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The effects of IL2R γ knockout on depression and contextual memory

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Abstract

Interleukin (IL)-2R γ shows robust upregulation in neuroinflammatory states associated with clinical depression. We tested the hypothesis that mice lacking IL2R γ would have decreased depressive-like behavior. Contrary to this expectation, these knockout mice showed increased immobility in both the Porsolt forced swimming and Nomura water wheel tests. By comparison, the auditory fear conditioning test showed increased retention of contextual freezing. Thus, intact IL2R γ combats depressive-like behavior.

Keywords

Interleukin-2; Interleukin-15; IL2R γ ; Depression; Fear conditioning; Hippocampus; Cytokines; Receptor knockout

The interleukin (IL)-2 family of cytokines consists of at least six members that all share the common gamma chain (γ_c). IL15 binds to this shared gamma chain (IL2R γ) and another co-receptor IL2R β [26]. By comparing IL2 ligand KO and IL2R β receptor KO mice, Petitto and colleagues showed that the absence of IL2R β leads to a different spectrum of behavioral deficits than occurs in the IL2 KO mice. Specifically, IL2 KO mice show impaired spatial learning and memory in the Morris water maze but no change in the open field and elevated plus maze tests for anxiety or auditory startle responses [22]. The IL2R β KO mice show reduced fearfulness in the elevated plus-maze test without changes in locomotor activity. They also have deficits in prepulse inhibition of the acoustic startle reflex with significant reduction of reactivity [21]. The differential effects of IL2, IL2R β , and IL15R α KO on neurobehavior suggest redundancy of the cytokine signaling system.

IL2R γ is unique among the receptor heterocomplex in that its gene is located on the X-chromosome in mammals [6]. Defects in the γ_c chain cause X-linked severe combined immunodeficiency in humans [25]. The immunological phenotype of the IL2R γ KO mice is better characterized than the neurobehavior. These mice have low B cell number, absent NK cells, and decreased T cell proliferation [3,16]. Despite severe immunodeficiency, IL2R γ KO mice are fertile although the females show irregularity of the estrous cycle [15]. Thus,

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the changes in the IL2R γ KO mice probably result from altered neuroimmune interactions that affect CNS functions.

The importance of studying the functions of IL2R γ in the brain is emphasized by the observation that neuroinflammation induces a robust upregulation of IL2R γ as well as its co-receptors in the brain and blood-brain barrier. This is seen after challenge with the proinflammatory cytokine tumor necrosis factor α (TNF) or lipopolysaccharide [19,20], and in mouse models of experimental autoimmune encephalomyelitis [9,28]. Neuroinflammation and CNS autoimmunity are often associated with sickness behavior and depression [5], and IL2 administration is associated with depression in patients [4]. Accordingly, we tested the general hypothesis that IL2R γ deletion affects behavior and the specific hypothesis that this knockout results in less depressive-like behavior than controls. The results could have direct implications in health and disease.

Animals

All studies were conducted following a protocol approved by the Institutional Animal Care and Use Committee. B6.129S4-IL2rg^{tm1Wjl}/J (IL2R γ KO) male and female mice and their littermate controls (Jackson Laboratories, Bar Harbor, ME) were housed in the animal care facility for at least 2 weeks before the study. In IL2R γ KO mice, a neomycin resistance cassette replaces part of exon 3 and all of exons 4–8 of the gene, resulting in the loss of most of the extracellular domain and all of the transmembrane and cytoplasmic domains of the protein [3]. The mice were subjected to behavioral tests when 2.5 – 3.5 month old. Individual behavioral tests were spaced at intervals of one week or longer. Both male and female were studied because IL2R γ is located on X-chromosome in mice [6] and its deletion may differentially affect mouse behavior.

Forced swimming test

The forced swimming test was conducted according to the method described by Porsolt et al. [24]. To assess immobility, the mice were placed individually in a glass cylinder (height 40 cm, diameter 18 cm) containing 20 cm of water at $25 \pm 1^\circ\text{C}$. The immobile time of the mice was recorded for 5 min.

Nomura water wheel test

The water wheel test is a modified Porsolt forced swim test more suitable for mice [11,17] with minor modifications. The apparatus consists of a plexiglass water tank (19×10×13.5 cm) with a water wheel in its center. The water wheel is made of a plexiglass shaft (diameter 3 cm, length 10 cm) with 8 paddles (width 0.5 cm). The tank was filled with water ($25 \pm 1^\circ\text{C}$) to a height of 9 cm, with the paddle just resting on the surface. After the mouse was placed in the water tank, the turns of the water wheel were counted for 5 min. The last 2 min were used to determine immobility, as described by the established test criteria.

Fear conditioning test

The fear conditioning test was conducted in an automated video fear conditioning system (Med-Associates, St. Albans, VT) as previously described [8]. The test duration was 10 min, divided into twenty blocks of 30 sec performed on three consecutive days. On day 1, the mice were placed in the chambers to acclimate for 5 min, followed by stimulation of 5 pairings of a 30 sec tone (85 db, 4 KHz) that co-terminated with a shock (0.5 mA, 1 sec). On day 2, the mice were returned to the same chambers where no stimuli were applied. The activity level (percent freezing) measured in 30 sec intervals reflects contextual fear. On day 3, the mice returned to the chambers that had been modified with different floor, roof, and

an acetic acid odor to produce a novel environment. Five min later, a continuous tone (85 db, 4 KHz) was applied for 5 min, and the percent of freezing was recorded.

Statistical analyses were conducted with GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The effects of gender and strain on depressive-like behavior were determined by two-way analysis of variance (ANOVA), followed by Bonferroni post-hoc tests. Repeated-measures ANOVA was performed to assess strain effects in the fear conditioning test. The overall effect of treatment (KO vs wildtype) in the fear conditioning test was assessed by Student's t-test. All graphs show mean $\pm$ standard error of the mean.

Reduced performance in Porsolt forced swimming test (Fig.1A)

Both male and female KO and control mice were subjected to the Porsolt forced swimming test ($n = 7$ each for wildtype males and females, and $n = 8$ each for IL2R γ KO males and females). There were significant effects of both strain and gender ($p < 0.001$) on the immobility time, and there was no interaction between the two variables. The KO mice spent more time floating on the surface of the water. The male KO mice were more immobile than the female KO mice ($p < 0.001$), though there was no gender difference in the wildtype controls.

Reduced performance in Nomura water wheel test (Fig.1B)

Since the performance of a mouse on the Porsolt forced swimming test may be affected by body composition (adiposity and body weight) and propensity to avoid water, a modified swimming test was conducted by use of the Nomura water wheel. There were significant effects of both strain and gender on the time paddling the wheel, a measure inversely correlated with immobility. The KO mice showed reduced effort in paddling ($p < 0.001$). Female KO mice did worse than the male KO mice ($p < 0.01$). There was no interaction between the strain and gender. In the first 3 min pretest, there were no differences between KO and wildtype mice, indicating that the later immobility was not explained by a locomotor deficit in the KO mice.

Improvement in contextual memory during fear conditioning test (Fig.2)

The memory to tone-paired foot shock was tested 24 h after the initial stimulation. The male KO mice showed increased freezing as analyzed by repeated measures ANOVA ($p < 0.05$, Fig.2A). The female KO mice showed a trend ($p = 0.08$) toward an increase. In contrast to this unexpected enhancement in response to context-shock conditioned stimulus, there was no significant difference between the KO and wildtype mice on auditory conditioned stimulus-shock association tested 48 h after the initial test in either male (Fig.2B) or female mice.

In two different tests of immobility reflecting depressive-like behavior, the IL2R γ KO mice were more immobile than the wildtype controls. Contrary to the expectation from upregulation of IL2R γ in neuroinflammatory states associated with depression and from increased depressive symptoms in patients receiving IL2 [4], this indicates a depressive-like phenotype in these KO mice. Although a gender difference might be expected because of the X-chromosome location of the IL2R γ gene [6], the results do not seem to support an X-linked basis for the depressive behavior.

Depression is typically associated with deterioration of memory. However, we unexpectedly found an increase in contextual fear conditioning in the male KO mice. This is opposite to the findings in IL15R α KO mice which have decreased contextual memory and spatial memory [8], although they also show depressive-like behavior. The distinctive effect of

IL2R γ KO on behavior, therefore, is unlikely to be mediated by IL15 but rather affected by other cytokines in this family.

Contextual learning is hippocampus and amygdala dependent [13], and the dorsal hippocampus is particularly important for forming contextual associations [7,12,14]. Cued conditioning involves many of the same brain regions as contextual fear conditioning but not the hippocampus [23]. The preservation of tone fear performance (unconditioned response on day 3 of the fear conditioning test) indicates that amygdaloid function remains grossly intact in the KO mice. Therefore, we speculate that the hippocampus is mainly involved in the altered behavior in these mice. Consistent with this, male IL2 KO mice have increased neurogenesis in both the infrapyramidal and suprapyramidal limbs of the granule cell layer of the dentate gyrus of the hippocampus, whereas female mice do not show this difference [2]. Male mice may also have more granule cells in the dentate gyrus than female mice [27]. The dentate gyrus is one of the few regions in the adult brain where neurogenesis occurs. The improvement of the male IL2R γ KO mice in contextual fear conditioning may be explained by increased neurogenesis in the adult hippocampus, similar to that seen in male IL2 KO mice.

Synaptic connectivity may be impaired as a result of IL2 or IL2R γ deletion. IL2 KO mice have reduced mossy fiber length in the infrapyramidal region of the hippocampus and altered cytoarchitecture in both hippocampus and septum [1]. It is possible that similar changes underlie the depressive-like behavior in the IL2R γ KO mice. Preliminary data also show that IL2R γ KO mice have decreased 5-HT_{1A} immunoreactivity in the retrosplenial gyrus and hippocampus (Wu X et al., unpublished observations). As a dysfunctional serotonergic system plays a crucial role in major depressive disorders [10], and 5-HT_{1A} signaling affects other neurotransmitters such as the release of glutamate and acetylcholine [18], the mechanism mediating the neurobehavioral deficits in the IL2R γ KO mice may involve defective serotonin transmission in the hippocampus but is probably multifactorial.

In summary, contrary to expectations, IL2R γ KO mice showed more depressive-like behavior than controls in both genders, and male mice showed improved contextual fear memory. The results indicate an important role of cerebral IL2R γ in behavior.

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Fig.1A

Fig.1B

Fig 1. IL2R γ KO mice exhibited depressive-like behavior in the (A) Porsolt forced swimming and (B) Nomura water wheel tests (n = 7 for control groups and n = 8 for IL2R γ KO groups). *: p < 0.05; **: p < 0.01; ***: p < 0.001.

Fig.2A

Fig.2B

Fig.2. Fear conditioning test of male IL2R γ KO (n = 8) and control (n = 7) mice. (A) In the contextual test, IL2R γ KO male mice showed a significantly increased percent freezing time. *: p < 0.05. (B) In the tone test, no significant difference was found between male IL2R γ KO and control mice.