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Neuroinflammation in Ischemic Stroke: Focusing on the Critical Role of Microglia

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Abstract

Ischemic stroke remains a major cause of mortality and persistent neurological deficits worldwide, with its pathophysiology deeply intertwined with neuroinflammation and various cell death mechanisms. This review focuses on the pivotal role of microglia in mediating the neuroinflammatory response during ischemic stroke and highlights the complex interactions between microglia and diverse programmed cell death pathways, including necroptosis, autophagy, pyroptosis, and ferroptosis, and the newly proposed efferocytosis. Furthermore, we discuss the significant influence of the gut microbiota on stroke outcomes through the gut-brain axis. Recent advancements in understanding these interactions offer promising insights into novel therapeutic approaches that could enhance neuroprotection and aid neurological recovery. The review concludes with perspectives on future research directions, stressing the importance of integrating advanced omics technologies and sophisticated animal models to explore the underlying molecular mechanisms and develop rapid, effective therapeutic interventions within the limited time window available post-stroke.

Keywords: Ischemic Stroke, Neuroinflammation, Microglia, Programmed Cell Death, Efferocytosis, Gut-Brain Axis.

1. Introduction

Stroke remains a leading cause of death and disability worldwide, exerting a significant burden on healthcare systems(1). Ischemic stroke, accounting for approximately 75-80% of all stroke cases, is particularly challenging due to its sudden onset and the severe neurological deficits it can cause(2,3). This type of stroke results from an obstruction within a blood vessel supplying blood to the brain, leading to a cascade of debilitating events that can severely impact a patient's quality of life(3). The primary treatment for acute ischemic stroke involves the intravenous administration of tissue-type plasminogen activator (t-PA), which can restore cerebral perfusion by dissolving blood clots and potentially rescuing dying cells within the ischemic penumbra(4). However, the effectiveness of t-PA is constrained by a narrow therapeutic window of 3-4.5 hours post-onset, after which the risk of hemorrhagic complications increases significantly, limiting its widespread use(3,4). As an alternative, intra-arterial thrombectomy has emerged, offering promise but also presenting significant challenges and potential complications(5). These treatments, while critical, are frequently hampered by their timing requirements and the intricate pathophysiology of the disease.

The development and progression of ischemic stroke involve complex, interrelated pathophysiological processes including excitotoxicity, oxidative stress, mitochondrial dysfunction, and extensive neuroinflammation, all contributing to the disruption of the blood-brain barrier (BBB) and subsequent neuronal damage(6,7). Prolonged and intense inflammatory responses play a pivotal role in exacerbating brain damage. Extensive research has linked post-stroke inflammation with BBB breakdown, vasogenic edema, hemorrhagic transformation, and the restoration of neurological functions(8–10). Neuroinflammation is a pervasive feature at all stages of ischemic stroke, initiated by the activation of damage-associated molecular patterns (DAMPs) released from injured or dying cells(9,11). These DAMPs, including adenosine, heat shock proteins, high mobility group box 1 (HMGB1), and interleukin-33, are recognized by immune cells, triggering a variety of downstream signaling pathways(6,9). During the inflammatory process, various immune cells such as microglia, macrophages, and T lymphocytes are activated, producing inflammation-related cytokines, interferons, and chemokines(6). The upregulation of adhesion molecules promotes the adhesion of leukocytes on the vascular surface, intensifying the infiltration of immune cells. An abundance of pro-inflammatory stimulates endothelial cells and pericytes, leading to the disruption of the BBB(12). These factors collectively facilitate the adhesion and infiltration of immune cells into the CNS, culminating in the breakdown of the BBB and the formation of cytotoxic edema.

Microglia, central to the neuroinflammatory response in ischemic stroke, are the principal immune cells within the CNS and play pivotal roles in its pathophysiology. They play multifaceted roles not only in regulating neurogenesis, the fate of oligodendrocyte precursor cells, and myelination but also in influencing neuronal survival through critical functions(7,13). Microglia phagocytose cellular debris, release neurotrophic factors and reactive oxygen species, and regulate synaptic pruning controlled by the complement system. Furthermore, microglia engage in complex interactions with various neuronal and non-neuronal cell types, such as astrocytes(14). Following ischemic events, microglia are activated, releasing pro-inflammatory cytokines that enhance phagocytosis and exhibit antigen-presenting capabilities(7). While the activation of microglia plays a beneficial role in clearing dead cells and tissue debris and in promoting the release of growth factors, it also exacerbates brain tissue damage through the release of pro-inflammatory cytokines

and oxidants(14–16). This dual role highlights the complexity and significance of microglial function, which is also a critical determinant of neuronal death in cerebral ischemia.

In light of recent findings, our discussion explores the complex dynamics of microglial activation in ischemic stroke, examining their dual roles in both exacerbating damage and facilitating repair. We discuss the latest insights into microglial activation and its modulation by various cell death mechanisms, including necroptosis, autophagy, pyroptosis, and ferroptosis. Furthermore, we explore emerging areas such as the influence of the gut microbiome on microglial function, highlighting how systemic factors can impact brain health. This review seeks to illuminate the multifaceted roles of microglia and suggest future research and therapeutic directions that may enhance neuroprotection and aid neurological recovery.

1. Microglia

Overview

Microglia, which comprise about 5-20% of all glial cells and 10% of total brain cells in adults, play an essential role in maintaining brain homeostasis(17–19).. In a healthy brain, microglia bolster neural precursor cell proliferation and are instrumental in sculpting neuronal circuits. They regulate synaptic structures and prune non-functional synapses via the complement system, interacting intricately with various neuronal and non-neuronal cell types, including astrocytes(12,19). Upon acute brain injury, such as ischemic stroke, microglia transform rapidly from a ramified resting state, characterized by extensive branching processes, to an activated, motile ameboid form(20). Known as brain macrophages in this state, microglia clear cellular debris through phagocytosis and emit a wide array of signaling molecules, including cytokines, neurotransmitters, and extracellular matrix proteins, which regulate neuronal and synaptic activity and their functional plasticity(9). Microglial activation is a hallmark of the early stages of neuroinflammation in ischemic stroke; these cells can be detected in affected areas as early as two hours post-ischemia and up to one week after the initial brain injury(21,22). Activated microglia release cytotoxic factors such as superoxide, nitric oxide (NO), TNF-α, IL-1β and IL-6 in both in vitro and in vivo models, initiating shortly after the insult and potentially exacerbating injury over the following days(23,24). However, during the chronic stage (several days post-onset), microglia can also produce protective cytokines, such as the neurotrophic factor IGF-1, which contributes significantly to neural repair and survival(25,26). This dual nature of microglia, both as a mediator of damage and a facilitator of healing, underscores their complex and critical role in the pathogenesis of stroke.

Microglial Activation

Following an ischemic stroke, microglial activation initiates the inflammatory response to brain injury. Immediately after ischemia sets in, resident microglia are rapidly mobilized to the site of injury. At this location, they undergo significant morphological changes in response to decreased cerebral blood flow and energy deprivation, including hypertrophy of the cell body, development of motile branches, and soma migration(27,28)(28,29). The activation of microglia post-ischemia is a complex process influenced by various factors that disrupt the BBB. Post-ischemia, changes in junctional proteins increase BBB permeability, while microglia release matrix metalloproteinase (MMP)-9, promoting further breakdown(30,31). Additionally, microglia produce cytokines and chemokines, enhancing endothelial adhesion and leukocyte infiltration which contribute to further BBB breakdown(12). As reperfusion begins, activated microglia engage in phagocytosis of endothelial cells, facilitating the entry of serum components(32). Microglial activation is largely driven by DAMPs such as

HMGB1(33), Peroxiredoxin-1(34), and galectin-3(35). These activate microglia through pathways involving toll-like receptors, particularly TLR4(36), and other receptors linked to signal transduction pathways like MAPK(37) and NF-κB(38). However, the continuum of microglial activation extends beyond initial protective responses, evolving into a phase that can exacerbate pathology(14). As the ischemic environment persists, microglia adopt a pro-inflammatory phenotype, secreting cytokines and chemokines that, while essential for signaling and recruiting peripheral immune support, also amplify the inflammatory milieu, contributing to secondary neuronal injury(39).

Microglial Polarization

Microglial polarization, akin to macrophages, reflects their adaptive response to environmental stimuli and is crucial in modulating the progression and resolution of neuroinflammation(23). The patterns of microglial activation vary substantially between the infarcted core and peri-infarcted area(40). Historically, microglial activation has been categorized into two primary phenotypes: classically activated (M1) and alternatively activated (M2)(41). M1 microglia, induced by factors like LPS and pro-inflammatory cytokines, produce high levels of pro-inflammatory agents and oxidative metabolites, exacerbating neuronal damage(23,42,43). In contrast, the M2 phenotype, associated with anti-inflammatory cytokines and neurotrophic factors, promotes tissue repair and inflammation resolution(23,44). The polarization of microglia is regulated by pathways like the Nrf2, which under oxidative stress conditions, favors an anti-inflammatory M2 state by enhancing AMP-activated protein kinase (AMPK) activity(45) (46). However, recent insights from single-cell RNA sequencing suggest a more complex spectrum of microglial activation, revealing diverse subtypes within the traditionally binary M1/M2 classification, each playing unique roles in tissue repair and wound healing(41). (47). (48).

These insights underscore the potential for modulating microglial activation and polarization to alter their phenotypes, opening new research avenues for how cell death mechanisms like necroptosis, autophagy, pyroptosis, and ferroptosis interact with microglial functions. Additionally, the emerging roles of efferocytosis and the gut microbiome in influencing microglial activity present exciting new opportunities for research.

Cell Death Mechanisms

Overview

Physiologically, cell death is a highly regulated and crucial homeostatic mechanism required to maintain tissues, organ size, and function(49). Within the CNS, programmed cell death (PCD) is pivotal in developing functional circuitry, helping to regulate neuron numbers and establish precise neural connections(50,51). PCD encompasses several types, each with distinct molecular mechanisms and functions, including apoptosis, necroptosis, autophagy, and more recently recognized forms such as pyroptosis and ferroptosis(50–53). The brain tissue area affected by ischemic stroke can be conceptually divided into the core and penumbra, with the core being the central area experiencing the most severe ischemia, and typically becoming rapidly necrotic, whereas the penumbra is the surrounding areas experiencing a lower level of ischemia in which neurons are functionally depressed but still viable. Initially, neurons in the penumbra activate signaling pathways that promote survival, potentially lasting several hours to days(50). Over time, however, neuronal density in the penumbra adjacent to the infarct core decreases, coinciding with the activation of glial cells and potential expansion of infarct lesions at the expense of the penumbra(29). Thus, salvaging penumbra neurons is considered a crucial target for post-stroke therapies. Apoptosis, the most common form of PCD, is a non-inflammatory mode of cell death that is characterized by the overexpression of both apoptotic and anti-apoptotic proteins in the

penumbra(54). The fate of cells in this region is influenced by the balance between these opposing forces, with caspases playing a central role in both regulating neuroinflammation and executing apoptosis. Traditionally, caspases are classified based on their function into apoptotic (caspase-2, -3, -6, -7, -8, -9, and -10) or inflammatory categories (caspase-1, -4, -5, -11, and -12)(55). This section of the review will focus on exploring the various ways in which these cell death mechanisms regulate the inflammatory response in microglia.

Necroptosis

Necroptosis is identified as a lytic form of PCD, orchestrated by the activation of the kinases RIPK1 and RIPK3(49). These kinases interact through their RIP homotypic interaction motif (RHIM) domains to form a cytosolic complex known as the necrosome. Activation of the necrosome leads to the phosphorylation of MLKL by RIPK3. Once phosphorylated, MLKL forms a multimeric complex that disrupts the plasma membrane, causing cell lysis and the release of intracellular contents(56), which starkly contrasts with apoptosis, as it lacks the orderly packaging of cellular components. In cells undergoing necroptosis, RIPK1 and RIPK3 play crucial roles in neuroinflammation by promoting the release of DAMPs, which involves the nuclear translocation of activated RIPK1 and its interaction with the BAF complex, leading to upregulated chromatin modification and inflammatory gene expression, thereby triggering inflammation(57). As mentioned above, DAMPs being one of the well-known activators of microglia.

Multiple lines of evidence support a role for necroptosis in ischemic stroke(58). For instance, TNF-α derived from microglia mediates endothelial necroptosis, which significantly exacerbates BBB disruption following ischemic events(59). Specifically, this process is driven by perivascular M1-like microglia, where the interaction of TNF-α secreted by these microglia and its receptor is crucial for inducing endothelial necroptosis and subsequent BBB disruption. Moreover, treatment with anti-TNF-α agents significantly ameliorates endothelial necroptosis, reduces BBB destruction, and improves overall stroke outcomes. TRAF2 has been identified as a key suppressor of necroptotic cell death, which is significantly upregulated in ischemic brains, particularly within neurons and microglia. One study (60)reveals that knockdown of TRAF2 exacerbates brain damage following ischemic events by increasing infarction size and enhancing microglial activation along with pro-inflammatory responses. Furthermore, in vitro experiments show that TRAF2 knockdown leads to increased cell death in microglia and neuronal cells under stress. The application of necrostatin-1, a necroptosis inhibitor, has shown potential in mitigating these effects, suggesting that strategies targeting necroptosis pathways may offer new therapeutic opportunities for managing ischemic stroke.

Autophagy

Autophagy is a crucial cellular mechanism in eukaryotes, characterized by a panel of autophagy-related (ATG) gene products orchestrates the formation of a double-membrane vesicle, known as the autophagosome, which encapsulates cellular cargo and fuses with lysosomes, resulting in the degradation of its contents through the activities of lysosomal hydrolases(61,62). Three forms of autophagy have reported: macroautophagy, microautophagy and chaperone-mediated autophagy, each of these processes is distinct(63), however, macro autophagy is the most widely studied and well described of the three types, and macroautophagy is the main pathway of intracellular degradation(64). Autophagy-related processes is fundamental not only for modulating the development, differentiation, and death of cells and tissues but also for maintaining inflammation and immune homeostasis, and adapting to metabolic demands(62). Autophagy can be triggered by

various stress conditions including hypoxia, starvation, oxidative stress, endoplasmic reticulum stress, protein aggregation, and mammalian target of rapamycin (mTOR) inhibitors(62). The regulation of autophagy involves complex signaling pathways, primarily through the mTOR and adenosine-monophosphate activated protein kinase (AMPK)(62). While mTOR serves as an inhibitor of autophagy by phosphorylating Unc-51-like kinase (ULK), AMPK promotes autophagy. It does this by inhibiting mTOR activity and directly activating ULK, thereby inducing autophagy. Notably, ischemia propagates autophagy through AMPK activation(65).

Autophagy extensively crosstalks with inflammatory signaling cascades(62), numerous studies have found that microglial autophagy can regulate inflammatory responses by affecting their functional phenotypes in cerebrovascular diseases(66). The transformation from the M1 to the M2 phenotype can protect the body from excessive inflammatory injury, and one of the mechanisms affecting macrophage polarization is autophagy(67). In mammalian cells, microglial autophagy has been demonstrated to be critical for microglial activation in vitro, and the inhibition of microglial autophagy results in the upregulation of proinflammatory cytokines, causing increased M1 microglial activation(68). In ischemic stroke, autophagy and inflammation are intricately connected processes that play crucial roles in microglial function, displaying a finely balanced interaction. In studies utilizing BV-2 cells to simulate the effects of oxygen-glucose deprivation/reperfusion (OGD/R), findings indicate dynamic changes in microglial activity over time(69). In the initial stages of OGD/R, there is a simultaneous increase in both pro-inflammatory and anti-inflammatory microglial activities. However, as the condition progresses to later stages, specifically by 72 hours post-OGD/R, a notable shift occurs: autophagic activity within the microglia is inhibited, leading to an increase in pro-inflammatory microglia and a concurrent decrease in anti-inflammatory microglia. Nonetheless, phagocytosis is a therapeutic target yet to be explored in stroke and other brain disorders. Several intracellular molecules such as PARP14 (a member in the Poly (ADP-ribose) polymerase superfamily) and PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α) have been demonstrated to limit microglial activation and promote neurological recovery after stroke by inducing microglial autophagy(70,71).

Pyroptosis

Pyroptosis, also known as 'fiery death,' is a distinct form of inflammatory PCD characterized by the activation of caspase-1 through inflammasomes, sharing features with both necroptosis and apoptosis and involving rapid cell lysis and inflammation(53,72). Like necroptosis, pyroptosis leads to plasma membrane pore formation and cellular swelling, culminating in cell rupture and the release of pro-inflammatory cytokines and intracellular contents. However, it uniquely mirrors apoptosis through mechanisms such as caspase dependence, chromatin condensation, and DNA fragmentation(73). Inflammasomes, central to pyroptosis, are multiprotein complexes that detect pathogenic microorganisms and damaged cell signals through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs). These complexes respond to pathogen-associated molecular patterns (PAMPs) and DAMPs, leading to the activation of caspase-1, which then cleaves gasdermin D (GsdmD)(72). Under both in vitro and in vivo ischemic conditions, microglia release components and activation-dependent pro-inflammatory cytokines via the inflammasome, with the NLRC4 inflammasome complex playing a pivotal role in mediating pyroptotic cell death in BV2 microglial cells(74). Pyroptosis distinguishes itself from necroptosis by its reliance on GsdmD as the primary executor of cell death(75). In the canonical pathway, pyroptosis is triggered by the cleavage of gasdermin D at

D275—based on human GsdmD numbering—into N- and C-termini by caspase-1. In the noncanonical pathway, caspases 4, 5 in humans, and caspase 11 in mice are responsible for this cleavage. The N-terminus of GsdmD then forms a transmembrane pore that facilitates the release of pro-inflammatory cytokines such as IL-1β and IL-18. This pore formation disrupts ion and water regulation, leading to intense inflammation and cell death.

Like other forms of PCD, accumulating evidence has suggested targeting microglia pyroptosis may offer preventive and therapeutic benefits for stroke management(76). Edaravone dexborneol is a traditional prescription known for its synergistic antioxidant and anti-inflammatory effects, and it effectively inhibits the activation of the NLRP3 inflammasome-induced microglial pyroptosis in experimental ischemic stroke by modulating the NF-κB/NLRP3/GSDMD signaling pathway. Complementarily, studies involving CX3CL1 show that its exogenous administration, particularly recombinant CX3CL1 (rCX3CL1), not only reduces neurological deficits and infarct size in mice post-middle cerebral artery occlusion (MCAO) but also diminishes GSDMD-dependent pyroptosis in microglia(77). Similarly, bone marrow mesenchymal stem cell-derived exosomes have been shown to attenuate cerebral ischemia-reperfusion injury by modulating microglial M1/M2 phenotypes, thus reducing neuroinflammation and pyroptosis(78). Additionally, intermittent theta-burst stimulation has demonstrated improvements in motor function in cerebral ischemic mice by inhibiting neuronal pyroptosis and regulating microglial polarization through the TLR4/NFκB/NLRP3 signaling pathway(79).

Ferroptosis

Ferroptosis refers to a distinct modality of PCD driven by iron-dependent phospholipid peroxidation(80). The research field of ferroptosis has experienced exponential growth since the term was first coined in 2012(81). Morphologically, ferroptosis exhibits smaller than normal mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of mitochondria crista, and outer mitochondrial membrane rupture, yet lacks many of the defining characteristics of other PCD, such as blebbing, lysis, or DNA fragmentation(53,80). Ferroptosis, predominantly occurring in the brain(81), is primarily triggered by the generation of ROS from various sources including iron metabolism, mitochondrial electron transport chain activity, and reactions involving the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) protein family, all contributing to a continuous and complex process of cellular oxidative stress and lipid peroxidation(80). Additionally, the system xc– glutathione (GSH)–glutathione peroxidase 4 (GPX4) axis serves as a key regulatory mechanism for cellular resistance against the formation of lipid hydroperoxides, which are fundamental characteristics of ferroptosis(82). Disruption of this axis can weaken cellular antioxidant defenses, leading to increased ROS levels and heightened sensitivity to ferroptosis(83).

The mechanisms of ferroptosis highlight its potential roles and functional implications, ever since iron is essential for catalyzing various redox reactions vital to cellular metabolism. Increasing evidence suggested that beyond regulating infection responses through ferroptotic immune cells such as T and B cells, ferroptosis-mediated sterile inflammation also plays a pathological role in the progression of ischemia-reperfusion injury across various tissues(84). Notably, brain cells display varying sensitivities to ferroptosis, with microglia being the most susceptible, whereas neurons are less affected. Interestingly, in a mixed-culture system, cells exhibit greater resistance to ferroptosis compared to when cultured individually(85,86). As a result, targeting microglia has emerged as a primary strategy to regulate ferroptosis in ischemic conditions. Cui et al. (86) demonstrated that cell ferroptosis-conditioned medium significantly

triggered inflammation in microglia and peritoneal macrophages, and RSL3 inhibited LPS-induced inflammation to protect cells from ferroptosis by upregulating Nrf2 expression in microglia and macrophages. Acyl-CoA synthetase long-chain family member 4 (ACSL4), crucial for polyunsaturated fatty acids metabolism, modulates ferroptosis sensitivity. Knockdown of ACSL4 not only protects against brain ischemia but also reduces proinflammatory cytokine production in microglia(87). Moreover, recent studies underline the protective roles of progranulin and caffeic acid against cerebral ischemia-induced ferroptosis in MCAO models(88,89). Progranulin not only reduces neuronal ferroptosis post-stroke but also modulates inflammatory responses. Similarly, caffeic acid offers protection by engaging the Nrf2 pathway, renowned for its dual role in promoting anti-oxidation and anti-inflammation. In summary, these findings suggest that ferroptosis inhibition is a viable therapeutic strategy for ischemic stroke. Developing specific ferroptosis inhibitors that have minimal side effects on other organs remains a crucial area for future research.

2. Efferocytosis

Dead cells can become secondarily necrotic, potentially leading to autoimmunity, tissue necrosis, and pathological inflammation(90,91). This raises a crucial question: how do phagocytes that engulf dead cells effectively manage the substantial metabolic cargo resulting from the degradation of these ingested cells to prevent such inflammatory responses? Accumulating evidence suggests that efferocytosis may address this issue through a reciprocal interaction between dead or dying cells and phagocytes(92). Phagocytes, both professional (such as macrophages and dendritic cells) and non-professional (like epithelial cells), are drawn to sites of cell death by "Find-me" signals. They then recognize and engulf dying cells via "Eat-me" signals, leading to their internalization for degradation and processing. Notably, genetic, biochemical, and imaging techniques have revealed morphological and mechanistic distinctions between efferocytosis and classical forms of phagocytosis(93). In the brain, efferocytosis is mainly performed by microglia and is essential for maintaining tissue homeostasis and immune tolerance, as well as for resolving inflammation and facilitating tissue repair(91). To effectively regulate inflammation, efferocytosis not only sequesters dead or dying cells but also reduces the release of DAMP(94). Furthermore, this process involves several key metabolic-sensing molecules, including LXRα, LXRβ, PPARγ, PPARδ, RXRα, and the CD73–adenosine receptor A2a axis. These molecules play crucial roles in modulating inflammatory responses within phagocytes and promote the resolution of inflammation(92,95).

The mechanisms of efferocytosis outlined above clearly demonstrate that dysregulated or impaired efferocytosis could potentially contribute to the etiology of a variety of inflammatory diseases. However, the specific signaling pathways that govern the phagocytic behavior of microglia and macrophages in ischemic brains, and their phenotypic transformation following efferocytosis, are still not well understood. Addressing this gap, Cai and colleagues have conducted studies exploring these critical aspects(96). They have identified the STAT6/Arg1 signaling axis as crucial for regulating microglia/macrophage responses in ischemic brains, showing that STAT6 activation is essential for effective efferocytosis, reducing inflammation, and improving stroke outcomes with experiments demonstrating that STAT6 deficiency leads to increased neuronal death and larger infarct volumes, while adoptive transfer of wild-type macrophages into STAT6-deficient mice significantly ameliorated these effects. Following these findings, a recent study underscored the role of the Sigma-1 receptor (Sig-1R) in macrophage/microglia-mediated efferocytosis during ischemic stroke. Using Sig-1R knockout mice and bone marrow-derived macrophages,

researchers found that Sig-1R deficiency impaired phagocytic function, exacerbating brain damage and neurological deficits. However, adoptive transfer of Sig-1R intact macrophages not only restored neuronal clearance but also reduced neuroinflammation and enhanced recovery, with Rac1 activation identified as a key mechanism in Sig-1R-mediated efferocytosis(97). Beyond these foundational insights, additional studies have revealed other factors influencing efferocytosis in ischemic stroke. For example, research indicates that a high-salt diet impairs the efferocytic capabilities of macrophages, worsening inflammation and neurological outcomes post-stroke(98). Additionally, osteopontin-derived peptides, particularly those containing the RGD motif, have been demonstrated to reduce brain damage and promote recovery by enhancing anti-inflammatory responses and the efferocytic activity of microglia(99). Moreover, blocking the CD300a receptor, highly expressed on myeloid brain cells, significantly enhances efferocytosis, thereby reducing inflammation and improving recovery outcomes in stroke models(100). Although there have been advances, a deeper understanding of the molecular and cellular mechanisms and impacts of efferocytosis is required in this emerging field.

3. Gut microbiome

Ischemic stroke initiates an inflammatory cascade that leads to microglial activation and the recruitment of peripheral immune cells to the ischemic hemisphere (101). Studies have shown that some of these immune cells originate from the intestine and are influenced by the gut microbiota(102,103). The gut microbiota, primarily located in the large intestine, comprises tens of trillions of microorganisms, including bacteria, viruses, fungi, and archaea(104). This complex ecosystem plays a crucial role in several physiological functions, such as digestion, vitamin synthesis, and fortifying the gut barrier, which protects against pathogens and maintains intestinal integrity(105). Functionally, the microbiome is vital for the maturation and functioning of the host's immune system, influencing the balance between pro-inflammatory and anti-inflammatory responses(106). Additionally, gut bacteria are critical for the maturation and function of microglia under both steady-state conditions and following disturbances. For instance, mice raised in germ-free environments, which lack all commensal bacteria, display an immature microglial phenotype(107).

Studies have demonstrated that gut microbiota-derived metabolites, particularly short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate, significantly influence microglial activation(108). Produced by bacterial fermentation of dietary fibers, SCFAs can cross the BBB and enter systemic circulation. Once within the brain, SCFAs impact microglial function by modulating histone deacetylase activity, which in turn regulates the expression of genes associated with inflammation and immune response(109). Post-stroke, SCFAs alter microglial morphology to a less activated, more ramified state, shown by changes in Iba-1 immunohistochemistry and morphology analysis. This modulation reduces microglia numbers and the expression of CD68, an activation marker, thereby mitigating microglial hyperactivation and decreasing the invasion of pro-inflammatory lymphocytes into the brain(110). These actions suggest SCFAs play a comprehensive role in regulating both microglial response and broader immune reactions post-stroke, highlighting a critical aspect of the gut-brain connection. Following ischemic stroke, significant reductions in fecal SCFAs have been observed in both humans and experimental animals(111,112), accompanied by concurrent decreases in plasma SCFAs in experimental animals(113). Post-stroke changes in SCFAs have prompted studies on how dietary or probiotic interventions targeting gut microbiota might influence stroke outcomes. Pre-stroke supplementation of drinking water with SCFAs for four weeks significantly enhanced recovery and

cortical reorganization in mice, altering gene expression related to microglial function and activation in affected brain regions(113). SCFA-treated mice also displayed reduced activation and lower numbers of microglial cells and T lymphocytes in the cortical tissue, with further evidence of SCFAs' beneficial effects on post-stroke recovery observed in aged mice transplanted with SCFA-producing bacteria combined with inulin(114). Although these studies primarily examined overall SCFA levels, one study found that both in vivo and in vitro, butyrate induced morphological and functional shifts in microglia towards a homeostatic state and restrained proinflammatory changes(115).

Additionally, other gut-derived metabolites like Trimethylamine N-oxide (TMAO) have been associated with adverse cardiovascular outcomes, including stroke(116). A systematic review and meta-analysis revealed a positive dose-response relationship between TMAO levels and major adverse cardiovascular and cerebrovascular events(117). However, research on TMAO's role in inflammation, particularly its effect on microglial regulation, is still sparse. One study demonstrated that TMAO worsens ischemic stroke outcomes by inducing the release of inflammatory cytokines in the brains of MCAO/R mice and by activating the OGD/R microglial inflammasome via the modulation of FTO/IGF2BP2 and NLRP3 inflammasome activation(118). As the gut microbiota becomes increasingly central in biomedical research, the deepening understanding of the gut–brain axis is driving the development of innovative research methods aimed at reducing stroke severity and enhancing neurological recovery.

4. Conclusion and future perspectives

This review has elucidated the pivotal role of microglia in mediating neuroinflammation during ischemic stroke and highlighted the intricate interactions between various cell death mechanisms, including efferocytosis. It also emphasizes the significant impact of the gut microbiota through the gut-brain axis on both systemic and CNS-specific inflammation, underlining the potential of gut-derived metabolites like SCFAs to modulate microglial activation and promote neuroprotective environments.

Recent insights into PANoptosis, a newly proposed category of cell death, integrate molecules from pyroptosis, apoptosis, and necroptosis within complex PANoptosome structures(119), representing a significant advancement in our understanding of cellular death mechanisms. This conceptual integration offers a comprehensive perspective on the interconnections between various forms of cell death and inflammation, particularly in the context of ischemic stroke. Furthermore, the role of metabolic reprogramming in immune cell activation presents a valuable area for further research. Given the diverse metabolic requirements of immune cells based on their phenotypes, exploring these metabolic pathways could lead to new methods for enhancing reparative functions while mitigating harmful inflammation post-stroke(120).

Looking ahead, leveraging advanced omics technologies and sophisticated animal models will be crucial for deepening our understanding of how metabolic alterations and novel cell death pathways, such as PANoptosis, impact the gut-brain axis and influence stroke outcomes. Future research should also focus on elucidating the specific molecular mechanisms through which metabolic reprogramming and PANoptosis affect microglial functions and on identifying modifiable risk factors within these pathways. Ischemic strokes can be extremely acute, allowing only a short time window for intervention. Therefore, it is essential to consider how therapies based on advanced mechanisms such as PCD, gut microbiota, the PANoptosome, or metabolic reprogramming can be applied timely to exert their effects rapidly enough.

In summary, while significant progress has been

made in delineating the roles of microglia, PCD mechanisms such as ferroptosis, efferocytosis, and the gut microbiota in ischemic stroke, the exploration of PANoptosis and metabolic reprogramming is opening new therapeutic frontiers. Comprehensive clinical studies and the development of innovative therapeutics based on these insights are imperative to fully capitalize on this knowledge for the benefit of stroke patients worldwide.

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