Journal of Neuroimmunology 285 (2015) 137–142

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Peripheral challenge with a viral mimic upregulates expression of the complement genes in the hippocampus

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ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 5 June 2015
Accepted 10 June 2015

Keywords:
Polyinosinic–polycytidylic acid
Inflammation
Hippocampus
Complement system
Transcriptome
Immunofluorescence

ABSTRACT

Peripheral challenge with a viral mimetic, polyinosinic–polycytidylic acid (PIC) induces hippocampal hyperexcitability in mice. Here, we characterized this hippocampal response through a whole genome transcriptome analysis. Intraperitoneal injection of PIC resulted in temporal dysregulation of 625 genes in the hippocampus, indicating an extensive genetic reprogramming. The bioinformatics analysis of these genes revealed the complement pathway to be the most significantly activated. The gene encoding complement factor B (CfB) exhibited the highest response, and its upregulation was commensurate with the development of hyperexcitability. Collectively, these results suggest that the induction of hippocampal hyperexcitability may be mediated by the alternative complement cascades.

1. Introduction

It has been well established that peripheral inflammation exerts profound effects on the brain. A classic example is the induction of sickness behavior, an assembly of behavioral traits that alter the priorities of the affected individual to promote recovery (Dantzer, 2006; Dantzer and Kelley, 2007; Quan and Banks, 2007). The underlying mechanisms involve the activation of local innate immune cells that mount a fulminant inflammatory response characterized by the production of a slew of cytokines, chemokines and other factors. Subsequently, these peripheral inflammatory signals are relayed to the brain whereby they induce a "mirror" inflammatory response. The cerebrally-generated inflammatory factors, as well as the peripherally-generated factors that gain access to the brain parenchyma, interact with specific neuronal circuits and change their activities, leading to behavioral changes such as fever, depression, anhedonia, malaise, anorexia, adipsia, lethargy and fatigue. Although sickness behavior has evolved as a protective mechanism, the activation of cerebral inflammatory pathways carries a risk for exacerbating other neuropathologies. For example, systemic administration of a viral mimic, polyinosinic–polycytidylic acid (PIC), heightens prion-related neurodegeneration (Field et al., 2010), and autoimmune retinal damage (Ren et al., 2011). The administration of a bacterial mimic, lipopolysaccharide (LPS) increases the formation of neurofibrillary tangles in a transgenic model of Alzheimer's disease (Kitazawa et al., 2005), degeneration of the nigrostriatal dopaminergic system in a model of Parkinson's disease (Machado et al., 2011) and degeneration of motor axons in a model of amyotrophic lateral sclerosis (Nguyen et al., 2004). LPS challenge also reactivates focal autoimmune lesions in a model of multiple sclerosis (Serres et al., 2009), increases post-stroke mortality (Denes et al., 2011) and hampers post-stroke regeneration (Yousuf et al., 2013). These effects are congruent with clinical evidence that the burden of peripheral infections aggravates dementia in Alzheimer's disease (Holmes et al., 2003), increases the severity of functional detriments in Parkinson's disease (Ferrari and Tarelli, 2011) and in amyotrophic lateral sclerosis (Zhang et al., 2009), and exacerbates relapses in multiple sclerosis (Edwards et al., 1998; Buljevac et al., 2002). However, the underlying mechanisms remain elusive.

We have recently demonstrated that intraperitoneal injection of PIC renders the brain hyperexcitable as seen from a profound increase in the susceptibility of C57BL/6 mice to kainic acid (KA)-induced seizures (Kirschman et al., 2011; Michalovicz and Konat, 2014). Hyperexcitability is a critical component and a putative mediator in various neuropathologies, including epilepsy (McNamara, 1999), Alzheimer's disease (Khedr et al., 2011), Parkinson's disease (Ikoma et al., 1994), amyotrophic lateral sclerosis (Bae et al., 2013), multiple sclerosis (Caramia et al., 2004; Rossi et al., 2012), stroke (Carmichael, 2003; Huynh et al., 2013) and traumatic brain injury (Nardone et al., 2011). Consequently, hyperexcitability is likely to provide a mechanistic link between peripheral inflammation and the progression of neurodegeneration. In our studies (Kirschman et al., 2011; Michalovicz and Konat, 2014), PIC-induced hyperexcitability manifested as a several-fold increase in the intensity and duration of KA-induced status epilepticus that persisted for three days. Because the hippocampus is the ictal onset region for

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http://dx.doi.org/10.1016/j.jneuroim.2015.06.003
0165-5728/

KA-induced seizures (Ben-Ari and Cossart, 2000), we evaluated changes in the expression of selected hippocampal genes induced by PIC challenge (Michalovicz and Konat, 2014). In concordance with the previous studies in the whole brain (Konat et al., 2009; Fil et al., 2011), we found the upregulation of a battery of genes encoding cytokines, chemokines and chemokine receptors in the hippocampus. Moreover, PIC challenge altered the expression of several genes related to glutamatergic and GABAergic neurotransmission, as well as several microRNAs associated with seizure pathology and/or with modulation of neuro-immune functions. This polygenic response warranted further, more comprehensive genetic studies to delineate molecular/cellular pathways that govern the development of hippocampal hyperexcitability.

In the present study, we use a transcriptome-based microarray approach to identify putative regulatory pathways in the hippocampus that may underlie development of the hyperexcitable phenotype instigated by PIC-induced peripheral inflammation. We found the complement pathway to be the primary pathway upregulated by PIC challenge. Because the cerebral complement system emerges as a õpotent regulator of neuronal excitability (Schafer et al., 2012), PIC-induced complement upregulation may provide a mechanistic link between peripheral inflammation and hippocampal hyperexcitability.

2. Materials and methods

2.1. Animals

Eight-week old C57BL/6 mice were procured from Charles River Laboratories (Wilmington, MA) and housed under 12-h light/dark conditions (lights on at 6 am) with unrestricted access to food and water. The animals were intraperitoneally (i.p.) injected with 12 mg/kg of high molecular weight PIC (Invivogen, San Diego, CA) in 100 μl of saline. Control mice were injected with saline only. After 3 h, the development of sickness behavior was assessed by the rearing test (Michalovicz and Konat, 2014) to confirm successful i.p. injection. All procedures were approved by the West Virginia University Animal Care and Use Committee and conducted in compliance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Microarray analysis

At 6, 24 and 48 h after PIC challenge, mice were deeply anesthetized with 65 mg/kg of pentobarbital (Fatal Plus, Vortech Pharmaceutical, Dearborn, MI) administered i.p., sacrificed by pneumothorax, and transaortically perfused with saline. The hippocampi were dissected out, homogenized in a TRI reagent (Molecular Research Center, Inc., Cincinnati, OH), and total RNA was isolated per manufacturer's protocol. RNA integrity was verified by the Agilent bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). The microarray analysis was performed using the Illumina BeadChip mouse WG-6 format (Illumina, Inc., San Diego, CA). The BeadChips were scanned using the Illumina iSCAN system and analyzed by Illumina's GenomeStudio 2011.1 Gene expression analysis module 1.9.0 (Illumina, Inc., San Diego, CA). The RNA integrity and microarray analysis work for this publication was performed in the Genomics Research Core at the University of Pittsburgh. Data analysis was performed using the caGEDA web application (Patel and Lyons-Weiler, 2004). Briefly, fluorescence intensities were median normalized and $\log_2$ transformed. Subsequently, differential gene expression between time points (0, 6, 24 and 48 h) was evaluated using the J5 test (Patel and Lyons-Weiler, 2004) with a threshold cutoff of 6.0. The J5 test identified DEGs based on the average difference between controls and 6, 24 and 48 h for a particular gene over the average difference for all genes on the array. In addition to individual time point comparisons, a gene expression profile was created for a comparison of 0 h to all other time points (0–ALL) which comprised only those genes whose average expression across 6, 24 and 48 h was above threshold. Each individual time point comparison, i.e. 0–6, 0–24 and 0–48, was tested for overlap with the 0–ALL comparison, using the Overlap4 tool (http://bioinformatics.pitt.edu/GE2/Overlap4.html) and only the overlapping genes were included in later analysis. Gene ontology analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003). Pathway analysis was performed using the Pathway Express application (Draghici et al., 2007).

2.3. qRT-PCR

Total RNA was isolated as above. cDNA was synthesized using a SuperScript III First-strand Synthesis kit (Invitrogen, Carlsbad, CA) and quantified using RT^2 SYBRGreen (Qiagen, Valencia, CA). qRT-PCR was performed in an ABI7500 real-time PCR system (Applied Biosystems, Foster City, CA). Glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA was used as an internal control. The ΔΔCt method was used for quantitation. Primer sequences are available upon request.

2.4. Immunofluorescence

Mice were sacrificed as described above and transaortically perfused with saline followed by 4% paraformaldehyde. Brains were removed from the skull and infused in 4% paraformaldehyde for a minimum of 24 h at 4 °C. The brains were cryoprotected in 30% sucrose for 24 h at 4 °C before sectioning. 35 μm slices were cut on a freezing microtome and stored in 4% paraformaldehyde. For immunofluorescent staining, free-floating sections were blocked in PBS with 5% FBS (Atlanta Biologicals, Lawrenceville, GA) and 0.5% Triton-X 100 (Fisher Scientific, Waltham, MA) for 1 h at room temperature and then incubated in primary antibody overnight at 4 °C. After washing in PBS, sections were incubated in secondary antibody for 1 h at room temperature. Sections were then washed and mounted on slides using a Vectashield hard set mounting medium (Vector Laboratories, Burlingame, CA). Confocal imaging was performed at the WVU Microscope Imaging Facility with a Zeiss LSM 510 laser scanning confocal on a LSM Axio Imager upright microscope (Zeiss, Jena, Germany). Primary antibodies were: goat-anti-Cfb (N-14; Santa Cruz Biotechnology, Dallas, TX) and mouse-anti-NeuN (Millipore, Billerica, MA), rabbit-anti-GFAP (Dako; Carpinteria, CA), rabbit-anti-Iba1 (Wako; Richmond, VA). Secondary antibodies were anti-goat, anti-mouse and anti-rabbit conjugated to Alexa Fluor 488 or Alexa Fluor 555 (Invitrogen, Carlsbad, CA), respectively. Images were analyzed using the ZEN 2012 image analysis software (Zeiss, Jena, Germany).

2.5. Statistical analysis

Data were analyzed by ANOVA and expressed as means ± SD. Statistical comparisons between groups were performed using Student's *t* test. Differences between groups were considered significant at $p \leq 0.05$.

3. Results

3.1. Genome-wide expression analysis

Previously, we have demonstrated that peripheral challenge with PIC results in the alteration of a plethora of inflammatory, neurotransmission-related and miRNA genes in the mouse hippocampus (Michalovicz and Konat, 2014). To gather a more global perspective of this genomic response, we performed a genome-wide array analysis of the hippocampi at 0, 6, 24 and 48 h following PIC challenge. As shown in Fig. 1 (left panel), a total of 625 differentially expressed genes (DEGs) were identified across all time points when compared with control (0 h). While the expression of many DEGs was restricted to particular intervals after PIC challenge, 98 DEGs were altered at all of the time points.

Taking advantage of our microarray time course, we interrogated the dataset for genes that showed dynamic (changing) expression over all