Brain, Behavior, and Immunity 44 (2015) 176–186

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Advancing the understanding of behaviors associated with Bacille Calmette Guérin infection using multivariate analysis

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ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form 25 September 2014
Accepted 28 September 2014
Available online 7 October 2014

Keywords:
Sickness
Depression-like indicator
Cluster analysis
Principal component analysis
Discriminant analysis

ABSTRACT

Behavioral indicators in the murine Bacille Calmette Guérin (BCG) model of inflammation have been studied individually; however, the variability of the behaviors across BCG levels and the mouse-to-mouse variation within BCG-treatment group are only partially understood. The objectives of this study were: (1) to gain a comprehensive understanding of sickness and depression-like behaviors in a BCG model of inflammation using multivariate approaches, and (2) to explore behavioral differences between BCG-treatment groups and among mice within group. Adult mice were challenged with either 0 mg (saline), 5 mg or 10 mg of BCG (BCG-treatment groups: BCG0, BCG5, or BCG10, respectively) at Day 0 of the experiment. Sickness indicators included body weight changes between Day 0 and Day 2 and between Day 2 and Day 5, and horizontal locomotor activity and vertical activity (rearing) measured at Day 6. Depression-like indicators included duration of immobility in the forced swim test and in the tail suspension test at Day 6 and sucrose consumption in the sucrose preference test at Day 7. The simultaneous consideration of complementary sickness and depression-like indicators enabled a more precise characterization of behavioral changes associated with BCG-treatment and of mouse-to-mouse variation, relative to the analysis of indicators individually. Univariate and multivariate analyses confirmed differences between BCG-treatment groups in weight change early on the trial. Significant differences between BCG-treatment groups in depression-like behaviors were still measurable after Day 5. The potential for multivariate models to account for the correlation between behavioral indicators and to augment the analytical precision relative to univariate models was demonstrated both for sickness and for depression-like indicators. Unsupervised learning approaches revealed the complementary information provided by the sickness and depression-like indicators considered. Supervised learning approaches using cross-validation confirmed subtle differences between BCG-treatment groups and among mice within group identified by the consideration of sickness and depression-like indicators. These findings support the recommendation for multivariate and multidimensional analyses of sickness and depression-like indicators to augment the systemic understanding of the behavioral changes associated with infection.

1. Introduction

The relationship between inflammation and depression in humans and in animal models is well-established. Individuals receiving immunotherapies have a higher incidence of depressive symptoms (Capuron and Miller, 2011). Patients with major depressive disorders have higher levels of serum pro-inflammatory cytokines than healthy controls (Maes, 2011). Likewise, depressive phenotypes were observed in response to bacterial challenge (Brydon et al., 2008). These associations suggested that inflammation may result in depressive symptomatology mediated by neuroimmune mechanisms. Designed experiments using animal models are offering insights into the relationship between infection, inflammation, and depression-like indicators. Mice injected live

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http://dx.doi.org/10.1016/j.bbi.2014.09.018
0889-1591/

attenuated Bacille Calmette-Guérin (BCG) displayed high circulatory pro-inflammatory cytokines and indoleamine 2,3-dioxygenase activity. These mice exhibited sickness behaviors encompassing reduction in body weight and locomotor activity from Day 5 to Day 7. Likewise, challenged mice demonstrated depressive-like behaviors including lower mobility in the tail suspension test and in the Porsolt forced swim test, and lower sucrose intake in the sucrose preference test from Day 7 to Day 30 after treatment (Moreau et al., 2008; O'Connor et al., 2009). In addition, substantial mouse-to-mouse variation in response to BCG treatment was reported, including up to 30% of treated mice failing to exhibit adverse mobility effects (Platt et al., 2013).

Reductionist approaches based on the analysis of individual components have dominated the study of complex behavioral responses to infection. However, these reductionist approaches could have hindered the identification and characterization of systemic responses across multiple and typically correlated behaviors.

Six studies reported associations between BCG-treatment and sickness and depression-like behaviors in mice (Moreau et al., 2008; O'Connor et al., 2009; Kelley et al., 2013; Painsipp et al., 2013; Platt et al., 2013; Vijaya Kumar et al., 2014). In these studies, behavioral indicators were analyzed separately. This univariate approach could lead to substantially lower precision to detect differences between treatment groups, especially when the number of mice per group is low. Consideration of the covariation between indicators through multivariate approaches could enhance the precision to characterize smaller differences between treatment groups and the statistical significance of the BCG-treatment effect. Furthermore, the consideration of multiple indicators simultaneously could enhance the characterization of mouse-to-mouse variation and strengthen the identification of behavioral outliers. However, higher precision could come at the expense of higher number of parameters that need to be specified or estimated. Whether this trade-off results in a net benefit of better understanding the relationship between infection and sickness and depression indicators needs to be investigated.

The objectives of this study were: (1) to gain a comprehensive characterization of behavioral indicators in the BCG model of inflammation using multivariate approaches, and (2) to uncover behavioral differences associated with BCG-treatment level. Supporting activities include the consideration of complementary multidimensional approaches and study of mouse-to-mouse variation.

2. Materials and methods

2.1. Samples

Adult (12–14 weeks old) male C57BL/6J mice from the Charles River Laboratory were studied. Mice were housed in individual cages under a normal 12:12 h light/dark cycle in a temperature- (23 °C) and humidity- (45%) controlled room. Mice were offered water and food *ad libitum* (Teklad 8640 chow, Harlan Laboratories, Indianapolis, IN, USA) and handled daily for one week prior to the trial to ensure adaptation. Within the light cycle (lights on 10:00 PM–10:00 AM), behavioral tests began during the start of the dark phase under red lighting (O'Connor et al., 2009).

Three doses of the BCG strain of *Mycobacterium bovis* were studied. Live attenuated mycobacteria TICE BCG (50 mg wet weight of lyophilized culture containing 1×10^8 colony forming units or CFU/vial) was used (Organon USA Inc., USA). Vial reconstitution prior to inoculation used preservative-free saline and followed the provider's instructions. Individual mice were challenged once with either 10 mg/mouse (BCG10 group, $n = 5$), 5 mg/mouse (BCG5 group, $n = 6$) or sterile saline solution (BCG0 group, $n = 7$) at Day 0 of the experiment. Treatments were standardized to 0.3 ml/mouse and administered via intraperitoneal injection. Each mice was measured for the same set of sickness and depression-like indicators and thus, the measurements from 18 mice (5 mice BCG10 + 6 mice BCG5 + 7 mice BCG0) were analyzed. Experiments and measurements were implemented in accordance with the Animal Care and Use Program established by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee.

2.2. Sickness and depression-like indicators

The behavioral measurements are described in the sequence they were obtained. The measurements started early on the dark phase of the light cycle and behavioral experiments were performed during the first 7 h of the dark phase of the light cycle and followed established protocols (O'Connor et al., 2009; Lawson et al., 2013). Body weight changes following the BCG challenge was one indicator of sickness. Changes in body weight between Day 0 and Day 5 reflected the impact of infection on sickness through anorexia and modifications to metabolic homeostasis. Recovery from sickness was inferred from the subsequent increase in weight and similarity in locomotor activity and rearing between BCG treated and untreated mice at Day 6. Body weight was the first measurement and was recorded early in the dark phase of the light cycle. Daily measurements started on Day −1 to record the baseline weight. Locomotor activity measurements reflected the complementary impact of infection on sickness through fatigue and apathy for exploration. Horizontal movements (termed locomotor activity) and vertical locomotor activity (termed rearing) were measured at Day 6 in a novel cage using an established protocol for the open field method (O'Connor et al., 2009). Briefly, individual mice were placed in a standard acrylic cage including opaque walls and an insert dividing the floor into quadrants. The movements of the mice during 5 min were video recorded and counted by a trained observer that was blind to the treatment assignments. Locomotor activity was measured as the number of times the mouse crossed one of the grid lines with all four paws and rearing was measured as the number of times the mice stood on their hind legs either along a wall or independently (Brown et al., 1999).

Complementary depression-like indicators that reflect despair- and reward-based behaviors were measured. The duration of immobility in the tail suspension test and in the forced swim test at Day 6 were used as indicators of despair-based behaviors (Castagné et al., 2011). Sucrose intake in the sucrose preference test at Day 7 was used as indicator of anhedonia and a reward-based behavior (Strekalova et al., 2011).

The forced swim test followed the locomotor activity test (O'Connor et al., 2009). In the forced swim test, mice were placed in a cylinder containing 15-cm-high water that is approximately 23 °C. After placing the mice in the water, the activity was recorded for 6 min. The duration of immobility was measured during the final 5 min by a trained observer (O'Connor et al., 2009). Applying published protocols, the tail suspension test followed the forced swim test (O'Connor et al., 2009). Mice were suspended by their tails from a hanger linked to a load cell for 10 min. The force transducer detected movements and the seconds spent motionless or immobile per minute were automatically recorded using the Mouse Tail Suspension package (MED-TSS-MS; Med Associates Inc., St. Albans, VT, USA). The average time that a mouse remained motionless per minute between 3 and 8 min post suspension was used as an indicator of immobility to remove extreme behaviors at the start and end of the trial. The spacing between the sickness locomotor and depression despair measurements within a mouse was on average 2.4 h. The sucrose preference test was