Biochimica et Biophysica Acta 1862 (2016) 339–351

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Signal transduction and epigenetic mechanisms in the control of microglia activation during neuroinflammation☆

Bozena Kaminska [a,*], Mariana Mota [b], Marina Pizzi [b,c,**]

[a] Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland
[b] Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia and Istituto Nazionale di Neuroscienze, Brescia, Italy
[c] IRCCS, San Camillo Hospital, Venice, Italy

ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 12 October 2015
Accepted 28 October 2015
Available online 30 October 2015

Keywords:
Neuroinflammation
Microglia
Signaling pathways
Transcription regulation
Epigenetics
HDAC inhibitors

ABSTRACT

Activation of microglia is a common denominator and a pathophysiological hallmark of the central nervous system (CNS) disorders. Damage or CNS disorders can trigger inflammatory responses in resident microglia and initiate a systemic immune system response. Although a repertoire of inflammatory responses differs in those diseases, there is a spectrum of transcriptionally activated genes that encode various mediators such as growth factors, inflammatory cytokines, chemokines, matrix metalloproteinases, enzymes producing lipid mediators, toxic molocules, all of which contribute to neuroinflammation. The initiation, progression and termination of inflammation requires global activation of gene expression, postranscriptional regulation, epigenetic modifications, changes in chromatin structure and these processes are tightly regulated by specific signaling pathways. This review focuses on the function of "master regulators" and epigenetic mechanisms in microglia activation during neuroinflammation. We review studies showing impact of epigenetic enzyme inhibitors on microglia activation *in vitro* and *in vivo*, and critically discuss potential of such molecules to prevent/moderate pathological events mediated by microglia under brain pathologies. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

1. Overview of major signaling pathways involved in microglia activation

1.1. Microglia are instigators of neuroinflammation

Microglia are the innate immune cells in the central nervous system (CNS). Microglia actively survey the CNS microenvironment [118] and maintain homeostasis [47] under normal physiological conditions and participate in the inflammatory response [93]. Microglia contribute to normal CNS function by mechanisms such as fine tuning of neural circuits [115] and phagocytosis of apoptotic debris [116]. Microglia are highly reactive to insult or injury. The classical M1 type activation is associated with cytotoxicity and inflammatory responses, whilst the alternative activation M2 type is regarded as being beneficial, and can be further subdivided into M2a, involved in repair and regeneration, the immunoregulatory M2b, and an acquired-deactivation M2c type [23].

Activation of microglia is a common denominator and a pathophysiological hallmark of virtually all neurodegenerative disorders. CNS infections, massive trauma, post-ischemic or toxicity-related necrosis, hemorrhage or accumulation of neurotoxic fibers can trigger inflammatory responses in resident microglia. Inflammation is a feature of several neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and multiple

Abbreviations: AP-1, activator protein 1; APC, antigen-presenting cells; ARE, AU-rich element; ASK, Apoptosis Signal regulating Kinase; ATF-2, Activating Transcription Factor 2; CRE, Cyclic AMP Responsive Element; CBP, CREB binding protein; CsA, ciclosporin A; ERK, Extracellular signal Regulated Kinase; iNOS, inducible Nitric Oxide Synthase; ISRE, interferon-stimulated response element; JNK, c-Jun N-terminal Kinase; LPS, lipopolysaccharide; MCAO, middle cerebral artery occlusion; MCP-1, monocyte chemoattractant protein 1; MAPKAP-K2, MAP kinase Activated Protein Kinase 2/3; MEK, MAP/ERK kinase; MKK, MAP Kinase Kinase; MAP3K, MAP Kinase Kinase Kinase; MLK, Mixed Lineage Kinase; MMPs, metalloproteinases; MyD88, myeloid differentiation factor 88; NIK, NF-κB-inducing kinase; PAK, p21 activated kinase; STAT, Signal Transducers and Activators of Transcription; TAK1, Transforming growth factor Activated Kinase 1; TBP, TATA box binding protein; TGFβ, Transforming growth factor; TLR, Toll-like receptors; TNF, Tumor Necrosis Factor; TRAF, TNF receptor associated factor; TRIF, Toll/IL-1R domain-containing adaptor-inducing IFN-beta.

☆ This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

* Correspondence to: B. Kaminska, Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, 02-093 Warsaw, Pasteur 3 str., Poland.
** Correspondence to: M. Pizzi, Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, V.le Europa, 11, Brescia, Italy.
E-mail addresses: bozenakk@nencki.gov.pl (B. Kaminska), marina.pizzi@unibs.it (M. Pizzi).

http://dx.doi.org/10.1016/j.bbadis.2015.10.026
0925-4439/

sclerosis (MS) [47]. Neuroinflammation can be divided into acute and chronic phases [13]. Inflammatory stimuli activate microglia, which release inflammatory cytokines and phagocyte debris and dead cells, in an attempt to initiate tissue repair and, thus, resolve the inflammatory process. However, if the resolution mechanisms fail or the inflammatory stimulus persists, there is a self-propagating and persistent stage of chronic inflammation, which leads to neurotoxicity and neuronal death [13,22]. Although a repertoire of inflammatory responses differs in various diseases, there is a spectrum of transcriptionally activated genes which encode various endogenous mediators such as growth factors, inflammatory cytokines interleukin 1 (IL-1β), tumor necrosis factor (TNF)-α, interleukin (IL)-6, chemokines Fractalkine (CX3CL1), Macrophage Inflammatory Factor (MIP)-1/CCL3, IL-8, matrix metalloproteinases (MMPs), enzymes producing lipid mediators, nitric oxide and free radicals, all of which contribute to neuroinflammation [1,39,71,98].

Despite different etiology of brain diseases associated to neuroinflammation there are several primary triggers which are recognized by an array of receptors on microglia. After an ischemic insult, damaged neurons release different molecules: ATP, heat shock protein (HSP), adenine dinucleotide (NAD), hyaluronic acid and fibronectin produced by extracellular matrix degradation, nucleic acids, mannose residues proteolytic enzymes and high-mobility group box 1 protein (HMGB1) [84,106]. DAMPs activate immune cells, including microglia [65] which release pro-inflammatory cytokines toxic to already vulnerable neurons [136]. Excessive neuronal release of glutamate, which directly contributes to neuronal death, activates microglia expressing metabotropic glutamate receptors [154,155,112]. Damaged or overactive neurons release or leak purines, including ATP and UTP that activate corresponding receptors. Surveying microglia show a constitutive expression of most receptors (P1 adenosine, P2 ionotropic P2X and metabotropic P2Y receptors) but alter their set of purinergic receptors upon activation. Microglial activation and morphological transformation into amoeboid phagocytic cells after damage require mostly $P2Y_{12}$ and A_3 receptors; A_2A receptors induce amoeboid transformation, $P2Y_{12}$, $P2X_4$, and A_1 receptors interact to induce recruitment to lesion site [90]. The damage signals released from injured cells are collectively called danger-associated molecular patterns (DAMPs) and are recognized and bind by pattern recognition receptors (PRRs).

1.2. TLRs and cytokine signaling in microglia activation upon neuroinflammation

1.2.1. Signaling via Toll-like receptors (TLR) in inflammatory microglia

Toll like receptors (TLRs) recognize a wide variety of danger signals and consequently activate inflammatory cascades [43]. TLRs (with the exception of TLR3) initiate intracellular signaling via recruitment of the intracellular adaptor proteins containing intracellular Toll-IL-1 receptor (TIR) domain. These adaptors include: MyD88 (myeloid differentiation factor 88); TIRAP (TIR-domain-containing adaptor protein), TRIF (TIR-domain-containing adaptor protein inducing IFN-β), and TRAM (TRIF-related adaptor molecule), and can be differentially recruited to the TLR or IL-1 receptors and determine the specificity of signaling (Fig. 1A).

Toll-IL-1 receptors recruit an intracellular adaptor protein MyD88 (myeloid differentiation factor 88) containing TIR domain. When MyD88 or other adaptors are recruited to activated TLRs, either directly (TLRs 5 and 11) or indirectly (TLRs 1, 2, 4, 6), MyD88 engages members of the IRAK (IL-1R-associated kinase) family, to perform auto- and cross-phosphorylation. Phosphorylated IRAKs dissociate from MyD88, and bind TRAF6 (TNF receptor-associated factor 6) and ubiquitin E3 ligases. TRAF6 activates TAK1 (transforming growth factor β-activated kinase) which activates the IKK complex and MAPKK (mitogen activated kinase kinases) as shown in the Fig. 1. The IKK complex phosphorylates IκB proteins which is necessary for the ubiquitination and proteosomal degradation of IκBs and the subsequent nuclear translocation of the transcription factor NF-κB [76,77]. Members of the MAPK family phosphorylate and activate components of the transcription factor AP-1. The endosomal receptors TLR7 and TLR9 can recruit MyD88 which further activates members of the IRAK family that bind TRAF3. Activation of TRAF3 leads to phosphorylation and activation of the interferon responsive factors: IRF3, IRF5, and IRF7. The endosomal TLR3 recruits the Toll-interleukin 1 receptor domain-containing adaptor inducing interferon β (TRIF) which binds kinases TBK1 and IKKε, which activate IRF3. Further, TRIF recruits TRAF6 and RIP-1, which results in activation of MAPK and IKKα/β [76,77].

TLRs and their downstream signaling molecules control microglial behavior during neurodegeneration. TLR4 defects reduced levels of TNFα, IL-1β, IL-10 and IL-17 in the brains of APP (APPswe/PSEN1)-mice [68], diminished amyloid β (Aβ)-induced IL-1β, CCL3, and CCL4 expression in monocytes, reduced microglia activation and increased Aβ deposits which was associated with impairment in cognitive functions [145]. TLR2 deficiency (TLR2-KO) in APPswe/PSEN1 mice increased soluble Aβ in the brain and exacerbated cognitive impairments [132]. MyD88 deficiency reduced brain amyloid β pathology and microglial activation [103,145], although a recent observation using same transgenic mice showed that MyD88 signaling does not significantly affect Aβ-induced microglial activation and cerebral Aβ-deposits [168]. Reconstitution of the immune system of irradiated APP(swe)/PSEN1 mice with MyD88-deficient cells significantly accelerated memory deficits [113]. In a cellular model of PD, microglia cultured from TLR2 KO mice or cells treated with blocking antibody against TLR2a showed reduced production of inflammatory mediators after the exposure to the conditioned media from SH-SY5Y cells overexpressing human α-synuclein. These data implicate TLR2 as a receptor for α-synuclein released from damaged neurons, responsible for microglial activation observed in PD [89].

A growing body of evidence shows that TLRs and their downstream signaling molecules modulate microglial responses during acute neuroinflammation induced by nerve transection injury, intracerebral ischemia and hemorrhage, traumatic brain injury, and hippocampal excitotoxicity [45]. TLR-4 deficiency protected mice against ischemia and retinal ganglion cell axotomy-induced degeneration [86]. TLR4-deficient mice had smaller cerebral ischemia–reperfusion injury and reduced TNF-α and IL-6 levels [45]. Further studies revealed that TLR4-KO (but not TLR3- or TLR9-KO) mice had reduced infarct area in a middle cerebral artery occlusion (MCAo) model. TLR4 was expressed in $CD11b^+$ microglial cells in the ischemic striatum and $CD11b^+$ cell accumulation was reduced in TLR4-KO mice [64]. In ischemic brains, TLR2 expression was induced in lesion-associated microglia and TLR2-deficient mice had decreased brain injury after focal cerebral ischemia [97]. Systemically administered TLR ligands induce tolerance to subsequent ischemic injury and it has been proposed that stimulation of TLRs prior to ischemia reprograms TLR signaling which leads to reduced expression of pro-inflammatory molecules and enhanced expression of anti-inflammatory mediators [108]. Investigation of stroke patients demonstrated that upregulated expression of TLR4 in monocyte subpopulations correlates with severity of acute cerebral infarction [160,172]. TLR2 and TLR4 expression on monocytes were independently associated to poor outcome and correlated with higher serum levels of IL1β, IL6, TNFα, and VCAM1 [10].

Findings in animal models and in humans suggest interactions between TLRs and complement. Microglial cells constitutively express the receptors for complement cascade components: C1q and for cleavage products of C3, that mediate phagocytosis and stimulate cytokine production by microglia. CR3 can regulate the signaling activity of TLR2 and TLR4 via an adaptor protein TIRAP, which acts as a sorting adaptor and facilitates the recruitment of the signaling adaptor MyD88 to either TLR2 or TLR4. Microglia also respond to C1q with a pro-inflammatory activation in CNS diseases with blood–brain barrier impairment [52].

1.2.2. Signaling via a TNF receptor family

TNF receptor family includes lymphotoxin (LT) receptor, Fas, CD40, the low affinity nerve growth factor receptor, TRAIL receptors, RANK