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Review

Microglia in the TBI brain: The good, the bad, and the dysregulated

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ABSTRACT

As the major cellular component of the innate immune system in the central nervous system (CNS) and the first line of defense whenever injury or disease occurs, microglia play a critical role in neuroinflammation following a traumatic brain injury (TBI). In the injured brain microglia can produce neuroprotective factors, clear cellular debris and orchestrate neurorestorative processes that are beneficial for neurological recovery after TBI. However, microglia can also become dysregulated and can produce high levels of pro-inflammatory and cytotoxic mediators that hinder CNS repair and contribute to neuronal dysfunction and cell death. The dual role of microglial activation in promoting beneficial and detrimental effects on neurons may be accounted for by their polarization state and functional responses after injury. In this review article we discuss emerging research on microglial activation phenotypes in the context of acute brain injury, and the potential role of microglia in phenotype-specific neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and regeneration. We also describe some of the known molecular mechanisms that regulate phenotype switching, and highlight new therapeutic approaches that alter microglial activation state balance to enhance long-term functional recovery after TBI. An improved understanding of the regulatory mechanisms that control microglial phenotypic shifts may advance our knowledge of post-injury recovery and repair, and provide opportunities for the development of novel therapeutic strategies for TBI.

Contents

1. Introduction

Neuroinflammation is a prominent feature of many neurodegenerative diseases (Eikelenboom et al., 2010; Perry et al., 2010), and is increasingly being recognized as an important pathophysiological mechanism underlying chronic neurodegeneration following traumatic brain injury

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(TBI) (Faden and Loane, 2015; Johnson et al., 2013; Smith et al., 2013, 2012). As the primary mediators of the innate immune response in the central nervous system (CNS), microglia play a critical role in neuroinflammation and secondary injury after TBI. The understanding of the functional role of microglia in the injured brain and spinal cord has developed significantly in recent years, with strong support for dual beneficial and detrimental roles for microglia, resulting in tissue repair or neurodegeneration respectively (David and Kroner, 2011; Kumar and Loane, 2012). By removing cellular debris by phagocytosis and releasing neurotrophic factors and anti-inflammatory cytokines microglia can prevent neuronal injury and restore tissue integrity in the injured brain. However, it is clear that the development of a disinhibited and highly reactive microglial activation state results in the release of high levels of pro-inflammatory and cytotoxic mediators that contribute to neuronal dysfunction and cell death (David and Kroner, 2011; Kumar and Loane, 2012). Immune cells within the CNS milieu such as microglia and infiltrating macrophages appear to be heterogeneous with diverse functional phenotypes that range from pro-inflammatory (M1-like) phenotypes to immunosuppressive (M2-like) phenotypes. The "M1/M2" paradigm has been increasingly studied in neurodegenerative diseases in an attempt to uncover mechanisms of immunopathogenesis, and advances in understanding of molecular and functional states of microglia and macrophages may provide a framework to dissect out and interrogate the dual beneficial and destructive roles of these cells after TBI.

In this review, we focus on microglia and macrophage phenotypes in the context of acute CNS injury and post-traumatic repair. We discuss their role in phenotype-specific neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and regeneration, and the extracellular and molecular signals that regulate phenotype switching. We also highlight new therapeutic approaches that alter M1-/M2-like balance following TBI and enhance long-term functional recovery via promotion of tissue repair processes and suppression of neurodegeneration.

2. Microglia: origin and function in the uninjured brain

Microglia represent the major cellular component of the innate immune system of the brain, and constitute up to 10% of the total cells in the adult CNS (Kettenmann et al., 2011). Up until recently microglia were considered to be macrophages of the CNS, however a series of recent findings has established that microglia have a distinctive lineage and molecular signature as compared with circulating monocytes (Butovsky et al., 2014; Ginhoux et al., 2010; Hickman et al., 2013). Fate mapping analysis revealed that adult microglia derive from primitive erythromyeloid progenitors that leave the yolk sac on E8.5–E9.0, enter the neural tube via the primitive blood stream, and journey to the CNS where they gain lineage specific gene expression and differentiate into mature microglia (Ginhoux et al., 2010). Microglia development is dependent on Pu.1 and Irf8 transcription factors (Kierdorf et al., 2013), whereas essential bone-marrow derived macrophage transcription factors Myb and colony-stimulating factor 1 (CSF1) are not required (Schulz et al., 2012). In addition, microglial development is primarily regulated by CSF-1 receptor (CSF-1R) (Erblich et al., 2011) and its alternate ligand interleukin (IL)-34 (IL-34) (Greter et al., 2012), and transforming growth factor-β (TGFβ) has been recently identified as a critical differentiation factor beginning at E14.5 (Butovsky et al., 2014). Yolk-sac-derived microglia are maintained independently of circulating monocytes throughout life (Ajami et al., 2007), with the population being maintained by self-renewal in the healthy CNS, perhaps by a progenitor source within the CNS (Elmore et al., 2014). Microglia and brain macrophages express a number of common protein markers such as CD11b and CX_3CR1, however recent transcriptomic analyses have revealed that microglia have a unique molecular signature as compared to circulating monocytes and other tissue-resident macrophages derived from primitive yolk-sac (Butovsky et al., 2014; Hickman et al., 2013). This unique cluster of microglial-specific genes, which includes p2yr12, Fcrls, Tmem119, Offml3, Tgfbr1 (Butovsky et al., 2014; Hickman et al., 2013), can now be used to accurately distinguish microglia from macrophages and monocytes.

Up until recently highly ramified microglia were presumed to be resting and inactive in the healthy brain, but elegant in vivo imaging studies revealed that microglia are in fact highly active, and their long cellular processes undergo continuous cycles of extension, withdrawal and de novo formation to continuously scan their environment for disruptions in homeostasis (Davalos et al., 2005; Nimmerjahn et al., 2005). This physiological activity enables microglia to patrol the brain microenvironment and clear accumulated metabolic products or tissue debris by phagocytosis. More than a case of solely maintaining CNS homeostasis, it is now clear that microglia play a critical role in brain development, activity-dependent synaptic plasticity, and learning in the adult CNS, via activities in regulating cell death, synapse elimination, neurogenesis and neuronal surveillance (for review see Katsumoto et al., 2014; Salter and Beggs, 2014; Tremblay et al., 2011). Thus, the latest research indicates that microglia refine neuronal circuits and connectivity, and contribute to plasticity. Therefore, in addition to responding to CNS injury and disease, microglia also play an active role in shaping activity in the healthy brain (Salter and Beggs, 2014).

In the healthy CNS, microglia remove cellular debris without changing their ramified phenotype. In contrast, in response to injury or infection microglia become activated which results in dramatic morphological transformation and gene expression changes. Similar to peripheral cells microglia express pathogen recognition receptors such as toll-like receptors (TLRs) and NOD-like receptors (NLRs), and therefore respond to pathogen-associated molecular patterns (PAMPs) and endogenously produced danger-associated molecular patterns (DAMPs), which are secreted by damaged neurons and others cells of the CNS (Hanisch and Kettenmann, 2007). They also express receptors for a number of other factors that are released by damaged neurons, including ATP, glutamate, growth factors and cytokines. Microglia are antigen presenting cells and communicate with T lymphocytes, and upon activation upregulate cell surface markers such as MHC II and CD86 among others, as well as adhesion molecules and complement receptors (Hanisch and Kettenmann, 2007). A key to maintaining microglia in a normal physiological state is the immunosuppressive potential of the CNS. In the healthy brain neurons express a number of immunosuppressive proteins that act as "off" signals, that when paired with specific microglial receptors maintain microglia in a non-activated state (Harrison et al., 1998; Hoek et al., 2000). Such neuronal–microglial signaling mechanisms include fractalkine–CX3CR1, CD200–CD200R1, CD47–CD172a/SIRPα, among others (Hanisch and Kettenmann, 2007). In addition, soluble factors such as neurotrophins, anti-inflammatory cytokines and prostaglandins released locally by neurons, astrocytes and microglia downregulate the immune response thereby maintaining microglia in a surveillant state (Hanisch and Kettenmann, 2007).

3. Heterogeneity of microglial activation

It is now recognized that there is considerable heterogeneity of microglial activation in the brain. Macrophages in non-neuronal tissues have remarkable plasticity that allows them to efficiently respond to environmental signals and change their phenotype and function following pathogen exposure or tissue damage (Gordon, 2003; Sica and Mantovani, 2012). This process is called macrophage polarization, and enables the adaptive responses of innate immunity to take place. Two distinct macrophage polarization states have been described, termed M1 and M2, which represent both ends of a spectrum of functional macrophage activation (Gordon, 2003; Sica and Mantovani, 2012). Within this activation spectrum there is one M1 and three M2 polarization subtypes, M2a, M2b and M2c, each with a specific function and pattern of phenotypic marker expression (Gordon, 2003; Sica and Mantovani, 2012). The presence of multiple activation phenotypes for microglia is a relatively new concept (Colton, 2009), and it should not be assumed

that phenotypes and functions of peripheral macrophages will translate accurately to microglia in the brain. In fact, the latest research indicates that the stimulus-induced transcriptional plasticity of microglia does not directly correspond to the M1 or M2 plasticity observed with macrophages (Butovsky et al., 2014). However, despite their different ontogeny, microglia also have the capacity to become polarized into 'M1-like' and 'M2-like' activation phenotypes, although their roles in brain injury progression and repair have yet to be fully determined.

Microglia and macrophages respond to pro-inflammatory molecules, such as bacterial lipopolysaccharide (LPS) or the T_H1 cytokine, interferon-γ (IFNγ), to adopt a 'classical' M1-like phenotype, which produces high levels of pro-inflammatory cytokines (IL-1β, IL-12, tumor necrosis factor-α (TNFα)), chemokines (CCL2, CXCL9, CXCL10), and reactive oxygen species (ROS) that are essential for host defense (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M1-like phenotype markers include CD16, CD32, CD86, MHC II, and iNOS. M1-like activation is associated with phagocytosis, ability to kill pathogens by iron restriction, phagosome acidification, and ROS release (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). In many cases, the M1-like response is protective and is downregulated once the damage or pathogen has been removed; however, a dysregulated or excessive M1-like activation in the brain can induce neurotoxicity due to release of pro-inflammatory factors and neurotoxic mediators that set off vicious cycles of microglial-mediated neurodegeneration (Gao et al., 2003; Qin et al., 2004). Microglia and macrophages also respond to T_H2 cytokines such as IL-4 and IL-13 to induce an 'alternative' M2a-like state that is associated with immunity against parasites, Th2 cell recruitment, tissue repair, and growth stimulation (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). In addition to producing anti-inflammatory cytokines (IL-10), M2a-polarized cells upregulate several phenotypic markers such as arginase 1, CD206, Ym1, Fizz1, inhibit NFκB isoforms, and increase production of scavenger receptors for phagocytosis (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). Microglia/macrophages adopt a 'deactivated' M2c-like phenotype in response to IL-10, glucocorticoids, or uptake of apoptotic cells, and this phenotype appears to be involved in tissue remodeling and matrix deposition after inflammation has been downregulated (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M2c-polarized cells upregulate phenotypic markers such as CD163, CD206, TGFβ, and Sphk-1. Finally, microglia/macrophages can adopt an intermediate M2b-like phenotype in response to immune complex exposure and toll like receptor (TLR) ligands (Colton, 2009; Gordon, 2003; Sica and Mantovani, 2012). M2b has either pro- or anti-inflammatory function and is associated with memory immune responses (B-cell class switching and recruitment of Treg cells). The M2b phenotype has characteristics of both M1 (MHCII, CD86) and M2 (IL-10^{high}, IL-12^{low}) cells,

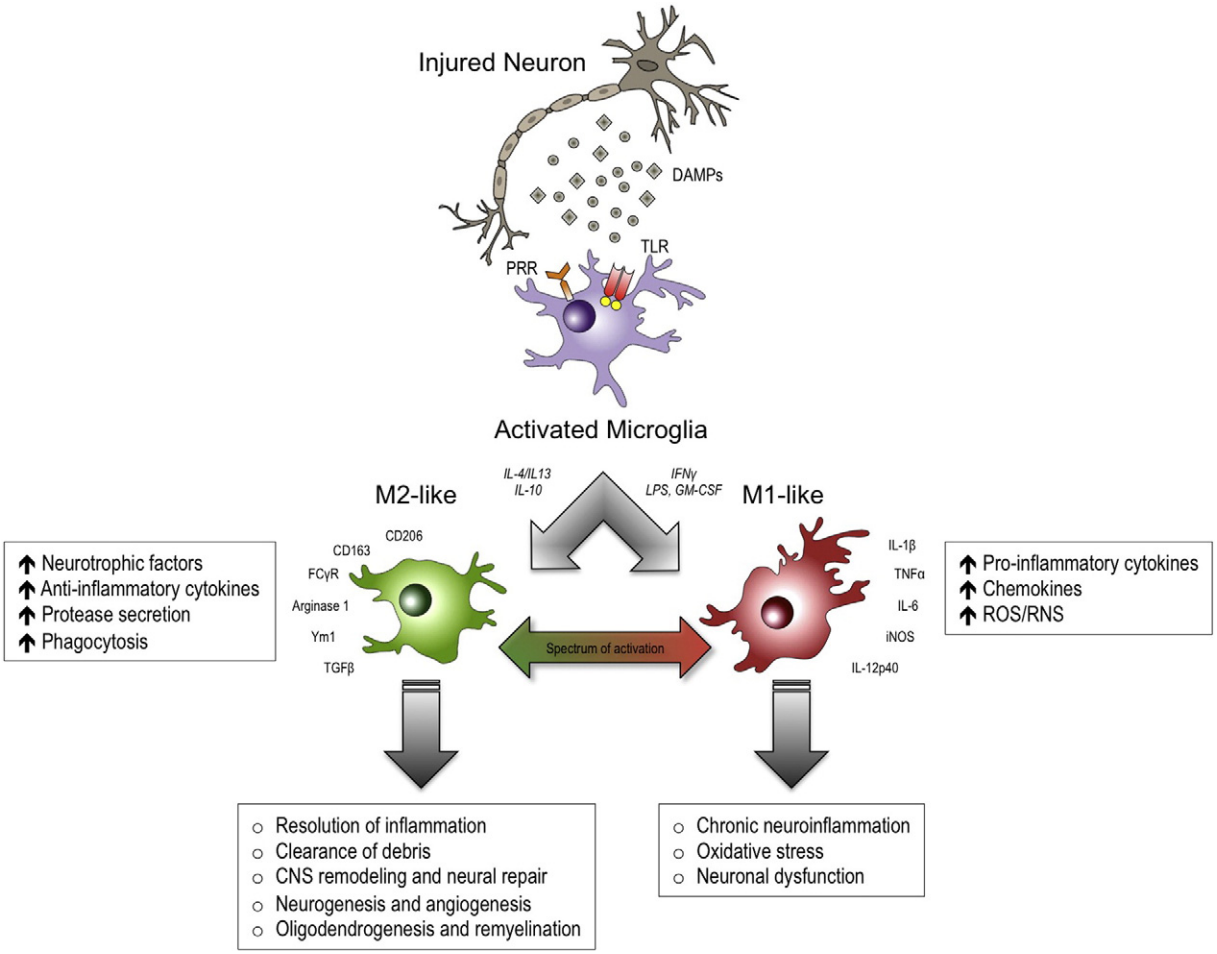

Fig. 1. Microglial phenotypic and functional responses after TBI. In response to DAMPs and other extracellular signals released by injured neurons microglia can become polarized towards M1-like and M2-like activation states that can have distinct roles in neurodegeneration and tissue repair. M1-like microglia are characterized by upregulated expression of phenotypic protein markers such as IL-1β, TNFα, IL-6, iNOS, and IL-12p40. They release pro-inflammatory cytokines, chemokines and free radicals that impair brain repair and contribute to chronic neuroinflammation, oxidative stress and long-term neurological impairments. In contrast, M2-like microglia upregulate protein markers such as CD206, CD163, FCγR, arginase 1, Ym1, and TGFβ. M2-like microglia release anti-inflammatory cytokines, neurotrophic factors and proteases, and they have increased phagocytic activity. M2-like microglia promote immunosuppression and resolution of M1-mediated neuroinflammation, and participate in CNS remodeling and repair by modulating neurorestorative processes such as neurogenesis, angiogenesis, oligodendrogenesis and remyelination. Abbreviations: DAMPs, danger-associated molecular patterns; PRR, pathogen recognition receptors; TLR, toll-like receptors.

and when M2b macrophages stimulate T cells they are biased towards a Th2 response (Filardy et al., 2010), which suggests that they may be a potential regulator of the M2 response in general.

Phenotypic markers for each polarization state were established using in vitro model systems, and defining specific phenotypes in vivo is much more challenging due to various environmental and tissue specific stimuli that drive polarization (Novak and Koh, 2013). After CNS injury or in neurodegenerative disorders microglia or infiltrating macrophages often present mixed phenotypes indicating their plastic nature and their ability to acquire multiple activation phenotypes in response to local environmental signals and dynamic changes in the inflammatory milieu (Sica and Mantovani, 2012). Clearly, the M1–M2 paradigm is an oversimplified model that only represents two extreme states within an activation continuum. However, given the limited data on the roles of microglia and infiltrating macrophage subsets after TBI, their classification into M1-like and M2-like provides a framework to dissect out and interrogate the dual beneficial and destructive roles of resident microglia and infiltrating macrophages after TBI (Fig. 1), and for exploration of new therapeutic strategies.

4. The immune system response to TBI

Within minutes of TBI there is a robust neuroinflammatory response that is mediated by complex molecular and cellular inflammatory events. The temporal profile of these events have been studied using animal models of TBI, as well as human surgical and post-mortem tissue samples and analysis of cerebrospinal fluid (CSF) and plasma from TBI patients (Ziebell and Morganti-Kossmann, 2010). Depending on the intensity of the injury and whether it is penetrating or not, TBI induces instantaneous cell death at the site of impact (primary injury). Damaged cells release DAMPs that signal to other resident and infiltrating immune cells via pattern recognition receptors, and astrocytes, microglia and damaged neurons at the site of injury secrete cytokines and chemokines. These potent immune mediators activate microglia and astrocytes at the site of injury and recruit peripheral immune cells that traffic through the damaged blood–brain barrier during the acute post-traumatic period.

Neutrophils are the first peripheral cells to accumulate in the brain after TBI (Clark et al., 1994), and they attempt to clear cell debris by phagocytosis. However, neutrophils also contribute to ongoing tissue damage by releasing toxic mediators such as ROS (Rhodes, 2011). Experimental studies indicate that neutrophil infiltration in the TBI brain is maximal at 1 day post-injury, and is followed by accumulation of leukocyte subsets that peak at about 3 days post-injury (Soares et al., 1995). Monocytes are recruited to the damaged brain in response to local chemokine signals (e.g. CCL2, CXCL10 and CCL5), and once in the brain they differentiate into macrophages. Two subpopulations of monocytes have recently been defined based on their relative cell-surface expression of the chemokine receptors CCR2 and CX3CR1, with CCR2+ cells representing 'inflammatory' ($CD11b^+CD45^{hi}CCR2^+Ly6C^{hi}$) monocytes and the CX3CR1+ cells representing 'patrolling' ($CD11b^+CD45^{hi}CX3CR1^+$) monocytes (Auffray et al., 2007). It is the inflammatory monocytes that are preferentially recruited to the TBI brain (Hsieh et al., 2013), and they predominate the lesion site at 3 days post-injury. Dendritic cells (DCs), T lymphocytes and natural killer (NK) cells are also recruited to the TBI brain during this period (Jin et al., 2012), but at much lower numbers than those of infiltrating monocytes. The exact functional role of DCs and T cells in TBI pathology has yet to be established, but distinct T cell subsets may modulate local inflammatory responses to be either harmful or protective (Walsh et al., 2014). Within this time frame brain resident glial cells become highly activated. Astrocytes surrounding the lesion are reactive and up-regulate GFAP and produce cytokines and chemokines that contribute to additional recruitment and activation of resident microglia and peripheral immune cells. Microglia transform from a ramified to an amoeboid morphology and when activated they are morphologically indistinguishable from recruited blood-derived macrophages. They secrete pro-inflammatory cytokines and free radicals that are cytotoxic to neurons and can contribute to neurodegeneration after TBI. Thus, the inflammatory response to TBI is highly complex with several interrelated molecular and cellular events initiated after injury to activate resident microglia and astrocytes, recruit additional peripheral immune cells, and damage neurons. Determining the relative contribution and functional role of M1- and M2-like polarized microglia and infiltrating macrophages in this complex tissue injury environment is challenging, but experimental studies are beginning to shed light on these phenotypic responses.

5. M1- and M2-like polarization after TBI

It would be expected that M1- and M2-like microglia and macrophages would work in concert to fine-tune inflammatory responses, scavenge debris, and promote remodeling and repair after TBI; thereby contributing to successful wound healing and promoting a return towards homeostasis after injury. However, experimental and clinical studies demonstrate a chronic and persistent M1-like phenotype for months to years after a single moderate-level TBI or repeated mild TBI (Acosta et al., 2013; Aungst et al., 2014; Johnson et al., 2013; Loane et al., 2014a; Mouzon et al., 2014; Nagamoto-Combs et al., 2007; Nonaka et al., 1999; Ramlackhansingh et al., 2011; Smith et al., 2013), with limited capacity for tissue repair, particularly after moderate-to-severe TBI.

Experimental studies in models of spinal cord and ischemic brain injury have shown that the majority of microglia and recruited macrophages at the site of injury have mixed M1- and M2-like activation profiles, but that the M2-like response is short-lived and there is a phenotypic shift towards an M1-like dominant response within one week of injury (Hu et al., 2012; Kigerl et al., 2009). M1-like cells that dominate the lesion have reduced phagocytic activity, and increased secretion of pro-inflammatory and neurotoxic mediators that can exacerbate injury and contribute to pathology (David and Kroner, 2011; Hu et al., 2012; Kigerl et al., 2009). Furthermore, M1-like macrophages have also been shown to impair axon regeneration via upregulation of chondroitin sulfate proteoglycans that are a potent inhibitor of axon regrowth (Galtrey and Fawcett, 2007; Martinez et al., 2006).

Similar M1- and M2-like activation dynamics have been reported in the TBI brain. Focal contusion TBI induced by controlled cortical impact (CCI) results in extensive local tissue damage, activation of microglia ($CD11b^+/CD45^{low}$), as well as a rapid and robust cellular infiltration of neutrophils ($CD11b^+/CD45^+/Ly6G^+$) and inflammatory monocytes ($CD11b^+/CD45^{hi}/CCR2^+/Ly6C^{hi}$) in the injured cortex within 24 h of CCI (Hsieh et al., 2013; Jin et al., 2012). Using flow cytometry in combination with markers for M1- and M2-like activation Jin et al. (2012) described a bimodal microglial activation response after CCI, with an initial peak at 7 days post-injury characterized by $CD206^+/CD45^{low}/CD11b^+$ M2-like activation and a secondary peak at 28 days post-injury characterized by $CD86^+/CD45^{low}/CD11b^+$ M1-like activation (Jin et al., 2012). Such temporal dynamics are supported by histological studies that showed transient CD206+ M2-like activation that peaked at 5 days after CCI followed by a transition to a dominant CD16/32+ M1-like activation at later time points that was associated with increased white matter injury (Wang et al., 2013b). These data indicate that different subsets of microglia and/or macrophages are being activated in the acute and chronic phases after CCI.

Recently, pharmacological studies combined with transgenic mouse models have provided important information on the relative contribution of resident microglia versus infiltrating monocytes to M1/M2-like polarization after TBI. Morganti and colleagues took advantage of $CX3CR1^{GFP/+}CCR2^{RFP/+}$ reporter mice (Saederup et al., 2010) to study the temporal kinetics of TBI-induced CCR2+ monocyte accumulation in the brain (Morganti et al., 2015). Using this model it is possible to

distinguish between peripheral inflammatory monocytes ($CCR2^{RFP/+}$) and resident microglia ($CX3CR1^{GFP/+}$) based on their relative expression of CCR2 because microglia do not express CCR2 natively or following CNS injury (Saederup et al., 2010; Schilling et al., 2009). CCL2 is the cognate ligand for CCR2, and its expression is significantly upregulated within hours of head trauma as detected in surgically resected samples and CSF from TBI patients (Semple et al., 2010; Stefini et al., 2008), or in rodent TBI models (Dalgard et al., 2012; Israelsson et al., 2008; Rhodes et al., 2009; Semple et al., 2010). The functional consequences of CCL2/CCR2 signaling after TBI has been evaluated using knockout mice, and deficiency of either CCL2 or CCR2 results in improved neurological and histological outcomes. When compared to wildtype mice, CCL2 −/− mice subjected to a closed head TBI model had increased neurological function, reduced lesion volume, astrogliosis and accumulation of F4/80 + macrophages at delayed time points post-injury (Semple et al., 2010). Similarly, CCR2 −/− mice subjected to CCI had reduced lesion volumes, decreased pro-inflammatory gene expression, and decreased macrophage infiltration ($CD45^{hi}CCR2^{+}$ and $F4/80^{+}$ cells) in the injured cortex when compared to wildtype mice (Hsieh et al., 2014; Israelsson et al., 2014), and CCR2 −/− CCI mice also had improved cognitive performance in the Morris water maze test and reduced hippocampal neurodegeneration at 8 weeks post-injury (Hsieh et al., 2014). Studies using CCR2 antagonists have also improved outcomes in experimental TBI models. When the CCR2 antagonist RS504393 was administered after weight-drop TBI in rats a high dose treatment reduced early apoptotic cell death and improved cognitive function in the Morris water maze test (Liu et al., 2013). More recently Morganti and colleagues evaluated a new CCR2 antagonist (CCX872) in $CX3CR1^{GFP/+}CCR2^{RFP/+}$ reporter mice using the CCI model, and demonstrated that CCX872 pre-treatment resulted in reduced macrophage accumulation ($CD45^{hi}CCR2^{+}$ $F4/80^{+}$ cells) acutely after CCI (Morganti et al., 2015). CCR2 antagonism also improved cognitive function recovery after TBI as demonstrated by increased performance of CCX872-treated mice in a radial arm water maze test at 28 days post-injury (Morganti et al., 2015). They also showed that CCR2 + infiltrating macrophages in the brain expressed both M1- and M2-like markers acutely after CCI, with sequential activation of M1-, followed by M2a-, and finally M2c-marker expression (Morganti et al., 2015). The M1-/M2-like activation profile of CCR2 + brain macrophages after CCI was similar to the damage response of wound-healing macrophages outside the CNS, such that proinflammatory and proteolytic macrophages accumulate rapidly after injury, followed by macrophages that promote wound-healing and anti-inflammatory responses (Deonarine et al., 2007; Gensel and Zhang, 2015). However, the accumulation of CCR2 + macrophages into the TBI brain ultimately resulted in upregulation of pro-inflammatory M1-like cells with neurotoxic potential at chronic time points, and CCR2 antagonism robustly reduced this pro-inflammatory profile and ameliorated the long-term cognitive dysfunction induced by TBI (Morganti et al., 2015). In another interesting study Hsieh and colleagues used a reporter mouse model for M2-polarized macrophages (eYFP arginase 1; YARG) to investigate the role of infiltrating brain macrophage subsets after CCI, and demonstrated robust infiltration of arginase 1 (Arg1 +)-positive macrophages that differentiated into at least two distinct subpopulations with unique chemokine expression patterns (Hsieh et al., 2013). Expression profiling of Arg1 + and Arg1 – macrophage subpopulations that infiltrated the TBI brain revealed that they do not separate into true M1- or M2-like transcriptional profiles, but rather adopt mixed molecular phenotypes, and the ratio of macrophage subsets change over time after TBI (Hsieh et al., 2013). These later two studies highlight the plasticity of the macrophages response to TBI, and how phenotypes and/or functions may switch over time in response to changing stimuli within the local lesion microenvironment.

It is important to note that the majority of existing data on M1-/M2-like phenotypes after TBI have been developed using focal TBI models that have significant tissue damage/loss and robust cellular infiltration. Diffuse brain injury models such as the midline fluid percussion injury (mFPI) model also cause chronic microglial activation that have M1-like characteristics but without significant contributions from infiltrating macrophage populations (Fenn et al., 2014a). Following diffuse brain injury IL-1β and TNFα are transiently increased in the cortex and hippocampus as early as 4 h post-injury, before returning to baseline levels by 72 h (Fenn et al., 2014a), and enriched microglia show increased expression of M1-like markers (IL-1β, CD14, iNOS) and M2-like markers (arginase 1) at 24 h post-injury when compared to sham-injured controls (Fenn et al., 2015). At chronic time points up to 30 days post-mFPI there is increased MHC II expression on isolated microglia, and this 'primed' phenotype results in a hyperinflammatory response to secondary immune challenges such as LPS that persist for weeks to months, and is associated with development depressive-like behavior (Fenn et al., 2014a). There are subtle changes in microglial morphology in the cortex and hippocampus following diffuse brain injury; one study described microglia with a unique rod-like morphology and train arrangement along axon tracks that were predominantly detected in the primary sensory barrel fields of mFPI rats (Ziebell et al., 2012). These cells may participate in circuit reorganization following diffuse brain injury, but whether they contribute to detrimental (M1-like) or beneficial (M2-like) responses has yet to be determined. Interestingly, rod-like microglia have neuroprotective properties in in vitro model systems (Tam and Ma, 2014).

Several other factors influence M1-/M2-like polarization in the injured brain, such as injury severity, location within gray or white matter, or aging. Models of ischemic injury demonstrate that microglia/macrophages in the ischemic core are phenotypically different from those in the penumbra. For example, M2-like markers Ym1 and CD206 are solely expressed in amoeboid-like cells in the lesion core, while CD16/32 (M1-like marker) is highly expressed in amoeboid-like cells in the ischemic core, but also in ramified cells in the penumbral regions (Hu et al., 2012; Perego et al., 2011). Interestingly, Ym1 is differentially expressed after focal TBI; it is highly expressed in amoeboid cells that lack CD68 expression in the cortical contusion zone, but it is also expressed in ramified cells in subcortical regions (Kumar et al., 2013). In addition, microglial phenotypes can vary depending on tissue type, with a more rapid transition from M2- to M1-dominant phenotypes in the white matter after TBI when compared to phenotypic switches in gray matter tissue (Wang et al., 2013b). Therefore, injury severity and location greatly influence morphological and phenotypic changes in microglia/macrophages after TBI, and future research needs to investigate the functional role of microglia in different CNS regions following a diverse range of injury severities and in different TBI models (e.g. focal versus diffuse models).

With aging, microglia develop an altered profile consistent with a heightened inflammatory or primed state (Norden et al., 2014). Primed microglia have higher baseline expression of inflammatory markers and mediators, a lower threshold to be activated and switched to a pro-inflammatory (M1-like) state, and an exaggerated inflammatory response following immune activation. Microglial priming in the aged brain can result in development of cognitive deficits, impaired synaptic plasticity and accelerated neurodegeneration (Norden et al., 2014). Several studies investigating phenotypic changes in microglia in the aged brain identified an overall increase in pro-inflammatory (M1-like) gene expression, with diminished alternative (M2-like) gene expression (Cribbs et al., 2012; Lee et al., 2013). However, a recent study using direct RNA sequencing demonstrated that primed microglia in the aged brain are M2 polarized and change the microglial phenotype towards a more neuroprotective state (Hickman et al., 2013). Interestingly, phenotypic analysis of post-mortem frontal cortex and cerebellar tissue from early and late Alzheimer's disease (AD) revealed that early AD samples were biased towards the M1 phenotype, while patients with later stage AD had an M2a bias (Wilcock, 2014). Given the lack of consensus, and the complexities involved in analyzing phenotypic and functional roles of microglia in the brain, further studies are needed to determine the effects of aging on microglia phenotypes in CNS injury and neurodegenerative

disease. Nonetheless, when aged mice (24 month old) are subjected to CCI they have an exaggerated neuroinflammatory response in the hippocampus and cortex when compared with young TBI mice (3 month old), that is associated with increased neurodegeneration and worse outcomes (Kumar et al., 2013; Sandhir et al., 2008). Microglia from aged mice migrate more slowly to sites of laser-induced focal injury, and remain at the lesion site for more extended periods than those of microglia in young mice (Damani et al., 2011). Aged microglia also have less phagocytic capacity and clear less myelin after toxin-induced demyelination injury than young microglia (Zhao et al., 2006). Phenotypically, aged TBI mice have a significantly increased M1-like gene expression profile (IL-1β, TNF, iNOS) when compared to young TBI mice, and the M2-like gene expression profile in aged TBI mice appears to be dysregulated, with several M2-like genes significantly upregulated (arginase 1, Ym1), and others downregulated (Fizz-1, TGFβ, IL-4Rα), when compared to young TBI mice (Kumar et al., 2013). The blunted IL-4Rα and TGFβ expression in aged TBI mice suggests that aged microglia have an impaired M2-like regulatory phenotype, and are less sensitive to feedback from anti-inflammatory pathways. This data is supported by other studies in which aged microglia fail to upregulate IL-4Rα after LPS injection or following isolated SCI (Fenn et al., 2014b, 2012), resulting in reduced sensitivity to IL-4 dependent programming towards an M2a-like or pro-repair microglial phenotype in aged mice.

6. Phenotype-specific roles of microglia and macrophages in CNS repair and regeneration

A significant body of in vitro and in vivo research now supports a role for M2-polarized microglia and macrophages in promoting phenotype-specific CNS repair and regeneration. Therefore, a greater understanding of the molecular mechanisms that regulate the functional responses of microglia/macrophages after TBI may guide the development of future therapeutic interventions that target phenotypic switching to improve functional recovery after TBI. In the following sections we review how phenotypic changes in microglial/macrophage activation can impact multiple steps in CNS repair and regeneration.

6.1. Neurogenesis

In the adult mammalian brain neurogenesis occurs predominantly in the subgranular zone (SGZ) of the dentate gyrus in the hippocampal formation and in the forebrain subventricular zone (SVZ) of the lateral ventricle (Gage, 2000). Ramified microglia are an essential component of the neurogenic niche in the SGZ of the adult hippocampus, and direct the migration and differentiation of neural stem/progenitor cells (NSC) by secreting soluble factors that promote neurogenesis (Walton et al., 2006). Brain injury induces endogenous adult neurogenesis in the SVZ and SGZ, as well as in non-neurogenic regions such as the striatum and cerebral cortex adjacent to the injury (Arvidsson et al., 2002; Bengzon et al., 1997). However, only a fraction of new neurons will survive long-term, and this is largely due to the pathological environment encountering the newborn neurons.

Neuroinflammation is a key component of the pathological environment, and depending on cell types involved or activation status, post-traumatic neuroinflammation can be detrimental for or supportive of adult neurogenesis (Ekdahl et al., 2009). For example, LPS-induced inflammation, which drives a pronounced M1-like activation profile in microglia, strongly impairs basal hippocampal neurogenesis levels in adult rats (Ekdahl et al., 2003; Monje et al., 2003); an effect that can be restored when anti-inflammatory drugs, such as indomethacin or minocycline, are administered to LPS-treated rats. Further, LPS-stimulated microglia or their conditioned medium, reduces NSC survival and prevents differentiation in vitro (Cacci et al., 2008). Numerous additional studies have demonstrated that several pro-inflammatory cytokines produced by M1-like microglia and macrophages, such as IFNγ, IL-1β, TNFα, and IL-6, can also suppress neurogenesis (Ben-Hur et al., 2003; Cacci et al., 2005; Iosif et al., 2008; Koo and Duman, 2008; Monje et al., 2003; Vallieres et al., 2002). In contrast, IL-4 stimulation of microglia, which polarizes microglia towards an M2-like phenotype, promotes neurogenesis of NSCs in co-culture systems (Butovsky et al., 2006b), by increasing levels of insulin-like growth factor-1 (IGF-1), a cytokine that has been correlated with increased hippocampal proliferation and neurogenesis (Aberg et al., 2003; Choi et al., 2008; O'Kusky et al., 2000; Trejo et al., 2001). Further support for a beneficial role of microglial in adult neurogenesis is provided by in vitro studies of NSCs co-cultured with microglia, or grown in conditioned media from microglia containing neurotrophic factors such as basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Aarum et al., 2003; Morgan et al., 2004; Walton et al., 2006). For example, microglia and microglia-conditioned medium rescued the in vitro formation of neuroblasts from SVZ NSCs, which otherwise would have been lost with continued culture (Aarum et al., 2003; Walton et al., 2006). Therefore these, and other protective factors released by microglia (e.g. protease serine 2; Nikolakopoulou et al., 2013), can increase neural precursor cell proliferation, neuroblast migration and functional integration of new born neurons into the SGZ of the adult hippocampus (Ekdahl et al., 2009).

TBI increases the rates of NSC proliferation in focal and diffuse injury models without increasing neurogenesis in the adult hippocampus (Bye et al., 2011; Gao and Chen, 2013). In addition, TBI promotes cell proliferation of glial cells (astrocytes and microglia) that are robustly activated at chronic time points (Acosta et al., 2013; Bye et al., 2011; Gao and Chen, 2013). These studies suggest that TBI activates through promotion of NSC proliferation an innate repair response and/or plasticity mechanism in the brain. However, long-term upregulation of chronic pro-inflammatory pathways may suppress functional integration of newborn neurons into niche zones, such that additional support/intervention is required to increase endogenous neurogenesis to successfully repair the damaged brain after TBI. Indeed, various treatments that increase endogenous neurogenesis lead to improved post-TBI recovery in adult rodents (Han et al., 2011; Zhang et al., 2012). Further, it would appear that IGF-1 plays an important role in neurogenesis after TBI as conditional IGF-1 overexpression in regions of neuronal damage results in a marked increase in immature neuron densities in the SGZ at 10 days after CCI (Carlson et al., 2014), and this results in increased cognitive function recovery after TBI (Madathil et al., 2013). Interestingly, when CCI mice undergo a delayed voluntary exercise program using in-cage running wheels, chronic M1-like microglial activation is robustly reduced after TBI, and IL-10, IGF-1, CREB and BDNF levels are significantly increased in the hippocampus, which results in enhanced neurogenesis in the SGZ and improved cognitive function recovery at chronic time points (Piao et al., 2013).

6.2. Angiogenesis

The adult CNS vasculature is extremely stable under physiological conditions, but is activated upon injury (Greenberg and Jin, 2005). Adult vascular remodeling includes angiogenesis by mature endothelial cells and vasculogenesis by endothelial progenitor cells (EPCs). EPCs are present in the bone marrow and peripheral blood, and mobilize in the blood and accumulate in the brain after TBI (Guo et al., 2009). Angiogenesis may play a significant role in mediating functional recovery following TBI (Xiong et al., 2010), and several pharmacological agents that increase angiogenesis have been shown to improve functional outcome following experimental TBI (Wu et al., 2008; Zhang et al., 2009).

In the injured brain angiogenic remodeling occurs in the ischemic penumbra within days of ischemic insult (Chen et al., 1994; Wei et al., 2001), and angiogenic vessels are often surrounded by microglia and macrophages (Jander et al., 1998; Manoonkitiwongsa et al., 2001), suggesting that activated microglia/macrophages may be instrumental in promoting the angiogenic response to cerebral ischemia. Although the phenotype-specific role of microglia in post-traumatic angiogenesis

and vascular repair is currently unknown, M2-polarized macrophages play a critical role in vascular repair and wound healing (Jetten et al., 2014; Medina et al., 2011; Willenborg et al., 2012; Zajac et al., 2013). M2 macrophages promote angiogenesis by producing proangiogenic cytokines and growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2) (Jetten et al., 2014). Both factors have dose dependent proangiogenic effects and have been shown to act synergistically in vitro (Xue and Greisler, 2002), and they induce proliferation and migration of endothelial cells and formation of vascular sprouts (Pakala et al., 2002; Polverini et al., 1977; Sunderkotter et al., 1991). In addition, a recent study unveiled a molecular mechanism for high angiogenic capacity whereby M2-like macrophages release matrix metalloproteinase 9 propeptide (proMMP9) via downregulation of tissue inhibitor of metalloproteinases 1 (TIMP-1) to induce angiogenesis and vasculature repair in complex pathological microenvironments (Zajac et al., 2013). Importantly, overexpression of proangiogenic factors (VEGF and FGF) after experimental TBI is neuroprotective, and increases post-traumatic angiogenesis and neurogenesis resulting in improved functional recovery (Siddiq et al., 2012; Thau-Zuchman et al., 2010, 2012a, 2012b).

The activation state of microglia has been shown to regulate brain endothelial cell proliferation, an early event in cerebral angiogenesis, by altering the balance of the M1-like cytokine TNFα and the M2-like cytokine TGFβ (Welser et al., 2010). In addition, treatment of brain endothelial cells with conditioned medium collected from metformin-polarized M2 microglia, promotes angiogenesis in vitro (Jin et al., 2014). Furthermore, chronic metformin treatment of mice subjected to middle cerebral artery occlusion (MCAO) enhances M2-polarization of microglia/macrophages, increases angiogenesis and neurogenesis, and results in improved functional recovery after ischemic injury (Jin et al., 2014). Taken together, the in vitro and in vivo data support a potential role of M2-polarized microglia and macrophages in post-traumatic angiogenesis and vascular repair following TBI.

6.3. Oligodendrogenesis and remyelination

Recent studies indicate that M2-polarized microglia and macrophages are essential for efficient remyelination after CNS injury. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, there is a switch from an M1- to an M2-dominant phenotype during the remyelination phase of disease, whereas the relapsing phase is characterized by a dominant M1 response and suppression of immunomodulatory M2-polarized cells at the lesion site (Miron et al., 2013). Oligodendrocyte differentiation is also modulated by phenotype, because conditioned media from M2-polarized microglia promote differentiation of precursors into mature oligodendrocytes in vitro, and oligodendrocyte differentiation is significantly impaired when M2-polarized cells are depleted in EAE mice (Miron et al., 2013). These data suggested that M2-polarized microglia/macrophages support CNS remyelination by driving oligodendrocyte differentiation. Additional studies support these findings and show that microglia/macrophage phenotypes influence oligodendrocyte survival and fate. Using an in vitro model of hypoxia and ischemia, Wang and colleagues demonstrated that conditioned media from M1-polarized microglia exacerbated oxygen glucose deprivation (OGD)-induced oligodendrocyte death (Wang et al., 2013b, 2015), whereas M2-conditioned media protected oligodendrocyte viability after OGD (Wang et al., 2015). Inflammation also impacts oligodendrogenesis in the adult CNS, such that M1-polarized microglia block oligodendrogenesis via a TNFα-dependent mechanism (Butovsky et al., 2006b), whereas M2-polarized microglia overcome this blockage, and promote oligodendrocyte formation via mechanisms that include the release of IGF-I by microglia (Butovsky et al., 2006a). Therefore, the latest research suggests that microglia and macrophage polarization plays an important role in oligodendrocyte fate and remyelination after CNS injury.

7. Therapeutic strategies to alter M1-/M2-like balance and improve outcomes after TBI

As mentioned earlier the lesion microenvironment plays a critical role in determining the polarization state of microglia and macrophages after CNS injury, with extracellular signals from within the lesion biochemical milieu driving phenotypic shifts and functional cellular responses. For example, when conditioned medium from ischemic neurons is added to cultured microglia, the microglia adopt an M1-like phenotype with increased production of pro-inflammatory mediators (TNFα, nitric oxide) that inhibit microglial phagocytic activity (Hu et al., 2012), indicating that injured neurons release soluble factors that drive an M2 to M1 phenotypic shift. Several soluble factors found in the lesion microenvironment, such as TNFα, IFNγ and lipocalin-2, can drive this M2 to M1 transition (Jang et al., 2013; Kroner et al., 2014). T cell subsets can also infiltrate the lesion and release T-cell-derived factors that can drive microglial/macrophage phenotypic changes after CNS injury (Hooten et al., 2015; Walsh et al., 2014). Furthermore, a complex network of signaling pathways, transcription factors, epigenetic mechanisms, and post-transcriptional regulators control microglial/macrophage polarization. These include IRF/STAT/SOCS signaling, NFκB activation, nuclear receptors (PPARγ, PPARδ, RXR), redox signaling (Nrf2, NOX2, HIF-1α), histone demethylases (Jmdj3), and microRNAs (miR; miR-155/miR-124), among others (for review see: Sica and Mantovani, 2012). Therefore, both extracellular signals from the lesion microenvironment and intracellular molecular mechanisms control phenotypic switching of microglia and macrophages. From a therapeutic perspective targeting factors within the lesion microenvironment and/or intracellular signaling pathways may dynamically alter phenotypic and functional responses of microglia/macrophages after TBI, and offer novel therapeutic approaches.

Cell-based therapies are being developed for TBI, and given that M2-like microglia and macrophages are anti-inflammatory and can promote regeneration and repair, transplant of M2-polarized cells to counteract the M2 to M1 transition after TBI might be an appealing strategy to reduced neurodegeneration and promote long-term functional recovery. This approach has had mixed results in CNS injury models. In one study adoptive transfer of M2 macrophages into adult rats after SCI resulted in a shift in inflammatory profile from M1-dominated to M2-like through the production of anti-inflammatory cytokines (IL-10, TGFβ), which in turn created a local microenvironment that was conducive to the rescue of residual myelin and neurons, and preservation of locomotor function (Ma et al., 2015). However, despite showing promise in in vitro models, adoptive transfer of M2 macrophages into ischemic rat brain at 3 days post-MCAO failed to improve functional or histological outcomes at 2 weeks post-injury (Desestret et al., 2013). To date, adoptive transfer of M2 macrophages in TBI models has not been evaluated, however, when human bone marrow mesenchymal stem cells (MSC) are infused into TBI mice they promote robust M2-like polarization of microglia and alter the lesion microenvironment, resulting in M2-mediated repair responses and long-term neurological recovery (Zanier et al., 2014). These effects may be mediated in part by STAT3/NFκB signaling because MSC induce M2 polarization of macrophage via activation of STAT3 and inhibition of NFκB pathways (Gao et al., 2014). Despite encouraging findings in some experimental models it is unclear how plastic M2-polarized cells will respond when transplanted into a reactive injury environment, which may contain a vast array of extracellular signals that could reverse or reduce the M2-properties required for immunosuppression, resolution and repair. Further investigation is needed to determine the true potential of adoptive transfer strategies to promote M2-mediated neuroprotection and repair for TBI.

Several classes of drugs have been demonstrated to alter M1-/M2-like balance of microglia and macrophages, and may be useful as therapeutic agents for TBI. The nuclear hormone receptor peroxisome proliferator-activated receptors (PPAR) act as master regulators of the M2-like phenotype in macrophages (Chawla, 2010), and PPARγ activation primes

macrophages into an M2-like state with broad anti-inflammatory and tissue repair properties (Bouhlel et al., 2007). PPARγ activation produces similar effects in microglia (Storer et al., 2005), and PPAR activation of microglia promotes increased phagocytic uptake of amyloid-β plaques and is neuroprotective in mouse models of AD (Mandrekar-Colucci et al., 2012; Yamanaka et al., 2012). Considering that TBI produces large amounts of myelin and cell debris, PPAR-induced M2-like polarization of microglia/macrophages may be beneficial by promoting removal of debris. PPAR agonists are neuroprotective in TBI models, and the PPARα receptor agonist, fenofibrate, reduced post-traumatic neuroinflammation, oxidative stress and cerebral edema, and improved neurological function when administered after TBI (Besson et al., 2005; Chen et al., 2007). Similarly, the PPARγ receptor agonists, pioglitazone and rosiglitazone, reduced post-traumatic microglial activation and promoted anti-oxidant (Mn-SOD) and neuroprotective chaperone protein (HSP27/70) expression that resulted in reduced neurodegeneration and improved functional recovery after TBI (Sauerbeck et al., 2011; Thal et al., 2011; Yi et al., 2008). Rosiglitazone also induces IL-4 expression in the brain, and reduces age-dependent M1-like microglial activation in an IL-4 dependent manner (Loane et al., 2009a). As such, the anti-inflammatory and neuroprotective properties of PPARγ agonists in TBI models may be mediated in part via M2-like polarization of microglia/macrophages.

Selective activation of metabotropic glutamate receptor 5 (mGluR5) on microglia is a novel mechanism to attenuate M1-like microglial activation and associated microglial-mediated neurotoxicity (Byrnes et al., 2009a; Loane et al., 2009b). Activation of mGluR5 has powerful neuroprotective effects in experimental models of CNS injury (Bao et al., 2001; Byrnes et al., 2012, 2009b; Chen et al., 2012; Loane et al., 2013; Wang et al., 2013c), and the mGluR5 orthosteric agonist, CHPG, attenuates chronic M1-like microglial activation following TBI via inhibition of microglial NOX2 (Byrnes et al., 2012; Loane et al., 2013, 2009b). Interestingly, NOX2 has been proposed to act as a molecular switch between M1-like and M2-like microglial activation states (Choi et al., 2012), as it serves to promote the former and suppress the latter. Recently it has been demonstrated that a positive allosteric modulator for mGluR5, VU0360172, repolarizes cultured microglia towards an M2-like phenotype following LPS or IFNγ stimulation (Loane et al., 2014b). Furthermore, in addition to attenuating chronic NOX2 expression in activated microglia, VU0360172 alters the M1-/M2-like balance following TBI. VU0360172 treatment results in decreased iNOS expression (M1-like marker) and increased expression of arginase 1 (M2-like marker) after TBI, and this predominant M2-like microglial activation profile is associated with reduced neurodegeneration and improved long-term motor function recovery in VU0360172-treated TBI mice (Loane et al., 2014b). VU0360172 is also neuroprotective in an experimental subarachnoid hemorrhage model in rats via attenuation of microglial activation and the upregulation of the Bcl-2 anti-apoptotic pathway (Zhang et al., 2015).

Macrophage phenotypic transitions can be regulated by epigenetic mechanisms (Ivashkiv, 2013). For example, IL-4 induces the upregulation of the histone demethylase Jmjd3 in macrophages, which alters chromatin modifications to promote M2 gene expression and inhibition of M1 genes (Ishii et al., 2009; Satoh et al., 2010). Similar mechanisms control microglia polarization; Jmjd3 enhances the polarization of M2-like microglia by modifying histone H3K27me3, and suppression of Jmjd3 in the MPTP model of Parkinson's disease results in microglial overactivation and exacerbated dopaminergic neuron cell death in the substantia nigra (Tang et al., 2014). Histone deactylases (HDAC) remove acetyl groups from ε-N-acetyl lysine amino acids on a histone to allow DNA to be wrapped more tightly around histones. This blocks gene transcription and opposes histone acetyltransferase activity that promotes transcription. Several HDAC inhibitors provide neuroprotection against TBI (Dash et al., 2010; Lu et al., 2013; Shein et al., 2009; Wang et al., 2013a; Zhang et al., 2008). Scriptaid, a novel inhibitor of class I/II HDACs, offers dose-dependent neuroprotection that results in long-lasting functional recovery following focal TBI, even when administered up to 12 h post-injury (Wang et al., 2013a). Moreover, it has been shown that Scriptaid prevents white matter injury by polarizing microglial/macrophage towards the M2-like phenotype (Wang et al., 2015). Specifically, HDAC inhibition promotes an M1- to M2-like phenotypic shift by upregulating glycogen synthase kinase 3 beta (GSK3β), which inactivates phosphatase and tensin homolog (PTEN) through phosphorylation to initiate PI3K/Akt signaling. (Wang et al., 2015). The GSK3β-dependent M2-like phenotype exerts potent anti-inflammatory effects that protect myelin-forming oligodendrocytes resulting in diminished white matter injury. Thus, inhibition of HDACs in microglia alters M1-/M2-like balance via the GSK3β/PTEN/Akt axis, and offers a novel therapeutic target to promote tissue repair and functional recovery after TBI.

Several other drugs have been used effectively to alter M1-/M2-like polarization of microglia and macrophages following acute brain injury. These include drugs that modulate endogenous endocannabinoid system (Tchantchou et al., 2014; Tchantchou and Zhang, 2013), CB2 inverse agonists (Reiner et al., 2015), α-7 nicotinic acetylcholine receptor agonists (Han et al., 2014a, 2014b), endogenous antagonists of bone morphogenetic proteins (Shin et al., 2014), and CCR2 antagonists (Morganti et al., 2015), among others. Therefore, given the role for M2-like polarized microglia/macrophages in promoting phenotype-specific brain injury repair, drug treatment strategies that alter phenotypic and functional responses of microglia and/or macrophages after TBI offer considerable promise as new treatment options for TBI.

8. Considerations for future development of therapeutic strategies that alter microglial/macrophage phenotypes

Historically, broad-spectrum anti-inflammatory therapies for TBI have translated poorly to the clinic for human head injury (Kumar and Loane, 2012; Maas et al., 2010), which may in part reflect loss of beneficial M2-like effects in microglia. It is likely that efficient tissue repair after TBI requires both M1-like and M2-like functional responses, including coordinated transitions between phenotypes that fine-tune the sequential inflammatory, proliferative and remodeling phases of active repair (Gurtner et al., 2008; Novak and Koh, 2013). Therefore caution is advised against initiating a poorly timed M1- to M2-like phenotypic shift because the M1-like response plays a critical role in early repair processes, and additional beneficial roles for M1-like microglia following TBI may still be discovered. Now that microglia are known to play an active role in sculpting neuronal circuits and synapse remodeling (Salter and Beggs, 2014), it is not inconceivable that M1-like microglia may also drive the clearance of cellular debris from damaged synapses early after brain injury. Therefore, when developing therapeutic strategies to modulate M1-/M2-like phenotypes, timing the transitions between polarization states to facilitate efficient tissue repair needs to be carefully considered, as may tapering M2-like augmenting strategies to avoid chronic immunosuppressive effects and maladaptive repair processes. A long-term repair phase after a rapid pro-inflammatory response that is driven by M2-like macrophages has been demonstrated to result in fibrosis and other aberrant repair (Hesse et al., 2001), because elevated arginase activity shifts the metabolism of L-arginine significantly to produce increased ornithine and proline that simulate cell division leading to hyperplasia and fibrosis. In addition, a long-term increase in arginase activity leads to an uncoupled decrease in NO production, which has been shown to precipitate endothelial dysfunction (Horowitz et al., 2007). Thus, it is critical to boost, and diminish, the correct microglial and/or macrophage phenotypes at the right time in order to drive endogenous repair pathways following acute brain injury and to avoid maladaptive responses. Finally, a note of caution is needed with regard to the translation of M1-/M2-like polarization states developed in rodents to humans because several commonly used markers of the M2-like phenotype, such as arginase 1 and Ym1, are not expressed in human myeloid cells (Raes et al., 2005). However, others such as CD206 and CD163, appear to be consistent (Vogel et al., 2013). Therefore, if markers for M2-like polarization are carefully selected then it may be possible to identify M2-like microglia

and macrophages in human brain injury and translate promising therapeutic strategies to the clinic.

9. Conclusion

The latest research implicates M1/M2-like polarization of microglia and/or macrophages in acute brain injury, and activated microglia/macrophages with distinct molecular phenotypes can either promote neurorestorative processes, or hinder CNS repair and expand tissue damage leading to chronic neurodegeneration after TBI. In the context of CNS remodeling, the M2-like phenotype can support enhanced functional recovery after TBI via the induction of key neurorestorative processes, such as neurogenesis, angiogenesis, oligodendrogenesis and remyelination. Future therapeutic approaches that target post-traumatic neuroinflammation should avoid broad suppression of microglial activation, and instead should alter the balance between different phenotypes to promote maximal CNS remodeling and repair. This concept is supported by recent experimental studies that modulated M1/M2-like polarization to protect against post-traumatic neurodegeneration and enhance CNS repair. Therefore, future research is needed to determine key regulatory mechanisms that control phenotype switching in microglia in order to boost their "good" and suppress their "bad" activation states, and promote maximal functional recovery following TBI.

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References

Aarum, J., Sandberg, K., Haeberlein, S.L., Persson, M.A., 2003. Migration and differentiation of neural precursor cells can be directed by microglia. Proc. Natl. Acad. Sci. U. S. A. 100, 15983–15988.

Aberg, M.A., Aberg, N.D., Palmer, T.D., Alborn, A.M., Carlsson-Skwirut, C., Bang, P., Rosengren, L.E., Olsson, T., Gage, F.H., Eriksson, P.S., 2003. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. Mol. Cell. Neurosci. 24, 23–40.

Acosta, S.A., Tajiri, N., Shinozuka, K., Ishikawa, H., Grimmig, B., Diamond, D., Sanberg, P.R., Bickford, P.C., Kaneko, Y., Borlongan, C.V., 2013. Long-term upregulation of inflammation and suppression of cell proliferation in the brain of adult rats exposed to traumatic brain injury using the controlled cortical impact model. PLoS One 8, e53376.

Ajami, B., Bennett, J.L., Krieger, C., Tetzlaff, W., Rossi, F.M., 2007. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat. Neurosci. 10, 1538–1543.

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., Lindvall, O., 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat. Med. 8, 963–970.

Auffray, C., Fogg, D., Garfa, M., Elain, G., Join-Lambert, O., Kayal, S., Sarnacki, S., Cumano, A., Lauvau, G., Geissmann, F., 2007. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science 317, 666–670.

Aungst, S.L., Kabadi, S.V., Thompson, S.M., Stoica, B.A., Faden, A.I., 2014. Repeated mild traumatic brain injury causes chronic neuroinflammation, changes in hippocampal synaptic plasticity, and associated cognitive deficits. J. Cereb. Blood Flow Metab. 34, 1223–1232.

Bao, W.L., Williams, A.J., Faden, A.I., Tortella, F.C., 2001. Selective mGluR5 receptor antagonist or agonist provides neuroprotection in a rat model of focal cerebral ischemia. Brain Res. 922, 173–179.

Ben-Hur, T., Ben-Menachem, O., Furer, V., Einstein, O., Mizrachi-Kol, R., Grigoriadis, N., 2003. Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol. Cell. Neurosci. 24, 623–631.

Bengzon, J., Kokaia, Z., Elmer, E., Nanobashvili, A., Kokaia, M., Lindvall, O., 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc. Natl. Acad. Sci. U. S. A. 94, 10432–10437.

Besson, V.C., Chen, X.R., Plotkine, M., Marchand-Verrecchia, C., 2005. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, exerts neuroprotective effects in traumatic brain injury. Neurosci. Lett. 388, 7–12.

Bouhlel, M.A., Derudas, B., Rigamonti, E., Dievart, R., Brozek, J., Haulon, S., Zawadzki, C., Jude, B., Torpier, G., Marx, N., Staels, B., Chinetti-Gbaguidi, G., 2007. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab. 6, 137–143.

Butovsky, O., Jedrychowski, M.P., Moore, C.S., Cialic, R., Lanser, A.J., Gabriely, G., Koeglsperger, T., Dake, B., Wu, P.M., Doykan, C.E., Fanek, Z., Liu, L., Chen, Z., Rothstein, J.D., Ransohoff, R.M., Gygi, S.P., Antel, J.P., Weiner, H.L., 2014. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat. Neurosci. 17, 131–143.

Butovsky, O., Landa, G., Kunis, G., Ziv, Y., Avidan, H., Greenberg, N., Schwartz, A., Smirnov, I., Pollack, A., Jung, S., Schwartz, M., 2006a. Induction and blockage of oligodendrogenesis by differently activated microglia in an animal model of multiple sclerosis. J. Clin. Invest. 116, 905–915.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A.E., Pluchino, S., Martino, G., Schwartz, M., 2006b. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol. Cell. Neurosci. 31, 149–160.

Bye, N., Carron, S., Han, X., Agyapomaa, D., Ng, S.Y., Yan, E., Rosenfeld, J.V., Morganti-Kossmann, M.C., 2011. Neurogenesis and glial proliferation are stimulated following diffuse traumatic brain injury in adult rats. J. Neurosci. Res. 89, 986–1000.

Byrnes, K.R., Loane, D.J., Stoica, B.A., Zhang, J., Faden, A.I., 2012. Delayed mGluR5 activation limits neuroinflammation and neurodegeneration after traumatic brain injury. J. Neuroinflammation 9, 43.

Byrnes, K.R., Stoica, B., Loane, D.J., Riccio, A., Davis, M.I., Faden, A.I., 2009a. Metabotropic glutamate receptor 5 activation inhibits microglial associated inflammation and neurotoxicity. Glia 57, 550–560.

Byrnes, K.R., Stoica, B., Riccio, A., Pajoohesh-Ganji, A., Loane, D.J., Faden, A.I., 2009b. Activation of metabotropic glutamate receptor 5 improves recovery after spinal cord injury in rodents. Ann. Neurol. 66, 63–74.

Cacci, E., Ajmone-Cat, M.A., Anelli, T., Biagioni, S., Minghetti, L., 2008. In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. Glia 56, 412–425.

Cacci, E., Claasen, J.H., Kokaia, Z., 2005. Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. J. Neurosci. Res. 80, 789–797.

Carlson, S.W., Madathil, S.K., Sama, D.M., Gao, X., Chen, J., Saatman, K.E., 2014. Conditional overexpression of insulin-like growth factor-1 enhances hippocampal neurogenesis and restores immature neuron dendritic processes after traumatic brain injury. J. Neuropathol. Exp. Neurol. 73, 734–746.

Chawla, A., 2010. Control of macrophage activation and function by PPARs. Circ. Res. 106, 1559–1569.

Chen, H.H., Chien, C.H., Liu, H.M., 1994. Correlation between angiogenesis and basic fibroblast growth factor expression in experimental brain infarct. Stroke 25, 1651–1657.

Chen, T., Zhang, L., Qu, Y., Huo, K., Jiang, X., Fei, Z., 2012. The selective mGluR5 agonist CHPG protects against traumatic brain injury in vitro and in vivo via ERK and Akt pathway. Int. J. Mol. Med. 29, 630–636.

Chen, X.R., Besson, V.C., Palmier, B., Garcia, Y., Plotkine, M., Marchand-Leroux, C., 2007. Neurological recovery-promoting, anti-inflammatory, and anti-oxidative effects afforded by fenofibrate, a PPAR alpha agonist, in traumatic brain injury. J. Neurotrauma 24, 1119–1131.

Choi, S.H., Aid, S., Kim, H.W., Jackson, S.H., Bosetti, F., 2012. Inhibition of NADPH oxidase promotes alternative and anti-inflammatory microglial activation during neuroinflammation. J. Neurochem. 120, 292–301.

Choi, Y.S., Cho, H.Y., Hoyt, K.R., Naegele, J.R., Obrietan, K., 2008. IGF-1 receptor-mediated ERK/MAPK signaling couples status epilepticus to progenitor cell proliferation in the subgranular layer of the dentate gyrus. Glia 56, 791–800.

Clark, R.S., Schiding, J.K., Kaczorowski, S.L., Marion, D.W., Kochanek, P.M., 1994. Neutrophil accumulation after traumatic brain injury in rats: comparison of weight drop and controlled cortical impact models. J. Neurotrauma 11, 499–506.

Colton, C.A., 2009. Heterogeneity of microglial activation in the innate immune response in the brain. J. NeuroImmune Pharmacol. 4, 399–418.

Cribbs, D.H., Berchtold, N.C., Perreau, V., Coleman, P.D., Rogers, J., Tenner, A.J., Cotman, C.W., 2012. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. J. Neuroinflammation 9, 179.

Dalgard, C.L., Cole, J.T., Kean, W.S., Lucky, J.J., Sukumar, G., McMullen, D.C., Pollard, H.B., Watson, W.D., 2012. The cytokine temporal profile in rat cortex after controlled cortical impact. Front. Mol. Neurosci. 5, 6.

Damani, M.R., Zhao, L., Fontainhas, A.M., Amaral, J., Fariss, R.N., Wong, W.T., 2011. Age-related alterations in the dynamic behavior of microglia. Aging Cell 10, 263–276.

Dash, P.K., Orsi, S.A., Zhang, M., Grill, R.J., Pati, S., Zhao, J., Moore, A.N., 2010. Valproate administered after traumatic brain injury provides neuroprotection and improves cognitive function in rats. PLoS One 5, e11383.

Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8, 752–758.

David, S., Kroner, A., 2011. Repertoire of microglial and macrophage responses after spinal cord injury. Nat. Rev. 12, 388–399.

Deonarine, K., Panelli, M.C., Stashower, M.E., Jin, P., Smith, K., Slade, H.B., Norwood, C., Wang, E., Marincola, F.M., Stroncek, D.F., 2007. Gene expression profiling of cutaneous wound healing. J. Transl. Med. 5, 11.

Desestret, V., Riou, A., Chauveau, F., Cho, T.H., Devillard, E., Marinescu, M., Ferrera, R., Rey, C., Chanal, M., Angoulvant, D., Honnorat, J., Nighoghossian, N., Berthezene, Y., Nataf, S., Wiart, M., 2013. In vitro and in vivo models of cerebral ischemia show discrepancy in therapeutic effects of M2 macrophages. PLoS One 8, e67063.

Eikelenboom, P., van Exel, E., Hoozemans, J.J., Veerhuis, R., Rozemuller, A.J., van Gool, W.A., 2010. Neuroinflammation – an early event in both the history and pathogenesis of Alzheimer's disease. Neurodegener. Dis. 7, 38–41.

Ekdahl, C.T., Claasen, J.H., Bonde, S., Kokaia, Z., Lindvall, O., 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc. Natl. Acad. Sci. U. S. A. 100, 13632–13637.

Ekdahl, C.T., Kokaia, Z., Lindvall, O., 2009. Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158, 1021–1029.

Elmore, M.R., Najafi, A.R., Koike, M.A., Dagher, N.N., Spangenberg, E.E., Rice, R.A., Kitazawa, M., Matusow, B., Nguyen, H., West, B.L., Green, K.N., 2014. Colony-stimulating factor 1

receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82, 380–397.
Erblich, B., Zhu, L., Etgen, A.M., Dobrenis, K., Pollard, J.W., 2011. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One 6, e26317.
Faden, A.I., Loane, D.J., 2015. Chronic neurodegeneration after traumatic brain injury: Alzheimer disease, chronic traumatic encephalopathy, or persistent neuroinflammation? Neurotherapeutics 12, 143–150.
Fenn, A.M., Gensel, J.C., Huang, Y., Popovich, P.G., Lifshitz, J., Godbout, J.P., 2014a. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biol. Psychiatry 76, 575–584.
Fenn, A.M., Hall, J.C., Gensel, J.C., Popovich, P.G., Godbout, J.P., 2014b. IL-4 signaling drives a unique arginase+/IL-1beta+ microglia phenotype and recruits macrophages to the inflammatory CNS: consequences of age-related deficits in IL-4Ralpha after traumatic spinal cord injury. J. Neurosci. 34, 8904–8917.
Fenn, A.M., Henry, C.J., Huang, Y., Dugan, A., Godbout, J.P., 2012. Lipopolysaccharide-induced interleukin (IL)-4 receptor-alpha expression and corresponding sensitivity to the M2 promoting effects of IL-4 are impaired in microglia of aged mice. Brain Behav. Immun. 26, 766–777.
Fenn, A.M., Skendelas, J.P., Moussa, D.N., Muccigrosso, M.M., Popovich, P.G., Lifshitz, J., Eiferman, D.S., Godbout, J.P., 2015. Methylene blue attenuates traumatic brain injury-associated neuroinflammation and acute depressive-like behavior in mice. J. Neurotrauma 32, 127–138.
Filardy, A.A., Pires, D.R., Nunes, M.P., Takiya, C.M., Freire-de-Lima, C.G., Ribeiro-Gomes, F.L., DosReis, G.A., 2010. Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)IL-10(high) regulatory phenotype in macrophages. J. Immunol. 185, 2044–2050.
Gage, F.H., 2000. Mammalian neural stem cells. Science 287, 1433–1438.
Galtrey, C.M., Fawcett, J.W., 2007. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res. Rev. 54, 1–18.
Gao, H.M., Liu, B., Hong, J.S., 2003. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J. Neurosci. 23, 6181–6187.
Gao, S., Mao, F., Zhang, B., Zhang, L., Zhang, X., Wang, M., Yan, Y., Yang, T., Zhang, J., Zhu, W., Qian, H., Xu, W., 2014. Mouse bone marrow-derived mesenchymal stem cells induce macrophage M2 polarization through the nuclear factor-kappaB and signal transducer and activator of transcription 3 pathways. Exp. Biol. Med. 239, 366–375.
Gao, X., Chen, J., 2013. Moderate traumatic brain injury promotes neural precursor proliferation without increasing neurogenesis in the adult hippocampus. Exp. Neurol. 239, 38–48.
Gensel, J.C., Zhang, B., 2015. Macrophage Activation and its Role in Repair and Pathology After Spinal Cord Injury. Brain Res.
Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M.F., Conway, S.J., Ng, L.G., Stanley, E.R., Samokhvalov, I.M., Merad, M., 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330, 841–845.
Gordon, S., 2003. Alternative activation of macrophages. Nat. Rev. Immunol. 3, 23–35.
Greenberg, D.A., Jin, K., 2005. From angiogenesis to neuropathology. Nature 438, 954–959.
Greter, M., Lelios, I., Pelczar, P., Hoeffel, G., Price, J., Leboeuf, M., Kundig, T.M., Frei, K., Ginhoux, F., Merad, M., Becher, B., 2012. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. Immunity 37, 1050–1060.
Guo, X., Liu, L., Zhang, M., Bergeron, A., Cui, Z., Dong, J.F., Zhang, J., 2009. Correlation of CD34+ cells with tissue angiogenesis after traumatic brain injury in a rat model. J. Neurotrauma 26, 1337–1344.
Gurtner, G.C., Werner, S., Barrandon, Y., Longaker, M.T., 2008. Wound repair and regeneration. Nature 453, 314–321.
Han, X., Tong, J., Zhang, J., Farahvar, A., Wang, E., Yang, J., Samadani, U., Smith, D.H., Huang, J.H., 2011. Imipramine treatment improves cognitive outcome associated with enhanced hippocampal neurogenesis after traumatic brain injury in mice. J. Neurotrauma 28, 995–1007.
Han, Z., Li, L., Wang, L., Degos, V., Maze, M., Su, H., 2014a. Alpha-7 nicotinic acetylcholine receptor agonist treatment reduces neuroinflammation, oxidative stress, and brain injury in mice with ischemic stroke and bone fracture. J. Neurochem. 131, 498–508.
Han, Z., Shen, F., He, Y., Degos, V., Camus, M., Maze, M., Young, W.L., Su, H., 2014b. Activation of alpha-7 nicotinic acetylcholine receptor reduces ischemic stroke injury through reduction of pro-inflammatory macrophages and oxidative stress. PLoS One 9, e105711.
Hanisch, U.K., Kettenmann, H., 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. 10, 1387–1394.
Harrison, J.K., Jiang, Y., Chen, S., Xia, Y., Maciejewski, D., McNamara, R.K., Streit, W.J., Salafranca, M.N., Adhikari, S., Thompson, D.A., Botti, P., Bacon, K.B., Feng, L., 1998. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc. Natl. Acad. Sci. U. S. A. 95, 10896–10901.
Hesse, M., Modolell, M., La Flamme, A.C., Schito, M., Fuentes, J.M., Cheever, A.W., Pearce, E.J., Wynn, T.A., 2001. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. J. Immunol. 167, 6533–6544.
Hickman, S.E., Kingery, N.D., Ohsumi, T.K., Borowsky, M.L., Wang, L.C., Means, T.K., El Khoury, J., 2013. The microglial sensome revealed by direct RNA sequencing. Nat. Neurosci. 16, 1896–1905.
Hoek, R.M., Ruuls, S.R., Murphy, C.A., Wright, G.J., Goddard, R., Zurawski, S.M., Blom, B., Homola, M.E., Streit, W.J., Brown, M.H., Barclay, A.N., Sedgwick, J.D., 2000. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290, 1768–1771.
Hooten, K.G., Beers, D.R., Zhao, W., Appel, S.H., 2015. Protective and Toxic Neuroinflammation in Amyotrophic Lateral Sclerosis. Neurotherapeutics.
Horowitz, S., Binion, D.G., Nelson, V.M., Kanaa, Y., Javadi, P., Lazarova, Z., Andrekopoulos, C., Kalyanaraman, B., Otterson, M.F., Rafiee, P., 2007. Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease. Am. J. Physiol. Gastrointest. Liver Physiol. 292, G1323–G1336.
Hsieh, C.L., Kim, C.C., Ryba, B.E., Niemi, E.C., Bando, J.K., Locksley, R.M., Liu, J., Nakamura, M.C., Seaman, W.E., 2013. Traumatic brain injury induces macrophage subsets in the brain. Eur. J. Immunol. 43, 2010–2022.
Hsieh, C.L., Niemi, E.C., Wang, S.H., Lee, C.C., Bingham, D., Zhang, J., Cozen, M.L., Charo, I., Huang, E.J., Liu, J., Nakamura, M.C., 2014. CCR2 Deficiency Impairs Macrophage Infiltration and Improves Cognitive Function After Traumatic Brain Injury. J. Neurotrauma.
Hu, X., Li, P., Guo, Y., Wang, H., Leak, R.K., Chen, S., Gao, Y., Chen, J., 2012. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. Stroke 43, 3063–3070.
Iosif, R.E., Ahlenius, H., Ekdahl, C.T., Darsalia, V., Thored, P., Jovinge, S., Kokaia, Z., Lindvall, O., 2008. Suppression of stroke-induced progenitor proliferation in adult subventricular zone by tumor necrosis factor receptor 1. J. Cereb. Blood Flow Metab. 28, 1574–1587.
Ishii, M., Wen, H., Corsa, C.A., Liu, T., Coelho, A.L., Allen, R.M., Carson, W.F.t., Cavassani, K.A., Li, X., Lukacs, N.W., Hogaboam, C.M., Dou, Y., Kunkel, S.L., 2009. Epigenetic regulation of the alternatively activated macrophage phenotype. Blood 114, 3244–3254.
Israelsson, C., Bengtsson, H., Kylberg, A., Kullander, K., Lewen, A., Hillered, L., Ebendal, T., 2008. Distinct cellular patterns of upregulated chemokine expression supporting a prominent inflammatory role in traumatic brain injury. J. Neurotrauma 25, 959–974.
Israelsson, C., Kylberg, A., Bengtsson, H., Hillered, L., Ebendal, T., 2014. Interacting chemokine signals regulate dendritic cells in acute brain injury. PLoS One 9, e104754.
Ivashkiv, L.B., 2013. Epigenetic regulation of macrophage polarization and function. Trends Immunol. 34, 216–223.
Jander, S., Schroeter, M., D'Urso, D., Gillen, C., Witte, O.W., Stoll, G., 1998. Focal ischaemia of the rat brain elicits an unusual inflammatory response: early appearance of CD8+ macrophages/microglia. Eur. J. Neurosci. 10, 680–688.
Jang, E., Lee, S., Kim, J.H., Kim, J.H., Seo, J.W., Lee, W.H., Mori, K., Nakao, K., Suk, K., 2013. Secreted protein lipocalin-2 promotes microglial M1 polarization. FASEB J. 27, 1176–1190.
Jetten, N., Verbruggen, S., Gijbels, M.J., Post, M.J., De Winther, M.P., Donners, M.M., 2014. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo. Angiogenesis 17, 109–118.
Jin, Q., Cheng, J., Liu, Y., Wu, J., Wang, X., Wei, S., Zhou, X., Qin, Z., Jia, J., Zhen, X., 2014. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. Brain Behav. Immun. 40, 131–142.
Jin, X., Ishii, H., Bai, Z., Itokazu, T., Yamashita, T., 2012. Temporal changes in cell marker expression and cellular infiltration in a controlled cortical impact model in adult male C57BL/6 mice. PLoS One 7, e41892.
Johnson, V.E., Stewart, J.E., Begbie, F.D., Trojanowski, J.Q., Smith, D.H., Stewart, W., 2013. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 136, 28–42.
Katsumoto, A., Lu, H., Miranda, A.S., Ransohoff, R.M., 2014. Ontogeny and functions of central nervous system macrophages. J. Immunol. 193, 2615–2621.
Kettenmann, H., Hanisch, U.K., Noda, M., Verkhratsky, A., 2011. Physiology of microglia. Physiol. Rev. 91, 461–553.
Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E.G., Wieghofer, P., Heinrich, A., Riemke, P., Holscher, C., Muller, D.N., Luckow, B., Brocker, T., Debowski, K., Fritz, G., Opdenakker, G., Diefenbach, A., Biber, K., Heikenwalder, M., Geissmann, F., Rosenbauer, F., Prinz, M., 2013. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat. Neurosci. 16, 273–280.
Kigerl, K.A., Gensel, J.C., Ankeny, D.P., Alexander, J.K., Donnelly, D.J., Popovich, P.G., 2009. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J. Neurosci. 29, 13435–13444.
Koo, J.W., Duman, R.S., 2008. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc. Natl. Acad. Sci. U. S. A. 105, 751–756.
Kroner, A., Greenhalgh, A.D., Zarruk, J.G., Passos Dos Santos, R., Gaestel, M., David, S., 2014. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuron 83, 1098–1116.
Kumar, A., Loane, D.J., 2012. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav. Immun. 26, 1191–1201.
Kumar, A., Stoica, B.A., Sabirzhanov, B., Burns, M.P., Faden, A.I., Loane, D.J., 2013. Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. Neurobiol. Aging 34, 1397–1411.
Lee, D.C., Ruiz, C.R., Lebson, L., Selenica, M.L., Rizer, J., Hunt Jr., J.B., Rojiani, R., Reid, P., Kammath, S., Nash, K., Dickey, C.A., Gordon, M., Morgan, D., 2013. Aging enhances classical activation but mitigates alternative activation in the central nervous system. Neurobiol. Aging 34, 1610–1620.
Liu, S., Zhang, L., Wu, Q., Wu, Q., Wang, T., 2013. Chemokine CCL2 induces apoptosis in cortex following traumatic brain injury. J. Mol. Neurosci. 51, 1021–1029.
Loane, D.J., Deighan, B.F., Clarke, R.M., Griffin, R.J., Lynch, A.M., Lynch, M.A., 2009a. Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain. Neurobiol. Aging 30, 920–931.
Loane, D.J., Kumar, A., Stoica, B.A., Cabatbat, R., Faden, A.I., 2014a. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. J. Neuropathol. Exp. Neurol. 73, 14–29.
Loane, D.J., Stoica, B.A., Byrnes, K.R., Jeong, W., Faden, A.I., 2013. Activation of mGluR5 and inhibition of NADPH oxidase improves functional recovery after traumatic brain injury. J. Neurotrauma 30, 403–412.
Loane, D.J., Stoica, B.A., Pajoohesh-Ganji, A., Byrnes, K.R., Faden, A.I., 2009b. Activation of metabotropic glutamate receptor 5 modulates microglial reactivity and neurotoxicity by inhibiting NADPH oxidase. J. Biol. Chem. 284, 15629–15639.

Loane, D.J., Stoica, B.A., Tchantchou, F., Kumar, A., Barrett, J.P., Akintola, T., Xue, F., Conn, P.J., Faden, A.I., 2014b. Novel mGluR5 Positive Allosteric Modulator Improves Functional Recovery, Attenuates Neurodegeneration, and Alters Microglial Polarization After Experimental Traumatic Brain Injury. Neurotherapeutics.

Lu, J., Frerich, J.M., Turtzo, L.C., Li, S., Chiang, J., Yang, C., Wang, X., Zhang, C., Wu, C., Sun, Z., Niu, G., Zhuang, Z., Brady, R.O., Chen, X., 2013. Histone deacetylase inhibitors are neuroprotective and preserve NGF-mediated cell survival following traumatic brain injury. Proc. Natl. Acad. Sci. U. S. A. 110, 10747–10752.

Ma, S.F., Chen, Y.J., Zhang, J.X., Shen, L., Wang, R., Zhou, J.S., Hu, J.G., Lu, H.Z., 2015. Adoptive transfer of M2 macrophages promotes locomotor recovery in adult rats after spinal cord injury. Brain Behav. Immun. 45, 157–170.

Maas, A.I., Roozenbeek, B., Manley, G.T., 2010. Clinical trials in traumatic brain injury: past experience and current developments. Neurotherapeutics 7, 115–126.

Madathil, S.K., Carlson, S.W., Brelsfoard, J.M., Ye, P., D'Ercole, A.J., Saatman, K.E., 2013. Astrocyte-specific overexpression of insulin-like growth factor-1 protects hippocampal neurons and reduces behavioral deficits following traumatic brain injury in mice. PLoS One 8, e67204.

Mandrekar-Colucci, S., Karlo, J.C., Landreth, G.E., 2012. Mechanisms underlying the rapid peroxisome proliferator-activated receptor-gamma-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J. Neurosci. 32, 10117–10128.

Manoonkitiwongsa, P.S., Jackson-Friedman, C., McMillan, P.J., Schultz, R.L., Lyden, P.D., 2001. Angiogenesis after stroke is correlated with increased numbers of macrophages: the clean-up hypothesis. J. Cereb. Blood Flow Metab. 21, 1223–1231.

Martinez, F.O., Gordon, S., Locati, M., Mantovani, A., 2006. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J. Immunol. 177, 7303–7311.

Medina, R.J., O'Neill, C.L., O'Doherty, T.M., Knott, H., Guduric-Fuchs, J., Gardiner, T.A., Stitt, A.W., 2011. Myeloid angiogenic cells act as alternative M2 macrophages and modulate angiogenesis through interleukin-8. Mol. Med. (Cambridge Mass.) 17, 1045–1055.

Miron, V.E., Boyd, A., Zhao, J.W., Yuen, T.J., Ruckh, J.M., Shadrach, J.L., van Wijngaarden, P., Wagers, A.J., Williams, A., Franklin, R.J., ffrench-Constant, C., 2013. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat. Neurosci. 16, 1211–1218.

Monje, M.L., Toda, H., Palmer, T.D., 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302, 1760–1765.

Morgan, S.C., Taylor, D.L., Pocock, J.M., 2004. Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. J. Neurochem. 90, 89–101.

Morganti, J.M., Jopson, T.D., Liu, S., Riparip, L.K., Guandique, C.K., Gupta, N., Ferguson, A.R., Rosi, S., 2015. CCR2 antagonism alters brain macrophage polarization and ameliorates cognitive dysfunction induced by traumatic brain injury. J. Neurosci. 35, 748–760.

Mouzon, B.C., Bachmeier, C., Ferro, A., Ojo, J.O., Crynen, G., Acker, C.M., Davies, P., Mullan, M., Stewart, W., Crawford, F., 2014. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. Ann. Neurol. 75, 241–254.

Nagamoto-Combs, K., McNeal, D.W., Morecraft, R.J., Combs, C.K., 2007. Prolonged microgliosis in the rhesus monkey central nervous system after traumatic brain injury. J. Neurotrauma 24, 1719–1742.

Nikolakopoulou, A.M., Dutta, R., Chen, Z., Miller, R.H., Trapp, B.D., 2013. Activated microglia enhance neurogenesis via trypsinogen secretion. Proc. Natl. Acad. Sci. U. S. A. 110, 8714–8719.

Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.

Nonaka, M., Chen, X.H., Pierce, J.E., Leoni, M.J., McIntosh, T.K., Wolf, J.A., Smith, D.H., 1999. Prolonged activation of NF-kappaB following traumatic brain injury in rats. J. Neurotrauma 16, 1023–1034.

Norden, D.M., Muccigrosso, M.M., Godbout, J.P., 2014. Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. Neuropharmacology.

Novak, M.L., Koh, T.J., 2013. Phenotypic transitions of macrophages orchestrate tissue repair. Am. J. Pathol. 183, 1352–1363.

O'Kusky, J.R., Ye, P., D'Ercole, A.J., 2000. Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. J. Neurosci. 20, 8435–8442.

Pakala, R., Watanabe, T., Benedict, C.R., 2002. Induction of endothelial cell proliferation by angiogenic factors released by activated monocytes. Cardiovasc. Radiat. Med. 3, 95–101.

Perego, C., Fumagalli, S., De Simoni, M.G., 2011. Temporal pattern of expression and colocalization of microglia/macrophage phenotype markers following brain ischemic injury in mice. J. Neuroinflammation 8, 174.

Perry, V.H., Nicoll, J.A., Holmes, C., 2010. Microglia in neurodegenerative disease. Nat. Rev. Neurol. 6, 193–201.

Piao, C.S., Stoica, B.A., Wu, J., Sabirzhanov, B., Zhao, Z., Cabatbat, R., Loane, D.J., Faden, A.I., 2013. Late exercise reduces neuroinflammation and cognitive dysfunction after traumatic brain injury. Neurobiol. Dis. 54, 252–263.

Polverini, P.J., Cotran, P.S., Gimbrone Jr., M.A., Unanue, E.R., 1977. Activated macrophages induce vascular proliferation. Nature 269, 804–806.

Qin, L., Liu, Y., Wang, T., Wei, S.J., Block, M.L., Wilson, B., Liu, B., Hong, J.S., 2004. NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J. Biol. Chem. 279, 1415–1421.

Raes, G., Van den Bergh, R., De Baetselier, P., Ghassabeh, G.H., Scotton, C., Locati, M., Mantovani, A., Sozzani, S., 2005. Arginase-1 and Ym1 are markers for murine, but not human, alternatively activated myeloid cells. J. Immunol. 174, 6561 author reply 6561–6562.

Ramlackhansingh, A.F., Brooks, D.J., Greenwood, R.J., Bose, S.K., Turkheimer, F.E., Kinnunen, K.M., Gentleman, S., Heckemann, R.A., Gunanayagam, K., Gelosa, G., Sharp, D.J., 2011. Inflammation after trauma: microglial activation and traumatic brain injury. Ann. Neurol. 70, 374–383.

Reiner, A., Heldt, S.A., Presley, C.S., Guley, N.H., Elberger, A.J., Deng, Y., D'Surney, L., Rogers, J.T., Ferrell, J., Bu, W., Del Mar, N., Honig, M.G., Gurley, S.N., Moore 2nd, B.M., 2015. Motor, visual and emotional deficits in mice after closed-head mild traumatic brain injury are alleviated by the novel CB2 inverse agonist SMM-189. Int. J. Mol. Sci. 16, 758–787.

Rhodes, J., 2011. Peripheral immune cells in the pathology of traumatic brain injury? Curr. Opin. Crit. Care 17, 122–130.

Rhodes, J.K., Sharkey, J., Andrews, P.J., 2009. The temporal expression, cellular localization, and inhibition of the chemokines MIP-2 and MCP-1 after traumatic brain injury in the rat. J. Neurotrauma 26, 507–525.

Saederup, N., Cardona, A.E., Croft, K., Mizutani, M., Cotleur, A.C., Tsou, C.L., Ransohoff, R.M., Charo, I.F., 2010. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5, e13693.

Salter, M.W., Beggs, S., 2014. Sublime microglia: expanding roles for the guardians of the CNS. Cell 158, 15–24.

Sandhir, R., Onyszchuk, G., Berman, N.E., 2008. Exacerbated glial response in the aged mouse hippocampus following controlled cortical impact injury. Exp. Neurol. 213, 372–380.

Satoh, T., Takeuchi, O., Vandenbon, A., Yasuda, K., Tanaka, Y., Kumagai, Y., Miyake, T., Matsushita, K., Okazaki, T., Saitoh, T., Honma, K., Matsuyama, T., Yui, K., Tsujimura, T., Standley, D.M., Nakanishi, K., Nakai, K., Akira, S., 2010. The Jmjd3–Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nat. Immunol. 11, 936–944.

Sauerbeck, A., Gao, J., Readnower, R., Liu, M., Pauly, J.R., Bing, G., Sullivan, P.G., 2011. Pioglitazone attenuates mitochondrial dysfunction, cognitive impairment, cortical tissue loss, and inflammation following traumatic brain injury. Exp. Neurol. 227, 128–135.

Schilling, M., Strecker, J.K., Ringelstein, E.B., Schabitz, W.R., Kiefer, R., 2009. The role of CC chemokine receptor 2 on microglia activation and blood-borne cell recruitment after transient focal cerebral ischemia in mice. Brain Res. 1289, 79–84.

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., Prinz, M., Wu, B., Jacobsen, S.E., Pollard, J.W., Frampton, J., Liu, K.J., Geissmann, F., 2012. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336, 86–90.

Semple, B.D., Bye, N., Rancan, M., Ziebell, J.M., Morganti-Kossmann, M.C., 2010. Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2−/− mice. J. Cereb. Blood Flow Metab. 30, 769–782.

Shein, N.A., Grigoriadis, N., Alexandrovich, A.G., Simeonidou, C., Lourbopoulos, A., Polyzoidou, E., Trembovler, V., Mascagni, P., Dinarello, C.A., Shohami, E., 2009. Histone deacetylase inhibitor ITF2357 is neuroprotective, improves functional recovery, and induces glial apoptosis following experimental traumatic brain injury. FASEB J. 23, 4266–4275.

Shin, J.A., Lim, S.M., Jeong, S.I., Kang, J.L., Park, E.M., 2014. Noggin improves ischemic brain tissue repair and promotes alternative activation of microglia in mice. Brain Behav. Immun. 40, 143–154.

Sica, A., Mantovani, A., 2012. Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. 122, 787–795.

Siddiq, I., Park, E., Liu, E., Spratt, S.K., Surosky, R., Lee, G., Ando, D., Giedlin, M., Hare, G.M., Fehlings, M.G., Baker, A.J., 2012. Treatment of traumatic brain injury using zinc-finger protein gene therapy targeting VEGF-A. J. Neurotrauma 29, 2647–2659.

Smith, C., Gentleman, S.M., Leclercq, P.D., Murray, L.S., Griffin, W.S., Graham, D.I., Nicoll, J.A., 2013. The neuroinflammatory response in humans after traumatic brain injury. Neuropathol. Appl. Neurobiol. 39, 654–666.

Smith, C., Gentleman, S.M., Leclercq, P.D., Murray, L.S., Griffin, W.S.T., Graham, D.I., Nicoll, J.A.R., 2012. The neuroinflammatory response in humans after traumatic brain injury. Neuropathol. Appl. Neurobiol.

Soares, H.D., Hicks, R.R., Smith, D., McIntosh, T.K., 1995. Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. J. Neurosci. 15, 8223–8233.

Stefini, R., Catenacci, E., Piva, S., Sozzani, S., Valerio, A., Bergomi, R., Cenzato, M., Mortini, P., Latronico, N., 2008. Chemokine detection in the cerebral tissue of patients with post-traumatic brain contusions. J. Neurosurg. 108, 958–962.

Storer, P.D., Xu, J., Chavis, J., Drew, P.D., 2005. Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis. J. Neuroimmunol. 161, 113–122.

Sunderkotter, C., Goebeler, M., Schulze-Osthoff, K., Bhardwaj, R., Sorg, C., 1991. Macrophage-derived angiogenesis factors. Pharmacol. Ther. 51, 195–216.

Tam, W.Y., Ma, C.H., 2014. Bipolar/rod-shaped microglia are proliferating microglia with distinct M1/M2 phenotypes. Sci. Rep. 4, 7279.

Tang, Y., Li, T., Li, J., Yang, J., Liu, H., Zhang, X.J., Le, W., 2014. Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease. Cell Death Differ. 21, 369–380.

Tchantchou, F., Tucker, L.B., Fu, A.H., Bluett, R.J., McCabe, J.T., Patel, S., Zhang, Y., 2014. The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury. Neuropharmacology 85, 427–439.

Tchantchou, F., Zhang, Y., 2013. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J. Neurotrauma 30, 565–579.

Thal, S.C., Heinemann, M., Luh, C., Pieter, D., Werner, C., Engelhard, K., 2011. Pioglitazone reduces secondary brain damage after experimental brain trauma by PPAR-gamma-independent mechanisms. J. Neurotrauma 28, 983–993.

Thau-Zuchman, O., Shohami, E., Alexandrovich, A.G., Leker, R.R., 2010. Vascular endothelial growth factor increases neurogenesis after traumatic brain injury. J. Cereb. Blood Flow Metab. 30, 1008–1016.
Thau-Zuchman, O., Shohami, E., Alexandrovich, A.G., Leker, R.R., 2012a. Combination of vascular endothelial and fibroblast growth factor 2 for induction of neurogenesis and angiogenesis after traumatic brain injury. J. Mol. Neurosci. 47, 166–172.
Thau-Zuchman, O., Shohami, E., Alexandrovich, A.G., Leker, R.R., 2012b. Subacute treatment with vascular endothelial growth factor after traumatic brain injury increases angiogenesis and gliogenesis. Neuroscience 202, 334–341.
Trejo, J.L., Carro, E., Torres-Aleman, I., 2001. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J Neurosci 21, 1628–1634.
Tremblay, M.E., Stevens, B., Sierra, A., Wake, H., Bessis, A., Nimmerjahn, A., 2011. The role of microglia in the healthy brain. J. Neurosci. 31, 16064–16069.
Vallieres, L., Campbell, I.L., Gage, F.H., Sawchenko, P.E., 2002. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J. Neurosci. 22, 486–492.
Vogel, D.Y., Vereyken, E.J., Glim, J.E., Heijnen, P.D., Moeton, M., van der Valk, P., Amor, S., Teunissen, C.E., van Horssen, J., Dijkstra, C.D., 2013. Macrophages in inflammatory multiple sclerosis lesions have an intermediate activation status. J. Neuroinflammation 10, 35.
Walsh, J.T., Watson, N., Kipnis, J., 2014. T cells in the central nervous system: messengers of destruction or purveyors of protection? Immunology 141, 340–344.
Walton, N.M., Sutter, B.M., Laywell, E.D., Levkoff, L.H., Kearns, S.M., Marshall 2nd, G.P., Scheffler, B., Steindler, D.A., 2006. Microglia instruct subventricular zone neurogenesis. Glia 54, 815–825.
Wang, G., Jiang, X., Pu, H., Zhang, W., An, C., Hu, X., Liou, A.K., Leak, R.K., Gao, Y., Chen, J., 2013a. Scriptaid, a novel histone deacetylase inhibitor, protects against traumatic brain injury via modulation of PTEN and AKT pathway: Scriptaid protects against TBI via AKT. Neurotherapeutics 10, 124–142.
Wang, G., Shi, Y., Jiang, X., Leak, R.K., Hu, X., Wu, Y., Pu, H., Li, W.W., Tang, B., Wang, Y., Gao, Y., Zheng, P., Bennett, M.V., Chen, J., 2015. HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3beta/PTEN/Akt axis. Proc. Natl. Acad. Sci. U. S. A. 112, 2853–2858.
Wang, G., Zhang, J., Hu, X., Zhang, L., Mao, L., Jiang, X., Liou, A.K., Leak, R.K., Gao, Y., Chen, J., 2013b. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. J. Cereb. Blood Flow Metab. 33, 1864–1874.
Wang, J.W., Wang, H.D., Cong, Z.X., Zhang, X.S., Zhou, X.M., Zhang, D.D., 2013c. Activation of metabotropic glutamate receptor 5 reduces the secondary brain injury after traumatic brain injury in rats. Biochem. Biophys. Res. Commun. 430, 1016–1021.
Wei, L., Erinjeri, J.P., Rovainen, C.M., Woolsey, T.A., 2001. Collateral growth and angiogenesis around cortical stroke. Stroke 32, 2179–2184.
Welser, J.V., Li, L., Milner, R., 2010. Microglial activation state exerts a biphasic influence on brain endothelial cell proliferation by regulating the balance of TNF and TGF-beta1. J. Neuroinflammation 7, 89.
Wilcock, D.M., 2014. Neuroinflammatory phenotypes and their roles in Alzheimer's disease. Neurodegener. Dis. 13, 183–185.
Willenborg, S., Lucas, T., van Loo, G., Knipper, J.A., Krieg, T., Haase, I., Brachvogel, B., Hammerschmidt, M., Nagy, A., Ferrara, N., Pasparakis, M., Eming, S.A., 2012. CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair. Blood 120, 613–625.
Wu, H., Lu, D., Jiang, H., Xiong, Y., Qu, C., Li, B., Mahmood, A., Zhou, D., Chopp, M., 2008. Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. J. Neurotrauma 25, 130–139.
Xiong, Y., Mahmood, A., Chopp, M., 2010. Angiogenesis, neurogenesis and brain recovery of function following injury. Curr. Opin. Investig. Drugs 11, 298–308.
Xue, L., Greisler, H.P., 2002. Angiogenic effect of fibroblast growth factor-1 and vascular endothelial growth factor and their synergism in a novel in vitro quantitative fibrin-based 3-dimensional angiogenesis system. Surgery 132, 259–267.
Yamanaka, M., Ishikawa, T., Griep, A., Axt, D., Kummer, M.P., Heneka, M.T., 2012. PPARgamma/RXRalpha-induced and CD36-mediated microglial amyloid-beta phagocytosis results in cognitive improvement in amyloid precursor protein/presenilin 1 mice. J. Neurosci. 32, 17321–17331.
Yi, J.H., Park, S.W., Brooks, N., Lang, B.T., Vemuganti, R., 2008. PPARgamma agonist rosiglitazone is neuroprotective after traumatic brain injury via anti-inflammatory and anti-oxidative mechanisms. Brain Res. 1244, 164–172.
Zajac, E., Schweighofer, B., Kupriyanova, T.A., Juncker-Jensen, A., Minder, P., Quigley, J.P., Deryugina, E.I., 2013. Angiogenic capacity of M1- and M2-polarized macrophages is determined by the levels of TIMP-1 complexed with their secreted proMMP-9. Blood 122, 4054–4067.
Zanier, E.R., Pischiutta, F., Riganti, L., Marchesi, F., Turola, E., Fumagalli, S., Perego, C., Parotto, E., Vinci, P., Veglianese, P., D'Amico, G., Verderio, C., De Simoni, M.G., 2014. Bone marrow mesenchymal stromal cells drive protective M2 microglia polarization after brain trauma. Neurotherapeutics 11, 679–695.
Zhang, B., West, E.J., Van, K.C., Gurkoff, G.G., Zhou, J., Zhang, X.M., Kozikowski, A.P., Lyeth, B.G., 2008. HDAC inhibitor increases histone H3 acetylation and reduces microglia inflammatory response following traumatic brain injury in rats. Brain Res. 1226, 181–191.
Zhang, Y., Chopp, M., Mahmood, A., Meng, Y., Qu, C., Xiong, Y., 2012. Impact of inhibition of erythropoietin treatment-mediated neurogenesis in the dentate gyrus of the hippocampus on restoration of spatial learning after traumatic brain injury. Exp. Neurol. 235, 336–344.
Zhang, Y., Xiong, Y., Mahmood, A., Meng, Y., Qu, C., Schallert, T., Chopp, M., 2009. Therapeutic effects of erythropoietin on histological and functional outcomes following traumatic brain injury in rats are independent of hematocrit. Brain Res. 1294, 153–164.
Zhang, Z.Y., Sun, B.L., Liu, J.K., Yang, M.F., Li, D.W., Fang, J., Zhang, S., Yuan, Q.L., Huang, S.L., 2015. Activation of mGluR5 attenuates microglial activation and neuronal apoptosis in early brain injury after experimental subarachnoid hemorrhage in rats. Neurochem. Res. 40, 1121–1132.
Zhao, C., Li, W.W., Franklin, R.J., 2006. Differences in the early inflammatory responses to toxin-induced demyelination are associated with the age-related decline in CNS remyelination. Neurobiol. Aging 27, 1298–1307.
Ziebell, J.M., Morganti-Kossmann, M.C., 2010. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics 7, 22–30.
Ziebell, J.M., Taylor, S.E., Cao, T., Harrison, J.L., Lifshitz, J., 2012. Rod microglia: elongation, alignment, and coupling to form trains across the somatosensory cortex after experimental diffuse brain injury. J. Neuroinflammation 9, 247.

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Activation of the Hypothalamo-Pituitary-Adrenal Axis by the Growth Hormone (GH) Secretagogue, GH-Releasing Peptide-6, in Rats

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ABSTRACT

GH-releasing hexapeptide (GHRP-6) is a synthetic secretagogue that stimulates the release of GH by acting at both hypothalamic and pituitary sites. GHRPs also consistently elicit small, but significant, increases in plasma concentrations of ACTH and adrenal steroids. As these secretagogues do not release ACTH directly, they probably interact with the hypothalamic peptidergic systems controlling ACTH release, such as CRH and arginine vasopressin (AVP). We have now examined the activation of the hypothalamo-pituitary-adrenal axis by GHRP-6 in conscious rats. In a series of experiments, rats were injected iv with 10 μg GHRP-6, 2 μg CRH, 0.5 μg AVP, or saline, alone or in combination, and serial plasma samples withdrawn and assayed for ACTH, corticosterone, and GH. CRH and AVP increased plasma ACTH levels in all rats, whereas ACTH and corticosterone responses to GHRP-6 were variable and were dependent on the prevailing activity of the hypothalamo-pituitary-adrenal axis. GHRP-6 stimulated the largest ACTH responses in rats that had the lowest basal plasma ACTH and corticosterone levels before GHRP-6 administration. GHRP-6 given in combination with CRH did not increase ACTH levels beyond the response to CRH alone (change in ACTH, 1570 $\pm$ 207 *vs.* 1714 $\pm$ 245 pg/ml), whereas the combination of GHRP-6 and AVP markedly increased ACTH levels compared with the effects of AVP alone (change in ACTH, 5587 $\pm$ 669 *vs.* 2338 $\pm$ 451 pg/ml; $P < 0.05$). The GH responses to GHRP-6 were significantly greater in rats with low basal plasma ACTH and corticosterone levels than in rats with elevated ACTH and corticosterone levels (change in GH response, 119 $\pm$ 27 *vs.* 29 $\pm$ 7 ng/ml; $P < 0.01$). CRH alone significantly inhibited GH release (pre- *vs.* 40 min post-CRH, 11.9 $\pm$ 3.8 *vs.* 1.7 $\pm$ 0.4 ng/ml; $P < 0.05$), whereas AVP alone had no effect on GH levels. Neither CRH nor AVP had any effect on the GH response to GHRP-6. We suggest that GHRP-6 acts via the hypothalamus to mediate the release of ACTH, and that these effects are probably mediated at least in part via the release of endogenous CRH and are subject to regulation by circulating glucocorticoids. (*Endocrinology* **138:** 1585–1591, 1997)

THERE IS increasing interest in the use of GH-releasing peptides (GHRPs) and their nonpeptide analogs to release GH in animals and man (1–3). These molecules have all been derived from synthetic strategies, and although endogenous GHRPs have long been postulated, none has yet been identified, although the search for them has recently been invigorated by the identification and cloning of an endogenous G protein-coupled receptor, specifically activated by GHRPs (4, 5).

Initial studies with the synthetic hexapeptide GHRP-6 suggested that this compound acted primarily on the pituitary gland and was absolutely specific for GH release (6). More recent studies have qualified both of these assumptions. In conscious animals, the GH responses to GHRPs are complex and can best be explained by effects exerted at both pituitary and hypothalamic sites, interacting with both GRF and somatostatin (1, 7–9). Furthermore, when more recently developed GHRP analogs were tested in man, small but consistent elevations in ACTH and cortisol were observed (3, 10, 11). The clinical significance of these small rises in cortisol is unclear, although they are consistently seen with a variety of GHRP analogs and in different species (12–15).

GHRPs do not release glucocorticoids from the adrenal glands directly, but via stimulation of ACTH secretion (16), and several lines of evidence suggest that this effect is exerted indirectly via a hypothalamic action. For example, GHRPs do not release ACTH directly from isolated pituitaries (17, 18), nor do they synergize with GRF to release more ACTH *in vivo* as they do to release GH (19). Furthermore, the ACTH response to GHRPs *in vivo* is abolished if the pituitary stalk is transected (15). As one *in vivo* effect of GHRP on GH release is to release endogenous GRF (20), which it then synergizes with at the level of the pituitary to release GH (7, 8), we reasoned that GHRPs might have an analogous action on the hypothalamo-pituitary-adrenal (HPA) axis, by stimulating the release of, or by synergizing with, one or other of the endogenous ACTH secretagogues.

ACTH release is primarily controlled by CRH and arginine vasopressin (AVP) (21). Both peptides stimulate ACTH release when given alone and produce a synergistic response when given together (22). We have now explored the effects of giving GHRP-6 alone or in combination with CRH or AVP on ACTH, corticosterone, and GH release in chronically catheterized conscious rats. Some of these results has been presented in abstract form (23).

Materials and Methods

Animals and surgery

All experimental procedures were carried out in accordance with institutional and national guidelines for animal experiments. Normal male AS rats (220–250 g) were obtained from the National Institute for Medical Research colony (Mill Hill, London, UK). They were housed

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individually in metabolic cages in a light- and temperature-controlled room (14 h of light/day; 23–25 C) and allowed access to food and water *ad libitum*. After 7 days to acclimatize to their surroundings, a catheter was inserted into the right jugular vein under halothane anesthesia as previously described (24). After a period of recovery, which varied from 2–6 days in different experiments, the catheters were connected to a computer-controlled blood microsampling system (25).

Animal experiments

Exp 1. The aim of this experiment was to ascertain whether administration of GHRP-6 increased plasma corticosterone concentrations in the rat and to compare the response to that of CRH. Two group of four rats that had been catheterized 6 days previously were randomly injected iv at 1000 h with either 10 μg GHRP-6 or saline (100 μl), and blood samples (20 μl) were collected 15 and 0 min before and 15, 30, 45, and 60 min after injection. The same rats were injected again at 1500 h, but with the treatments reversed. On the following day, using the same injection and sampling protocol, the rats were randomly injected with either 2 μg CRH or saline. At the end of the experiment, the diluted plasma aliquots were assayed for corticosterone by RIA.

Exp 2. The aim of this experiment was to characterize the ACTH response that underlies the increase in plasma corticosterone concentrations induced by GHRP-6 in the rat. A group of 12 rats was implanted with jugular venous catheters under halothane anesthesia. After 2 days of recovery, all animals were connected to the computer-controlled blood-sampling apparatus and injected iv with 10 μg GHRP-6. Beginning 1 h before injection, serial blood samples (30 μl) were withdrawn every 10 min for 3 h and collected into ice-cold tubes containing a 70-μl solution of heparinized saline (5 U/ml), 50 mM EDTA, and 0.1 trypsin inhibitor unit aprotinin (Sigma Chemical Co., St. Louis, MO). The samples were centrifuged at 4 C, and the plasma supernatants were divided into aliquots and stored at −20 C until assayed for ACTH and GH. Samples collected at −10, 0, 20, 30, 40, and 50 min relative to the time of injection were also assayed for corticosterone.

Exp 3. The aim of this experiment was to determine whether GHRP-6 interacted with CRH or AVP to release ACTH or GH in the rat. As the results from Exp 2, begun 2 days after surgery, showed that some rats still had elevated plasma ACTH and corticosterone concentrations before GHRP-6 administration, all rats were left for 4 days to acclimatize to the automatic blood-sampling system before the beginning of this experiment. Groups of rats (n = 5–11) were then randomly injected iv with 10 μg GHRP-6, 2 μg CRH, 0.5 μg AVP, or saline (100 μl), alone or in combination. Blood samples were collected as before, and the plasma supernatants were assayed for ACTH and GH.

Peptides

GHRP-6 was provided by Ferring (Malmo, Sweden). Rat CRH and AVP were purchased from Bachem (Saffron Walden, UK). All peptides were dissolved in sterile heparinized (5 IU/ml) saline immediately before injection.

RIAs

The concentration of ACTH was measured in plasma by direct RIA (26), using an antiserum (no. 8514/2) supplied by Dr. G. B. Makara, Institute of Experimental Medicine (Budapest, Hungary). Rat ACTH (Peninsula Laboratories, St. Helens, UK) was used as the assay standard. Under our sampling conditions, the sensitivity of the assay was 40 pg/ml. Rat GH concentrations were measured in diluted plasma samples by RIA as previously described (24), using reagents generously supplied by the NIDDK (Bethesda, MD). The sensitivity of the assay was 0.2 ng/ml, and results are expressed in terms of the reference preparation rGH RP-2. Plasma corticosterone concentrations were measured using a double antibody RIA kit purchased from ICN Biomedicals (High Wycombe, UK).

Statistical analysis

All data are shown as the mean ± SEM, and statistical comparisons were made using ANOVA followed by Duncan's multiple range test when a significant ($P < 0.05$) interaction was found.

Results

Intravenous injection of 10 μg GHRP-6 into conscious undisturbed male rats resulted in a 4-fold increase ($P < 0.001$) in plasma concentrations of corticosterone 30 min later (Fig. 1). On the following day, the same rats exhibited a similar sized increase in corticosterone levels after the administration of 2 μg CRH, whereas saline injections had no effect.

In Exp 2, this dose of GHRP-6 was given to a larger group of rats, and their ACTH and GH responses were measured. Plasma levels of ACTH varied markedly between rats in this experiment, which was begun after only 2 days of recovery from surgery, and the individual responses to GHRP-6 were highly variable, with both increases and decreases observed. Inspection of these data suggested that the variability was related to the prevailing ACTH levels. This was confirmed by plotting the ACTH responses to GHRP-6 administration against the mean plasma ACTH concentrations 1 h before injection in individual rats (Fig. 2), which revealed a highly significant negative correlation ($r = -0.89$; $P < 0.001$).

All data from this experiment are presented in Fig. 3, in which animals with high initial plasma corticosterone levels (>700 ng/ml; n = 6) were analyzed separately (Fig. 3b) from those with low initial plasma corticosterone levels (<350 ng/ml; n = 6) before GHRP-6 injection (Fig. 3a). Both plasma ACTH and corticosterone levels increased markedly ($P < 0.001$) in response to GHRP-6 in those rats that had low corticosterone levels 1 h before GHRP-6 administration (Fig. 3a), whereas in the rats with high initial corticosterone levels (Fig. 3b), plasma ACTH and corticosterone levels showed a significant decrease ($P < 0.05$) 20–30 min after GHRP-6 injection. GHRP-6 induced GH release in all rats, but the animals with low initial corticosterone levels exhibited a much greater ($P < 0.01$) GH response than those with high initial corticosterone levels (ΔGH, 119 ± 27 *vs.* 29 ± 7 ng/ml; *bottom panel*, Fig. 3, a *vs.* b).

In the next experiment, all animals were left to recover for 4 days before sampling began, and basal ACTH levels were uniformly low in all rats at the beginning of each sampling

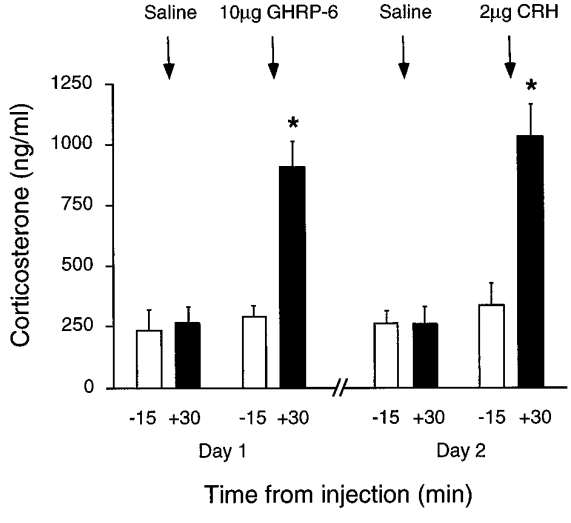

FIG. 1. Effect of iv injection of GHRP-6 and CRH on plasma corticosterone levels in conscious male rats. Values are the mean ± SEM (n = 8). *, $P < 0.001$ *vs.* saline control.

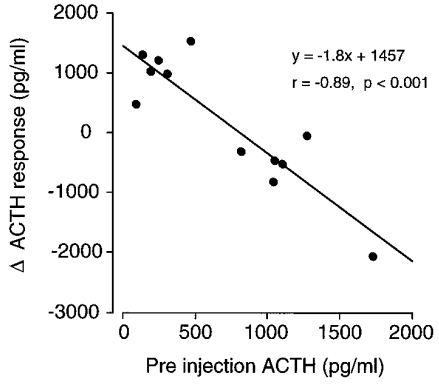

FIG. 2. The mean plasma level of ACTH 1 h before GHRP-6 administration is negatively correlated with the net change in plasma ACTH after GHRP-6 administration in individual rats.

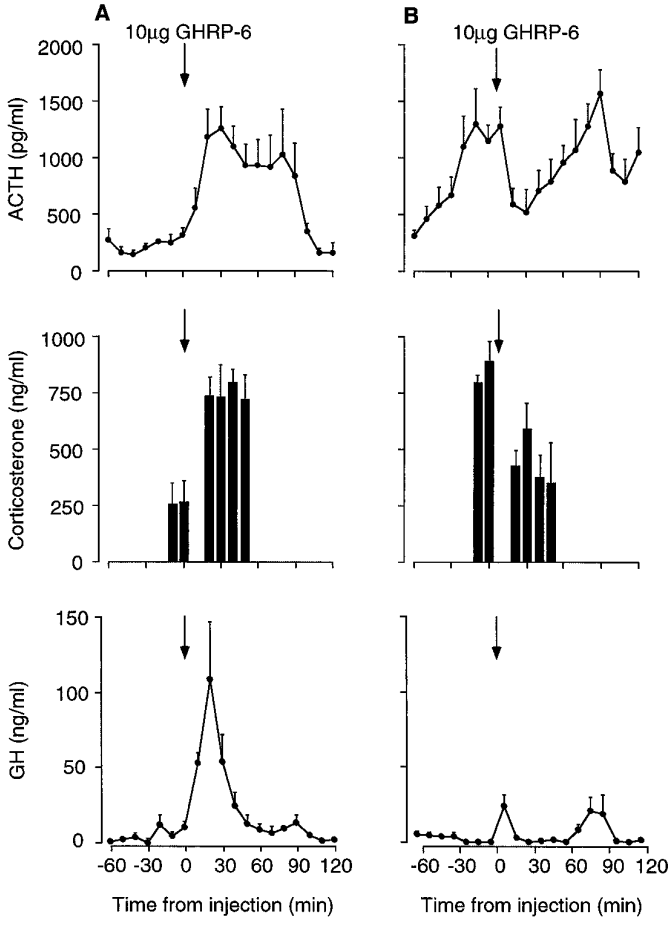

FIG. 3. Effect of iv injection of GHRP-6 on plasma concentrations of ACTH, corticosterone, and GH in conscious male rats. The animals were separated into two groups based on their plasma concentrations of corticosterone before GHRP-6 treatment: A) rats with less than 350 ng/ml plasma corticosterone (n = 6), and B) rats with more than 700 ng/ml plasma corticosterone (n = 6). Values are the mean ± SEM for each time point.

period (Fig. 4). The administration of GHRP-6, CRH, or AVP significantly ($P < 0.05$) increased plasma concentrations of ACTH. The mean data are shown in Fig. 4, and the net changes in ACTH release (ΔACTH response above baseline) are shown in Fig. 5. Both GHRP-6 and CRH when given individually released similar amounts of ACTH, and their combination did not increase plasma ACTH levels beyond the response to CRH alone (ΔACTH, 1570 ± 207 *vs.* 1714 ± 245 pg/ml). However, the combination of GHRP-6 and AVP produced a significantly ($P < 0.05$) greater increase in ACTH than the effects of AVP alone (ΔACTH, 5587 ± 669 *vs.* 2338 ± 451 pg/ml). Saline injection had no effect on plasma ACTH levels.

The same samples were also assayed for GH, and the results are shown in Figs. 6 and 7. As expected, GHRP-6 stimulated GH release 6- to 7-fold, whereas saline injections had no effect. Injection of AVP alone had no significant effect on plasma GH concentrations, whereas CRH alone inhibited ($P < 0.05$) GH release for up to 90 min (pre- *vs.* 40 min post-CRH; 11.9 ± 3.8 *vs.* 1.7 ± 0.4 ng/ml). When given in combination with GHRP-6, neither AVP nor CRH affected the GH response compared with the effects of GHRP-6 alone (Figs. 6 and 7).

Discussion

Although GHRPs have been shown to activate the HPA axis in other species (3, 10–14), this is the first study to characterize this effect of GHRP-6 in the conscious rat. Acute administration of GHRP-6 resulted in a significant stimulation of plasma corticosterone concentrations. This is likely to be mediated via central control of ACTH release rather than any direct effect on the adrenals, because GHRPs do not release adrenal steroids in animals following hypophysectomy (16), nor do they release ACTH after pituitary stalk transection (15) or from pituitary cells *in vitro* (17, 18).

We have now documented the increase in plasma ACTH after GHRP-6 treatment in the rat, confirming data from other secretagogues used in other species (11, 12, 14). In one experiment performed only 2 days after surgery, we found that ACTH release in response to GHRP-6 was highly variable and dependent on the prevailing activity of the HPA axis. Rats with high initial corticosterone levels, reflecting an underlying pulsatility of ACTH secretion before GHRP-6 administration, did not show an increase in ACTH release in response to GHRP-6, whereas those rats with lower initial corticosterone and ACTH levels did respond. Much less variability in the ACTH response to GHRP-6 was seen in animals left for a longer recovery period after surgery and in animals that had much lower ACTH levels at the beginning of the experiments. It would, therefore, seem likely that high circulating levels of glucocorticoids feed back to reduce the ACTH response to GHRP-6, as they do for other ACTH secretagogues (27).

Although all rats responded with an increase in GH, this was also blunted in those animals with high initial corticosterone levels. This is in good agreement with other data showing that the GH response to GHRP-6 is significantly reduced after chronic dexamethasone treatment in rats (28) and that the GH response to a low dose (0.2 μg/kg) of L-692,429 is attenuated after glucocorticoid therapy in man (29). Cushing patients also show a reduced response to GHRP-6 (30), but Giustina *et al.* (31) found that the GH response to hexarelin was not affected in acromegalic patients with glucocorticoid excess. Much less is known about

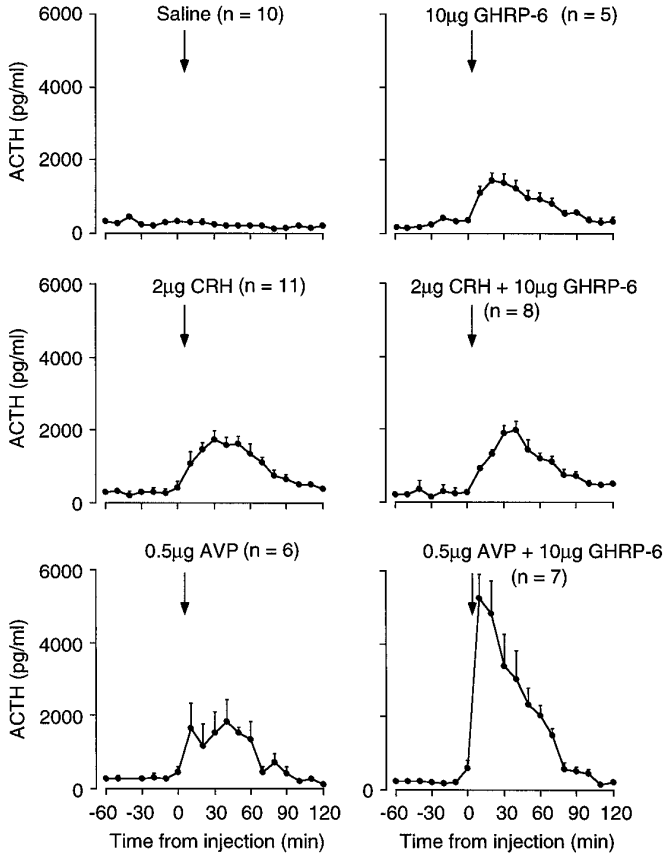

FIG. 4. Effect of iv injection of GHRP-6, CRH, and AVP on plasma concentrations of ACTH in conscious male rats. Values are the mean ± SEM for each time point.

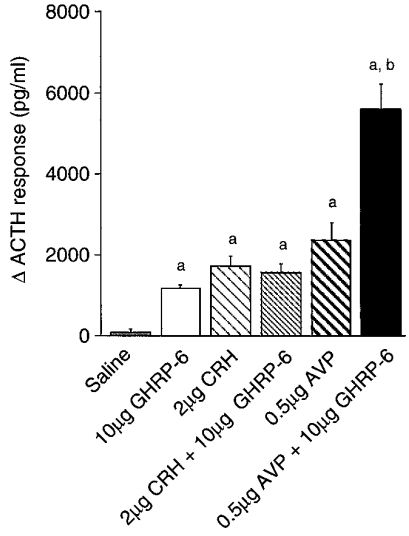

FIG. 5. The net change in plasma ACTH concentrations in response to GHRP-6, CRH, and AVP in conscious male rats. Values are the mean ± SEM (n = 5–11). a, $P < 0.05$ *vs.* saline control; b, $P < 0.05$ *vs.* 0.5 μg AVP.

glucocorticoid feedback on GHRP-induced activation of the HPA axis in man, because the rises in cortisol secretion are of much smaller magnitude than those for GH, and their significance is usually discounted. Nevertheless, as GHRP-6 is clearly an ACTH secretagogue in man (3, 10, 11), its actions on this axis are likely to be subject to physiological feedback regulation by glucocorticoids.

The mechanism of glucocorticoid feedback could be exerted at both the pituitary and hypothalamic levels. It has been suggested that elevated glucocorticoids may reduce GH release in rats by stimulating somatostatin release from the hypothalamus (32, 33). Furthermore, central somatostatin has been shown to inhibit the effects of GHRP-6 on GH release (34), whereas antibodies to somatostatin increase the GH response to GHRP-6 (7). Thus, the reduced GH response to GHRP-6 in rats with high circulating corticosterone levels may reflect an increased somatostatin tone in these animals.

As GHRPs do not release ACTH directly, their most likely mechanism of action is to stimulate the release of and/or synergize with endogenous ACTH secretagogues, such as CRH or AVP. We, therefore, tested the effects of GHRP-6, given alone or in combination with these peptides, on both ACTH and GH release. We found a markedly greater increase in plasma ACTH when GHRP-6 was given in combination with AVP, but not when it was given in combination with CRH. This interaction was specific for ACTH, because in the same experiments we found no synergism on GH release when GHRP-6 was given together with AVP or CRH. Although our results identify a synergism between GHRP-6 and AVP on ACTH release, they do not necessarily imply a direct interaction between these peptides. Although GHRPs are not direct ACTH secretagogues (17, 18), the possibility that GHRP-6 amplifies the responses to AVP or CRH at the level of the corticotroph has not been tested. However, we have not observed any additive effect of GHRP-6 on AVP- or CRH-induced ACTH release from cultured rat pituitary cells *in vitro* (Samsø-Schmidt, L., and I. C. A. F. Robinson, unpublished results), suggesting that this interaction with AVP must occur at the hypothalamic level.

It is well established that CRH and AVP synergize at the pituitary to release ACTH in the rat (22). The most logical explanation for our data, therefore, is that GHRP-6 stimulates CRH release, which then synergizes with AVP at the pituitary. Hickey *et al.* (12) also considered CRH release induced by GHRPs to be one of several possible mechanisms for the central effects of nonpeptide GH secretagogues to release ACTH in dogs. Our data, however, do not exclude the possibility that GHRP-6 could also release AVP, but we were unable to demonstrate any synergism between GHRP-6 and CRH in these studies. It is also possible that GHRP-6 may interact with ACTH secretagogues other than AVP or CRH (*e.g.* oxytocin or catecholamines) or may even act via an unidentified factor, as has been postulated for its GH-releasing activity (7).

There is a strong precedent for our hypothesis that GHRP-6 acts within the hypothalamus to release an ACTH secretagogue such as CRH. It is now well established that an important action of GHRPs to release GH involves a hypothalamic effect to release GRF, which then synergizes with GHRPs at the level of the somatotroph to release GH (7, 8). There is direct evidence for release of GRF into hypophysial portal blood after hexarelin administration (20), and recent preliminary evidence from sampling four sheep using this model has confirmed a rise in CRH levels in portal blood after

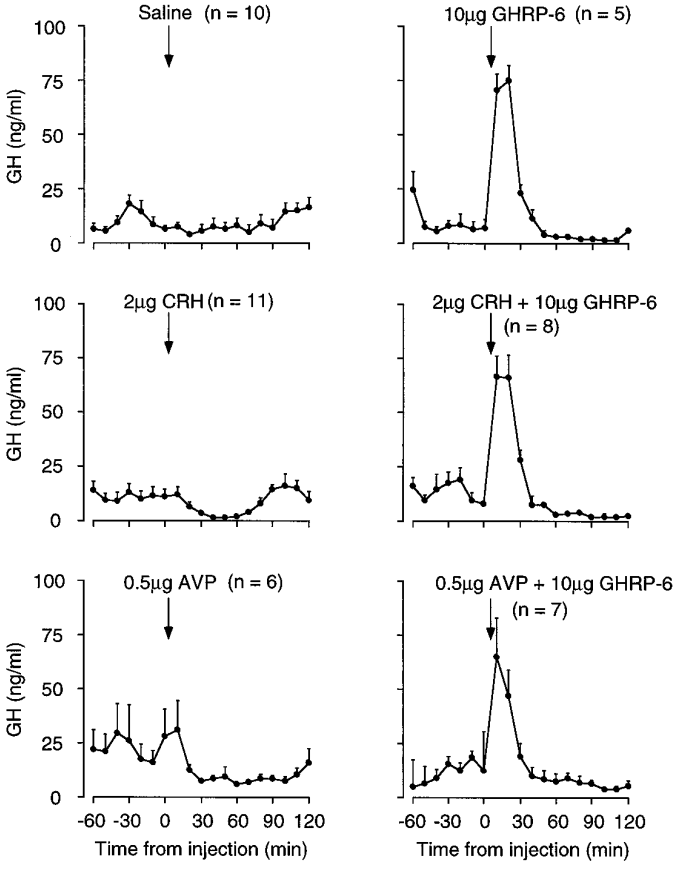

FIG. 6. Effect of iv injection of GHRP-6, CRH, and AVP on plasma concentrations of GH in conscious male rats. Values are the mean ± SEM for each time point.

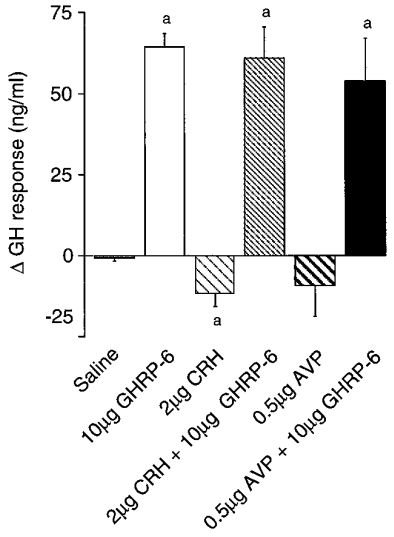

FIG. 7. The net change in plasma GH concentrations in response to GHRP-6, CRH, and AVP in conscious male rats. Values are the mean ± SEM (n = 5–11). a, $P < 0.05$ *vs.* saline control.

GHRP-6 administration (Guillaume, V., C. Oliver, G. B. Thomas, and I. C. A. F. Robinson, unpublished results).

A dependence on the endogenous peptides governing normal ACTH secretion could also explain why the ability of GHRPs to release ACTH is most manifest when the underlying activity of the HPA axis is low. A rise in glucocorticoid tone would then blunt the effect of GHRP-6 by negative feedback effects on CRH or AVP (27). In the rats with high ACTH levels before injection, administration of GHRP-6 was associated with a transient fall in ACTH. This could reflect an inhibitory effect of GHRP-6 under conditions of high CRH tone. However, an alternative explanation could simply be that the fall in ACTH levels reflects the negative feedback action of the high circulating corticosterone levels, which masks the initial stimulation by GHRP-6.

ACTH-releasing activity has been demonstrated for all classes of peptide or nonpeptide GHRPs reported to date, and these activities are probably mediated via the recently described GHRP receptor (4, 5). Although this receptor has been localized to the hypothalamus in man and swine (5), its distribution in the rat hypothalamus is not yet known. Our data suggest that it might be profitable to look for GHRP receptor expression in the parvocellular region of the paraventricular nucleus (PVN), which is the site of the cell bodies expressing CRH and AVP (35). On the other hand, the effect of GHRPs need not be exerted directly on CRH cell bodies. GHRPs activate Fos protein expression and electrical activity in neurons in a restricted area of the arcuate nucleus, and no GHRP-6-induced Fos protein expression was seen in the PVN (36). However, many of the arcuate cells activated by GHRP-6 are neuropeptide Y (NPY)-positive cells (37), and it is known that the PVN receives a major projection from arcuate NPY neurons (38). It is, therefore, conceivable that a single action of GHRPs on NPY cells in the arcuate nucleus could affect both arcuate GRF neurons and PVN CRH neurons.

Although combinations of CRH or AVP with GHRP-6 had no effect on the ability of the latter to release GH, we noted that injections of CRH alone produced a marked suppression of basal GH release. Central administration of CRH results in an inhibition of spontaneous GH secretion in the rat (39, 40), although in both of these studies, peripheral injections of CRH did not lower mean GH levels. The only obvious difference between our study and those previously reported is that our basal GH levels were very much lower [*e.g.* 12 *vs.* 50 ng/ml (39) or 190 ng/ml (40)], which may have made the inhibition of basal GH release easier to detect. It has also been shown that CRH treatment blunts the nocturnal rise in GH secretion in man (41). These inhibitory effects of CRH may well be mediated by a direct stimulation of somatostatin release (40, 42, 43).

These studies have largely focussed on the mechanism of acute activation of the HPA axis by GHRP-6 in the rat. It is clear that the GH-releasing effects of GHRPs are highly dependent on the pattern of exposure to secretagogues and can fade markedly with continuous exposure (44, 45). As glucocorticoids themselves can inhibit the growth-promoting activity of GH (33), it would be desirable, at least on theoretical grounds, to attempt to minimize the ACTH-releasing activity of GHRPs. On the other hand, the changes in adrenal steroid levels are relatively small, and it is unclear whether they persist with continued GHRP-6 treatment or will also desensitize in parallel with GH, as has been shown for nonpeptide GHRPs in man (45). Whatever the biological signif-

icance of GHRP-induced activation of the HPA axis, it is important to understand the hypothalamic mechanisms involved, as they will presumably be shared by the unknown endogenous ligand(s) for the recently cloned GHRP receptor (5).

In conclusion, we have shown that GHRP-6 mediates the release of ACTH in conscious rats, and that the magnitude of the release depends on the prevailing activity of the HPA axis. GHRP-6 increases the effects of AVP on ACTH release, and we suggest that this may be a hypothalamic effect via the stimulation of CRH, which then synergizes with AVP at the corticotroph to release ACTH. Furthermore, these effects are probably subject to regulation by glucocorticoids.

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References

1. **Bowers CY, Veeraragavan K, Sethumadhavan K** 1994 Atypical growth hormone peptides. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Springer-Verlag, New York, pp 203–222
2. **Smith RG, Cheng K, Schoen WR, Pong SS, Hickey G, Jacks T, Butler B, Chan W, Chaung L, Judith F, Taylor J, Wyvratt MJ, Fisher MH** 1993 A nonpeptidyl growth-hormone secretagogue. Science 260:1640–1643
3. **Gertz BJ, Barrett JS, Eisenhandler R, Krupa DA, Wittreich JM, Seibold JR, Schneider SH** 1993 Growth hormone response in man to L-692,429, a novel nonpeptide mimic of growth hormone-releasing peptide-6. J Clin Endocrinol Metab 77:1393–1397
4. **Pong S-S, Chaung L-YP, Dean DC, Nargund RP, Patchett AA, Smith RG** 1996 Identification of a new G-protein-linked receptor for growth hormone secretagogues. Mol Endocrinol 10:57–61
5. **Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin MJ, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu K, McKee KK, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJS, Dean DC, Mellio DG, Patchett AA, Nargund R, Griffin PR, De Martino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LHT** 1996 A receptor in the pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977
6. **Bowers CY, Momany F, Reynolds GA, Hong A** 1984 On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. Endocrinology 114:1537–1545
7. **Bowers CY, Sartor AO, Reynolds GA, Badger TM** 1991 On the actions of the growth hormone-releasing hexapeptide, GHRP. Endocrinology 128:2027–2035
8. **Clark RG, Carlsson LMS, Trojnar J, Robinson ICAF** 1989 The effects of a growth hormone-releasing peptide and growth hormone-releasing factor in conscious and anaesthetized rats. J Neuroendocrinol 1:249–255
9. **Cheng K, Chan WWS, Barreto A, Convey EM, Smith RG** 1989 The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3′,5′-monophosphate accumulation in rat primary pituitary cell culture. Endocrinology 124:2791–2798
10. **Bowers CY, Reynolds GA, Durham D, Barrera CM, Pezzoli SS, Thorner MO** 1990 Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. J Clin Endocrinol Metab 70:975–982
11. **Frieboes RM, Murck H, Maier P, Schier T, Holsboer F, Steiger A** 1995 Growth hormone-releasing peptide-6 stimulates sleep, growth hormone, ACTH and cortisol release in normal man. Neuroendocrinology 61:584–589
12. **Hickey G, Jacks T, Judith F, Taylor J, Schoen WR, Krupa D, Cunningham P, Clark J, Smith RG** 1994 Efficacy and specificity of L-692,429, a novel nonpeptidyl growth-hormone secretagogue, in beagles. Endocrinology 134:695–701
13. **Chang CH, Rickes EL, McGuire L, Cosgrove S, Taylor J, Chen H, Feighner S, Clark JN, de Vita R, Schoen W, Wyvratt M, Fisher M, Smith RG, Hickey GJ** 1995 Activity of a novel nonpeptidyl growth hormone secretagogue, L-700,653, in swine. Endocrinology 136:1065–1071
14. **Jacks T, Hickey G, Judith F, Taylor J, Chen H, Krupa D, Feeney W, Schoen W, Ok D, Fisher M, Wyvratt M, Smith R** 1994 Effects of acute and repeated intravenous administration of L-692,585, a novel-peptidyl growth hormone secretagogue, on plasma growth hormone, IGF-1, ACTH, cortisol, prolactin, insulin, and thyroxine levels in beagles. J Endocrinol 143:399–406
15. **Hickey GJ, Drisko J, Faidley T, Chang C, Anderson LL, Nicolich S, McGuire L, Rickes E, Krupa D, Feeney W, Friscino B, Cunningham P, Frazier E, Chen H, Laroque P, Smith RG** 1996 Mediation by the central nervous system is critical to the *in vivo* activity of the GH secretagogue L-692,585. J Endocrinol 148:371–380
16. **Schleim K-D, Jacks T, Cunningham P, Feeney W, Frazier EG, Niebauer GW, Zhang D, Chen H, Smith RG, Hickey G,** Effect of MK-677 on growth hormone, IGF-1 and cortisol in beagles before and after hypophysectomy. 10th International Congress of Endocrinology, San Francisco CA, 1996 (Abstract P1–603)
17. **Cheng K, Chan WWS, Butler B, Wei L, Smith RG** 1993 A novel non-peptidyl growth hormone secretagogue. Horm Res 40:109–115
18. **Elias KA, Ingle GS, Burnier JP, Hammonds RG, McDowell RS, Rawson TE, Somers TC, Stanley MS, Cronin MJ** 1995 *In vitro* characterization of four novel classes of growth hormone-releasing peptide. Endocrinology 136:5694–5699
19. **Massoud AF, Hindmarsh PC, Pringle PJ, Brook CGD,** Low dose hexarelin and GHRH leads to massive GH release without significant effects on cortisol and prolactin. 10th International Congress of Endocrinology, San Francisco CA, 1996 (Abstract P1–593)
20. **Guillaume V, Magnan E, Cataldi M, Dutour A, Sauze N, Renard M, Razafindraibe H, Contedevolx B, Deghenghi R, Lenaerts V, Oliver C** 1994 Growth-hormone (GH)-releasing hormone-secretion is stimulated by a new GH-releasing hexapeptide in sheep. Endocrinology 135:1073–1076
21. **Antoni FA** 1986 Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of a 41-residue corticotropin releasing factor. Endocr Rev 7:351–378
22. **Rivier C, Vale W** 1983 Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion *in vivo*. Endocrinology 113:939–942
23. **Thomas GB, Fairhall KM, Robinson ICAF,** GH, ACTH and corticosterone responses to GHRP-6, CRF and AVP in conscious rats. 10th International Congress of Endocrinology, San Francisco CA, 1996 (Abstract P1–592)
24. **Clark RG, Robinson ICAF** 1985 Growth hormone (GH) responses to multiple injections of a fragment of human GH-releasing factor in conscious male and female rats. J Endocrinol 106:281–289
25. **Clark RG, Chambers G, Lewin J, Robinson ICAF** 1986 Automated repetitive microsampling of blood: growth hormone secretion in conscious male rats. J Endocrinol 111:27–35
26. **Kovacs KJ, Antoni FA** 1990 Atriopeptin inhibits stimulated secretion of adrenocorticotropin in rats: evidence for a pituitary site of action. Endocrinology 127:3003–3008
27. **Keller-Wood M, Dallman M** 1984 Corticosteroid inhibition of ACTH secretion. Endocr Rev 5:1–24
28. **Voltz DM, Piering AW, Magestro M, Giustina A, Wehrenberg WB** 1995 Effect of GHRP-6 and GHRH on GH secretion in rats following chronic glucocorticoid treatment. Life Sci 56:491–497
29. **Gertz BJ, Sciberras DG, Yogendran l Christie K, Bador K, Krupa D, Wittreich JM, James I** 1994 L-692,429, a novel nonpeptide growth hormone (GH) secretagogue, reverses glucocorticoid suppression of GH secretion. J Clin Endocrinol Metab 79:745–749
30. **Leal-Cerro A, Pumar A, Garcia-Garcia E, Dieguez C, Casanueva FF** 1994 Inhibition of growth hormone release after the combined administration of GHRH and GHRP-6 in patients with Cushing's syndrome. Clin Endocrinol (Oxf) 42:649–654
31. **Giustina A, Bussi AR, Deghenghi R, Imbimbo B, Licini M, Poiesi C, Wehrenberg WB** 1995 Comparison of the effects of growth-releasing hormone and hexarelin, a novel growth hormone-releasing peptide-6 analog, on growth hormone secretion in humans with or without glucocorticoid excess. J Endocrinol 146:227–232
32. **Wehrenberg WB, Janowski B, Piering AW, Culler F, Jones KL** 1990 Glucocorticoids: potent inhibitors and stimulators of growth hormone secretion. Endocrinology 126:3200–3203
33. **Giustina A, Wehrenberg WB** 1992 The role of glucocorticoids in the regulation of growth hormone secretion: mechanisms and clinical significance. Trends Endocrinol Metab 3:306–311
34. **Fairhall KM, Mynett A, Robinson ICAF** 1995 Central effects of growth hormone-releasing hexapeptide (GHRP-6) on growth hormone release are inhibited by central somatostatin action. J Endocrinol 144:555–560
35. **Swanson LW, Sawchenko PE, Rivier J, Vale WW** 1983 Organization of ovine corticotropin-releasing factor immunoreactive cells and fibres in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186
36. **Dickson SL, Leng G, Robinson ICAF** 1993 Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons. Neuroscience 53:303–306
37. **Dickson SL, Luckman SM** 1997 Induction of c-*fos* mRNA in neuropeptide Y and growth hormone-releasing hormone neurones in the rat arcuate nucleus following systemic injection of the growth hormone secretagogue, GHRP-6. Endocrinology 138: 771–777
38. **Baker RA, Herkenham M** 1995 Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus; neuropeptidergic identity and conse-

quences of adrenalectomy on mRNA levels in the rat. J Comp Neurol 358:518–530
39. **Rivier C, Vale W** 1984 Corticotropin-releasing factor (CRF) acts centrally to inhibit growth hormone secretion in the rat. Endocrinology 114:2409–2411
40. **Katakami J, Arimura A, Frohman LA** 1985 Involvement of hypothalamic somatostatin in the suppression of growth hormone secretion by central corticotropin-releasing factor in conscious male rats. Neuroendocrinology 41:390–393
41. **Holsboer F, von Bardeleben U, Steiger A** 1988 Effects of intravenous corticotropin-releasing hormone upon sleep-related growth hormone surge and sleep EEG in man. Neuroendocrinology 48:32–38
42. **Hisano S, Daikoku S** 1991 Existence of mutual synaptic relations between corticotropin-releasing factor-containing and somatostatin-containing neurons in the rat hypothalamus. Brain Res 545:265–275
43. **Peterfreund RA, Vale WW** 1983 Ovine corticotropin-releasing factor stimulates somatostatin secretion from cultured brain cells. Endocrinology 112:1275–1278
44. **McDowell RS, Elias KA, Stanley MS, Burdick DJ, Burnier JP, Chan KS, Fairbrother WJ, Hammonds RG, Ingle GS, Jacobsen NE, Mortensen DL, Rawson TE, Won WB, Clark RG, Somers TC** 1995 Growth hormone secretagogues: Characterization, efficacy, and minimal bioactive conformation. Proc Natl Acad Sci USA 92:11165–11169
45. **Copinschi G, Van Onderbergen A, L'Hermite-Baleriaux M, Mendel CM, Caufriez A, Leproult R, Bologenese JA, De Smet M, Thorner MO, Van Cauter E** 1996 Effects of a 7-day treatment with a novel, orally active, growth hormone (GH) secretagogue, MK-677, on 24-hour GH profiles, insulin-like growth factor I, and adrenocortical function in normal young men. J Clin Endocrinol Metab 81:2776–2782