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Behavioral evaluation of transgenic mice with CNS expression of IFN- α by elevated plus-maze and Porsolt swim test

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Abstract

Chronic IFN- α treatment as an antiviral or anti-cancer therapy can lead to severe psychiatric complications, including depression and anxiety in patients. In many animal models of IFN- α -induced behavioral dysfunction, the opposite results have frequently been reported. In an attempt to overcome the limitation of pharmacological studies, IFN- α -transgenic mice with CNS-targeted expression of the IFN- α transgene were used to study depression- and anxiety-like behaviors by Porsolt swim and elevated plus-maze assays, respectively. Interestingly, chronic stimulation of IFN- α signaling in mouse brains did not cause depression or anxiety as measured by these tests in comparison with wild-type littermates. This observation suggests that factors other than IFN- α may be necessary for the development of psychiatric complications following IFN- α therapy in patients.

Keywords

Interferon- α ; transgenic mice; brain; behavior; elevated plus-maze; Porsolt swim test

Interferon-alpha (IFN- α) is an innate immune mediator with pleiotropic biological activities [5]. It is used in treatments for chronic hepatitis C viral infection and a number of malignancies, including hairy cell leukemia, chronic myelogenous leukemia (CML) and malignant melanoma [10]. In addition to its therapeutic effects, clinical investigations have reported that chronic treatment with IFN- α can have severe neuropsychiatric consequences, mainly depression and anxiety, in up to 45% of patients [31]. This finding indicates a possible role of this antiviral cytokine in behavioral regulation as well as in the pathogenesis of relevant idiopathic psychiatric disorders [29]. Animals, especially rodents, have been used in the search to determine the cellular and molecular basis for behavioral regulation by IFN- α in humans, however, the substrate for IFN- α -induced behavioral dysfunctions remains obscure.

The behavioral impact of pharmacological intervention by IFN- α has been highly controversial in both mice [19,23,35] and rats [9,17,20]. A recent study from our laboratory revealed that murine, but not human, IFN- α activates the expression of IFN-regulated genes in the brain and peripheral tissues following systemic IFN- α administration in mice [34].

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This finding not only confirmed the species' specificity of IFN- α originally identified from studies *in vitro* [37], but also indicated the necessity of using murine IFN- α for such studies in mice. The behavioral studies in rodents have also been limited by the cost of high doses and long-term treatment of the expensive recombinant murine IFN- α protein. Nonetheless, given the direct activity of IFN- α on the brain following systemic administration [34], genetically modified mice with CNS-targeted expression of the IFN- α transgene may provide a useful model in such an investigation to overcome the limitations of pharmacological studies. Hence, behavioral characterization of such IFN- α -transgenic mice will be invaluable.

In the present study, the line of GFAP-IFN α transgenic mice named GIFN12 was kindly provided by Dr. Iain L. Campbell at the Scripps Research Institute (La Jolla, CA). The genetically modified mice were generated on a mixed C56BL/6J $\times$ BALB/c background as previously described [1]. To establish a congenic strain for behavioral studies, the heterozygous transgenic mice were backcrossed to the wild-type C57BL/6J strain (The Jackson Laboratories, Bar Harbor, Maine) for 9 generations to generate C57BL/6J GFAP-IFN α mice. The genotype of the transgenic animals was determined by tail DNA polymerase chain reaction (PCR) as described previously [36]. Mice were group-housed in a vivarium ($22 \pm 1^\circ\text{C}$) under 12-12 light cycle conditions (lights on 7:00 AM) with free access to food and water. Both male and female transgenic mice and their wild-type littermates were used in the study. All experiments were conducted in accordance with the National Institutes of Health (NIH) guidelines for animal care and use, and an in-house protocol approved by the University of Missouri-Kansas City Institutional Animal Care and Use Committee (IACUC).

Poly(A⁺) RNA was extracted from freshly dissected snap-frozen brain and liver using oligo-dT (Ambion, Austin, TX). RNase protection assays (RPAs) for the detection of IFN-regulated genes and cytokine RNAs were performed as described previously [34]. Two probe sets, one for cytokines (ML11) and the other for IFN-stimulated genes (ISG1), were also used as previously described [13,36].

Elevated plus-maze (EPM) test was conducted as described previously [16]. The automated EPM apparatus from Kinder Scientific (Poway, CA) was based on that described by Lister [16]. The mouse was placed individually in the center platform facing one of the open arms and allowed to freely explore the maze for a total of 5 min. Movements through the maze were detected by equally spaced photocells. Entries into each arm and times spent in each arm were captured by a Dell computer using MotorMonitor[®] software provided by the vendor. The following measures were calculated: percentage of time spent in open arms, percentage of entries into open arms, total number of arm entries and total number of beam breaks. The total number of arm entries and beam breaks represents locomotor activity while the percentage of time spent in open arms and percentage of entries into open arms is indicative of the anxiety profile, independent of the locomotor activity effect [14]. A significant increase in the percentage of time spent in open arms and percentage of entries into open arms was revealed in the mice treated with the anxiolytic Buspirone (1.0 mg/kg by ip) as previously reported by others [3].

Porsolt forced swimming test (FST) was performed as described originally by Porsolt et al [25,26]. An automated forced swim station FS2000 from Kinder Scientific (Poway, CA) equipped with two 4 $\times$ 4 photo beam arrays that allows for the monitoring of swimming behaviors was used. For testing, mice were individually gently placed in a transparent Plexiglas cylinder (30 cm high, 10 cm in diameter; light intensity, 300 lux) filled with water at 22–24°C to a depth of 18 cm, and allowed to swim for 5 min. The resting time in seconds, indicating the duration of immobility and basic movements (total beam breaks), were

acquired through MotorMonitor software after data reduction. Water was changed between every two to four subjects.

To validate the sensitivity of the measurements for depression- and anxiety-like behavior by FST and EPM tests, respectively, we treated mice the lipopolysaccharide (LPS) as a depressogenic and anxiogenic agent according to previous studies [11,15]. Consistent with these previous reports, a significant increase in immobility (the measure of depression-like behavior by FST) and a decrease in the percentage of time spent in open arms and the percentage of entries into open arms (the measures of anxiety-like behavior by EPM) were observed following ip administration of 5 μ g LPS.

The intensity of light was 50 lux for EPM and 300 lux for FST, respectively. For all behavioral studies, mice were transported into the testing room from the housing facility at least 2 hr before testing. All tests were performed between 12:30 p.m. and 4:30 p.m. to avoid circadian variation. All results are presented as the mean $\pm$ S.E.M (standard error of mean). Unpaired student's t-tests were utilized for statistical analysis. Significance was set at a p value less than or equal to 0.05 using two-tailed tests.

Previous studies demonstrated that expression of IFN- α transgene was detected only in the brain, but not peripheral organs in GFAP-IFN α transgenic mice [1,33]. The CNS-targeted expression of IFN- α was also evidenced by highly increased expression of IFN-regulated genes, especially STAT1 (signal transducer and activator of transcription 1) and ISG15 (IFN-induced 15 kDa protein), in the brain, but not in the liver of GIFN12 mice (Fig. 1 left). Despite similar IFN- α expression across the brain regions in transgenic mice [1], *in situ* hybridization revealed higher expression of IFN-regulated genes in the granular neuron layer of the cerebellum, hippocampus, and olfactory bulb [1,33], indicating that neurons are highly responsive to IFN- α . Additionally, similar to that previously reported [36], chronic cerebral production of IFN- α did not significantly alter the expression of the cytokines that are putatively involved in behavioral dysfunctions [8], such as TNF- α , IL-1 β , IL-2, IFN- γ and IL-6 (Fig. 1 right).

To evaluate the behavioral consequences of chronic stimulation of IFN- α in the brain, GIFN12 mice and wild-type littermates were evaluated for anxiety and depression profiles by elevated plus-maze (EPM) and forced swimming test (FST), respectively. EPM and FST tests were performed sequentially, separated by 4–5 days between the two tests. Independent groups of mice at different ages (8, 16 and 40 weeks) were used for behavioral tests to avoid possible adaptation with behavioral measurements from repeated testing [32].

Interestingly, the GIFN12 IFN- α -transgenic mice displayed no significant changes in anxiety profile (reflected by percentage of time spent in open arms and percentage of entries into open arms) or locomotor activities (measured by total arms entries and total beam breaks) in EPM tests compared with wild-type littermates at both 8 and 16 weeks of age (Fig. 2A&B). Although a small increase in the percentage of the time in open arms (11–15%) was observed in transgenic mice at both 8 and 16 weeks of age, the increase did not reach statistical significance ($p > 0.05$). In contrast to the results from the mice at 8 and 16 weeks of age, GIFN12 mice showed a 20% decrease in the percentage of time in the open arms at 40 weeks of age. Again, the decrease was not significant ($p > 0.05$). Nevertheless, a small, but statistically significant decrease in total beam breaks, a key measurement for locomotion by EPM, was found in the 40-week-old GIFN12 mice (Fig. 2C).

In addition to measuring anxiety-like behavior, the depression profile was also evaluated by FST using a standard 5-minute session. To our surprise, the immobile time, a measure of depression-like behavior by the Porsolt swim test in rodents, was not significantly changed between the IFN- α transgenic mice and wild-type controls in all age groups examined (Fig.

3). Comparable numbers of total beam breaks, a measurement of general activity by the Porsolt swim test, were recorded between transgenic and wild-type littermates at 8, 16, and 40 weeks of age. An increase of 15% in resting time was observed in GIFN mice at 40 weeks of age; however, the change did not reach statistical significance ($p = 0.08$).

The present report showed that chronic IFN- α stimulation in the mouse brain by local expression of the IFN- α transgene did not alter depression or anxiety measures by either forced swimming or elevated plus-maze test, indicating no change in depression and anxiety profile in IFN- α transgenic mice. The results are consistent with those of direct IFN- α treatment by intraperitoneal injection [9,17]. However, such observation contrasts with the depressive and anxiogenic effects of chronic systemic IFN- α treatment in humans. The reason for such discrepancy is currently unknown. Nevertheless, while 21% to 45% of patients developed depression and other psychiatric complications following chronic IFN- α therapy [28], factors other than IFN- α treatment itself contribute to IFN- α -triggered psychiatric abnormality. Of note, the risk for depression following IFN- α treatment has been demonstrated to be influenced by genetic polymorphisms of a number of genes, including serotonin transporter and IL-6 genes [6,18]. It should also be pointed out that all relevant clinical studies were carried out in cohorts of patients with chronic viral infection or malignancies [22,24,29]. The preexisting imbalanced immune status in these patients (including immune stimulation by virus invasion or cancer cells) could dramatically change the neurochemical and behavioral responses to IFN- α compared to healthy subjects.

Additionally, a recent study revealed a synergistic effect of psychological stressor with acute systemic IFN- α treatment on their neurochemical, neuroendocrine, immunoregulatory and behavioral actions in mice [2]. The results suggest a psychological factor in the development of psychiatric abnormalities in the patients following chronic IFN- α treatment. In this regard, it will be important to determine whether such synergistic behavioral impact occurs in IFN- α transgenic mice (with chronic cerebral IFN- α expression) when a similar psychological distress (e.g., social disruption) is imposed.

On the other hand, evidence indicates that peripheral cytokines can signal the brain by a number of mechanisms, including blood-brain barrier (BBB)-dependent and -independent pathways [27]. Early pharmacokinetic studies estimate that only a small fraction of peripherally administered molecules gain access to the CNS [4]. Hence, while the peripheral administration of IFN- α can directly activate receptors in the brain and mediate alterations in neurobiological activities [34], potential differences between IFN- α in the periphery versus IFN- α in the CNS remain. In this regard, studies are necessary to determine whether or not IFN- α -induced depression is associated with higher CNS IFN- α levels in humans.

The unaltered depression and anxiety profile found in GIFN12 transgenic mice is in agreement with the findings reported in recent pharmacological studies in rodents following direct IFN- α administration [9,17,35]. Nevertheless, as regular transgenic models constitutively express the gene of interest from the onset of embryogenesis, potential compensatory mechanisms may be developed during development in transgenic mice [7]. Therefore, the findings from such transgenic animals need be interpreted with caution. This may not be the case for GIFN12 mice since persistent IFN- α expression and signaling is detected in the brains of these transgenic mice and leads to progressive neuropathological consequences at a late age [1]. The GIFN12 mice might, however, have developed tolerance, or compensatory alterations, to counteract depressive and anxiogenic effects of IFN- α . In this regard, future development and application of conditional IFN- α transgenic mice will help to clarify such a possibility.

HIV-associated dementia (HAD) represents the most severe form of HIV-related neuropsychiatric impairment in patients with the late stage of HIV infection [12]. It is characterized by cognitive, behavioral and locomotor abnormalities. Whereas the pathogenesis of HAD remains obscure, highly activated expression and signaling of IFN- α is consistently detected in HIV-induced encephalopathy [21,30]. Considering the neuropathological outcome of chronic IFN- α signaling demonstrated in GFAP-IFN α transgenic mice [1,36], a pathogenic role of IFN- α for HAD is suggested. Given the fact that decreased motor activity is among the major clinical manifestations in HAD patients [12], attenuated locomotor activity found in IFN- α transgenic mice at 40 weeks of age in the present study is of significance. Such a behavioral deficit developed in the late stages of life in IFN- α transgenic mice is in line with the neuroinflammation and neurodegeneration developed in the brains of GFN12 mice after 8 months of age [1,36]. Therefore, the IFN- α transgenic mice may potentially be used as an animal model for HAD in humans. Further behavioral characterization of these mice, including cognitive behavior tests in relationship with the neuropathological change, will determine the importance of IFN- α in behavioral regulation, as well as for relevant neurological and neuropsychiatric diseases.

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Fig 1.

Expression of IFN-regulated genes and cytokine genes in GIFN12 versus control mice. RPA analysis of IFN-regulated (left panel) and cytokine (right panel) genes using poly(A⁺) RNA (2 μ g per sample) extracted from the brain and liver of 3-month-old mice. Samples in each lane are derived from individual animals.

Fig. 2.

Performance of GIFN12 mice in elevated plus-maze (EPM) test. Naïve IFN- α transgenic mice and wild-type (WT) littermates at 8 weeks (**A**; $n = 22$), 16 weeks (**B**; $n = 20$ – 23), and 40 weeks (**C**; $n = 10$ – 14) of age were tested for 5 minutes in the EPM. The percentage of time spent in the open arms, the percentage of entries into the open arms, the total number of arm entries, and the total number of beam breaks were measured. * Indicates significant difference from wild-type control mice ($p < 0.05$).

Fig. 3.

Performance of GIFN12 mice in forced swimming test (FST). Naïve IFN- α transgenic mice and wild-type (WT) littermates at 8 weeks (A; $n = 22$), 16 weeks (B; $n = 20-23$), and 40 weeks (C; $n = 10-14$) of age were tested in the FST. The resting time (immobility in seconds) and the total number of beam breaks from a 5-minute session were measured. No significant differences were observed at any age.