Bacillus Calmette–Guérin vaccine induces a selective serotonin reuptake inhibitor (SSRI)–resistant depression like phenotype in mice

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Abstract
Preclinical studies have shown that administration of Bacillus Calmette–Guérin (BCG) vaccine induces depression-like behaviors in mice; however, the effect of antidepressant drug treatment has not been reported earlier. In the present study, we induced depression-like behavior by administering BCG vaccine to BALB/c mice. BCG treatment produced robust serum sickness as shown by a decrease in body weight, reduced spontaneous locomotor activity and reduced voluntary wheel running activity. BCG treatment also elevated plasma IL6 and IFNγ levels and produced a marked activation of lung IDO activity. At a time point when serum sickness-related behaviors had fully recovered (i.e., day 14) BCG-treated mice showed a significant increase in immobility in the forced swim test (FST) and tail suspension test (TST) indicative of a pro-depressant phenotype. We observed significant increase in [3H]PK11195 binding in cortex and hippocampus regions of BGC-treated mice in comparison to saline-treated mice indicating prominent neuroinflammation. Pharmacological evaluation of FST behavior in BCG-treated mice demonstrated selective resistance to the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and escitalopram. In contrast the tricyclic antidepressant imipramine, the dual serotonin/norepinephrine reuptake inhibitor (SNRI) duloxetine, and the dual dopamine/norepinephrine reuptake inhibitor (DNRI) nomifensine retained antidepressant efficacy in these mice. The lack of efficacy with acute treatment with SSRIs could not be explained either by differences in drug exposure or serotonin transporter (SERT) occupancy. Our results demonstrate that BCG-vaccine induced depression like behavior is selectively resistant to SSRIs and could potentially be employed to evaluate novel therapeutic agents being developed to treat SSRI-resistance in humans.

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1. Introduction

Major depressive disorder (MDD) is one of the leading causes of disability worldwide, and is associated with persistent feelings of sadness, depressed mood, anhedonia and suicidal thoughts (Samuels et al., 2011). With current treatment approaches many (67%) patients achieve only a partial therapeutic response and 33% are resistant to antidepressant treatment, a condition referred to as treatment resistant depression (TRD) (Lapidus et al., 2013). The etiology of MDD is multi-factorial and includes a role for inflammation as a potential driver of the disturbances in brain monoamine and glutamate signaling thought to underlie this disorder (Miller et al., 2009; Piser, 2010). Significant elevation of serum pro-inflammatory cytokines has been reported in MDD patients (Janelidze et al., 2011) and the serum cytokine profile has been proposed as a potential patient stratification biomarker, although further work is needed in this area (Schmidt et al., 2011).

Anti-cytokine therapy used in the treatment of cancer, multiple sclerosis, rheumatoid arthritis, and hepatitis can lead to the development of depressive symptoms in patients (Yirmiya, 2000), and precipitate symptoms after acute administration in healthy

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volunteers (Dellagioia and Hannestad, 2010). Various preclinical approaches have also established the concept of cytokine-induced depression-like behaviors in rodents using agents like interferon-α (Orsal et al., 2008), lipopolysaccharide (Yirmiya et al., 2001), or Bacillus Calmette–Guerin (BCG) vaccine (Moreau et al., 2008 and O’Connor et al., 2009a). A major challenge for cytokine mediated rodent models of depression is the separation of the serum sickness phase from the expression of depression-like behaviors (Dantzer et al., 2008). This is particularly important for the testing of novel pharmacological agents in order to avoid potential confounds in the interpretation of effects on behavioral measures.

In the present study we have focused on the BCG vaccine model, a cytokine-driven model where the development of depression-like behaviors is clearly distinguishable from the acute serum sickness phase. In the past considerable amount of work has been carried out by other laboratories to develop and validate BCG-induced depression models (Moreau et al., 2008; O’Connor et al., 2009a,b). All these studies were focused only on characterizing the BCG-induced depression phenotype using CD1 mice. However, to the best of our knowledge this is the first report to show pharmacological validation of BCG-induced pro-depressant phenotype involving acute treatment with different classes of antidepressants like SSRI, SNRI, TCA and DNRI in FST. In the preliminary studies we have evaluated the sensitivity of CD1 and BALB/c mice to the BCG effects and found that BALB/c mice are more responsive. Hence, we used BALB/c strain for model development and pharmacological validation and also previous reports indicated that BALB/c is more sensitive to BCG-vaccine induced cytokine response than other strains of mouse (Converse et al., 2011). The goals of the current study were two fold: (i) to differentiate serum sickness and depression phase, and to establish the association between depression and neuroinflammation, (ii) to examine the effect of acute antidepressant drug treatments on attenuation of increased immobility duration induced by BCG treatment in FST.

2. Materials and methods

2.1. Subjects

Adult male BALB/c mice were obtained from Harlan (Netherlands). Mice were group housed (n = 4/cage) for all studies except for voluntary when running test (VWR). Animals used for VWR were individually housed. All the animals were kept in a AAALAC accredited animal holding facility maintained at controlled temperature (23 ± 1°C) and humidity (50 ± 20%) under a 12:12 h light: dark cycle (lights on at 07:00 h). Food and water were provided ad libitum. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with procedures set by the Committee For The Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Mice were handled once daily for 3 days prior to the start of the study to reduce handling stress.

2.2. Injection of Bacillus Calmette–Guerin

A commercial preparation of live attenuated strain of Mycobacterium bovis (Onco-BCG, Serum Institute of India Ltd., India) was used to induce the depression-like phenotype in mice. On the inoculation day, the contents of the vial were suspended in sterile physiological saline to attain a concentration of 10^7 CFU/ml, and administered intraperitoneally (i.p.) in a volume of 1 ml per mouse. The dose of BCG-vaccine was determined based on the dose response validation study in which the selected dose elicited significant changes in body weight, locomotor activity and an increase in immobility duration in FST (Supplementary Fig. 1). Control mice were administered the same volume of saline.

2.3. Behavioral characterization of BALB/c mice after BCG administration

All behavioral testing was conducted between 9:00 and 13:30 h except for voluntary wheel running activity (VWR) which was measured from 18:00 to 06:00 h. The forced swim test (FST) and tail suspension tests (TST) were conducted under low noise conditions and dim light (100 Lux) and locomotor activity (LMA) was evaluated under dark conditions. Body weight, LMA and VWR were used to examine the extent of serum sickness; the emergence of depression-like behaviors was assessed using the FST and TST.

VWR was determined in mice housed individually in cages containing a Low Profile “BioServ” Wireless Running Wheel (ENV-044, Med Associates Inc., St. Albans, VT, USA). Mice were allowed to acclimate to the wheels in their home cage for 1 week with wheel access available from 18.00 to 06.00 h. Animals were then treated with BCG and VWR was recorded daily for up to 21 days post treatment. The data was acquired through a USB interface hub (DIG-804, Med Associates Inc., USA) recorded and analyzed for the total distance rotated using wheel manager software (SOF-860, Med Associates Inc.; USA). Wheels were cleaned daily during the light phase of the dark: light cycle. A dedicated cohort of animals was used for measuring VWR.

FST behavior was assessed in mice placed in individual glass cylinders (46 cm height × 20 cm diameter) containing water (20 cm deep, maintained at 24–25 °C) at a level that prevents the tail from touching the cylinder base. Mice were tested for 6 min and then cleaned and dried with a cloth before they were returned to their home cage and placed on a thermal blanket. Immobility was defined as the time spent floating, without struggling, and with only minimal movement necessary to keep the head above the water. All subjects were tested only once with the test session videotaped and the total duration of immobility quantified using automated software (Forced Swim Scan, Version 2.0; Clever Systems Inc., USA).

TST was performed using the Mouse Tail Suspension apparatus (MED-TSS-MS; Med Associates, St. Albans, VT). Adhesive tape was attached (2 cm away from the tip of the tail) to suspend the mouse from a strain gauge and the apparatus was thoroughly cleaned after each subject. Mice evaluated in TST show a well characterized behavioral response that includes several escape attempts interspersed with immobility periods during which they are completely motionless. The duration of immobility observed in a 6 min test session was quantified using Tail Suspension Software (SOF-821, Med Associates, St. Albans, VT).

2.4. Measurement of plasma cytokines

Blood samples were collected by retro orbital bleeds into EDTA coated tubes. Plasma was separated by centrifugation at 5000g at 4°C for 10 min, and aliquots were frozen at −80 °C. Plasma IL6 and IFNγ were measured by sandwich ELISA based methods according to the manufacturer instructions (BD Opt EIAkit set, Cat No. 555240 and 551866 respectively). Cytokine levels were detected by measuring the optical density at 450 nM (Spectramax,
Molecular Devices, USA) using a calibration curve of standards in the range of 15.6–1000 pg/ml.

2.5. Measurement of lung indoleamine 2,3 dioxygenase (IDO) Activity

Lung indoleamine 2,3 dioxygenase (IDO) activity was determined as described in Moreau et al. (2005) and Moreau et al. (2008). Briefly, lung tissue was homogenized in ice-cold 20 mM phosphate buffer containing 140 mM KCl (pH 7.0) followed by centrifugation at 13,000 g, 30 min, 4 °C. Supernatant was added to the incubation mixture containing 0.4 mM L-Tryptophan, 20 mM ascorbate, 10 μM methylene blue, 100 μg catalase in 50 mM phosphate buffer (pH 6.5) and reaction continued for 3 h at 37 °C. The reaction was terminated by adding 30% Trichloroacetic acid and incubated further at 60 °C for 30 min followed by centrifugation at 15,000g for 15 min at 4 °C. The amount of kynurenine in the assay mixture was determined by treating supernatants with Ehrlich’s reagent followed by absorbance at 480 nM. One unit of enzyme activity was defined as nmol of Kynurenine/h/mg of protein. The amount of protein in the homogenates was determined by Bradford kit as per the vendor’s instructions (Sigma Aldrich, India).

2.6. In vitro \(^{3}H\)PK11195 autoradiography

Frozen brain tissue collected on day 14 post BCG or saline treatment was sectioned (20 μm) on a cryostat and mounted on slides. Slides were then processed for in vitro binding autoradiography by incubation with 2 nM \(^{3}H\)PK11195 for 40–60 min at 22–24 °C. The incubation solution contained (in mM) 50 HEPES, 10 MgCl₂, and 200 EGTA (pH 7.4). Non-specific binding was defined by incubation of adjacent sections with 1 μM PK11195. After incubation, the slides were washed in ice-cold phosphate-buffered saline (PBS; pH 7) and subsequently dried under a stream of cold air. The slides were placed in cassettes against \(^{3}H\)-sensitive storage phosphor-imaging screens (PerkinElmer, Waltham, MA) then scanned using Cyclone Plus Phospho Imager (PerkinElmer, USA). The data was analysed using Opti Quant software (PerkinElmer, USA). The range for pixel intensity is 0–359 and the range is determined automatically by analysis software. The radioisogland binding value in a given brain region was measured as digital light units per millimeter squared (DLU/mm²), and the specific binding density was calculated by subtraction of the non-specific binding from the total binding values. Data represented as percentage change from saline-treated control mice.

2.7. Effect of acute treatment with antidepressants in forced swim test

Mice were dosed acutely with antidepressant agents on day 14 post-BCG or saline treatment. All drugs were formulated in milli-Q water (10 ml/kg) and administered 30 min prior to testing except for nomifensine which was administered 1 h prior to the test. The antidepressants tested were as follows: Fluoxetine HCl (30 mg/kg, i.p.) and Escitalopram HBr (10 mg/kg, i.p.) (Jai Radhe Sales, India); Imipramine HCl (10 mg/kg, i.p.) and Nomifensine maleate (10 mg/kg, po) (Sigma–Aldrich, U.S.A); Duloxetine HCl (10 mg/kg, sc; Nosch Labs, Hyderabad, India). All the doses and routes of administration were chosen based on initial dose response studies conducted in naive BALB/c mice. In all the subsequent studies vehicle was administered intraperitoneally as either oral and subcutaneous routes of administration did not show any effect on immobility duration of mice (data not shown). Immediately after FST, brain and plasma samples were collected from representative animals to measure SERT occupancy and drug levels in brain and plasma compartments.

2.8. Determination of plasma and brain concentrations of escitalopram and fluoxetine

The concentrations of escitalopram and fluoxetine in plasma and brain samples were measured using an API 4000 LC-MS/MS triple quadrupole mass spectrometer (AB-Sciex, Ontario, Canada) using propranolol as internal standard. Plasma/brain tissue samples were treated with six times the sample volume of acetonitrile containing internal standard (IS), vortexed (3 min), centrifuged (3100 rpm for 3 min at 4 °C), and clear supernatant was collected in a collection plate. Analytes were chromatographically separated on Acquity (Waters, USA) BEH, 50 × 2.1 mm 1.7 μm column set at 40 °C using mobile phase A, consisting of 0.1% formic acid in water (MilliQ water) and mobile phase B, consisting of 0.1% formic acid in acetonitrile in gradient mode. Suitable ionization was achieved with Electro Spray Ionization in the positive ion mode at the ion spray interface temperature of 500 °C, using nitrogen as the nebulizing and heating gas. The ion spray voltage was 5500 kV for both the analytes. Escitalopram and fluoxetine were analyzed in the MRM mode using the transitions (Q1 → Q3 amu) m/z 325.3 → m/z 262.3 and m/z 310.3 → m/z 44, respectively. The concentrations of the analytes in plasma and brain samples were established by interpolation from the standard curve.

2.9. Measurement of serotonin (SERT) transporter occupancy

Brain tissue collected from FST subjects was cut into three pieces (two hemispheres and cerebellum-brain stem), snap frozen in dry ice-chilled isopentane and stored at –80 °C until assayed. Brain SERT occupancy was determined by incubating the brain homogenates with 4.5 nM \(^{3}H\)Citalopram for 10 min at 4 °C as described by Lengyel et al. (2008). Non-specific binding was determined using 10 μM of citalopram.

2.10. Statistical analysis

All data are presented as the mean ± SEM. Results from behavioral studies and body weight measurements were analyzed by either Two-Way ANOVA (FST, TST, LMA) or Two-Way ANOVA with repeated measures (body weight, VWR) followed by Bonferroni’s multiple comparison test. Results from SERT occupancy, SSRI exposure analysis, lung IDO activity and \(^{3}H\)PK11195 autoradiography were analyzed by unpaired t-test. All statistical analysis was done using Graph Pad Prism software version 5.0.2.

3. Results

3.1. Phenotypic characterization of mice after BCG administration

Administration of BCG to mice produced a marked decrease in body weight that was significant on day 1, maximal on days 2–3 and fully recovered by day 12 post BCG treatment (treatment: F(1,396) = 462.22, p < 0.0001; time: F(17,396) = 27.60, p < 0.001; treatment × time interaction: F(17,396) = 26.79, p < 0.001; Fig. 1A). Spontaneous LMA was also markedly reduced 24 h post-BCG treatment (treatment: F(1,53) = 76.45, p < 0.001; time: F(2,53) = 15.62, p < 0.001; treatment × time interaction: F(2,53) = 15.19, p < 0.001) an effect that was still observed on day 7 but fully recovered by day 14 post-BCG (Fig. 1B). Finally BCG treatment produced an immediate and profound decrease in VWR activity which was still significant on day 7 but fully recovered by day 14 post-BCG (treatment: F(1,156) = 32.17, p < 0.001; time: F(12,156) = 32.49, p < 0.001; treatment × time interaction: F(12,156) = 16.94, p < 0.001; Fig. 1C). These results indicate that BCG produces a rapid serum sickness response that
requires a 2 week period for body weight and spontaneous motor-related behaviors to fully recover to a level seen in control subjects. At this time point (day 14), BCG treatment produced a depression-like phenotype as indicated by a significant increase in the duration of immobility in the FST assay (Fig. 1D). Elevated immobility was also observed on days 21 and 28 but was no longer apparent on day 35 post BCG treatment (treatment: \(F(1,76) = 35.52, p < 0.001\); time: \(F(3,76) = 1.21, p = 0.31\); treatment \times time interaction: \(F(3,76) = 1.14, p = 0.34\)). Consistent with the effects seen in FST, BCG treatment also produced increased immobility in the TST assay on days 14 and 21 post-treatment (treatment: \(F(1,30) = 13.76, p < 0.001\); time: \(F(1,30) = 0.63, p = 0.43\); treatment \times time interaction: \(F(1,30) = 1.04, p = 0.75\); Fig. 1E).

While a detailed time course analysis of plasma cytokines was not conducted, an assessment of plasma IL6 and IFN-\(\gamma\) was made at selected days post-BCG treatment. We observed a significant increase in plasma IL6 on day 7 which was no longer significant on day 14 (treatment: \(F(1,20) = 103.94, p < 0.001\); time: \(F(1,20) = 73.95, p < 0.001\); treatment \times time interaction: \(F(1,20) = 73.95, p < 0.001\)). In contrast, a significant elevation of plasma IFN-\(\gamma\) was observed on both days 14 and 21 post-treatment (treatment: \(F(1,40) = 145.5, p < 0.001\); time: \(F(1,40) = 0.070, p = 0.79\); time \times treatment interaction: \(F(1,40) = 0.075, p = 0.79\); Table 1). We observed a significant elevation (12-fold; \(p < 0.01\)) (2.64 vs 31.7 nmoles/h/mg protein) in lung IDO activity on day 14, consistent with previous reports showing a correlation between the time course of development of depression-like behaviors and lung IDO activity (Moreau et al., 2008; Wichers et al., 2005).

3.2. Increased brain \([\text{H}]\text{PK11195}\) binding in BCG-treated mice

PK11195 is a ligand which binds selectively to the peripheral benzodiazepine receptor (also known as the mitochondrial 18 kDa translocator protein or TSPO). Radioisotope-labeled PK11195 has been used to examine brain glial activation and
neuro-inflammation in clinical and preclinical settings (Banati, 2002). As illustrated in Fig. 2, BCG-treated mice showed a significant \((p < 0.05)\) increase of \([3H]PK11195\) binding in the hippocampus and cortex regions (45%) indicating prominent neurino-inflammation on day 14 after BCG administration.

3.3. Effect of acute antidepressant treatment on BCG-induced depression-like behavior

A series of studies were conducted to examine the effects of acute treatment with different antidepressant drugs on the increased FST immobility behavior observed on day 14 post-BCG treatment. Consistent with the initial results reported above, significant increase in FST immobility were observed in BCG-treated mice compared to controls indicating the reproducibility of this effect (Fig. 3). In the first study the effects of treatment with the selective serotonin reuptake inhibitors (SSRIs) escitalopram (10 mg/kg, i.p.) and fluoxetine (30 mg/kg, i.p.) or the norepinephrine/dopamine reuptake inhibitor (NDRI) nomifensine (10 mg/kg, po) were examined. The dose of each antidepressant was chosen based on the literature support and our in-house dose response (DRC) studies. From the DRC a single minimum effective dose was chosen for subsequent pharmacological validation in BCG-treated mice studies. In control subjects, acute administration of all the three agents significantly reduced the duration of FST immobility compared to vehicle treatment (Fig. 3A). In contrast, while nomifensine retained efficacy in BCG-treated mice, a significant decrease in immobility was not observed in BCG mice treated with escitalopram or fluoxetine (treatment: \(F(3,89) = 34.38, p < 0.001\); BCG condition: \(F(1,89) = 40.44, p < 0.001\); treatment × BCG condition interaction: \(F(3,89) = 3.68, p < 0.05\); Fig. 3A).

In order to rule out any differences in drug exposure or target engagement, plasma and brain drug concentrations and serotonin transporter (SERT) occupancy were determined in a subset of behaviorally tested mice. The results show that drug concentrations and SERT occupancy were similar in both controls and BCG-treated mice and are unlikely to explain the differences in behavioral response to SSRIs (Table 2).

To further examine the lack of efficacy of acute SSRI treatment, we treated mice with fluoxetine (30 mg/kg, i.p.) and tested them in the TST assay. Consistent with previous results, BCG-treatment significantly increased immobility in mice tested 14 days after BCG treatment compared to controls (Fig. 3B). Further, while the expected antidepressant effect was observed in control mice, acute fluoxetine treatment failed to significantly decrease TST immobility in BCG-treated subjects consistent with the effects seen in

Table 1: Elevated plasma IL6 and IFN\(\gamma\) in BCG-treated mice.

<table>
<thead>
<tr>
<th>Plasma cytokines (pg/ml)</th>
<th>Group</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6</td>
<td>Saline</td>
<td>&lt;LLQ</td>
<td>&lt;LLQ</td>
<td>&lt;LLQ</td>
</tr>
<tr>
<td>BCG</td>
<td>100 ± 10***</td>
<td>8.3 ± 3.0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>IFN(\gamma)</td>
<td>Saline</td>
<td>ND</td>
<td>&lt;LLQ</td>
<td>&lt;LLQ</td>
</tr>
<tr>
<td>BCG</td>
<td>ND</td>
<td>354.3 ± 44.2***</td>
<td>371 ± 46***</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as the mean ± SEM plasma level of IL6 or IFN\(\gamma\) (\(n = 6–12\) /group) and were analyzed by 2-way ANOVA followed by Bonferroni’s multiple comparison test; ***\(p < 0.001\) compared to saline treatment. LLQ = Lower limit of quantification ND = not determined.

Fig. 2. Increased \([3H]PK11195\) binding in BCG-treated mouse. Representative brain sections showing \([3H]PK11195\) binding in brain samples collected 14 days after (A) saline or (B) BCG treatment. (C and D) Mean ± SEM \([3H]PK11195\) binding in prefrontal cortex and hippocampus regions respectively, (\(n = 4\) /group); * \(p < 0.05\) compared to saline group. Results were analyzed by unpaired t-test.
SSRI exposure and SERT occupancy are unchanged in BCG-treated mice.

Approximately 30% of MDD patients exhibit resistance to SSRIs. Despite advances in the depression research, mechanisms responsible for SSRI resistance are not fully understood, however, various reports have shown increased levels of proinflammatory cytokines in SSRI resistant patients (O’Brien et al., 2007; Maes, 1999; Eller et al., 2008). The condition of SSRI-resistant depression has become a highly unmet medical need and novel drug needs to be developed to treat this condition. A major challenge to these efforts is lack of preclinical models displaying resistance to SSRI-resistance associated with clinical features like elevated plasma cytokines. Results of the current study demonstrate that BCG-vaccine induces depression-like behaviour which is selectively resistant to SSRIs, which could potentially be employed to evaluate newer antidepressants in a cytokine mediated SSRI-resistant mouse model of depression.

Numerous reports demonstrate that either direct administration or induced elevation of pro-inflammatory cytokines elicits behavior in rodents that are often considered correlates of the symptoms seen in patients with major depressive disorder (O’Connor et al., 2009a,b). Using CD1 mice, Moreau et al. (2008) have demonstrated that acute injection of BCG-vaccine elicits brief period of serum sickness followed by delayed depression-like behavior. However, the effect of anti-depressants to alleviate the inflammation-induced depression-like behavior is not reported in pre-clinical studies. Our studies extend the neuroinflammation driven depression phenotype to BALB/C mice and establish the efficacy of various classes of antidepressants to attenuate the BCG-vaccine induced depression-like behavior in mice. We have observed the resolution of serum-sickness and depression phases by day-14 post-BCG treatment. Hence, the interpretation of effects of anti-depressants on day-14 post-BCG in FST could not be

FST (treatment: $F(1,35) = 13.5$, $p < 0.001$; BCG condition: $F(1,35) = 62.11$, $p < 0.001$; treatment $\times$ BCG condition interaction: $F(1,35) = 1.45$, $p = 0.24$; Fig. 3B).

Finally, we examined the effects of treatment with the tricyclic antidepressant imipramine (10 mg/kg, i.p.) or the serotonin/norepinephrine reuptake inhibitor (SNRI) duloxetine (10 mg/kg, sc). Both agents produced a significant decrease in FST immobility in control and BCG-treated mice (treatment: $F(2,74) = 29.12$, $p < 0.001$; BCG condition: $F(1,74) = 40.64$, $p < 0.001$; treatment $\times$ BCG condition interaction: $F(2,74) = 0.43$, $p = 0.653$; Fig. 4). The results suggest that the loss of acute FST antidepressant effect in BCG-treated mice is specific for the SSRI class of antidepressants.

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confounded symptoms of serum sickness phase. Our pharmacological validation demonstrated that BCG-vaccine induced depression-like behavior in mice is resistant to acute SSRI treatment but not to other classes of antidepressants.

Any challenge to the immune system produces an initial serum sickness effect driven by the elevation of pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β (Kelly et al., 2003; Moreau et al., 2008). In our study, we observed an early serum sickness behavior in mice characterized by decreased body weight, decreased spontaneous LMA and voluntary wheel running activity and robustly elevated plasma IL6. The dose of BCG-vaccine used in our study was relatively higher than earlier publications (Moreau et al., 2008 and O’Connor et al., 2009c). The earlier experimenters used 10² or 10⁶ CFU/mouse dose of BCG-vaccine. However, we did not observe significant serum sickness or depression phenotype with the 10⁶ CFU/mouse dose of BCG. This might be due to the difference in the mouse strain as well the source of BCG-vaccine used in this study. Complete recovery of sickness-related behaviors were relatively slow in the BCG-treated mice, requiring at least 2 weeks time to behave similar to saline treated control mice. In addition to IL6, we also observed a significant elevation of plasma IFNγ on days 14 and 21 post-BCG injection. IFNγ is thought to play a key role in the link between activation of the immune system and the emergence of depression-like behaviors (Moreau et al., 2005). It was hypothesized that elevated IFNγ leads to marked activation of peripheral and brain IDO levels (Maes et al., 2011). This leads to increased degradation of L-tryptophan via the kynurenine pathway resulting in decreased 5-HT synthesis and increased plasma kynurenine to tryptophan ratio (O’Connor et al., 2009c). In the current study we did not measure IDO activity, kynurenine and 5-HT in brain and looked at only lung IDO activity. However, an earlier preclinical report by Moreau et al. (2005) indicated a positive correlation between lung and Brain IDO activity. There are earlier reports linking increased neuroinflammation to brain IDO activity associated with increased plasma kynurenine to tryptophan ratio (Dobos et al., 2012; O’Connor et al., 2009c). We have observed increased [3H]PK11195 binding in BALB/c mice indicating prominent neuroinflammation. Therefore, in line with this literature precedence we hypothesize that increased lung IDO activity would have resulted in increased brain IDO activity followed by increased kynurenine to tryptophan ration in the brain.

BCG treatment reproduced a reproducible increase in immobility in both the FST and TST assays, effects occurring at a time when full recovery from serum sickness was achieved (day 14). Furthermore, investigation of the acute effect of antidepressant drug treatment showed that the SSRIs, escitalopram and fluoxetine, no longer produced a significant antidepressant effect in BCG-treated mice at doses that were effective in saline treated controls. In contrast other monoamine based antidepressants, namely duloxetine, norfipine and imipramine, retained efficacy in BCG-treated mice. Additional follow up studies clearly demonstrated that fluoxetine and escitalopram levels in plasma and brain samples were not significantly different in BCG-treated mice indicating that pharmacokinetic effects cannot explain these results. Furthermore, the levels of SERT occupancy achieved in BCG-treated mice dosed with escitalopram were not different from controls, achieving a level (i.e., > 80%) associated with antidepressant efficacy in MDD patients (Meyer, 2007). While SERT binding per se cannot explain these results, the impact of SERT inhibition on extracellular 5-HT levels was not investigated in these studies. However, the exact mechanism for SSRI-resistance may not be explained with existing data, but will be pursued upon. A recent report from Pratt et al. (2013) indicated attenuation of BCG-depression phenotype in tail suspension test (TST) using acute treatment with fluoxetine and desipramine in CD1 mice. Numerous methodological differences exist between these studies including mouse strain, behavioral testing methods, time points examined post-BCG treatment, and the dose of fluoxetine (56 mg/kg, i.p.) used. All classes of antidepressants are effective in acute treatment during pre-clinical studies, however, they require 2–3 weeks of treatment to elicit therapeutic effect in patients. Hence, our efforts are continued to evaluate the BCG-induced SSRI-resistance upon chronic treatment with SSRIs.

In summary, the present results demonstrate that single dose of BCG vaccine produces an early serum sickness response followed by the emergence of a pro-depressant behavioral phenotype that is associated with neuroinflammation. Preliminary characterization of antidepressant drug treatment indicates that the pro-depressant behaviors seen in BCG treated mice are selectively resistant to acute SSRIs administered at pharmacologically relevant doses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2014.06.205.

References


