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Microglial activating transcription factor 3 upregulation: An indirect target to attenuate inflammation in the nervous system

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Activating Transcription Factor 3 (ATF3) is upregulated in reaction to several cellular stressors found in a wide range of pathological conditions to coordinate a transcriptional response. ATF3 was first implicated in the transcriptional reaction to axotomy when its massive upregulation was measured in sensory and motor neuron cell bodies following peripheral nerve injury. It has since been shown to be critical for successful axon regeneration in the peripheral nervous system and a promising target to mitigate regenerative failure in the central nervous system. However, much of the research to date has focused on ATF3's function in neurons, leaving the expression, function, and therapeutic potential of ATF3 in glia largely unexplored. In the immunology literature ATF3 is seen as a master regulator of the innate immune system. Specifically, in macrophages following pathogen or damage associated molecular pattern receptor activation and subsequent cytokine release, ATF3 upregulation abrogates the inflammatory response. Importantly, ATF3 upregulation is not exclusively due to cellular stress exposure but has been achieved by the administration of several small molecules. In the central nervous system, microglia represent the resident macrophage population and are therefore of immediate interest with respect to ATF3 induction. It is our perspective that the potential of inducing ATF3 expression to dampen inflammatory microglial phenotype represents an unexplored therapeutic target and may have synergistic benefits when paired with concomitant neuronal ATF3 upregulation. This would be of particular benefit in pathologies that involve both detrimental inflammation and neuronal damage including spinal cord injury, multiple sclerosis, and stroke.

KEYWORDS

microglia, ATF3, activating transcription factor 3, innate immune system, central nervous system, adaptive stress response

1. Introduction

Activating Transcription Factor 3 (ATF3) is a basic leucine zipper (bZIP) transcription factor (Hai et al., 1989) that is immediately upregulated in response to cellular stress (Liang et al., 1996; Hai et al., 1999; Kristensen et al., 2013) to regulate target gene expression. Since ATF3's precise DNA binding site depends on which bZIP transcription factor it dimerizes with (Tsukada et al., 2011; Rodríguez-Martínez et al., 2017), ATF3's effect on cellular phenotype is dependent on its context. Given the wide range of stimuli that can trigger a cellular stress response, ATF3 is relevant in a number of pathologies (Allen-Jennings et al.,

2002; Wu et al., 2010; Li et al., 2017). ATF3 was first identified in the injured nervous system when its robust upregulation was observed following peripheral nervous system injury in the cell bodies of motor and primary afferent neurons (Tsujino et al., 2000). Since then, it has been shown to contribute to the successful regeneration of axotomized neurons in the peripheral nervous system (PNS) (Gey et al., 2016; Holland et al., 2019); classifying it as a regeneration associated gene. Despite the failure of ATF3 overexpression to initiate a successful neuronal regenerative response in the central nervous system (CNS) (Seijffers et al., 2007), it may still have important prosurvival functions in neurons (Kole et al., 2020; Seijffers et al., 2014). The majority of research done to date on ATF3 and its effect in the nervous system has focused on its neuronal functions, leaving the role of ATF3 in glia largely unexplored (see Figure 1).

2. ATF3 as an immune regulator

In the immunologic context, ATF3 is considered a negative regulator of innate immune activation (Gilchrist et al., 2006; Suganami et al., 2009). Macrophages, following damage associated molecular pattern (DAMP) or pathogen associated molecular pattern activation (PAMP) of toll-like receptor 4 (TLR4), initiates a signal transduction cascade that ultimately results in a pro-inflammatory phenotype and the secretion of inflammation propagating signals (Gong et al., 2019; Orecchioni et al., 2019). ATF3 is upregulated in response to TLR4 activation where it functions to suppress pro-inflammatory gene expression, at least partially, through the regulation of histone acetylation (Nguyen et al., 2020). The importance of ATF3's immunosuppressive function is highlighted in several the disease models where a loss of ATF3 is

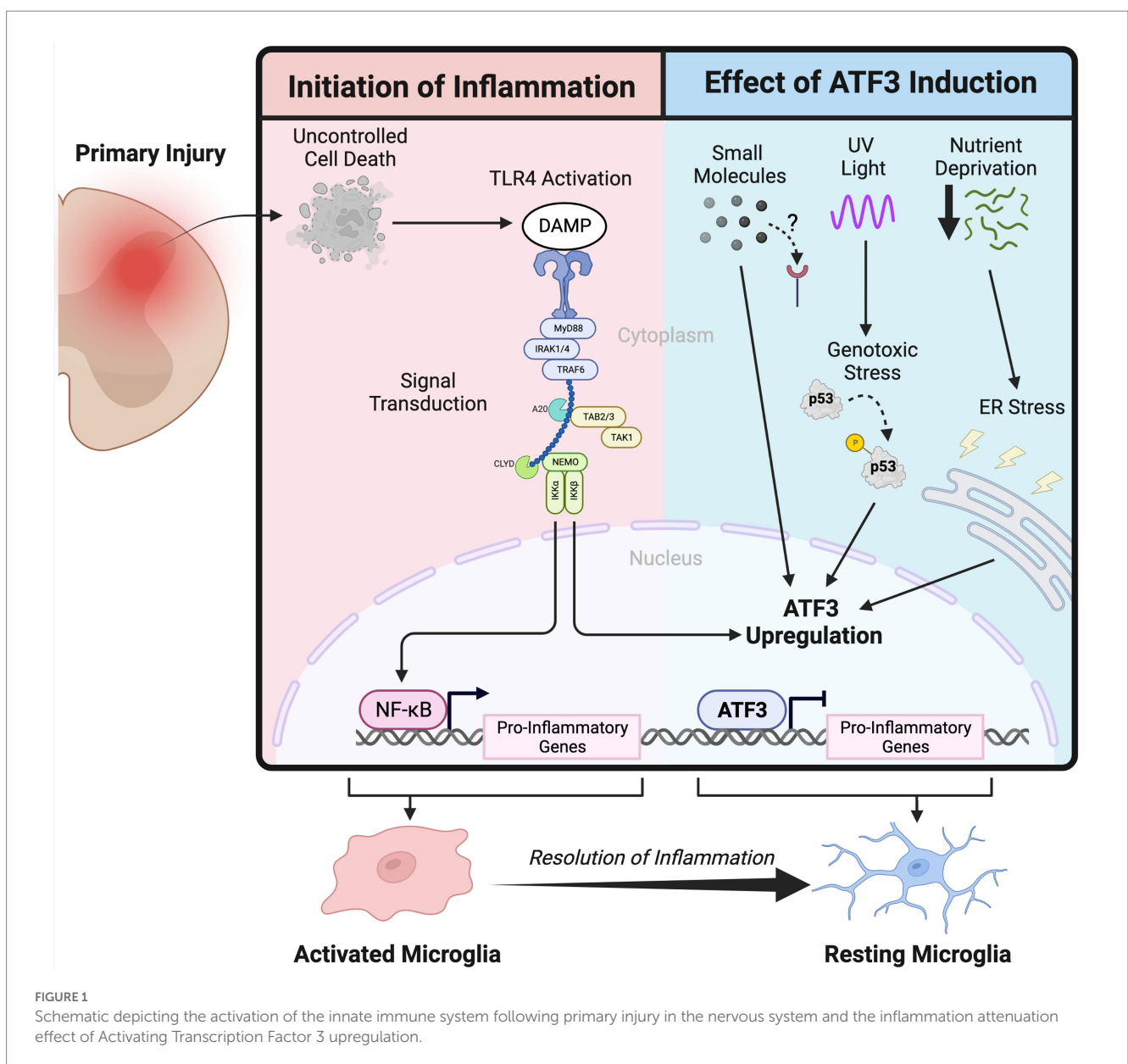


FIGURE 1 Schematic depicting the activation of the innate immune system following primary injury in the nervous system and the inflammation attenuation effect of Activating Transcription Factor 3 upregulation.

detrimental including endotoxic shock (Hoetzenecker et al., 2011), atherosclerosis (Gold et al., 2012), and ischemia reperfusion injury (Rao et al., 2014).

ATF3 is also implicated in regulating the innate immune reaction to microbial infection by coordinating the macrophage interferon (IFN) response. ATF3 deficient macrophages following TLR3 or STING stimulation increase IFN β production compared to wildtype cells (Labzin et al., 2015). IFN β also increases ATF3 expression (Labzin et al., 2015), suggesting that much like in TLR4 signaling, ATF3 functions as part of an IFN negative feedback loop. Interestingly, using a neuronal cell line that is deficient in IFN I synthesis ATF3 upregulation is observed in response to viral infection (Sood et al., 2017); implying the IFN signaling is not strictly required for inducing ATF3 expression. The demonstration of ATF3 upregulation *via* multiple pattern recognition receptors (TLR4, TLR3, STING) further supports the idea that ATF3 is a hub gene that broadly functions to mitigate an excessive macrophage inflammatory response.

The immunomodulatory function of ATF3 however is not limited to macrophages: ATF3 deficient neutrophils produce excessive CCL2 (a chemokine) but paradoxically are deficient in chemotaxis (Boespflug et al., 2014). Mice with ATF3 deficient natural killer (NK) cells control murine cytomegalovirus better than wildtype NK cells, likely through increased IFN γ production (Rosenberger et al., 2008). In T cells, ATF3 is upregulated upon CD4+ Th1 but not Th2 differentiation; notably a knockdown of ATF3 function in these differentiated cells correspond to decreased IFN γ production (Filén et al., 2010). ATF3 regulates the phenotype of a variety of immune cells however its precise function is dependent on the exact cell type and context.

3. Means of ATF3 upregulation

PAMP or DAMP activation is one avenue by which ATF3 can be upregulated, but given its role responding to cellular stress there are additional signals that will induce ATF3. Ultraviolet and ionizing radiation induce ATF3 expression in response to DNA damage through both p53 dependent and independent pathways (Fan et al., 2002). Given its association with DNA damage it is no surprise that ATF3 is overexpressed in cancer (Yan et al., 2017) although its function is dependent on the degree of malignancy (Yin et al., 2008). Amino acid or glucose deprivation, and the latter's subsequent stress on the endoplasmic reticulum is another pathway by which ATF3 upregulation has been achieved (Pan et al., 2003). The multiple avenues that lead to its upregulation has given rise to the idea that ATF3 is the hub of an adaptive response network that ultimately functions to counteract inflammation (Hai et al., 2010).

In the literature there have been reports of small molecules that induce ATF3 expression with varying results. One of the first uses of an exogenous molecules demonstrated to upregulate ATF3 was anisomycin (Liang et al., 1996), an antibiotic and partial protein synthesis inhibitor, and at low concentrations is used to activate stress response kinases (Kallunki et al., 1994). Utilizing an unbiased screen, the topoisomerase I inhibitor camptothecin was identified as another small molecule capable of upregulating ATF3, and when tested *in vivo* was found to enhance peripheral nerve regeneration

(Cheng et al., 2021). Dimethyl itaconate, a cell permeable electrophile was used to upregulate ATF3 and shown to attenuate inflammation in activated macrophages (Bambouskova et al., 2018). These examples prove the principle that ATF3 upregulation is achievable through the administration of small molecules; making ATF3 a potential indirect drug target.

4. Aberrant innate immune activation in the CNS

In the CNS microglia are effectively the resident macrophages and represent the primary regulators of the innate immune response (Li and Barres, 2017; Bachiller et al., 2018). While microglial function includes synapse pruning and modulation, they are classically known to survey their environment and initiate an innate immune response if infection or damage is detected. This innate immune reaction can be paradoxically detrimental as it may propagate the initial insult and contribute to a subsequent wave of injury further damaging the parenchyma. An aberrant activation of the innate immune system is a unifying underlying pathology in several diseases. Following a spinal cord injury (SCI), the initial damage to the neural tissue results in uncontrolled cell death that triggers an inflammatory response thought to contribute to the larger secondary wave of injury (Donnelly and Popovich, 2008); mitigating this inflammatory response has been identified as a potential neuroprotective strategy (Kwon et al., 2004). Multiple Sclerosis is an autoimmune disease that targets the myelin sheath resulting in focal inflammatory lesions throughout the CNS (Filippi et al., 2018); several immunomodulatory drugs are currently in use to mitigate the damage and functional impairment exacerbated by the uncontrolled inflammation (Faissner et al., 2019). Much like in SCI, acute ischemic stroke and intracerebral hemorrhage begins as an initial insult that propagates to a secondary injury, a component of which is caused by uncontrolled inflammation (Shi et al., 2019). A strategy to mitigate the microglial inflammatory response would be broadly applicable to a number of CNS diseases by targeting a common pathology.

5. Discussion

It is the authors' perspective that targeting microglial ATF3 upregulation to mitigate inflammation is an unexplored therapeutic avenue across a range of CNS disease. Utilizing small molecules to induce ATF3 in addition to the canonical PAMP/DAMP signaling has been shown to be feasible and blood brain barrier permeability may not be necessary as it is already compromised in many of the applicable conditions. While ATF3 may already be elevated in sterile microglial activation, targeting additional ATF3 upregulation may result in a faster resolution of inflammation, more cells ceasing to release pro-inflammatory cytokines, or providing a different cellular context to alter the precise downstream regulatory effects. Achieving microglial ATF3 induction may also have synergistic benefits when paired with concomitant neuronal ATF3 upregulation given its well-established role as a regeneration associated gene.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Figure 1 was created with BioRender.com.

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