IL-10 modulates depressive-like behavior

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Abstract

The role of pro-inflammatory cytokines in psychiatric disorders has been the focus of great research attention in recent years. Paradoxically, the same is not true for anti-inflammatory cytokines. In the present study, we assessed the behavioral profile of animals with altered expression of the anti-inflammatory cytokine IL-10.

We performed a battery of tests to assess anxiety, depressive-like and cognitive behaviors in mice overexpressing IL-10 (PMT10) and IL-10$^{-/-}$ animals; in the later mice we also tested the behavioral effect of IL-10 administration.

In the forced-swimming test, IL-10$^{-/-}$ females displayed increased depressive-like behavior; importantly, this phenotype was reverted by the injection of IL-10. Moreover, mice overexpressing IL-10 presented a decreased depressive-like behavior. Despite the presence of a similar trend, male animals did not reach significant differences in depressive-like behavior. Assessment in the open-field showed that the absence of IL-10 decreased the percentage of time spent in the center of the arena in both male and female mice, while male animals overexpressing IL-10 revealed an opposite behavior. For both sexes, imbalance in IL-10 levels did not affect spatial reference memory.

In conclusion, variations in IL-10 expression are associated with an altered depressive-like behavior, but do not influence cognitive performance. Interestingly, IL-10 imbalance produced more profound behavioral changes in females than in male animals. This is in accordance with clinical data demonstrating an increased susceptibility of women to mood disorders, suggesting an interplay between anti-inflammatory cytokines and sexual steroids.
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1. Introduction

The cross-talk between the central nervous system (CNS) and the peripheral immune system has been a subject of great research interest in recent years. Since the discovery of cytokine receptors in both glial cells and neurons (Araujo et al., 1989; Breder et al., 1988; Cunningham and De Souza, 1993; McGeer and McGeer, 1995). The influence of cytokines in brain function, resulting either from signaling by cytokines produced peripherally or within the CNS (mainly by microglia and astrocytes) (Araujo et al., 1989; Breder et al., 1988; Cunningham and De Souza, 1993; McGeer and McGeer, 1995), has been characterized. Several studies have highlighted the role of cytokines in neuropathogenesis, particularly in mood disorders (Papanicolaou et al., 1998; Schiepers et al., 2005; Yirmiya et al., 2000). Indeed, one of the most consistent observations in the field is the increased levels of pro-inflammatory cytokines in depression (Lanquillon et al., 2000; Maes et al., 1995b; Mikova et al., 2001; Sluzewska et al., 1995; Tuglu et al., 2003).

In an attempt to integrate the above described findings, it has been proposed that the release of pro-inflammatory cytokines (mostly IL-1β, IL-6, IFN-γ, TNF-α) is a major

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determinant for the behavioral, neuroendocrine and neuro-chemistry alterations associated with depressive disorders (Maes et al., 1993). Whether these changes in cytokine expression are the cause or consequence of depression is still a matter of dispute, but the demonstration that the immune dysregulation precedes the development of depression (Yirmiya et al., 2000) is of notice. In support of the role of cytokines in mood disorders are the observations of early depressive symptoms in patients receiving interferon and IL-2 therapy (Capuron et al., 2000); interestingly, subsequent studies have also shown that these depressive symptoms can be relieved by the administration of antidepressants (Musselman et al., 2001; Yirmiya et al., 2001). The role of cytokines in mood disorders is further strengthened by the demonstration that pro-inflammatory cytokines are able to activate the hypothalamus–pituitary–adrenal (HPA) axis (Crane et al., 2003). The overactivation of the HPA is one of the most recognized findings in mood disorders and considered to be an important trigger of these psychopathologies (Carroll et al., 1968; Plotsky et al., 1998). Moreover, increases in both IL-1β (Linthorst et al., 1995; Merali et al., 1997; Shintani et al., 1993; Song et al., 1999) and TNF (Brebner et al., 2000; Hayley et al., 1999) have been associated with alterations at the neurochemical level, predominantly in the serotoninergic system (Dunn et al., 1999), in several brain regions known to be implicated in depression. As a consequence, animals exposed to increase levels of pro-inflammatory cytokines display signs of depressive-like behavior (De La Garza, 2005; Dunn et al., 2005) and increased anxiety (Silverman et al., 2007).

While several reports implicate pro-inflammatory cytokines in behavior, less is known on the influence of anti-inflammatory cytokines. Among the few studies on the subject, administration of IL-10 prior to LPS injection has been shown to revert the behavioral effects of LPS injection (Bluthe et al., 1999), including the effects upon mobility, rearing activity and social exploration and interaction (Leon et al., 1999; Nava et al., 1997; Smith et al., 1999). Several authors have hypothesized that the behavioral effects of IL-10 are a consequence of an inhibitory effect on IL-1, INF-γ and TNF production and not a direct effect of this anti-inflammatory molecule; in fact, it is known that IL-10 is important on the down-modulation of these pro-inflammatory cytokines (Fiorentino et al., 1991; Harvey et al., 2006; Moore et al., 2001). However, more recently, it was demonstrated that IL-10 administration to animals without exposure to inflammatory challenge induces increased motor activity and abnormal exploratory patterns (Harvey et al., 2006), which indicates that this cytokine might directly influence behavior. In this study, we aimed to further investigate whether manipulation of IL-10 levels could modulate behavior. To achieve this goal, we examined the behavioral profile of IL-10$^{-/-}$ and transgenic mice that overexpress this anti-inflammatory cytokine (PMT10); moreover, to further assess the influence of this cytokine in modulating behavior, we analyzed the effects of IL-10 administration to IL-10$^{-/-}$ mice.

2. Methods and materials

2.1. Animals

The IL-10$^{-/-}$ animals on a Balb/c background were bred in our animals facilities from a breeding pair provided from Dr. A. O'Garra (National Institute for Medical Research, London, UK). For behavioral characterization of these knock-out animals, we used 40 IL-10$^{-/-}$ (23 females and 17 males) on a Balb/c background and 31 wild-type Balb/c (17 females and 14 males) as respective controls. All animals were genotyped by PCR. As some IL-10$^{-/-}$ animals develop spontaneous inflammatory bowel disease, analysis of bowels was carefully performed throughout the experimental period and diarrheic animals were excluded from this study.

PMT10 animals on a C57BL/6 background were produced by Drs. P Vieira and AG Castro. A mouse IL-10 cDNA sequence was cloned in the p169ZT vector (Sousa et al., 2002), which carries the sheep metallothioneinc (MT) Ia promoter (Peterson and Mercer, 1986), a β-globin splice site and the SV40 polyadenylation signal. The resulting vector (pMT-IL10) was injected in C57BL/6 eggs and transgenic founders were identified by PCR using MT-specific primers. The presence of the transgene was confirmed by Southern blot analysis encompassing the sheep MT-promoter. PMT10 mice were bred at our animal facilities. In this experiment, ten PMT10 animals and ten wild-type C57BL/6 as respective controls were used. IL-10 overexpression was induced by giving a 2% sucrose solution with 50 mM of zinc sulfate to animals ad libitum. As the IL-10 promoter is associated with a metalloprotein, the presence of zinc in this solution induces its activation. Wild type animals were also supplied with the same drinking solution.

Serum levels of IL-10, which range between 3 and 5 ng/ml, could be measured 3 days after induction and remained stable while the animals were drinking the zinc solution. IL-10 was never detected in the serum of non-transgenic littermates or in non-induced transgenic mice. IL-10 overexpression was induced 1 week before the behavioral testes were initiated.

All mice were kept in an animal facility in a 12 h light:12 h dark cycle, with food and water available ad libitum. Males and females were kept separately. At 3 months of age all animals were behaviorally tested between 10 a.m. and 5 p.m. in a counterbalanced order; IL-10$^{-/-}$ mice were compared to wild-type Balb/c, whereas PMT10 animals were compared to wild-type C57BL/6 mice. At the end of the experiment, animals were sacrificed by decapitation; decapitation was performed by trained operators. All experimental procedures were conducted in accordance to the European Communities Council Directive, 86/609/EEC.

2.2. IL-10 injection

Another subset of 20 females and 16 males of IL-10$^{-/-}$ mice were used in a supplementary experiment in which

they were injected with recombinant mouse IL-10 (R&D systems, Minneapolis, USA). Half of these animals received a daily i.p. injection of 40 ng of IL-10 for 11 days (6 days prior the behavioral analysis followed by 5 more days concomitant with the behavioral assessment), while control animals were injected with a saline solution.

2.3. Cytokine measurements

Serum levels of INF-γ were measured by a two-side sandwich ELISA with the anti-IFN-γ specific affinity-purified mAbs (R4-6A2 as capture and biotinylated AN-18 as detecting mAbs), and a standard curve was generated with known amounts of IFN-γ (Peprotech, Rocky Hill, NJ, USA). The sensitivity of the assay was 16 pg/ml.

Quantification of TNF-α was done by ELISA (Duo Set; R&D Systems, Minneapolis, USA). The sensitivity of the assay was 32 pg/ml.

Serum levels of IL-10 were determined by ELISA (Quantikine; R&D Systems, Minneapolis, USA). The sensitivity of the assay was 4 pg/ml.

2.4. Behavioral analysis

2.4.1. Open-field test

Animals were placed in the center of the arena (43.2×43.2 cm transparent acrylic walls and white floor) and their position was monitored and recorded by a two 16-beam infrared system (MedAssociates, Vermont, USA), over a period of 5 min. Time in the predefined central and peripheral areas was recorded and used to evaluate anxious-like behavior, while the total distance traveled assessed spontaneous activity. The number and duration of the "*rearings*" (vertical activity) were also recorded as a measure of exploratory behavior.

2.4.2. Elevated-plus maze

Animals were placed in a elevated-plus maze (EPM) apparatus consisting of two opposite open arms (50.8×10.2 cm) and two opposite closed arms ($50.8 \times 10.2 \times 40.6$ cm) raised 72.4 cm above the floor (ENV-560, MedAssociates, Vermont, USA). The number of entries and the time spent in each arm was registered by an infrared system over a total test period of 5 min.

2.4.3. Forced-swimming test

Animals were placed in a cylinder (diameter: 37 cm; 55 cm of height) filled with water (25 °C) to 35 cm depth such as that they had no solid support for the rear paws nor for the tail. Animals were tested in a 5 min session 24 h after being exposed to the test for the same time period. Only the second session was recorded and later scored by two independent researchers which were blind to the experimental conditions. Time of immobility and latency to immobility were computed and used as a measure of depressive-like behavior.

2.4.4. Morris water maze

This maze consisted of a tank (diameter: 170 cm; depth: 50 cm), divided into quadrants by imaginary lines and filled with opaque water to a depth of 31 cm. During testing, a platform (12×12 cm; invisible to the mice) was placed in the same quadrant during five consecutive days. Each test session consisted of four trials which lasted in maximum for 2 min. Time spent swimming to reach the hidden platform was recorded and used to evaluate learning and memory performances.

2.5. Statistical analysis

All the results from the behavioral tests are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago/IL). The effect of gender and IL-10 expression, per se, in the Open Field and the forced swimming tests were analyzed by independent samples t-test, while the overall effects were studied by two-way analysis of variance (ANOVA).

Data from the Morris water maze task was analyzed using a repeated measures ANOVA analysis throughout the 5 days test. Each day is a mean of the four consecutive trials. All behavioral results are expressed as means ± SE and statistical significance was considered for p values $\leqslant 0.05$.

3. Results

3.1. Biometric parameters

Analysis of the relative weight of thymus and adrenal glands from IL-10$^{-/-}$ animals compared with wild-type animals showed a significant reduction of the thymus in both male and female mice ($t = 3.9$; $p \leqslant 0.001$ and $t = 2.9$; $p < 0.05$, respectively) (Fig. 1A and B). Two-way
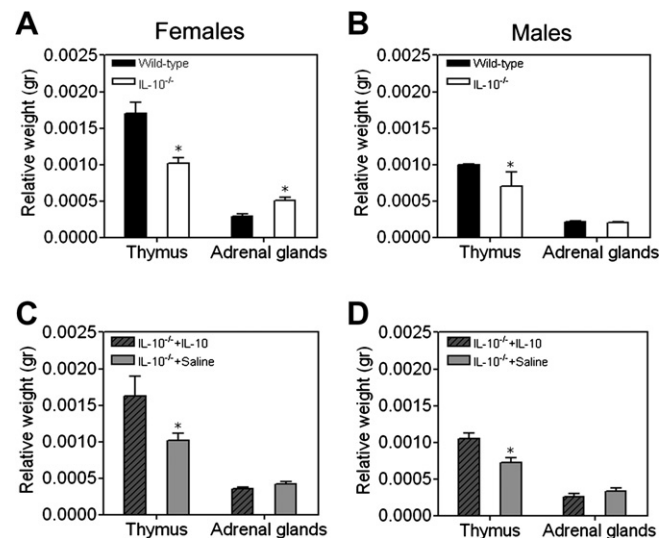

Fig. 1. Thymus and adrenal glands weight relative to the body weight of Wild-type and IL10$^{-/-}$ females and males (A and B) and IL-10$^{-/-}$ receiving IL-10 (IL-10$^{-/-}$ + IL-10) and IL-10$^{-/-}$ receiving saline (IL-10$^{-/-}$ + Saline) animals (C and D). Values are means ± SE and $^*p < 0.05$.

ANOVA revealed a gender effect on adrenals weight ($F_{1,27} = 24.1$; $p < 0.001$), with interaction between gender and IL-10 factors. Interestingly, IL-10 administration reverted the atrophy of thymus in IL-10$^{-/-}$ mice ($t = -3.1$; $p < 0.05$ for females and $t = -3.5$; $p < 0.05$ for males) (Fig. 1C and D).

Regarding the adrenals weight, a significant increase was found in females IL-10$^{-/-}$ ($t = -4.5$; $p < 0.001$) (Fig. 1A). Once more, IL-10 administration showed to be effective in restoring the adrenals weight in IL-10$^{-/-}$ females ($t = 2.5$; $p < 0.05$; Fig. 1C). No statistical significance was found in males (Fig. 1B and D). Two-way ANOVA showed a gender effect on both biometric parameters ($F_{1,31} = 13.8$; $p < 0.05$ for thymus and $F_{1,31} = 9.3$; $p < 0.05$ for adrenal glands).

3.2. IL-10 production influences depressive-like behavior in female mice

In the forced-swimming test (FST) we evaluated the ability of mice to cope with a stressful and inescapable situation (learned helplessness). In this test, animals displaying decreased latency to immobility and longer immobilization periods are considered to have increased helplessness, which is a sign of depressive-like behavior. When tested in the FST, IL-10$^{-/-}$ female showed decreased activity ($t = 6.1$, $p < 0.001$) during the 5 min of the test and stopped swimming earlier than wild-type mice ($t = 3.3$ $p < 0.005$ (Fig. 2A). No significant differences were observed between male animals (Fig. 2B). Two-way ANOVA revealed a significant effect of gender, being the females more affected than males in latency to immobility ($F_{1,67} = 14.0$, $p \leqslant 0.001$) and activity times ($F_{1,67} = 5.2$, $p \leqslant 0.05$).

Interestingly, IL-10 administration was able to reverse the depressive-like phenotype of IL-10$^{-/-}$ females, in as much as after administration of this cytokine there was an increased activity time namely when compared with animals receiving saline injection ($t = -2.9$, $p < 0.05$) (Fig. 2C). Although this difference was only observed in females, a similar trend was also found in male mice (Fig. 2D).

Further supporting the anti-depressant action of IL-10, PMT10 female mice showed increased activity time when compared with their respective counterparts ($t = -5.5$; $p \leqslant 0.001$), which reveals a decreased susceptibility to depressive-like behavior (Fig. 2E). Although male animals showed a similar behavioral pattern, no statistical significant differences were observed in any of the parameters analyzed (Fig. 2F). Again, two-way ANOVA showed a significant effect of gender in the activity time ($F_{1,16} = 6.5$, $p \leqslant 0.05$), being the females more vulnerable to IL-10 imbalances.

3.3. Variations in IL-10 levels affect anxious-like behavior in the open-field

The open-field test (OF) was performed to assess general locomotor activity and exploratory behavior. In terms of
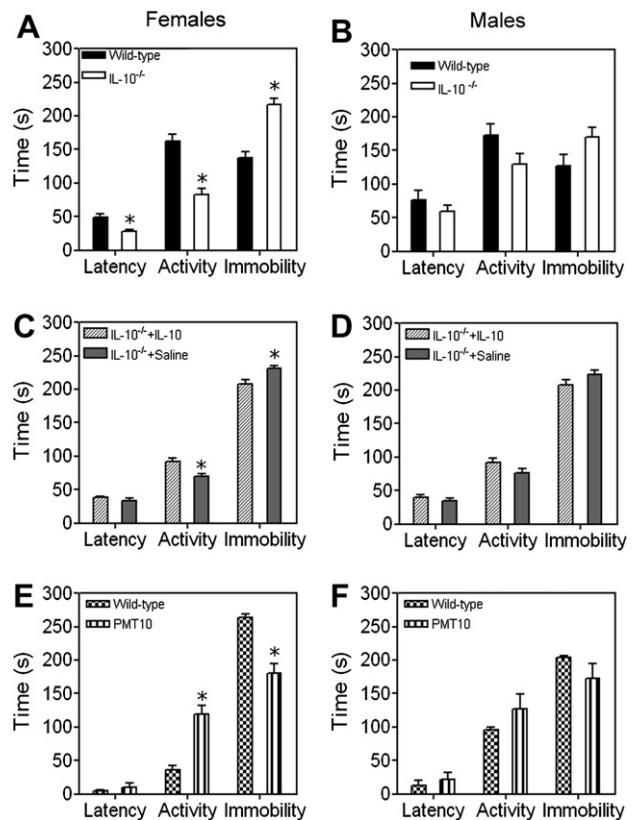

Fig. 2. Depressive-like behavior assessed with the forced-swimming test (FST). IL-10$^{-/-}$ females displayed increased immobility time and decrease latency to immobility compared with their wild-type controls (A). No significant effect was observed in male mice (B). Administration of IL-10 was only able to increase activity time in female IL-10$^{-/-}$ mice, when compared with IL-10$^{-/-}$ mice receiving saline injection (C and D). Female animals overexpressing IL-10 (PMT10) revealed an increase activity time in the FST (E). The same tendency was obtained for male PMT10 animals (F). Values are means ± SE and $^*p < 0.05$.

spontaneous activity, assessed by the total distance traveled throughout the 5 min of the test, no differences were observed between IL-10$^{-/-}$ and wild-type animals. Moreover, both IL-10 administration and overexpression failed to induce any change in this behavior both in males and females.

Analysis of the exploratory behavior in terms of animals' vertical activity (number and duration of rearings) revealed a significant increase in both measurements in IL-10$^{-/-}$ female mice compared to their wild-type counterparts (Fig. 3A). No significant differences were observed in male animals, although there was a trend for increased exploratory behavior in IL-10$^{-/-}$ animals (Fig. 3B). Both the administration and the overexpression of IL-10 failed to show any effect in vertical activity (Fig. 3C, D and E, F), despite a tendency for reduction after IL-10 administration in male IL-10$^{-/-}$ mice.

Although the OF is not the most widely used test to assess anxious-like behavior, the percentage of time spent in center of the arena over the total time provides an indicative measure of anxiety-behavior (Boguszewski and Zagrodzka, 2002; Ramos et al., 1997; Simen et al., 2006).
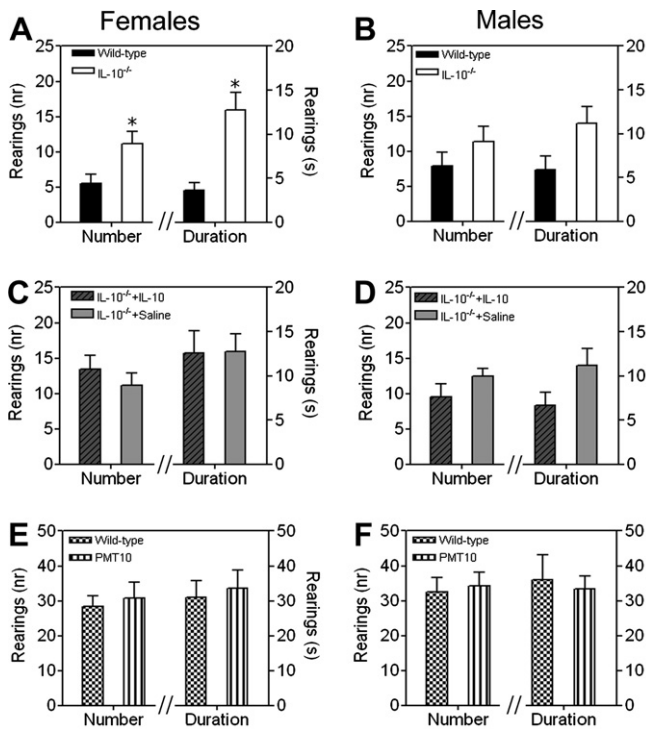
Fig. 3. Vertical activity assessed by the number and duration of rearings in the open-field test (OF), showed increased exploratory behavior in IL-$10^{-/-}$ female but not in males (A and B); IL-10 administration did not change rearing activity neither in male or female mice (C and D). No significant differences in rearing activity were observed between PMT10 and respective control animals (E and F). Values are means ± SE and $^*p < 0.05$.
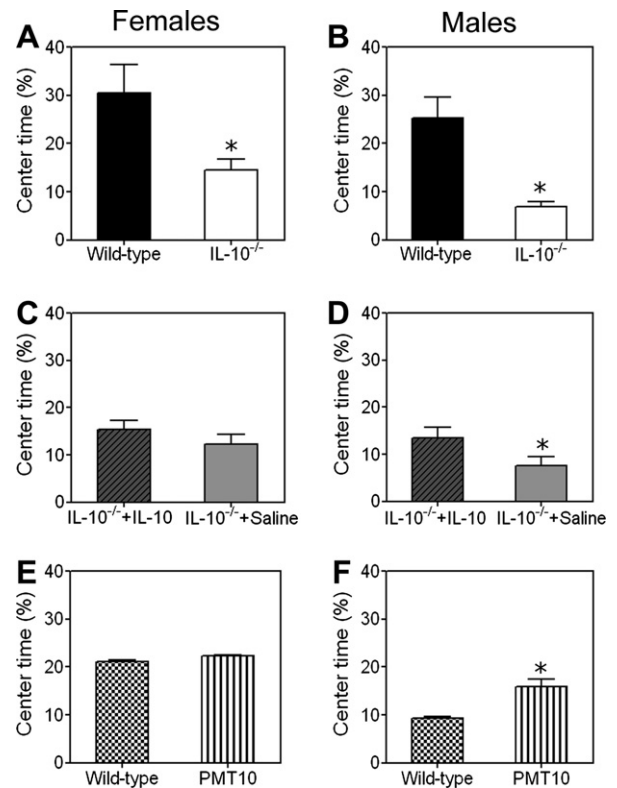

Fig. 4. Anxious-like behavior assessed by the time spent in the center of the OF arena, showed increased anxiety in IL-$10^{-/-}$ (A and B). However, administration of IL-10 only reversed the phenotype in IL-$10^{-/-}$ male (C and D). Male PMT10 spent more time in the center than control animals a sign of decreased anxious-like behavior (E and F). Values are means of the percentage of time spent in the center over the total time ± SE and $^*p < 0.05$.

Analysis of this parameter demonstrated that both female and male IL-$10^{-/-}$ mice spent significantly less time in the center than in the periphery when compared to wild-type animals ($t = 2.6$, $p < 0.05$, $t = 4.0$, $p \leqslant 0.001$, respectively) (Fig. 4A and B).

Interestingly, the IL-10 treatment was able to reverse the IL-$10^{-/-}$ mice phenotype, in males, resulting in increased time spent in the center of the OF arena ($t = -2.9$, $p < 0.05$). Two-way ANOVA revealed a gender effect in this parameter ($F_{1,32} = 4.6$, $p < 0.05$) (Fig. 4C and D).

The same trend was observed for PMT10 animals which spent significantly more time in the center of the OF, even though differences were only significant in males ($t = -4.3$, $p \leqslant 0.05$; Fig. 4E and F).

However, care must be taken in the interpretation of these results as no differences were found in the EPM in neither IL-$10^{-/-}$ nor in PMT10 mice when compared with respective control animals (data not shown).

3.4. IL-10 production did not affect hippocampal-dependent spatial memory

To investigate whether changes in IL-10 "milieu" influence hippocampal-dependent learning and memory, the Morris water maze (MWM) test was performed. No differences were found in both male and females regarding the time and the distance swam to find the hidden platform (Fig. 5), thus showing that neither the overexpression nor the absence of IL-10 seem to affect spatial memory.

3.5. Changes in IL-10 expression did not induce detectable changes in TNF-α and IFN-γ levels

In order to investigate whether genetic manipulation of IL-10 expression influences the production of two relevant pro-inflammatory cytokines, the serum levels of TNF-α and INF-γ were measured. No differences were observed between PMT10 and IL-$10^{-/-}$ and their respective wild-type controls as determinations for both cytokines in the serum were below the detection levels (16 pg/ml for TNF-α and 32 pg/ml for INF-γ).

4. Discussion

By studying emotional behavior in mice lacking or over-expressing IL-10, we herein show that this anti-inflammatory cytokine influences depressive-like behavior. Mice lacking IL-10 displayed signs of depressive-like behavior, assessed by immobilization time and latency to immobility, when compared to their strain-matched wild-type counterparts. In contrast, both PMT10 and IL-$10^{-/-}$ receiving this
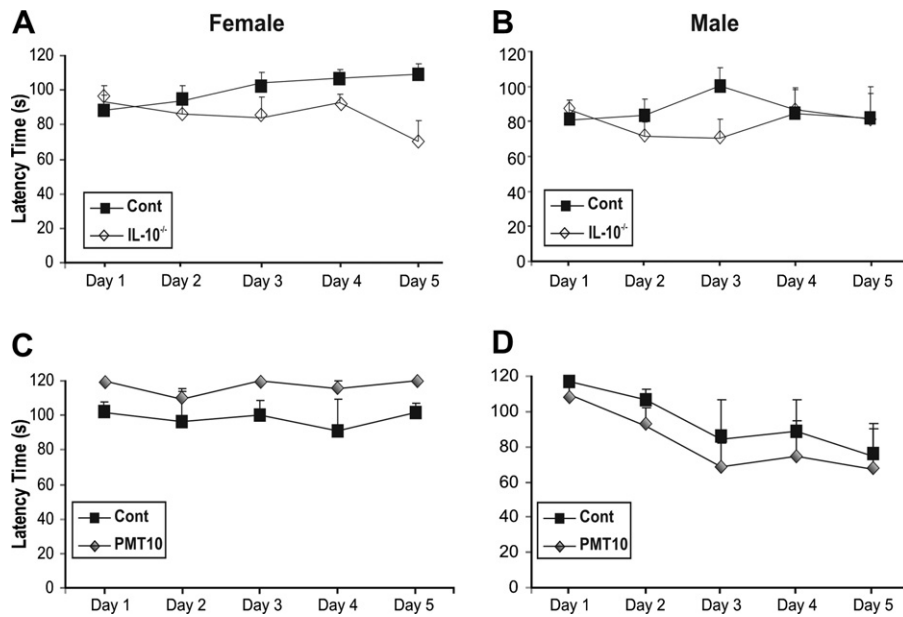
Fig. 5. Spatial memory evaluation in the classical Morris water maze (MWM) paradigm failed to show any significant difference between IL-10$^{-/-}$ and wild-type animals for both female (A) and male (B). The same results were observed for PMT10 animals (C and D). Values are expressed as means of the four trials/day $\pm$ SE and $^{*}p < 0.05$.

cytokine displayed an opposite behavioral phenotype. Remarkably, variation in IL-10 levels affected more profoundly females, which correlates with the recognized higher susceptibility of women to depression. Taken together, these results reveal, for the first time, that this anti-inflammatory cytokine is an important mediator in depression.

This observation adds to the so-called "cytokine hypothesis of depression", that was built on the evidence that pro-inflammatory cytokines have a trigger effect on the pathogenesis of depression (Maes et al., 1995a; Maes et al., 1993). This effect was proposed to be mediated by neuroendocrine and neurotransmitter systems involved in vulnerability to affective disorders (Maes, 1999). In this respect it has been demonstrated that IL-1β and TNF-α stimulate the expression/release of corticotrophin-releasing hormone (CRH) in the paraventricular nucleus (PVN) of the hypothalamus (Hayley et al., 2001; Tilders and Schmidt, 1998), the control center of the HPA axis and alters the turnover of norepinephrine and serotonin (5-HT) in the hypothalamus, amygdala, prefrontal cortex, and hippocampus (Ando and Dunn, 1999; Brebner et al., 2000; Dunn et al., 1999; Hayley et al., 1999). Further evidence for the role of pro-inflammatory cytokines in depression was gathered from studies with TNF receptors (TNFR) knock-out mice, in which it was shown that both TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice were more active in the FST than wild-type animals (Simen et al., 2006). Importantly, the mechanisms underpinning the behavioral changes in these mice models are similar and include alterations in neurotransmission in regions of the brain implicated in emotional behavior. Of notice, is also the evidence of the immunomodulatory effects of antidepres-

sants that act preferentially in the noradrenergic and serotoninergic system. Kubera and co-workers (Kubera et al., 2001) demonstrated the ability of different drugs to decrease the levels of INF-γ, while increasing the levels of the anti-inflammatory cytokine IL-10. These data were also corroborated by studies using other antidepressants in stimulated human blood cells (Maes et al., 1999).

While the involvement of pro-inflammatory cytokines in many aspects of depressive illness is now indisputable, we clearly demonstrate in this study that anti-inflammatory cytokines also influence emotional behavior in rodents. Work from Bluthe and collaborators (Bluthe et al., 1999) had already suggested that IL-10 administration could abrogate the behavioral effects of LPS injection in sickness behavior in rats. It was postulated that IL-10 inhibits the expression of pro-inflammatory cytokines (IL-1, INF-γ and TNF-α) produced in response to LPS and its behavioral consequences, increasing the duration of social interaction. The present data reveals that variations in IL-10 expression influence mood behavior, even in the absence of detectable variations in the serum levels of INF-γ and TNF-α. This fact, however, does not exclude the possibility of an inhibition of pro-inflammatory cytokines secretion in the CNS; in accordance, IL-10 deficient mice have increased brain levels of TNF-α and IL-6 (Agnello et al., 2000).

Another relevant issue raised from our study is the increased susceptibility to variations in IL-10 expression in females. Of relevance is the fact that the same gender effect is observed in the clinical practice, where the increased susceptibility to depression in women in conditions of decrease estrogen secretion (e.g., premenstrual, during the postpartum period and perimenopausely) is

well-known (Osterlund et al., 2005). This increased susceptibility has been attributed to fluctuations in estrogen secretion. In fact, evidence derived from experimental and clinical studies demonstrated an important role of (decreased) estrogens in the pathogenesis of depression but also in the production and bioactivity of a variety of cytokines (Nordell et al., 2003; Suuronen et al., 2005). Estrogens depletion increases the levels of pro-inflammatory cytokines (Bismar et al., 1995), and studies using murine microglia cells showed that estrogens are able to increase IL-10 levels (Dimayuga et al., 2005). Accordingly, our results suggest that in animals that cannot express IL-10, the possible protective effects induced by estrogens are lost since IL-10$^{-/-}$ female mice displayed increased signs of depressive-like behavior when compared with male animals. In contrast, the higher levels of IL-10 present in PMT10 animals were probable bolstered by estrogens, potentiating their protective effects and anti-depressive effects in female mice. Moreover, clinical studies showed that estrogens not only trigger antidepressant-like actions (for review, see (Halbreich and Kahn, 2001)) but also improve the therapeutic action of antidepressants (Soares et al., 2001), specially of those acting in the serotoninergic system(Chang and Chang, 1999; Estrada-Camarena et al., 2006; Lu and Bethea, 2002). Besides the influence of estrogens, other sexual steroids, such as testosterone, might also be implicated in the gender difference observed in depressive-like behavior after IL-10 imbalances. In fact some human and animal studies have shown that testosterone increases IL-10 expression (Liva and Voskuhl, 2001; Malkin et al., 2004) and reduces the expression of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α (D'Agostino et al., 1999). Taken together, these data highlight a possible interaction between sexual steroids and cytokines actions.

The association between anxiety and depressive disorders is well-known, and there are several common factors involved in both conditions (Cameron, 2006; Cameron et al., 2004; Gulley and Nemeroff, 1993). Therefore, the exploration of anxiety-like signs in experimental models influencing depressive-like behavior becomes relevant. Interestingly, data from the open-field test suggested that IL-10$^{-/-}$ animals display a hyperanxious phenotype both in males and females. However, this phenotype could not be confirmed in the EPM, a more robust test to assess anxiety-like behavior. A possible explanation for these paradoxical findings could be the increased exploratory behavior evinced by IL-10$^{-/-}$ animals. As the EPM is based on the conflict between the innate exploratory behavior and fear of height and exposed environments, it is likely that the increased tendency for exploration in IL-10$^{-/-}$ might be a confounding effect in this behavioral paradigm and blunt the hyperanxious phenotype. In further support of this view are the findings of decreased anxiety evinced by PMT10 mice. Further studies, using other behavioral tests, are needed to better explore the influence of IL-10 levels in anxiety behavior both in basal and under stressful condition (an approach currently under study in our laboratory).

While the influence of IL-10 on anxiety behavior needs further experimental work, the present data rule out any influence of this anti-inflammatory cytokine in reference memory. In fact, the hippocampus-dependent task used to assess spatial reference memory failed to reveal differences between the performance of both genetically modified mice models and their respective wild-type controls. These findings are of relevance, in the sense that they reveal the specificity of the influence of IL-10 levels in affective/mood conditions.

In conclusion, our behavioral data demonstrate that IL-10, an anti-inflammatory cytokine, is an important molecule in the modulation of depressive-like behavior. This finding calls for a reappraisal of the "cytokine hypothesis of depression", in the sense that imbalances of both pro- or anti-inflammatory cytokines might modulate mood behavior. Furthermore, the present observations might be of relevance in all those conditions (autoimmune, malignant and infectious disease) associated to polymorphisms of the IL-10 family gene clusters in which depression seems to be more prevalent (Nery et al., 2007; van Boxel-Dezaire et al., 1999; Zorzon et al., 2001).

Conflict of interest

There are no financial or other conflicts of interest.

Contributors

Mesquita AR, Correia-Neves M and Sousa N designed the study, wrote the protocol, analyzed the data and wrote the first draft of the manuscript; Mesquita AR performed all behavior experiments and Roque S the ELISA procedures; Pedrosa J and Palha JA discussed the results and provided very important comments for the final version of the manuscript; Castro G and Vieira P produced the PMT10 animals and also provided important comments for interpretation of results. All authors contributed to and have approved the final manuscript.

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