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RESEARCH ARTICLE

Epigenetic Alterations of IL-1ß Pathway Mediates Microglial Neuroinflammation in Neurodegenerative Diseases

Ashley C. Ellis

Academy of the Sacred Heart, 4521 St. Charles Ave, New Orleans, LA, 70115, USA; Acellis408@gmail.com

ABSTRACT: Neurodegenerative diseases (NDDs), including Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), and Amyotrophic lateral sclerosis (ALS), are characterized by detrimental, chronic neuroinflammation primarily generated by the aberrant inflammatory response of microglia, the resident immune cells of the brain. The inflammatory signature of microglia is mediated by pro-inflammatory cytokines, including Interleukin 1beta (IL-1ß). Although upregulated IL-1ß is speculated to be a key component of microglia's aberrant immune response in NDDs, the mechanisms causing IL-1ß upregulation are unclear; however, alterations in the microglial epigenetic profile are emerging as a possible contributor to increased IL-1ß. This review summarizes updated discoveries on the role of epigenetic alterations in influencing microglial IL-1ß pathways in NDDs. It specifically focuses on elucidating the impact of microRNAs, histone acetylation agents, histone methylation agents, and DNA methylation agents on the inflammatory profile of microglia. The findings summarized below emphasize epigenetics as a key component of neuroinflammation in NDDs and suggest that future therapeutic developments target the dysregulation of epigenetic agents in microglia.

KEYWORDS: Biomedical and Health Sciences; Genetics and Molecular Biology of Disease; Neurodegenerative Diseases; Microglia; Epigenetics; Interleukin 1-beta.

Introduction

Pathology and Neuroinflammatory Signature: Introduction to Neurodegenerative diseases :

Neurodegenerative diseases (NDDs) are chronic, progressive neurological disorders that inhibit or degrade normal cognitive and motor functions of the healthy brain.[1] Neurodegenerative diseases such as; Alzheimer's Disease (AD), Amyotrophic lateral sclerosis (ALS), Parkinson's Disease (PD), and Huntington's Disease (HD) are characterized by neuronal and synaptic loss, neuroinflammation, and consequential cognitive or motor decline.[2] Although AD, ALS, PD, and HD progress in various brain regions and generate diverse clinical symptoms, they share some similarities in basic pathology and pathogenesis. They are classified as proteinopathies – abnormal intracellular or extracellular protein aggregation within specific central nervous system (CNS) regions. ALS, for example, is characterized by intra-neuronal inclusions comprised of an accumulation of transactivation response DNA binding protein 43 (TDP-43), which affects the brain and spinal cord's upper and lower motor regions, causing a loss of motor function.[2] PD, the second most common NDD worldwide, is characterized by the accumulation of cytoplasmic Lewy bodies in the substantia nigra resulting from the aggregation of the protein α-synuclein. In HD, intranuclear inclusions of the protein huntingtin (HTT) appear throughout the striatum and cortex, resulting in progressive atrophy of these distinct regions of the brain.[3] There are multiple risk factors for the development of neurodegenerative diseases, including hereditary genetic mutations, previous illness, and aging.[4,5]

The most common neurodegenerative disease worldwide is AD, accounting for an estimated 60-80% of all dementia cases.[6] AD is uniformly characterized by the abnormal aggregation of two proteins: amyloid-beta (Aß) and tau. As Aß accumulates in the extracellular matrix of the CNS, it is initially concentrated around the hippocampus and neocortex and gradually permeates other areas of the brain as the pathology progresses. Tau aggregates in intraneuronal inclusions in AD, forming neurofibrillary tangles that compromise neuronal function and lead to neuronal death.[2] These protein aggregates cause a progressive loss of memory and general cognitive function, making the disease the sixth leading cause of death in the United States.[7]

Microglia and Neuroinflammation :

The immune response in the CNS plays a crucial role in regulating the pathology and progression of neurodegenerative diseases. Specifically, microglia, the resident immune cells in the CNS, have been identified as an essential and complex component of NDD progression.[3] In the healthy brain, microglia are responsible for an extensive range of functions, including neuronal monitoring, homeostasis, phagocytosis, and responding to invasion or injury.[8,9] They are highly interactive with their extracellular surroundings, as exhibited by their high concentration of membrane signal receptors and cellular processes. In response to a homeostatic shift in the brain parenchyma due to injury or invasion, microglia will assume an "activated" role characterized by functional, genotypic, and phenotypic changes.[8] When activated, microglia typically polarize to an M1 state defined by the reduction of cellular processes, an enlarged soma, a shifted transcriptome, and increased production of pro-inflammatory markers.[9,10] These alterations primarily serve to kill off any invading specimens and provide a protective response to immune challenges in the brain; however, in NDDs and aging, this response is exacerbated, resulting in a

DOI: 10.36838/v4i2.2

in the initial stages of AD development, microglia assume a predominately beneficial role in ameliorating AD pathology by surrounding Aß plaques to phagocytize the aggregated protein.[10] However, microglial regulation is impaired as the disease progresses, resulting in chronic neuroinflammation and the detrimental upregulation of pro-inflammatory cytokines such as TNF-α, IL-1ß, IL-6, IL-8, IL-33, and more. This pro-inflammatory shift in microglial function contributes to neuronal death and neurodegeneration, which in turn further augments neuroinflammation, resulting in dangerous and inevitable cycles of disease progression.[10,12]

Epigenetic Alterations Impact Neurodegeneration:

Although it was previously determined that microglia undergo a pro-inflammatory shift in NDDs and aging, the mechanisms behind this phenomenon remain elusive.[3,9-11,13] Some researchers have begun studying age-related epigenetic modifications to elucidate a process that causes the aberrant microglial immune response commonly observed in neurodegenerative diseases.[13] In recent years, the field of epigenetics has experienced a sharp increase in attention as groundbreaking studies reiterate the importance of epigenetic mechanisms in mediating genetic function and disease pathologies.[13-15] The term epigenetics refers to the mechanisms that influence gene expression without altering or involving DNA sequences. These mechanisms include DNA methylation and histone modifications such as acetylation, methylation, phosphorylation, ubiquitination, and more. Another mechanism involves the use of microRNA or non-coding RNA in silencing the mRNA expression of certain genes.[13-15] These alterations have been implicated in many diseases, including cancers, autoimmune disorders, and more recently, neurodegenerative diseases.[13,14] Indeed, aging was determined as the primary risk factor in developing AD, and multiple studies have linked aging with aberrant epigenetic regulation.[5,16-18]

In the case of neurodegenerative diseases like AD, epigenetic abnormalities are believed to impact disease pathology via altering the processes by which microglia produce and release specific pro-inflammatory cytokines in response to immune challenges.[13] In many studies involving microglia, the bacterial immune challenge lipopolysaccharide (LPS) is used to activate microglia; however, in NDDs, immune challenges that activate microglia originate from the excess of protein aggregates that are abnormally accumulating throughout the CNS, including Aß, tau, α-synuclein, and TDP-43. Pro-inflammatory cytokines that are upregulated in activated microglia include those of the interleukin family, such as interleukin 1-Beta (IL-1ß), along with tumor necrosis factor-alpha (TNF-α), and many others.[13] Increasing evidence has supported the argument that epigenetic alterations may contribute to the elevation of these cytokines seen in many NDDS.18 Although these potent cytokines have begun to be studied more in-depth, knowledge concerning the specific molecular mechanisms and pathways contributing to the production of IL-1ß at the epigenetic level must be elucidated to expand our understanding of the complex role of microglia in NDDs.

■ Discussion

Epigenetic changes in IL-1ß pathways:

IL-1ß: Overview

While IL-1ß is a crucial contributor to neuroinflammatory pathology seen in NDDs, it is also vital for an appropriate immune response of microglia in the healthy brain. It has even been found to have neuroprotective, tissue-remodeling, and reparative roles within the CNS.[19] Indeed, in specific contexts, temporary neuroinflammation resulting from IL-1ß may be primarily beneficial in responding to immune challenges; however, IL-1ß is currently understood as having a neurotoxic, deleterious role in neurodegenerative diseases.[12,19] For example, the rapid induction of IL-1ß production was found to lead to neuronal damage and death, and additional clinical studies have identified increased IL1ß expression in the postmortem brain tissue of individuals with AD or PD.[20] This deleterious role is essentially a consequence of the aberrant increase in IL-1ß production experienced in microglia in aging and NDDs, which results in chronic neuroinflammation.[21]

While some specific aspects of IL-1ß secretion remain unknown, scientists have elucidated a conventional, NLRP3-dependent two-step pathway for myeloid IL-1ß production.[22,23] Initially, a primary signal indicating an immune challenge (i.e., LPS) is received by a pattern recognition receptor such as toll-like receptor 4 (TLR4), which then stimulates a cascade of processes that activate the nuclear factor kappa-b (NF-kB) complex. NF-kB will induce transcriptional activity to produce components of the NLRP3 inflammasome (i.e., the adapter apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and the effector protease caspase-1), as well as pro-IL-1ß.[22,24] Upon stimulation by a secondary signal (pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)), the NLRP3 inflammasome will be activated and cleave pro-IL-1ß via Caspase-1, thereby producing mature IL-1ß to be secreted by the cell.[22] Like most neurological processes, IL-1ß production in the CNS is not yet fully understood. For example, although the primary IL-1ß production pathway is dependent on NLRP3, the synthesis of the active Caspase-1 that is necessary to cleave pro-IL-1ß can be performed by multiple inflammasomes, including NLRC4, NLRP6, AIM2, and NLRP1b.[25] Despite this, our current grasp provides valuable insight into the molecular mechanisms allowing for the IL1ß expression that is observed in NDDs, and recent findings have implicated epigenetic changes as a driving factor of IL-1ß-mediated neuroinflammation in the CNS.

Acetylation:

Histone acetylation is the addition or removal of acetyl groups from lysine tails of histones to modulate alterations in chromatin folding. Histone acetyltransferases (HATs) loosen chromatin structures to create euchromatin, thus allowing transcription factors easier access to DNA binding regions and increased gene expression. By adding an acetyl group to lysine tails, the positive charge of histones is neutralized, and the attraction between histones and the negatively charged phosphate backbone of DNA is weakened.[26] Opposite from HATs, histone deacetylases (HDACs) remove acetyl groups

DOI: 10.36838/v4i2.2

from lysine residues to increase the charged attraction between positive histones and negative DNA strands, thus condensing chromatin structure into heterochromatin. This process inhibits transcription factors' access to DNA binding regions and reduces gene expression. Both HATs and HDACs are crucial for mediating gene expression and, therefore, cellular protein synthesis. The HAT-HDAC equilibrium is vital in maintaining proper expression; a deficiency in either protein would offset the careful balance of histone acetylation, resulting in hyper- or hypo- acetylation of histones. Such a shift would have severe implications on cellular homeostasis.[27]

The impairment of the HAT-HDAC equilibrium seen in some NDDs is primarily a consequence of irregular HDAC regulation. Certain HDACs, such as histone deacetylase 4 (HDAC4), have been shown to impact IL-1ß pathways; however, the exact mechanism has not yet been elucidated.[28] Some evidence suggests that HDACs can result in the upregulation of pro-inflammatory proteins, including *IL-1ß*, through a signaling cascade involving TLR4. Upon stimulating murine macrophages with LPS, a TLR4 agonist, IL1ß expression was reduced in cells treated with the HDAC inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA).[29] IL-1ß was also significantly reduced in LPS-stimulated macrophages treated with the HDAC4-specific siRNA.29 These results indicate that the upregulation of HDACs – particularly HDAC 4 – may contribute to the sensitization of the TLR4 pro-inflammatory pathway that produces aberrant *IL1ß* expression. Another proposed role of HDACs in contributing to disease pathology is their involvement in regulating microRNA activity. One study found that HDAC inhibition increased the expression of MiR-146a, a negative regulator of IL-1ß.[30] Histone deacetylase inhibitors SAHA and LBH589 were able to significantly upregulate MiR-146a expression by reducing deacetylation of its promoter. Consequently, MiR-146a downregulated the IL-1ß inflammatory response by suppressing interleukin-1 receptor-associated kinase-1 (IRAK1), a molecule that interacts with other components to activate *IL1β* transcription. Although these results were observed in fibroblast-like synoviocytes, their shared mesenchymal origination with microglia may indicate that a similar mechanism is at work in NDD-associated microglia.[30]

While studies involving HDAC inhibitors have provided promising results, therapeutic options for mitigating IL-1ß production may also concern facilitating the upregulation of certain class II HDACs, such as Sirtuin 1 (SIRT1), an HDAC that acts by deacetylating other proteins in addition to histones. Unlike other HDACs, SIRT1 deficiency is widely associated with cognitive dysfunction in aging and NDDs because of its inhibitory effects on IL-1ß production in the healthy brain. One role of SIRT1 in microglia is to activate DNA methyltransferase 1 (DNMT1), a protein that, in tandem with other DNA methyltransferases, is responsible for the methylation of the *IL1ß* promoter at specific CpG sites. When SIRT1 is present, its activation of DNMT1 allows for higher methylation of the *IL1ß* promoter, resulting in decreased expression of *IL1ß*. DNMT1 inhibition by 5-Azacytidine decreased methylation at a CpG site 215 bp upstream from the *IL1ß* transcriptional start site, which correlated with decreased cognitive function in mice.[31] SIRT1 upregulation has also demonstrated a neuroprotective role by deacetylating lysine 310 on the RelA/p65 segment of NF-kB, resulting in the reduced production of pro-inflammatory cytokines activated by the NF-kB cascade, including IL-1ß.[32,33]

Despite the hope that HDAC inhibitors will be developed as a clinical treatment in NDDs, there remains uncertainty regarding their exact impacts on pro-inflammatory cytokines. For example, results suggesting the effectiveness of the comprehensive HDAC inhibitor Trichostatin A (TSA) in ameliorating NDD-associated neuroinflammation have varied among studies. A study by Hsing et al. demonstrated that TSA mitigated neuroinflammation and cognitive dysfunction in LPS-treated mice by reducing multiple pro-inflammatory cytokines, including IL-1ß.[34] Another study observed a positive correlation between TSA and IL-1ß production in monocytes, and still, another indicated that TSA had no significant impact on IL-1ß production.[29,35] These varied outcomes indicate a flawed or incomplete understanding of the comprehensive role of HDACs in regulating IL-1ß production. In the future, elucidating this role should be a major focus to develop therapeutic options for treating neuroinflammation.

Along with that histone deacetylation, histone acetylation homeostasis is also impaired in neurodegenerative diseases. A global reduction of HATs in the diseased brain is associated with the irregular acetylation of specific proteins involved in neuroinflammation. While not widely observed in microglia, the reduction of acetyltransferases such as p300 and CBP was especially relevant in propagating neuronal loss via apoptosis induced by the activation of the inflammatory protein caspase-6.[36] Along with regulating CBP/p300, caspase-6 is a mediator of NLRP3 inflammasome activation, and caspase-6-/- mice exhibited significantly reduced IL-1ß levels.[37] One study determined that the TTK210-mediated activation of p300 and CBP promoted neurogenesis and memory duration in mice models, suggesting that this therapeutic target might help treat cognitive loss seen in aging and NDDs.[38] The underlying mechanisms causing acetylation dysregulation are not fully understood; however, many have looked towards age as a major risk factor. Peleg *et al*. demonstrated the significance of aging in a study that suggested a role for age-related acetylation irregularities at histone 4 lysine 12 (H4L12) in contributing to impaired memory consolidation in mice.[39]

MicroRNAs:

MicroRNAs (miRNAs) are a family of non-coding RNA molecules that influence gene expression by assembling into an RNA-induced silencing complex (RISC). When mature miRNA assembles into a RISC, the complex will bind with the base pairs of target mRNA, allowing the RISC to inhibit gene expression by cleaving mRNA or repressing mRNA translation.[40] It was recently determined that miRNAs play an influential role in the CNS through the regulation of microglial activation, and there is evidence to suggest that miRNA dysregulation in neurodegenerative diseases may contribute to chronic neuroinflammation and NDD pathology via the aberrant regulation of IL-1ß production in microglia.[13] In a study

by Cardoso *et al*., miR-155 was a pro-inflammatory mediator that was upregulated after introducing an LPS challenge to an N9 murine microglial cell line and primary microglia cultures. This upregulation of mi-155 led to a substantial decrease of the negative inflammatory regulator SOCS-1, resulting in an excess of microglial IL-1ß.[41] The positive correlation between mi-155 and IL-1ß was especially relevant in the pathogenesis of PD, as α-synuclein induced inflammation was significantly attenuated in MiR-155$^{-/-}$ mice.[42] Although there exists many biological factors that may contribute to the observed results, these findings suggest that miR-155 might be a feasible target for future therapeutic approaches, specifically those targeting PD.

Some miRNAs can influence IL-1ß production by mediating the expression of other epigenetic agents influencing IL1ß genes, including the deacetylase, Sirtuin 1 (SIRT1).[43] As previously indicated, SIRT1 is a critical epigenetic mediator of IL-1ß. In SIRT1 deficiency – as observed in aging and NDDS -- IL-1ß is upregulated due to hypomethylation of this cytokine's promoter.[18] In a study carried out by Li *et al*., the microRNA miR-204 was believed to inhibit SIRT1 expression and therefore negatively correlate with the methylation of the IL1ß promoter, resulting in the upregulation of IL-1ß and other pro-inflammatory cytokines in N9 cells.[43] Another study demonstrated that the microRNA miR-129-5p improved cognitive function in mouse models of AD by inhibiting SOX6 expression, which correlated with decreased IL-1ß.[44] Similarly, overexpression of miR-181-c attenuated inflammation in LPS-stimulated macrophages by inhibiting the TLR4/NF-kB signaling pathway and thereby decreasing *IL1ß* expression.[45] MiR-181-c was found to be under-expressed in the blood, brain, and cerebrospinal fluid of AD patients, a discovery that may implicate miR-181-c deficiency as a potential contributor to IL-1ß upregulation and neuroinflammation.[46] Interestingly, one study found that miR-181-c deficiency increased the enzyme Serine palmitoyltransferase (SPT). Upregulated SPT might account for an increase in Aß levels since SPT is known to positively correlate with Aß aggregation in sporadic AD.[47] Taken together, the current research on microRNAs as a critical regulator of the microglial IL-1ß response in neurodegenerative diseases supports the idea that targeting microRNAs in next-generation therapeutics would likely be a valuable tool in alleviating NDD neuroinflammation.

Methylation:

DNA Methylation :

At the epigenetic level, methylation can refer to DNA methylation or histone methylation. DNA methylation is characterized by the covalent binding of methyl groups to the C5 position of cytosine-guanine dinucleotide (CpG sites) of DNA.[48] Methyltransferases (DNMTs) link methyl groups to CpG rich regions, called CpG islands, which are typically located upstream of gene promoters. The methylation of CpG islands restricts the binding of transcription factors and polymerases to gene promoters and thus reduces gene expression.[49,50] It is generally understood that genes corresponding with DNA promoters that have dense arrangements of methylated CpG islands will be inactive. Although there are many complex factors at play regarding protein synthesis, high promoter methylation typically correlates with silenced gene transcription, which results in decreased protein production. In NDDs, global DNA methylation is significantly altered.[50] Recently, studies linking DNA methylation alterations in NDD-associated microglia and worsened cognitive deficits have begun to shed light on the role of methylation agents in microglia-induced neuroinflammation.[18,19]

One study in which adult and aged mice were injected with an LPS challenge found that mRNA levels of methylating agents DNMT1, DNMT3a, and DNMT3b were reduced in isolated microglia, while IL-1ß production was heightened. Further, IL-1ß levels were highest in microglia treated *in vitro* with both LPS and the DNMT-inhibiting agent, 5-Azacytidine (5-aza-dC).[18] These observations indicate that, although the IL1ß promoter does not overlap a CpG island, the methylation of CpG islands near the *IL1ß* promoter may still notably impact the expression of *IL1ß*.[31] Interestingly, while 5-aza-dC upregulated *IL1ß* expression, it reduced the expression of Casp 1 and NLRP3 – proteins necessary in forming the NLRP3 inflammasome that cleaves pro-IL-1ß to produce mature IL-1ß. These results indicate that there remain uncertainties about how various proteins involved in the IL-1ß inflammatory pathway interact. However, these findings indicate that the reduction of DNMTs and consequent hypomethylation of the proximal IL1ß promoter is positively correlated with upregulated IL-1ß production. Moreover, aged and LPS-stimulated mice with decreased DNMT levels exhibited reduced cognitive function -- as determined through an administered locomotor test -- compared to younger and control mice samples, which was attributed to upregulated IL1ß expression.[18]

There are few studies available that further expand on the role of DNA methylation in IL-1ß regulation of NDD-associated microglia; however, the importance of DNA methylation in regulating *IL1ß* expression is further demonstrated in several studies that focus on non-microglia cells, including chondrocytes and THP-1 cells. In one study, human chondrocytes were treated with an immune challenge, and *IL1ß* and DNMT1 expression levels were measured. While control chondrocytes did not express significant IL1ß mRNA, chondrocytes treated with 5-aza-dC experienced a 5-fold increase in *IL1ß* mRNA levels, which translated into the IL-1ß protein. The % methylation at CpG sites specific to the *IL1ß* promoter in 5-aza-dC-treated chondrocytes was also considerably reduced.[51] Interestingly, another study determined Zaluzanin D (ZD), a type of sesquiterpene lactone from the leaves of *Vernonia Arborea*, as a possible mediator of DNA methylation of CpG islands found in promoter regions coding for the transcription of matrix metallopeptidase 9 (MMP-9). MMP-9 is a known activator of IL-1ß -mediated inflammation, and multiple studies have supported the ability of MMP-9 to cleave proIL-1ß in an NLRP3 and Caspase-1-independent pathway in numerous immune cell variances.[52,53] Consequently, the ZD-mediated decrease in MMP-9 expression also correlated with reduced IL-1ß production

DOI: 10.36838/v4i2.2

in THP-1 cells[54], which may suggest an epigenetic-based therapeutic role for ZD in ameliorating IL-1ß inflammation.

While the cell variances observed in the aforementioned studies differ from microglia in function and phenotype, their shared mesenchymal-derived origination and the similar results determined from microglia-specific studies involving IL-1ß indicate that the conclusions extrapolated from studies including varying cell types may also pertain to microglia stimulated by NDD-associated immune challenges.[18,51-54] Still, more reliable insight into the impact of MMP-9, ZD, DNMTs, and other methylation-associated agents on microglial IL-1ß production can and should be elucidated in future research. Such knowledge would likely provide a more comprehensive understanding of the role of DNA methylation in microglia-induced neuroinflammation and neurodegeneration.

Histone Methylation:

Differing from DNA methylation, histone methylation is the covalent binding of methyl groups to positively charged histone tail residues, including arginine and lysine.[13,55] This binding is primarily facilitated by histone methyltransferases (HMTs), which can be categorized by histone tails and further by degree: lysine tails can be mono-, di-, or tri-methylated, and arginine tails can be mono-, symmetrically, or asymmetrically di-methylated. Unlike histone acetylation, histone methylation does not operate via altering the electronegative attraction between DNA and histones; instead, it utilizes complex enzymatic and catalytic activities to influence chromatin structure. Like histone acetylation, histone methylation remains in a critical balance between histone methyltransferases, such as H3K27me3, and histone demethylases, such as Jumonji domain-containing 3 (Jmjd3), which remove methyl groups from histone tails.[56] This balance is believed to be interrupted in NDDs and is implicated in aberrant production of IL-1ß in the microglial immune response.[57,58]

Jumonji domain-containing 3 (Jmjd3) -- a type of demethylase that removes the trimethyl group from histone H3K27 -- is an essential regulator of both pro-inflammatory and anti-inflammatory markers, and it is reduced in age and PD.[59] Jmjd3 is a crucial two histone demethylase because its expression prompts microglia to polarize to an anti-inflammatory (M2) activation state, which can combat adverse effects of pro-inflammatory (M1) microglia that often proliferate aberrantly in NDDs. One study determined that the suppression of Jmjd3 in LPS-treated microglia cell cultures results in exaggerated M1 polarization and was associated with the upregulation of pro-inflammatory cytokines, including IL-1ß.[58] Jmjd3 suppression and its impact on *IL1ß* expression were also evaluated in murine macrophages stimulated with LPS and interferon-γ (IFN-γ). Interestingly, this study found that Jmjd3-mediated methylation of the IL1ß promoter increased upon stimulation, a phenomenon that positively correlated with *IL1ß* expression.[57]

The opposing conclusions presented in these studies may be attributed to Jmjd3's role as a mediator of both M1 and M2 markers. As demonstrated in the two studies, Jmjd3 is thought to impact gene expression of *IL1ß* by regulating the methylation level of its promoter; however, Jmjd3 also regulates the methylation of promoters coding for the transcription of M2 markers, including arginase 1 and CD206. Therefore, the suppression of Jmjd3 not only attenuates *IL1ß* expression but may also result in the hypermethylation of genes transcribing anti-inflammatory proteins, effectively reducing their expression and subsequently reducing the potential of microglia to resist M1 polarization. Ultimately, a significant downregulation of anti-inflammatory M2 proteins that generally oppose the proliferation of pro-inflammatory cytokines like IL-1ß could result in a disruption of protein homeostasis that cannot be controlled solely by the reduction of IL-1ß production, as seen in Jmjd3 suppression.[57-59] The impact of DNA methylation on M2 markers in worsening IL-1ß associated inflammation is also apparent through the methylase H3K27me3. H3K27me3 is negatively correlated with brain-derived neurotrophic factor (BDNF), an important anti-inflammatory molecule and M1 antagonist. H3K27me3 was upregulated in aged mice, which contributed to impairments in memory consolidation.[60] Further, BDNF-injected mice exhibited a decrease in IL-1ß levels.[61] These findings indicate that the hypermethylation of the BDNF promoter through an increase in H3K27me3 might contribute to upregulated IL-1ß levels in microglia.[12,61]

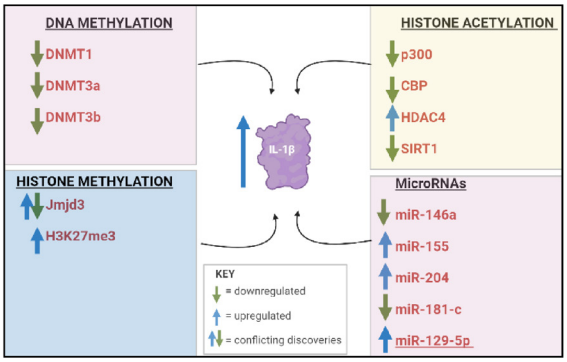

Figure 1: Dysregulated epigenetic agents possibly contributing to increased IL-1ß. Epigenetic agents that are abnormally downregulated in NDD-associated, activated microglia include MicroRNAs miR-146a and miR-181-c, histone acetylases p300 and CBP, histone deacetylase SIRT1, and DNA methylases DNMT1, DNMT3a, and DNMT3b. Epigenetic agents that are abnormally upregulated in NDD-associated, activated microglia include MicroRNAs miR-155, miR-129-5p, and miR-204, histone deacetylase HDAC4, and histone methylase H3K27me3. Histone demethylase Jmjd3 has been observed correlating with increased IL-1ß in NDD-associated microglia when both upregulated and downregulated.

■ Conclusion

Despite the steps that have been taken to elucidate the processes of microglial neuroinflammation in NDDs, the exact pathogenesis of IL-1ß-associated neuroinflammation remains a complex and unclear topic within the field of neurology. The prospect that multiple, intricate molecular mechanisms can generate a singular biological effect has made the neuroinflammatory process challenging to study and observe, especially through *in vivo* models. Many of these epigenetic molecular mechanisms have been implicated in the pathology of NDDs, including in microglial neuroinflammation. Systems influencing microRNAs, histone acetylation, DNA methylation, and histone methylation have been observed as having a particular impact on the production of the cytokine IL-1ß, a critical pro-inflammatory marker of microglia in

NDDs. Such systems were often dysregulated in NDD-associated mice models or microglial cultures/cell lines, which contributed to the aberrant production of IL-1ß and, in some studies, consequent cognitive decline.

While the studies discussed in this review advance our understanding of the role of epigenetics in NDDs, they also demonstrate a significant lack of knowledge concerning specific epigenetic mechanisms and their impact on microglia. For example, many studies have observed an important link between epigenetic alterations and IL-1ß production in various cell lines, including chondrocytes, synoviocytes, THP-1 cells, and more; however, the conclusions drawn from such studies have yet to be observed in microglia. Indeed, a lack of evidence linking epigenetic alterations and IL-1ß production in microglia may be preventing the development of NDD-specific therapies targeting the regulation of epigenetic systems impacting neuroinflammation. The constantly evolving classification of microglial activation presents an additional challenge in applying the insights regarding epigenetics to developing therapies that aim to alleviate neuroinflammation; recent reports have called into question the reliability of the M1/M2 polarization scheme of activated microglia, arguing that such classification may oversimplify the true variability of microglia behavior amid immune challenges.[62] Another limitation is whether studies involving murine microglia or live mice models can be applied to human models, as the two have significant biological and neurological differences. Despite such obstacles, this updated review presents a compilation of evidence suggesting that the dysregulation of multiple epigenetic mechanisms in microglia contributes to aberrant IL-1ß production and consequent chronic neuroinflammation, a major hallmark of neurodegenerative diseases such as AD, PD, HD, and ALS. These conclusions present epigenetic changes as a critical subject of future studies aimed at clarifying the molecular mechanisms responsible for NDD-associated microglia activation and neuroinflammation.

Acknowledgements

The creation of this review would not have been possible without the Horizon Academic Research Program team and the guidance of Mrs. Paula Martorell at the Department of Neurodegenerative Disorders at the University Hospital Bonn.

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Author

Ashley Ellis is a senior at The Academy of Sacred Heart in New Orleans, Louisiana. She enjoys learning about biological processes and volunteering in her community. At college, she plans to major in biomedical engineering.

DOI: 10.36838/v4i2.2