Peripheral viral infection induced microglial sensome genes and enhanced microglial cell activity in the hippocampus of neonatal piglets

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Although poorly understood, early-life infection is predicted to affect brain microglial cells, making them hypersensitive to subsequent stimuli. To investigate this, we assessed gene expression in hippocampal tissue obtained from a previously published study reporting increased microglial cell activity and reduced hippocampal-dependent learning in neonatal piglets infected with porcine reproductive and respiratory syndrome virus (PRRSV), a virus that induces interstitial pneumonia. Infection altered expression of 455 genes, of which 334 were up-regulated and 121 were down-regulated. Functional annotation revealed that immune function genes were enriched among the up-regulated differentially expressed genes (DEGs), whereas calcium binding and synaptic vesicle genes were enriched among the down-regulated DEGs. Twenty-six genes encoding part of the microglia sensory apparatus (i.e., the sensome) were up-regulated (e.g., IL1R1, TLR2, and TLR4), whereas 15 genes associated with the synaptosome and synaptic receptors (e.g., NPTX2, GABRA2, and SLC5A7) were down-regulated. As the sensome may foretell microglia reactivity, we next inoculated piglets with culture medium or PRRSV at PD 7 and assessed hippocampal microglia morphology and function at PD 28 when signs of infection were waning. Consistent with amplification of the sensome, microglia from PRRSV piglets had enhanced responsiveness to chemoattractants, increased phagocytic activity, and secreted more TNFα in response to lipopolysaccharide and Poly I:C. Immunohistochemical staining indicated PRRSV infection increased microglia soma length and length-to-width ratio. Bipolar rod-like microglia not evident in hippocampus of control piglets, were present in infected piglets. Collectively, this study suggests early-life infection alters the microglia sensome as well as microglial cell morphology and function.

1. Introduction

Neuroimmune factors are thought to play important roles in the etiology of psychiatric disorders such as autism spectrum disorders and schizophrenia. The supposition is predicated on the prenatal and early postnatal brain being vulnerable to infectious insults that, if present, affect development and increase risk for behavioral disorders later (Andersen, 2003; Rees and Inder, 2005). Pro-inflammatory cytokines produced by immune cells, rather than the pathogen per se are strongly implicated (Dantzler et al., 2008). Therefore, an important role for microglia seems likely as they are resident immune cells in the brain, produce pro-inflammatory cytokines in response to infection, and play a key role in development by phagocytizing neural progenitors and engaging in synaptic pruning. Indeed, untimely activation of microglia has been shown to reduce prenatal cortical neurogenesis, resulting in fewer cortical neurons in adulthood (Cunningham et al., 2013). Furthermore, rat pups infected with Escherichia coli to induce acute neuroinflammation, showed lower cognitive resilience as adults when exposed to another immune stressor (Bilbo et al., 2006; Bilbo and Schwarz, 2009). A working hypothesis is that early-life infection alters microglial cells in a manner that renders them hypersensitive to subsequent stimuli (Meyer et al., 2011). However, there is a gap of knowledge regarding the effects of early
postnatal viral infection on microglia activity and their sensitivity to subsequent insults.

Recently, we have conducted studies using a neonatal domestic piglet model. The piglet immune system is similar to human as is growth and development of its gyrencephalic brain (Conrad and Johnson, 2015). In our previous studies with neonatal piglets, infection with porcine reproductive and respiratory syndrome virus (PRRSV) resulted in reduced neurogenesis and altered pyramidal neuron structure in hippocampus (Conrad et al., 2015), reduced gray and white matter in several brain regions (Conrad et al., 2014), and compromised performance in a hippocampal-dependent spatial T-maze task (Elmore et al., 2014). In addition, peripheral infection was associated with robust activation of microglia in the hippocampus (Elmore et al., 2014). These studies indicated PRRSV infection induced a neuroinflammatory response and affected brain development.

To significantly extend these findings and to address the gap of knowledge regarding infection and microglia activity and subsequent sensitivity, the present study first assessed gene expression patterns in hippocampal tissue obtained from our previously published study reporting increased microglial cell activation and reduced hippocampal-dependent learning in piglets infected with PRRSV (Elmore et al., 2014). Results of this analysis suggested infection up-regulated a number of genes that are part of the newly defined microglia sensome (Hickman et al., 2013). As this sensory apparatus may foretell microglia reactivity, we subsequently assessed the effects of peripheral infection on hippocampal microglia morphology and function. The important findings suggest early-life infection alters the sensome, rendering microglia more responsive to subsequent immune stimuli.

2. Materials and methods

2.1. Animals, housing, virus inoculation and detection

All animal care and experimental procedures were in accordance with the NRC Guide for the Care and Use of Laboratory Animals and approved by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee. A detailed description of animals and housing can be found in Elmore et al. (2014) and Conrad et al. (2015). Briefly, naturally farrowed crossbred piglets obtained at PD 2 were assigned to either control (control, sham-inoculated) or PRRSV treatment balancing for sex, body weight and litter of origin. Piglets were housed individually in cages (0.87 m\(\times\)0.49 m; \(C2\)) and fed a pelleted crossbred diet (Advance Liqui-Wean, Milk Specialties). Milk was supplied at 1 mL/C2 at Purdue University (West Lafayette, Indiana). PRRSV, a single-stranded RNA virus (PRRSV) resulted in reduced neurogenesis and altered pyramidal neuron structure in hippocampus (Conrad et al., 2015), reduced gray and white matter in several brain regions (Conrad et al., 2014), and compromised performance in a hippocampal-dependent spatial T-maze task (Elmore et al., 2014). In addition, peripheral infection was associated with robust activation of microglia in the hippocampus (Elmore et al., 2014). These studies indicated PRRSV infection induced a neuroinflammatory response and affected brain development.

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2.2. hippocampal gene expression

2.2.1. RNA extraction, cDNA synthesis, and RNA-sequencing library preparation

To assess the neuroimmune effects of early life peripheral infection, gene expression in hippocampal tissue collected at PD 28 (21 dpi) from our previously published study wherein piglets were experimentally infected with PRRSV was assessed (Elmore et al., 2014). The experimental design, animal housing, feeding, virus inoculation and tissue collection protocol in the study by Elmore et al. (2014) was the same as in the current study. RNA was extracted using an AllPrep DNA/RNA Mini Kit (Qiagen) following the manufacturer’s protocol. RNA concentrations were determined using a NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.). Samples were analyzed by the Carver High-Throughput DNA Sequencing and Genotyping Unit (HTS lab, University of Illinois, Urbana, IL, USA) using an Agilent bioanalyzer DNA7500 DNA chip (Agilent Technologies) to determine RNA integrity as well as the presence/absence of genomic DNA (gDNA). Only RNA samples with an RNA integrity number (RIN) greater than 7 were used for sequencing. This high-quality RNA was sent to the HTS lab (University of Illinois, Urbana, IL, USA) for the generation of TruSeq Stranded RNA-seq libraries using the TruSeq Stranded RNA Sample Preparation Kit (Illumina Inc.) following standard protocols. Briefly, messenger RNA was selected from 1 ug of high quality, DNase treated, total RNA and first-strand synthesis performed with a random hexamer and SuperScript II (Life Technologies). Double stranded DNA was blunt-ended, 3’-end A-tailed, and ligated to indexed adaptors. The adaptor-ligated double-stranded cDNA was amplified by PCR for 10 cycles with the Kapa HiFi polymerase (Kapa Biosystems) to reduce the likelihood of multiple identical reads due to preferential amplification. The final libraries were quantified using a Qubit (Life Technologies) and the average size was determined on the bioanalyzer and diluted to 10 nM. The 10 nM dilution was further quantified by qPCR on an ABI 7900 (Applied Biosystems), which resulted in a high accuracy quantification for consistent pooling of barcoded libraries and maximized the number of clusters in the Illumina flowcell.

2.2.2. Illumina RNA-sequencing

RNA-seq Illumina sequencing was performed by the HTS lab (University of Illinois, Urbana, IL, USA). Briefly, the libraries were multiplexed and loaded onto 8-lane flowcells for cluster formation and sequenced on an Illumina HiSeq2000 (Illumina, Inc.). One of the lanes was loaded with a PhiX Control library that provides a balanced genome for the calculation of the matrix, phasing and prephasing, which are essential for accurate base calling. The libraries were sequenced from both ends (paired-end sequencing) of the molecules to a total read length of 100 bp from each end. The run generated .bcl files which were converted into demultiplexed, compressed fastq files using Casava 1.8.2 (Illumina Inc.).

2.2.3. RNA-sequencing data analysis

An average of 35.7 million raw paired-end reads were produced for each sample, ranging from 29.7 to 43 million. Raw reads were trimmed three times sequentially for adapter contamination: A-tails, minimum quality score (20), and minimum length (20 bp) using Trim Galore v.0.3.3 (http://www.bioinformatics.babraham. cam.ac.uk/projects/trim_galore/).
expression level (row pig (control or PRRSV), and each row represents the relative DEG dataset using the heatmap.2 function of the R package for expressed genes (DEGs).

The RNA-seq data was mapped in the swine genome, whereas 384 were mapped in the human genome. Default settings of annotation categories and were selected as the reference set because only 122 of the 455 DEGs and down-regulated DEG were uploaded and analyzed separately for each individual pig. Functional enrichment analysis was performed using cufflinks v.2.2.1 (Trapnell et al., 2010). First, Cufflinks was used to assemble transcripts for each sample using the fr-firststrand option, followed by Cuffmerge to merge the assembled transcripts from all samples with the reference transcripts. Cuffquant was used to pre-compute gene expression levels for each sample using the u option, which more accurately weights reads mapping to multiple locations, and the fr-firststrand option. Finally Cuffdiff was used for differential gene expression analysis using the u and fr-firststrand options. The RNA-seq data sets supporting the results of this article are available in the European Nucleotide Archive and are available under accession number PRJEB11625 (www.ebi.ac.uk/ena/data/view/PRJEB11625).

2.2.4. Visualization and functional enrichment analysis of differentially expressed genes (DEGs)

For visualization of DEGs, a heatmap was created based on the DEG dataset using the heatmap2 function of the R package for visualization. Each column represents the DEGs of an individual pig (control or PRRSV), and each row represents the relative expression level (row z-score) of one gene across all pigs. The columns were clustered based on similarity of expression patterns of each individual pig. Functional enrichment analysis was performed using DAVID v6.7 (Huang et al., 2009). Official gene symbols of up- and down-regulated DEG were uploaded and analyzed separately using the human genome as the background. The human genome was selected as the reference set because only 122 of the 455 DEGs were mapped in the swine genome, whereas 384 were mapped in the human genome. Default settings of annotation categories and sources for DAVID were accepted. The default stringency settings were used for Gene Ontology enrichment analysis. Enriched GO terms (cellular component, molecular function, and biological process) and KEGG pathways were reported.

2.3. Microglia activity and morphology after infection

2.3.1. Immunostaining of microglia and assessment of soma morphology

Free floating coronal hippocampal sections (40 μm) were prepared as described previously (Conrad et al., 2015). Three sections of each hippocampus were used for immunostaining of microglia with a primary antibody, rabbit anti-IBA1 (1:1200, Wako Chemicals) and a biotinylated goat anti-rabbit secondary antibody (1:250, Jackson ImmunoResearch Laboratories). The ABC system (Vector Laboratories) and diaminobenzidine kit (DAB; Sigma) were used for the chromogen. Digital images were captured using a Nanozoomer Digital Pathology System at 40× magnification (Hamamatsu Photonics). Consecutive images of the CA1 region of each section were exported at 40× digital zoom. Microglial soma morphology (length, width, and total area) was measured using Imagej. Soma length was defined as the direct distance connecting the two farthest points of the soma, while soma width was the widest distance of the soma orthogonal to the soma length. Approximately 170 cells from hippocampal CA1 region of each piglet were measured for soma morphology in both treatment group (n = 6 piglets/treatment).

2.3.2. Isolation of hippocampal microglia and flow cytometry

Hippocampal CD11b+ cells were isolated using the Neural Tissue Dissociation Kit P (Miltenyi Biotec) based on the protocol described by Nikodemova and Watters (2012). Briefly, both hippocampi of a piglet were dissected, homogenized/dissociated using a gentleMACS Dissociator (Miltenyi Biotec) and enzymatically digested at 37 °C for 35 min. Aggregates were removed by passing the cell suspension through a 40 μm cell strainer to yield a single cell suspension. Myelin was removed by passing cells through 25 mL of a 30% percoll-plus (GE Healthcare) solution. Cells were incubated in buffer (1 × PBS with 2 mM EDTA and 0.5% BSA) containing anti-CD11b magnetic MicroBeads at 4 °C for 15 min. CD11b+ cells were separated in a magnetic field using MS columns (Miltenyi Biotec). Isolated cells (~6 × 10⁶) from both hippocampi of the same pig were resuspended in DMEM and pooled for subsequent in vitro assays. The initial plan was to isolate a pure microglia fraction for in vitro assays by staining with CD45 antibody, which is a surface marker that is widely used to differentiate microglia (CD11b+/CD45low to intermediate) from macrophages (CD11b+/CD45high) in rodents (Hickman et al., 2013; Nikodemova and Watters, 2012; Sedgwick et al., 1991). However, microglial yield and viability was low. Therefore, CD11b+ cells were used for in vitro assays. Microglia (CD45low to intermediate) made up 80–90% of the CD11b+ cells for both control and PRRSV piglets (Fig. S2.B).

2.3.3. Chemotaxis assay

Migration of CD11b+ cells towards chemoattractants (recombinant porcine CCL-2; Innovative Research Inc.; and recombinant porcine IL-8; R&D systems) was measured using the Neuro Probe ChemoTx system (101-8; Neuro Probe, Inc.) following the manufacturer’s protocol. In brief, DMEM (control), CCL-2 (100 ng/mL DMEM), or IL-8 (100 ng/mL DMEM) were added to the bottom chamber of a 96-well plate of a chemotaxis system and 30 μL of DMEM with CD11b+ cells (1 × 10⁵ cell/mL) were added to the upper chamber. After 3 h at 37 °C with 5% CO₂, plates were centrifuged at 300 × g for 10 min to separate cells that had migrated through the filter paper. Adherent cells were stained with Hoechst 33342 solution (5 μg/mL; Life technologies) and relative fluorescence was determined.

2.3.4. Phagocytosis assay

Phagocytic activity of CD11b+ cells was analyzed using the Vybrant Phagocytosis Assay Kit (Life technologies) following the manufacturer’s protocol with minor modifications. Cells were diluted (~6 × 10⁶ cells/mL) in DMEM containing 10% heat-inactivated FBS, 1% penicillin-streptomycin, and 10 ng/mL recombinant porcine GM-CSF (R&D Systems) and plated with 5 intended replicates in a 96-well cell culture plate (100 μL/well). Cells were incubated for 24 h at 37 °C 5% CO₂ and then given fresh DMEM, LPS (1 ng/mL DMEM; Escherichia coli 0127:B8, Sigma), or Poly I:C (1 μg/mL DMEM; GE Healthcare/Amersham Biosciences). After 3 h incubation, 100 μL of fluorescein-labeled BioParticles were added to each well to assess phagocytosis. After a 90 min incubation, the BioParticle suspension was removed by aspiration and 100 μL of a 1 × trypsin blue suspension was added for 15 min at room temperature to quench the fluorescence of extracellular (non-engulfed) BioParticles. The plate was read at an excitation of 485 nm and an emission of 528 nm.

2.3.5. Cytokine production and microglial gene expression

CD11b+ cells were diluted (~5 × 10⁶ cells/mL) in DMEM containing 10% heat-inactivated FBS, 1% penicillin–streptomycin, and 10 ng/mL recombinant porcine GM-CSF, and plated in duplicate in 24-well plates (~5 × 10⁶ cells/well). After 24 h at 37 °C 5% CO₂, DMEM, LPS (1 ng/mL DMEM), or Poly I:C (1 μg/mL DMEM)
was added to wells. Following 8 h incubation, medium was collected and assayed for TNFα using a commercially available porcine-specific ELISA kit (R&D systems). Conditioned supernatants were assayed for lactate dehydrogenase using a commercially available detection kit (Roche Diagnostics Corporation) following the manufacturer’s instructions to verify treatments were not cytotoxic. Total RNA from microglia was isolated using TRI Reagent protocol (Sigma), and cDNA was synthesized using a Quantitect Reverse Transcription Kit (QIAGEN). The real-time PCR was performed using the Applied Biosystems Taqman Gene Expression Assay Protocol. Genes of interest (Table S1) were compared with and expressed as fold change relative to the reference gene (GAPDH, Ss03374854_g1, Applied Biosystem).

2.3.6 Statistical analysis

Data were analyzed using the mixed procedure of SAS 9.4. Body temperature, feeding score, body and brain weight, serum cytokines, MHCII expression, microglia morphology were analyzed as a complete randomized design with PRRSV infection as a fixed effect and pig within treatment as a random term. For variables measured over time (e.g., body temperature), a repeated statement was used in the model. Data from the in vitro studies were analyzed as a split-plot design with PRRSV infection as a fixed effect of the main plot and in vitro treatment as a fixed effect of the subplot. Significant difference was declared at $P < 0.05$, tendency at $P < 0.10$.

3. Results

3.1. Respiratory viral infection affects the hippocampal transcriptome in neonatal piglets

We first assessed gene expression patterns in hippocampal tissue obtained from our previously published study (Elmore et al., 2014). A total of 455 DEGs were revealed in the hippocampus in response to PRRSV ($P < 0.05$, Benjamini-Hochberg adjusted for multiplicity; Fig. 1A), of which 334 were up-regulated and 121 were down-regulated. The hierarchical clustering heatmap of the overall DEG pattern suggested a consistent effect of PRRSV infection on hippocampal transcriptomic profiles (Fig. 1B). The Database for Annotation, Visualization and Integrated Discovery (DAVID v6.7) tool was used for functional enrichment analysis. Using DAVID, only 122 of the 455 DEGs were mapped with the swine genome, whereas 384 were mapped with the human genome. Due to limited gene annotation of the swine genome in the DAVID database, the human genome was selected as background for functional annotation.

On the one hand, Gene Ontology (GO) Biological Process (BP) terms significantly enriched for up-regulated DEGs (Bonferroni-adjusted $P < 0.05$) were predominantly involved in immune response (e.g., adaptive and innate immune responses and classical complement activation pathway; Fig. 2A, B and S1), indicating robust activation of neuroimmune defense mechanisms. Cytokine receptors (IL1R1, IL10RA, FAS and IL13RA1), chemokine receptors (CCR2/CRS5), complement (ITGB2), toll-like receptors (TLR3 and TLR4), integrins (ITGAS and ITGB2), Fc receptor (CD32), scavenger receptor (MSR1), leukocyte antigens (CD37, CD53, CD86, and PTPRC), and transmembrane proteins associated with the immune response (IFITM3 and TMEM173) were coordinately up-regulated in the hippocampus of PRRSV piglets (Fig. 2C). The protein products of these genes were previously defined as being part of the microglial “sensome” (Hickman et al., 2013), which serves as a “sensory apparatus” to pathogen-associated molecular patterns and danger-associated molecular patterns. This was surprising because there was little evidence that the brain was infected by PRRSV: First, with the >33.5 M sequenced cDNA fragments from hippocampal tissue of PRRSV piglets, a BLAST search of the PRRSV genome revealed on average only 8 hits, a number so small it is most likely explained by contaminating blood; second, virus was not detected in microglia isolated from PRRSV piglets even though PRRSV preferentially infects mononuclear myeloid cells (data not shown). In addition to immune-related genes, GO BP terms for extracellular matrix (ECM) organization, blood vessel development, and adhesion molecules were significantly enriched (Bonferroni-adjusted $P < 0.05$). Of note, adhesion molecules mediate migration and recruitment of immune cells across the endothelial barrier, although we have not seen evidence of brain infiltration of leukocytes in this model (Conrad et al., 2015).

On the other hand, GO Cellular Component (CC) and Molecular Function (MF) terms significantly enriched for down-regulated DEGs (unadjusted $P < 0.05$) were mainly associated with synapse and calcium channel, respectively (Fig. 2D and E). A closer look at the 21 DEGs encoding synaptic proteins that were manually identified revealed 15 were down-regulated and only 6 were up-regulated (Fig. 2F).
3.2. PRRSV infection causes sickness and affects microglia phenotype and morphology

The RNA sequencing data suggested peripheral viral infection induced a robust neuroimmune response. Furthermore, the up-regulation of genes that are part of the microglia sensome suggested peripheral infection altered microglial cell activity, although this had not been previously established. Therefore, in a subsequent study piglets were inoculated (PD 7) intranasal with PRRSV or given sterile culture medium and euthanized 21 d later (PD 28) to assess microglial cell phenotype, morphology, and function. All experimental procedures were identical to that described by Elmore et al. (2014), except spatial learning was not assessed. Repeated-measures ANOVA of body temperature, feeding activity, body weight (Fig. 3A–C), and serum TNF-α and IL-10 (Fig. 3E–F) revealed main effects of day and treatment, and a day × treatment interaction (at least $P < 0.03$ for all except the main effect of treatment for body weight was $P < 0.06$). In general, piglets showed clinical signs of infection beginning 3 d after inoculation and showed signs of recovery by the end of the study. At the end of the study, whole brain weight was less in PRRSV piglets ($P < 0.001$; Fig. 3D).

Microglia were isolated from hippocampal tissue at PD 28 following a procedure involving enzymatic digestion of tissue and separation of CD11b+ cells by antibody-coated magnetic beads as was used in rodents (Nikodemova and Watters, 2012). CD11b+ cells were subsequently stained for CD45, which differentiates microglia (CD11b+CD45low to intermediate) from infiltrating macrophage/monocytes (CD11b+CD45high) (Hickman et al., 2013; Nikodemova and Watters, 2012; Sedgwick et al., 1991). Three populations that differed in CD45 expression (low, intermediate, or high) were identified in CD11b+ cells by flow cytometry (Fig. 4A). The three populations exhibited a similar side scatter, but the forward scatter of the CD45low fraction differed from that of CD45intermediate and CD45high, suggesting the CD45low cells were larger. This is consistent with a study in rats that found neonatal infection resulted in larger CD11b+ cells in brain (Bilbo et al., 2012). In PRRSV piglets the percentage of cells CD11b+CD45low decreased ($P = 0.028$) and CD11b+CD45intermediate increased ($P = 0.051$). Consistent with this, hippocampal expression of PTPRC, which encodes CD45, increased in response to infection. These results suggest respiratory viral infection shifted CD45low cells towards CD45intermediate and affected cell morphology (Fig. S2B). No differences were observed for the CD45high fraction, suggesting infection did not result in infiltration of peripheral monocytes, although it should be noted that infiltrating monocytes can downregulate CD45 after entering the brain parenchyma (Wohleb et al., 2013). Consistent with an earlier study (Elmore et al., 2014), infection dramatically increased the proportion of isolated cells that stained for MHC-II,
a marker of microglial cell activation (Fig. 4B and C). The increase in MHC-II was evident in CD45+ cells (low, intermediate and high; Fig. 4B).

Coronal sections of the hippocampus from a subset of control and PRRSV piglets were stained with Iba-1 to assess microglial cell morphology. Representative photomicrographs of microglia in the CA1 region of the hippocampus of a control and PRRSV piglet are shown in Fig. 5, as are summary graphs showing effects of treatment on soma length, width, length-to-width ratio, and overall size. PRRSV infection increased soma length and the length-to-width ratio (P < 0.05), and tended to increase overall soma size (P < 0.10). An increased in microglial cell volume was also observed in adult rats that was suffered from bacterial infection at neonatal age (Bland et al., 2010). In healthy brain, microglia can show heterogeneous shapes, ranging from amoeboid to spindle or rod-shaped (Taylor et al., 2014). Herein we observed more bipolar rod-like microglia in PRRSV piglets.

3.3. PRRSV infection affects microglia function

The effects of PRRSV infection on the transcriptional signature of hippocampal tissue as well as the phenotype and morphology...
of microglia suggested microglial cell function was altered. Therefore, we next sought to assess in microglia isolated from control and PRRSV piglets chemotaxis, phagocytosis, and cytokine production in response to LPS or Poly I:C. The yield of viable CD11b+-microglia from control and PRRSV piglets (63±, scale bar = 10 µm). Inserts highlight a typical microglial cell seen in controls and a rod-like microglial cell seen in PRRSV piglets (63±, scale bar = 10 µm). (C, D, E, F) Soma length (C), width (D), length-to-width ratio (E), and soma size (F). Statistical analysis: one-way ANOVA; *P < 0.05, #P < 0.10; n = 6).

Fig. 5. PRRSV infection increased the number of microglia in CA1 region displaying an elongated soma at 21 dpi. (A and B) Representative photomicrographs of Iba1-stained microglia in a control (A) and PRRSV (B) piglet (40×, scale bar = 100 µm). Inserts highlight a typical microglial cell seen in controls and a rod-like microglial cell seen in PRRSV piglets (63×, scale bar = 10 µm). (C, D, E, F) Soma length (C), width (D), length-to-width ratio (E), and soma size (F). Statistical analysis: one-way ANOVA; *P < 0.05, #P < 0.10; n = 6).

Contrary to the up-regulation of microglia sensome genes in homogenized hippocampal tissue from PRRSV piglets (Fig. 2C), isolated microglia from PRRSV and control piglets expressed similar levels of TLR3 and TLR4 mRNA when unstimulated (Fig. 6D and E). This may have been due to microglia being cultured for more than 24 h prior to isolating RNA, but we cannot exclude that the expression profile of hippocampus is also impacted by other cell types (i.e., astrocyte). Nonetheless, a significant interaction between in vivo PRRSV treatment and in vitro treatment affected microglia expression of TLR3 (P < 0.003) and TLR4 (P < 0.023) mRNA. Compared to microglia from control piglets, microglia from PRRSV piglets expressed more TLR3 and TLR4 mRNA after LPS (Fig. 6D and E). Poly I:C increased TLR3 and TLR4 mRNA similarly in microglia from control and PRRSV piglets (Fig. 6D and E). Finally, microglia from PRRSV piglets tended to have lower expression of the neurotrophic factor BDNF (P = 0.099) regardless of in vitro treatment. Collectively, these findings suggest respiratory viral infection heightens the activity of hippocampal microglial cells.

4. Discussion

The hippocampal transcriptome induced by PRRSV revealed a gene expression pattern indicative of (a) neuroinflammation as the up-regulated DEGs were predominantly involved in the immune response; and (b) reduced plasticity as synaptic genes were mostly down-regulated. This was evident 21 d after inoculation at a time piglets showed signs of recovery and in the absence of compelling evidence that virus entered the brain. By isolating microglia from adult mice and direct RNA sequencing, Hickman et al. (2013) reported microglia express a cluster of transcripts encoding proteins for sensing changes in the brain's milieu, including endogenous ligands and microbes. In the present study, although the transcriptome was not assessed for isolated microglia, the hippocampus transcriptome showed up-regulation of 26 genes that are consistent with the microglia sensome, suggesting peripheral viral infection up-regulates this sensory apparatus. It warrants future research to characterize potential long-lasting consequences associated with up-regulated sensome genes. Nonetheless, these data are interesting in light of the proposed neuroimmune mechanism of psychiatric disorders and a recent transcriptome analysis of brain samples acquired through the Autism Tissue Program (Gupta et al., 2014). The analysis implicated dysregulated microglial responses in concert with altered neuronal activity-related genes in autism brains. The analysis further suggested activation of the type I interferon signaling pathway in autism brain, which is notable because type I interferons are part of the innate immune response to viruses and type I interferon genes were up-regulated in the present study. These findings are largely consistent with another study that used gene co-expression network analysis and identified two network modules highly correlated with autism: (a) a down-regulated neuronal module including genes involved in synaptic function; and (b) an
Collectively, the results presented herein suggest early-life infection alters the sensome, rendering microglia more responsive to subsequent stimuli. Several limitations should be noted, however. First, enhanced activity was determined ex vivo with microglia isolated from control and infected piglets. If microglia of infected and recovered piglets behave similarly to a secondary in vivo challenge is not currently known. Second, all assessments related to microglia activity were done at a single time point after inoculation. A more complete picture of the induction, maintenance, and cessation of microglia activity would be useful, as would knowing if changes to microglia persisted into adulthood. Clearly more work is needed to understand how this might impact brain development and associated behavioral outcomes. Nonetheless, the results highlight a new potential role for the microglial sensome in response to peripheral infection.

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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.2016.02.010.

References


