# NEUROBIOLOGY OF DISEASE WORKSHOP:

## The Role of Innate Immunity in CNS Disorders Throughout the Lifespan

Organized by Gwenn Garden, MD, PhD,
Stuart Lipton, MD, PhD, and
John Neumaier, MD, PhD

SfN Society for Neuroscience

Neuroscience 2018

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# NEUROBIOLOGY OF DISEASE WORKSHOP

## The Role of Innate Immunity in CNS Disorders Throughout the Lifespan

Organized by Gwenn Garden, MD, PhD; Stuart Lipton, MD, PhD; John Neumaier, MD, PhD

Friday, November 2, 2018

8 a.m.–5 p.m.

Location: San Diego Convention Center • Room: 6A

| TIME | TOPIC | SPEAKER |
|---|---|---|
| 7:30–8 a.m. | CHECK-IN | |
| 8–8:10 a.m. | Opening Remarks | Gwenn Garden, MD, PhD • University of Washington<br>Stuart Lipton, MD, PhD • Scripps Research Institute<br>John Neumaier, MD, PhD • University of Washington |
| 8:10–8:40 a.m. | Patient Presentation | Josep Dalmau, MD, PhD • University of Pennsylvania, JSD Hospital Barcelona |
| 8:40–9:20 a.m. | Introduction to Innate Immunity in the CNS Context | Chris Glass, MD, PhD • University of California, San Diego |
| 9:20–10 a.m. | Microglia: Phagocytosing to Clean, Sculpt, and Destroy | Beth Stevens, PhD • Harvard Medical School |
| 10–10:40 a.m. | Microglia and Neurodegeneration | Joseph El-Khoury, MD • Massachusetts General Hospital |
| 10:40–11 a.m. | MORNING BREAK | |
| 11–11:40 a.m. | The Emerging Role of the Immune System in Depression and other Psychiatric Disorders | Andrew Miller, MD • Emory University |
| 11:40–12:20 p.m. | Neuroimmune Interactions that Drive Chronic Itch | Diana Bautista, PhD • University of California, Berkeley |
| 12:20–1 p.m. | Brain Injury, Inflammation and Impulse Control | Catharine Winstanley, PhD • University of British Columbia |
| 1–2 p.m. | LUNCH — ROOM 6DE | |

**AFTERNOON BREAKOUT SESSIONS • PARTICIPANTS SELECT FIRST DISCUSSION GROUPS AT 2 P.M.**

| TIME | BREAKOUT SESSIONS | SPEAKERS | ROOM |
|---|---|---|---|
| 2–3:30 p.m. | Group 1:<br>Neuroinflammation, Reward, and Depression | Scott Russo, PhD and Andrew Miller, MD | 1A |
| | Group 2:<br>Neuroinflammation and Disorders of Impulse Control | Catharine Winstanley, PhD and John Neumaier, MD, PhD | 1B |
| | Group 3:<br>How Cancer and Cancer Treatment Impact CNS Function | Gwenn Garden, MD, PhD and Joseph El-Khoury, MD | 2 |
| | Group 4:<br>iPSC Derived Microglia as Models of Neuroinflammation and Human Disease | Nicole Coufal, MD, PhD, Stuart Lipton, MD, PhD, Dorit Trudler, PhD, and Chris Glass, MD, PhD | 4 |
| | Group 5:<br>The Gut-Brain Axis: Microbiome Metabolites and CNS Function | Elaine Hsiao, PhD, Francisco Javier Quintana, PhD, and Diana Bautista, PhD | 5A |
| | Group 6:<br>Psychosis Involving Innate and Adaptive Immune Dysfunction | Beth Stevens, PhD and Josep Dalmau, MD, PhD | 5B |

**AFTERNOON BREAKOUT SESSIONS • PARTICIPANTS SELECT SECOND DISCUSSION GROUPS AT 3:30 P.M.**

| TIME | BREAKOUT SESSIONS | SPEAKERS | ROOM |
|---|---|---|---|
| 3:30–5 p.m. | Same as sessions above | | |

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# Introduction

Neuroscience has evolved to include the exploration of communication between the nervous system and biological signals that originate in other organs. This evolution has dramatically influenced our understanding and knowledge of the neurobiology of disease, enabling translational neuroscientists to explore disorders of the nervous system in a more holistic manner. A prime example is immunological dysfunction, which is a major contributor to many nervous system disorders.

The immune system can be divided into two components: innate and adaptive immunity. Adaptive immunity is the response by lymphocytes to generate populations of cells that specifically target defined antigens. Innate immunity generally involves macrophages and microglia: it is the system of cells and molecules involved in the response to injury or infection (known as inflammation) that operates without targeting specific antigens. As such, inflammation often regionally disrupts tissue homeostasis, causing bystander injury. In the nervous system, neuroinflammation is an important mechanism contributing to a wide spectrum of disorders that start during neurodevelopment and extend to age-related neurodegenerative disease. This Neurobiology of Disease Workshop (NDW) will address the mechanisms by which neuroinflammation contributes to the pathophysiology of nervous system disorders.

The course book consists of articles from the faculty covering topics related to the NDW lectures and the research being conducted in faculty laboratories. Dr. Glass and colleagues review the transcriptional regulatory mechanisms involved in microgliogenesis and activation and how these processes are involved in disease processes. Dr. Stevens describes how microglia remodel brain cells and synaptic circuits via phagocytosis in both health and disease states—from developmental disorders to neurodegenerative diseases. Dr. El Khoury reviews the inflammatory mechanisms influencing neurodegenerative disease pathogenesis. Dr. Miller and colleagues explain how neural–immune pathway interactions play a major role in stress disorders. In fact, they have been recently identified as targets for therapy in depression, diverging from traditional neurotransmitter targets. Drs. Walsh and Bautista describe how neural and immune cells and molecular signals interact to produce chronic itch. This particularly distressing hallmark of a variety of skin disorders can induce a vicious cycle of scratching that further exacerbates itch involving both the central and peripheral nervous systems. And finally, Dr. Winstanley discusses the role of neuroinflammation after traumatic brain injury in the subsequent development of behavioral and cognitive abnormalities involving impulsivity.

These chapters will supplement your access to information on this emerging area of study in translational neuroscience. The goals of the faculty organizing this NDW are to inspire participants' long-term interest in this area of research. The faculty invite your questions about the information in this course book and throughout the NDW lectures, both during the event and at follow-up webinars.

# Transcriptional Regulation of Microglia

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## Introduction

Microglia are the main resident macrophage population of the CNS and perform numerous functions required for CNS development, homeostasis, immunity, and repair. Many lines of evidence indicate that dysregulation of microglia contributes to the pathogenesis of neurodegenerative and behavioral diseases. These observations motivate a clearer definition of the mechanisms that control microglia identity and function in health and disease. In this chapter, we present a conceptual framework for how different classes of transcription factors (TFs) interact to select and activate regulatory elements that control microglia development and their responses to internal and external signals. We then describe functions of specific TFs in both normal and pathological contexts and conclude with a consideration of open questions to be addressed in the future.

Microglia derive from a unique lineage of erythromyeloid precursors (EMPs) in the yolk sac (Hoeffel et al., 2015). These EMPs infiltrate the brain during early development, differentiate into microglia, and maintain their population by self-renewal (Nayak et al., 2014). Microglia distribute themselves throughout the CNS, adopt a heavily ramified morphology, and continuously scan their surroundings (Nimmerjahn et al., 2005). Microglia share many traits with other subsets of tissue-resident macrophages, including a dependence on the CSF1 receptor (CSF1R) for differentiation and survival, a requirement for PU.1 as an essential lineage-determining transcription factor (LDTF), the ability to efficiently phagocytose tissue debris, and the ability to quickly trigger an inflammatory response following detection of pathogens or tissue damage (Davies et al., 2013). In addition to responding to injury and infection, microglia carry out functions that are specific to the CNS environment, including secretion of neurotrophic factors and developmental refinement of synaptic networks (Salter and Beggs, 2014). Upon activation, microglia can acquire a range of phenotypes that can either contribute to disease progression or ameliorate it. Although dysregulated microglia are implicated in the pathogenesis of several neurodegenerative and psychiatric conditions, the mechanisms controlling developmental, homeostatic, and pathogenic programs of microglia gene expression remain poorly understood.

Insights into the transcriptional regulation of cell-type-specific functions can be obtained by analyzing transcriptional regulatory elements (Heinz et al., 2015). Promoters provide the obligatory transcriptional start sites necessary for RNA synthesis and are often sites of signal-dependent regulation. However, they are occupied primarily by broadly expressed TFs such as SP1 and GABP, and by themselves are insufficient to confer the specific regulatory control necessary to generate cell-type-specific programs of gene expression. This additional information is provided by distal regulatory elements called enhancers (Andersson et al., 2014). Enhancers represent the most numerous binding sites for LDTFs and signal-dependent transcription factors (SDTFs) and are major sites for the integration of internal and external signals. Enhancers exhibit distinctive patterns of modifications to adjacent histones that can be detected by chromatin immunoprecipitation sequencing (ChIP-seq), and these patterns can be used to putatively classify enhancers as inactive, primed, or active (Ernst et al., 2011).

*In vitro* studies of elicited peritoneal macrophages provided the basis for a collaborative/hierarchical model of enhancer selection and activation involving interactions between LDTFs and SDTFs (Heinz et al., 2010, 2013). According to this model, the initial steps of enhancer selection are driven by collaborative interactions of LDTFs and other "pioneer" factors that recognize factor-specific DNA sequences in closed chromatin and generate nucleosome-free regions (Zaret et al., 2008) (Fig. 1). Initial occupancy of enhancers by LDTFs and their collaborative partners can result in histone modifications associated with a primed state of activity. The transition from an inactive or primed state to an active enhancer can be induced by SDTFs in response to stimuli (Kaikkonen et al., 2013). These SDTFs bind mainly to enhancers previously established by LDTFs (Heinz et al., 2010). This process is frequently hierarchical, such that SDTF binding is dependent on LDTF binding, whereas loss of the SDTF does not influence the binding of the LDTF (Kaikkonen et al., 2013). The observation that SDTFs are directed to a predetermined set of cell-specific enhancers at least partly explains how a broadly expressed TF can regulate specific transcriptional responses in different cell types. However, the distinction between LDTFs and SDTFs is not always clear-cut, as some TFs that regulate the ontogeny of a cell type can also serve as mediators for environmental stimuli. In this chapter, we examine microglia development and function in the context of this model, with a focus on roles of the major LDTFs and SDTFs that are expressed in microglia.

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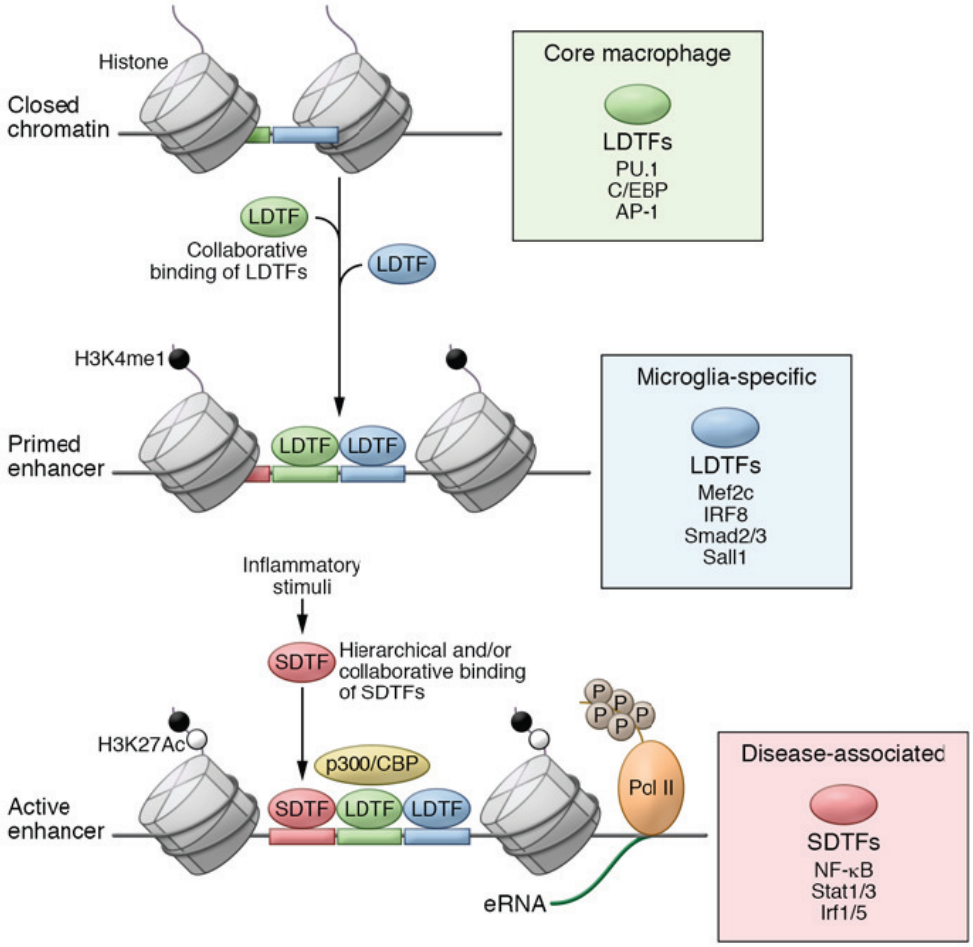


**Figure 1.** Different classes of TFs interact and regulate cellular identity. Top, Initial steps of enhancer selection in closed chromatin consist of regularly positioned nucleosomes. Green and blue shading, Closely spaced binding sites for LDTFs. Middle, Collaborative interactions between LDTFs generate primed enhancers, characterized by a nucleosome-free region and monomethylated H3K4 (H3K4me1). Bottom, Active SDTFs localize to primed enhancers, resulting in recruitment of coactivators and RNA polymerase II (Pol II) (thought to be important for both enhancer expression and activation) and generation of enhancer RNAs (eRNAs). Reprinted with permission from Holtman et al. (2017), Fig. 2. Copyright 2018, American Society for Clinical Investigation.

## Transcriptional Control of Microglia Identity

In mice, EMPs exclusively express the TF runt-related TF-1 (RUNX1) between embryonic day (E) 6.5 and E8.0. Microgliogenesis is dependent on the TFs PU.1 and interferon regulatory factor-8 (IRF8 (Kierdorf et al., 2013). In the yolk sac, EMPs differentiate into pre-macrophages (pMacs) that express a core macrophage program that includes the receptors CX3CR1 and CSF1R, as well as the TFs MAF, BATF3, PPARG, IRF8, and zinc-finger E-box-binding homeobox-2 (*ZEB2*) (Mass et al., 2016), a program that persists when these precursors infiltrate various organs to establish resident macrophage populations. Microglia development has been proposed to progress in three developmental stages (Matcovitch-Natan et al., 2016): early microglia (until E14), pre-microglia (from E14 to the first weeks after birth), and adult microglia (from a few weeks after birth onward). Early microglia, like EMPs, express genes associated with cell-cycle signaling pathways and the TF E2F6 and DNA methyltransferase-1 (DNMT1), which may help them populate the brain. The genes expressed by pre-microglia, including the TFs early growth response-1 (EGR1) and spalt-like TF-1 (SALL1), are associated with neural maturation and synaptic pruning (Buttgereit et al., 2016). Later, as the brain matures, microglia acquire a surveilling phenotype and express the TFs JUN, FOS, MEF2A, and MAFB as well as a set of genes that is highly expressed in microglia, such as the characteristic purinergic receptor P2RY12, transmembrane protein TMEM119, and the chemokine receptor CX3CR1 (Butovsky et al., 2014), the expression of which is mostly conserved in humans (Galatro et al., 2017; Gosselin et al., 2017).

Systematic analyses of human microglia gene expression from postmortem (Galatro et al., 2017) and surgical tissues (Gosselin et al., 2017) indicate broad similarities with mouse, but also a number of significant differences, including genes implicated

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in neurodegenerative diseases. Further, age-related changes in gene expression differed significantly between mice and humans. Both human and mouse microglia enhancers (Gosselin et al., 2014, 2017) are enriched for motifs associated with PU.1, IRF, RUNX, MEF2, CEBP, AP-1, SMAD, and MAF, indicating a conserved set of microglia LDTFs. However, SALL2, SALL3, and SMAD were expressed much more highly in mice, whereas class II major histocompatibility complex transactivator (CIITA), PPARG, EGR3, and RUNX2 were preferentially expressed in human microglia. These findings suggest that the differences between mouse and human microglia gene expression are driven mainly by species-specific organization of regulatory elements, but some differences also result from differential expression of TFs.

The importance of the brain environment in maintaining microglia identity is suggested by the rapid and extensive changes in gene expression that occur when they are transferred from the brain to an *in vitro* environment (Butovsky et al., 2014). Substantial subsets of genes associated with risk alleles for neurodegenerative or behavioral diseases exhibited environment-dependent expression (Gosselin et al., 2017). Genes that were downregulated *in vitro* were highly correlated with genes that are upregulated in primitive macrophages following their migration into the fetal brain (Matcovitch-Natan et al., 2016; Gosselin et al., 2017). These changes in gene expression are associated with downregulation of numerous putative microglia LDTFs, such as SALL1, and corresponding alterations in enhancer landscapes. Collectively, these findings provide evidence for an important role of brain-derived signals in controlling a conserved network of signal-dependent and lineage-determining TFs in mouse and human microglia.

## LDTFs That Establish Physiological Microglia

PU.1 is a master regulator of the myeloid lineage that drives microgliogenesis and is a major factor in selecting the microglia enhancer landscape (Goldmann et al., 2016). PU.1-deficient mice exhibit multiple hematopoietic abnormalities, and PU.1 deficiency ablates microglia (Kierdorf et al., 2013). Moreover, PU.1 binds to most enhancers in both mouse (Gosselin et al., 2014) and human microglia (Gosselin et al., 2017), highlighting its central role in establishing the microglia enhancer landscape.

SALL1 is a zinc-finger transcriptional repressor that belongs to the spalt-like family of TFs that function in tissue morphogenesis. Mutations in SALL1 are associated with Townes–Brocks Syndrome. Human and mouse microglia uniquely and highly express SALL1 compared with other macrophage populations, suggesting that it may function as an LDTF (Gosselin et al., 2017). *Sall1*-deficient animals have normal microglia colonization in the brain, but the microglia exhibit an abnormal amoeboid morphology (Koso et al., 2016). Moreover, in the absence of *SALL1*, microglia are more proinflammatory and phagocytic and show downregulation of the microglia-specific gene-expression signature (Buttgereit et al., 2016), which has detrimental consequences for neurogenesis and tissue homeostasis. Taken together, these studies suggest that SALL1 inhibits reactive microgliosis while promoting surveilling homeostatic microglia.

MAFB is a member of the large MAF subfamily of TFs that bind to the MAF recognition element and form heterodimers with cMAF, Jun, and Fos. Motif mutation analysis suggests that MAFB functions as an LDTF in collaboration with PU.1 (Gosselin et al., 2014). In microglia, the expression of MAFB increases during microglia development and its deletion disrupts gene expression in adult microglia more severely than in premature microglia (Matcovitch-Natan et al., 2016). MAFB regulates the expression of immune and antiviral genes.

MEF2C is a member of the MEF2 family of TFs that mediate the stress response. Microglia-specific enhancers are enriched for the MEF2-binding motif, and MEF2C is the highest expressed transcription factor that can bind to this motif (Gosselin et al., 2014). Microglia-targeted *Mef2C* knockout mice exhibit an exaggerated inflammatory response to lipopolysaccharide (LPS), which leads to an adverse effect on behavior and cognition (Deczkowska et al., 2017). Interestingly, an age-related downregulation of MEF2C in microglia was observed that was negatively correlated with increased type I interferon response. It is suggested that Mef2C functions as a microglial "off switch" that keeps microglia inflammatory behavior in check, that is downregulated in aging, and that could thereby contribute to age-related neurodegenerative diseases.

## Transcriptional Control of Disease Phenotypes

In disease conditions, microglia can adopt various phenotypes that exert both beneficial and deleterious effects, including tissue support, excessive pruning of synapses, induction of neuropathic pain, and exacerbation of neurodegeneration. New studies are beginning to shed light on how these phenotypes

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are transcriptionally regulated (Fig. 2). The collaborative/hierarchical model suggests that, in disease, a unique combination of pathological and inflammatory factors activates SDTFs that regulate the expression of specific microglia-response programs. A major obstacle to understanding the relationship among the different classes of TFs is that the environmental factors and pathological signals are complex and interact on multiple levels. In addition, the observation that culturing microglia *in vitro*, where they no longer receive brain-derived signals, results in dramatic changes to their enhancer landscape (Butovsky et al., 2014) calls into question the extent to which findings about SDTFs obtained *in vitro* are translatable to microglia *in vivo*. Given these observations, findings regarding the roles of SDTFs from *in vitro* studies should be interpreted with caution until they can be confirmed in *in vivo* conditions. Nevertheless, an increasing number of studies have identified transcriptional regulators of microglia activation and microglia phenotypes in pathological conditions.

## SDTFs That Mediate Inflammation

As the resident macrophages of the CNS, microglia are the first to respond to inflammatory insults in the brain, which they do by expressing a wide array of cytokines, chemokines, nitric oxide (NO), and various reactive oxygen species. Several transcriptional families have been identified that regulate the expression of proinflammatory mediators in macrophages and microglia, such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), signal transducer and activator of transcription (STAT), and IRF.

Members of the NF-κB family are pleiotropic regulators that play key roles in inflammation (Lanzillotta et al., 2015). NF-κBB1 (p105/p50), NF-κBB2 (p100/p52), RELA (p65), RELB, and C-REL form homodimers and heterodimers. Without stimulation, NF-κBB is sequestered in the cytoplasm; however, once activated, it translocates to the nucleus, where it binds to NF-κB binding sites exposed in open chromatin regions and induces

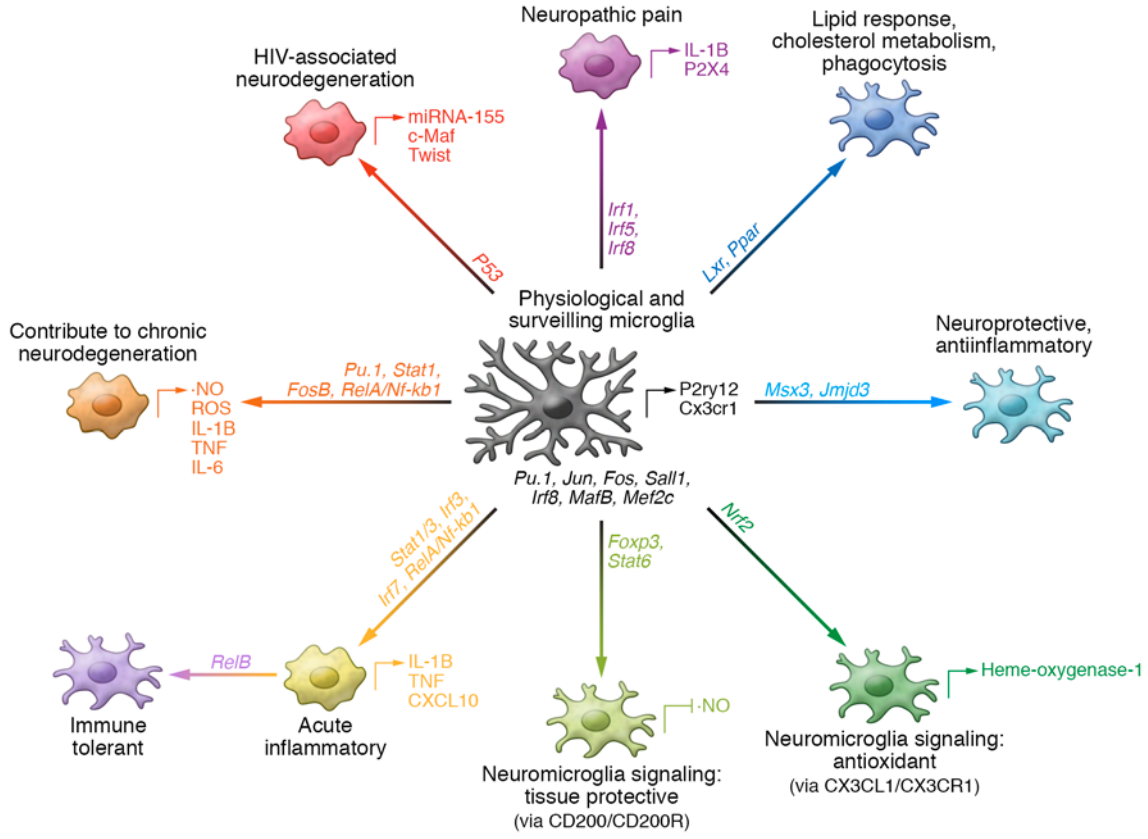


**Figure 2.** Transcription factors that regulate different microglia phenotypes. Microglia ontogeny is crucially dependent on LDTFs, such as PU.1 and SALL1. Microglia are diverse cells that acquire different functional phenotypes in response to the environment in which they reside. Several transcriptional regulators have been identified that regulate different microglia phenotypes. For example, proinflammatory microglia are regulated by IRF7, RelA, STAT1/3, and FosB. CXCL10, chemokine (C-X-C motif) ligand 10 (CXCL10); IL, interleukin; miRNA, micro RNA; ROS, reactive oxygen species; TNF, tumor necrosis factor. Reprinted with permission from Holtman et al. (2017), Fig. 3. Copyright 2018, American Society for Clinical Investigation.

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the expression of target genes (Bhatt and Ghosh, 2014). Many studies have examined the role of NF-κB in brain aging and neurodegenerative conditions (Lanzillotta et al., 2015). However, since expression of NF-κB is not limited to microglia, most of these studies have focused on its role in other cell types or its generic expression in the brain. In microglia, NF-κB is activated by signal transduction pathways responding to the presence of saturated fatty acids (Wang et al., 2012), α-synuclein (Couch et al., 2011; Hoenen et al., 2016), and amyloid-β (Chen et al., 2005). The induced response can be either protective or deleterious depending on the context of the stimulation.

The IRF family consists of nine members that bind to genomic loci known as interferon-sensitive response elements. IRF3, IRF5, and IRF7 mediate type I interferon responses induced by various toll-like receptor ligands (Zhao et al., 2015), whereas IRF1 is essential for the type II interferon response (Kaminska et al., 2016). IRF8 is a key regulator that appears to function as both an LDTF and an SDTF in microglia. *Irf8* deletion substantially reduces microglia numbers (Kierdorf et al., 2013), microglia ramification, and surface area and alters the expression of several cell-surface markers, indicating increased microglia immune activation (Minten et al., 2012). In zebrafish, *Irf8*-null mutants showed a complete absence of microglia (Shiau et al., 2015). In spinal cord microglia, IRF8 (in conjunction with IRF1 and IRF5) contributes to the induction of neuropathic pain after peripheral nerve injury (Masuda et al., 2012).

## Anti-inflammatory and Tissue-Supportive Mediators

Excessive inflammation in the CNS is detrimental to neuronal health (Perry and Holmes, 2014). Not surprisingly, several transcriptional mechanisms have been identified that limit the inflammatory responses of microglia and induce neuroprotective behavior. These include NR4A2 (nuclear receptor subfamily 4, group A, member 2, also known as NURR1), estrogen receptors (ERs), and nuclear respiratory factor 2 (NRF2).

In microglia, NR4A2 inhibits LPS-induced expression of proinflammatory cytokines (Saijo et al., 2009). Reduced NR4A2 expression results in exaggerated inflammatory responses in microglia and increases dopaminergic neuron death. NR4A2 exerts an anti-inflammatory effect by binding to RELA on inflammatory gene promoters and recruits the CoREST (corepressor of repressor element 1–silencing transcription factor) corepressor complex, which clears RELA and represses transcription. Additionally, activation of NR4A2 blocks inflammatory gene expression (De Miranda et al., 2015) and reduces infarct volume in acute cerebral ischemia (Xie et al., 2017). These studies suggest that NR4A2 is an anti-inflammatory regulator that exhibits neuroprotective features.

ERα and ERβ are a subclass of the nuclear receptor superfamily and function as estrogen-dependent TFs. Estrogens exert neuroprotective actions by signaling through ERs that are widely distributed in the male and female brain (Arevalo et al., 2015). In microglia, estrogen signaling has been shown to exert anti-inflammatory effects (Bruce-Keller et al., 2000). For example, androstenediol is an estrogenic steroid produced in the brain that binds to ERβ Androstenediol activation of ERβ suppresses the inflammatory responses of microglia by recruiting C-terminal binding protein–corepressor complexes to AP-1-dependent promoters (Saijo et al., 2011). Conversely, reduction of androstenediol or ERβ expression results in exaggerated LPS responses, and administration of androstenediol *in vivo* prevents encephalomyelitis. These findings provide evidence for an ERβ signaling pathway that controls inflammatory responses in microglia.

## Conclusions and Future Perspectives

Many transcriptional regulators that mediate microglia in health and disease have been identified, but much remains unknown about their functional consequences, how they guide microglia phenotypic diversity, the effects of natural genetic variation, and their responses to specific signals in the local microenvironment. One challenge is that many studies are purely descriptive or are performed on cultured microglia. The recent development of effective and inducible drivers of Cre-recombinase, such as *Cx3cr1-CreER*, enable microglia-targeted knockout and overexpression of genes of interest. These tools should provide stronger and more detailed experimental evidence as well as qualitatively new insights into the functional roles of TFs in regulating microglia *in vivo*.

Microglia can exhibit a diverse range of phenotypes in response to their surroundings, but these phenotypes are much more complex than the simple M1/M2 dichotomy originally proposed for macrophages (Ransohoff, 2016). Nor are these phenotypic states

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easily classified on a one-disease/one-phenotype basis. The proper framework for cataloging microglia diversity across pathological conditions, brain regions, local microenvironment, and developmental stages remains elusive. New technologies such as single-cell RNA sequencing and mass cytometry should facilitate a framework for microglia phenotype classification. Finally, the degree to which findings about transcriptional regulation in mice and zebrafish translate to humans is critical for gaining insight into the pathophysiology of neurodegenerative and neuropsychiatric diseases but remains mostly unaddressed. Further studies of human microglia are essential to identify the strengths and weaknesses of animal and cell-culture models.

## Acknowledgments

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# Microglia: Phagocytosing to Clean, Sculpt, and Destroy

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## Introduction

Microglia are the resident macrophages and primary phagocytes of the CNS. Unlike other phagocytes, which function primarily in immunity, microglia are immune cells that are heavily involved in not only supporting brain tissue, but also shaping it. Using phagocytosis, they destroy excess functional connections between neurons (synaptic pruning) to sculpt neuronal and synaptic circuits during development and throughout adulthood. They use classical immune molecules, such as complement, to signal to neurons and glia, and they survey their microenvironment using their dynamic processes. We now appreciate that microglia are key modulators of neuronal development and plasticity, yet details about their normal homeostatic role in the healthy brain and how they contribute to disease remain elusive. Several neurodegenerative disorders involve synapse loss, and emerging evidence from several mouse models suggests that microglia mediate this loss. Although pruning is not their only role, understanding how microglia recognize and prune synapses during development is providing new insight into synapse loss and dysfunction in disease, potentially nominating new therapeutic candidates.

## What Defines Microglia

Microglia were first characterized by del Rio-Hortega as a population of migratory phagocytic cells within the CNS. A long-standing mission has been to determine what defines microglia and to assess how they vary—not only across cell populations, but also across time, space, and individuals. It is becoming increasingly clear that microglia are a distinct macrophage population, differentiated and specialized from other tissue macrophages by microenvironment cues unique to the CNS (Gosselin et al., 2014; Lavin et al., 2014). Even so, the transcriptional profiles of microglia are remarkably diverse. Their profiles appear to vary by cell age, developmental stage, resident brain region, sex, and even gut microbiota (Butovsky et al., 2013; Hickman et al., 2013; Grabert et al., 2016; Matcovitch-Natan et al., 2016), suggesting that the cells' functional roles are shaped by complex regulatory networks in their local milieu. An intriguing question is whether this microglial heterogeneity shapes circuit wiring (or vice versa) in the developing brain and whether this underlies region-specific vulnerability in disease. Further studies are needed to obtain a more comprehensive profile of microglia, and particularly to assess how that profile changes in disease. This would provide insight into their remarkable plasticity in the living brain and the molecular pathways that underlie it.

## Phagocytic Functions in the Brain

Microglia are the local phagocytes of brain parenchyma, where they rapidly and efficiently clear dead or dying cells and debris. They have many roles, but being our brain's innate immune cells, they react to damage signals (Ransohoff and Cardona, 2010). They migrate to injury, extend their processes to it, and produce cytokines, chemokines, and other proinflammatory and anti-inflammatory signals for repairing injury and maintaining homeostasis. In addition, because they enter the brain early in development (embryonic day ~9.5 in mouse) (Ginhoux et al., 2010), they are well poised to impact the developing brain. Indeed, deletion of microglia-related genes or dysregulation of inflammatory markers leads to altered brain wiring and produces behavioral deficits associated with neuropsychiatric or neurodevelopmental disorders (Frost and Schafer, 2016). Microglia are also involved in spatial patterning, engulfing cells undergoing apoptosis (programmed cell death) as the embryonic brain matures and clearing apoptotic cells during adult neurogenesis. Together, these findings suggest that microglia play an important role in shaping the brain; however, signaling mechanisms underlying the crosstalk between microglia and other cell types, including neurons and astrocytes, still remain unclear.

## Microglia Shape Brain Wiring by Targeting Synapses

Microglia and immune-related proteins are critical for the refinement of neuronal connectivity in the developing brain. Manipulating microglia or microglia-related functions leads to sustained defects in synaptic connectivity, brain wiring, and plasticity-associated tasks (Hong et al., 2016b). Under healthy basal conditions, microglia connect with synapses using their highly motile processes (Fig. 1). The frequency of these connections is regulated by neuronal activity or sensory (visual) experience, suggesting that these contacts have functional implications. Indeed, microglia sculpt synaptic connectivity by engulfing the neuronal terminals that form the synapse, a process known as synaptic pruning (Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012).

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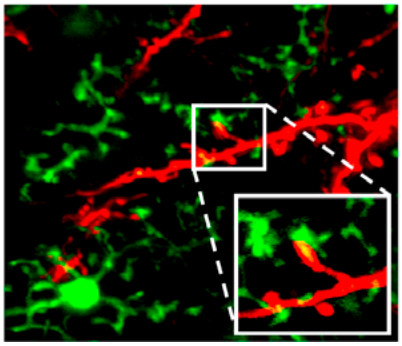

**Figure 1.** Microglia dynamically interact with synaptic elements in the healthy brain. Two-photon imaging in the olfactory bulb of adult mice shows processes of CX3CR1-GFP-positive microglia connecting to tdTomato-labeled neurons. Image courtesy of Jenelle Wallace at Harvard University. Reprinted with permission from Hong and Stevens (2016), Fig. 1. Copyright 2016, Elsevier.

## Complement as an "Eat Me" Signal for Synaptic Engulfment

How do microglia target synapses for phagocytosis? One mechanism involved in both neural development and models of neurodegeneration is the classical complement cascade (Stevens et al., 2007; Schafer et al., 2012; Hong et al., 2016a; Lui et al., 2016). In the peripheral immune system, classical complement proteins are "eat me" signals that promote rapid clearance of invading pathogens or cellular debris, which is done in part by macrophages expressing complement receptors (CRs) including CR3. In the developing visual thalamus, C1q (the initiating protein of the cascade) and C3 (a downstream protein) localize to subsets of immature synapses, likely marking them for elimination (Stevens et al., 2007). Microglia, which express CR3, phagocytose these synaptic inputs through the C3–CR3 signaling pathway (Schafer et al., 2012). Significantly, mice deficient in C1q, C3, or CR3 have sustained defects in synaptic connectivity. This complement-dependent synaptic pruning is significantly downregulated in the mature brain, suggesting that it is a highly regulated process, likely restricted to refinement stages of development.

## When Synapses Are (Wrongly) Marked as Debris

Reactive microglia and neuroinflammation are hallmarks of Alzheimer's disease (AD) and other neurodegenerative disorders, including Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (Ransohoff, 2016). Long considered to be events secondary to neurodegeneration, microglia-related pathways have been identified by emerging genetic and transcriptomic studies as central to AD risk and pathogenesis (Guerreiro et al., 2013; Jonsson et al., 2013; Lambert et al., 2013; Zhang et al., 2013; Karch and Goate, 2015; Villegas-Llerena et al., 2016; Efthymiou and Goate, 2017). Large-scale genome-wide association studies (GWAS) have identified more than 20 loci that are causally linked to AD. Of these, approximately half are expressed or exclusively expressed in microglia or myeloid cells, including TREM2 (triggering receptor expressed on myeloid cells 2), CD33 and members of the classical complement cascade, apolipoprotein J (ApoJ)/Clusterin, and complement receptor 1 (CR1) (Colonna and Wang, 2016; Villegas-Llerena et al., 2016). These findings (Griciuc et al., 2013) implicate microglia as critical or even causal players in AD pathogenesis; however, their biological significance remains elusive.

Region-specific synapse loss and dysfunction are early hallmarks of AD. Microglia and immune-related pathways have been implicated in AD pathogenesis through GWAS, but their role in synapse loss and cognitive impairment is poorly understood (Hong et al., 2016b). Complement proteins are often upregulated in AD and localize to neuritic plaques along with microglia, but these processes have been regarded largely as secondary to plaque-related neuroinflammation. However, in multiple AD mouse models, C1q and C3 have been associated with synapses before overt plaque deposition and are localized to brain regions vulnerable to synapse loss (Hong et al., 2016a). In addition, microglia in adult mice phagocytose synaptic material in the presence of soluble oligomeric amyloid-β, a key synaptotoxic species in AD. This engulfment is dependent on CR3, and blocking the complement cascade (C1q, C3, and CR3) protects the synapses (Hong et al., 2016a). Similarly, complement activation and microglia-mediated synaptic pruning are drivers of neurodegeneration caused by progranulin deficiency in mice (Lui et al., 2016). Together, these results suggest that a key developmental pruning pathway involving complement and microglia is reactivated early in the disease process to "mark" vulnerable synapses for destruction (Fig. 2).

It is important to understand what other pathways, besides complement, are critical for pruning. For example, CX3C chemokine receptor 1 (CX3CR1, also known as the microglial fractalkine receptor) is necessary for synapse maturation, elimination, and functional connectivity (Paolicelli et al., 2011, Zhan et al., 2014); however, whether or how fractalkine signaling regulates synaptic engulfment is not yet clear.

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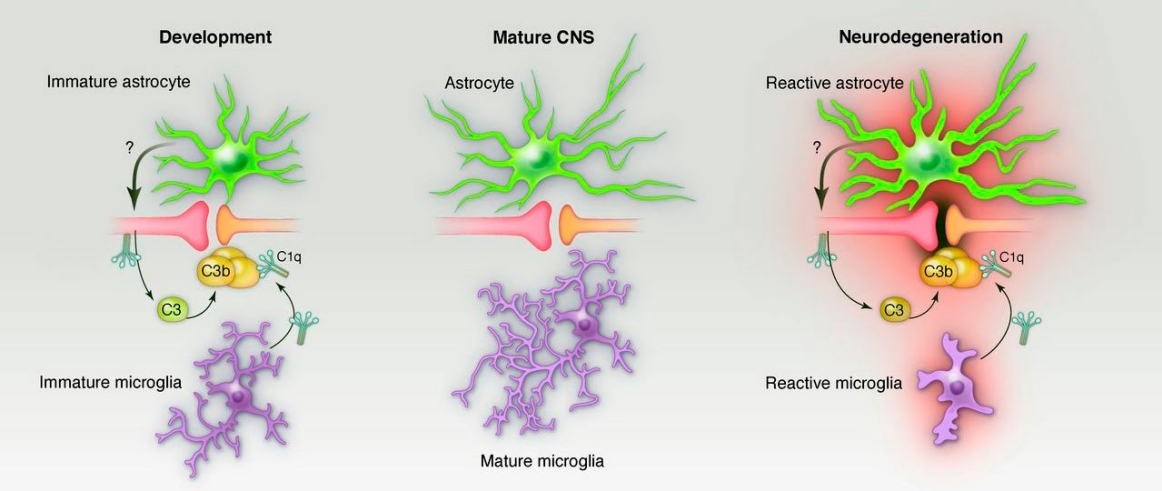


**Figure 2.** Complement-mediated synapse elimination during development and in neurodegenerative diseases. Left, In the developing brain, astrocytes induce the production of C1q in neurons through a molecular signal (?) that was recently identified as TGF-α (Bialas and Stevens, 2013). Neuronal and microglia-derived C1q tags the weak or superfluous synapses for removal through the classical complement pathway, resulting in C3 cleavage and synaptic C3b deposition. Complement-tagged synapses are removed through phagocytosis by microglia. Center, In the absence of activated complement, synapses remain stable. Right, We propose that complement-mediated synapse elimination drives the development and/or progression of neurodegenerative diseases. As observed in the developing brain, reactive astrocytes release signal(s) (?) that induce C1q production in neurons. Neuronal and microglia-derived C1q is then recruited to synapses, which triggers the activation of downstream classical complement components, produced in excess by reactive astrocytes, microglia, and neurons, resulting in microglia-mediated synapse elimination. Modified with permission from Stephan et al. (2012), Fig. 2. Copyright 2012, Annual Reviews.

Another pathway could involve neuronal activity that modulates synaptic engulfment in the developing brain (Schafer et al., 2012). Several other immune-related molecules have recently been identified as mediators of synaptic refinement and plasticity in the developing and mature brain (Boulanger 2009). These include neuronal pentraxins (NP1, NP2), neuronal activity–regulated pentraxin (Narp), and components of the adaptive immune system (e.g., the major histocompatibility class I [MHCI] family of proteins and receptors) (Huh et al., 2000; Bjartmar et al., 2006; Syken et al., 2006; Lee et al., 2014).

It is intriguing to speculate that components of the complement pathway may be interacting with one of several of these immune-related molecules to mediate CNS synapse elimination in health and disease. In neurodegenerative diseases, there is aberrant neuronal activity in distinct brain networks (Seeley et al., 2009), perhaps triggering region-specific microglial phagocytosis of synapses. Knowing the positive and negative signals that regulate pruning, as well as those that guide microglia to specific synapses, will be important for nominating therapeutic candidates.

## Redefining the Role of Microglia in Health and Disease

Loss of synaptic integrity has been linked to a host of developmental and neurodegenerative diseases, potentially implicating microglia-mediated pruning. Indeed, data from multiple models of neurological diseases, including AD, frontotemporal dementia, glaucoma, and West Nile virus–induced memory impairment, suggest that region-specific activation of the microglia–complement signaling pathway leads to synapse loss (Stevens et al., 2007; Hong et al., 2016a; Lui et al., 2016; Vasek et al., 2016). In addition, C4 (complement component 4) is a strong risk factor for schizophrenia (Sekar et al., 2016), indicating that this pathway could also underlie neurodevelopmental and neuropsychiatric diseases such as autism and schizophrenia. Microglia-mediated pruning may thus be involved in a variety of diseases, but its activation may differ in time, place, and magnitude. As such, it is imperative that we catalog pruning activity in the healthy brain and understand how microglia recognize and engulf specific synapses. This knowledge could not only lead to novel biomarkers for disease severity (e.g., of

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cognitive decline) but could also identify the time and place where intervention to protect synapses may be the most efficient. Further, learning how to modulate microglial functions may lead to drug candidates with broad therapeutic potential across multiple diseases.

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# The Emerging Role of the Immune System in Depression and Other Psychiatric Disorders

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## Inflammation and Depression

Depression is a devastating disease affecting 10% of the U.S. population and is the leading cause of disability worldwide. Data supporting the role of inflammation in depression are extensive and include findings that span experimental paradigms. Patients with depression exhibit all the cardinal features of an inflammatory response, including increased proinflammatory cytokines and their receptors and increased acute-phase reactants, chemokines, and soluble adhesion molecules in peripheral blood and CSF (Miller and Raison, 2016). Researchers have also described peripheral blood gene-expression profiles consistent with a proinflammatory M1-like macrophage phenotype and an overrepresentation of interleukin (IL)-6, IL-8, and type-I interferon (IFN)–related signaling pathways (Brambilla et al., 2014; Drago et al., 2015). In addition, increased expression of a variety of innate immune genes and proteins, including toll-like receptors, as well as microglial and astroglial activation in multiple brain regions have been found in postmortem tissue from depressed suicide victims (Steiner et al., 2008; Pandey et al., 2014; Torres-Platas et al., 2014; Nagy et al., 2015). Peripheral blood IL-1β, IL-6, tumor necrosis factor (TNF), and C-reactive protein (CRP) appear to be the most reliable biomarkers of inflammation in patients with depression (Miller and Raison, 2016). Polymorphisms in inflammatory cytokine genes, including those encoding IL-1β, TNF, and CRP, have also been associated with depression and its response to treatment (Barnes et al., 2017). Administration of inflammatory cytokines (e.g., IFN-α) or their inducers (e.g., endotoxin or typhoid vaccination) to otherwise nondepressed individuals causes symptoms of depression (Eisenberger et al., 2010; Capuron et al., 2012; Harrison et al., 2016). Moreover, blockade of cytokines (e.g., TNF) or inflammatory signaling pathways (e.g., cyclooxygenase-2) reduces depressive symptoms in patients with autoimmune or inflammatory disorders as well as in patients with depression (Kohler et al., 2014; Kappelmann et al., 2018).

As the field has matured, it has become increasingly apparent that inflammatory markers are elevated only in a subgroup of depressed patients (Miller and Raison, 2016) and also occur in patients with other stress-related and psychiatric disorders, including anxiety disorders and schizophrenia (Goldsmith et al., 2016; Michopoulos et al., 2016). Moreover, inflammation has specific effects on behavior across diagnoses that align with the National Institutes of Health's Research Domain Criteria. These include positive and negative valence systems related to altered motivation and motor activity (anhedonia, fatigue, and psychomotor retardation) and increased threat sensitivity (anxiety, arousal, and alarm) (Miller and Raison, 2016; Miller et al., 2017). The impact of inflammation on these specific symptom dimensions may trace its origins to evolutionary adaptations of withdrawal and hyperarousal that, respectively, may have subserved energy conservation for healing wounds and fighting infection while providing vigilance against attack in an otherwise vulnerable, wounded, and/or infected animal (Miller and Raison, 2016). Of note, a greater likelihood of increased inflammation is found in patient populations with a history of childhood maltreatment, medical illnesses, metabolic syndrome, and treatment resistance (Miller et al., 2017).

## Immune Pathways Involved in Depression

### The inflammasome: stress in translation

Exposure to psychosocial stress is a robust and reproducible predictor of developing depression and other psychiatric disorders in humans and represents the primary experimental pathway to produce depressive-like behavior in laboratory animals. Thus, the observation that a psychosocial laboratory stressor can activate an inflammatory response in humans was a major breakthrough in linking inflammation to depression (Bierhaus et al., 2003). An important question for the field has been, How does this occur? Recent data suggest that the inflammasome may represent a critical immunological interface between stress and inflammation (Fig. 1) (Fleshner et al., 2017). The inflammasome is made up of cytosolic protein complexes that form in myeloid cells in response to pathogenic microorganisms and nonpathogenic or "sterile" stressors. Assembly of the inflammasome leads to activation of caspase-1, which then cleaves the precursor forms of IL-1β and IL-18 into the active cytokines (Fleshner et al., 2017). Given the relatively sterile nature of psychosocial stress, inflammasome activation in depression may by triggered by endogenous damage-associated molecular patterns (DAMPs), including ATP, heat shock proteins (HSPs), uric acid, high mobility group box 1 (HMGB1), and a variety of molecules related to oxidative stress. All of these DAMPs are induced by the typical stressors used in animal models of depression (Fleshner et al., 2017), an effect that is in part mediated by the stress-induced release of catecholamines. Moreover, studies in laboratory animals indicate that chronic mild stress activates the NLRP3 (NOD-, LRR-, and pyrin domain–containing 3) inflammasome, which is well known to respond to DAMPs (Zhang et al., 2015). Blockade of NLRP3 reverses stress-induced increases in IL-1β in the peripheral blood and

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brain, while abrogating depressive-like behavior in rodents (Zhang et al., 2015). Interestingly, NLRP3 inflammasome upregulation and caspase-mediated cleavage of the glucocorticoid receptor can cause resistance to glucocorticoids, one of the most potent anti-inflammatory hormones in the body (Paugh et al., 2015). Glucocorticoid resistance is also a well-characterized biologic abnormality in patients with depression and has been associated with increased inflammation (Miller and Raison, 2016).

Increased expression of NLRP3 and caspase-1 has been found in peripheral blood mononuclear cells of depressed patients in association with increased blood IL-1β and IL-18, which in turn correlated with depression severity (Alcocer-Gomez et al., 2014). In addition, DAMPs that are known to activate NLRP3 are increased in patients with mood disorders (Stertz et al., 2015). Finally, there is increasing interest in the role of the gut microbiome in mood regulation, which may be mediated in part by the inflammasome (Mayer et al., 2014). Indeed, nonpathogenic commensal bacteria and related microbial-associated molecular patterns (MAMPs) from the gut can leak into the peripheral circulation during stress and activate the inflammasome, a process mediated by the sympathetic nervous system and catecholamines (Fleshner et al., 2017). Of note, stress-induced increases in IL-1β and IL-18 were attenuated by treating animals with antibiotics or neutralizing lipopolysaccharide (LPS), demonstrating the importance of the composition of the gut microbiome and gut permeability in stress-induced inflammatory responses (Fleshner et al., 2017).

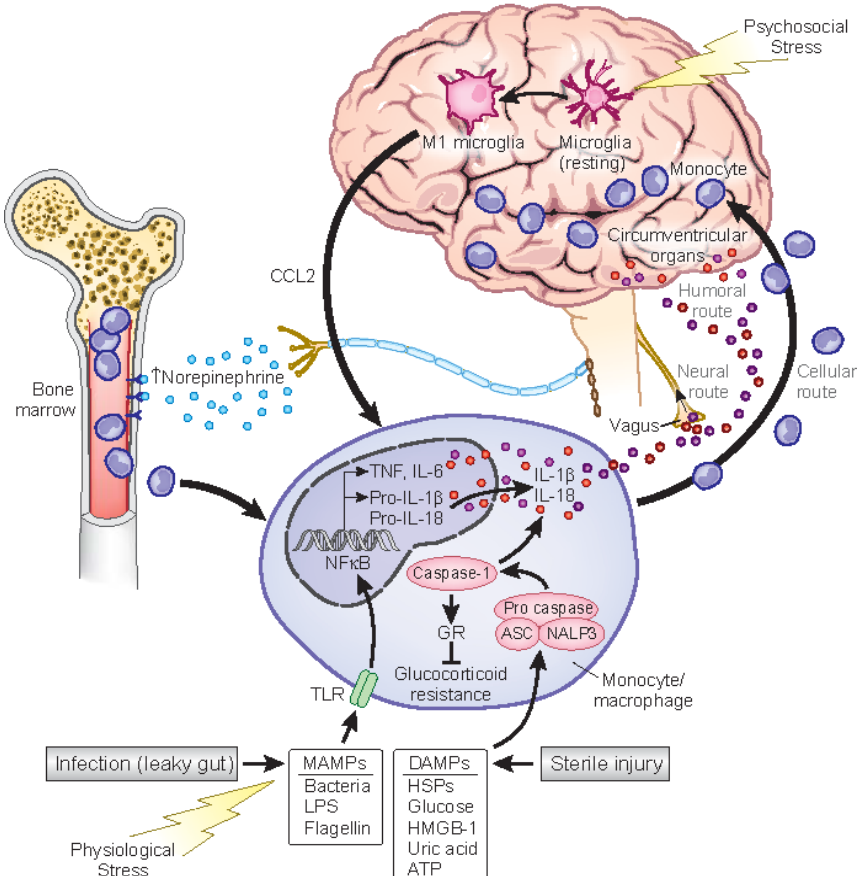


**Figure 1.** Transmitting stress-induced inflammatory signals to the brain. Stress-induced catecholamines stimulate bone-marrow release of myeloid cells (e.g., monocytes), which enter the periphery and encounter stress-induced DAMPs and MAMPs leaked from the gut. These molecules subsequently activate inflammatory signaling pathways such as NF-κB and the NLRP3 inflammasome. Stimulation of NLRP3 in turn activates caspase-1, which leads to mature IL-1 and IL-18 while cleaving the glucocorticoid receptor (GR), thereby contributing to glucocorticoid resistance. Activation of NF-κB stimulates the release of other proinflammatory cytokines, which, together with IL-1β and IL-18, can access the brain through humoral and neural routes. Psychosocial stress can also lead to the activation of microglia that release CCL2, which in turn attracts activated myeloid cells to the brain. Activated macrophages in vasculature and meninges stimulate a central inflammatory response. ASC, apoptosis-associated speck-like protein containing a CARD; NALP3: NACHT-, LRR-, and PYD domain–containing protein 3. Adapted with permission from Miller and Raison (2016), Fig. 2. Copyright 2016, Springer Nature.

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## Transmitting inflammatory signals to the brain

Work from laboratory animal studies has elucidated several pathways through which inflammatory signals can be transmitted from the periphery to the brain (Fig. 1). These data support the idea that inflammatory responses in peripheral tissues may drive inflammation in the brain, leading to behavioral changes including depression. Major pathways include the "humoral pathway," which involves cytokine passage through leaky regions in the blood-brain barrier (BBB), such as the circumventricular organs, and the binding of cytokines to saturable transport molecules on the BBB; and the "neural pathway," which involves the binding of cytokines to peripheral afferent nerve fibers, such as the vagus nerve, that in turn communicate cytokine signals to the brain (Miller and Raison, 2016). Interestingly, recent data suggest that the humoral pathway may be uniquely involved in stress, whereby stress-induced decreases in claudin-5 (a key molecule involved in BBB integrity) lead to direct passage of IL-6 into the brain proximate to the nucleus accumbens (Menard et al., 2017). Decreased claudin-5 was also found in the nucleus accumbens of postmortem samples from depressed patients (Menard et al., 2017). A third pathway, referred to as the "cellular pathway," involves the trafficking of activated immune cells (typically monocytes) to the brain vasculature and meninges. For example, release of TNF from inflamed peripheral tissues has been found to stimulate microglial cell production of chemokine (C-C motif) ligand 2 (CCL2), which then attracts monocytes to the brain (Wohleb and Delpech, 2017). Blockade of monocyte infiltration of the brain using antibodies to adhesion molecules abrogates depressive-like behavior. To illustrate, during social-defeat stress, labeled monocytes have been found to coalesce in multiple brain regions associated with the detection of threat (e.g., amygdala). This effect was dependent on CCL2 and facilitated by the mobilization of monocytes from the bone marrow as a result of stress-induced release of catecholamines and glucocorticoids (McKim et al., 2018) (Fig. 2). In postmortem brain samples from depressed patients, increased numbers of perivascular macrophages in association with increased gene expression of *CCL2* further support the cellular pathway (Torres-Platas et al., 2014). Taken together, these data support the existence of a central inflammatory response in human depression that is driven primarily by peripheral inflammatory events. Moreover, data demonstrate that antibodies specific to TNF that do not cross the BBB can block stress-induced depression in mice (Krugel et al., 2013). These findings indicate that peripheral inflammatory responses may serve as both biomarkers and targets of immune-based therapies for depression and other stress-related disorders.

## Cytokines and neurotransmitters

Given the pivotal importance of neurotransmission to mood regulation, attention has been paid to the impact of inflammation on the monoamines serotonin (5-HT), norepinephrine (NE), and dopamine (DA) as well as on the excitatory amino acid glutamate (Fig. 2). There are several pathways through which inflammatory cytokines can lead to reduced synaptic availability of the monoamines (Miller et al., 2017). For example, IL-1β and TNF induction of mitogen-activated protein kinases (MAPKs) has been shown to increase the expression and function of the transporters for 5-HT, NE, and DA (SERT, NET, and DAT, respectively), leading to decreased synaptic availability of monoamines and depressive-like behavior in laboratory animals (Zhu et al., 2010). Through the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), inflammatory cytokines can also decrease tetrahydrobiopterin (BH4), a key enzyme cofactor in the synthesis of all monoamines, which is highly sensitive to oxidation (Neurauter et al., 2008). Activation of the enzyme indoleamine 2,3-dioxygenase (IDO) is also believed to be involved in cytokine-induced neurotransmitter alterations, in part by diverting the metabolism of tryptophan (the primary amino-acid precursor of serotonin) into kynurenine, a compound that can be converted into the neurotoxic metabolite quinolinic acid by activated microglia and infiltrating macrophages in the brain (Miller et al., 2017). Of note, increased levels of quinolinic acid have been found in microglia in the anterior cingulate cortex of suicide victims who suffered from depression (Steiner et al., 2011). Quinolinic acid directly activates receptors for glutamate NMDA while stimulating glutamate release and blocking glutamate reuptake by astrocytes (Haroon et al., 2017). The effects of quinolinic acid converge with the direct effects of inflammatory cytokines on glutamate metabolism, which include decreasing the expression of astrocyte glutamate reuptake pumps and stimulating astrocytic glutamate release (Haroon et al., 2017). Ultimately, these effects contribute to excessive glutamate both within and outside the synapse. Excessive extrasynaptic glutamate can bind to extrasynaptic NMDA receptors, which leads to decreased production of brain-derived neurotrophic factor (BDNF) and increased excitotoxicity and may contribute to disruption of local network integrity (Haroon et al., 2017). Of note, in stress-induced

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animal models of depression, BDNF is also reduced by IL-1β and TNF and their downstream signaling pathway NF-κB (Koo et al., 2010). Increased levels of glutamate in the basal ganglia and dorsal anterior cingulate cortex (dACC), as measured by magnetic resonance spectroscopy (MRS), have been described in patients receiving IFN-α (Haroon et al., 2017). More recent data indicate that increased inflammation (as reflected by CRP level) is also associated with increased basal ganglia glutamate in depressed patients in association with anhedonia and decreased psychomotor speed (Haroon et al., 2016). Interestingly, blocking glutamate receptors with ketamine or inhibiting IDO activity protects mice from LPS-induced or stress-induced depressive-like behavior but leaves the inflammatory response intact (O'Connor et al., 2009; Walker et al., 2013). Of note, conventional antidepressant medications act by increasing the synaptic availability of monoamines and increasing neurogenesis through the induction of BDNF (Miller et al., 2017). Therefore, cytokines such as IL-β and TNF serve to undermine these activities as they decrease the synaptic availability of monoamines while decreasing BDNF and increasing extracellular glutamate, which is not a target of conventional antidepressant therapy. These cytokine-driven effects may explain the association of increased inflammation with treatment resistance (Miller et al., 2017).

## Effects of inflammation on neurocircuitry

Given the impact of cytokines on neurotransmitter systems that subserve functional activity of neurocircuits, it is no surprise that neuroimaging studies have revealed cytokine-induced alterations in regional brain activity (Fig. 2).

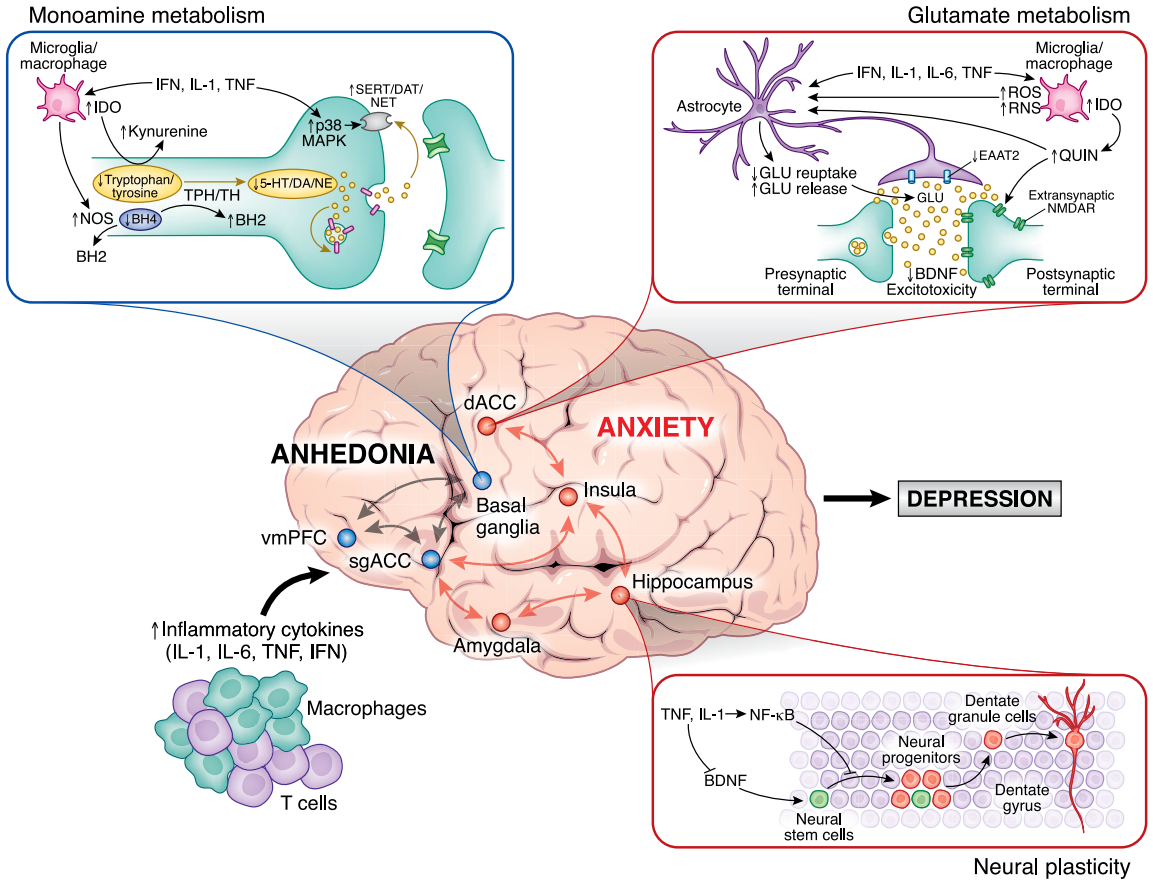


**Figure 2.** Cytokine targets in the brain: neurotransmitters and neurocircuits. Once in the brain, the inflammatory response can impact metabolic and molecular pathways that influence neurotransmitter systems. These effects can ultimately disrupt neurocircuits that regulate behavior, especially those relevant to decreased motivation (anhedonia), avoidance, and alarm (anxiety), which characterize multiple neuropsychiatric disorders (e.g., depression). BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; EAAT2, excitatory amino acid transporter-2; GLU, glutamate; NMDAR, NMDA receptor; QUIN, quinolinic acid; sgACC, subgenual anterior cingulate cortex; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase. Adapted with permission from Miller and Raison (2016), Fig. 3. Copyright 2016, Springer Nature.

DA plays a fundamental role in motivation and motor activity, and cytokines have been shown to decrease DA release in the basal ganglia in association with decreased effort-based motivation as well as reduced activation of reward circuitry in the basal ganglia (in particular, the ventral striatum) (Capuron et al., 2012; Felger et al., 2013). Demonstrating the validity and reproducibility of these cytokine-mediated effects on the brain, inflammation has been associated with reductions in reward responsiveness in the striatum across multiple neuroimaging platforms (positron emission tomography, functional magnetic resonance imaging [fMRI], MRS, and quantitative magnetization transfer imaging) and inflammatory stimuli (IFN-α, endotoxin, or typhoid vaccination) (Miller and Raison, 2016). Interestingly, recent fMRI studies suggest that inflammation-induced decreases in responsiveness to positive reward are also associated with increased sensitivity to aversive stimuli (negative reinforcement) (Harrison et al., 2016). fMRI findings have been expanded to patients with depression in whom increased plasma CRP was associated with decreased functional connectivity within reward-related circuits, including the ventral striatum and the ventromedial prefrontal cortex (vmPFC), which in turn mediated the relationship between CRP and anhedonia (Felger et al., 2016).

fMRI studies have demonstrated that increased inflammation is also associated with increased activation of threat-related and anxiety-related neurocircuitry, including the dACC as well as the insula and amygdala (Slavich et al., 2010). Of note, the dACC and amygdala are regions that exhibit increased activity in patients with high trait anxiety and neuroticism—conditions that often accompany depression and are associated with increased inflammation. For example, increased concentrations of oral IL-6 and soluble TNF receptor-2 in response to a public-speaking stressor were significantly correlated with the response of the dACC to a social rejection task (Slavich et al., 2010). In addition, increased oral IL-6 in response to social evaluation was correlated with amygdala activation, with subjects exhibiting the highest IL-6 responses to stress, demonstrating the greatest connectivity within threat circuitry, including the amygdala and dorsomedial prefrontal cortex (Muscatell et al., 2015).

## Translational Considerations

Based on the extant literature, the following guidelines can inform clinical trials testing the role of inflammation in relevant psychiatric disorders. First, clinical trials should enrich for patient populations with evidence of increased inflammation, which has been shown to identify depressed patients who respond to anticytokine therapy (Raison et al., 2013; Miller et al., 2017). Researchers should also consider that anti-inflammatory drugs may harm patients without increased inflammation. Inflammatory cytokines and the innate immune response play pivotal roles in synaptic plasticity, neurogenesis, long-term potentiation (a fundamental process in learning and memory), and possibly, antidepressant response (Warner-Schmidt et al., 2011; Miller et al., 2017). Second, primary behavioral outcome variables should include measures of anhedonia and anxiety. Inflammation targets neurocircuits in the brain that regulate motivation and reward as well as anxiety, arousal, and alarm (Miller et al., 2017). In limited studies, related symptoms have been shown to respond to anticytokine therapy. Third, drugs that specifically target inflammatory cytokines and/or their signaling pathways are preferable. Most clinical trials to date have used anti-inflammatory drugs (nonsteroidal anti-inflammatory agents and minocycline) that have multiple off-target effects, making the data difficult to interpret. Finally, target engagement must be established. Protein and gene-expression markers of inflammation in the peripheral blood can serve as relevant proxies for inflammation in the brain, especially given the evidence that activated peripheral immune cells mobilize to the brain and that BBB integrity is reduced in stress-induced animal models of depression. Relevant therapeutic interventions should decrease peripheral inflammatory markers in concert with improving specific depressive symptoms. In future clinical trials, neuroimaging strategies may ultimately serve as the most direct measure of target engagement in the brain and the impact of anti-inflammatory or other (e.g., neurotransmitter-directed) therapies (Miller et al., 2017).

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# Neuroimmune Interactions in Chronic Itch

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## Introduction

Chronic itch is a debilitating disorder that affects millions of people worldwide. This condition is driven by cooperative interactions among neurons, epithelial cells, and immune cells. However, the molecular mechanisms underlying these interactions are not well understood. Here we review the current understanding of the molecular and cellular mechanisms in the periphery that contribute to itch disorders and discuss open questions about the neuroimmune interactions that trigger inflammatory itch pathogenesis.

## Acute Itch

Acute itch is an extremely common experience: it can be caused by an insect bite, contact with an irritating plant such as poison ivy, or even a wool sweater. While unpleasant, the sensation of itch, like pain, is thought to play a fundamental role in survival by teaching us to avoid irritating elements in our environment. Itch triggers the protective reflex of scratching, which promotes removal of the by-products of irritating plants as well as insects and helminths capable of transmitting diseases such as Zika or malaria. In contrast to acute itch, chronic itch is a profoundly debilitating condition that affects ~10% of the adult population (Wilson et al., 2013). Chronic itch remains poorly understood and difficult to treat, making it a tremendous clinical problem.

Itch is mediated by a subset of somatosensory neurons, a highly heterogeneous group of cells that transduce touch, itch, and pain sensations. Somatosensory neurons have their cell bodies in the trigeminal and dorsal root ganglia (DRG); each neuron has a single bifurcated axon with one branch innervating the skin and the other innervating the dorsal horn of the spinal cord (Ross et al., 2014). The peripheral afferents that innervate the skin transduce environmental stimuli into electrical signals that travel to the central afferent of the neuron and synapse and onto a variety of second-order interneurons in the spinal cord (Fig. 1).

Projection neurons in the spinothalamic tract project from the spinal cord to the parabrachial nucleus and the thalamus and activate third-order neurons that project to the somatosensory cortex. While itch signaling in the CNS remains very poorly understood, functional magnetic resonance imaging data reveal that itchy stimuli activate both unique areas and some of the pain regions of the primary somatosensory cortex. They also activate a myriad of brain areas involved in emotional processing, reward, evaluation, and motor control (Dong and Dong, 2018).

### Cells and molecules of acute itch

Studies have demonstrated two broad classes of itch neurons (termed pruriceptors): histamine-dependent and histamine-independent. Whereas many forms of acute and allergic itch are caused in part by histamine signaling, chronic itch tends to be dominated by nonhistaminergic mechanisms, and therefore does not respond to antihistamines. An astonishing array of pruritogens leads to activation of distinct subsets of pruriceptors, including factors derived from keratinocytes, a wide variety of immune cells, and exogenous triggers (Table 1).

Recent studies have identified TRPA1 (transient receptor potential ankyrin 1) and TRPV1 (transient receptor potential vanilloid 1) as two of the primary ion channels responsible for driving acute

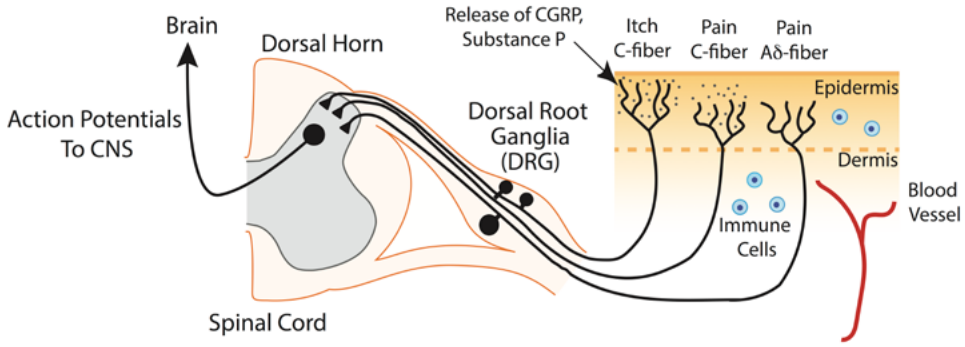


**Figure 1.** Transmission of itch signals from the periphery to the CNS. Distinct subsets of somatosensory neurons mediate acute itch, chronic itch, and pain. Somatosensory neurons have cell bodies in the DRG. These neurons are pseudounipolar and have two axonal branches: one branch innervates the epidermis, where environmental stimuli are transduced into action potentials, and the other innervates interneurons in the dorsal horn of the spinal cord, where the signals are processed and sent to the brain. Somatosensory neurons are diverse, with itch and pain neurons innervating the epidermis and dermis and interacting with immune cells in multiple layers of the skin.

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## Table 1. Peripheral Mediators and Neuronal Receptors of Itch

| Molecular Mediator | Main Cellular Source | Neuronal Receptor | Ion Channel | DRG Neuron Subtypes | Cause of itch |
|---|---|---|---|---|---|
| Histamine | mast cells | H1R, H4R | TRPV1, TRPV4 | NP2, NP3 | insect bites, dermatitis |
| Serotonin (5-HT) | mast cells, keratinocytes | HTR7, HTR2 | TRPA1, TRPV1, TRPV4 | NP3 | atopic dermatitis |
| Proteases | mast cells, plants | PAR2, MrgprC11 | TRPA1, TRPV1 | NP2 | cowhage, dermatitis |
| TSLP | keratinocytes | TSLP receptor (IL-7Rα + TSLPR) | TRPA1 | | atopic dermatitis |
| IL-31 | Th2 T helper cells | IL-31 receptor (IL-31Rα + OSMR) | TRPA1, TRPV1 | NP3 | atopic dermatitis, T cell lymphoma |
| IL-33 | keratinocytes | IL-33 receptor (IL-1RAcP + ST2) | TRPA1, TRPV1 | NP2 | allergic contact dermatitis |
| IL-4 and IL-13 | Th2 cells, ILC2, basophils | IL-4Rα, IL-13Rα1 | TRPA1, TRPV1 | NP1, NP2, NP3 | atopic dermatitis, chronic idiopathic pruritis |
| Poly I:C, Imiquimod | pathogens, drug | TLR3, TLR7 | | | psoriasis, xerosis (dry skin) |
| BAM8-22 peptide | keratinocytes | MrgprC11 | TRPA1, TRPV1 | NP2 | xerosis (dry skin) |
| Chloroquine | medicine in circulation | MrgprA3 | TRPA1, CNO1 | NP2 | drug-induced itch |
| β-alanine | medicine in circulation | MrgprD | | NP1 | drug-induced itch |

**Table 1.** Peripheral mediators and neuronal receptors of itch. A remarkable variety of pruritogens derived from epithelial cells, immune cells, and the environment activate somatosensory neurons and evoke itch sensations and scratching behaviors. BAM, bovine adrenal medulla; CNO, clozapine N-oxide; HTR, 5-hydroxytryptamine (serotonin [5-HT]) receptor; IL-1RAcP, interleukin-1 receptor accessory protein; ILC, innate lymphoid cell; NP, natriuretic peptide; OSMR, oncostain receptor; PAR, protease-activated receptor; TLR, toll-like receptor; TSLP, thymic stromal lymphopoietin. Adapted with permission from Dong and Dong (2018), Table 1. Copyright 2018, Elsevier.

itch excitability. Activation of these channels promotes the opening of voltage-gated ion channels that trigger action potential firing and synaptic transmission to activate spinal neurons in the dorsal horn. Several molecules have been shown to play a key role in itch transmission within the spinal cord, including glutamate, Gastrin-releasing peptide (GRP), natriuretic peptide B (NPPB), and dynorphin (Kardon et al., 2014; Hoon, 2015; Dong and Dong, 2018). Glutamate is believed to have a specific role in pain signaling and may inhibit itch: genetic deletion of the glutamate transporter VGLUT2 from nociceptive neurons was found to enhance itch behaviors (Dong and Dong, 2018). DRG neurons transmit itch signals to second-order spinal cord neurons via neuropeptides, including NPPB. NPPB-positive DRG neurons synapse onto neurons that express the NPPB receptor NPRA, as well as the neuropeptide GRP. These neurons then release GRP onto a group of interneurons in the dorsal horn that express the GRP receptor, GRPR (Dong and Dong, 2018). Conversely, some groups have found evidence that DRG neurons are able to release GRP directly onto GRPR-positive spinal interneurons. Itch signaling in the spinal cord is highly complex and is an area of ongoing debate and investigation (Fig. 2).

## Chronic Itch Disorders

While many of the molecules and neurons that drive acute itch have been elucidated in the past decade, the molecular and cellular mechanisms that trigger chronic itch have remained enigmatic. Chronic itch arises from the dysfunction of neurons, immune cells, and epithelial cells at multiple sites, including the periphery, spinal cord, and brain. The inflammatory skin diseases psoriasis and atopic dermatitis (AD) are two of the most prevalent chronic itch disorders. Neuronal and immune dysregulation leads to the distinct clinical phenotypes observed in these disorders. As such, psoriasis and AD have been the target of contemporary studies aimed at defining molecular and cellular mechanisms that contribute to disease phenotypes.

### Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by defects in keratinocyte differentiation, epidermal thickening, and raised scaly plaques. Severe itching is a hallmark symptom of this disorder, which affects 2–3% of the population. It is considered an "immune-centric" disorder owing to the fact that disease clearance is observed after immunosuppressive therapies (Golden et al., 2013).

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The T cell–derived cytokines interleukin (IL)-17 and IL-23 are believed to be particularly important, and biologic therapies that block these cytokines have recently been developed, with excellent results in patients (Guttman-Yassky and Krueger, 2017).

### Atopic dermatitis

AD is an allergic inflammatory disorder that typically begins in childhood. It affects roughly 10–20% of the population worldwide (Bantz et al., 2014). AD is characterized by skin barrier dysfunction, thickened lesions, and extreme chronic itch (Spergel and Paller, 2003). It is believed to be caused by a complex interplay between genetic and environmental factors, and is often the first step in a process known as the "atopic march," in which children with AD are significantly more likely to develop asthma and allergic rhinitis (Spergel and Paller, 2003). AD is difficult to treat and causes tremendous psychological and financial burdens for the patients and families affected. The need is urgent for a better understanding of the pathology of this disorder as well as improved therapies.

## Pathophysiology of Chronic Itch

Until recently, research has focused mainly on the roles of epithelial cells and immune cells in driving itch and inflammation. However, contrary to the long-held viewpoint in which immune cells release mediators that trigger activation in somatosensory neurons, leading to chronic itch, a number of contemporary studies have demonstrated that short-term and long-term signaling processes go both ways between neurons and immune cells in the periphery. Furthermore, several receptors canonically believed to be expressed only in immune

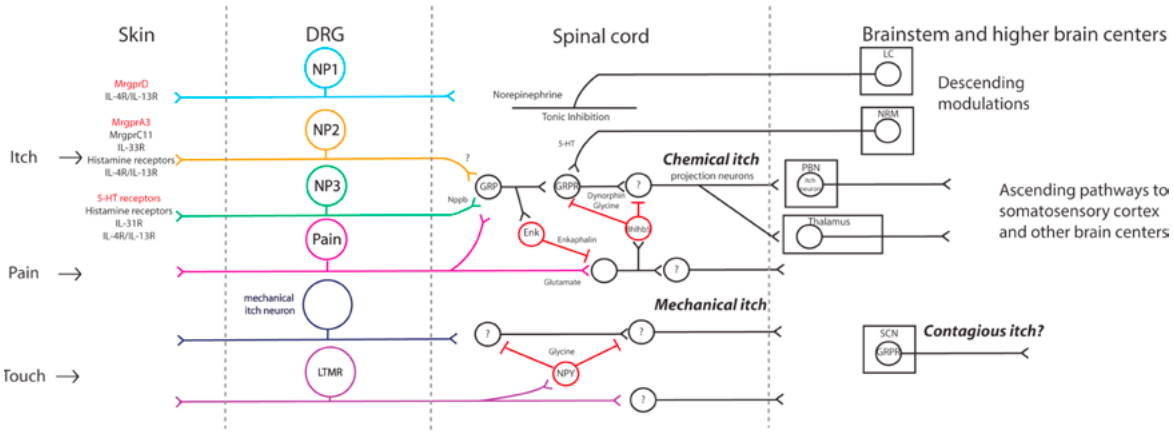


**Figure 2.** Hypothetical schematic of peripheral and central itch circuits based on current knowledge. Itch processing in the spinal cord is highly complex. This schematic depicts a hypothetical relationship of circuits based on findings from current literature. Forked and flat synaptic endings indicate excitatory and inhibitory connections, respectively. Primary somatosensory neurons in the DRG can be categorized by their gene-expression profiles. Single-cell expression profiling revealed three subtypes of itch neurons termed NP1, NP2, and NP3. NP1 neurons are characterized by their expression of MrgprD, NP2 by MrgprA3, and NP3 by serotonin (5-HT) receptors. The MrgprA3-positive NP2 neurons have been shown to be itch specific, since activating these neurons elicits only itch behaviors. Itch sensory neurons make synaptic connections with GRP-positive ($GRP^+$) interneurons in the spinal cord, which are in turn connected with $GRPR^+$ neurons. Ablation of GRPR signaling or $GRPR^+$ neurons completely abolished responses to itchy stimuli, indicating vital importance of this pathway in itch transmission. Information about itch stimuli in the periphery is eventually conveyed to higher brain centers by projection neurons. The parabrachial nucleus (PBN) in the brainstem serves as an important relay center in the process. Itch interacts with other sensory modalities in the spinal cord. $GRP^+$ neurons receive both itch and pain stimuli but will inhibit their own responses to high-intensity pain sensations through enkephalin signaling. Meanwhile, a painful stimulus will activate both itch and pain sensory neurons, but only pain will be perceived because itch is inhibited by pain via inhibitory interneurons in the spinal cord. One group of these interneurons is marked by the transcription factor BHLHB5 (basic helix-loop-helix, class B, 5). These neurons are activated by a variety of anti-itch stimuli and likely inhibit itch via the release of dynorphin and glycine. Similarly, a group of inhibitory interneurons labeled by neuropeptide Y (NPY) were shown to mediate the inhibition of mechanical itch by light touch (although the sensory neurons and further transduction circuits of mechanical itch remain to be identified). These $NPY^+$ interneurons receive input from touch-transducing LTMRs (low-threshold mechanoreceptors). The spinal cord dorsal horn also receives descending neuromodulation from higher brain centers. The nucleus raphe magnus (NRM) sends serotoninergic projections to the spinal cord and can stimulate $GRPR^+$ neurons and potentiate itch by coactivating HTR1A and GRPR receptors. The dorsal horn also receives tonic noradrenergic inhibition, likely from the locus coeruleus (LC). $GRPR^+$ neurons in the suprachiasmatic nucleus (SCN) of the hypothalamus have been suggested to play a role in contagious itch. Reprinted with permission from Dong and Dong (2018), Fig. 3. Copyright 2018, Elsevier.

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cells or neurons have been found to play key roles in multiple cell types. These findings demonstrate that an ongoing conversation between immune cells and somatosensory neurons is required to drive itch and inflammation in the AD disease state. Future therapies will depend on furthering our understanding of this conversation.

## Immune cells modulate neuronal function

The direct activation of neurons by immune cells in the context of AD remains an important avenue through which itch and inflammation are induced. In addition to simply activating neurons, immune cells change the signaling behavior of somatosensory neurons in a number of complex ways, including by lowering excitation thresholds, evoking neurite outgrowth, and facilitating immune cell–neuron contacts in the periphery. Four immune cell types have been shown to directly influence neuronal excitability, sensitization, and branching in the context of chronic itch: Langerhans cells, T cells, eosinophils, and mast cells (Fig. 3). A consistent theme in the story of immune cell signaling to neurons is the neuronal expression of receptors long believed to function only in immune cells. These include a variety of canonical immune cell receptors, e.g., IL-31Rα, IL-4Rα, and IL-13Rα1 (Table 1), among others, which are expressed and active on somatosensory neurons, as described below.

### *Langerhans cells*

Langerhans cells are dendritic cells located in the epidermis that are important for initiating cutaneous immune responses. Their dendritic processes are located in the stratum corneum, the most superficial layer of the skin, and promote skin barrier homeostasis (Jaitley and Saraswathi, 2012; Seneschal et al., 2012; Yoshida et al., 2014). Like other types of dendritic cells, Langerhans cells respond to antigens from the external and internal environment and, upon activation, influence T-cell activation and migration (Ding et al., 2008). A role for Langerhans cells in AD stems from both human and animal studies. Human atopic skin contains significantly higher numbers of Langerhans cells that display an altered morphology, with more dendritic branches that extend through tight junctions toward the superficial epidermis. These branches have the molecules that capture exogenous antigens, including haptens or viral antigens. These antigens are presented to T cells, triggering activation (Dubrac et al., 2010; Nakajima et al., 2012). Cultured murine Langerhans cells produce nitric oxide in response to the potent inflammatory agents lipopolysaccharide and interferon-gamma (Qureshi et al., 1996). Nitric oxide is a well-known agonist of the TRPA1 and TRPV1 ion channels, which have been shown to play key roles in many forms of acute and chronic itch (Bautista et al., 2014; Kittaka and Tominaga, 2017). Furthermore, one study (Morita et al., 1995) showed decreases in pruritis among AD

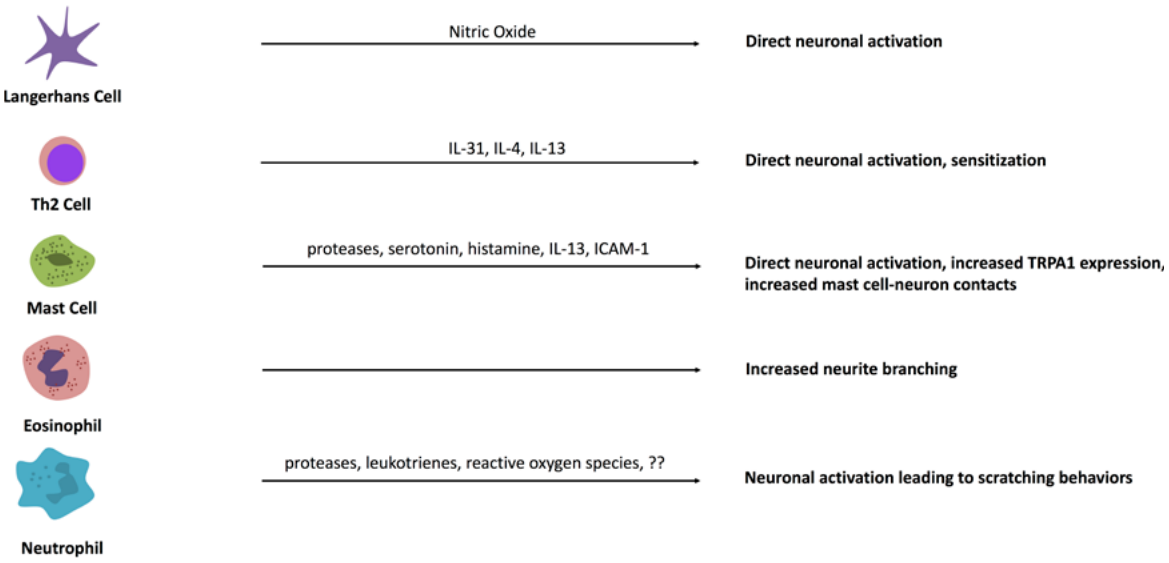


**Figure 3.** The "most wanted" immune cell offenders in chronic itch. Immune cells modulate neuronal activity to promote chronic itch. Langerhans cells, Th2-type $CD4^+$ T cells, mast cells, and eosinophils have been shown to modulate neuronal function in chronic itch disorders.

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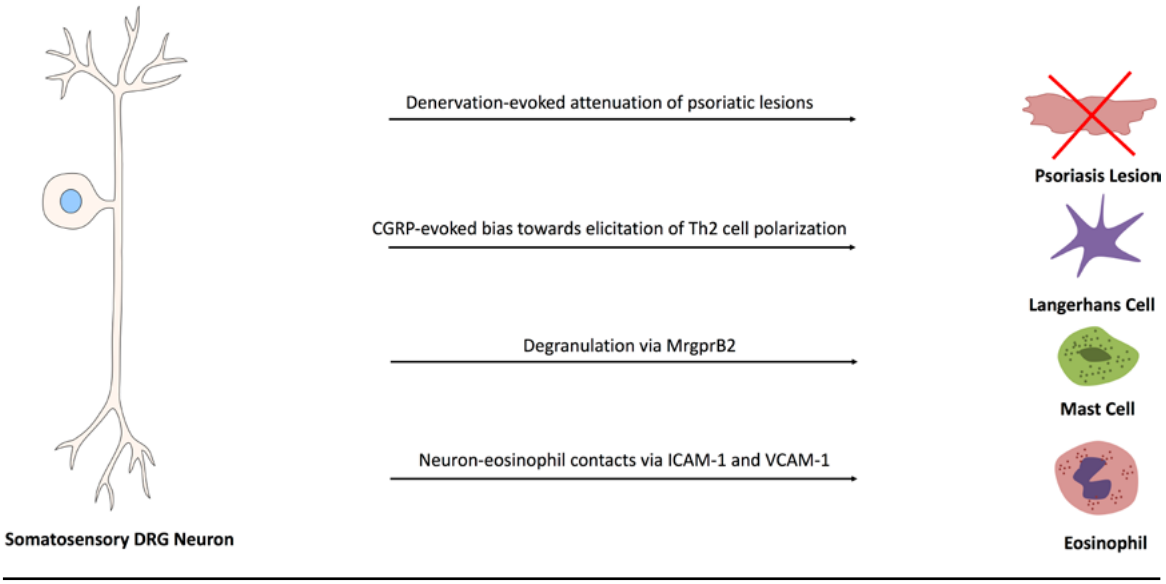


**Figure 4.** The "most wanted" neuron offenders in chronic itch. Neurons modulate the activity of various immune cells in chronic itch disorders by evoking degranulation, increasing neuron–immune cell contacts, and affecting T-cell polarization.

patients in response to a topical nitric oxide synthase inhibitor, suggesting a role for this molecule in AD pathogenesis. More direct evidence for a role of Langerhans cells comes from mouse models of AD in which depletion of Langerhans cells significantly decreases skin inflammation and lesions (Elentner et al., 2009).

### *T cells*

T cells play a key role in AD. T cells have long been known to communicate with other immune cells and epithelial cells, but until recently, their interactions with somatosensory neurons under chronic itch conditions were unknown. There are multiple kinds of T cells, including cytotoxic T cells, which kill cells that are infected with bacteria or viruses, and T helper (Th) cells, which secrete cytokines that then act on other types of cells. T helper cells are further specialized into subtypes such as Th1, Th2, Th17, etc., depending on their mode of activation (e.g., viral or bacterial infection, allergen exposure). The Th17 subtype of T cells is a key mediator of psoriasis (Guttman-Yassky and Krueger, 2017). Th17 cells secrete a variety of inflammatory interleukins, which in turn alter the activity of many genes involved in skin barrier function that trigger the psoriatic phenotype. In contrast, the Th2 subtype of T cells is activated in essentially all AD patients, whereas Th17 and Th1 activation is seen in only a subset of AD patients (Guttman-Yassky and Krueger, 2017). Cyclosporine is a potent immunosuppressant that inhibits T cells by inhibiting signal transduction downstream of T cell–receptor activation. Cyclosporine very effectively suppresses the AD disease phenotype in the short term (Khattri et al., 2014), but owing to relapse and renal toxicity, is not an effective long-term therapy.

IL-31, a cytokine largely derived from Th2 cells, is highly upregulated in AD patients (Nobbe et al., 2012). Cevikbas and colleagues discovered that IL-31 evokes calcium transients in cultured DRG neurons and triggers itch in mice upon injection into the skin (Cevikbas et al., 2014). These results were dependent on the ion channels TRPV1 and TRPA1, which play key roles in immune cell function. Moreover, IL-31 has been implicated in neurite outgrowth (Feld et al., 2016). Increases in nerve fiber density are observed in the skin of AD patients, likely contributing to their itch and inflammation symptoms (Tominaga and Takamori, 2014). Indeed, overexpression of IL-31 in lymphocytes or intradermal injection of the protein in mice triggers dramatic sprouting of sensory nerve fibers in the skin (Feld et al., 2016).

### *Eosinophils*

In addition to Th2 cells, eosinophils are thought to be key regulators of sensory neuron innervation in chronic itch. Eosinophils are white-blood-cell granulocytes that mediate viral, bacterial, and fungal defense and immunoregulation (Simon et al., 2004; Radonjic-Hösli and Simon, 2014). In AD patients, eosinophil counts and eosinophil granule proteins are elevated in peripheral blood and correlate with disease severity (Simon et al., 2004). The number of eosinophils is also increased in the skin in multiple

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chronic itch mouse models (Li et al., 2006; Foster et al., 2011). A recent study demonstrated that eosinophils contribute to skin barrier dysfunction in a mouse model of AD (Naidoo et al., 2018), underscoring the importance of these cells in AD pathology. Eosinophils were recently demonstrated to increase somatosensory neurite branching in mouse neuron–eosinophil co-cultures as well as when sensory neurons were treated with medium from these co-cultures. These results suggest that the close functional interaction between eosinophils and neurons is necessary for full eosinophil-mediated increase in neurite branching (Foster et al., 2011). Whether such interactions drive hyperinnervation in AD *in vivo* has yet to be directly examined. Although psoriatic lesions also display hyperinnervation (Taneda et al., 2011), it is unclear whether eosinophils and/or T cells contribute to this phenotype.

***Mast cells***

Mast cells are tissue-resident granulocytes that contribute to inflammatory chronic itch via degranulation and release of inflammatory cytokines. In human AD, mast cells are more abundant in lesional skin and display a high prevalence of degranulation (Liu et al., 2011). A number of studies have shown that mast cells can both directly activate and sensitize somatosensory neurons in the skin. The products of mast-cell degranulation, including serotonin, histamine, Bam8-22, and proteases, potently and directly activate their cognate receptors on somatosensory neurons to drive itch behaviors and promote neurogenic inflammation via the release of inflammatory peptides. Furthermore, mast cell–neuron interactions have been shown to be strengthened in a mouse model of AD. Hagiyama and colleagues demonstrated that mast cells upregulate expression of intercellular adhesion molecule 1 (ICAM-1), which mediates mast cell–sensory neuron interactions (Hagiyama et al., 2013). These data suggest that the strengthening of mast cell–neuron interactions may contribute to the increased degranulation and neuronal activation observed in AD.

Mast cells may also regulate the expression of excitatory signaling molecules within sensory neurons, thereby promoting hyperexcitability. Expression of the ion channel TRPA1 is markedly increased in skin biopsies from AD patients compared with normal skin and in the IL-13 mouse model of AD (Oh et al., 2013). TRPA1 is also required for itch behaviors in multiple mouse models of AD (Oh et al., 2013; Morita et al., 2015). Interestingly, mast cell–deficient mice do not display increased expression of TRPA1 in lesional skin. IL-13 also directly activates and sensitizes DRG neurons and increases itch-evoked scratching behaviors in mice following injection of a variety of pruritogens (Oetjen et al., 2017). This cytokine is released by both Th2 cells and mast cells, although the relative contributions of mast cells and Th2-derived IL-13 to neuronal excitation is unknown.

***Neutrophils***

Neutrophils are well known as mediators of psoriatic inflammation (Mitchell et al., 1982; Fleekop et al., 1987). Neutrophils migrate early to psoriatic lesions and secrete a number of inflammatory mediators and cytokines, including IL-17 and IL-23, which drive activation and hyperproliferation of immune cells and keratinocytes (Schön et al., 2017). Likewise, neutrophils have been shown to migrate to sites of allergic inflammation and to increase their production of reactive oxygen species in response to allergen challenge (Fleekop et al., 1987; Shalit et al., 1987). However, the role of these neutrophils in AD has been somewhat controversial, with mixed reports about the presence of neutrophils and neutrophilic markers in AD lesions (Choy et al., 2012; Dhingra et al., 2013). A role for neutrophils in AD itch stems from studies showing that neutrophils produce a number of mediators, including proteases, reactive oxygen species, and leukotrienes, that act as pruritogens (Liu and Ji, 2012; Akiyama et al., 2015; Mollanazar et al., 2016; Schön et al., 2017). Along these same lines, IL-8, a canonical neutrophil chemoattractant, predisposes human patients to recurrent AD bouts (Suárez-Fariñas et al., 2013; Mansouri and Guttman-Yassky, 2015). Indeed, our preliminary data suggest that protease activation of keratinocytes may result in increased expression of neutrophil chemoattractants, which recruit neutrophils to the skin to jump-start itch and inflammation during AD.

## Neurons recruit and activate immune cells

Far from being passive recipients of messages from immune cells, neurons actively participate in driving inflammation in a variety of chronic itch disorders. Psoriasis provides an excellent example of this phenomenon: it has long been understood as an "immune-centric" disease, driven by the action of Th17 cells. However, multiple lines of evidence support the requirement for neuronal signaling in this disorder, highlighting the complex, multicellular nature of the disease.

Psoriatic plaques have a high expression of neuropeptides and are hyperinnervated by somatosensory neurons (Ostrowski et al., 2011). Clinically, spontaneous remission of psoriasis plaques has been reported in patients following nerve

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injury, and two pilot studies have demonstrated that injections of botulinum toxin have beneficial effects in psoriasis patients (Ward et al., 2012; Campanati et al., 2017). These observations were recently validated in a mouse model. Treatment with botulinum toxin ameliorated psoriasis-like symptoms in the *KC-Tie2* genetic mouse model of psoriasis (Ward et al., 2012), which displays the immunological, epithelial, and neural characteristics of human disease. Surgical cutaneous denervation in this model resulted in significant reductions in epidermal thickening and T-cell infiltrate. These deficits could be rescued either by intradermal injection of calcitonin gene-related peptide (CGRP), a potent vasodilator and inflammatory neuropeptide produced by somatosensory nerves, or by activating the receptor for substance P, another inflammatory neuropeptide, with a selective agonist (Ostrowski et al., 2011). These data suggest that, in addition to T cells, neuropeptide signaling from sensory neurons drives epithelial cell dysfunction and inflammation in psoriasis.

Recent studies have begun to explore the role somatosensory neurons play in AD pathogenesis. Neurons signal to and regulate multiple types of immune cells in AD. Indeed, inflammatory neuropeptide release by somatosensory neurons has been shown to dramatically influence Langerhans cell activity. Langerhans cells play a key role in driving the Th2 response in AD. In a series of *in vitro* experiments, Ding and colleagues demonstrated that treating cultured Langerhans cells from mouse epidermis with CGRP upregulated their expression of Th2 cytokines. Furthermore, CD4-positive ($CD4^+$) T cells to which these CGRP-treated Langerhans cells presented antigens demonstrated Th2-type polarization (Ding et al., 2008). This work indicates that somatosensory neuronal signaling actually promotes a Th2 response, which is central to the pathology of AD.

Eosinophils, noted above for their remarkable ability to evoke neurite outgrowth, also respond to neuronal signaling. Cultured DRG neurons produce the potent and specific eosinophil chemokine, eotaxin-1, as well as the adhesion molecules ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) (Foster et al., 2011). ICAM-1 functions as a ligand for leukocyte function-associated antigen 1 (LFA-1), whereas VCAM-1 binds to very late antigen 4 (VLA-4). Both VLA-4 and LFA-1 are expressed by eosinophils (Lima et al., 2007). Moreover, eotaxin levels are elevated in AD lesions (Owczarek et al., 2010). These data suggest that under AD conditions, neurons call eosinophils to lesions, and eosinophils promote additional neuronal sprouting. Clearly, understanding neuron–eosinophil bidirectional signaling is fundamental to increasing our knowledge of these enigmatic cells.

Somatosensory nerve endings have been shown to be in close proximity to mast cells in both chronic itch and pain. Previous work has established that neuron–mast cell interactions are required for pain hypersensitivity (Chatterjea and Martinov, 2015). Somatosensory neurons secrete the membrane-type 5 matrix metalloprotease (MT5-MMP), which can trigger mast-cell degranulation and regulate neuron–mast cell contact via N-cadherin (Folgueras et al., 2009). Mice lacking this protease display heightened sensitivity to painful stimuli (Folgueras et al., 2009), but chronic itch has not yet been examined in these animals. Within the field of chronic itch, neuron–mast cell interactions are proving to be equally crucial.

Substance P is an inflammatory neuropeptide released by somatosensory neurons that are implicated in both chronic itch and pain (Chiu et al., 2012; Azimi et al., 2017). Substance P acts as a robust pruritogen in both humans and rodents. Interestingly, inhibition of the canonical substance P receptor, neurokinin-1 receptor (NK-1R), has no effect on substance P–evoked scratching (Azimi et al., 2017). Recent studies have identified a member of the Mas-related G-protein coupled receptor (Mrgpr) family, MrgprB2, as a new target for substance P. Indeed, substance P–evoked itch behaviors are inhibited by MrgprB2 antagonists in both wild-type and *NK-1R* knockout animals (Azimi et al., 2016).

While the Mrgpr family is well known for mediating neuronal responses to pruritogens (Liu and Dong, 2015), MrgprB2 is exclusively expressed on mast cells. Recent studies have shown that MrgprB2 is required for mast-cell activation by a variety of secretagogues, including substance P (McNeil et al., 2014). In fact, substance P does not evoke degranulation in cells transfected with missense mutants in the human orthologue of MrgprB2, MRGPRX2 (Alkanfari et al., 2018). These findings highlight a key role for neurons in regulating mast-cell function.

## Beyond the periphery

Interactions between neurons and immune cells in the skin are clearly fundamental to the development of chronic itch and pain, but neuroimmune signaling in the CNS also plays a key role (Romero-Sandoval et al., 2008). Indeed, persistent activation of microglia in the CNS is believed to drive chronic

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pain, suggesting that glial cells in the CNS may also contribute to chronic itch pathogenesis (Hulsebosch, 2012). Although microglia have not been examined in AD or psoriasis mouse models, they may play a key role in a contact hypersensitivity model of chronic itch, DNFB (dinitrofluorobenzene) (Zhang et al., 2015). This is sure to be an active area of research moving forward.

Another recent study focused on the contribution to chronic itch of reactive astrogliosis: a change in the morphology of spinal astrocytes resulting in increased arborization and enlarged cell bodies. Astrogliosis and expression of the astrocytic marker GFAP (glial fibrillary acidic protein) were demonstrated in the dorsal spinal cord in a mouse model of AD (Shiratori-Hayashi et al., 2015). This effect was dependent on TRPV1-expressing peripheral itch fibers that innervate the dorsal horn and observed in areas of the spinal cord that connect to scratched areas of the skin. Furthermore, scratching behaviors and astrogliosis were partially dependent on the astrocyte transcription factor STAT3 (signal transducer and activator of transcription 3). Perhaps most intriguing, whereas STAT3-knockout animals displayed reduced chronic scratching behavior, their acute itch responses remained intact, suggesting that this transcription factor plays a key role in the transition to a chronic itch state. Lipocalin 2 (LCN2), a protein involved in intracellular transport, was identified as a possible downstream target of STAT3 responsible for mediating its effects (Shiratori-Hayashi et al., 2015). The mechanisms by which changes in spinal and central signaling mediate the development of chronic itch remain a fascinating and active area of ongoing research.

## Conclusion

In recent years, a great deal of progress has been made in understanding the neuroimmune mechanisms of chronic itch. However, a number of open questions remain: What are the relative contributions of neurons and immune cells to chronic itch pathophysiology? Some of the same cell types implicated in chronic itch are also linked to chronic pain. What makes these cells behave differently in one disease context versus another? Are the neuroimmune signaling pathways in AD and psoriasis conserved in other inflammatory itch conditions? Will effective therapeutics require the blockade of both neuronal and immune cell pathways?

Early therapeutics, such as cyclosporine and steroids, have focused on broad immunosuppression. However, incomplete itch relief, remission, lack of penetrance, toxicity, and side effects make these types of treatments poor options for long-term use. Fortunately, a number of exciting recent advances have been made in the area of biologics, which block the actions of key cytokines that drive chronic itch pathology. Recent studies have shown that IL-17-targeting or IL-23-targeting antibodies are effective for reducing symptoms in psoriasis patients (Guttman-Yassky and Krueger, 2017). Although antibody targeting has proven less successful for atopic disease thus far (Sheridan, 2018), early studies show that dupilimab, which inhibits both IL-4 and IL-13 signaling by blocking IL4Rα, looks quite promising for treating moderate-to-severe AD (Simpson et al., 2016). Perhaps the effectiveness of these treatments stems from their ability to inhibit signaling in both immune and neuronal cell types. The groundbreaking work being done in the neuroimmune interactions driving chronic itch is sure to provide crucial insights that pave the way for new therapies.

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# Integrating Assays of Impulsivity with Experimental Models of Brain Injury to Explore Neurophysiological Causes of Cognitive Impairment

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## Introduction

Traumatic brain injury (TBI) affects more than 2.5 million people annually in the United States alone (U.S. Centers for Disease Control and Prevention, 2010a,b,c). In addition to the motor and memory impairments evident shortly after impact, which are the focus of most experimental therapeutics, many TBI patients experience serious cognitive and emotional problems that persist long after the injury (Reeves and Panguluri, 2011). These are significantly understudied by neuroscientists, potentially because the relevant expertise has traditionally been fostered in distinct branches of the field. Similar to numerous other clinical conditions that lie at the boundaries of psychology, psychiatry, and neurology, collaboration across disciplines will be essential for progress to be made in TBI. However, the challenge can be daunting, given the rich literature on all sides that has rarely interfaced. In this chapter, we summarize some experimental approaches that could be combined to address one particular area of concern: the emerging relationship between impulsivity and TBI that may contribute to elevated risk of substance use disorder in TBI patients (Kolakowsky-Hayner et al., 2002; Miller et al., 2013).

Conditions hallmarked by poor impulse control (e.g., bipolar disorder, impulsive aggression, and suicidality) affect a significant subset of TBI patients (Perry et al., 2015), and clinical reports indicate that even mild TBI can lead to impulse control deficits (Olson-Madden et al., 2012; Depue et al., 2014) Given that high levels of impulsivity are linked to vulnerability to substance abuse and other addiction disorders, TBI-induced impulse control deficits may significantly contribute to the increased risk of addiction in this patient group. Although clinical documentation of impulsivity and addiction after a TBI is an essential foundation for research, it is difficult to resolve issues of causation from these datasets: Does TBI precipitate, or result from, high impulsivity and drug-taking? Do the same neurobiological changes that precipitate impulsivity contribute to addiction risk post-TBI, or do these symptoms interact at the behavioral, but not neurophysiological, level?

Valid animal models can play an important role in this field of study and significantly catalyze investigations into underlying neurobiological processes. Although the neurophysiological basis of psychiatric complications caused by TBI remains underresearched, a synthesis of emerging data across neurology and psychiatry indicates that neuroinflammation may lie at the nexus of impulse control disorders, addiction, and TBI. Activation of proinflammatory immune signaling cascades has been well documented in the immediate aftermath of brain injury, and it is increasingly recognized that neuroinflammatory processes may contribute to the resulting cognitive impairments (Hellewell et al., 2013; Corser-Jensen et al., 2014; Juengst et al., 2014; Sharp et al., 2014). Elevated levels of circulating proinflammatory cytokines, as well as central and peripheral markers of oxidative stress, have also been associated with bipolar disorder, even in the absence of comorbid brain injury (Jones and Thomsen, 2013; Munkholm et al., 2013; Najjar et al., 2013; Bauer et al., 2014; Brown et al., 2014; Rosa et al., 2014). In addition, a growing body of data suggests that addiction processes are mediated, at least in part, by a neuroinflammatory mechanism (Crews et al., 2011; Clark et al., 2013; Rodrigues et al., 2014). A number of extensively studied and well-validated rodent models highlight different facets of impulsivity and addiction, yet only recently have they been combined with experimental models of brain injury. Moreover, experimenters with the necessary skills to study cell signaling or pathophysiology in TBI may struggle to interact with behavioral neuroscientists, who have expertise in studying cognitive behaviors. Here, we summarize some of the relevant models of TBI and impulsivity, with the hope of bridging that gap.

## Defining Impulsivity as a Cognitive Behavior of Interest

Impulsivity can be broadly defined as acting, or making decisions, without appropriate forethought, thereby enhancing the potential for negative consequences. It has become clear that many forms of impulse control exist and that the term "impulsivity" encompasses a range of behaviors, from motor disinhibition to maladaptive decision-making (Brunner and Hen, 1997; Evenden, 1999; Moeller et al., 2001). Deficits involving many types of impulsivity coexist in psychiatric populations, and using behavioral tests to assess more than one impulsive behavior has been shown to increase the accuracy of psychiatric diagnosis (Solanto et al., 2001). Therefore, in researching the relationship between an experimental model of pathology such as TBI and high levels of impulsivity, it is becoming increasingly apparent that multiple domains of impulsive behavior should be measured. Factor analysis of self-reported questionnaires such as the Barratt Impulsiveness Scale (BIS-11) (Patton et al., 1995) suggest three major dimensions of impulsivity: motor, nonplanning, and attentional (Patton et al., 1995; Moeller et al., 2001). The attentional domain reflects the degree to which an individual can focus on the task at hand, the motor component reflects

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spontaneity or action without due consideration, whereas nonplanning impulsivity reflects a lack of regard for the future. In translating these subjective measurements into objective behavioral tests that can be adapted across species, the attentional dimension has been the most problematic to isolate because attention is required for a broad range of executive functions. Thus, the extent to which the allocation of cognitive effort overlaps with impulse control is currently unclear. However, attempts to model both motor and nonplanning impulsivity have been more fruitful.

## Behavioral models of motor impulsivity

The stop-signal task (SST), the go/no-go task, and the continuous performance test (CPT) are the most commonly used behavioral tests of motor impulsivity for the assessment of clinical populations. In all these paradigms, the subject is required to withhold from making a prepotent motor response. However, there are some seemingly minor, yet structurally significant, differences among them. In the SST, the individual is required to respond as fast as possible to a particular target (Verbruggen and Logan, 2009). On a subset of trials, a stop signal is presented at varying delays after this go signal, and the subject must then cancel his or her already initiated response. The greater the delay between the stop and go signals (and therefore, the closer the timing of the stop signal to the mean go response time), the more difficult it becomes to withhold from making the go response. A reaction time measure may then be calculated to indicate how capable the subject is of canceling an action once it has already been initiated.

Although go/no-go paradigms likewise use two signals—one indicating that a go response is required, and the other that this response should be inhibited—only one signal is presented on any trial (Hogg and Evans, 1975). The go signal is considerably more frequent, thereby priming the motor response, but the signal occurs at the beginning of each trial, before any action has been initiated. Hence, in a go/no-go task, the subject must withhold from responding until the go signal is detected, whereas in the SST, the subject must always initiate the go response but inhibit its completion if the stop signal is detected. Both SST and go/no-go tasks have been developed for use in rodents and nonhuman primates (SST: Feola et al., 2000; Eagle and Robbins, 2003; Liu et al., 2009) (go/no-go: Iversen and Mishkin, 1970; Terman and Terman, 1973). Data from both human and animal models indicate that the types of inhibition taxed by these tasks—action cancellation in the SST, and action restraint in the go/no-go—can be dissociated in terms of both the supporting neural circuitry and the pharmacology of their regulation (Rubia et al., 2001; Eagle et al., 2008; Eagle and Baunez, 2010).

The CPT is very different in design from the other two tasks, in that subjects are required to scan a five-digit sequence and respond when the numbers match a target stimulus. "False alarm" errors occur when the subject responds positively to a sequence that is identical to the target, with the exception of the final number (Rosvold et al., 1956; Wilkinson, 1963). When making such errors, the subject is responding prematurely before processing the full sequence. To avoid making such impulsive responses, the subject needs to wait for the correct signal. The five-choice serial reaction time (5CSRT) task was designed as a rodent analogue of the CPT and provides somewhat independent measures of attention and motor impulsivity (Carli et al., 1983). In testing rodents, the visual stimulus is a simple cue light rather than a complex series of digits, but the cue can be presented in any one of five spatial locations and is illuminated for only a very brief time. The rodent is required to respond at the correct location in order to earn a food reward. Responses made prematurely, before the light has been presented, provide a reliable index of a rodent's inability to wait for the correct stimulus and are thought to be analogous to the errors of commission made on the CPT. Compared with the other models of impulsive action, the surface structure of the 5CSRT task probably deviates the most from the clinical test it is based on. Nevertheless, the neural circuitry and neurochemistry underlying performance of the CPT and 5CSRT task suggest a fair degree of correlation between the two species-dependent paradigms (Robbins, 2002), and the rodent 5CSRT task has since been successfully back-translated into human subjects (Sanchez-Roige et al., 2014; Voon et al., 2014).

## Behavioral models of "nonplanning," or impulsive decision-making

Despite the plethora of tasks that have been developed to study behavioral disinhibition, relatively few selectively target aspects of impulsive decision-making, or nonplanning impulsivity. Delay-discounting tasks have probably been the most successful in terms of modeling the inability to prioritize future rewards over satisfying the desire for more immediate gratification (Ainslie, 1975). In these tasks, every reward loses some of its subjective appeal as the delay to its delivery increases, that is, the delay discounts its value. In delay-discounting paradigms, subjects choose between smaller rewards

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available immediately and larger rewards available after a varying delay. On this measure, individuals that generate steeper discounting curves, such that each unit of time-delay has a greater negative effect on the valuation of the reward, are described as more impulsive. The majority of delay-discounting data from human subjects has been obtained using a computerized questionnaire involving a hypothetical choice between two fictional rewards, as per Rachlin's original studies (Rachlin et al., 1991).

Numerous delay-discounting paradigms have been developed in rats, yet none is an exact analogue of the methodology used most frequently in humans. Nevertheless, a general concordance has emerged in the output, in that nearly all variants of the task can distinguish between high and low impulsive subgroups and can be used to estimate a delay-discounting curve. However, the paradigms also vary considerably in terms of equipment used (e.g., operant chamber vs T-maze) and task structure (e.g., adjusting vs set delays, within-session vs between-session shifts in delay, relative size of large vs small reward). Although seemingly superficial, these differences in methodology can be crucial factors that influence drug effects (Winstanley, 2010; Winstanley et al., 2010). A valid goal of most research in disease models is, at some stage, to administer a candidate pharmacological treatment in the hopes of improving cognitive performance. Because of this goal, and perhaps due to the ease of implementing it relative to other methodologies, many studies have used a within-session shift in delays (Evenden and Ryan, 1996) so that a full curve may be evaluated in a single session.

## Modeling TBI in Animals

An ideal injury model generates behavioral and anatomical consequences that are congruent with those observed in patients. However, human TBI is complex and multimodal, making it difficult to replicate all its aspects in a single animal model (Finnie and Blumbergs, 2002; O'Connor et al., 2011). Because of this complexity, and to develop therapeutic strategies to treat TBI, many models of injury have been created that generate everything from highly specific injury to global brain damage across a spectrum of severities, and for species ranging from mice to nonhuman primates. Here, we will describe some of the most common models for TBI, but it should be noted that this list is not complete, and many additional models are available.

### Controlled cortical impact and fluid percussion injury models

Rodents are the most common species for TBI research because they represent a reasonable compromise in terms of cost and anatomical similarity to humans. Within rodent TBI, the two most often used models are controlled cortical impact (sometimes seen as cortical contusion injury [CCI]) and fluid percussion injury (FPI), with at least 1361 and 489 publications, respectively, as of this writing. Both CCI and FPI are surgical models that generate a focal injury, require a stereotaxic surgery frame, and involve performing a craniotomy. For CCI, a craniotomy is performed, and a stainless-steel piston is lined up with the dural surface. That piston is then driven at a prescribed velocity to a specific depth in order to generate a highly focal injury (Osier and Dixon, 2016). In the FPI, a lure with a membrane is loosely attached to the craniotomy with dental cement, and then the animal is moved to the FPI device and attached. A hammer on a pendulum then swings, impacting a fluid-filled tube and causing rupture of the lure membrane, resulting in direct compression of cortical tissue and a diffuse pressure wave as the water contacts the brain (Thompson et al., 2005). This results in an injury with both focal characteristics and diffuse axonal injury at more distal locations.

### Blast injury models

Blast injury has generated considerable interest recently due to its prevalence in wartime situations. Whereas lung damage due to blast has been investigated since the 1940s, blast injury models are a relatively recent addition to the experimental TBI literature and are caused through direct exposure to blast waves. Most models use either a high-pressure wave of gas or actual explosives to generate the pressure wave. This wave is then funneled through a tube to the anesthetized animal (Cernak and Noble-Haeusslein, 2010). Although realistic, the blast injury profile is complex, with many simultaneous systemic, local, and cerebral responses that can all interact. In humans, blast involves primary (blast pressure wave), secondary (air pressure and projectiles), tertiary (impact due to individual being thrown), and quarternary (burns, crush, and respiratory) events, whereas animal models concern themselves mainly with primary blast injury.

### Acceleration injury models

A final class of animal models of TBI reproduce impact and nonimpact acceleration injury. Collectively, these represent one of the earliest

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types of experimental injuries in the TBI field, and were initially designed to replicate conditions seen in car accidents where rapid deceleration occurs (Ommaya et al., 1973). In nonimpact acceleration models, animals are strapped to a moving device with the head either restrained or unrestrained and then subjected to a sudden deceleration, allowing the inertia of the brain to cause damage as it impacts the skull (Finnie and Blumbergs, 2002; Morales et al., 2005). These experiments are frequently performed in gyrencephalic animals (e.g., ferret, pig) because damage is maximized with both sulci and gyri, and when brain orientation is perpendicular to the brain stem, as in humans. To adapt this model to rodents, impact acceleration models have been adopted that generate rotational forces (commonly, coronal plane) as a result of an impact event (Kilbourne et al., 2009; Mychasiuk et al., 2014). More recent work has attempted to create a standardized model to minimize the considerable within-experiment and between-experiment variation that has come to characterize the field (Namjoshi et al., 2014). This standardization allows investigators to study these concussive forces in smaller laboratory animals.

### Mild TBI models

A final note should be made with regard to injury severity. Because the experimental TBI field initially emerged mainly from neurosurgery, much of the focus was on the effects of severe injuries. In recent years, however, interest in mild TBI has increased considerably, and a great debate is ongoing as to which of these models is ideal for investigating it. Each of the above models has been adapted in various manners for inducing milder forms of TBI (e.g., reduced impact force from CCI or FPI, weaker blast pressures, and milder accelerations), and each has achieved some success. However, many difficulties inherent in investigating mild TBI have limited the rate of discovery (Schultz et al., 2011). Notably, the secondary injury cascade may be considerably different, contributing to the heterogeneous outcomes in mild TBI.

## Concluding Remarks

The aim of this short primer was to summarize some of the different approaches that experimentalists have used to measure impulsivity, which is one aspect of cognitive behavior of considerable psychiatric relevance. We also reviewed the variety of models of TBI currently used for preclinical discovery. An appreciation of the diversity of possible approaches and the reason behind their development could help to catalyze improvements in experimental design and encourage more researchers to get involved in studying cognitive-behavioral changes resulting from brain injury.

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# Microglia in Neurodegeneration

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## Introduction

The neuroimmune system is involved in the development, normal functioning, aging, and injury of the CNS. Microglia, first described a century ago, are the main neuroimmune cells and have three essential functions: a sentinel function involved in constant sensing of changes in their environment, a housekeeping function that promotes neuronal well-being and normal operation, and a defense function necessary for responding to such changes and providing neuroprotection. Microglia use a defined armamentarium of genes to perform these tasks. In response to specific stimuli, or with neuroinflammation, microglia also have the capacity to damage and kill neurons. Injury to neurons in Alzheimer's, Parkinson's, Huntington's, and prion diseases, as well as in amyotrophic lateral sclerosis, frontotemporal dementia, and chronic traumatic encephalopathy, results from disruption of the sentinel or housekeeping functions and dysregulation of the defense function and neuroinflammation. Pathways associated with such injury include several sensing and housekeeping pathways, such as the Trem2, Cx3cr1, and progranulin pathways, which act as immune checkpoints to keep the microglial inflammatory response under control, and the scavenger receptor pathways, which promote clearance of injurious stimuli. Peripheral interference from systemic inflammation or the gut microbiome can also alter progression of such injury. Initiation or exacerbation of neurodegeneration results from an imbalance between these microglial functions; correcting such an imbalance may be a potential mode for therapy.

Recent research into microglia provides unprecedented insight into their roles in health, aging, and neurodegenerative diseases. These advances started 100 years ago in 1918, when Pio del Río Hortega published a method for staining microglia and distinguishing them from neighboring cells of the CNS (Río Hortega, 1918). Hortega named microglia the "third element" of the CNS, describing their phagocytic function, plasticity, regional distribution, and heterogeneity. For a century, microgliologists have been validating Hortega's observations.

Development of methods to isolate and culture neonatal microglia (Giulian and Baker, 1986) ascertained their functions, including phagocytosis and response to amyloid-β (Aβ), and supported their roles in neurodegeneration. Generation of mice with green fluorescent protein (GFP)–labeled microglia (Jung et al., 2000) allowed *in vivo* visualization by two-photon microscopy and showed that microglia continually survey and sense their microenvironment, respond rapidly to focal injury (Haynes et al., 2006), are involved in synaptic pruning and remodeling (Yang et al., 2013), and contribute to various neurodegenerative diseases. Novel methods to isolate adult microglia (Hickman et al., 2008) allowed transcriptomic analyses by RNA-sequencing (RNA-seq), thus identifying expression signatures that help define these cells (Hickman et al., 2013). Recently, single-cell RNA-seq has provided insight into potential microglial subpopulations in neurodegenerative diseases (Keren-Shaul et al., 2017).

In this chapter, we summarize the current knowledge of the roles of microglia in neurodegeneration. To better understand such roles, we introduce a revised functional and transcriptomic definition of microglia, discuss their roles in individual neurodegenerative diseases, and review common pathways involved in neurodegeneration. This chapter is based on a recent review by the authors (Hickman et al., 2018).

## A Functional and Molecular Definition of Microglia

Microglia constitute 5–12% of CNS cells, depending on the region (Lawson et al., 1990). They are the principal resident immune cells of the brain and are involved in homeostasis and in host defense against pathogens and CNS disorders (El Khoury, 2010; Ransohoff and El Khoury, 2015). Ontological studies of microglia confirmed Hortega's suspicion that they are mesenchymal, myeloid (Kierdorf et al., 2013), originating in the yolk sac, and capable of self-renewal independent of hematopoietic stem cells (Tay et al., 2017). Microglial survival and maintenance depend on cytokines, including colony-stimulating factor 1 (CSF1) and interleukin (IL)-34 (Wang et al., 2012), and on transcription factors such as interferon regulatory factor 8 (IRF8) (Kierdorf et al., 2013). Reprogramming stem cells or monocytes to develop into microglia-like cells is possible (Muffat et al., 2016; Abud et al., 2017; Ryan et al., 2017) and is dependent on their environment (Gosselin et al., 2017).

Until recently, a simplistic definition of microglia described them as innate immune cells of the CNS of myeloid origin that express Cx3cr1, CD11b, Iba1, and F4/80 (Ransohoff and El Khoury, 2015). Based on comprehensive gene-expression profiling and functional studies (Hickman et al., 2013; Ransohoff and El Khoury, 2015), we propose a functional and molecular definition of microglia that correlates their gene expression with their functions. RNA-seq analysis identified a new set of microglia-specific markers

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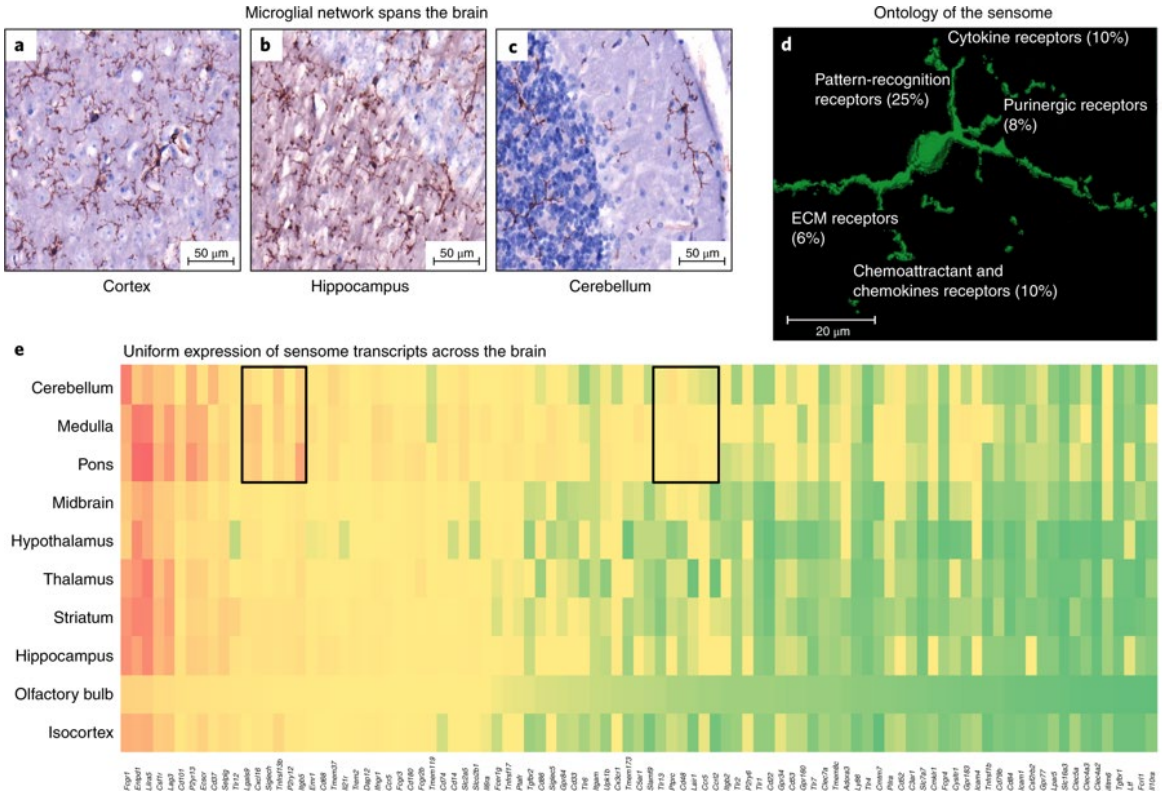


**Figure 1.** Microglia in a normal mouse brain. ***a–c***, Mouse microglia, stained here with anti-CD11b, have distinct processes that are constantly moving in the area around the cell body, and form a network of cells that spans most of the CNS, including the cortex (***a***), hippocampus (***b***), and cerebellum (***c***). Scale bars, 50 μm. ***d***, Three-dimensional image of mouse microglia with a summary of gene ontology analysis of the sensome genes. Scale bar, 20 μm. ***e***, Heat map showing comparative expression of microglial sensome genes identified by RNA-seq data using the Allen Brain Atlas *in situ* hybridization dataset. Most of the genes are similarly expressed in most areas of the brain, except for two small clusters that appear to have differential expression in the brain stem. ECM, extracellular matrix.

in the healthy brain that includes HexB, P2ry12, S100A8, S100A9, Tmem119, Gpr34, SiglecH, TREM2, and Olfml37. Microglial transcriptomes allow them to perform three essential functions: (1) sense their environment, (2) conduct physiological housekeeping, and (3) protect against modified-self and non-self injurious agents. These normal functions are important in various stages of development, from embryonic stages to adulthood and aging.

## Sensing

Microglia form a network spanning the CNS (Lawson et al., 1990). Their thin processes are dynamic and in constant motion, allowing them to scan the area surrounding their cell body every few hours and rapidly polarize toward focal injury (Figs. 1*a–c*; Supplementary Video 1). They use the products of nearly 100 genes to sense changes in their microenvironment (their sensome) including *P2yr12*, *AXL*, and *MER* (Haynes et al., 2006; Hickman et al., 2013; Fourgeaud et al., 2016) (Figs. 1*d,e*). Sensome messenger RNAs (mRNAs) are uniformly expressed in microglia in various areas of the brain, suggesting that all microglia are capable of performing their sensing function (Fig. 1*e*). Sensing is a prerequisite for microglia to perform their housekeeping and host-defense functions.

## Housekeeping

Physiological housekeeping functions include synaptic remodeling (a function critical for CNS development, homeostasis, and neurodegeneration) (Zhan et al., 2014; Lui et al., 2016; Vasek et al., 2016); migration to sites of neuronal death to phagocytose dead or dying cells (Fuhrmann et al., 2010; Krasemann et al., 2017) or debris; and maintaining myelin homeostasis (Healy et al., 2016). Interacting with astrocytes is another important microglial function involved in homeostasis, inflammation, and possibly neurodegeneration (Liddelow et al., 2017). Among the genes involved in housekeeping are those encoding chemokine and chemoattractant receptors, genes involved in phagocytosis (scavenger receptors and *Trem2*), and genes involved in synaptic

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pruning and remodeling (*C1q* and *Cx3cr1*) (Fig. 2) (Hickman et al., 2013). Aberrant housekeeping can lead to neurodegeneration.

## Protection against injurious self and non-self stimuli

Microglia mediate host defense against infectious pathogens, injurious self-proteins such as Aβ, aggregated α-synuclein, mutant huntingtin, mutant or oxidized superoxide dismutase (SOD), or prions, as well as primary or metastatic CNS tumors. To perform these functions, microglia express Fc receptors, toll-like receptors (TLRs), viral receptors, and antimicrobial peptides (Fig. 2) (Hickman et al., 2013). In response to such stimuli, microglia can initiate a neuroinflammatory response. Like peripheral inflammation, this response includes production of cytokines such as tumor necrosis factor (TNF) and IL-1 (El Khoury et al., 2003; Hickman et al., 2008), and possibly chemokines such as Ccl2 (El Khoury et al., 2007), to recruit additional cells and induce them to clear injurious agents and maintain brain homeostasis. Neuroinflammation, however, unlike peripheral inflammation, can also be limited to microglia without recruiting circulating leukocytes. Persistent neuroinflammation in turn induces neurotoxicity, leading to neurodegeneration.

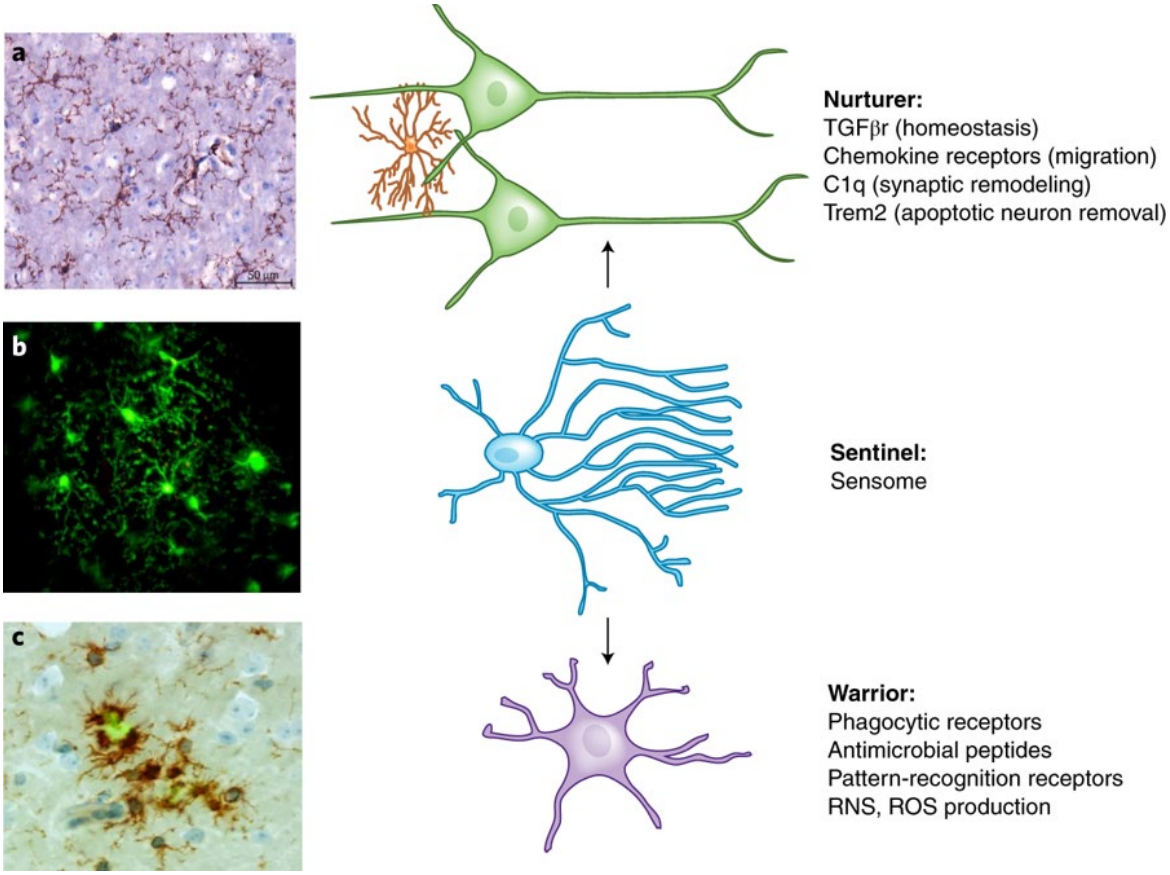


**Figure 2.** Three proposed functional states of microglia. ***a***, Nurturer state: microglia (left) stained for Cd11b (brown) in a normal brain are highly ramified and evenly spaced throughout the brain parenchyma. In their nurturer role, they maintain milieu homeostasis, participate in synaptic remodeling and migration, and remove apoptotic neurons, all mediated by specific receptors and receptor-linked pathways. Scale bar, 50 μm. ***b***, Sentinel state: micrograph taken from a video using two-photon microscopy from a Cx3cr1-GFP mouse with a cranial window shows a cluster of green microglia with abundant processes. The video from which this micrograph was taken (Supplementary Video 1) shows that microglia (green) processes are in constant motion, surveilling their surroundings. Focal laser-induced injury initiates microglia response, with those microglia closest to the site of injury displaying polarization of surveilling processes toward the area of injury. Microglia sensing is mediated by proteins encoded by sensome genes, which are portals for microglia to perform their housekeeping and host-defense functions. ***c***, Warrior state: microglia (left) stained for Cd11b (brown) accumulate around Aβ deposits stained with thioflavin-S (green), where they are observed to be twofold to fivefold denser than in neighboring areas. The warrior morphology becomes stockier and less ramified, and defense against infectious pathogens and injurious self-proteins, including Aβ, is mediated through microglial Fc receptors, TLRs, viral receptors, and antimicrobial peptides. Sensing is a prerequisite for microglia to perform their housekeeping and host-defense functions.

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A take-home message is that there are no resting microglia (Fig. 2). Their sensing, housekeeping, and protecting functions keep them constantly engaged, and most microglia in healthy brains are capable of performing such functions. Dysregulation of any of these functions results in an imbalance that initiates or propagates neurodegeneration. Next we summarize what we know about microglia and what happens to their functions in various neurodegenerative diseases.

## Alzheimer's Disease

Alzheimer's disease (AD) is characterized by formation of Aβ-containing plaques, neurofibrillary tangles comprising intracellular hyperphosphorylated tau protein, and neuronal loss (Selkoe and Hardy, 2016). An accepted sequence of events is that accumulation of Aβ leads to a microglial response, which promotes tau hyperphosphorylation and formation of neurofibrillary tangles, leading to neurodegeneration and cognitive impairment. In AD patients and animal models, microglia accumulate around senile plaques (Fig. 2*c*), where their density is twofold to fivefold higher than in normal parenchyma (Frautschy et al., 1998). They contain intracellular Aβ, suggesting phagocytosis (D'Andrea et al., 2004), show proinflammatory morphological changes such as somatic swelling and process shortening (Fig. 2*c*), and have increased proinflammatory markers including major histocompatibility complex II, CD36, IL-1, IL-6, and TNF (Tooyama et al., 1990; Martin et al., 2017). So how do microglia contribute to AD pathogenesis?

### Genome-wide association studies

Evidence for a direct microglial role in AD came from genome-wide association studies. Mutations in triggering receptor expressed on myeloid cells 2 (*Trem2*) were associated with a 3.0-fold to 4.5-fold increased AD risk, almost as high as that associated with *ApoE* ε4 (Jonsson et al., 2013; Lambert et al., 2013). Mutations in other microglial genes, such as *CR1, HLA–DRB1, CD33, MS4A6A*, and *BIN1*, were associated with more modest AD risks (Lambert et al., 2013). Since these genes regulate key microglial functions, understanding how they affect AD will impact all AD patients whether they have these mutations or not.

### Aβ clearance

Aβ deposition is regulated by equilibrium between Aβ production and clearance. Small changes in this equilibrium result in abnormal accumulation. Aβ clearance involves, in part (El Khoury and Hickman, 2009), phagocytosis and endocytosis via microglial scavenger receptors (SRs) (El Khoury et al., 1996; Frenkel et al., 2013) and extracellular degradation by Aβ-degrading enzymes (AβDEs) (Hickman et al., 2008; El Khoury and Hickman, 2009). Decreased clearance contributes to Aβ accumulation in late-onset AD. In support of this concept, microglia from a mouse model of Aβ deposition (Aβ-mice) have reduced expression of Aβ-phagocytic receptors and AβDEs, but their ability to produce proinflammatory cytokines is maintained (Hickman et al., 2008). These results suggest that Aβ accumulation is in part due to failure of microglia to clear this toxic peptide.

### Aβ-induced inflammation

Microglia–Aβ interactions lead to early synapse loss (Hong et al., 2016), production of neurotoxic reactive oxygen species (ROS) and reactive nitrogen species (RNS), NLRP3 (NOD-, LRR-, and pyrin domain–containing 3) inflammasome activation, and production of proinflammatory cytokines and TNF (Coraci et al., 2002; El Khoury et al., 2003; Gold and El Khoury, 2015; Venegas et al., 2017). This requires Aβ interaction with microglial pattern recognition receptors (PRRs) including TLRs, SRs, and complement receptor 3 (CR3) (Hickman and El Khoury, 2013; Hickman et al., 2013).

### Microglia in AD: a double-edged sword

Based on these findings, microglial–Aβ interaction is a double-edged sword. While monitoring the brain environment, microglial sensing of Aβ peptides results in Aβ clearance and removal of the injurious agent (Fig. 3). However, persistent production of Aβ and its chronic interaction with microglia drive further amyloid deposition. Indeed, Aβ-induced proinflammatory cytokines reduce microglial Aβ clearance ability, and NLRP3 activation releases microglial apoptosis-associated speck-like protein containing a CARD (ASC) which binds Aβ, causing its aggregation and leading to further amyloid "seeding" and spreading of amyloid pathology (Venegas et al., 2017). Similarly, Aβ-induced cytokines promote tau hyperphosphorylation and pathology, thus initiating a self-perpetuating loop that culminates in worsening disease (Oddo et al., 2003; Villemagne et al., 2017). The double-edged sword metaphor refers to various stages of a single microglia in AD. At a certain time point during disease progression, microglia assume a useful role, and then progress into a dysfunctional cell that ultimately becomes deleterious. In support of this concept, recent transcriptomic studies of microglia in normal and Aβ-mice identified subpopulations defined as disease-associated microglia (DAM) (Keren-Shaul et al., 2017; Krasemann et al., 2017).

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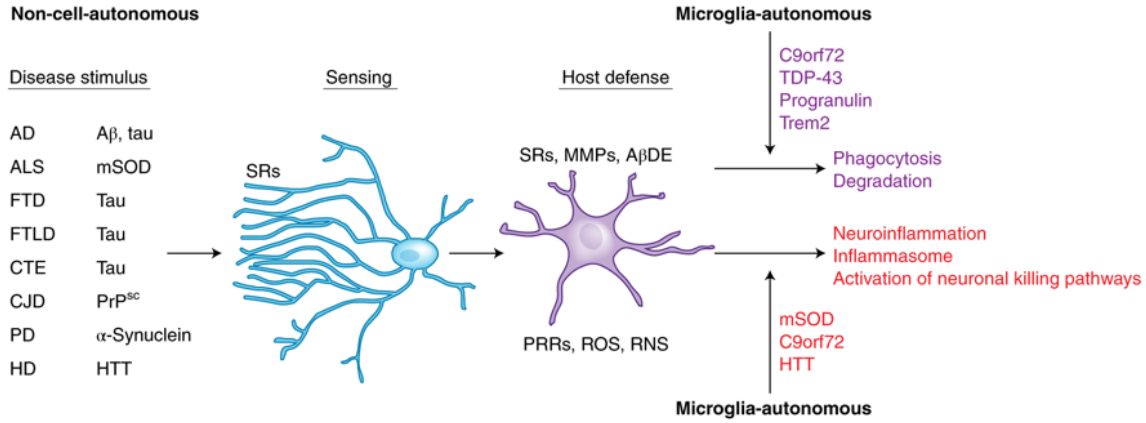


**Figure 3**. Effectors of microglia function associated with neurodegeneration. Two common themes for microglia's roles in neurodegenerative diseases emerge. First, as microglia perform their normal sentinel function, they encounter aberrant or misfolded proteins such as Aβ, aggregated α-synuclein, oxidized or mSOD1, or $PrP^{Sc}$. In response to these toxic stimuli, microglia perform their host-defense function, attempting to clear these agents via SRs and other PRRs. The nature of the aberrant proteins or their persistent production disrupts microglial housekeeping functions and dysregulates microglial host-defense functions, leading to an exaggerated proinflammatory response, neurotoxicity, and neurodegeneration. A second theme is that in some neurodegenerative diseases, such as AD, ALS, and HD, mutations in specific genes cause self-autonomous dysregulation of host defense, thereby initiating or exaggerating proinflammatory responses, resulting in neurotoxicity and neurodegeneration. For example, mutations in TDP-43, progranulin, and Trem2 affect phagocytosis and associated degradation pathways (purple), whereas mutations in mSOD and HTT affect inflammasome activation and neuronal killing pathways (red). Mutations in C9orf72 affect both phagocytosis and inflammasome pathways.

DAMs are located around Aβ plaques and have dysregulated expression of sensing, housekeeping, and host-defense genes. It is not clear how DAMs differ from "dark microglia" associated with Aβ deposits, which exhibit condensed cytoplasms and nucleoplasms and express high levels of CD11b and Trem2 (Bisht et al., 2016). These findings support a direct link between aberrant microglial functions and AD and suggest that a subset of microglia transitions from homeostatic microglia to DAMs in AD.

## Tauopathies

Tauopathies are neurodegenerative diseases characterized by hyperphosphorylated and aggregated tau and neurofibrillary tangles. Tauopathies include AD, progressive supranuclear palsy, corticobasal degeneration, frontotemporal dementia (FTD), and chronic traumatic encephalopathy (CTE) associated with repetitive concussive head injuries. Most tauopathies have neuronal and glial accumulations of toxic insoluble tau, neuronal loss, and proinflammatory microglia (Ferrer et al., 2014).

Microglia can engulf, degrade, and clear tau (Asai et al., 2015; Bolós et al., 2016). In contrast, when activated, proinflammatory microglia increase tau phosphorylation (Lee et al., 2010) and drive the spread of tau pathology (Asai et al., 2015). This finding is supported by human studies showing elevated levels of microvesicle-associated tau in the cerebrospinal fluid and blood of AD patients and by brain imaging studies showing proinflammatory microglia in FTD, progressive supranuclear palsy, and CTE (Saman et al., 2012; Fiandaca et al., 2015; Cherry et al., 2016). Proinflammatory microglia precede visible tau pathology in transgenic mice, and their activation is attenuated by the immunosuppressant drug FK506, which attenuates tau pathology and extends lifespan in these mice, suggesting that microglia can mediate tau neurotoxicity (Yoshiyama et al., 2007).

The double-edged sword concept of microglia's role in AD also applies to tauopathies (Fig. 3). Microglia first sense and clear tau in an attempt to protect from tau toxicity, but dysregulation of microglial sensing and housekeeping pathways, such as the Cx3cr1 and Trem2 pathways, lead to dysregulation of the host-defense pathway, resulting in a neuroinflammatory response gone awry, causing neuronal damage and loss (Bhaskar et al., 2010; Nash et al., 2013; Bemiller et al., 2017).

## Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 1.2% of individuals over age 65. Most cases are sporadic, and 5–10% are inherited (Deng et al., 2018). Neurodegeneration occurs in the substantia nigra, with dopaminergic denervation of the striatum and accumulation of Lewy bodies containing aggregated α-synuclein (Dickson, 2018). Reactive microglia expressing HLA-DR are abundant in the substantia nigra of PD patients (McGeer et al., 1988). Positron

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emission tomography (PET) studies show widespread proinflammatory microglia, but this response does not correlate with clinical severity, suggesting it occurs early in the disease (Gerhard et al., 2006).

The mechanism(s) by which microglia participate in PD may be similar to those in AD (Fig. 3). Microglia internalize and degrade α-synuclein, possibly to clear it. A defect in this process leads to accumulation of extracellular α-synuclein similar to Aβ (Halliday and Stevens, 2011). Microglia accumulate near α-synuclein deposits and become proinflammatory in a manner dependent on receptors that also bind Aβ, such as CD36 and TLR2 (Croisier et al., 2005; Su et al., 2008; Kim et al., 2013). These findings, which need to be validated in animal models of PD, suggest that AD and PD have similar pathogenic pathways, raising the possibility that microglia may also be a double-edged sword in PD.

## Multiple Sclerosis

Multiple sclerosis (MS) patients have demyelinated plaques in the white and gray matter. Ongoing disease leads to progressive neurodegeneration, resulting in brain atrophy. Neuroinflammation is present in all stages of MS, and a proposed classification of MS lesions relies in part on the presence or absence of microglia in the lesions (Kuhlmann et al., 2017).

Microglia in MS may be detrimental or beneficial. In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, microglia release proteases, proinflammatory cytokines, ROS, and RNS, and they recruit reactive T lymphocytes, thereby causing toxicity to neurons and oligodendrocyte precursors. Targeted deletion of the transforming growth factor (TGF)-β-activated kinase 1 in microglia in EAE reduced CNS inflammation and axonal and myelin damage by cell-autonomous inhibition of the nuclear factor-κB (NF-κB), JNK, and ERK1/2 pathways (Ransohoff and El Khoury, 2015). These results suggest that microglia promote tissue injury in EAE. However, at disease onset, microglia promote axonal regeneration, remyelination, clearance of inhibitory myelin debris, and the release of neurotrophic factors, suggesting a beneficial role (Yamasaki et al., 2014).

These observations are likely to be relevant to MS stages, as microglia are closely associated with lesions with active demyelination (Zrzavy et al., 2017). It is possible that the detrimental and beneficial roles of microglia in MS depend on the stage of the disease or of specific lesions. What is clear is that essential microglial functions are altered in EAE and possibly in MS, including their ability to sense and clear debris and mount a neuroprotective response.

## Huntington's Disease

Huntington's disease (HD) is characterized by progressive atrophy of the striatum and cortex in advanced disease (Ghosh and Tabrizi, 2018). Microscopically, there are intranuclear inclusions containing the protein huntingtin (HTT) and neurodegeneration of medium-size spiny, enkephalin-containing inhibitory neurons (Ghosh and Tabrizi, 2018). HD is caused by mutations in *HTT* (mHTT) that lead to expansion of the trinucleotide CAG stretch, which translates into a polyglutamine stretch in the HTT protein (Ghosh and Tabrizi, 2018).

HTT mRNA is expressed in microglia at a relatively high level, similarly to TLR47. Proinflammatory microglia are seen early in HD by PET. In presymptomatic HD gene carriers, their presence correlates with a higher probability of developing HD in 5 years (Tai et al., 2007). In HD patients, the presence of proinflammatory microglia correlates with HD severity (Sapp et al., 2001; Pavese et al., 2006).

HD is associated with altered microglial function and mRNA profile. Expression of mHTT in microglia confers a cell-autonomous increase in proinflammatory genes (Crotti et al., 2014) (Fig. 3). Levels and transcriptional activities of the myeloid lineage-determining factors PU.1 and CCAAT/enhancer-binding protein (C/EBP)-α,β are increased and exhibit enhanced binding at thousands of genomic locations in HD patients and mouse models (Crotti et al., 2014). This correlates with increased expression of *IL6* and *TNF*. These changes are unique to microglia and not observed in other myeloid cells (Crotti et al., 2014). Functionally, several of the genes that are increased in mHTT microglia are involved in sensing their milieu, such as *Tlr2*, *Cd14*, *Fcgr1*, *Clec4d*, *Adora3*, *Tlr9*, *Tnfrsf1b*, and others (Hickman et al., 2013; Crotti et al., 2014), suggesting a possible increase in the capacity to sense extracellular stimulants. This was associated with increased microglial neurotoxicity (Crotti et al., 2014). Upregulation of *IL6* and *TNF* mRNA (Crotti et al., 2014) suggest that the microglial response is an exaggerated host-defense response to rid the brain of mHTT that went awry, thereby exacerbating neurodegeneration.

## Amyotrophic Lateral Sclerosis

Most patients with amyotrophic lateral sclerosis (ALS; also called Lou Gehrig's disease) have sporadic ALS, but ~10% of patients have mutations in specific genes including *SOD1*, *C9orf72*, *TDP43*, and *FUS* (Lall and Baloh, 2017). The disease presents with

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loss of motor neurons in the cortex, brainstem, and spinal cord. Microglia expressing proinflammatory markers are found near injured neurons in autopsies (Henkel et al., 2004) and seen by PET in the brains of live ALS patients (Turner et al., 2004).

Transgenic mice overexpressing mutant human SOD1 (mSOD) develop a progressive motor neuron disease similar to ALS (Gurney et al., 1994). mSOD1 expression in microglia accelerates disease onset (Yamanaka et al., 2008), and microglial activation exacerbates motor neuron death (Apolloni et al., 2013). Microglia change their phenotype with disease progression. Some proinflammatory microglia are seen in the spinal cord before clinical disease develops, increase with disease progression, and persist into end-stage disease (Hall et al., 1998). Microglia isolated from mSOD1 mice at disease onset were neuroprotective, in contrast to microglia isolated at end-stage disease (Liao et al., 2012). Neurotoxicity of mSOD1 microglia is NF-κB-dependent (Frakes et al., 2014) and partly mediated by IL-1β (Meissner et al., 2010). These findings directly implicate microglia with mSOD1 in ALS progression.

Pathways leading to microglial activation and neurotoxicity in ALS are both cell-autonomous and dependent on exogenous stimuli (Fig. 3). Expression of mSOD1 in microglia disrupts regulation of NADPH oxidase, leading to excessive neurotoxic superoxide production (Harraz et al., 2008). Intraneuronal or extracellular misfolded SOD1 is sensed by microglia similarly to their sensing of Aβ or α-synuclein through TLRs and SRs, rendering them proinflammatory (Zhao et al., 2010). These findings suggest that the two major microglial functions altered in ALS include the sensing of exogenous stimuli and danger signals, and the host response. They also point to shared neurodegenerative pathways between ALS, AD, and PD, as ALS microglia also change their phenotype with disease progression from neuroprotective to neurotoxic (Liao et al., 2012).

Transgenic mice carrying the human *C9orf72* gene with disease-associated expansion repeats display pathological features of ALS, without behavioral abnormalities or neurodegeneration (O'Rourke et al., 2015). In contrast, mice deficient in C9orf72 exhibited enhanced TNF and IL-1 production when stimulated and defective maturation of phagosomes to lysosomes (O'Rourke et al., 2016). While these findings seem contradictory, they suggest that C9orf72 is required for normal microglial function and that altering microglial ability to clear aggregated proteins by altering phagosome-to-lysosome maturation, an important step in host defense, may contribute to neurodegeneration in patients carrying the *C9orf72* expansion (Fig. 3). Future functional studies with microglia from *C9orf72* patients would help clarify their complex role in this subset of ALS.

Transgenic mice expressing inducible human TDP-43 (hTDP-43) exhibit progressive motor neuron loss but only subtle microglial changes (Spiller et al., 2018). Following suppression of hTDP-43 transgene expression, microglia selectively cleared the existing neuronal hTDP-43. When microgliosis was blocked during the early recovery phase using a CSF1R and c-Kit inhibitor, these mice failed to regain full motor function, suggesting a neuroprotective role for microglia (Spiller et al., 2018). Interestingly, conditional deletion of *TDP43* in microglia promotes their phagocytic functions and leads to enhanced synapse loss (Paolicelli et al., 2017). While additional work is required to establish a clear pathway linking TDP-43, microglia, and ALS pathogenesis, these findings suggest dysregulation of microglial phagocytic function in ALS patients with TDP-43 mutations.

We propose that several genes with ALS-associated mutations regulate microglial host-defense functions, including production of ROS (mutant *SOD1*), cytokines (*C9orf72*), and phagocytosis (*C9orf72* and *TDP43*) (Fig. 3). These findings support a key role for microglia in ALS pathogenesis but indicate that targeting microglia for potential ALS therapy should be tailored to the specific pathway(s) affected and that a gunshot approach is not a useful therapeutic strategy.

## Prion Diseases

Prion diseases are genetic, sporadic, or acquired neurodegenerative disorders resulting from sustained aggregation of $PrP^{Sc}$, the proteinase-resistant form of the prion protein. Examples of prion diseases include Creutzfeldt–Jakob disease (CJD), scrapie, and chronic wasting disease. Prion-related neurodegeneration includes neuronal loss, increased proinflammatory microglia, and spongiform changes (Iaccarino et al., 2018). Microglia phagocytose $PrP^{Sc}$ as early as 60 days postinfection (Yamasaki et al., 2017), and their depletion increases prion titers and susceptibility to prion infection (Falsig et al., 2008), suggesting they help control prion disease.

The double-edged sword metaphor also applies to microglia in prion diseases. Microglia produce ROS in response to the PrP106-126 fragment and enhance its neurotoxicity. Ablation of superoxide-producing enzymes protected mice from PrP

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toxicity, further suggesting that microglia mediate prion neurodegeneration (Sorce et al., 2014). Upregulation of proinflammatory IL-1β, IL-6, inducible nitric oxide synthase (iNOS), NF-κB, cyclophilin A, matrix metalloproteinases (MMPs), and NLRP3 inflammasome components have all been demonstrated in prion disease microglia (Hafner-Bratkovič et al., 2012; Aguzzi and Zhu, 2017). Whether this proinflammatory response affects disease progression is unclear, since deletion of NLRP3 or the inflammasome adaptor Pycard did not markedly affect the clinical course of scrapie in mice (Aguzzi and Zhu, 2017).

Prion infection affects microglial sensing and housekeeping ability, in part by disrupting the Cx3cr1–fractalkine pathway (Xie et al., 2013). $PrP^{Sc}$ also reduces microglial phagocytosis of aberrant proteins, including $PrP^{Sc}$ and apoptotic debris or cells, despite production of proinflammatory mediators, suggesting dysregulated host defense (Hughes et al., 2010). As in AD, PD, and ALS, the effects of $PrP^{Sc}$ on microglia appear to be mediated by SRs and TLRs in an Src-kinase-dependent manner (Kouadir et al., 2012; Sakai et al., 2013). It is plausible that microglia try initially to clear $PrP^{Sc}$, leading to their persistent activation and dysregulated functions in another example of a host-defense response gone awry, and resulting in neurotoxicity and subsequent disease progression.

Two common themes for microglia's roles in neurodegenerative diseases emerge (Fig. 3). First, while microglia are performing their normal sentinel function, they sense the presence of an aberrant or misfolded protein such as Aβ, aggregated α-synuclein, oxidized or mutant SOD1, or $PrP^{Sc}$. In response to these toxic stimuli, microglia perform their host-defense function, attempting to clear these agents via SRs and other PRRs. The nature of the aberrant proteins or their persistent production disrupts microglial housekeeping functions and dysregulates microglial immune checkpoints and pathways that keep inflammation in check, such as the Cx3cr1 or progranulin pathways, leading to an exaggerated proinflammatory response, neurotoxicity, and neurodegeneration. A second theme is that in some neurodegenerative diseases, mutations in specific genes, such as *Trem2*, *HTT*, and *TDP43*, cause a self-autonomous dysregulation of sensing, housekeeping, or host defense, thereby initiating or exaggerating proinflammatory responses and leading to neurotoxicity and neurodegeneration.

## Common Pathways to Neurodegeneration: Microglial Immune Checkpoints

### How do microglia damage and kill neurons?

A recurring theme in neurodegenerative diseases is the damaging and killing of neurons by microglia using several direct and indirect tools (Fig. 4). When activated by ligands such as an infectious pathogen, Aβ, $PrP^{Sc}$, aggregated α-synuclein, or mSOD1, NADPH produces superoxide, which is released and either is transformed into $H_2O_2$ by extracellular SOD or reacts with NO to produce peroxynitrite (Simonian and Coyle, 1996). These cause cellular necrosis (Simonian and Coyle, 1996) or apoptosis (Brown and Vilalta, 2015) (Fig. 4). Microglia also cause excitotoxic neuronal death either by overexpressing iNOS or directly releasing glutamate (Maezawa and Jin, 2010; Brown and Vilalta, 2015). Microglial proteases such as cathepsins are released in response to Aβ, leading to neuronal apoptosis (Gan et al., 2004), and MMPs can cause neuronal injury in hypoxia-ischemia (Fig. 4) (Leonardo et al., 2009). Microglia can also damage neurons indirectly, either by releasing TNF or by reducing production of nutritive or neuroprotective brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF), thereby increasing neuronal apoptosis (Fig. 4) (Brown and Vilalta, 2015).

It is evident that the microglial host-defense function provides microglia with the tools for fratricide to become neuronal killers. This does not happen continuously because microglia have several immunological checkpoints or pathways that prevent their overreaction to external stimuli. These include the Trem2, Cx3cr1–fractalkine sensing, and housekeeping pathways, the progranulin pathways that keep their inflammatory response in check, and the SR pathways that promote clearance of injurious stimuli. Dysregulation of any of these pathways or disruption of the sentinel and housekeeping functions initiates or exacerbates neurodegeneration (Box 1).

## Trem2

The *Trem2* gene encodes an innate immune receptor of the immunoglobulin family located on chromosome 6 in humans and chromosome 17 in mouse (Hickman and El Khoury, 2014). Trem2 is expressed on macrophages, dendritic cells, osteoclasts, and microglia and is part of the microglial sensome (Hickman et al., 2013). Trem2 ligands include ApoE, phosphatidylserine, sphingomyelin,

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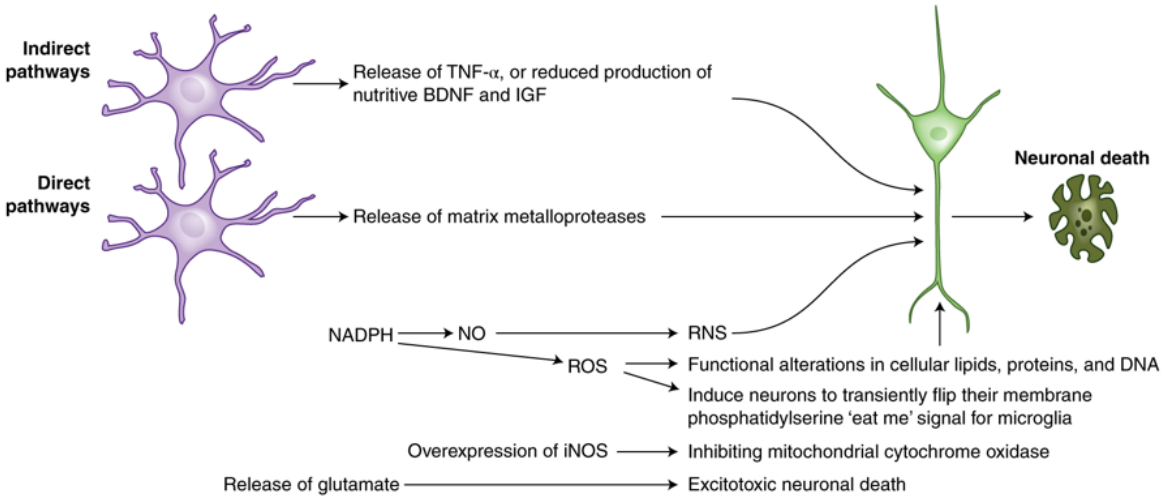


**Figure 4.** How microglia damage or kill neurons. There are several direct and indirect tools used by microglia to perform this task. When microglia interact with ligands, such as an infectious pathogen, Aβ, $PrP^{Sc}$, aggregated α-synuclein, or mSOD1, several pathways are activated. NADPH oxidase produces superoxide and derivative oxidants. Nitric oxide and its derivatives are produced by iNOS. Glutamate, cathepsin B, and other proteases are released, or phagocytic killing of stressed neurons occurs. Oxidative lipid damage reduces membrane fluidity and membrane potential and increases ion permeability, resulting in organelle swelling, loss of membrane depolarization, and rupture of the plasma membrane, leading to necrosis. Microglia also use indirect means to kill and damage neurons, including release of TNF, which stimulates NMDA receptor activity, or reduced production of nutritive BDNF and IGF.

Aβ, dead neurons, and damaged myelin (Wang et al., 2015; Yeh et al., 2016; Zhao et al., 2018).

Trem2 forms a signaling complex with the adaptor tyrosine kinase–binding protein (TyroBP or DAP12) (Hickman and El Khoury, 2014). Ligand binding to Trem2 triggers phagocytosis and chemotaxis and negatively regulates TLR-induced inflammatory responses (Hickman and El Khoury, 2014). The extracellular domain of Trem2 can be released as a soluble protein (sTrem2) and increases with age and with MS, AD, and FTD (Suárez-Calvet et al., 2016a). How is Trem2 involved in specific neurodegenerative disease?

## Alzheimer's disease

Genome-wide association studies identified mutations in *Trem2* as major risk factors in late-onset AD (Jonsson et al., 2013). Trem2 expression increases in plaque-associated microglia and infiltrating monocytes, suggesting a role in the microglial response to Aβ (Hickman and El Khoury, 2014). *Trem2* deletion decreased microglial phagocytosis, proliferation, and survival and increased proinflammatory cytokines and RNS (Takahashi et al., 2005). *Trem2*$^{-/-}$Aβ-mice have increased Aβ deposition, reduced numbers of myeloid cells around plaques, reduced Aβ phagocytosis, and greater neuritic dystrophy in early disease (Wang et al., 2016). This reduction of microglia around plaques was attributed to lower proliferation, decreased metabolic fitness (Ulland et al., 2017), and increased death (Wang et al., 2016). Since microglia form a physical barrier to prevent plaque expansion and protect neurons (Condello et al., 2015), increased accumulation of dystrophic neurons in *Trem2*$^{-/-}$Aβ-mice was attributed to decreased clearance by microglia rather than increased neuronal death. Collectively, these results implicate Trem2 in microglial recruitment to Aβ plaques and restricting exposure of neurons to toxic Aβ.

*Trem2* variants comprise amino-acid substitutions, frameshift and nonsense mutations, and splice-site alterations that likely result in loss of function (Jay et al., 2017). The *Trem2* variant with the strongest AD association is R47H (three to four times increased risk), a single amino-acid substitution in the extracellular domain (Jay et al., 2017). In transgenic Aβ-mice in which endogenous *Trem2* was replaced with the human common variant of normal *Trem2* or the variant R47H (Song et al., 2018), RH47 was associated with reduced Aβ-induced microglial responses, further supporting that R47H reduces Trem2 function *in vivo*.

Single-cell RNA-seq identified three microglial subpopulations in AD: homeostatic, intermediate, and DAM (Keren-Shaul et al., 2017). Transition from the homeostatic to the DAM state appears to be a two-step process characterized by a Trem2-

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independent homeostatic-to-intermediate state and a Trem2-dependent intermediate-to-DAM state. DAMs are neuroprotective and clear Aβ. Another subtype of DAM identified in aged mice, models of AD, ALS, and MS is dependent on a Trem2–ApoE pathway and is induced by phagocytosis of apoptotic neurons (Krasemann et al., 2017). This subtype exhibits decreased housekeeping and sensing genes and increased neurodegeneration-associated genes, suggesting that the Trem2–ApoE pathway regulates a switch from the homeostatic to a neurodegenerative phenotype. More work is needed to reconcile the role of Trem2 in each DAM subtype. However, there is consensus that Trem2 is a key regulator of microglia functions and phenotype in AD.

## Nasu–Hakola disease

*Trem2* variants are also associated with increased risk for polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, also called Nasu–Hakola disease (NHD) (Paloneva et al., 2003). NHD patients develop axonal degeneration, loss of white matter, and cortical atrophy. They exhibit increased microglial density and activation suggesting a dysregulated proinflammatory response (Satoh et al., 2011).

## Tauopathies, ALS, and PD

Trem2 may regulate tau pathology. sTrem2 correlates with tau levels in cerebrospinal fluid early in clinical AD (Suárez-Calvet et al., 2016b) and with tangle score and paired helical filament levels in postmortem AD brains (Lue et al., 2015). *Trem2* variants were identified in families with FTD or frontotemporal lobar degeneration (FTLD) (Supplementary Table 1). Studies assessing the risk of FTD or FTLD in nonfamilial cases are conflicting, although *Trem2* variants appear to influence clinical manifestations of these diseases (Lill et al., 2015). One study found R47H to be a significant risk for ALS (Cady et al., 2014), but this needs to be replicated. Attempts to correlate R47H with increased PD risk have had mixed results (Lill et al., 2015).

Studies in mice are also conflicting. In one study, Trem2 deficiency exacerbated tau pathology, neurodegeneration, and spatial learning deficits (Jiang et al., 2015). In another study, Trem2 deficiency reduced microgliosis, brain atrophy, and levels of inflammatory cytokines without affecting tau levels (Leyns et al., 2017). Similarly, in a CTE model, injured *Trem2*$^{-/-}$ mice showed less hippocampal atrophy, reduced inflammatory transcripts, and improved behavioral deficits compared with controls. Additional studies are needed to definitively assess the role of Trem2 in tauopathies and reconcile conflicting results.

The association of *Trem2* variants with AD, NHD, FTD, and possibly ALS and PD suggests that Trem2 is a major immune checkpoint that regulates an important microglial homeostatic pathway. Disruption of this pathway by *Trem2* variants leads to neurodegeneration (Box 1). However, the diversity of pathologies associated with these diseases suggests that additional genetic and/or epigenetic factors interact with Trem2 to cause a specific disorder. Therefore, understanding the biology of Trem2 and how it regulates microglial functions will be useful not only for helping patients with *Trem2* variants but also for understanding the role of microglia in neurodegeneration in general. Ongoing work should

**Box 1**. Microglia immune checkpoints disrupted in neurodegeneration.

As with lymphocytes, microglia have several immunological checkpoints that prevent their overreaction to external stimuli. These checkpoints are different from those of lymphocytes. They include the microglial Trem2, Cx3cr1–fractalkine sensing, and housekeeping pathways and the progranulin pathways, which keep their inflammatory response in check. Dysregulation of any of these pathways or disruption of the sentinel, defense, or housekeeping functions initiates or exacerbates neurodegeneration.

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|---|
| **Trem2** regulates all three microglial functions (sensing, housekeeping, and host defense), and dysregulation of Trem2 increases the risk for AD, FTLD, FTD, and possibly ALS. |
| **Cx3cr1** regulates sensing and housekeeping functions. However, disruption of this pathway has not been shown to increase the risk for neurodegenerative diseases, although it does alter disease courses in animal models. |
| **The progranulin pathway** regulates housekeeping, and its disruption may increase risk for AD, FTLD, FTD, and ALS. |
| As with T-cell immune checkpoints, microglial immune checkpoints may be important targets for therapy. |

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determine whether Trem2 is a realistic therapeutic target for neurodegenerative diseases, at what stage therapy would work best, and whether sTrem2 is a potential biomarker for efficacy.

## Cx3cr1–Fractalkine

Interactions between neuronal fractalkine (Cx3cl1) and its microglial receptor, Cx3cr1, define another immune checkpoint and pathway that regulates microglial function, likely because fractalkine and Cx3cr1 promote reciprocal neuron–microglial signaling. Membrane-bound fractalkine or its soluble form, (s)fractalkine, are the only known ligands for Cx3cr1 (Hickman and El Khoury, 2010).

Fractalkine–Cx3cr1 interactions regulate microglial homeostatic functions and temper microglial response to inflammatory and injurious stimuli. Blocking such interactions upregulates microglial TNF production, causing neurotoxicity (Zujovic et al., 2001). In neurons, these interactions regulate synaptic maturation (Zhan et al., 2014) and promote neuronal survival (Zujovic et al., 2001). However, because Cx3cr1 is also expressed on perivascular macrophages, studies using *Cx3cr1*$^{-/-}$ mice should be cautiously interpreted; the effects observed may also be attributed to perivascular macrophages.

### Alzheimer's disease

In Aβ and tau mouse models, Cx3cr1 deficiency enhances Aβ clearance (Liu et al., 2010) with a gene-dosage effect, but worsens tau pathology (Bhaskar et al., 2010). In support of such a dual role, overexpression of (s)fractalkine in tau mice but not in Aβ-mice led to substantial improvement (Morganti et al., 2012). The reason for this dichotomy is unclear, but since Cx3cr1 is important for microglial sensing and homeostatic functions, dysregulation of these functions is implicated. Because of this dichotomy, the role of fractalkine–Cx3cr1 in AD remains ambiguous and needs to be tested in mice with combined Aβ and tau pathology.

### Parkinson's disease

In the MPTP (a dopaminergic neurotoxic compound) model of PD, *Cx3cr1*$^{-/-}$ mice showed more extensive neuronal loss than *Cx3cr1*$^{+/-}$ mice (Cardona et al., 2006). In support of this neuroprotective role, overexpression of (s)fractalkine reversed MPTP toxicity for dopaminergic neurons (Morganti et al., 2012). In contrast, in transgenic mice overexpressing α-synuclein, *Cx3cr1*$^{-/-}$ microglia were less activated by aggregated α-synuclein, leading to reduced loss of dopaminergic neurons (Thome et al., 2015). In mice deficient in *LRRK2*, another PD-associated gene (Martin et al., 2014), Cx3cr1 was upregulated and microglia had reduced responsiveness to lipopolysaccharide. These studies suggest that altered Cx3cr1-mediated microglial sensing and housekeeping functions play a direct role in PD.

### ALS

In the mSOD1G93A mouse model of ALS, *Cx3cr1*$^{-/-}$ mice showed more neuronal loss than *Cx3cr1*$^{+/-}$ or *Cx3cr1*$^{+/+}$ mice, indicating a gene-dosage neuroprotective effect (Cardona et al., 2006). In support of this role, the loss of function of Cx3cr1 seen with the V249I rs3732379 Cx3cr1 variant was associated with accelerated progression and reduced survival in some ALS patients (Lopez-Lopez et al., 2014) but not in others (Calvo et al., 2018). In both studies, Cx3cr1 variants did not increase risk for ALS. Although these opposing findings are confusing, they suggest that Cx3cr1 is a disease-modifying factor in ALS and raise the need for studies with more patients.

### Prion diseases

Scrapie-infected hamsters' brains show progressive downregulation of fractalkine (Xie et al., 2013). The same effect is observed in response to PrP106-126 in mixed neuron–glia cultures (Xie et al., 2013). Infection of *Cx3cr1*$^{-/-}$ mice reduced their time to disease onset compared with wild-type mice, suggesting that Cx3cr1 may be protective in prion disease. No differences were seen in the pattern and localization of microglia or in chemokine and cytokine levels (Grizenkova et al., 2014). This was not fully duplicated in *Cx3cr1*$^{-/-}$ mice with a different genetic background (Striebel et al., 2016). Repeating these studies and using fractalkine-deficient (*Cx3cl1*$^{-/-}$) mice would help clarify the discrepancy.

There is consensus that the Cx3cr1–fractalkine immune checkpoint is overall a neuroprotective pathway that regulates sensing, housekeeping, and host-defense functions of microglia (Box 1). However, there are multiple knowledge gaps in our understanding of the roles of this pathway in neurodegeneration.

## Scavenger Receptors

SRs are innate immune PRRs that promote removal of non-self or altered-self ligands and elimination of degraded or harmful substances (PrabhuDas et al., 2017). They have roles in AD, PD, ALS, and prion diseases.

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### Alzheimer's disease

SR-A1 is an Aβ-phagocytic receptor (El Khoury et al., 1996; Frenkel et al., 2013) expressed on microglia surrounding Aβ plaques in humans and Aβ-mice. SR-A1 deficiency decreases microglial Aβ uptake by 60% (Frenkel et al., 2013; Cornejo et al., 2018) and increases mortality and Aβ accumulation (Frenkel et al., 2013; Cornejo et al., 2018), whereas upregulation of SR-A leads to reduced Aβ burden in Aβ-mice (Frenkel et al., 2013). Therapeutically, it would be beneficial to upregulate microglial expression of SRA1 to increase Aβ clearance.

SR-B2 (also called CD36) is also a microglial Aβ receptor that forms a complex with TLR-4 and TLR-6, leading to cytokine, chemokine, and ROS production, as well as microglial migration, inflammasome activation, and neurotoxicity (Coraci et al., 2002; El Khoury et al., 2003). Polymorphism (rs3211892) that increases CD36 levels is associated with increased AD risk (Šerý et al., 2017). Therapeutically, it may be advantageous to inhibit CD36–Aβ binding or signaling to reduce microglial neurotoxicity (Wilkinson et al., 2011).

Binding of Aβ to the microglial SR-J1 (also called RAGE) activates mitogen-activated protein kinase (MAPK) and NF-κB and contributes to synaptic dysfunction through IL-1β release in 2- to 3-month-old animals (Origlia et al., 2010). Six-month-old *RAGE*$^{-/-}$ Aβ-mice have reduced Aβ deposition and more AβDEs (Vodopivec et al., 2009). However, no change in cognition or microglial recruitment to plaques was seen in 12-month-old mice, suggesting that RAGE is not essential for microglial recruitment but could affect Aβ processing in early disease (Vodopivec et al., 2009).

These reports indicate that various SRs play complementary roles in microglia–Aβ interactions that may have therapeutic implications for AD. Pharmacological upregulation of SR-A1 expression or function may be helpful for AD treatment, whereas blocking CD36–Aβ or RAGE–Aβ interactions or reducing the expression of these SRs may stop or delay AD progression.

### Parkinson's disease, ALS, and prion diseases

Similarly to the roles they play in AD, SRs may also be involved in PD, ALS, and prion diseases through their interactions with misfolded proteins. SR-B2 promotes α-synuclein–microglial interactions (Su et al., 2008) and senses misfolded mutant or oxidized SOD1 in diseased motor neurons or in the extracellular space, rendering these cells proinflammatory (Zhao et al., 2010). Aggregated prion peptides also interact with microglial SRs, leading to uptake and/or neurotoxin production (Kouadir et al., 2012).

Cumulatively, these reports suggest a potential common pathway for handling misfolded proteins in neurodegenerative diseases, involving SRs (Fig. 3). Because of their ability to bind a diverse class of ligands, SRs are important sensors of misfolded proteins including Aβ, mSOD1, aggregated α-synuclein, and $PrP^{Sc}$, possibly to clear them. With disease progression, SR expression decreases, leading to defective clearing and subsequent accumulation of these misfolded proteins. Some of these receptors, such as CD36, form receptor complexes with other PRRs (e.g., TLRs) and initiate an inflammatory response that leads to neurotoxicity and neurodegeneration.

## Progranulin

Progranulin is a secreted glycoprotein with neuroimmunomodulatory properties and autocrine neurotrophic functions important for long-term neuronal survival (Chitramuthu et al., 2017). Progranulin deficiency leads to age-dependent, progressive upregulation of lysosomal and innate immunity genes, increased complement production, and enhanced C1q-dependent synaptic pruning by microglia, suggesting that progranulin is an important immune checkpoint that suppresses aberrant microglial activation in aging (Lui et al., 2016). Dysregulation of this pathway in microglia may occur in neurodegenerative diseases. Interestingly, similar results were obtained in a mouse model of adrenomyeloneuropathy. Deficiency in the very-long-chain fatty-acid transporter ABCD1 increased complement activation and synapse loss and aggravated microglial phagocytosis of neurons, suggesting a novel pathway for "death by microglia" (Gong et al., 2017).

### Frontotemporal lobar degeneration and Alzheimer's disease

Haploinsufficiency caused by autosomal dominant mutations in the progranulin gene leads to FTLD (Chitramuthu et al., 2017). Progranulin polymorphism is also linked to late-onset AD (Chen et al., 2015). In Aβ-mice, progranulin overexpression inhibits Aβ deposition and protects from Aβ toxicity. Selective reduction of microglial expression of progranulin in Aβ-mice also impairs phagocytosis, increases plaque load, and exacerbates cognitive deficits. Thus, increasing progranulin expression has been proposed as a potential therapy for FTLD and AD (Minami et al., 2014).

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### Parkinson's disease

Progranulin polymorphism is associated with increased PD risk, but the mechanism is not clear (Chen et al., 2015). In the MPTP mouse model of PD, upregulation of progranulin expression in nigrostriatal neurons was accompanied by reductions in markers of MPTP-induced inflammation and apoptosis, and it protected these neurons from MPTP toxicity and preserved striatal dopamine content and turnover. This was associated with preservation of locomotor function. Dysregulation of the progranulin pathway may therefore contribute to PD pathogenesis, and restoring progranulin levels may have therapeutic potential for PD (Van Kampen et al., 2014).

### ALS

Five progranulin mutations (four missense and one 5′ regulatory variant) are associated with reduction in age at onset and shorter survival after onset of ALS, suggesting that progranulin modifies ALS disease course (Sleegers et al., 2008). The mechanism of this association is not clear. Based on mouse and human studies, progranulin appears to be an important immune checkpoint that modulates the microglial response to external stimuli and prevents microglia from overreacting to such stimuli and thereby causing neurodegeneration (Box 1).

## Peripheral Regulation of Microglia in Neurodegeneration

Despite the anatomical separation between the CNS and the gastrointestinal system, a bidirectional connection between the two, known as the "brain–gut axis," exists. Intestinal bacterial colonization regulates immune system maturation and development in the CNS. Eradication of gut microbiota also alters microglial numbers, size, transcriptomes, and surveillance functions and downregulates host-defense genes (Erny et al., 2015). Recolonization with a complex microbiota or with microbiota-derived products partially restores microglial properties (Erny et al., 2015).

Human studies show associations between gut microbiota and neurodegenerative diseases (Vogt et al., 2017). In Aβ-mice, manipulating gut microbiota influences Aβ deposition and alters microglial reactivity and morphology by unknown mechanisms (Minter et al., 2016). In a PD model, antibiotic treatment ameliorates PD pathology, whereas oral administration of specific microbial metabolites promotes neuroinflammation and motor symptoms (Sampson et al., 2016). These studies add another layer to how microglial functions are regulated in normal and diseased states, suggesting that gut-to-brain signaling modulates neurodegeneration.

## Conclusions

The understanding of microglial biology has increased exponentially in the past decade. Microglial gene-expression profiles are being defined at the single-cell level and correlated with specific functions. We are also beginning to understand microglial roles in neurodegeneration and to explore pathways that regulate their response to injury, including neuroprotective immune checkpoint pathways that keep the microglial proinflammatory response in check and pathways that promote clearance of injurious stimuli. We are also exploring how peripheral influences from the gut microbiome can alter progression of such injury. Yet significant knowledge gaps exist. Analyzing the transcriptomes and epigenetic profiles in various disease states, understanding how aging and disease progression alter these profiles at the single-cell level, and correlating such changes with microglial behavior are necessary. Expanding the studies from mouse models to human patients remains a major limiting factor and requires development of new reliable *in vitro* cellular models derived from patient samples as well as additional technologies for *in vivo* imaging and analysis. In this regard, the ability to isolate primary microglia from fresh postmortem brain tissues or to reprogram human stem cells or monocytes to develop into microglia-like cells, as well as the ability to generate such cells from patients with various neurodegenerative diseases, are steps in the right direction (Muffat et al., 2016; Abud et al., 2017; Ryan et al., 2017). However, because correct programming is dependent on the cell's environment (Gosselin et al., 2017), devising new techniques to incorporate these cells into three-dimensional organoids, or improving existing techniques for organotypic brain-slice culture while maintaining their *in vivo* transcriptomic and epigenetic profiles, are crucial next breakthroughs waiting to be achieved. These steps are necessary for any successful effort to harness the power of microglia for the treatment of neurodegenerative disease.

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