Neurobiology of Aging 34 (2013) 1610–1620

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

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

Aging enhances classical activation but mitigates alternative activation in the central nervous system

Daniel C. Lee [a,d], Claudia R. Ruiz [f], Lori Lebson [e], Maj-Linda B. Selenica [a,d], Justin Rizer [b,d], Jerry B. Hunt Jr [a,d], Rahil Rojiani [a,d], Patrick Reid [a,d], Sidharth Kammath [b], Kevin Nash [b,d], Chad A. Dickey [c,d], Marcia Gordon [b,d], Dave Morgan [b,d,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL, USA
[b] Department of Molecular Pharmacology and Physiology, College of Medicine, University of South Florida, Tampa, FL, USA
[c] Department of Molecular Medicine, College of Medicine, University of South Florida, Tampa, FL, USA
[d] University of South Florida Byrd Alzheimer's Institute, Tampa, FL, USA
[e] Department of Neurology and Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[f] Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 31 December 2011
Received in revised form 19 December 2012
Accepted 20 December 2012

Keywords:
Microglia
Cytokines
Inflammation
Macrophage
Alternative activation
Classical activation
Microarray

ABSTRACT

The roles of microglia and macrophages during neuroinflammation and neurodegenerative diseases remain controversial. To date, at least 2 activations states have been suggested, consisting of a classical response (M1) and the alternative response (M2). Identifying selective biomarkers of microglia that representative their functional activation states may help elucidate disease course and enable a better understanding of repair mechanisms. Two cocktails containing either tumor necrosis factor (TNF)–α, interleukin (IL)–12, and IL-1β (referred to as CKT-1) or IL-13 and IL-4 (referred to CKT-2) were injections into the hippocampus of mice aged 6, 12, or 24 months. Microarray analysis was performed on hippocampal tissue 3 days postinjection. Gene transcripts were compared between CKT-1 versus CKT-2 stimulator cocktails. Several selective transcripts expressed for the CKT-1 included CXCL13, haptoglobin, MARCO, and calgranulin B, whereas a smaller subset of genes was selectively induced by the CKT-2 and consisted of FIZZ1, IGF-1, and EAR 11. Importantly, selective transcripts were induced at all ages by CKT-1, whereas selective gene transcripts induced by CKT-2 decreased with age suggesting an age-related reduction in the IL-4/ IL-13 signaling pathway.

1. Introduction

Microglia are typically viewed as tissue macrophages of the central nervous system, although their embryonic origin may be different from circulating monocytes (Chan et al., 2007, Ginhoux et al., 2010, Prinz et al., 2011, Ransohoff and Cardona, 2010). Microglia constantly survey the central nervous system (CNS) and, when necessary, clear cellular debris and contribute to tissue remodeling after CNS injury. They respond to environmental changes with a variety of activation states, differing in morphology and gene expression. Environmental signals such as cytokines, chemokines, and pattern recognition signals can regulate the activation state of the microglia (Graeber, 2010; Morgan et al., 2005; Rivest, 2009).

Peripheral macrophages have been classified as having at least 2 polarized activation states consisting of classical (M1) and alternative (M2) activation states, analogous to the Th1 and Th2 activation states of T cells (Gordon and Martinez, 2010; Mantovani et al., 2004). M1, or classical activation, is associated with elevations of interferon (IFN)–γ, interleukin (IL)–1, IL-6, IL-12, and tumor necrosis factor (TNF)–α, and is often characterized as inflammatory. This response pattern causes pathogen destruction but can also result in tissue damage. M2, or alternative activation, is associated with elevations of IL-4 and/or IL-13. This response is associated with parasitic infections and tends to dampen the M1 activation response; in some circumstances this is associated with tissue remodeling and healing (Gordon, 2003; Taylor et al., 2005). In addition, a regulatory macrophage state has recently been described, associated with cytokines such as IL-10 and immune complexes (Martinez-Pomares, 2003; Tierney et al., 2008). Although much of the literature supports polarized responses for peripheral macrophages, various activation states of microglia during CNS diseases are less well understood. For example, a recent

This work is supported by NIH AG04418 and AG15470.
* Corresponding author at: 4001 E. Fletcher Avenue, MDC36, Tampa, FL 33613, USA. Tel.: +1-813-974-3949; fax: +1-813-866-1601.
E-mail address: scientist.dave@gmail.com (D. Morgan).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.12.014

report described roles for TNF-α in brain repair, preventing neuronal apoptosis, oligodendrocytic proliferation, and remyelination of neurons (Arnett et al., 2001; Park and Bowers, 2010). Microglial activation is prominent in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Akiyama et al., 2000). In some circumstances, activated microglia are thought to promote the disease, and many attempts to diminish CNS inflammation have been attempted in Alzheimer's disease patients, so far without benefit (Aisen et al., 2000; Aisen et al., 2003; Breitner et al., 1995). In murine models, a complex impact of microglial activation on amyloid versus tau pathology is being observed, with microglial activation by lipopolysaccharide, fractalkine receptor deletion, or IL-1 overexpression benefiting amyloid pathology, yet exacerbating tau pathology (DiCarlo et al., 2001; Lee et al., 2010; Liu et al., 2010; Shaftel et al., 2007). The studies described here were intended to address several questions. The first study regarded the degree to which brain microglia express distinct M1 and M2 phenotypes after exposure to cytokine cocktails intended to elicit these phenotypes. The second study regarded identification of additional markers to characterize the distinct phenotypes to better understand their role during disease progression. A final question was to determine the degree to which these responses vary over the lifespan of the mouse. Because neurodegeneration occurs on a background of brain aging, we believed that age-associated changes in the response of microglia to cytokine signals might help to explain divergent results regarding the role of inflammation in young to middle-aged mice compared to the observations in the aged human brain.

We chose 2 cocktails to begin the examination of microglial responses. The first comprised IL-1, IL-12, and TNFα. We specifically excluded IFN-γ from this cytokine mixture for 2 reasons. The first is that IFN-γ is a T-cell product and T cells are not normally found in the brains of Alzheimer's disease patients (Akiyama et al., 2000). The second is that the response to IFN-γ in preliminary studies to define cocktail constituents was so massive that no dose of M2 cocktail components could produce a comparable reaction. To permit comparisons, we opted to exclude IFN-γ and induce the classical activation response with a combination of IL-1, IL-12, and TNFα. For the M2 cytokine cocktail, we used both IL-4 and IL-13. Although both appear to use the same signaling system and share receptors (Martinez et al., 2009), we believed that there might still be some unique responses to IL-13 that would be important to monitor.

2. Methods

2.1. Stereotaxic intracranial injections

All mice were nontransgenic mice derived from our breeding colony producing a mixed genetic background of 60% C57BL/6; 20% DBA, 10% SJL, and 10% SW. Although arguments can be made in either direction, we believed that this hybrid background, which we have bred stably for 12 years, may avoid well-described idiosyncratic macrophage responses associated with individual inbred mouse strains. The only selection in this breeding has been the elimination of the retinal degeneration mutation (*rd1*) contributed by SJL and SW lines. Young (6 months old), middle-aged (12 months), or aged (24 months) mice were injected bilaterally with 2 μL per site (4 sites in total; 8 μL in total) of an M1 cytokine cocktail (CKT-1) containing TNF-α (667 ng), IL-12 (26 ng), and IL-1β (26 ng), with an M2 cytokine cocktail (CKT-2) containing IL-4 (800 ng) and IL-13 (240 ng), or with vehicle control (phosphate-buffered saline). The cytokine concentrations represent total mass delivered in 8 μL. Mice were injected with a 10-μL Hamilton syringe with a 26-gauge needle in the right and left cortex and in the right and left hippocampus. The bilateral hippocampus and anterior cortex paradigm was used to generate a greater overall repsonse to the activation profile versus a single injection. Based on previous determinations, we found that bilateral injections into the hippocampus and anterior cortex produces a greater overall activation response than single or unilateral injections. Previously determined coordinates from bregma were as follows: frontal cortex, anteroposterior, +1.7 mm, lateral, ±2.5 mm, vertical, −3.0 mm; hippocampus, anteroposterior, −2.7 mm; lateral, ±2.7 mm, vertical, −3.0 mm. The solution was dispensed at a constant rate of 0.5 μL/min.

2.2. Tissue collection and histochemical procedures

Three days after cytokine injections, mice were weighed and injected with 100 mg/kg of pentobarbital. Mice were then intracardially perfused with 25 mL of 0.9% saline. The brain was removed, the right hemisphere was dissected on ice and frozen at −80 °C, and the left hemisphere was immersion fixed in 4% paraformaldehyde in 100 mM PO_4 buffer (pH 7.4) for 24 hours. The tissue was cryoprotected in a series of 10%, 20%, and 30% sucrose solutions. Horizontal sections were cut at 25 μm using a sliding microtome and stored at 4 °C in Dulbecco's phosphate buffered saline containing 100 mM sodium azide for immunohistochemistry.

2.3. Microarray analysis and quantitative real-time PCR

Total RNA was extracted from mouse hippocampus (15–30 mg wet tissue) using rotor-stator emulsification (Tissuemizer) and applied to the RNeasy mini-spin columns kit (Qiagen, Valencia, CA, USA) followed by DNase treatments to removed genomic DNA. All total RNA was reverse transcribed using the Superscript II kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. For microarray analysis only, an aliquot of total mRNA from each of 2 mice of the same group and same age were pooled together for a total of 1 μg per sample, yielding 3 samples per treatment group. Ocean Ridge Biosciences performed microarray analysis. Arrays were subjected to slide format with a total of 38,467 spots. Raw data was filtered to remove manufacturer and visual QC flags, resulting in 38,338 spot intensities. Raw data were background subtracted, Log2 transformed, and normalized, resulting in 34,954 mouse probes. After true positive thresholds (TPTs) were calculated from negative control probe data, spiking controls, and constitutive mouse probes, 18,899 mouse probes scored above threshold from at least 1 group. Probes were ranked by false discovery rate (FDR) with corrected p values using analysis of variance (ANOVA) with a Bayesian error model. A total of 2,851 probes were significant at FDR <0.05.

Table 1
Primers used for quantitative real-time polymerase chain reaction

Gene	Source	Catalog no.
CXCL13	Qiagen	QT00107917
S100A8	Qiagen	QT01749958
S100A9	Qiagen	QT00105252
CCL5	Qiagen	QT01747165
TIMP1	Qiagen	QT00996282
MARCO	Qiagen	QT00102004
CHI3L3	Qiagen	QT00108829
IGF1	Qiagen	QT00154469
RETNLA	Qiagen	QT00254359
ARG1	Qiagen	QT00134288
EAR11	Qiagen	QT00266959
ERDR1	Qiagen	QT00318577
18S	Qiagen	QT01036875
GAPDH	Qiagen	QT01658692