a WT peripheral immune system. We found that congenic mice with normal brain IL-2 (two WT IL-2 alleles) that were immune reconstituted with autoreactive Treg-deficient T cells from IL-2KO mice developed the expected peripheral autoimmunity (splenomegaly) and had a comparable doubling of T cell trafficking into the septum and hippocampus. We also found that T cells from IL-2KO mice had an additional two-fold proclivity for the cerebellum over the septohippocampal regions, and that the increased homing of IL-2KO T cells to the cerebellum was independent of brain IL-2 gene expression. The results of this study showed that brain IL-2 deficiency induces endogenous changes in the brain that may contribute to the development of brain autoimmunity and that autoreactive T Reg-deficient IL-2KO T cells trafficking to the brain could have a proclivity to induce cerebellar neuropathology (e.g., cerebellar pathology in immune-related processes implicated in autism).

Given our previous finding of the apparent loss of approximately one-fourth of cholinergic cell bodies in the medial septum of IL-2KO mice [43], we recently completed a study that was designed to determine if loss of brain-derived IL-2, or autoimmunity stemming from loss of peripheral IL-2, was responsible for the alteration in ChAT expression in the medial septum of IL-2KO mice. To accomplish this objective, we compared ChAT-positive neurons between IL-2WT mice, IL-2KO, and congenic IL-2KO/RAG-2KO mice that lack both peripheral and brain-derived IL-2 (described above). We found that the IL-2KO and congenic IL-2KO/RAG-2KO mice had significantly lower numbers of ChAT-positive neurons than IL-2WT mice (approximately a 30% reduction, levels that coincided with our

previous work;[43]). Quantification of neurons labeled immunohistocytochemically with the pan-neuronal marker, neuronal class beta-III tubulin, demonstrated that the loss of ChAT staining did not coincide with an overall loss of cells in the medial septum, indicating that loss of brain IL-2 unrelated to autoimmunity results in a change in cholinergic phenotype unrelated to cell death. Surprisingly, we found no differences in the endogenous expression of cytokines and chemokines tested in the medial septum. Evaluation of BDNF and NGF levels between IL-2WT 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 IL-2WT mice. Together, the results of this series of experiments demonstrated that brain-derived IL-2 plays an essential role in the maintenance of septohippocampal projection neurons *in vivo*.

Brain-derived IL-2: discovery of inputs supporting medial septum circuitry

To evaluate the endogenous expression of IL-2 in the brain, we are currently using B6.Cg-Tg (Il2-EGFP) 17Evr (IL2-GFP) transgenic mice that reliably express green fluorescent protein (GFP) in immune cells known to produce IL-2 [48]. In light of the aforementioned pathology in the medial septum of IL-2KO mice, it was not surprising to find expression of the reporter in multiple limbic regions including those with direct projections to the septohippocampal system (lateral septum, medial septum, horizontal limb of the diagonal band of Broca, olfactory bulb, cingulate, and subiculum). The lateral septum, where robust expression of GFP was detected, projects mainly to the medial septum and hippocampus [49]. We also detected conservative expression of GFP from a subset of cells in the subiculum, the main output structure of the hippocampus that projects back to the septal nuclei [50,51]. This pattern of expression, in agreement with our earlier work, suggests IL-2 provides modulatory input to the septohippocampal system.

As demonstrated in IL-2KO mice, disruption of IL-2 signaling has significant effects on cholinergic phenotype in the medial septum. Nerve growth factor (NGF) has been well characterized and identified as the neurotrophic factor responsible for maintaining cholinergic phenotype in the septohippocampal system. For example, axotomized septohippocampal cholinergic neurons can be rescued by multiple strategies that deliver NGF to the injured system [2,52,53]. As noted above, we recently measured the relative expression of neurotrophic factors BDNF and NGF in the medial septum, and found that only NGF was elevated in the medial septum. Levels were found to be comparable to those we had detected previously in the hippocampus, the region from which NGF is transported to support cholinergic cell bodies in the septum [41]. Overall, we found there to be a substantial dysregulation of BDNF in the hippocampus and NGF in both regions [40]. Interestingly, one of the earliest observations of IL-2's effects on the septohippocampal system has been its trophic effects on fetal septal neurons in culture [29,30]. Considering IL-2's actions in vitro, and the dysregulation of neurotrophic factors we detected in IL-2KO mice, it seems plausible that IL-2 and NGF may work on converging pathways to maintain cholinergic phenotype in the medial septum.

In addition to the hippocampus, other areas positive for the reporter such as the olfactory bulb and cingulate also receive cholinergic input from the medial septum. It would be interesting to expand on our previous work with IL-2KO mice to include these projection fields to further evaluate the effects of loss of IL-2 on the cholinergic system.

Conclusions

These data and others illustrate the potential role of neuroimmunological processes in the brain that could advance our understanding of the pathophysiological processes involved in

human neurodegenerative disease. Our research showing that T cells may play a vital role in motoneuron viability and recovery post-injury, and the important actions of brain-derived IL-2 in the maintenance of septohippocampal cholingeric neuronal phenotype illustrate just two of many possible ways that the nervous and immune systems converge and how understanding these complex relationships explored in neuroimmunology could advance research relevant to clinical neurodegenerative diseases.

New clinical strategies are continually being sought to impact the progression of neurodegenerative diseases, intervene in brain injury and trauma. The research described in this review paper provides a plausible direction to devise potential new strategies to save or rejuvenate brain neurons that may have atrophied and/or lost their phenotype and function. It is possible, that the neurodevelopmental alterations associated with IL-2 dysregulation such as the progressive loss of the cholinergic phenotype of neurons that we have found [54], could conceivably contribute to a constellation of neurobiological changes that underlie the mild cognitive impairment which precede the overt onset of Alzheimer's disease. Interestingly, the imbalance that we see in the hippocampus of IL-2KO mice between BDNF and NGF levels (decreased BDNF and increased NGF concentrations) is also found in the post-mortem hippocampus of Alzheimer's disease brains [54].

Given the protective actions of T cells at facial motoneuron cell bodies following axotomy, it is conceivable that strategies using antigen-specific or otherwise augmented T cells could be used to home to injured cell bodies in a non-invasive and effective manner. It is known, for example, that following rubospinal axotomy, BDNF acting at the neuronal cell bodies, can induce reversal of atrophy up to one year later [3], but fails to elicit a response at the spinal injury site weeks after injury [55]. As the protective actions of T cells occur at facial motoneuron cell bodies following facial nerve axotomy, it is conceivable that strategies using antigen-specific or otherwise augmented T cells could be used to target neuropathology occurring in the CNS. Moreover, diminished T cell function associated with immunosenescence and normal aging could be involved in related processes of brain aging and repair. Manipulating the immune system of mice, such as we have with the RAG-2KO and IL-2KO strains, could address these and other important questions in the future. Neuroimmunological approaches could provide new insights into yet undiscovered factors that regulate neuronal integrity and loss of phenotype, as well as atrophy-induction and atrophy-reversal. Such findings could have important implications for one day discovering more effective strategies for treating patients with neurotrauma and vascular related brain damage as well as neurodegenerative diseases (e.g., Alzheimer's disease).

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