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Disruption of microglia histone acetylation and protein pathways in mice exhibiting inflammation-associated depression-like symptoms

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Highlights

- IDO1 deficiency is associated with changes in the abundance of 14-3-3 (YWHA) proteins that are also associated with the tryptophan pathway.
- Proteins associated with BCG challenge are also associated with the alcoholism and systemic lupus erythematosus pathways.
- Microglia phagocytosis and gap junction communication pathways are associated with BCG challenge and IDO1 deficiency.
- Histone acetylation is a prevalent epigenetic post-translational modification associated with BCG challenge and IDO1 deficiency.
- The present study furthers the understanding of the changes in protein levels, pathways and histone acetylation that correspond with inflammation-associated depression.

Abstract

Background: Peripheral immune challenge can elicit microglia activation and depression-related symptoms. The balance of inflammatory signals in the tryptophan pathway can skew the activity of indoleamine-pyrrole 2,3 dioxygenase (IDO1) towards the metabolization of tryptophan into kynurenine (rather than serotonin), and towards neuroprotective or neurotoxic metabolites. The proteome changes that accompany inflammation-associated depression-related behaviors are incompletely understood.

Methods: The changes in microglia protein abundance and post-translational modifications in wild type (WT) mice that exhibit depression-like symptoms after recovery from peripheral Bacille Calmette-Guerin (BCG) challenge were studied. This WT_BGG group was compared to mice that do not express depression-like symptoms after BCG challenge due to IDO1 deficiency by means of genetic knockout (BCG_KO group), and to WT Saline-treated (Sal) mice (WT_Sal group) using a mass spectrometry-based label-free approach.

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Results: The comparison of WT_BCG relative to WT_Sal and KO_BCG mice uncovered patterns of protein abundance and acetylation among the histone families that could influence microglia signaling and transcriptional rates. Members of the histone clusters 1, 2 and 3 families were less abundant in WT_BCG relative to WT_Sal whereas members in the H2A family exhibited the opposite pattern. Irrespective of family, the majority of the histones were less abundant in WT_BCG relative to KO_BCG microglia. Homeostatic mechanisms may temper the potentially toxic effects of high histone levels after BCG challenge to levels lower than Sal. Histone acetylation was highest in WT_BCG and the similar levels observed in WT_Sal and KO_BCG. This result suggest that histone acetylation levels are similar between IDO1 deficient mice after immune challenge and unchallenged WT mice. The over-abundance of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins (14-3-3 series) in WT_BCG relative to KO_BCG is particularly interesting because these proteins activate another rate-limiting enzyme in the tryptophan pathway. The over-representation of alcoholism and systemic lupus erythematosus pathways among the proteins exhibiting differential abundance between the groups suggest that these disorders share microglia activation pathways with BCG challenge. The over-representation of phagosome pathway among proteins differentially abundant between WT_BCG and KO_BCG microglia suggest an association between IDO1 deficiency and phagocytosis. Likewise, the over-representation of the gap junction pathway among the differentially abundant proteins between KO_BCG and WT_Sal suggest a multifactorial effect of BCG and IDO1 deficiency on cell communication.

Conclusions: The present study of histone acetylation and differential protein abundance furthers the understanding of the long lasting effects of peripheral immune challenges. Our findings offer insights into target proteins and mechanisms that provide clues for therapies to ameliorate inflammation-associated depression-related behaviors.

Keywords: indoleamine-pyrrole 2,3 dioxygenase, Bacille Calmette Guerin, epigenetics, mass spectrometry, phagosome, 14-3-3 proteins

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1. Introduction

Behavioral and molecular studies are uncovering the relationship between immune challenge, neuroinflammation, and depression behaviors (Dantzer et al., 2008; O'Connor et al., 2009). Peripheral immune challenge can activate microglia, the innate immune cells of the brain. Changes in cytokines and other cell signals can dysregulate the tryptophan-kynurenine metabolic pathway that is associated with depression-like behaviors. Mice challenged peripherally with Bacille Calmette-Guerin (BCG) exhibit sickness symptoms reflected by weight loss early in the first 2 days after challenge followed by recovery of weight by day 5 post challenge (Rodriguez-Zas et al., 2015). Despite sickness recovery, mice continue to display depressive-like behaviors up to one month post-challenge (O'Connor et al., 2009; Rodriguez-Zas et al., 2015). The microglia transcriptome remains dysregulated at day 7 after BCG challenge including genes associated with immune response and behavior consistent with symptoms of depression (Gonzalez-Pena et al., 2016a). All together, these findings support the use of BCG-challenged mice post-sickness recovery as a model to study the molecular mechanisms of inflammation-associated depression.

Indoleamine-pyrrole 2,3 dioxygenase (IDO1) is a tryptophan metabolizing enzyme that has major influence on inflammation-induced depression (Dantzer et al., 2008). IDO1 participates in the first step of the kynurenine pathway, catabolizing tryptophan into kynurenine instead of being available as precursor for serotonin production elsewhere in the brain. In turn, kynurenine can be metabolized into neuroprotective kynurenine acid or into neurotoxic 3-hydroxykynurenine and quinolinic acid (Kim et al., 2012). Inflammatory conditions enhance the activity of IDO1 in the microglia, skewing the metabolic pathways in the brain towards the production of neurotoxic metabolites rather than serotonin. Under these conditions mice exhibit depression-like behaviors (Dantzer et al., 2011).

Pharmacological inhibition of IDO1 as well as studies of genetic deletion such as IDO1 knockout have demonstrated that this enzyme plays a key role in the transition from sickness-related to depression behaviors (Nixon et al., 2015; O'Connor et al., 2009). Despite the similarity between wild type IDO1

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knockout mice in the display of sickness symptoms after peripheral lipopolysaccharide (LPS) challenge and in the transcriptome profile (Gonzalez-Pena et al., 2016b), IDO1 knockout mice do not display depression-like symptoms after immune challenge (Lawson et al., 2013). This feature makes IDO1-deficient mice a useful model to study the mechanisms associated with the development of inflammation-associated depression. We have previously compared the microglia transcriptome between wild type and IDO1-deficient mice and the transcriptome changes in response to BCG challenge (Gonzalez-Pena et al., 2016a; Gonzalez-Pena et al., 2016b). The corresponding disruptions in protein levels and post-translational modifications (PTMs) associated with inflammation-related depression remain partially understood.

The present study contributes a missing piece to understand the molecular mechanism linked with inflammation associated depression-like symptoms. Our objective was to detect and characterize persistent microglia changes in protein abundance and PTMs in response to BCG. The impact of BCG challenge after sickness recovery and of IDO1 deficiency in the mouse microglia proteins, acting alone or in combination, were evaluated. Functional analysis supported the identification of pathways and epigenetic mechanisms predominantly disrupted by these factors.

2. Materials and Methods

2.1. Animal experiments

Animal care and experimental procedures followed the guidelines listed in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health and were approved by the University of Illinois Institutional Animal Care and Use Committee. The present study characterized the proteins from the microglia of adult (approximately 22 weeks old) male C57Bl/6J wild type mice (WT genotype group) and IDO1-KO (C57Bl/6J background) mice (KO genotype group). A description of animal experiments and sample collection is provided in the supplementary materials Appendix. Mice management and BCG challenge protocols followed procedures described on the study of gene expression profiles from the same

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samples (Gonzalez-Pena et al., 2016a; Gonzalez-Pena et al., 2016b; Nixon et al., 2015). Succinctly, twelve WT and twelve KO mice were intraperitoneally injected 10 mg/mouse of BCG (BCG treatment group) and twelve WT mice were injected with sterile saline solution (Sal treatment group). Previous work offered evidence that WT mice display depression-like behaviors by day 7 post challenge (Rodriguez-Zas et al., 2015) whereas KO mice do not display these behaviors (Lawson et al., 2013). Following euthanasia by CO_2, mice were perfused, brains were excised and minced, and microglia was collected separately from each brain following established protocols (Nixon et al., 2015). Brains were trypsinized, dissociated, centrifuged, re-suspended in 30% Percoll (GE Healthcare, Princeton, NJ), and centrifuged to remove myelin. After the labelling of brain cells with anti-CD11b (integrin alpha M antibody) magnetized Miltenyi microbeads microglia cells were separated using a magnetic field and MS columns (Miltenyi Biotec, Germany). The $Cd11b^+$ fraction collected representing microglia cells was centrifuged, re-suspended, and stored at -80°C.

Three groups of mice representing two IDO1 genotypes (WT or KO) and two treatments (BCG or Sal) were studied: WT_Sal, WT_BCG, and KO_BCG. Prior analysis indicated negligible transcriptome differences between the WT_Sal and KO_Sal microglia and therefore the comparison of proteins is omitted (Gonzalez-Pena et al., 2016b). Each genotype-treatment group included individual microglia samples from 12 mice totaling 36 samples.

2.2. Proteomics experiment

A bottom-up proteomics approach was employed for protein detection and protein extraction followed our proven protocols using cold acetone precipitation. A detailed description of the proteomic experiments is provided in the supplementary materials Appendix. Briefly, the microglia from individual mice was thawed and centrifuged for 3 min at 200 $\times g$. The supernatant was removed, the pellet washed with Hank's Balanced Salt Solution (HBSS, Life Technologies Thermo Fisher Scientific, MA). SDS-PAGE loading buffer resuspension and regular single channel Gel Elution Liquid Fraction Entrapment

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Electrophoresis (GELFrEE 8100 Fractionation System, Expedeon, Cambridgeshire, UK) (Tran and Doucette, 2008) was used to fractionate the proteome to reduce the interference of high-abundance proteins and methanol/chloroform/water extraction was used to remove SDS. The first two fractions containing proteins of approximately 3.5 to 30 kDa were pooled into one fraction cluster and all the remaining fractions were pooled into another fraction cluster. Each fraction cluster from four mice within a genotype-treatment group were pooled to ensure adequate protein abundance for accurate detection, identification, and quantification. This protocol resulted in three independent protein samples per genotype-treatment group.

Overall, 18 samples corresponding to 3 genotype-treatment groups x 2 faction clusters x 3 independent samples were analyzed using a 12 T linear ion trap-Fourier transform ion cyclotron mass spectrometer (LTQ-FT ICR Ultra, Thermo Fisher Scientific, MA) interfaced with a 1D plus NanoLC pump from Eksigent Technologies (Dublin, CA). Proteins were identified from the mass spectra using the software PEAKS v7 (Zhang et al., 2012) and the *Mus musculus* genome (GRCm38.p4) database.

2.3. Differential Protein Abundance, Functional and Network Analyses

Protein abundance was quantified using the total number of mass spectra from all peptides corresponding to an identified protein in a sample. Negative Binomial and Poisson distributions of the spectra number were evaluated. Protein abundance was described using a Negative Binomial distribution and analyzed using a generalized linear model including the fixed effects of genotype-treatment group and fraction cluster and the random block effect of biological sample. The False Discovery Rate (FDR) method was used to adjust for multiple testing of genotype-treatment effects across proteins. The analysis of protein abundance was implemented using the PROC GLIMMIX procedure in SAS release 9.2 (SAS Institute, Cary, NC).

The identification of functional categories over-represented among differentially abundant proteins enabled the identification of molecular mechanisms predominantly disrupted among groups. Functional terms studied among the differentially abundant proteins without post-translational modifications included

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Gene Ontology (GO) biological processes (BPs) and molecular functions (MFs), and KEGG pathways. Proteins were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (Huang et al., 2009). The *Mus musculus* genome was used as background for testing and the Direct GO terms in DAVID were used to benefit from the enhanced category identification in the DAVID repository. Consideration of differential abundance at P-value<0.1 enabled the identification of mechanisms including strong and borderline changes in abundance. Enrichment of individual categories was assessed using Expression Analysis Systematic Explorer (EASE) score computed based on a one-tailed jackknifed Fisher hypergeometric exact test (Serao et al., 2011; Serão et al., 2013). The clustering of functional categories facilitated the interpretation of enriched processes. The statistical significance of the functional cluster corresponds to the geometric mean of the -log10 EASE scores of the categories within each cluster (Delfino and Rodriguez-Zas, 2013; Delfino et al., 2011; Gonzalez-Pena et al., 2016a; Gonzalez-Pena et al., 2016b).

Investigation of histone variants and PTMs was aided by the epigenetic resource Histome (Khare et al., 2012). Complementary investigation of the interactions between the proteins differentially abundant between genotype-treatment groups relied on the software STRING version 10 (Jensen et al., 2008). The *Mus musculus* genome background and default settings were used. The circular nodes in the network denote proteins that exhibit differential abundance between genotype-treatment group and the edges denote the relationships between these proteins in the STRING databases. The thicker the edge between proteins, the higher number of databases (including co-occurrence, co-expression, experiments, gene fusion, text mining repositories) supporting an interaction, and the higher confidence on the interaction between proteins (von Mering et al., 2005). The connectivity and modularity of the genotype-treatment contrast networks were compared using the metrics: a) average node degree (number of edges per node) in the network; b) clustering coefficient that indicates the degree of connectedness of the nodes in the network; and c) expected number of network edges when the nodes are selected at random.

3. Results

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Across groups, the mass spectrometry-based label-free analysis detected 1003 protein accession identifiers in the National Center for Biotechnology Information (NCBI) repository that corresponded to 501 non-redundant protein names. The number of proteins differentially abundant at FDR-adjusted P-value < 0.05 in the contrasts KO_BCG-WT_Sal, WT_BCG-KO_BCG, and WT_BCG-WT_Sal were 19, 18, and 9, respectively. Tables 1 to 3 list the top differentially abundant proteins (P-value < 0.05) in the contrasts: WT_BCG-WT_Sal (32 proteins), WT_BCG-KO_BCG (48 proteins), and KO_BCG-WT_Sal (65 proteins), respectively. Complementary confirmation of the statistical power of the relative quantitation was offered by housekeeping proteins that were not differentially abundant in either group contrast. Among suggested housekeeping markers also detected in the samples (Lee et al., 2016), albumin (ALB), serine and arginine rich splicing factor 1 (SRSF1), and vesicle-associated membrane protein, associated protein A (VAPA) were not differentially abundant between the three genotype-treatment groups compared. Proteins differentially abundant in a single genotype-treatment contrast are presented in Appendix A. The most frequent protein profiles spanning multiple contrasts are explored.

[Tables 1, 2, and 3]

3.1. Proteins more abundant in BCG-treated wild type mice relative to other groups

The microglia proteins exhibiting the pattern WT_BCG>WT_Sal>KO_BCG (Tables 1 to 3) included: actinin, alpha 1 (ACTN1), histone cluster 1 member HIST1H2AH, nucleolin (NCL), and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ). The transcriptome comparison between the WT_BCG and WT_Sal microglia samples (Gonzalez-Pena et al., 2016a) corroborated the profiles of NCL (log2 fold change = 0.16) and YWHAZ (log2 fold change = 0.08). Multiple other proteins in the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein family (YWHA proteins or 14-3-3 series) were more abundant in WT_BCG than KO_BCG. These proteins

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included YWHA–theta, -zeta, epsilon, and gamma polypeptide (YWHAQ, YWHAZ, YWHAE, and YWHAG).

Another group of proteins that exhibiting the pattern WT_BCG>KO_BCG and WT_Sal>KO_BCG (Tables 2 and 3) included: actinin, alpha 4 (ACTN4), calnexin (CANX), heterogeneous nuclear ribonucleoprotein A1-like 2, pseudogene 2 (GM5803 or Hnrnpa1l2-ps2), histone cluster 1 member HIST1H2AB, heat shock protein 90 members HSP90AB1 and HSP90B1, heat shock protein family A (HSPA8), karyopherin subunit beta 1 (KPNB1), keratin 77 (KRT77), lamin B1 (LMNB1), and tubulins TUBA1A, TUBB2A, and TUBB2B.

3.2. Proteins differentially abundant in saline- relative to BCG-treated mice

In the microglia, integrin alpha M *(*ITGAM) exhibited the pattern WT_BCG>WT_Sal and KO_BCG>WT_Sal with similar abundance between genotypes in BCG-treated mice (Tables 1 and 3). The proteins that exhibited the pattern WT_Sal>WT_BCG and WT_Sal>KO_BCG (Tables 1 and 3) included: histone cluster 1 members HIST1H2AD and HIST1H2AP, keratin 79 (KRT79), myelin basic protein (MBP), NUMA1. The WT_BCG-WT_Sal pattern of the latter proteins was corroborated by the corresponding mRNA pattern (Gonzalez-Pena et al., 2016a): Mbp (log2 fold change = -0.83) and Numa1 (log2 fold change = -0.06). The lower abundance of KRT79 in BCG relative to Sal-treated mice is consistent with the pattern of KRT2, KRT1 and KRT10 (Tables 1, 2, and 3). The WT_BCG<WT_Sal pattern of the latter keratins was confirmed by transcriptome analysis of the same mice (Gonzalez-Pena et al., 2016a): Krt10 (log2 fold change = -0.25) and Krt1 (log2 fold change = -0.59).

3.3. Differential abundance of histones between BCG challenge and IDO1 genotypes levels

Many members of various histone families exhibited differential abundance between the microglia groups studied (Tables 1, 2, and 3). Members of the histone clusters 1, 2 and 3 families tended to be less abundant in WT_BCG relative to WT_Sal whereas members in the H2A family exhibited the opposite

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profile. Overall, the majority of the histones across families (cluster 1, cluster 2, H2A) were more abundant in KO_BCG than in WT_BCG mice. These patterns were corroborated in a transcriptome analysis (Gonzalez-Pena et al., 2016a). The corroborating genes and expression fold change between WT_BCG and WT_Sal profiled on the same mice (Gonzalez-Pena et al., 2016a) were: Hist1h2br (log2 fold change = 2.40); Hist2h2aa2 (log2 fold change = -0.36); Hist3h2a (log2 fold change = -0.15); Hist1h2ae (log2 fold change = -0.26).

3.4. Additional proteins differentially abundant between levels of BCG challenge or IDO1 genotype

The proteins that exhibited lower abundance in WT_BCG relative to WT_Sal microglia (Table 1) included: protein disulfide isomerase 3 (PDIA3), Parkinson disease (autosomal recessive, early onset) 7 (PARK7), dihydropyrimidinase-like 2 (DPYSL2), actin gamma 2 (ACTG2). The WT_BCG - WT_Sal pattern of some of these proteins was consistent the mRNA patterns. The study the mRNA profiles the same samples supported the observed protein patterns for Park7 (log2 fold change = -0.18) and Dpysl2 (log2 fold change =-0.50).

The proteins that exhibited lower abundance in WT_BCG relative to KO_BCG microglia (Table 2) included: calmodulins (CALM 1, 2 and 3), SH3 domain binding glutamic acid-rich protein-like 3 (SH3BGRL3), and CDK5 regulatory subunit associated protein 2 (CDK5RAP2). The patterns of CALM, SH3BGRL3 and CDK5RAP2 are consistent with previous reports. CALM1 was less abundant in post mortem brains from patients suffering major depressive disorder relative to controls (Martins-de-Souza et al., 2012). The abundance of SH3BGRL3 was lower in the corpus callosum of patients with schizophrenia than control (Saia-Cereda et al., 2015). Likewise the expression level of the CDK5 gene modulates cAMP/protein kinase A (PKA) signaling, dopaminergic neurotransmission, and depression-related behaviors (Liu et al., 2016).

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The microglia proteins that exhibited the abundance pattern WT_BCG<WT_Sal<KO_BCG (Tables 1, 2, and 3) included: H2A histone family, member Y (H2AFY) and histone cluster 1 members HIST1H2AF, HIST1H2AI, and HIST2H2AA2. The proteins that exhibited a similar pattern with WT_BCG<WT_Sal and WT_BCG<KO_BCG (Tables 1 and 2) included: cofilin (CFL1), H2A histone family, member J (H2AFJ), histone cluster 1 members HIST1H2AL and HIST1H2AO, and keratin 10 (KRT10). Also exhibiting the comparable pattern with KO_BCG>WT_Sal and KO_BCG>WT_BCG (Tables 2 and 3) are the proteins: annexin A3 (ANXA3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), H2A histone family member X (H2AFX), histone cluster 2 member HIST2H2AB, high mobility group AT-hook 1B (HMGA1-RS1), myosin heavy chain 9 (MYH9), SET nuclear proto-oncogene (SET), and YWHAE.

3.5. Pathways and networks of proteins impacted by immune challenge and IDO1 genotype

A major finding from the functional analysis is the enrichment of the KEGG pathways "alcoholism" and "systemic lupus erythematosus" among the proteins differentially abundant on all three genotype-treatment contrasts (Table 4). The pathways "phagosome" and "gap junction" and the molecular function "structural molecule activity" were over-represented among the proteins differentially abundant between WT_BCG and KO_BCG, KO_BCG and WT_Sal, and WT_BCG and WT_Sal, respectively.

[Table 4]

The networks of differentially abundant proteins in the three contrasts were reconstructed and the topology studied to understand the association between BCG challenge, IDO1-deficiency, and protein interactions. Appendix Fig. A, B, and C depict the networks of differentially abundant proteins in the contrasts WT_BCG-WT_Sal, WT_BCG-KO_BCG, and KO_BCG-WT_Sal, respectively. Appendix Fig. D. summarizes the topology metrics that characterize each network.

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The differentially abundant proteins in the WT_BCG-WT_Sal microglia were both fewer (Table 1) and substantially less connected in absolute and relative terms (Fig. A) than the differentially abundant proteins in the contrast WT_BCG-KO_BCG (Fig. B). This finding is supported by network metrics including fewer number of edges, fewer average number of edges per node, higher local or modular clustering, and higher total expected number of edges in the WT_BCG-WT_Sal relative to the WT_BCG-KO_BCG network.

3.6. Post-translational modification of histones associated with immune challenge and IDO1 genotype

Histones are key epigenetic regulators and many of these proteins exhibited differential abundance between the three BCG challenge-IDO1 genotype groups studied (Tables 1 to 3). The present study is first to characterize the association between BCG treatment, KO genotype and the relative prevalence of different histone types and the incidence of the PTM acetylation using the molecular fragmentation patterns of the mass spectra. Table 5 summarizes the absolute (total) and relative (%) number of histone peptides detected and the number of histone peptide mass spectra supporting the detections partitioned by type of histone (replication-independent and -dependent), acetylation PTM evidence (yes or no) and genotype-treatment group. Table 6 presents the distribution of histone families by acetylation PTM and genotype-treatment group. The majority of the histones were more abundant in the microglia of WT_Sal relative to WT_BCG mice (Table 1) and in KO_BCG relative to WT_BCG mice (Table 2). Supporting these results, the total number of histone peptides and peptide mass spectra were higher in WT_Sal and in KO_BCG relative to BCG WT microglia (Table 5).

[Tables 5 and 6]

An unexpected finding was the opposite trend between the total number of histone peptides and the number of peptides and peptide mass spectra exhibiting acetylation among the replicate-dependent histones. The WT_BCG microglia had the highest number of acetylated peptides from replicate-dependent histones and lowest number of histone peptides relative to WT_Sal and KO_BCG microglia (Table 5). No

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acetylation of replicate-independent histones was observed in any of the three genotype-treatment groups. Tables 1 to 3 and 5 list the names of the replication dependent and independent and histones.

Most of the replication-dependent histones detected, irrespective of acetylation, pertained to the histone 1 cluster, followed by the histone 2 cluster, the histone 4 cluster and the histone 3 cluster (Table 6). No evidence of histone 3 cluster acetylation was detected in either genotype-treatment group. Due to the low number of histone 4 and histone 3 cluster peptides detected, further interpretation focuses on the first two clusters.

Notable, our novel results demonstrate an interaction between the effects of KO genotype and BCG challenge both on the acetylation level of each histone cluster and also on the relative abundance among the non-acetylated histone cluster peptides. The WT_BCG microglia had the lowest percentage of non-acetylated histone 1 cluster peptides yet the highest percentage of acetylated histone 1 cluster peptides. On the other hand, WT_BCG microglia had the highest percentage of non-acetylated and acetylated histone 2 cluster peptides. The microglia from WT_BCG mice had the lowest percentage of non-acetylated histone 4 cluster peptides whereas KO_BCG mice had the highest percentage of non-acetylated histone 3 cluster peptides.

4. Discussion

The present characterization of differences in microglia proteins between the genotype-treatment groups WT_BCG, KO_BCG and WT_Sal furthers the understanding of the molecular mechanisms underlying inflammation-associated depression-like behaviors. Depression-like behaviors were detected at day 7 after BCG treatment in WT mice (Rodriguez-Zas et al., 2015), whereas KO mice did not exhibit these behaviors after challenge (Lawson et al., 2013). The identification of differentially abundant proteins in the contrast WT_BCG-WT_Sal (Table 1) provides a baseline of protein levels that remain disrupted in the microglia after sickness recovery and could be associated with persistent depression-like symptoms (Rodriguez-Zas et al., 2015). The identification of differentially abundant proteins in the contrast

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WT_BCG-KO_BCG microglia that is deficient in IDO1 (Table 2) enhances the understanding of the association between IDO1 and depression-like symptoms after recovery from sickness. Lastly, the identification of differentially abundant proteins in the contrasts KO_BCG-WT_Sal (Table 3) facilitates the molecular characterization of the joint action of both factors, genotype and treatment.

4.1. Protein profiles characteristic of BCG-treated and IDO1 genotype groups

The highest number of differentially abundant proteins corresponded to the contrast KO_BCG-WT_Sal contrast (Table 3) and this result speaks to possible synergistic effect of IDO1 deficiency and BCG challenge. Many of these differentially abundant proteins including those pertaining to the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation and histone families have been associated with brain inflammation, microglia activation and depression-related symptoms in studies of neurological disorders including Alzheimer's disease and schizophrenia (Monji et al., 2009; Solito and Sastre, 2012).

Among the proteins more abundant in BCG-treated wild type mice relative to other groups, are members of the 14-3-3 (YWHA) series. This finding is directly relevant to the IDO1 deficient genotype studied, because 14-3-3 proteins activate tryptophan hydroxylases (TPH1 and TPH2) and tyrosine hydroxylase (TH). In the neuronal tryptophan pathway, TPH2 is a rate-limiting enzyme in the synthesis of serotonin whereas TH is a key enzyme in the synthesis of dopamine. The levels of 14-3-3 genes and of serotonin and dopamine have been associated with depression and neurological disorders that include depression comorbidities (Zainullina et al., 2016). Sixteen days post-BCG inoculation mice exhibited depression symptoms, higher kynurenine/tryptophan ratio and lower dopamine levels in the hippocampus relative to control. These changes were accompanied by no change in kynurenine/tryptophan and dopamine levels in the prefrontal cortex, and no change in serotonin levels in either brain region (Kwon et al., 2012). Also within the tryptophan pathway, IDO1 is a rate-limiting enzyme that metabolizes tryptophan into kynurenine products that can be neurotoxic and augment the likelihood and severity of depression-like symptoms in mice (O'Connor et al., 2009).

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In agreement with the present study, several 14-3-3 proteins (YWHAB, YWHAH, YWHAG, YWHAZ) exhibited lower abundance in the corpus callosum of schizophrenic patients relative to controls (Saia-Cereda et al., 2015). Schizophrenia diagnosis is frequently correlated with depression or mania and has been linked to microglia activation (Saia-Cereda et al., 2015). Moreover, the pattern observed in 14-3-3 proteins is confirmed by the similar patterns observed in heat shock proteins (HSPs) because 14-3-3 and HSP molecules tend to bind (Satoh et al., 2005). The over-abundance of HSP in WT_BCG relative to KO_BCG is in agreement with reports of higher levels of these proteins associated with depression and activated microglia (Miller and Raison, 2016; Van Noort, 2008). The abundance of damage-associated molecular pattern (DAMP) proteins including HSP and histones is consistent with response to BCG challenge.

An interpretation of a molecular mechanism associated with inflammation-associated depression is put forward; this interpretation builds on the observed protein profiles associated with BCG and IDO1 KO and in concert with reports of the association between 14-3-3 proteins, IDO1 and TPH2 activity, inflammation and depression. We propose that the balance between serotonin and kynurenine metabolites in the brain that is correlated with depression symptoms is also associated with the balance between the activity of the enzymes TPH in neurons and IDO1 in the microglia. A likely repercussion of the increase in 14-3-3 proteins triggered by BCG-related inflammatory signals is the increase in TH and THP2 activity that supports the brain's capability to compensate for the inflammation-induced decrease synthesis of monoamines in response to BCG. Moreover, the heightened activity of TPH and TH would not only compensate for the heightened activity of IDO1 triggered by inflammation signals but also for the dysregulation of additional factors in the dopamine or serotonin pathways triggered by inflammatory signals. Supporting this proposition, the metabolism of phenylalanine into tyrosine and of tyrosine into dopamine is impaired because of the decreased availability of tetrahydrobiopterin, an important cofactor for phenylalanine hydroxylase and tyrosine hydroxylase (Sperner-Unterweger et al., 2014). This bottleneck could be partly compensated by increased activity of TH bolstered by 14-3-3 proteins. In a similar way, an

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increase in TPH activity can compensate for deficits in the bioavailability of tryptophan (due to inflammation induced IDO1 hyperactivity) and can attenuate the reduction in serotonin levels.

The higher levels of 14-3-3 proteins in WT_BCG than in WT_Sal and KO_BCG microglia could be attributed to the competition for tryptophan between the 14-3-3 proteins and IDO1 shortly after BCG challenge. The activation of IDO1 in response to inflammation would reduce the availability of tryptophan for the 14-3-3 proteins. Instead, higher 14-3-3 protein levels are observed in this study because proteins were profiled 7 days after infection and at this stage IDO1 levels should be falling to pre-challenge levels. The same would be applicable for the KO_BCG group at day 7 post infection. Our data suggest that the abundance of the 14-3-3 activators of the TPH enzyme in the WT mice relative to IDO1 KO at day 7 after BCG challenge could be critical to the recovery from the depression-like symptoms by promoting serotonin over kynurenine production.

The higher abundance of NCL, LMNB1, KPNB1, and ACTN1 (Tables 1 to 3) in the microglia of WT_BCG mice displaying depression-like symptoms relative to KO_BCG is in accord with other studies of disorders correlated with depression. A duplication of the gene coding for LMNB1 is associated with a neurological disorder involving depression, autonomic dysfunction and cognitive impairment (Dos Santos et al., 2012). KPNB increases in response to treatment with Lithium treatment, a mood stabilizer that is used to treat, among others, depression (Bosetti et al., 2002). NCL interacts with protein p42IP4/centaurin-alpha 1 in the brains of Alzheimer's patients that often exhibit depression comorbidity (Reiser and Bernstein, 2004). Likewise, ACTN1 is differentially expressed in the frontal cortex of patients diagnosed with HIV and meningitis (Selvan et al., 2015) and these conditions exhibit depression comorbidity (Carmo et al., 2001).

The abundance of tubulins (tubulin, beta 2A, tubulin, beta 2B, tubulin, alpha 1A) in the WT_BCG-KO_BCG contrast is consistent with reports that Tuba1c (and other differentially abundant proteins in our study including ENO1B, ANXA3, HNRNPK, HSPs) were more abundant in the prefrontal cortex of a rat model of depression characterized by chronic unpredictable mild stress relative to control (Yang et al.,

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2013). Also, tubulin proteins were more abundant in the post mortem brain of patients diagnosed with major depressive disorder (Martins-de-Souza et al., 2012) and patients diagnosed with schizophrenia that can be accompanied by depression and microglia activation (Saia-Cereda et al., 2015).

Among the proteins that were differentially abundant in saline- relative to BCG-treated mice, the under-expression of ITGAM in Sal relative to both BCG-treated groups was in agreement with reports that inactivation of ITGAM hinders the phagocytosis of *S. aureus* in microglia (Weinstein et al., 2015). The lower abundance of keratins in BCG relative to Sal-treated groups is consistent with findings that microglia challenged with LPS exhibited lower keratin abundance than control (Kim et al., 2007; Pu et al., 2015). Likewise, the lower abundance of MBP in WT_BCG relative to WT_Sal microglia is consistent with the reports of MBP decline in the white matter of neonatal rats that exhibited acute brain inflammation after LPS exposure (Pang et al., 2010). Also, lower levels of MBP were reported in patients diagnosed with schizophrenia relative to controls (Saia-Cereda et al., 2015). Several histones, keratins and CFL were less abundant in WT_BCG whereas several actinins, integrins and NCL were more abundant in WT_BCG relative to WT_Sal and KO_BCG (Tables 1 and 2). For these proteins, the IDO1 deficiency may have an antagonistic effect, cancelling some of the effects of BCG at day 7 post challenge and thus the KO_BCG patterns are more similar to the WT_Sal microglia.

Histones exhibited differential abundance between BCG and Sal-treatment and between KO and Sal genotype groups. Histones are chromatin structural proteins that regulate DNA transcription. The participation of these proteins in inflammation-associated depression behaviors is likely because macrophages and microglia mount rapid response to signals elicited in response to immune challenge including the release of histones and HSPs (Perry et al., 2010). The observed protein profiles are consistent with reports that many histones (including HIST1H4A and H3F3C) exhibited lower abundance in the corpus callosum of patients with schizophrenia than control and this neurological disorder is associated with microglia activation (Saia-Cereda et al., 2015).

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Evidence that histones can be released into the extracellular space in response to stress or physical challenges is accumulating. This influx could have toxic and proinflammatory effects leading to cell damage or death. Conversely, treatments that inactivate or neutralize histones can have protective effects and ameliorate the possible negative effects of histones (Xu et al., 2015). The characterization in the present study suggests that BCG challenge is associated with accumulation of histone in the microglia. Responsive homeostatic mechanisms aiming to moderate the potentially toxic high levels of histone may bring histones to levels lower than Sal by day 7 post challenge until stabilization. Additional time may be required to balance the histone levels post-BCG challenge closer to baseline. In addition, the disruption of histone proximal to gene regulatory regions is expected to influence gene transcriptional rates (Kaminska et al., 2016).

4.2. Proteins differentially abundant between levels of BCG challenge or IDO1 genotype

The under-abundance of PDIA3, PARK7, DSYSL2, and ACTG2 in BCG-challenged relative to Sal mice is consistent with prior work. In fact, PDIA3 is used as a biomarker of microglial activation (Yoo et al., 2014); astrocytes from PARK7 knockout mice exhibit decreased neuroprotection to LPS challenge (Ashley et al., 2016); and DPYSL2 was under-abundant in the prefrontal cortex of a rat clomipramine model of depression characterized (Gellén et al., 2017). Oxidative stress has been associated with depression-related disorders and a study of oxidative stress in rat brains reported high abundance of ACTG2 and ACTG1 ACTG2 (Foley et al., 2016).

The proteins that exhibited differential abundance in the KO_BCG versus WT_Sal contrast exclusively can offer insights into the molecular mechanisms disrupted by the BCG challenge that are unique to IDO1 deficient microglia and are not observed in WT microglia. Among these, S100 calcium binding protein A9 (calgranulin B or S100A9), malate dehydrogenase 1, NAD (MDH1), neutrophilic granule protein (NGP), ribosome binding protein 1 (RRBP1), and lactate dehydrogenase B (LDHB) were more abundant in KO_BCG than in WT_Sal microglia (Table 3). The profile of S100A9 is consistent with

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the gene expression profiles reported in the transcriptome study of BCG-challenged relative to saline WT mice (Gonzalez-Pena et al., 2016a).

The higher abundance of MDH1 and S100A9 in KO_BCG relative to WT_Sal is consistent with the pattern of this molecule observed in two disorders associated with reactive microglia, schizophrenia and sclerosis. The gene coding for MDH1 was over-expressed in patients with amyotrophic lateral sclerosis and S100A9 was more abundant in the microglia of patients diagnosed with schizophrenia linked to cerebral inflammation (Trépanier et al., 2016). S100A9 also acts as intermediary between amyloid cascade and microglia activation parallel to Alzheimer's disease neuroinflammatory and plaque deposition processes (Gruden et al., 2016).

High expression levels of the gene coding for the antimicrobial peptide NGP have been reported in the microglia (Hickman et al., 2013). The over-abundance of NGP in KO_BCG relative to WT_Sal (Table 3) suggests that the microglia of BCG-challenged IDO1 deficient mice is primed for innate host defense at day 7 post challenge. Likewise, the over-abundance of RRBP in the KO_BCG relative to the WT_Sal microglia supports in vitro and in vivo studies that detected high expression levels of Rbp1 in microglia that was stimulated with LPS/IFN relative to macrophages (Schmid et al., 2009). Also, LDHB was more abundant in cultured mouse microglia that was exposed to HIV relative to control (Wang et al., 2008).

The higher abundance of heat shock proteins, histones, ATP-related proteins, and S100 molecules in KO_BCG relative to WT_Sal offers evidence that at day 7 post-BCG challenge and post sickness recovery, some microglia mechanisms remain activated in IDO1 deficient mice. These profiles correspond to the damage-associated molecular patterns (DAMPs) known to activate microglia in response to stressors in wild type mice (Reus et al., 2015). This result is consistent with our expectation that proteins in pathways other than the tryptophan metabolism pathway remain disrupted at day 7 after BCG challenge in IDO1 KO similarly to WT mice.

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The lower abundance of lymphocyte cytosolic protein 1 (LCP1), splicing factor proline/glutamine rich (SFPQ) and profilin 1 (PFN1) in KO_BCG relative to WT_Sal microglia is consistent with studies of disorders that exhibit microglia activation and depression-related behaviors. In association with cerebral inflammation, LCP1 exhibited lower abundance in the microglia of schizophrenic patients relative to control (Trépanier et al., 2016). The observed PFN1 pattern is consistent with reports that the expression of this gene decreases with aging activated microglia (Solito and Sastre, 2012) isolated at autopsy from the parietal cortex of human subjects with intact cognition (Galatro et al., 2017). Also, mutations that annul the expression of PFN1 have been associated with Alzheimer's incidence that has been connected with microglia activation (Dietrich, 2013). Transcript and immunoblot studies uncovered depletion of the nuclear splicing factor and transcriptional regulator SFPQ in the glia of Alzheimer's disease affected brain areas (Ke et al., 2012).

Consistent with the lower abundance of CFL1 in WT_BCG relative to other groups observed in this study, the inhibition of this protein during stresses such as ischemic stroke secondary injury may support neuronal and glial repairing and protection (Alhadidi et al., 2016). The protein levels of CFL1 levels were also lower after contusion injury in rat spinal cord injury (Yan et al., 2010) and CFL1 regulates actins such as those differentially abundant in our study (Tables 2 and 3).

4.3. Insights from the functional and network analysis of differentially abundant proteins

The enrichment of the KEGG pathways "alcoholism" and "systemic lupus erythematosus" among the proteins differentially abundant on all three contrasts between genotype-treatment groups supports the viewpoint that these two disorders share with depression-related disorders molecular mechanisms associated with microglia activation (Ercan et al., 2016; Kelley and Dantzer, 2011). Individuals diagnosed with alcoholism exhibit activated microglia and elevated immune responses to pathogenic challenge (Crews et al., 2006) and systemic lupus erythematosus is associated with microglia activation (Ercan et al., 2016). Moreover, depressive disorders are comorbidities associated with the chronic autoimmune disorder

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systemic lupus erythematosus (Palagini et al., 2013). Alcoholism, systemic lupus erythematosus and BCG challenge can trigger neuroinflammation and pro-inflammatory signals in the brain that elicit an increase in the expression of IDO1 and metabolization of tryptophan augmenting the levels of neurotoxins and of inflammatory processes associated with depression-like symptoms (Kelley and Dantzer, 2011).

The enrichment of "structural molecule activity" among the proteins differentially abundant between WT_BCG and WT_Sal microglia indicates disruption in molecules that contribute to the assembly or structural integrity of a complex. Consistent with our finding, the structural molecule activity was also enriched among the proteins differentially abundant between patients diagnosed with schizophrenia compared to mentally healthy controls (Saia-Cereda et al., 2015). The over-representation of "phagosome" pathway among the proteins differentially abundant in the WT_BCG and KO_BCG contrast suggests disturbance of molecules that participate in cell phagocytosis or digestion of large particles during processes such as inflammation. This pathway was also enriched in the microglia transcriptome of mice repeatedly challenged with LPS and profiled 19 days later (Bodea et al., 2014). Our findings offer evidence supporting the hypothesis that in addition to neurotoxic metabolites, immune challenges such as BCG activate the microglia phagosome pathway potentially inducing neurodegeneration and depression-like behaviors (Bodea et al., 2014). The prevalence of the "gap junction" pathway among the proteins differentially abundant between KO_BCG and WT_Sal microglia highlights the unbalance molecules that participate in the direct communication between cells, permitting the transfer of small molecules including ions and metabolites and supporting homeostasis and apoptosis. The enrichment of gap junction-related proteins is in agreement with suggestions that, in response to immune challenge, the activated microglia releases bioactive molecules that increase the activity of hemichannels and reduce gap junctional communication (Orellana et al., 2009).

The networks connecting the differentially abundant within the three contrasts provided supplementary insights on the impact of BCG-treatment and IDO1 genotype on molecular interactions. The proteins in the WT_BCG-WT_Sal network were less connected than the proteins in the KO networks. The

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higher connectivity of the IDO1-deficient networks could be associated with the targeted role of IDO1 on the tryptophan (and related) pathways whereas the effect of BCG is distributed across more pathways that can respond to immune challenge. Furthermore, the highly connected network of the contrast KO_BCG-WT_Sal suggests a synergistic effect of both factors whereas the proteins in the unifactorial contrasts (WT_BCG-KO_BCG and WT_BCG-WT_Sal) correspond to more disconnected processes.

4.4. Differential epigenetic histone acetylation among BCG-treated and IDO1 genotype groups

Histones participate in epigenetic mechanisms that influence cell signaling that, in turn can influence the initiation, progression and termination of inflammation processes (Kaminska et al., 2016). Epigenetic mechanisms are typically more persistent than transcriptional and translational modifications of signaling proteins and may persist after recovery from the original challenge. Histone acetylation is a PTM that could support long-term changes in molecular mechanisms associated with inflammation-associated depressive-like symptoms. Histone acetylation has been proposed as a mechanism that regulates the activation of microglia in response to pro-inflammatory signals (Kaminska et al., 2016).

This is the first study to investigate the association between BCG challenge, IDO1 deficiency and the relative predominance of different histone types and the incidence of the PTM acetylation. The higher number of acetylated peptides from replicate-dependent histones and lower number of histone peptides in WT_BCG relative to WT_Sal and KO_BCG microglia (Table 5) observed in this study may be associated with the paralog relationship between the coding genes. Replication-independent histones tend to be coded by single-copy genes that are distributed across the genome and are loosely conserved. Replication-dependent histones are concentrated in genome clusters, exhibit multiple copies, and are highly conserved (Povey et al., 2001).

The higher level of acetylation in WT_BCG microglia relative to the other groups supports our premise that histone acetylation is associated with immune response and with inflammation-associated depression-like symptoms. Studies of histone deacetylation mechanisms and immune response agree with our findings. Low expression of histone deacetylases both in primary cultures and in microglia isolated 4

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to 6 hrs after LPS-treated mice were reported (Kannan et al., 2013). In another study, the inhibition of histone deacetylase in LPS-challenged mice attenuated the higher levels of neurotoxic kynurenic metabolites in microglia (associated with depression-like behaviors) and the higher activity of kynurenine monooxygenase in the tryptophan pathway relative to unchallenged mice (Giorgini et al., 2008). Treatment with a neuroprotective histone-deacetylase inhibitor blocked the activation in the microglia of the kynurenine pathway in a study of mice associated with a mutation in the Huntington gene (Giorgini et al., 2008).

Our study uncovered a KO-by-BCG interaction effect on the acetylation level of each histone cluster and on the relative abundance among the non-acetylated histone cluster peptides. The non-additive effects of IDO1 deficiency and BCG treatment on acetylation and histone type and family could impact the effectiveness of deacetylation inhibitor therapies that are broadly used to ameliorate disorders associated with microglia activation.

Evidence supporting the hypothesis that the histone acetylation mechanisms in IDO1-deficient mice after immune challenge are similar to those in unchallenged WT mice can be drawn from the similarity in the number and percentage of detected acetylated and non-acetylated peptides and peptide spectra between the KO_BCG and WT_Sal microglia (Table 5). The KO_BCG and WT_Sal groups were also similar in the non-acetylated histone clusters and the acetylated histone 2 cluster within the replication-dependent group (Table 6).

Conclusions

The comparison of microglia between three genotype-treatment groups: WT_Sal, WT_BCG and KO_BCG mice furthers the elucidation of the molecular basis of inflammation-associated depression-like symptoms. Our proteome profiling confirmed patterns associated with immune-associated depression-like symptoms and detected unexpected molecular players. The higher abundance of several DAMP proteins

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such as heat shock proteins and histones in KO_BCG relative to WT_Sal offers evidence that some microglia molecular mechanisms remain activated post sickness recovery from BCG challenge.

Evidence was uncovered of the possible role of microglia proteins from the 14-3-3 series in the activation of TPH2. These proteins were significantly more abundant at day 7 post-challenge in WT_BCG than KO_BCG microglia. The TPH2 activation may compensate for the lower availability of tryptophan to produce serotonin in serotonergic neurons due to the higher activity of IDO1 on KO_BCG microglia. These process are probably necessary to compensate for inflammation-induced decreased synthetic pathways of biosynthesis of monoamines in WT mice challenged with BCG.

The enrichment of the pathways alcoholism and systemic lupus erythematosus among the proteins differentially abundant between WT_BCG and WT_Sal, WT_BCG and KO_BCG, and KO_BCG and WT-Sal supports the premise that protein disruption associated with microglia activation is shared between these two disorders and immune response to BCG. The enrichment of the phagosome pathway among the proteins differentially abundant between WT_BCG and KO_BCG suggests that IDO1 deficiency is associated with interference microglia phagocytosis in response to BCG challenge. The gap junction pathway was enriched among the proteins differentially abundant between KO_BCG and WT_Sal and this finding suggests that disruptions in cell-cell communication cannot be compensated by IDO1 deficiency.

The present proteomic profiling indicated that IDO1 deficiency or KO may have an antagonistic effect to BCG challenge on selected proteins, cancelling the effects of this immune challenge by day 7 post challenge. Many proteins exhibited similar differential patterns in WT_BCG-WT_Sal and WT_BCG-KO_BCG. For these proteins, the microglia of WT_Sal and KO_BCG mice are more similar to each other than to WT_BCG. Among these, many histones, keratins and CFL were least abundant in WT_BCG than in the other two groups whereas many actinins, integrins and NCL are more abundant in WT_BCG than in the other two groups.

A recognized paradigm encompassing BCG- or Sal- treated IDO1 KO and WT mice was used to unearth proteins, their pathways and histone acetylation events that accompany inflammation-associated

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depressive state. The proteins and histone PTM were evaluated at a single time point when mice still exhibit depression-like symptoms and soon after recovery from sickness. A longitudinal study encompassing additional time points until depression symptoms recede in WT mice would enable the profiling of subtle proteomic changes achieved when natural pro- and anti-inflammatory signaling homeostasis is restored. Also, the evaluation of anti-inflammatory treatments in WT and IDO1 KO mice would enable to compare the molecular mechanisms impacted by the treatment relative IDO1 deficiency.

The bottom-up proteomic approach used in this study supported the identification and relative quantitation of proteins. These profiles were key to the inference of protein networks in the three treatment-genotype contrasts and comparative network analysis. The intrinsic dynamic range of the proteomic analysis favored the detection of medium- and high-abundance proteins. A more complete characterization of the protein networks would benefit from complementary analysis that further increase the depth of the proteomic coverage. Low abundant proteins and corresponding modest changes in abundance may be critical in the development of inflammation-associated depression. Our findings lead us to propose the compensation between the processes that divert tryptophan for either kynurenine or serotonin. These metabolic pathways do not take place in the same cell types. Proteomic measurements on serotonergic neurons, such as neurons from the raphe, would increase our understanding of the complex relationship between the kynurenine and serotonin metabolism pathways and the distinct cells that manifest these pathways.

This is the first study to characterize the impact of BCG challenge and IDO1 deficiency on the relative predominance of the different histone types and their acetylation rate. The WT_BCG microglia had the highest number of acetylated peptides from replicate-dependent histones and lowest number of histone peptides relative to WT_Sal and KO_BCG mice. Most of the replication-dependent histones detected, irrespective of acetylation, pertained to histone 1 cluster, followed by histone 2 cluster and no acetylation of replicate-independent histones was observed. The similarity in the number and percentage of acetylated and non-acetylated peptides and peptide spectra between the KO_BCG and WT_Sal groups suggests that

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the histone acetylation mechanisms in IDO1 KO mice after immune challenge are similar to those in unchallenged WT mice.

The present study furthers the understanding of the persistent changes in protein levels, pathways, and histone acetylation triggered by immune challenge. This innovative investigation offers the opportunity to advance antidepressant prognostic and diagnostic tools and treatments. Several neurological disorders including schizophrenia exhibit both disruption of inflammation indicators and depression-related symptoms. Also, many immunotherapies for a wide range of diseases such as autoimmune and infectious disorders, diseases of aging, and cancer can trigger disruptions of inflammatory system and depression comorbidity. The protein and histone acetylation signatures specific to inflammation-associated depression uncovered in this study can emerge as innovative biomarkers for detection, risk assessment, prediction and the evaluation of the potential impact of treatments that interact with inflammatory signals and depression.

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Conflict of interest

RD has received honoraria from Danone Nutricia Research for work unrelated to this research. The other authors declare no conflict of interest.

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Table 1. Differentially abundant proteins between wild type BCG-challenged (WT_BCG) and saline (WT_Sal) mice.

Symbol	Protein Name	Log2(WT_BCG/ WT_Sal)[1]	SE[2]	P-value
HIST1H2AL*	histone cluster 1, H2al	-0.80	0.15	1.3E-07
HIST1H2AD*	histone cluster 1, H2ad	-0.88	0.21	3.0E-05
HIST3H2A*	histone cluster 3, H2a	-0.86	0.21	4.0E-05
ACTG2	actin, gamma 2, smooth muscle, enteric	-1.00	0.27	2.4E-04
KRT2	keratin 2	-0.82	0.22	2.4E-04
KRT79	keratin 79	-1.11	0.35	1.8E-03
NUMA1	nuclear mitotic apparatus protein 1	-2.20	0.75	3.2E-03
CFL1	cofilin 1, non-muscle	-0.64	0.23	5.0E-03
KRT10	keratin 10	-0.58	0.21	5.1E-03
KRT5	keratin 5(	-0.41	0.16	8.7E-03
NCL	nucleolin(	1.10	0.44	1.2E-02
KRT6B	keratin 6B(Krt6b)	-0.55	0.22	1.2E-02
HIST1H2AP*	histone cluster 1, H2ap	-0.88	0.36	1.5E-02
MBP	myelin basic protein	-0.69	0.29	1.7E-02
TPM3	tropomyosin 3, gamma	1.32	0.56	1.9E-02
H2AFJ^	H2A histone family, member J	-0.72	0.31	1.9E-02
HIST2H2AA2*	histone cluster 2, H2aa2	-0.64	0.27	2.0E-02
ACTN1	actinin, alpha 1	0.98	0.44	2.6E-02
HNRNPF	heterogeneous nuclear ribonucleoprotein F	-1.25	0.57	2.7E-02
HIST1H2AH*	histone cluster 1, H2ah	0.32	0.14	2.8E-02
PDIA3	protein disulfide isomerase associated 3	1.08	0.50	3.2E-02
H2AFY^	H2A histone family, member Y	-0.60	0.28	3.5E-02
HIST1H2BR*	histone cluster 1, H2br	0.68	0.32	3.6E-02
KRT6A	keratin 6A	-0.52	0.25	3.9E-02
HIST1H2AF*	histone cluster 1, H2af	-0.42	0.21	4.0E-02
HIST1H2AI*	histone cluster 1, H2ai	-0.42	0.21	4.0E-02
HIST1H2AO*	histone cluster 1, H2ao	-0.42	0.21	4.0E-02
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.76	0.38	4.6E-02
PARK7	Parkinson disease (autosomal recessive, early onset) 7	0.66	0.33	4.6E-02
DPYSL2	dihydropyrimidinase-like 2	-0.56	0.29	5.0E-02
ITGAM	integrin alpha M	2.08	1.06	5.0E-02
CYCS	cytochrome c, somatic	-1.39	0.71	5.0E-02

[1] Differential abundance in Log2 units.

[2] Standard error.

*Replication-dependent histone; ^Replication-independent histone.

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Table 2. Differentially abundant proteins between wild type BCG-challenged (WT_BCG) and IDO1 KO_BCG-challenged (KO_BCG) mice.

Symbol	Accession	Log2(WT_BCG /KO_BCG)[1]	SE[2]	P-value
HIST1H2AB*	histone cluster 1, H2ab	1.03	0.15	1.9E-11
HIST2H2AB*	histone cluster 2, H2ab	-0.80	0.14	3.3E-08
HIST1H2AL*	histone cluster 1, H2al	-0.75	0.14	1.2E-07
HIST2H2AA2*	histone cluster 2, H2aa2	-1.70	0.33	2.4E-07
HIST1H2AF*	histone cluster 1, H2af	-0.96	0.19	5.2E-07
HIST1H2AI*	histone cluster 1, H2ai	-0.96	0.19	5.2E-07
HSPA8	heat shock protein 8	1.41	0.29	9.5E-07
HIST1H2AH*	histone cluster 1, H2ah	0.79	0.16	9.7E-07
H2AFX^	H2A histone family, member X	-1.06	0.22	9.7E-07
H2AFY^	H2A histone family, member Y	-0.84	0.19	1.2E-05
NCL	nucleolin	2.40	0.56	1.9E-05
HSP90B1	heat shock protein 90, beta member 1	2.08	0.57	2.6E-04
ACTN1	actinin, alpha 1	2.08	0.65	1.3E-03
CFL1	cofilin 1, non-muscle	-0.71	0.23	1.7E-03
YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	0.85	0.28	2.3E-03
HIST1H2AO*	histone cluster 1, H2ao	-0.58	0.19	2.6E-03
KRT10	keratin 10	-0.61	0.22	5.4E-03
ACTN4	actinin, alpha 4	2.48	0.92	6.8E-03
HMGA1-RS1	high mobility group AT-hook I, related sequence 1	-1.10	0.41	7.1E-03
CALM2	calmodulin 2	-1.07	0.40	7.5E-03
CALM1	calmodulin 1	-1.07	0.40	7.5E-03
HSP90AB1	heat shock protein 90 alpha class B member 1	3.28	1.23	7.7E-03
MYH9	myosin, heavy polypeptide 9, non-muscle	-1.42	0.53	7.8E-03
YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, etha polypeptide	0.99	0.37	8.0E-03
CDK5RAP2	CDK5 regulatory subunit associated protein 2	0.99	0.37	8.0E-03
ANXA3	annexin A3	-0.62	0.23	8.1E-03
H2AFJ^	H2A histone family, member J	-0.71	0.27	8.9E-03
ITGB2	integrin beta 2	1.91	0.73	9.1E-03
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.53	0.20	9.2E-03
KPNB1	karyopherin (importin) beta 1	1.87	0.73	1.1E-02
YWHAG	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	0.87	0.34	1.2E-02

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ACTBL2	actin, beta-like 2	0.44	0.17	1.2E-02
TUBB2A	tubulin, beta 2A	1.10	0.47	1.9E-02
TUBB2B	tubulin, beta 2B	1.10	0.47	1.9E-02
ENO1B	enolase 1B, retrotransposed	0.74	0.32	2.1E-02
KRT77	keratin 77	0.73	0.32	2.2E-02
CALM3	calmodulin 3	-1.11	0.49	2.2E-02
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	-0.54	0.25	2.9E-02
TUBA1A	tubulin, alpha 1A	0.43	0.20	3.1E-02
SET	SET nuclear oncogene	-1.16	0.55	3.4E-02
HNRNPK	heterogeneous nuclear ribonucleoprotein K	0.79	0.38	3.5E-02
CANX	calnexin	3.33	1.59	3.6E-02
ATP5B	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	0.62	0.30	3.9E-02
SH3BGRL3	SH3 domain binding glutamic acid-rich protein-like 3	-1.13	0.55	4.0E-02
LMNB1	lamin B1	0.94	0.46	4.1E-02
KRT2	Keratin 2	-0.55	0.28	4.8E-02
RGS10	regulator of G-protein signalling 10	-0.52	0.26	5.0E-02
GM5803	predicted gene 5803	2.08	1.06	5.0E-02

[1] Differential abundance in Log2 units.

[2] Standard error.

*Replication-dependent histone; ^Replication-independent histone.

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Table 3. Differentially abundant proteins between IDO1 KO_BCG-challenged (KO_BCG) and wild type saline (WT_Sal) mice.

Symbol	UniProt	Log2(KO_BCG/WT_Sal)[1]	SE[2]	P-value
HIST1H2AB*	histone cluster 1, H2ab	-1.17	0.16	1.2E-12
HIST1H2AD*	histone cluster 1, H2ad	-0.93	0.17	6.6E-08
HIST3H2A	histone cluster 3, H2a	-0.91	0.17	1.1E-07
H2AFX^	H2A histone family, member X	1.14	0.22	1.5E-07
RRBP1	ribosome binding protein 1	2.59	0.61	1.9E-05
HIST1H2AF*	histone cluster 1, H2af	0.53	0.13	3.2E-05
HIST1H2AI*	histone cluster 1, H2ai	0.53	0.13	3.2E-05
HIST1H2AP*	histone cluster 1, H2ap	-1.32	0.32	3.2E-05
HIST1H2AG*	histone cluster 1, H2ag	-0.87	0.21	4.8E-05
HIST2H2AB*	histone cluster 2, H2ab	0.56	0.14	4.9E-05
HSP90B1	heat shock protein 90, beta (Grp94), member 1	-2.00	0.57	4.5E-04
HIST2H2AA2*	histone cluster 2, H2aa2	1.07	0.31	5.4E-04
MBP	myelin basic protein	-1.53	0.47	1.1E-03
LMNB1	lamin B1	-1.90	0.59	1.3E-03
KRT77	keratin 77	-0.80	0.28	4.3E-03
HIST1H2AH*	histone cluster 1, H2ah	-0.47	0.17	4.6E-03
ARHGDIB	Rho, GDP dissociation inhibitor (GDI) beta	1.14	0.41	4.8E-03
HSP90AB1	heat shock protein 90, alpha class B member 1	-3.61	1.30	5.4E-03
TUBA1B	tubulin, alpha 1B	-0.63	0.23	5.5E-03
NUMA1	nuclear mitotic apparatus protein 1	-0.94	0.36	8.0E-03
HMGA1-RS1	high mobility group AT-hook I, related sequence 1	1.23	0.48	9.6E-03
KPNB1	karyopherin (importin) beta 1	-1.95	0.76	1.0E-02
TUBB2A	tubulin, beta 2A	-1.09	0.43	1.0E-02
TUBB2B	tubulin, beta 2B	-1.09	0.43	1.0E-02
HSPA8	heat shock protein alpha 8	-0.98	0.39	1.1E-02
YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	-0.66	0.27	1.4E-02
ACTA1	actin, alpha 1, skeletal muscle	-0.48	0.20	1.4E-02
NGP	neutrophilic granule protein	1.58	0.64	1.4E-02
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	0.61	0.26	1.8E-02
GAPDH-PS15	glyceraldehyde-3-phosphate dehydrogenase, pseudogene 15	0.61	0.26	1.8E-02
KRT79	keratin 79	-0.75	0.32	1.8E-02
H2AFY^	H2A histone family, member Y	0.50	0.21	1.9E-02
SET	SET nuclear oncogene	1.22	0.52	1.9E-02
MYH9	myosin, heavy polypeptide 9, non-muscle	0.87	0.37	1.9E-02
LCP1	lymphocyte cytosolic protein 1	-1.04	0.45	2.0E-02

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TUBA1A	tubulin, alpha 1A	-0.51	0.23	2.5E-02
SFPQ	splicing factor proline/glutamine rich	-0.67	0.30	2.5E-02
ACTN4	actinin alpha 4	-1.85	0.83	2.6E-02
TUBA1C	tubulin, alpha 1C	-0.57	0.26	2.7E-02
ITGAM	integrin alpha M	2.23	1.02	2.8E-02
KRT1	keratin 1	-0.48	0.22	2.9E-02
LDHB	lactate dehydrogenase B	0.87	0.40	3.0E-02
HIST1H4M*	histone cluster 1, H4m	-0.44	0.20	3.2E-02
HIST2H4*	histone cluster 2, H4	-0.44	0.20	3.2E-02
HIST1H4H*	histone cluster 1, H4h	-0.44	0.20	3.2E-02
HIST4H4*	histone cluster 4, H4	-0.43	0.21	3.5E-02
HIST1H4D*	histone cluster 1, H4d	-0.43	0.21	3.6E-02
HIST1H4F*	histone cluster 1, H4f	-0.43	0.21	3.7E-02
HIST1H4I*	histone cluster 1, H4i	-0.43	0.21	3.7E-02
HIST1H4N*	histone cluster 1, H4n	-0.43	0.21	3.7E-02
HIST1H4A*	histone cluster 1, H4a	-0.43	0.21	3.7E-02
HIST1H4B*	histone cluster 1, H4b	-0.43	0.21	3.7E-02
HIST1H4C*	histone cluster 1, H4c	-0.43	0.21	3.7E-02
HIST1H4J*	histone cluster 1, H4j	-0.43	0.21	3.7E-02
HIST1H4K*	histone cluster 1, H4k	-0.43	0.21	3.7E-02
CANX	calnexin	-3.26	1.59	4.0E-02
ANXA3	annexin A3	0.58	0.28	4.2E-02
GM5803	predicted gene 5803	-2.08	1.03	4.4E-02
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zetha polypeptide	-0.42	0.21	4.4E-02
MDH1	malate dehydrogenase 1	0.75	0.38	4.6E-02
KRT2	keratin 2	-0.39	0.20	4.6E-02
ACTN1	actinin, alpha 1	-1.30	0.65	4.6E-02
NCL	nucleolin	-1.30	0.65	4.6E-02
S100A9	S100 calcium binding protein A9 (calgranulin B)	0.61	0.31	4.8E-02
PFN1	profilin 1	-0.53	0.27	4.9E-02

[1] Differential abundance in Log2 units.

[2] Standard error.

*Replication-dependent histone; ^Replication-independent histone.

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Table 4. Top functional categories enriched (> 5 genes, FDR-adjusted P-value < 0.05 and ≤ 5 molecular functions) among the genes coding for differentially abundant proteins in the WT_BCG versus WT_Sal, WT_BCG versus KO_BCG, and KO_BCG versus WT_Sal contrasts.

Contrast and Category[a]	GO Term or KEGG Pathway Name	Gene Count	FDR P-value
WT_BCG -WT_Sal			
MF GO:0005198	Structural molecule activity	10	1.1E-04
KEGG 05322	Systemic lupus erythematosus	9	1.1E-10
KEGG 05034	Alcoholism	8	1.1E-07
KEGG 05203	Viral carcinogenesis	5	6.9E-03
PFAM PF00125	Core histone H2A/H2B/H3/H4	15	4.4E-24
WT_BCG-KO_BCG			
BP GO:0006457	Protein folding	7	7.1E-03
MF GO:0003676	Nucleic acid binding	31	5.5E-07
MF GO:0097159	Organic cyclic compound binding	39	5.5E-07
MF GO:1901363	Heterocyclic compound binding	39	5.5E-07
MF GO:0003723	RNA binding	20	6.6E-06
MF GO:0019904	Protein domain specific binding	12	1.1E-05
KEGG 05322	Systemic lupus erythematosus	10	9.4E-11
KEGG 05034	Alcoholism	9	1.0E-07
KEGG 05203	Viral carcinogenesis	7	3.2E-04
KEGG 04145	Phagosome	6	9.5E-04
KEGG 04141	Protein processing in endoplasmic reticulum	5	7.9E-03
KEGG 04810	Regulation of actin cytoskeleton	5	1.8E-02
PFAM PF00125	Core histone H2A/H2B/H3/H4	13	3.3E-17
KO_BCG-WT_Sal			
BP GO:0071822	protein complex subunit organization	16	1.1E-02
BP GO:0051258	protein polymerization	5	4.7E-02
BP GO:0043933	macromolecular complex subunit organization	18	4.8E-02
MF GO:0003723	RNA binding	23	1.7E-06
MF GO:0005198	Structural molecule activity	14	1.7E-06
MF GO:0044822	Poly(A) RNA binding	19	1.4E-05
MF GO:0003676	Nucleic acid binding	30	1.7E-04
MF GO:0019899	Enzyme binding	19	2.4E-04
KEGG 05322	Systemic lupus erythematosus	9	1.8E-08
KEGG 05034	Alcoholism	8	8.7E-06
KEGG 04145	Phagosome	7	2.5E-04
KEGG 04540	Gap junction	5	1.4E-03
KEGG 04141	Protein processing in endoplasmic reticulum	5	1.8E-02
KEGG 05203	Viral carcinogenesis	5	3.3E-02
KEGG 04810	Regulation of actin cytoskeleton	5	3.8E-02
PFAM PF00125	Core histone H2A/H2B/H3/H4	10	1.1E-10
PFAM PF00091	Tubulin/FtsZ family, GTPase domain	5	2.7E-06

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PFAM PF03953	Tubulin C-terminal domain	5	2.7E-06

[a] Contrast: WT = wild type mice; KO = IDO1 KO mice; Sal = saline treatment; BCG = Bacillus Calmette–Guérin treatment. Categories: Gene Ontology (GO) biological process (BP) and molecular function (MF); KEGG pathway; and protein family PFAM.

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Table 5. Number (Count) and percentage (%) of histone peptides (Pep) and histone peptide mass spectra (Spec) without (No) and with (Yes) acetylation post-translational modification (PTM) by histone family and genotype-treatment group.

Group[a]	Histone family type	Peptide Count		Spectra Count		% Pep	% Spec
		No PTM	Yes PTM	No PTM	Yes PTM	Yes PTM	Yes PTM
WT_Sal	Replication-independent	294	0	949	0	0.0%	0.0%
WT_Sal	Replication-dependent	4882	111	16159	137	2.2%	0.8%
WT_BCG	Replication-independent	313	0	842	0	0.0%	0.0%
WT_BCG	Replication-dependent	4170	136	11981	216	3.2%	1.8%
KO_BCG	Replication-independent	509	0	1826	0	0.0%	0.0%
KO_BCG	Replication-dependent	6116	101	16798	157	1.6%	0.9%

[a] WT = wild type mice; KO = IDO1 KO mice; Sal = saline treatment; BCG = Bacillus Calmette–Guérin treatment.

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Table 6. Number and percentage (%) of replication-dependent histone peptides without (No) and with (Yes) acetylation post-translational modification (PTM) by genotype-treatment group and by histone cluster family.

Histone	Genotype-treatment[b]							Genotype-treatment					
cluster	WT_ Sal	WT_ BCG	KO_ BCG	Total	%		WT_ Sal	WT_ BCG	KO_ BCG	Total	%		
Family member	No PTM	No PTM	No PTM	No PTM	No PTM		Yes PTM	Yes PTM	Yes PTM	Yes PTM	Yes PTM		Total
Total Hist 1	4028	3258	5150	12436	98%		96	116	90	302	2%		12738
% Hist 1	83%	78%	84%				86%	85%	89%				
Total Hist 2	727	803	822	2352	99%		9	14	7	30	1%		2382
% Hist 2	15%	19%	13%				8%	10%	7%				
Total Hist 3	39	32	56	127	100 %		0	0	0	0	0%		127
% Hist 3	1%	1%	1%				0%	0%	0%				
Hist 4	88	77	88	253	94%		6	6	4	16	6%		269
% Hist 4	2%	2%	1%				5%	4%	4%				
Total	4882	4170	6116	15168	98%		111	136	101	348	2%		15516

[a] Hist #: Histone # cluster

[b]WT = wild type mice; KO = IDO1 KO mice; Sal = saline treatment; BCG = Bacillus Calmette–Guérin treatment.