Neuroscience 302 (2015) 59–73

REVIEW

M1 AND M2 IMMUNE ACTIVATION IN PARKINSON'S DISEASE: FOE AND ALLY?

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Abstract—Parkinson's Disease (PD) is a chronic and progressive neurodegenerative disorder of unknown etiology. Autopsy findings, genetics, retrospective studies, and molecular imaging all suggest a role for inflammation in the neurodegenerative process. However, relatively little is understood about the causes and implications of neuroinflammation in PD. Understanding how inflammation arises in PD, in particular the activation state of cells of the innate immune system, may provide an exciting opportunity for novel neuroprotective therapeutics. We analyze the evidence of immune system involvement in PD susceptibility, specifically in the context of M1 and M2 activation states. Tracking and modulating these activation states may provide new insights into both PD etiology and therapeutic strategies.

This article is part of a Special Issue entitled: Inflammation in Nervous System Disorders.

Key words: microglia, macrophage, monocyte, neurodegeneration, genetics, animal models.

Contents

Introduction 59
M1 activation state 60
M2 activation states 60
Microglia and macrophages in PD 61
Evidence of M1 activation in PD brain 63
Protein aggregates as M1 stimuli 63
Genetics of PD relevant to the M1 activation state 64
Animal models implicate M1 activation in dopaminergic neurodegeneration 65
M2 activation states in PD and neurodegeneration 65
Therapeutic manipulation of M1 and M2 responses in PD 66
Concluding remarks 67
Acknowledgements 67
References 67

INTRODUCTION

Parkinson's Disease (PD) is a chronic, progressive neurodegenerative disorder characterized by hallmark symptoms that include bradykinesia, ataxia, rigidity, and resting tremor. Pathologically, PD is characterized by the severe loss of melanated dopaminergic neurons in the substantia nigra pars compacta (SNpc), and deposition of α-synuclein into Lewy bodies and Lewy neurites in many remaining neurons (Spillantini et al., 1997; Spillantini et al., 1998). Markers of inflammatory responses have long been noted in and around the SNpc (Nagatsu et al., 2000; Hunot and Hirsch, 2003; Khandelwal et al., 2011). Initially, post-mortem examination using immunohistochemical techniques revealed a spectrum of different types of immune cells, as well as cytokines, in PD brain tissue (McGeer et al., 1988; Boka et al., 1994; Imamura et al., 2003). Later, ligands selective for activated immunological cells also demonstrated activation and inflammatory responses, both in early and late stages of disease (Gerhard et al., 2006; Bartels et al., 2010). Retrospective studies of anti-inflammatory therapeutics also implicate inflammation in some aspect of etiology (Gagne and Power, 2010). Several possibilities exist for understanding aspects of inflammation in PD: particular immunological responses are detrimental, benign, or beneficial. PD is not an acute disorder, so inflammatory responses may show temporal association with disease progression, where an initial response is beneficial and later becomes detrimental.

Therapeutic targeting of inflammation underlying disease pathogenesis represents an exciting approach for novel neuroprotective strategies. However, an incomplete understanding of the role of inflammation in PD will likely hinder successful implementation of rationally derived therapeutics. The canonical role of

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Abbreviations: AD, Alzheimer Disease; CD, cluster of differentiation; EAE, experimental autoimmune encephalitis; eQTL, expression of quantitative trait loci; GM-CSF, granulocyte-modifying colony-stimulating factor; GWAS, genome-wide association studies; HLA-DR, human leukocyte antigen-DR; IFNγ, interferon-gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; IRFs, interferon regulatory factors; JAK, Janus kinase; LPS, lipopolysaccharide; LRRK2, leucine-rich repeat kinase 2; MHCII, major histocompatibility complex 2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NSAID, non-steroidal anti-inflammatory drug; PBR, peripheral benzodiazepine receptor; PD, Parkinson's Disease; PET, positron emission tomography; TLR, toll-like receptor; TNF, tumor necrosis factor.

http://dx.doi.org/10.1016/j.neuroscience.2014.11.018
0306-4522/

microglia as predominant resident immune cell in the brain has led to the hypothesis that these cells underlie the inflammatory processes noted in PD (Qian and Flood, 2008; Long-Smith et al., 2009). However, there is emerging evidence that peripheral immune cells may also be changed in PD (Hisanaga et al., 2001; Saunders et al., 2012; Funk et al., 2013). Understanding inflammation in the context of M1 and M2 activation paradigms may help clarify interpretation of these complex and dynamic processes.

In this review, we will discuss a context for M1 and M2 microglia and macrophage activation states. Emerging evidence for a critical role for these cells and activation states in PD will also be discussed, along with predictions about how modulating or blocking activation might be beneficial for the treatment of PD.

M1 ACTIVATION STATE

Macrophage activation states are understood within a continuum of activation paradigms that mirrors the responses of lymphocytes. The M1, or classical activation state, is associated with pro-inflammatory and pro-killing functions defined by macrophage responses to microbes. The M1 response was defined through studying the antimicrobial activity of macrophages toward Bacillus and Listeria after secondary exposure to other bacteria (Mackaness, 1962). This study highlighted an antigen-dependent mechanism for macrophage activation, which has since been parsed into the prototypical M1 response.

The most common methods to track M1 responses include analysis of both secreted factors as well as cell surface and intracellular markers that increase in abundance. The M1 state causes the release of several pro-inflammatory cytokines including tumor necrosis factor (TNF), interleukin 6 (IL-6), IL-12, and IL-1β as well as several chemokines such as C-C motif ligand 2 (CCL2) and C-X-C motif ligand 10 (CXCL10). The production of these cytokines and chemokines is widely used as markers for the M1 state. Additional non-cytokine/chemokine markers of the M1 state include increased cell surface expression of major histocompatibility complex II (MHCII), increased cluster of differentiation marker 86 and 16/32 (CD86, CD16/32), and increased expression of inducible nitric oxide synthase (iNOS) (Nau et al., 2002; Martinez et al., 2006).

To induce a M1 state in macrophages *in vitro* and *in vivo,* more defined stimuli have been utilized to elucidate M1 responses in macrophages, including the cytokine interferon-gamma (IFNγ) and lipopolysaccharide (LPS), an outer membrane component of gram-negative bacteria. IFNγ signals through a dimer of the IFNγ receptor 1 and 2. Activated IFNγ receptors cause the recruitment of Janus kinase 1 and 2 (JAK1/2) which in turn phosphorylates and activates STAT1 and interferon regulatory factors (IRFs), mainly IRF1 (Hu and Ivashkiv, 2009). The signal transduction cascade induces transcriptional changes that up-regulate the expression of cytokines, receptors, and hundreds of other genes associated with the M1 response (Dalton et al., 1993; Huang et al., 1993; Waddell et al., 2010).

The other prototypical M1 stimulus, LPS, signals through a different class of pattern recognition receptors known as toll-like receptors (TLRs). LPS binds to TLR4 along with co-receptors MD2 and CD14. Other TLR4-independent LPS activation responses have also been described (Hagar et al., 2013; Kayagaki et al., 2013). TLR4 activation stimulates the transcription factors NFKβ, STAT5, AP1, and IRFs, through MyD88 and TRIF, which go on to cause a transcriptional up-regulation of a similar set of genes as IFNγ (Hu and Ivashkiv, 2009). Other TLRs show affinity for a variety of ligands. TLR2 binds a wide variety of microbial products including LTA. TLR3 binds dsDNA, TLR7 binds ssRNA, and TLR9 binds unmethylated CpG islands in DNA. These TLR activation cascades, through MyD88 or TRIF, skew macrophages toward the M1 state (Takeda and Akira, 2004; Yamamoto and Takeda, 2010; Casanova et al., 2011).

Granulocyte-modifying colony-stimulating factor (GM-CSF) is another, more recently described stimulus to the M1 activation paradigm (Lacey et al., 2012; Bayer et al., 2013). However, as opposed to LPS, GM-CSF can induce pleomorphic activation states that can show elements of both M1 and M2 activation states (Weisser et al., 2013). GM-CSF binds to a large receptor that is comprised of a dodecamer of subunits (Hansen et al., 2008). Intracellularly, GM-CSF utilizes many of the same effectors as that of the TLRs, but also utilizes ERK and AKT signal transduction pathways (Krausgruber et al., 2011). GM-CSF stimulation can produce similar cytokine responses to that of LPS, but to a much lesser extent as compared with other M1 stimuli (Lehtonen et al., 2007). GM-CSF function is understood through knockout studies in rodents as well as mutations in human populations, which highlight GM-CSF as a driver of hematopoietic (pre-cursors to myeloid lineage cells) cell differentiation and proliferation (Dranoff and Mulligan, 1994; Dirksen et al., 1997). The M1 activation state is graphically depicted in Fig. 1, and listed in Table 1.

M2 ACTIVATION STATES

The alternative M2 activation state encompasses a broad set of responses as compared to M1 responses. Generally, the M2 activation state is associated with healing and scavenging, opposing the pro-killing state of M1 activation states. The M2 state is further subdivided into M2a, M2b, and M2c. These three states have some biochemical overlap, but have distinct activation mechanisms as well as effector outputs.

The M2a category was the first alternative activation state described and was developed as a paradigm to understand host response to parasites, and, as such, is associated with encapsulation and killing of parasites as well as allergy. IL-4 is the prototypical M2a stimulus and can bind three different receptor pairs. Each receptor pair can activate JAK1 or JAK3 which activates STAT6 leading to transcriptional changes associated with the M2a state, including; CD206 (mannose receptor), scavenger receptors (SRs), and suppressor of cytokine release 1 (SOCS1) (Edwards et al., 2006; Martinez et al., 2013). M2a macrophages will secrete polyamines and IL-10, which will block pro-inflammatory (e.g., IFNγ, IL6, and TNF) cytokine production (Lu et al., 2013). With the exception of IL-10 secretion, which is released by all the