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REVIEW ARTICLE

Neutrophil Heterogeneity and its Roles in the Inflammatory Network after Ischemic Stroke

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Abstract: As the first peripheral immune cells to enter the brain after ischemic stroke, neutrophils are important participants in stroke-related neuroinflammation. Neutrophils are quickly mobilized from the periphery in response to a stroke episode and cross the blood-brain barrier to reach the ischemic brain parenchyma. This process involves the mobilization and activation of neutrophils from peripheral immune organs (including the bone marrow and spleen), their chemotaxis in the peripheral blood, and their infiltration into the brain parenchyma (including disruption of the blood-brain barrier, inflammatory effects on brain tissue, and interactions with other immune cell types). In the past, it was believed that neutrophils aggravated brain injuries through the massive release of proteases, reactive oxygen species, pro-inflammatory factors, and extracellular structures known as neutrophil extracellular traps (NETs). With the failure of early clinical trials targeting neutrophils and uncovering their underlying heterogeneity, our view of their role in ischemic stroke has become more complex and multifaceted. As neutrophils can be divided into N1 and N2 phenotypes in tumors, neutrophils have also been found to have similar phenotypes after ischemic stroke, and play different roles in the development and prognosis of ischemic stroke. N1 neutrophils are dominant during the acute phase of stroke (within three days) and are responsible for the damage to neural structures *via* the aforementioned mechanisms. However, the proportion of N2 neutrophils gradually increases in later phases, and this has a beneficial effect through the release of anti-inflammatory factors and other neuroprotective mediators. Moreover, the N1 and N2 phenotypes are highly plastic and can be transformed into each other under certain conditions. The pronounced differences in their function and their high degree of plasticity make these neutrophil subpopulations promising targets for the treatment of ischemic stroke.

Keywords: Neutrophils, ischemic stroke, heterogeneity, N1 neutrophils, N2 neutrophils, neuroinflammation.

1. INTRODUCTION

Ischemic stroke is a worldwide public health problem due to its high morbidity and frequency resulting in permanent disability [1]. Rescuing the ischemic penumbra, the area around the infarct core that is potentially salvageable if reperfused is the main objective of treatment during acute ischemic stroke. Currently, the most effective solutions are intravenous thrombolysis (IVT) using recombinant tissue plasminogen activator (rt-PA) and/or rapid recanalization by mechanical thrombectomy (MT) [2]. In addition to restoring blood supply during the initial time window, it is also necessary to tackle a series of pathophysiological mechanisms triggered by the acute ischemic episode. More and more studies have shown that the inflammatory response mediated by immune cells is one of the most important mechanisms responsible for injuries to the neural tissue following ischemia/reperfusion [3]. Neutrophils, one of the main immune cell types responsible for neuroinflammation after ischemic stroke, were thought to be the perpetrators of brain injuries following ischemia *via* disruption of the blood-brain barrier, edema, and oxidative stress [4]. However, clinical trials for the treatment of cerebral infarction by suppression of neutrophil infiltration into ischemic lesions have not been successful [5, 6]. One reason for this failure may be due to the heterogeneity of neutrophils: Those that cause damage ("harmful neutrophils") may be only a subgroup within the total population. Neutrophil heterogeneity has been confirmed in many diseases [7], but currently there is no unified standard for their classification. In recent years, the application of single-cell sequencing technology has allowed researchers to uncover the subpopulations of neutrophils in tumors and immune-related diseases [8, 9]. Unfortunately, this technique has not yet been applied to ischemic stroke. Studies so far

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have found that neutrophils play a dual role in ischemic stroke, participating both in the injury to the tissue and also in the protection mechanism against such injuries. This functional duality is based on two distinct functional phenotypes of neutrophils, N1 and N2. Neutrophils polarize into either the N1 or the N2 phenotype after ischemic stroke [10, 11]. The N1 phenotype promotes inflammation and aggravates brain injuries, while the N2 phenotype inhibits inflammation and promotes nerve repair. Therefore, promoting the polarization of neutrophils into the N2 phenotype is expected to become a new therapeutic strategy. Unfortunately, the temporal details and specific factors mediating the polarization into either of these two phenotypes after an ischemic attack are not yet known. In this article, we summarize the criteria used in the classification of neutrophil heterogeneity, then expound on the infiltration of neutrophils from the periphery to the brain after cerebral ischemia, as well as highlight the different roles of N1 and N2 neutrophils in ischemic lesions. Finally, we discuss the possible polarization mechanisms and locations during the entire inflammation process.

2. NEUTROPHILS: ORIGINS, FUNCTION, HETEROGENEITY, TRANSCRIPTOMIC ANALYSIS AND FUNCTIONAL DIFFERENCES

2.1. Origins of Neutrophils

Bone marrow is the main source of neutrophils. About 10^7 neutrophils in mice and 10^{11} in humans are produced per day [12]. Neutrophils originate from myeloid progenitors (myeloblasts) and differentiate into mature neutrophils through five stages: promyelocytic cells (PMs), bone marrow cells (MCs), mesenchymal cells (MMs), band neutrophils, and segmented neutrophils (BC/SCs), under the common regulation of transcription factors such as C/EBP a, β and ε, as well as PU.1 and Gfi-1 [13]. During this differentiation process, neutrophils gradually produce three kinds of granules and develop the ability to follow chemotactic cues and generate reactive oxygen species (ROS) in a phenomenon known as "respiratory burst" [14]. Neutrophils constantly undergo generation and maturation in the bone marrow, in a process mainly regulated by granulocyte colony-stimulating factor (G-CSF). G-CSF promotes the differentiation of myeloblasts into the myeloid lineage, the proliferation of neutrophil precursor cells, and the release of mature neutrophils from the bone marrow [15]. The retention and release of neutrophils in the bone marrow depend on the balance between the levels of chemokine receptors CXCR4 and CXCR2. Both CXCR4 and CXCR2 are expressed on the surface of mature neutrophils. CXCR4 is essential for neutrophil retention in the bone marrow [16]. Its ligand is CXCL12 (former stromal-derived factor 1 [SDF1]), which is expressed by the bone marrow stromal cells [16]. While CXCR2 promotes neutrophil influx to the blood by binding to its ligands CXCL1 and CXCL2, which are expressed by bone marrow endothelial cells [17]. G-CSF down-regulates the expression of SDF-1 and up-regulates the expression of CXCL-1 and CXCL2 in endothelial cells of the bone marrow, tilting the balance towards CXCR2 expression and thus promoting the release of neutrophils [17, 18]. Neutrophils can also be found in extramedullary tissues such as the spleen, lung, and liver. One possible explanation is that these organs function as reservoirs for mature neutrophils, which can then be quickly mobilized to the appropriate site in response to inflammation and infection [19].

2.2. Physiological and Pathological Functions of Neutrophils

As one of the main cell types of the innate immune system, neutrophils are involved in various immune and inflammatory processes. Accumulating evidence implicates neutrophils in the pathogenesis of a wide range of human diseases, including infectious diseases, sterile inflammation (trauma, ischemic stroke, chronic obstructive pulmonary disease (COPD), cardiovascular and autoimmune syndromes), and cancer [20]. The role of neutrophils varies according to the type of disease. Traditionally, neutrophils have been considered as the first line of defense against microbial invasion. When microorganisms (especially bacteria and fungi) invade the organism, neutrophils respond quickly by protecting the body through killing mechanisms (such as phagocytosis or respiratory burst) aimed at eliminating them [19]. The defective neutrophil function can lead to aggravation of the infection. In addition, the involvement of neutrophils in sterile inflammation is far beyond what was known in the past. In cases of acute sterile inflammation, such as ischemic stroke or myocardial infarction, peripheral hyperactivated neutrophils elicit an excessive inflammatory response and aggravate tissue damage *via* the release of proteases, ROS, and NETs [21]. However, in the later stages of inflammation, neutrophils create a favorable environment for tissue repair and remodeling through phagocytosis. The transition to an anti-inflammatory phenotype is regulated by anti-inflammatory mediators such as IL-10, inflammation-resolving factors such as lipoxins, and the release of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF), which promotes angiogenesis [22, 23]. In addition, persistently activated and infiltrated neutrophils are also involved in the pathogenesis of some chronic inflammations. For instance, the main pathological change of COPD is chronic bronchitis caused by the activation and recruitment of local neutrophils in response to external stimuli [24]. Neutrophils have also been shown to be involved in atherosclerosis and plaque rupture, with high numbers found in human atherosclerotic plaques. Histone H4, a major component of neutrophil-derived NETs, disintegrates smooth muscle cells and leads to plaque rupture [25]. Neutrophil-derived NETs also play a key role in autoimmune diseases. For example, NETs act as autoantigens that activate autoantibody production in systemic lupus erythematosus and in rheumatoid arthritis [26, 27]. Neutrophils also play a critical and complex role in tumor biology. Tumor-associated neutrophils (TANs) can exert both anti-tumor and pro-tumor effects in response to the tumor microenvironment. In the early stages of tumor development, neutrophils usually exhibit antitumor properties by releasing ROS and reactive nitrogen species (RNS), mediating antibody-dependent cellular cytotoxicity (ADCC), and recruiting other immune cell types such as T and natural killer (NK) cells. However, as cancer progresses, neutrophils gradually shift from an anti-tumor to a pro-tumor phenotype and promote tumor development and metastasis by inducing angiogenesis (through MMPs and VEGF) and by inhibiting the anti-tumor effect of T cells [28]. Neutrophils are also involved in the regulation of adaptive immunity: They regulate the proliferation, activation, chemotaxis and even polari-

zation of T and B cells (see section 7 for a more detailed description of the interaction between neutrophils and lymphocytes in ischemic stroke), and they can also act as antigen-presenting cells (APCs) to promote cellular immunity [29].

2.3. Neutrophil Heterogeneity and Lifespan

Neutrophils are the most abundant white blood cells in circulation. In humans, neutrophils account for 50-70% of all circulating white blood cells, and 10-25% in mice [30]. Although it is generally accepted that circulating neutrophils are short-lived cells with a half-life of seven to nine hours [31], current research suggests that the lifespan of neutrophils is extended. A study calculated that the lifespan of circulating neutrophils in humans is five days, whereas in mice, it is 18 hours [32]. Another study showed that the average life span of human neutrophils is approximately 5.4 days [33]. Neutrophils released from the bone marrow to the periphery undergo phenotypic changes. The newly-released neutrophils are characterized by high levels of L-selectin (CD62L) expression. Later, CD62L expression on the surface of neutrophils gradually decreases and CXCR4 expression gradually increases over time [34]. This process is known as neutrophil aging, it is accompanied by changes in transcriptional properties and promotes the transition of neutrophils to a pro-inflammatory phenotype. The process of neutrophil aging in healthy humans and mice follows circadian rhythms and is regulated by the microbiota [35]. The upregulation in CXCR4 expression levels attracts aging neutrophils back to the bone marrow to be cleared by macrophages [36]. Meanwhile, neutrophils that died while still at the vasculature are phagocytosed and cleared by Kupffer cells in the liver [37]. The IL-23/IL-17/G-CSF axis is involved in the regulation of the process described above. Macrophages that engulf neutrophils downregulate the expression of IL-23, thereby reducing the production of IL-17A by T cells, decreasingthe production of G-CSF and inhibits neutrophil maturation [38]. There is yet another form of neutrophil death in which neutrophils decompose and externalize nuclear contents as neutrophil extracellular traps (NETs). NETs contain complex components that lead to a range of inflammatory responses and contribute to the pathogenesis of multiple autoimmune diseases [39].

Due to a poor understanding of neutrophil lifespan and transcriptional activity,neutrophils have always been regarded as a homogeneous population in the past [40]. However, a large number of studies in recent years support the heterogeneity of neutrophils [33]. As mentioned, neutrophils are not the short-lived cells that have been identified in the past. Furthermore, under certain stimuli, including inflammation, tissue hypoxia, and exposure to cytokines and bacterial products, the lifespan of neutrophils can be abnormally prolonged [41-43]. This extended lifespan sets the foundation for phenotypic and functional changes in neutrophils [40]. Meanwhile, studies targeting the genome of neutrophils have shown extensive dynamic changes in transcription and epigenetic modifications through their entire developmental cycle [44]. The extent of transcriptional plasticity is particularly evident when neutrophils are exposed to inflammatory conditions [45].

2.3.1. Morphological Characteristics and Maturation Stages

Neutrophil heterogeneity has been demonstrated under both physiological and pathological conditions [46]. The original model for neutrophil development and maturation was initially established based on their morphological characteristics, starting from granulocyte-monocyte progenitor cells (GMPs) and progressing through a continuum of maturation stages that ranged from myeloblasts (MBs), promyelocytic cells (PMs), bone marrow cells (MCs), and mesenchymal cells (MMs) to band cells and segmented neutrophils (BC/SCs) [7]. Subsequently, it was discovered that circulating neutrophils from healthy humans and mice could be divided into three groups according to their relative positions in a density gradient after centrifugation: high-density (HDDs), normal density (NDNs), and low-density (LDNs) neutrophils. Moreover, these three subgroups were shown to have different functions in disease states such as tumors and autoimmune diseases [47]. However, these functional differences are not specific. For example, LDNs may exhibit pro-inflammatory and anti-inflammatory properties in different inflammatory diseases [48]. Therefore, the clustering of neutrophils based on density may not be ideal.

Several subpopulations of neutrophils with specific surface markers were found in the peripheral blood of healthy donors. For instance, a subpopulation of aging neutrophils that follows circadian rhythms and is characterized by high expression of CXCR4 and CD11b and low levels of CD62L has been shown to have increased anti-inflammatory activity in ischemic stroke and other inflammatory diseases. Another group of $CD49d^{+}$ neutrophils with high expression of VEGFR1 and CXCR4 demonstrated pro-angiogenic properties [49]. However, this classification method has a fatal weakness: it is unknown whether there is a overlap between the various subgroups and whether the entire neutrophil population is represented. A study focused on the cell cycle analysisusing mass cytometry described for the first time a proliferative neutrophil precursor population: pre-neutrophils (pre-Neu), which differentiate into non-proliferating immature and mature neutrophils [50]. Transcription analysis also indicated that the pattern of gene expression changes throughout the entire process, leading to differential responses to tumors and inflammation: pre-neutrophils are not mobilized to the peripheral circulation, whereas both immature and mature neutrophils are actively deployed to the injury site [51]. Although this has provided a new framework for the early development of neutrophils in healthy and diseased states, the functional differences between immature and mature neutrophils in different diseases are still poorly understood.

2.3.2. Transcriptome Sequencing Analysis

In recent years, the maturity of single-cell sequencing technology has allowed for the analysis of the whole transcriptome from a single neutrophil, which can be compared across species without bias [52]. This technology is currently considered to be the best way to explore the essence of neutrophil heterogeneity. Some recent single-cell sequencing studies have further characterized the subdivisions of neutrophils in healthy individuals and the context of cancer and inflammatory diseases. Under physiological conditions,

based on different transcriptional characteristics, neutrophils are considered to form a single continuum across tissues named neutrotime, which converges with chronological order. Neutrophils in the bone marrow represent the immature end of the continuum, while those in the spleen and peripheral blood represent the mature end [53]. Transcription-based unbiased and unsupervised clustering divides neutrophils into eight clusters: G0-4, mainly derived from the bone marrow, and G5a-c, located in the peripheral blood. The G0, G1, G2, G3 and G4 clusters correspond to BM GMP, neutrophil progenitors (pro-Neu), pre-Neu, immature and mature neutrophils, respectively. The G5a, G5b, and G5c clusters from the peripheral blood represent the most mature neutrophils, with transcriptomes that are significantly different from the rest of the clusters and remain unchanged during infection [54]. A different study described a classification system for neutrophils derived from humans and mice affected by cancer: Human neutrophils were resolved into five subsets (human N1-5 or hN1-5), while murine neutrophils were resolved into six subsets (mouse N1-6 or mN1-6) *via* cluster analysis [55]. N5 and mN5 are both tumor-specific and promote tumor growth. Unfortunately, single-cell analysis of neutrophils has not yet been reported in ischemic stroke.

2.3.3. Functional Differences

At present, the most extensively adopted system for neutrophil classification after ischemic stroke is based on the "N1" and "N2" phenotypes, defined by function. The concept of N1 and N2 neutrophils, similarly to M1/M2 macrophages, was first proposed in the field of cancer research [56]. The presence of transforming growth factor beta (TGF-β) in the tumor microenvironment induces the differentiation of TANs with the N2 pro-tumorigenic phenotype characterized by high expression of CCL2, CCL5, and arginase, whereas blockage of the TGF-β signal induces TANs to differentiate instead into the N1 anti-tumorigenic phenotype, with high expression of tumor necrosis factor alpha (TNF-α**)**, intercellular Adhesion Molecule 1 (ICAM-1) and ROS. The concept of N1 and N2 neutrophils was later extended to the pro- and anti-inflammatory phenotypes in other diseases such as myocardial infarction, infection, and ischemic stroke [57-59]. Some experimental stroke models have validated the existence of N1 and N2 phenotypes and their almost opposite effects. Conditioned medium from ischemic neurons has been shown to induce the polarization of neutrophils into the N1 phenotype, which enhances the neuronal damage caused by oxygen and glucose deprivation (OGD) [59]. A mouse model for permanent stroke proved that peroxisome proliferator-activated receptor-γ (PPAR-γ) could increase the number of N2 neutrophils in ischemic brain tissue [10]. Interestingly, this mouse model was also the first to reveal that the increase in phagocytic ability and clearance rate exhibited by N2 neutrophils plays a role in neuroprotection and ameliorates inflammation. It is noticeable that, due to the lack of specific surface markers, the research on N1/N2 polarization of neutrophils was performed using the same cell surface markers used to distinguish between M1 and M2 macrophages, and microglia. Despite the lack of clear phenotypic markers, the evident functional difference between the N1 and N2 phenotypes determines its value for clinical research in ischemic stroke: Both inhibiting the polarization of neutrophils to the N1 phenotype and promoting their polarization to the N2 phenotype have resulted in outcome improvements in animal models of stroke. This has therefore laid the foundation for a clinical treatment of ischemic stroke based on cell polarization.

3. DYNAMICS OF PERIPHERAL NEUTROPHILS AFTER ISCHEMIC STROKE: MOBILIZATION, PRESENCE IN BLOOD, CHEMOTAXIS

3.1. Neutrophil Mobilization

3.1.1. Mobilization of Neutrophils from the Peripheral Bone Marrow and Spleen and its Mechanisms

There is a large number of activated neutrophils in the peripheral circulation 24 hours after cerebral ischemia [60], and this is mainly due to mobilization from the bone marrow and the spleen in response to central nervous humoral-immune signals after cerebral infarction. After transient middle cerebral artery occlusion (tMCAO), the number of neutrophils in the mouse bone marrow increased between 10 minutes and four hours, which was accompanied by rapid activation, and then decreased again within 12 hours [61]. The number of neutrophils in the spleen reached a peak 12-24 hours after reperfusion and returned to baseline levels on days 2-7 [62, 63] (Table **1**).

3.1.1.1. Mechanism: Sympathetic Nervous System and Hypothalamic-Pituitary-Adrenal Axis

The main neural signaling pathways involved in ischemic stroke are the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis. Pro-inflammatory factors such as Interleukin 1 beta (IL-1β), TNF-α, and interleukin 6 (IL-6) released by ischemic brain tissue or peripheral immune cells directly induce the activation of the SNS and HPA axis [64]. The SNS and the HPA axis are activated and release norepinephrine (NE) and cortisol as main effectors, respectively [65, 66]. The two jointly mediate complex peripheral immune activities, including immediate immune activation and subsequent immune suppression, which is specifically manifested in the early neutrophil expansion in the bone marrow and spleen and subsequent spleen atrophy (Fig. **1**). Increased levels of NE in the bone marrow drive the proliferation of myeloid cells through β3 adrenergic receptors acting on stromal niche cells, resulting in a significant increase in the number of neutrophils and in their activation (mediated by a rapid change in the phosphorylation status of NF-κB, p65 and p38 mitogen-activated protein kinases [MAPK]) [67]. The spleen also releases monocytes and neutrophils to the periphery in response to the dual signal from the SNS and the HPA axis [68, 69]. The spleen volume gradually shrinks and lymphocytes decrease between 24 and 72 hours after permanent middle cerebral artery occlusion (pMCAO), due to the activation of α1 adrenergic receptor (α1-AR) in the splenic smooth muscle sac by catecholamines and glucocorticoids that continue to increase for several days in the peripheral blood [65].

3.1.1.2 Mechanism: Immune Signals from the Ischemic Brain

The bone marrow and spleen also respond to strong immune signals released by the ischemic brain (Fig. **1**). Vascular occlusion triggers neuronal ischemic damage, which

Table 1. Temporal and spatial changes of the total neutrophil count and N1/N2 phenotype proportion after ischemic stroke.

-	Bone Marrow /Spleen		Blood	Brain	References
	Bone Marrow	Spleen			
Total neutrophils	10 min-4 h: ↑ 4 h-1 d: ↓ 1 d-7 d: ↑	6 h-12 h: ↑ 12 h-24 h: peak 2 d-7 d: base line	0 d-3 d: ↑ 3 d-7 d: ↓ NLR: 6 h-12 h: ↑ 12 h-1 d: peak 1 d-5 d: ↓	15 min-1d: ↑ 1 d-3 d: Peak 4 d-7 d: ↓	[10, 11], [61, 62], [63, 67], [86, 125], [126]
N1	1 d: N1 ↓ 3 d: N1 ↓		1 d: N1 ↑ 3 d: N1 ↑	1d-2d: N1 ↑	[10, 191, 286]
N2	1 d: N2 ↓ 3 d: N2↓		1 d: N2 ↑ 3 d: N2 ↑	1d-2d: N2↑	[10, 191, 286]
N1/N2	1 d: N1/N2＜1	0 d-7 d: N1/N2 stable	pMCAO: 1 d: N1/N2≈1 tMCAO: 0 d-7 d: N1/N2 stable	pMCAO: 1 d-2 d: N1/N2≈3 tMCAO: 1 d: N1/N2≈1 1 d-3 d: N1/N2 ↑ 3 d: N1/N2≈3 3 d-7 d: N1/N2 ↓	[10, 11], [62, 140], [191]

Note: Changes in the amount of total neutrophils, N1 and N2, as well as the proportion of N1 and N2 in the bone marrow, spleen, peripheral blood and ischemic brain within 7 days after ischemic stroke; N1, N1 neutrophils; N2, N2 neutrophils.

releases ROS, chemokines (such as CXCL-1, CXCL-3, and CCL2), and damage-associated molecular patterns (DAMPs) including high mobility group protein 1 (HMGB1), heat shock protein 72 (HSP72), S100A9, and adenosine 5′ triphosphate (ATP) [70-73]. Soon afterward, the resident cells of the brain (microglia, astrocytes, and endothelial cells) are activated. This is followed by the release of a series of inflammatory signals, including proinflammatory cytokines (such as IL-1, TNF-α and granulocyte colony-stimulating factor [G-CSF]) and chemokines (such as IL-8 [KC in mice], CCL-2, leukotriene B4 [LTB4] and complement factor C5a) [74-77]. In addition to the activation of resident cells, the permeability of the blood-brain barrier (BBB) also increases significantly. The inflammatory signals previously mentioned cross the BBB barrier into the peripheral blood and induce an immune response in the bone marrow and spleen. DAMPs activate neutrophils directly by binding to the pattern recognition receptors (PRRs) on their surface, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). G-CSF promotes the release of CXCR2-positive neutrophils from the bone marrow by inhibiting the expression of CXCR4 on their surface and increasing instead the expression of CXCL1 and CXCL2 in bone marrow endothelial cells [78]. Leukotriene B4 and C5a also play a key role in bone marrow mobilization [77]. In addition, the expression of receptors for the chemokines CCL-2, CCL-3, and CXCL-1 is significantly increased in the spleen [79]. At the same time, pro-inflammatory mediators such as TNF-α, interferon γ (IFN-γ), and IL-6 are upregulated in the spleen, inducing the activation and release of neutrophils from this organ [80]. Triggering receptor expressed on myeloid cells 1 (TREM1), which is highly expressed by spleen neutrophils, also induces their release [81]. The proteins, lipids, and other immunogenic substances released by the ischemic brain are its endogenous ligands.

3.1.2. Mobilization of Neutrophils from Skull Bone Marrow

Traditionally, it was believed that peripheral immune cells should not exist in the central nervous system under physiological conditions due to the existence of the BBB, but recent studies have refuted this view. A study in mice demonstrated that under homeostatic conditions, there is a substantial pool of neutrophils and monocytes present at the border of the CNS (in particular, at the perivascular spaces and meninges), which are being continuously replenished from bone marrow niches in the adjacent skull or vertebrae [82]. Furthermore, thanks to the vascular connection between the bone marrow and the meninges, neutrophils located at the border of the CNS can be rapidly mobilized to the lesion area in response to CNS injury or inflammation [83]. To explore the contribution of different neutrophil subpopulations to neuroinflammation after ischemic stroke, Herisson and colleagues used different fluorescent membrane dyes to label bone marrow cells in the mouse skull and tibia and found that neutrophils derived from skull bone marrow entered the ischemic brain preferentially [84]. This recruitment bias may be due to the anatomical proximity between the skull bone marrow and the brain and to the direct vascular connection between the skull bone marrow and the meninges, which could provide a shortcut for neutrophil migration. In addition, it is unclear whether there are differences in terms of inflammatory stimulation, chemotactic signals, and chemotactic capacity between neutrophils from the peripheral blood and those originating in the skull bone marrow, but they may represent different neutrophil subpopulations and

therefore have distinct roles. Taken together, the studies described above suggest a novel pathway for neutrophil infiltration into the CNS and provide new ideas for therapeutic strategies targeted at neutrophils. Thus, it is necessary to further explore the differences between neutrophils derived from the skull and from blood.

3.2. Neutrophils in the Peripheral Blood after Ischemic Stroke

Thanks to the rapid mobilization from peripheral immune organs, the neutrophil count in the peripheral blood increases exponentially and reaches a peak 24 hours after ischemic stroke [85]. This high neutrophil count in the peripheral blood during the early stages of cerebral ischemia is associated with a larger infarct volume, worse clinical symptoms, and a poorer prognosis [60, 86].

As a marker of systemic inflammation, the neutrophil-to-lymphocyte ratio (NLR) is believed to be a better predictor for the series of events that take place after ischemic stroke. These include clinical outcomes, risk of death, and prognosis of the patients with or without endovascular treatment. A higher NLR (above 3.5) in patients with acute ischemic stroke at admission has been associated with a worse prognosis at three months [87]. It also has strong predictive value for special types of ischemic stroke. A high NLR within 48 hours of symptom onset in patients with acute supratentorial cerebral infarction is indicative of a poorer outcome and a higher risk of death after three months [88]. In addition, a higher NLR predicted stroke-associated pneumonia and delirium events in patients with cerebral infarction during hospitalization [89, 90]. Higher NLRs at admission in patients undergoing endovascular thrombectomy (EVT) were associated with cerebral hemorrhage, worse functional outcomes and a higher risk of death after three months [91], whereas another study demonstrated that the NLR measured between three and seven days after an EVT episode is an even better predictor for these parameters [92].

In addition to the surge in numbers, neutrophils in the peripheral blood are highly activated within a few hours after ischemic stroke. Adhesion molecules such as β2-integrin (CD11b/CD18), P-selectin glycoprotein ligand-1 (PSGL-1), and macrophage-1 antigen (Mac-1) are highly expressed in activated neutrophils, while expression of CD62L is low [4]. Cellular products derived from neutrophils such as elastase, lactoferrin and ROS are also significantly increased [93]. The activated state prepares neutrophils to infiltrate the ischemic brain and mediate inflammation.

3.3. Neutrophil Chemotaxis

3.3.1. Adhesion Cascade

The infiltration of neutrophils into the lesion relies on their ability to migrate along blood vessels and eventually through the vessel wall. This involves a complex interaction between neutrophils and vascular endothelial cells known as the adhesion cascade (Fig. **1**) [94]. The entire process includes capture, rolling adhesion, and crawling along the vessel wall. The activated vascular endothelium upregulates the expression of luminal surface adhesion molecules (E-, L- and P-selectin, ICAM-1, ICAM-2 and vascular cell adhesion protein 1 [VCAM-1]), which initiates the first step of the adhesion cascade [95]. P-selectin and E-selectin capture activated neutrophils by interacting with their respective ligands P-Selectin glycoprotein ligand-1 (PSGL-1) and E-selectin glycoprotein ligand (ESGL-1) expressed on their surface, thus mediating their rolling movement over endothelial cells [96]. Then, β2 integrin/lymphocyte function-associated antigen 1 (LFA-1) expressed by neutrophils initiates their adhesion to the vessel wall by binding to ICAM-1. Mac-1 and very late antigen-4 (VLA-4) expressed by mature $CXCR2^{+}$ neutrophils are also involved in this process [19, 97]. Firm adhesion is a prerequisite for crawling and migration of neutrophils into the vascular endothelium.

3.3.2. Neutrophils and Platelets

Platelets are also involved in neutrophil migration through the blood vessels. Stagnant blood flow in the vessel after arterial occlusion causes high shear stress on the endothelium and platelets resulting in the release of P-selectin, which is stored in the α-granules within the platelets [98]. Crawling neutrophils generate a domain known as uropod expressing PSGL-1 (a glycoprotein ligand for P-selectin) clustered in the plasma membrane. This region extends into the vessel lumen and "captures" platelets in the bloodstream by identifying and binding to P-selectin [99]. Platelet-specific GPIb (glycoprotein receptor Ib) also proved to be essential for the recruitment of neutrophils [100]. GPIb provides a binding site for Mac-1, which is expressed in activated neutrophils and acts as an important adhesion molecule (Fig. **1**) [101]. In addition, platelets can adhere to endothelial cells and stimulate them to secrete chemokines such as CXCL1 and CXCL5, which are strong chemoattractants for neutrophils [102]. The activated platelets themselves are one of the sources of neutrophil chemotactic factors since α-granules contain chemical attractants for neutrophils such as CXCL1, CXCL2, and CXCL5 [103].

4. INFILTRATION INTO THE BRAIN PARENCHYMA THROUGH BBB BREAKDOWN: ROLE OF NEUTROPHILS, MICROGLIA AND ASTROCYTES

Due to the differences in the time points for detection and processing of ischemic events between animal models, as well as the inherent differences between humans and animals, there is no consensus on the spatial and temporal distribution of neutrophils in the ischemic brain. Many experimental stroke models have nevertheless suggested that neutrophils are mainly accumulated in the pia mater vessels and the perivascular spaces, instead of entering the brain parenchyma [104]. However, more recent studies have refuted this hypothesis. The fact that neutrophils mainly infiltrate the brain parenchyma has been confirmed in permanent middle cerebral artery occlusion (pMCAO) models and also in stroke patients [105, 106].

4.1. Direct BBB Breakdown

The destruction of the BBB is the first step for the infiltration of neutrophils into the brain parenchyma. The BBB is a complex structure composed of capillary endothelial cells (ECs), astrocyte end-feet, pericytes, and extracellular matrix (ECM) [77]. Unlike endothelial cells at other sites, there is a junctional complex involving tight junctions (TJs) and adherens junctions (AJs) between brain endothelial cells. TJ

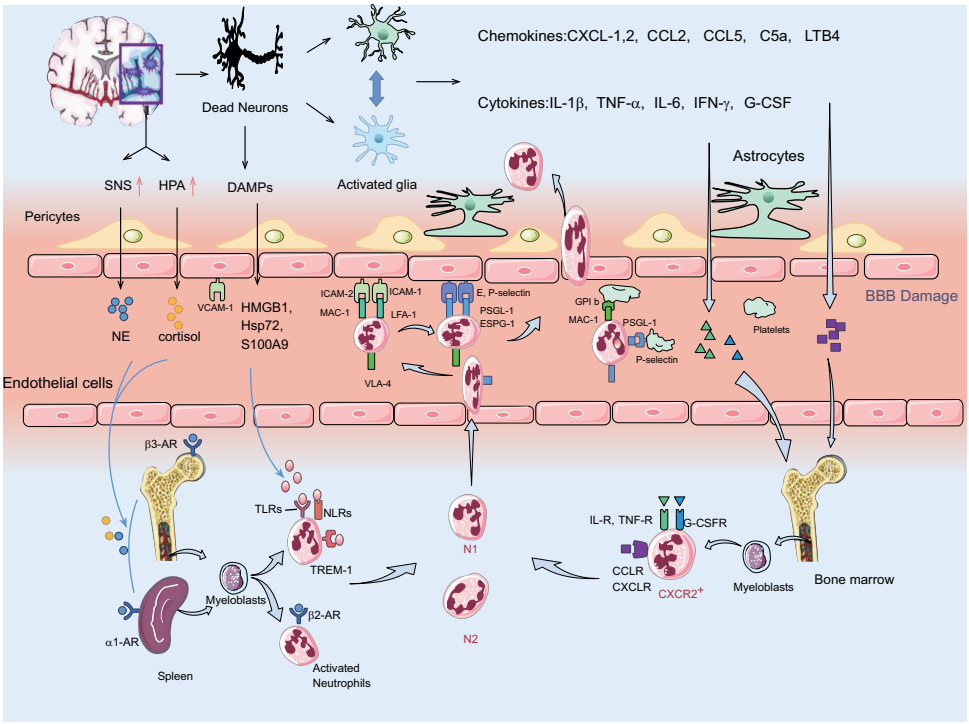

Fig. (1). Recruitment of neutrophils after ischemic stroke. After ischemic stroke, the SNS and the HPA axis are immediately activated and release norepinephrine (NE) and cortisol as main effectors, respectively. Dying neurons and activated glial cells release damage-associated molecular patterns (DAMPs), chemokines (CXCL-1, CXCL-2, CCL2, CCL5, C5a and LTB4), and cytokines (IL-1β, IL-6, TNF-a, INF-γ and G-GSF) to the periphery. Under the dual action of neuronal and immune signaling, $CXCR2^+$ neutrophils in the bone marrow and spleen are mobilized and released into the peripheral blood. Then neutrophils start to migrate within the blood vessels and eventually through the vessel walls. This involves a complex interaction between neutrophils and vascular endothelial cells known as the adhesion cascade, which includes sequential phases of capture, rolling adhesion, and crawling. Platelets are also involved in the migration of neutrophils along the blood vessels by releasing P-selectin and GPIb. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

proteins are composed of three complete protein types: claudin, occludin, and junctional adhesion molecules (JAMs), and the AJ proteins known as cadherins are anchored to the actin cytoskeleton by multiple accessory proteins (for example, zonula occludens [ZO]-1, -2 and -3) [107]. BBB disruption induced by cerebral ischemia is mainly manifested by loss of the structural integrity of endothelial TJs and degradation of the basal lamina. Under ischemic stimulation, the integrity of TJs is slightly damaged 30 to 60 minutes after ischemia/reperfusion (I/R) due to persistent actin polymerization and protein redistribution [108]. Redistribution of connexins is mainly mediated through enhanced endocytosis by endothelial cells. A study using the OGD model showed that caveolin-1 mediates the separation of occludin and claudin-5 from the cytoskeleton through endocytosis [109]. In this way, the paracellular pathway is temporarily loosened, allowing neutrophils to pass through the tight endothelial junctions. Hypoxia-derived events such as ATP depletion, a decrease of Na^+/K^+-ATPase, calcium overload, glutamate release and oxidative stress trigger endothelial swelling and BBB breakdown [110]. After ischemic stroke, the neural (nNOS) and inducible (iNOS) forms of nitric oxide synthase are upregulated in endothelial cells and neurons, resulting in increased NO production. NO reacts with oxygen to form peroxynitrite, which causes endothelial damage. Matrix metalloproteinase 9 (MMP9) and 2 (MMP2) have been regarded as the main proteases acting in the disruption of the BBB. After ischemic stroke, MMP9 is activated by inflammatory factors (such as TNF-α and IL-1β) and peroxides, whereas MMP2 is induced by hypoxia-inducible factor 1-alpha (HIF-1α) [111]. These proteases share the property of degrading endothelial junction proteins and basement membranes (*i.e.*, collagen IV, fibronectin and laminin), causing further severe and lasting disruption of the BBB [106]. As a result, large numbers of neutrophils readily infiltrate the brain. The migrating neutrophils in turn release effector molecules that aggravate the destruction of the BBB even further. Innate brain cell types such as astrocytes, microglia, and pericytes also participate in this process.

4.2. Role of Immune Cells in BBB Breakdown

4.2.1. Role of Neutrophils in BBB Breakdown

Acting together, the proteases, ROS, pro-inflammatory factors, and NETs produced by neutrophils are powerful destructive factors for the BBB. MMP9 disrupts endothelial connections and troponin to increase the extravasation of neutrophils, and it also degrades the parenchymal basement membrane to help neutrophils penetrate the brain parenchyma [108]. The massive amount of ROS produced by neutrophils can directly damage connexins (such as the E-cadherin/β-catenin complex and occludin) and the endothelial cytoskeleton [112]. Furthermore, pro-inflammatory factors such as IL-1β and TNF-α, and chemokines such as CCL2 and CCL3 produced by neutrophils can also destroy the BBB integrity [85]. These inflammatory mediators can also reduce

transendothelial electrical resistance and promote neutrophil extravasation in conjunction with the action of MMP2 and MMP9 [81]. NETs appear in the ischemic cerebral hemisphere a few hours after the stroke and also take part in the disruption of the structural integrity of the BBB due to the presence of myeloperoxidase (MPO) and neutrophil elastase (NE) in these structures [113].

4.2.2. Role of Microglia in BBB Breakdown

Microglia, as the innate immune cells in the brain, are rapidly activated after ischemic stroke, which is manifested in changes at the morphological and transcriptional levels. The microglial cells located in the penumbra respond to ischemic signals by stretching out flat protrusions to migrate towards blood vessels. The endothelial cells are captured and swallowed, causing endothelial dysfunction [114]. At the same time, microglial cells upregulate the expression of the pro-inflammatory mediators IL-1α, IL-1β, IL-6 and TNF-α due to the activation of the TLRs/NF-κB signaling pathway and the NLRP3 inflammasomes [115]. IL-1α and TNF-α are pivotal activators of reactive astrocytes, which play an important role in the disruption of the BBB [116]. In addition, IL-1β directly degrades and redistributes the connexins expressed by endothelial cells (such as ZO-1) [117]. TNF-α not only down-regulates the expression of occludin in endothelial cells but also causes endothelial necrosis directly, increasing BBB permeability [118]. Activated microglia can also produce a considerable amount of ROS and MMPs, which leads directly to the disintegration of the BBB [119].

4.2.3. Role of Astrocytes and Pericytes in BBB Breakdown

After ischemic stroke, DAMPs released by damaged neurons activate astrocytes, resulting in what is known as reactive astrogliosis. It is characterized by cell hypertrophy and overexpression of glial fibrillary acidic protein (GFAP) [120]. Reactive astrogliosis upregulates the expression of cytokines (such as IL-1 and IL-6), ROS, Lipocalin 2 (LCN-2), VEGF and MMP9, all of which are involved in BBB disruption. LCN-2 increases the permeability of the BBB by downregulating the expression of connexin [121]. As the downstream effector of hypoxia-inducible factor (HIF), VEGF derived from astrocytes is upregulated in the penumbra after ischemic stroke. It not only downregulates the tight junction proteins claudin-5 (CLN-5) and occludin of endothelial cells directly, but also activates MMP9 to further degrade connexins, which leads to BBB collapse [122]. Pericytes, as the main component of the BBB, respond quickly after ischemic stroke by detaching from the basement membrane, contracting and eventually undergoing cell death, thus increasing the permeability of the BBB [123]. Recent studies have found that pericytes can also potentially behave as immune cells, releasing pro-inflammatory factors, ROS and MMPs to mediate the disruption of the BBB after ischemic stroke [119].

5. ROLES OF NEUTROPHILS IN NEUROINFLAMMATION AFTER ISCHEMIC STROKE

Neutrophils begin to infiltrate the ischemic brain 15 minutes after ischemic stroke and reach a peak after 24 to 72 hours [124] (Table **1**). The number of neutrophils gradually decreases after four to seven days and becomes almost undetectable in the second week [85, 125]. In the acute phase of stroke (within three days of onset), the proportion of N1 neutrophils is significantly higher than that of N2 neutrophils [10, 62] (Table **1**). We will sequentially describe the role of N1 and N2 neutrophils after ischemic stroke (Fig. **2**).

5.1. Role of N1 Neutrophils in Neuroinflammation after Ischemic Stroke

5.1.1. Oxidative Burst

Neutrophils that enter the brain are highly activated and are one of the main sources of ROS [126]. In comparison to N2 neutrophils, N1 neutrophils produce higher levels of ROS. This has been supported by transcriptional analysis of neutrophils *in vitro* after polarization [127, 128]. The production of ROS in neutrophils depends on two enzymes: NADPH oxidase (Nox) and NOS [129]. There are seven known members in the Nox family, with $Nox\text{-}_2$ being prevalent in neutrophils. After ischemic stroke, the $Nox\text{-}_2$ present in neutrophils is rapidly activated and generates a series of free radicals such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) [130]. ·OH is considered a typical ROS generated by neutrophils. It reacts with unsaturated fatty acids to produce peroxyl radicals (ROOS), which trigger lipid peroxidation, further intensifying oxidative stress and causing neuronal apoptosis, brain edema, and BBB destruction [126]. NOS is another ROS-generating enzyme active in neutrophils. There are three different varieties known as nNOS, endothelial (eNOS) and iNOS. Neutrophil-derived iNOS is upregulated after cerebral ischemia due to the activation of the transcription factors NF-κB and STAT1, which have been shown to induce nerve damage [131]. iNOS is considered a marker for N1 neutrophils in experimental stroke models [59]. NO is generated *via* the conversion of L-arginine to L-citrulline [132]. NO reacts with O_2 to generate peroxynitrite (ONOO−), which leads to lipid peroxidation, protein nitration, and DNA damage, causing neuronal apoptosis and disrupting the BBB [133]. The NO/caveolin-1/MMP9 signaling cascade is the main mechanism for the production of MMP9 after ischemia-reperfusion. Using a rat model for experimental transient MCAO, it was proven that NO upregulation inhibits the expression of caveolin-1, which in turn activates iNOS and MMP9 [134]. MMP9 mediates BBB destruction, cerebral edema, and hemorrhagic transformation (HT) by breaking down the extracellular matrix. ROS can also upregulate the expression of endothelial cell adhesion molecules, which in turn promotes the infiltration of neutrophils and enhances inflammation [129]. In addition, ROS have been proven to be an important step in the formation of NETs [135], suggesting that N1 neutrophils may be the main cell type producing these structures.

5.1.2. Pro-Inflammatory Mediators

Compared with N2 neutrophils, N1 neutrophils show a general upregulation of pathways related to the immune response, which includes the release of pro-inflammatory mediators [136]. High mobility group box protein 1 (HMBG1) and ATP released from damaged brain tissues recognize Toll-like receptors on the surface of neutrophils and activate MyD88 and the transcription factor NF-κB, or activate inflammasomes through the NLRP3 pathway to promote the release of pro-inflammatory factors such as IL-1β and TNF-

α [137, 138], which are considered surface markers for N1 neutrophils [139]. A biphasic mode of release for IL-1β and TNF-α in ischemic tissue has been described in animal models for transient and permanent cerebral ischemia. The first peak appears one hour after the ischemic injury, and the second peak becomes evident at approximately 24 hours after injury [140, 141]. IL-1β and TNF-α are mainly produced by activated microglia, astrocytes, macrophages, and endothelial cells [107, 109]. They promote the expression of chemokines (including cytokine-induced neutrophil chemoattractant [CINC]) and adhesion molecules [142], together with the activation of astrocyte MMPs to induce the infiltration of neutrophils in the ischemic brain [143]. With the rapid increase of infiltration into brain tissue, N1 neutrophils become the main contributors to the second peak of IL-1β and TNF-α [19]. The IL-1β and TNF-α released by neutrophils not only attracts more neutrophils but also inhibits neutrophil apoptosis, enhancing the inflammatory response [4]. IL-1β can also upregulate the expression of the pro-inflammatory factor IL-6 [144]. The increase in the level of IL-6 in the blood and cerebrospinal fluid in stroke patients is one of the factors that determines the severity of stroke [145]. In addition, IL-1β and TNF-α can exert neurotoxic effects directly. IL-1β derived from neutrophils can disrupt the BBB, cause vascular edema, and increase HT [146, 147]. It can also promote calcium influx in neurons and increase their vulnerability to other injuries [148]. TNF-α has been shown to increase the neurotoxicity of glutamate by inhibiting clearance of extracellular excitatory glutamate [149].

5.1.3. Degranulation

Mature neutrophils can continuously form three different types of granules: primary (azurophil) granules containing MPO and NE, secondary (specific) particles containing lactoferrin, and gelatinase (level 3) granules containing MMP9 [46]. Activated neutrophils "degranulate" and release the above-mentioned proteases. Transcriptional analysis of N1 and N2 neutrophils polarized *in vitro* (using lipopolysaccharides and IL-4) showed that, compared to N2 neutrophils, N1 neutrophils exhibited increased MPO and MMP-9 activity [128].

MPO can be expressed by a variety of immune cells, including neutrophils, macrophages/microglia, and astrocytes, among others. MPO derived from neutrophils reaches a peak between one and three days after cerebral ischemia [150], and its levels are positively correlated with infarct volume and stroke severity. When neutrophils are depleted, there is a reduction in cerebral infarction accompanied by a decrease in MPO activity [151]. MPO mainly relies on the oxidants produced by itself and by the MPO-H_2O_2-Cl_2system to exert its biological function. Hypochlorous acid (HOCL) is the main toxic molecule that mediates oxidative damage after MPO activation [152]. Neutrophils adhere to cerebral blood vessels under inflammatory conditions. HOCL produced by MPO leads to BBB dysfunction in mice through lipid peroxidation in a process that involves the activation of the ERK1/2 and JNK1/2 signaling pathways [153]. HOCL can also aggravate oxidative stress, inducing the production of ROS and then damaging the brain tissue and the BBB [154]. In addition to inducing oxidative damage, MPO can also promote inflammation. The oxidants produced by MPO activation, such as H_2O_2, can promote the recruitment of neutrophils by upregulating β-integrin [155]. MPO can also increase the release of pro-inflammatory factors and inhibit the regression of inflammation by delaying neutrophil apoptosis [156, 157]. Since it is such an important part and active component of neutrophil-derived NETs, the role of MPO in NETs will be further elaborated below.

NE, the most abundant protease in neutrophils, is released from the primary granules together with MPO [46]. NE is generally considered to mediate tissue damage after stroke. The presence of NE was detected in the ischemic brain within one day after tMCAO in mice [11]. NE degrades the basement membrane and extracellular matrix and destroys the integrity of the BBB in parallel with the effects of MPO and MMP, leading to angiogenic edema and neuronal death [158]. NE is also involved in the adhesion of neutrophils to the vascular endothelium, enhancing it by upregulation of ICAM-1 on the latter [159]. When the NE gene was knocked out, mice subjected to ischemia-reperfusion showed weakened neutrophil infiltration and reduced infarct volume [160].

MMP9 (gelatinase B) is mainly produced by neutrophils [161]. In recent years, it has been associated with several neurological diseases, including Alzheimer's, multiple sclerosis, spinal cord injury, and ischemic stroke [162]. MMP9 plays a dual role after the onset of ischemic stroke. It mainly mediates injury in the acute phase after stroke, but participates in neurovascular remodeling in the delayed recovery phase of stroke [163]. Studies based on clinical data and on animal models for stroke have reported that expression of neutrophil-derived MMP9 in brain tissue increases within two days after cerebral infarction, and it is positively correlated with infarct volume and stroke severity [164, 165]. Resident microglia and astrocytes are the main sources of MMP9 within a few hours after cerebral infarction, which promotes the recruitment of peripheral neutrophils to the brain after BBB disruption [95]. The infiltrating neutrophils (mainly belonging to the N1 phenotype) also express MMP9, further increasing BBB permeability and causing vasogenic edema [166]. This vicious circle causes the BBB (the "gatekeeper") to almost collapse, accelerating the infiltration of peripheral immune cells into the lesion and promoting the release of inflammatory mediators that cause tissue damage. The destruction of the basement membrane by MMP9 (degradation of type IV collagen) allows extravasated red blood cells to enter the parenchyma, leading to hemorrhagic transformation after infarction [167]. MMP9 has also been shown to directly cause neuronal loss by excitotoxicity [168].

5.1.4. NETs

In addition to the direct release of proteases and cytokines, the activation of neutrophils leads to the decondensation of chromatin and the active release of granular contents to form network-like DNA structures known as neutrophil extracellular traps (NETs). NETs consist of histones, NE, MPO, cathepsin G, and other components [169]. NETs were originally considered as a defense mechanism of neutrophils against infection [170]. Then it was gradually recognized that NETs are also involved in the pathogenesis of aseptic inflammatory diseases (such as atherosclerosis, thrombus, myocardial infarction and ischemic stroke) [171]. Ischemic

stroke provides an ideal environment for the formation of NETs: Upregulation of pro-inflammatory factors [172], autophagy activation [173], the release of ROS [135] and DAMPs such as ATP [174] , HMGB1 [175] and mitochondrial DNA [176]. All of these have been shown to promote the release of NETs. The NADPH oxidase in neutrophils is activated after ischemic stroke, which in turn induces the production of massive ROS. ROS promotes the release of NE and MPO from primary granules. NE translocates first to the nucleus, where it promotes extensive chromatin decondensation. The late binding of MPO to chromatin enhances decondensation [177]. Accumulated ATP in the ischemic brain promotes Ca^{2+} influx and ROS production by activating P2X7R on the surface of neutrophils, leading to the subsequent upregulation and action of PAD4 [174]. PAD4 is another key enzyme that promotes chromatin decondensation. PAD4-mediated citrullination reduces the affinity between histones and negatively charged DNA, leading to the separation of histones from DNA, which leads to the compression of chromatin structure loss and deconcentration [178]. It was demonstrated using a MCAO model that HMGB1 induces the expression of citrullinated histone $H3^{+}$ (CitH3, a marker of NETs) *via* Toll-like receptor 4 (TLR4) and CXCR4 [175]. Using CitH3 as a specific marker, NETs were found in different locations of the ischemic brain (including the vascular cavity, vascular space, and brain parenchyma) as early as 24 hours after ischemic stroke, and reached a peak in the brain parenchyma approximately three days after stroke [98]. Circulating NETs were also significantly elevated in patients that had suffered an acute ischemic stroke, and their levels were correlated with stroke severity [160].

By releasing MPO, NE, and other proteases, NETs can directly damage the vascular endothelium and increase endothelial permeability, which is conducive to the further recruitment of inflammatory cells [179]. Another role of NETs that cannot be ignored is their participation in the formation of thrombosis after stroke. Some studies have reported the presence of NETs and neutrophils in almost all of the thrombosis sites in the brain of stroke patients [180-182]. The use of NET-targeting DNase 1 *in vitro* accelerates the dissolution of the thrombus [181]. NETs combine with von Willebrand factor (VWF) and fibrinogen to provide a stent for thrombosis and increase the stability of the thrombus. Moreover, Histones H3 and H4 are able to induce platelet aggregation through direct histone binding to platelet TLR2 and TLR4 [183], or indirectly through fibrinogen binding [184]. NETs can also promote thrombosis by activating the coagulation pathway [185]. Vascular endothelial damage caused by proteases released by NETs can also induce platelet activation and binding to promote thrombosis [186]. In turn, HMGB1 released by activated platelets also promotes the release of NETs [187].

There is no clear evidence supporting a differential ability of N1 and N2 neutrophils in the formation of NETs after cerebral infarction, but not all neutrophils produce NETs [188]. The ability to produce NETs may be restricted to a neutrophil subpopulation, which should have strong inflammatory activity. It is usually believed that N1 neutrophils are the main subgroup responsible for NET production after stroke based on the following reasons: First, the peak in their levels matches that of NETs. OGD experiments *in vitro* show that ischemic neurons drive neutrophils away from the N2 phenotype and promote the formation of NETs [11]. Another study confirmed that the application of all-trans retinoic acid (atRA), the ligand of retinoic acid receptors (RARs), promotes N2 phenotypic polarization and inhibits the formation of NETs by downregulating STAT1 [137]. Second, N2 neutrophils are considered to be a beneficial phenotype that inhibits inflammation and promotes nerve repair, which is the opposite of the harmful effects mediated by overexpression of NETs after stroke. The key mechanism for the release of NETs in aseptic inflammation is the production of ROS. Transcriptional analysis of N1 and N2 neutrophils showed that, compared with N2 neutrophils, N1 neutrophils produce much higher levels of ROS [189]. Of course, more in-depth studies are required to further elucidate the difference between N1 and N2 phenotypes in terms of their ability to generate NETs.

5.2. Role of N2 Neutrophils in Neuroinflammation after Ischemic Stroke

Three days after cerebral infarction, the pro-inflammatory effect of N1 neutrophils is gradually weakened due to a reduction in protease production and the levels of effector molecules [71]. The anti-inflammatory effect of N2 neutrophils slowly begins to dominate and tissue repair commences [59, 62] (Table **1**). IL-4 has been used to induce the *in vitro* expression of markers such as Arg1, CD206, Ym1, VEGF, TGF-β and IL-10 in N2 neutrophils, in a process analogous to the induction of M2 macrophages/ microglia [10, 57, 62, 190]. Similarly, a study in which stroke was elicited in mice found that activation of the transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ) promoted the polarization of neutrophils to the N2 phenotype and the expression of the above-mentioned molecules [10]. These molecules can be used not only as markers for N2 neutrophils but also as effectors to inhibit inflammation and mediate neuroprotection through these cells.

5.2.1. Arginase 1

The two arginase subtypes, arginase 1 (Arg1) and arginase 2 (Arg2), are expressed in the brain under physiological conditions [191]. Arg1 is present in neutrophils at higher levels than in any other white blood cells in the peripheral blood after stroke [192]. A rat model for stroke showed that the expression of the ARG1 gene is upregulated and the activity of Arg1 in the infarct area is increased after cerebral infarction, while the expression of Arg2 remains unchanged. Arg1 competes with NOS by using the same substrate (L-arginine), resulting in the inhibition of NOS activity. Therefore, high levels of Arg1 expression can inhibit the production of NO and reduce oxidative stress [193]. Arg1 also increases the formation of polyamines, therefore playing a positive role in neuroprotection and nerve regeneration [194]. In addition, A mouse model of ischemia-reperfusion showed that Arg1 restricts neurovascular degeneration by stimulating repair mediated by M2 macrophages [195].

5.2.2 Release of Anti-Inflammatory Mediators

As a typical anti-inflammatory factor, IL-10 has been confirmed to be secreted by neutrophils in response to stroke

[115]. Its effects are thought to be beneficial, and they involve regulation of the vascular endothelium, protection of neurons affected by ischemia, and downregulation of pro-inflammatory factors, among others. IL-10 can downregulate ROS levels to alleviate vascular endothelial disorders caused by oxidative stress. It can also inhibit the expression of endothelial adhesion molecules, reducing leukocyte infiltration and thus achieving endothelial protection [196]. *In vitro* cultures of cortical neurons derived from knockout mice for IL-10 were more vulnerable to OGD conditions, while exogenous IL-10 administration reversed this damage [197]. This neuroprotective effect is achieved through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and STAT3 signal transduction pathways [198]. IL-10 can activate the downstream signal molecule STAT3 to promote the upregulation of IL-1ra expression in neutrophils. IL-1ra acts as a natural antagonist of the pro-inflammatory factor IL-1β and competitively inhibits its effects [199]. Coincidentally, the mouse pMCAO model demonstrated that IL-$10^{-/-}$ mice show a higher infarct volume and increased expression of pro-inflammatory factors, whereas the infarct volume in transgenic mice overexpressing IL-10 is significantly reduced [200]. A clinical study also suggested that low serum IL-10 levels in the general population may be associated with an increased risk of ischemic stroke [201].

TGF-β is a family of proteins that can regulate cell growth and apoptosis, inflammation, and tissue repair, and it is overexpressed by N2 neutrophils [202]. TGF-β was upregulated in the brain tissue of rats after cerebral ischemia and reperfusion. It has also been shown that TGF-β can upregulate the Smd3 signaling pathway and down-regulate the JNK signaling pathway to reduce brain damage [203, 204]. Since it is downstream of the MAPK signaling pathway, the JNK signaling pathway mediates apoptosis after activation. TGF-β can also promote nerve repair. For example, TGF-β can induce neurite growth and their elongation in hippocampal neurons after injury. TGF-β has also been shown to be a key factor that mediates axon formation and migration in murine cortical neurons [205]. Importantly, the MCAO mouse model showed improved nerve function and obvious nerve regeneration between day 7 and day 28 after infarction in response to exogenous TGF-β [206]. The ability of TGF-β to promote neuroprotection and remodeling also translates as post-stroke enhancements in hippocampal memory and decreases in depression levels [207].

5.2.3. Enhanced Phagocytosis

A recent study revealed for the first time the increased phagocytic ability and clearance rate of N2 neutrophils [9]. On the one hand, the enhanced phagocytic capacity of N2 neutrophils accelerates the removal of debris from inflamed tissues to promote tissue repair. On the other hand, clearance of N2 neutrophils is accelerated by being preferentially phagocytosed by microglia and macrophages. *In vivo* and *in vitro* experiments have demonstrated the potential neuroprotective effect of microglia as the first line of immune defense after cerebral infarction [208, 209]. This effect is achieved by phagocytosis of infiltrating neutrophils and inhibition of their immunotoxicity. Macrophages that migrate from the peripheral blood to the brain play a similar role. The phagocytosis of N2 neutrophils by macrophages also inhibits their own production of IL-23, which in turn reduces the production of IL-17A by γδ T cells, leading to downregulation of G-CSF and finally to inhibition of neutrophil production [38]. In addition to the phagocytosis and clearing by the immune cell types mentioned above, a mechanism of "self-eating" has been described for neutrophils based on data obtained from the mouse gout model. The apoptotic neutrophils showed upregulation in the expression of TGF-β on their surface and downregulation of ROS production [210]. Since TGF-β is regarded as a marker of N2 neutrophils, this suggests that under certain inflammatory conditions this self-clearing mechanism may contribute to the polarization of neutrophils towards the N2 phenotype.

5.2.4. Neurovascular Recovery

The "self-rescue" behavior of the brain, a neurovascular recovery in the acute phase of ischemic stroke, begins four to seven days after the event [211]. This phenomenon is accompanied by an increase in the proportion of N2 neutrophils [11]. On the one hand, the enhanced phagocytosis ability of N2 cells creates an appropriate microenvironment for neurovascular repair and regeneration by contributing to the removal of dead cells and debris. On the other hand, N2 neutrophils directly express molecules or proteins that promote neurovascular regeneration and repair. Molecules such as TGF-β, IL-10, CD206, Ym1 and Arg1 (as mentioned above) have all been shown to promote neurovascular regeneration [212]. In addition, N2 neutrophils inhibit the death of ischemic neurons *via* the brain-derived neurotrophic factor/tropomyosin-related kinase B (BDNF/TrkB) signaling pathway, thereby promoting the spontaneous recovery of nerves in the late stage of cerebral ischemia. This effect has been verified *in vitro* in oxygen-glucose deprivation/reoxidation (OGD/R) conditions and *in vivo* in rat models for transient cerebral ischemia [11]. Brain-derived neurotrophic factor (BDNF) is thought to bind and activate tropomyosin-related kinase B (TrkB) receptors to affect synapse formation and neuronal development [213]. The VEGF overexpressed by N2 neutrophils is widely known to be involved in neurovascular regeneration and repair, and it has been shown to promote neurite outgrowth in cerebral cortex neurons *in vitro* by activating Rho/ROCK signals [214]. Overexpression of VEGF in mice increases neuron production in the subventricular zone (SVZ) and migration to the infarcted cortex after stroke [215]. VEGF also promotes the regeneration of blood vessels around the infarction site and increases the supply of oxygen and nutrients required for neurogenesis [216].

6. INTERPLAY BETWEEN NEUTROPHILS AND NEURAL CELLS

6.1. Neurons

Neurons are the cell type that is more affected by ischemic stroke. The interruption of the blood flow caused by vascular occlusion leads to the immediate death of neurons in the ischemic area due to nutritional and metabolic deficits. Necrotic neurons immediately release heat shock proteins, ATP, HMGB1 and other DAMPs to induce the infiltration of peripheral neutrophils into the brain. The correlation between neutrophil infiltration and neuronal death has been confirmed in some early studies. The number of neutrophils

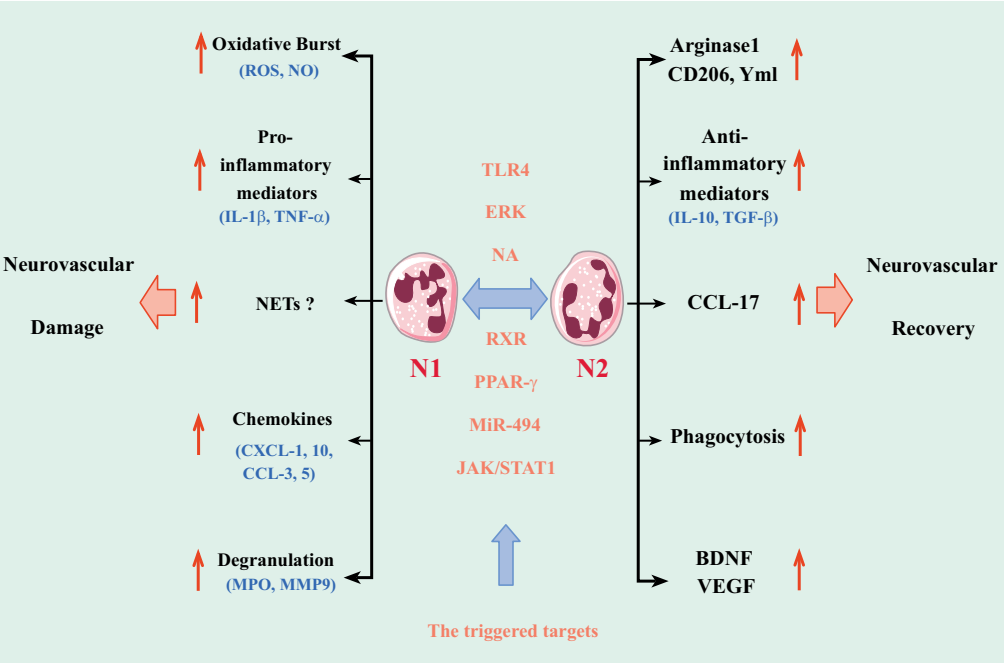

Fig. (2). The role of N1 and N2 neutrophils after ischemic stroke. N1 neutrophils are the predominant neutrophil subpopulation in the brain in the acute phase of stroke (within three days). N1 neutrophils aggravate neurovascular damage through oxidative stress (ROS, NO), production of pro-inflammatory cytokines (IL-1β, TNF-α), release of protease granules (MPO, MMP9), and generation of NETs. In later stages, the proportion of N2 neutrophils gradually increases. N2 neutrophils promote neurovascular repair and reconstruction by releasing Arg1, CD206, Ym1, BDNF, and VEGF, producing anti-inflammatory factors (IL-10, TGF-β) and displaying enhanced phagocytic ability. Recent studies have shown that the targets involved in neutrophil polarization include: NA, TLR4, ERK, JAK/STST1, MiR-494, PPAR-γ and RXR. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

in the peripheral blood is positively correlated with the infarct volume [49], and this volume is significantly reduced after the infiltration of neutrophils into the brain has been inhibited [217]. In addition, the upregulation of neutrophils on the ischemic side of the brain after experimental stroke is associated with the progressive neuronal loss in secondary neurodegeneration (SND) sites that are remote from the primary infarction site, but synaptically connected to it [218]. Current research evidence indicates that neutrophils can damage neurons both directly and indirectly. The stagnation and attachment of neutrophils to the capillaries during the migration process leads to the phenomenon known as "no-reflow", in which the blood flow fails to be completely re-established, aggravating the consequences of the infarction (Fig. **3**) [219]. The neutrophils present in the ischemic cerebral hemisphere are highly activated and express inflammatory factors, proteases, and free radicals that cause neuronal damage. The direct effects of neutrophils on neurons have been mainly studied *in vitro*. Co-cultures of neutrophils and mouse hippocampal neurons demonstrated that under physiological conditions, neutrophils do not affect the survival of neurons. However, neurons co-cultured with neutrophils showed more obvious damage (severe loss of axons and dendrites) in response to OGD conditions compared to neuronal monocultures [220]. Compared with the control group, LPS-induced activated neutrophils also exhibited stronger neurotoxicity *in vitro*. In addition, it was observed under the intravital microscope that neurons in contact with neutrophils frequently exhibit neurite destruction [221]. Endothelial cells treated with IL-β activate neutrophils and promote their transendothelial migration, triggering differentiation into the neurotoxic phenotype [222]. An *in vitro* study revealed that the neurotoxicity of neutrophils depends on the production of MMPs, ROS and TNF-α（Fig. **3**） [223]. Protease 3, produced by neutrophils, can also mediate neuronal death by inducing the generation of ROS [224].

6.2. Astrocytes

As mentioned earlier, astrocytes are transformed into a reactive state after ischemic stroke. It is currently believed that reactive astrocytes comprise two different subtypes that have been named "A1" and "A2". A1 astrocytes, but not A2 astrocytes, significantly upregulate the expression of complement C3 [120]. A1 astrocytes are the main astrocyte subtype present in the early stages after ischemic stroke [225]. They promote the infiltration of neutrophils by expressing chemokines and pro-inflammatory factors, by upregulating adhesion molecules in endothelial cells, and by enhancing the permeability of the BBB. A1 astrocytes upregulate the expression of IL-1, TNF-α, and the chemokines CXCL1, CXCL2 and CXCL5 by activating the NF-κB and the TGF-β-activated kinase 1 (TAK1) signaling pathways (Fig. **3**) [226, 227]. In addition to these indirect interactions, astrocytes contact neutrophils directly during migration. *In vitro* co-culture showed that neutrophils induced the death of astrocytes [228]. In turn, astrocytes extend the lifespan of neutrophils, promote the expression of its pro-inflammatory factors IL-1β, IL-6, TNF-α and enhance their phagocytic ability [229]. In summary, direct interaction with astrocytes enhances the inflammatory response of neutrophils.

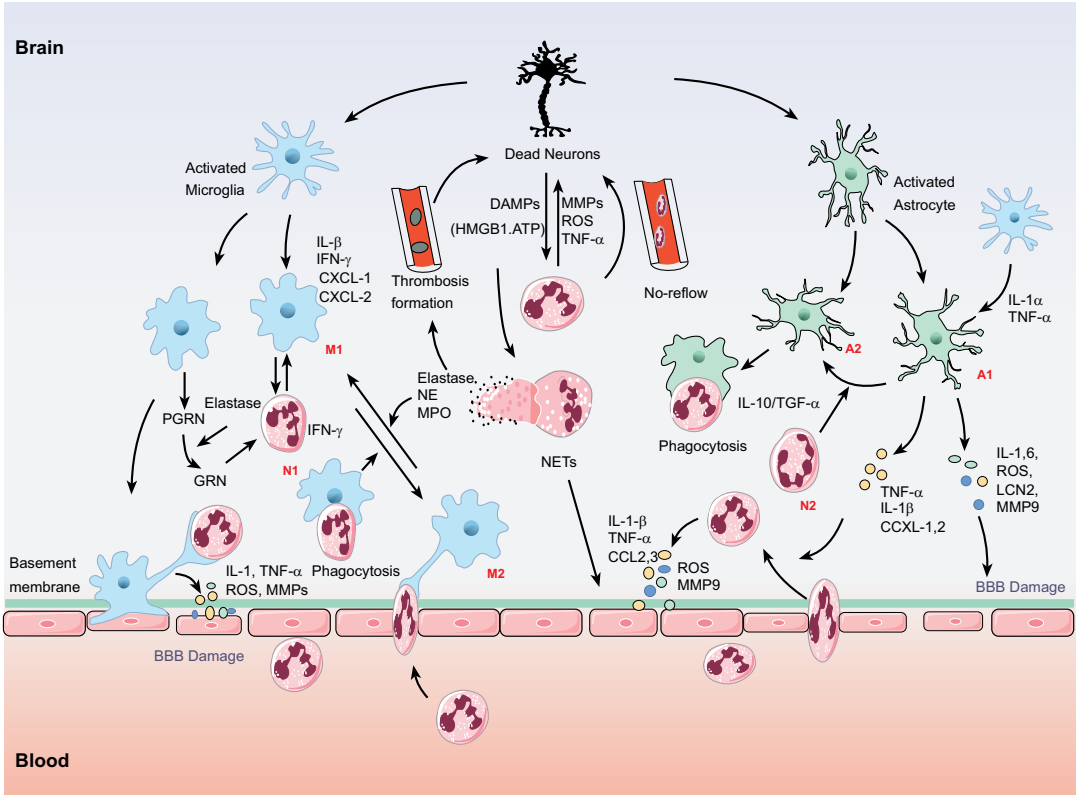

Fig. (3). The interplay between neutrophils and neural cells. Neutrophils can damage neurons directly or indirectly. The stagnation and attachment of neutrophils in the capillaries during the migration process leads to "no-reflow", which aggravates the damage in the infarction area. Neutrophils can also directly induce neurotoxicity by producing MMPs, ROS, and TNF-α. A1 astrocytes promote neutrophil infiltration by upregulating the expression of pro-inflammatory factors (IL-1β, TNF-α) and chemokines (CXCL1, CXCL2, CXCL5), and by accelerating BBB disruption through the release of ROS, MMP9, and LCN-2. A2 astrocytes limit the spread of neutrophils in the area around the infarction by forming glial scars (known as "glial hyperplasia") and phagocytosing neutrophils in the penumbra. Activated microglia undergo a morphological transition from a ramified to a round or amoeboid form and extend flat protrusions to the proximal luminal side to engulf neutrophils. The actions of the microglia are critical for protecting the ischemic penumbra. M1 microglia express the pro-inflammatory factor IFN-γ in large quantities, which induces the polarization of N1 neutrophils. Production of the anti-inflammatory factors IL-4, IL-10, and TGF-β by M2 microglia not only inhibits the expression of pro-inflammatory factors (TNF-α) and adhesion molecules (ICAM-1 and VCAM-1) but also induces the polarization of neutrophils to the N2 phenotype. NETs formed by neutrophils have also been shown to induce microglia to shift to a pro-inflammatory phenotype. Neutrophils induce the conversion of PGRN into the pro-inflammatory mediator GRN, enhancing the inflammatory response. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

The proportion of A2 astrocytes in the brain gradually increases 72 hours after ischemic stroke, when the proliferation and formation of glial scars ("glial hyperplasia") limits the spread of neutrophils in the area surrounding the infarction [230]. A2 astrocytes reduce tissue damage around the infarction site by swallowing neutrophils in the penumbra and restricting their further migration (Fig. **3**) [231]. The anti-inflammatory factors produced by N2 neutrophils and microglia play a key role in the transition of astrocytes from the A1 to the A2 phenotype by downregulating P2Y1R [232]. As the most abundant cells in the brain, astrocytes are bound to play diverse roles in stroke. Whether as source of nutrients or as immune cells, their functions are probably far more complex than what has been described so far. Therefore, the current evidence for the interaction of neutrophils with astrocytes is far from sufficient.

6.3. Microglia

Microglia are brain-intrinsic macrophages derived from the yolk sac. After ischemic stroke, activated microglia undergo a significant morphological transition from a ramified to a round or amoeboid form. Functionally, activated microglia exhibit enhanced phagocytosis and mediate inflammation [233]. When *in vivo* two-photon microscopy was used to study the vascular extravasation and active migration of neutrophils in the parenchyma it was at the same time observed that microglia extended flat protrusions to the proximal luminal side in response to endothelial injury signals to capture and engulf neutrophils [97]. The formation of an extensive cell to cell contact between neutrophils and microglia attracts more microglia to the contact site (Fig. **3**) [234]. Compared with neutrophils in the parenchyma, which are not phagocytosed, neutrophils in contact with microglia are almost immobile [235]. The morphology of microglia differs according to capillary blood flow. A decrease in the blood flow induces the de-ramification of microglia, while its complete blockage prevents microglial activation [236]. At day 1 and day 4 after pMCAO, the number of microglia in the ischemic core of the mouse brain is sparse and those cells that are present appear to be in a malnourished state, while the microglia

surrounding the infarct area is abundant in number, have been activated and have adopted an ameboid morphology. This directly leads to the accumulation of neutrophils in the ischemic core, while their number remains scarce in the ischemic penumbra [237]. Thus, the phagocytosis of neutrophils by microglia is of great significance in the rescue of the ischemic penumbra. Interestingly, neutrophils interacting with microglia in the brain parenchyma exhibit reverse transendothelial migration (rTEM) to the bloodstream, which is conducive to the resolution of brain inflammation. It has been postulated that the interaction with microglia triggers molecular modifications in the neutrophils that make them more prone to perform rTEM [234]. The neuroprotective effect of microglia is also achieved by the release of anti-inflammatory mediators such as progranulin (PGRN). Exogenous administration of recombinant PGRN inhibits the recruitment of neutrophils and the pro-inflammatory NF-κB signaling pathway [238]. PGRN production by microglia is significantly increased in rats after stroke, but NE released by neutrophils induces the conversion of PGRN into the pro-inflammatory mediator GRN, enhancing the inflammatory response [239]. Microglia are considered to be polarized into two distinct phenotypes following ischemic stroke: pro-inflammatory (M1) and anti-inflammatory (M2) [240]. Theoretically, there exists a crosstalk between these two phenotypes, and N1 and N2 neutrophils. M1 microglia expresses high amounts of the pro-inflammatory factor IFN-γ, which is an inducer of N1 neutrophils *in vitro*. In turn, N1 neutrophils also express IFN-γ to promote the polarization of microglia into the M1 phenotype [8]. NETs formed by neutrophils have also been shown to induce microglia to shift to a pro-inflammatory phenotype (Fig. **3**) [241]. A few days after the stroke episode, the microglia gradually shift to an M2 phenotype [242]. The production of the anti-inflammatory factors IL-4, IL-10 and TGF-β by M2 microglia not only inhibits the expression of pro-inflammatory factors such as TNF-α, and adhesion molecules such as ICAM-1 and VCAM-1, thereby limiting the infiltration of neutrophils, but also induces the polarization of neutrophils to the N2 phenotype [119].

7. INTERPLAY BETWEEN NEUTROPHILS AND OTHER IMMUNE CELLS

An intricate interplay between neutrophils and other immune cells is inevitable and cannot be ignored. Immune cells that reach the ischemic brain immediately after neutrophils include macrophages, lymphocytes, NK cells, and dendritic cells (DCs) [243]. The crosstalk between neutrophils and these cells is achieved through direct contact or the release of cell products.

7.1. Monocyte-Derived Macrophages

After ischemic stroke, monocyte-derived macrophages infiltrate the brain following neutrophils. Monocytes are mainly derived from bone marrow and are divided into two subgroups, pro-inflammatory (M1) and anti-inflammatory (M2). The former expresses C-C motif chemokine receptor 2 (CCR2), while the latter expresses C-X3-C motif chemokine receptor 1 (CX3CR1), a receptor for fractalkine (CX3CL1), but not CCR2 [240]. Monocyte chemoattractant protein-1 (MCP-1)/CCL2 released by N1 neutrophils, astrocytes, and endothelial cells is critical for monocyte-macrophage recruitment after ischemic stroke (Fig. **4**). By recognizing CCR2, M1 monocytes in the bone marrow or spleen are mobilized to the peripheral blood and eventually infiltrate the ischemic brain, becoming activated M1 macrophages [244]. The chemokines CXCL-1 and CCL-3 and the pro-inflammatory factors IL-1β and TNF-α produced by neutrophils promote macrophage infiltration [244]. The protease PR3 released by neutrophils enhances macrophage recruitment by upregulating the expression of endothelial ICAM-1 and CCL-2 [245]. NETs have been shown to promote cytokines production by macrophages *in vitro* [246]. M1 macrophages, in turn, cause nerve injury and neutrophil infiltration by releasing proinflammatory factors (TNF-α, IL-1β, IL-6) and chemokine CCL2 and CCL5 [240, 247]. Macrophages phagocytosis of apoptotic neutrophils can induce their polarization to the M2 phenotype and promote the release of lipid mediators known as specialized pro-degradation mediators (SPM), including resolvins, lipoxinA4 (LXA4), protectin (PD), maresins, among others (Fig. **4**) [248]. These mediators contribute to the amelioration of inflammation [188].

7.2. T Lymphocytes

Compared with neutrophils, lymphocytes (which include T cells, NK cells and B cells) enter the ischemic site at a later stage [249]. More and more studies have identified the vital role of T cells in the development of ischemic stroke. T cells are divided into two large subpopulations: $CD8^+$ cytotoxic T cells and $CD4^+$ T helper (Th) cells. As the main T cell subset infiltrating the ischemic brain, $CD4^+$ Th cells, including Th1, Th2 and Th17, and regulatory T cells (Treg) not only promote inflammation in the early stage of ischemic stroke but also participate on neural repair in later stages [250]. Th1 cells and Th17 cells have always been considered to be pro-inflammatory phenotypes and therefore to exacerbate ischemic damage, while Th2 cells and Treg cells are thought to be essential for neuroprotection and repair [251]. Early-arriving neutrophils contribute to the recruitment of T cells. The chemokines CXCL9 and CXCL10, or CCL2 and CCL20 (produced by neutrophils) attract Th1 or Th17 cells, respectively, to the ischemic site [252]. Treg cells are induced by CCL17 released by neutrophils [253]. N1 neutrophils exhibit a high expression of CXCL-10 and CCL2, while N2 neutrophils express CCL17 also at high levels [139]. Therefore, we speculate that N1 and N2 neutrophils might enhance their effects by interacting with pro-inflammatory and anti-inflammatory T cells, respectively (Fig. **4**). Activated $CD4^+$ and $CD8^+$ T cells mediate neutrophil activation and anti-apoptotic effects by releasing granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α and IFN-γ [254]. IL-17 released by Th17 cells and γδ T cells upregulates G-CSF and CXCL1 to promote neutrophil mobilization [255]. Th17 cells can also attract neutrophils directly by releasing CXCL-8 (Fig. **4**) [252]. *In vitro* co-culture demonstrated that $CD4^+$ and $CD8^+$ T cells can induce neutrophils to acquire DC-like identity (resulting in the expression of the surface markers CD11c, MHC II, CD80, and CD86), which in turn mediates T cell activation and differentiation through antigen presentation [256]. The NETs released by neutrophils contribute to T cell activation by lowering the activation threshold and inducing them to polarize towards the pro-inflammatory phenotype [257]. Of course, not only is there reciprocal promotion and amplifica-

tion between neutrophils and T cells, but also reciprocal inhibition. Arg1 produced by N2 neutrophils consumes extracellular L-arginine, thereby inhibiting the proliferation of T lymphocytes and reducing inflammation (Fig. **4**) [258]. The contribution of Treg cells to neural repair in the late stages of ischemic stroke has been revealed in recent years. Although the infiltration of Treg cells in the ischemic brain does not reach a peak until 14 to 30 days after MCAO, they have been shown to have an effect on stroke outcomes within the first days after ischemic stroke [259]. The anti-inflammatory factors IL-10 and TGF-β released by Treg cells not only inhibit the inflammatory response of immune cells with a pro-inflammatory phenotype such as N1 neutrophils and M1 microglia but also promote their transition to anti-inflammatory phenotypes (such as N2 neutrophils and M2 microglia) [260]. Treg cells can also upregulate the expression of programmed death ligand-1 (PD-L1) to inhibit the release of MMP9 by neutrophils (Fig. **4**) [261].

7.3. NK Cells

The infiltration of NK cells in the brain reaches a peak 24 hours after MCAO and gradually decreases after 72 hours. They are one of the culprits of neuronal damage in the acute phase of stroke [262]. Neutrophils are involved in regulating the differentiation and functional responsiveness of NK cells. Neutrophil function loss, either innate or mediated by an anti-Ly6G antibody, leads to defects in the maturation and functionality of NK cells [263]. Neutrophils, as one of the main sources of IL-18, activate NK cells and promote their secretion of a variety of cytokines, including IFN-γ, GM-CSF and TNF-α (Fig. **4**) [264]. Both ROS and prostaglandins produced by neutrophils can inhibit the survival and cytotoxic activity of NK cells [265]. In turn, the cytokines released by NK cells play a key role in neutrophil activation, recruitment, lifespan extension, and enhancement of phagocytosis [254].

7.4. B Lymphocytes

Compared with T lymphocytes, the contribution of B cells to inflammation associated to ischemic stroke is minor. B cells mainly play a neuroprotective effect in the acute phase of stroke through the release of anti-inflammatory factors such as IL-10 and TGF-β [266]. A specific subpopulation of neutrophils in the perifollicular area of the spleen in humans and mice has been defined as B cell helper neutrophils (NBH) because they mediate T cell-independent antibody responses by secreting a B cell-activating factor of the tumor-necrosis-factor family (BAFF), in addition to proliferation-inducing ligand (APRIL), IL-21 and CD40L (Fig. **4**) [267]. In autoimmune diseases and tumors, NBHs significantly increase the release of BAFF, APRIL, and NETs, inducing excessive B cell activation [268]. In neutrophils other than NBHs, a considerable amount of BAFF is also released under the stimulation of G-CSF, mediating the development and maturation of B cells during inflammation [269]. Unfortunately, the interaction between neutrophils and B cells in ischemic stroke has not been studied in detail.

7.5. DCs

DCs are divided into two subgroups according to their source: brain resident DCs (BDCs) and peripheral DCs. BDCs are not recruited within 6 hours after MCAO but their number reaches a peak at the edge of the infarction area after 24 hours. After 72 hours, peripheral DCs gradually infiltrate the brain and occupy the ischemic core [270]. The differences in the timing and the location of infiltration between DCs and neutrophils determine the nature of the interaction between the two cell types. The chemokines CCL3 and CCL5, produced by neutrophils, promote the migration of bone marrow DCs *in vitro* [271]. Both TNF-α and IFN-γ, highly expressed by N1 neutrophils, are involved in the activation of DCs [272]. Activated DCs mediate in turn the activation and differentiation of T cells, which are regulated by cell derivatives (MPO, NE, NETs, and endosomes) produced by neutrophils. MPO derived from neutrophils inhibits the activity of $CD4^+$ cells and the response to antigens by preventing the maturation of DCs [273]. NE induces the polarization of T cells to the Th17 phenotype by processing CXCL8 produced by DCs [274]. There is no consensus on the role of NETs on DCs. A study showed that NETs promote the activation of DCs and induce T cells to polarize towards the pro-inflammatory Th1 phenotype [275]. However, another study revealed that DCs treated with NETs inhibit the proliferation of T cells and drive them to polarize into the anti-inflammatory Th2 phenotype (Fig. **4**) [230].

8. POLARIZATION OF NEUTROPHILS

8.1. Location of Neutrophil Polarization

As mentioned above, the current definition of N1 and N2 neutrophils in experimental stroke is mainly based on whether the classic surface markers for M2 microglia such as Ym1 and CD206 are expressed [10, 62, 190]. Under physiological conditions, the relative proportions of N1 ($Ym1^-$) and N2 ($Ym1^+$) neutrophils in the bone marrow and peripheral blood of mice are comparable. However, the number and ratio of N1 and N2 neutrophils changes dramatically after pMCAO: The total number of neutrophils decreases in the bone marrow and increases dramatically in the periphery and in the brain between 24 and 48 hours after episode onset. The ratio between N1 and N2 neutrophils decreases significantly in the bone marrow but remains almost constant in the periphery. Moreover, almost two thirds of the total number of neutrophils in the ischemic brain belong to the N1 phenotype [10, 190]. This indicates that ischemic stroke induces neutrophil polarization, and that this process begins in the bone marrow. Furthermore, the polarization of neutrophils during this period is generally biased towards the N1 phenotype. As for the site of polarization, whether it is limited to the bone marrow is still unclear. Transcriptional analysis of N1 and N2 neutrophils suggests that N1 neutrophils exhibit enhanced chemotactic and adhesion capacity compared to N2 neutrophils. Specifically, N1 neutrophils express higher levels of the chemokines CCL3, CCL5, CXCL1, and CXCL10 as well as the adhesion molecules CD11b (Mac-1) and ICAM-1 [128, 139]. If neutrophil polarization only occurred in the bone marrow, the number of N1 neutrophils released to the periphery should be significantly higher than the number of N2 neutrophils. However, their enhanced chemotactic ability may significantly reduce the total time spent in the bloodstream, since they can infiltrate the brain more efficiently. Taking this into account, it makes sense that the number of N1 neutrophils is almost equal to that of N2 neutrophils in the peripheral blood, but significantly

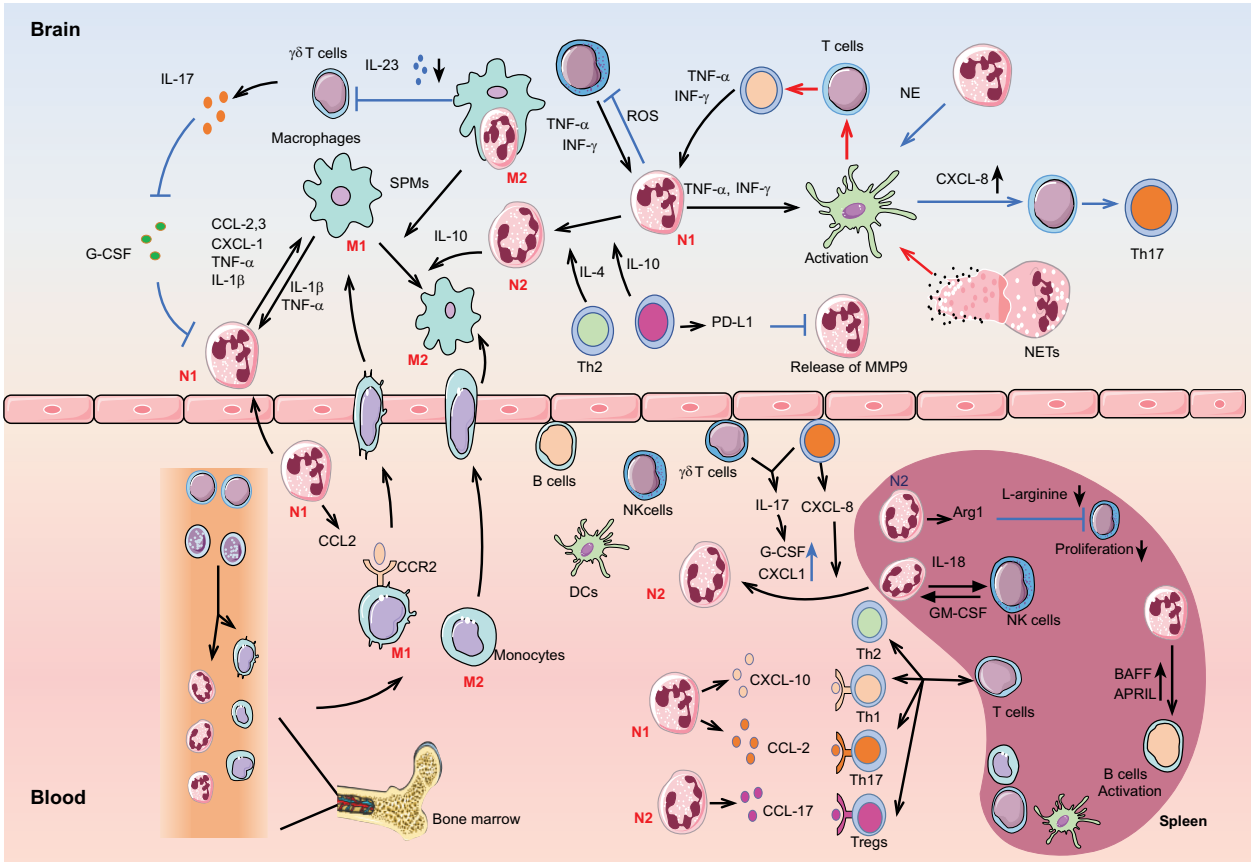

Fig. (4). The interplay between neutrophils and other immune cells. N1 neutrophils promote the migration of macrophages by releasing chemokines (CCL-2, CCL-3, CXCL-1) and proinflammatory factors (TNF-α, IL-1β). M1 macrophages, in turn, mediate nerve injury and neutrophil infiltration by releasing inflammatory factors, chemokines, and phagocytosis of neurons. The phagocytosis of apoptotic neutrophils by M2 macrophages can induce their polarization to the M2 phenotype and promote the release of lipid mediators known as specialized pro-degradation mediators (SPM), which contribute to the amelioration of inflammation. N1 neutrophils promote infiltration of Th1 and Th17 cells by expressing CXCL-10 and CCL-2. The NETs released by N1 neutrophils activate T cells by lowering the activation threshold and inducing them to polarize toward the pro-inflammatory phenotype. N2 neutrophils promote the infiltration of Treg cells by expressing CCL-17 and inhibit the proliferation of T lymphocytes by producing Arg1. Treg cells induce the transition of N1 neutrophils to the N2 phenotype by releasing IL-10 and TGF-β. Neutrophils activate NK cells and induce them to secrete a variety of cytokines (including IFN-γ, GM-CSF and TNF-α) by releasing IL-18. In turn, the cytokines released by NK cells play a key role in neutrophil activation, recruitment, lifespan extension, and enhancement of phagocytosis. Neutrophils located in the spleen activate B cells through the expression of high levels of BAFF, APRIL, and NETs. N1 neutrophils promote the activation and migration of DCs by producing TNF-α, IFN-γ, CCL3 and CCL5. Activated DCs mediate the activation and differentiation of T cells, which are regulated by cell derivatives (MPO, NE, NETs and endosomes) produced by neutrophils. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

exceeds this number within the brain. With the development of transcription analysis technology, it has gradually been recognized that the N1 and N2 phenotypes are not the product of two independent transcription programs but are likely to represent two different activation states [47] instead. The neutrophils mobilized from the bone marrow do not necessarily belong to the N1 or N2 subtype: There may be some "neutral" neutrophils present in the peripheral blood and ischemic brain as well, which may be polarized to the N1 or N2 phenotype under the right conditions. Moreover, the N1 and N2 neutrophils present in the peripheral blood and ischemic brain may also be transformed into each other in response to changes in the internal environment. This hypothesis is supported by studies on mouse models for stroke: In the subacute phase of ischemic stroke, the dominance of the pro-inflammatory N1 phenotype in brain neutrophils was reported to gradually decrease in favor of the anti-inflammatory N2 phenotype [48, 51]. Therefore, the peripheral blood and the brain may also be additional sites of neutrophil polarization, besides the bone marrow. Next, we will discuss the mechanism of neutrophil polarization and the process of reciprocal transformation between phenotypes.

8.2. Mechanisms of Neutrophil Polarization

8.2.1. Norepinephrine

Norepinephrine (NA) released in response to SNS activation is involved in the polarization of bone marrow neutrophils after cerebral ischemia. This induction is unbalanced, as confirmed by a recent study [276]: *In vitro* NA promotes neutrophil polarization towards the N2 phenotype, which

shows impaired chemotaxis. Similar results were observed in the mouse model for stroke, in which the SNS is in a state of pathological activation [276] (Fig. **5**).

8.2.2. Toll-Like Receptors

Neutrophils express almost all of the Toll-like receptors (with the exception of TLR3 and TLR7). As endogenous ligands of DAMPs, Toll-like receptors are essential for the activation, recruitment, and polarization of neutrophils in the bone marrow (Fig. **5**). An experimental model of myocardial infarction demonstrated that HMGB1 and heat shock protein 60 (HSP60) induce neutrophils to polarize to the N1 phenotype by binding to the TLR4 receptors on their surface [46]. The DAMPs released in the brain after ischemic stroke also include the alarmins S100A8 and S100A9, which have been shown to participate in the polarization of N1 neutrophils by activating the TLR4/MD2 receptor complex *in vitro* [128]. Moreover, the experimental stroke model confirmed that the neutrophils from mice deficient in TLR4 tend to polarize towards the N2 phenotype and to exhibit neuroprotective effects [190]. The polarization towards the N1 phenotype induced by TLR4 can be attributed to the downstream signaling pathways it activates, namely MyD88-dependent NF-κB and mitogen-activated protein kinases MAPKs (including extracellular signal-regulated kinases [ERKs], c-Jun N-terminal kinases [JNKs] and p38) [277]. These signaling pathways are involved in neutrophil activation and in promoting the expression of pro-inflammatory mediators. An analysis of the transcription characteristics of N1 and N2 neutrophils *in vitro* showed that, in contrast with N2 neutrophils, the signaling molecules ERK and the P65 subunit of NF-κB were activated in N1 neutrophils [113]. The transcription factor NF-κB promotes the expression of pro-inflammatory factors such as IL-1β, TNF-α and IL-6 by neutrophils [278]. ERK phosphorylation has been shown to promote the polarization of N1 neutrophils in inflammatory bowel disease, and the polarization is switched towards the N2 phenotype when it is inhibited [279]. The phosphorylation of ERK1/2 and p65 in N1 neutrophils is reduced in response to a S100A8/A9 antagonist, leading to a decrease in the production of the chemotactic factors CCL2, CCL3, and CCL5, and in the activity of NO, MPO and MMP-9. MARKs also promote the release of pro-inflammatory factors, ROS and MMPs [137].

8.2.3. Intracellular Signals

Studies have shown that several transcriptional regulators play a key role in the polarization of the N1 and N2 phenotypes. The signal transducer and activator of transcription (STAT) family play an important role in neutrophil function [280]. STATs are generally activated by Janus kinase (JAK), and it is well known that JAK/STAT1 can be inhibited by the suppressor of cytokine signaling 1 (SOCS1) [281]. atRA pretreatment of transient MCAO in mice promotes the polarization of neutrophils to the N2 phenotype and reduces brain damage, and this is mediated by the inhibition of JAK/STAT1 resulting from upregulation in the expression of SOCS1 [105] (Fig. **5**).

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is another transcription factor belonging to the nuclear receptor superfamily that can coordinate the conversion of macrophages and microglia from the M1 to the M2 phenotype [282]. It has been shown in a mouse model of permanent cerebral ischemia that the polarization of neutrophils to the N2 phenotype can be regulated by PPAR-γ activation [7]. Retinoid X receptors (RXR), as a heterologous partner of PPAR-γ, also play an important role in the differentiation and function of bone marrow cells, including the promotion of polarization of bone marrow cells towards the anti-inflammatory phenotype by inhibiting the activation of NF-κB [283]. Intravenous administration of the RXR agonist bexarotene to mice can significantly reduce the nerve damage caused by transient MCAO. This neuroprotective effect has been attributed to the activation of the RXR/PPAR-γ heterodimer, and it also extends to a decrease in the spleen atrophy caused by MCAO, an increase in the density of N2 neutrophils in the spleen, and an increase in their infiltration into the ischemic brain [284] (Fig. **5**).

MicroRNA 494 (miR-494) is another small intracellular molecule involved in the polarization of neutrophils. High levels of miR-494 derived from circulating neutrophils in patients with ischemic stroke are considered to be a clinical indicator of poor prognosis [285]. miR-494 regulates the expression of MMPs by binding to histone deacetylase 2 (HDAC2) in neutrophils. Intravenous administration of the miR-494 antagonist antagomiR-494 in a mouse model for stroke inhibited the shift of neutrophils towards the N1 phenotype and their infiltration into the brain, resulting in neuroprotection [286] (Fig. **5**). Therefore, miR-494 may be a key node in the complex network regulating neutrophil polarization.

8.2.4. Aging

Compared with healthy individuals, patients affected by acute ischemic stroke have an enlarged subpopulation of senescent peripheral neutrophils (with high expression of CXCR4 and β2 integrin, and low expression of CD62L), which are overactive and generate high levels of NO and NETs [287]. Aging is a key factor affecting the polarization of macrophages and microglia. It is believed that aging macrophages and microglia are more likely to be induced to M1, accompanied by upregulation of neurotoxicity [288]. *In vivo* analysis of aging shows that the pro-inflammatory activity of neutrophils is positively correlated with total time in circulation. Aging upregulates several molecules and pathways in neutrophils that are also enhanced during activation, including β2 integrin (Mac-1) and molecules related to leukocyte adhesion (CD11b, CD49d and ICAM-1), TLR, NOD-like receptor (NLR) and the NF-κB signaling pathway [289]. This suggests a high degree of overlap between senescent neutrophils and N1 neutrophils. In contrast, the expression pattern of N2 neutrophils is more similar to the one displayed by non-aged neutrophils [190]. Therefore, we speculate that senescent neutrophils may be part of the N1 neutrophil category. Neutrophils may also undergo a phenotypic transformation from the N2 to the N1 phenotype as they go through the process of senescence in the peripheral blood (Fig. **5**).

8.2.5. Threshold Hypothesis

The brain is very likely to be the site where the phenotype of neutrophils transforms, given that they are present

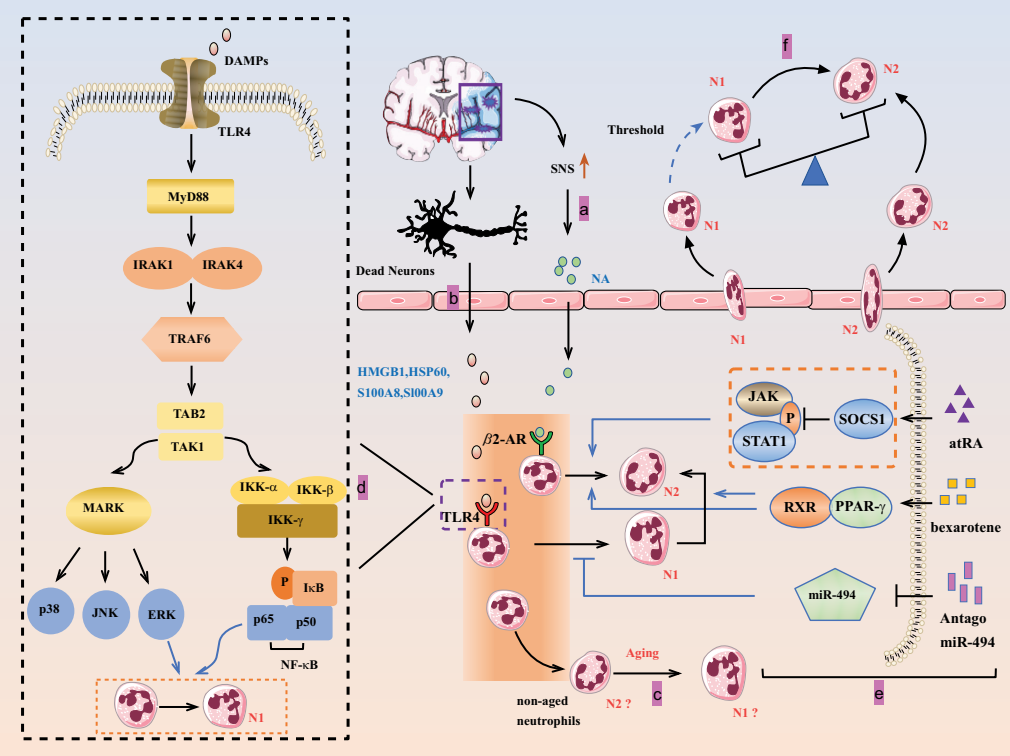

Fig. (5). Mechanisms of neutrophil polarization. (**a**) Norepinephrine (NA) released in response to SNS activation promotes neutrophil polarization towards the N2 phenotype after ischemic stroke. (**b**) DAMPs (including HMGB1, HSP60, S100A8 and S100A9) induce neutrophils to polarize to the N1 phenotype by binding to the TLR4 receptors on their surface. (**c**) Neutrophils may undergo a phenotypic transformation from the N2 to the N1 phenotype as they go through the process of senescence in the peripheral blood. (**d**) The polarization towards the N1 phenotype induced by TLR4 can be attributed to the downstream signaling pathways it activates, namely MyD88-dependent NF-κB and mitogen-activated protein kinases MAPKs. (**e**) Intracellular signals are targets that affect the polarization of the N1 and N2 phenotypes. (**f**) When the number of N1 neutrophils in the damaged tissue reaches a certain critical threshold, they respond by switching to the N2 phenotype. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

there in high enough numbers and for a sufficient period of time. N1 neutrophils are dominant in the brain during the hyperacute phase. This has been attributed, on the one hand, to the peripheral response to central pro-inflammatory signals, and on the other hand, to the fact that the brain experiences an inflammatory burst during this phase under the action of several different inflammatory cell types [290]. The neutrophils will therefore inevitably polarize to the N1 phenotype in response to these pro-inflammatory signals in order to eliminate necrotic tissues and cells. It has been proposed that when the number of N1 neutrophils in the damaged tissue reaches a certain critical threshold, they respond by switching to the N2 phenotype to mediate damage repair [291] (Fig. **5**). At the same time, experimental stroke models indicate that the neuroprotective effect of the N2 phenotype is based on an increase in neutrophil infiltration [7,159]. When this infiltration is reduced, the neuroprotective effect of the N2 phenotype is also weakened or even disappears. The increasing levels of N2 neutrophils thus mediate the transition between inflammation on the one hand, and nerve regeneration and repair on the other.

CONCLUSION AND FUTURE PERSPECTIVES

This article systematically describes the entire process of mobilization and migration of neutrophils from the periphery to the brain after ischemic stroke. The innovative aspect of it is that the role of N1 and N2 neutrophils and their crosstalk with other immune cells were individually discussed. In addition, we explored the potential mechanisms of neutrophil polarization and reciprocal transformation. In this way, it is easier to understand the role of neutrophils in ischemic stroke from a more comprehensive perspective. Overall, the predominance of N1 neutrophils in the early stage aggravates brain damage through overexpression of pro-inflammatory factors, proteases and ROS. In later stages, N2 neutrophils gradually begin to dominate and exert neuroprotective effects through overexpression of cytokines. The alternation of the two phenotypes implies a high degree of plasticity for this functional classification. Based on recent research results, we discussed the possible mechanisms regulating this phenotypic plasticity and concluded the following: After ischemic stroke, the polarization of neutrophils begins in the bone marrow and is biased towards the N1 phenotype. As the infarction progresses and the inflammatory environment changes, neutrophils may undergo a phenotypic transformation in the peripheral blood and ischemic brain. This process is co-regulated by both exogenous and intracellular signals. This model of neutrophil polarization provides more precision in terms of timing and location, and it may therefore prove useful for the specific targeting of the N1 and N2 neutrophil subpopulations to treat ischemic stroke.

Nevertheless, due to the lack of specific surface markers, the present conclusions are based on the expression of M1- and M2-like markers by neutrophils. Their specificity and accuracy remain to be verified. There are still many unsolved problems that need to be explored in depth: The differences in chemotactic adhesion between the N1 and N2 phenotypes have not been systematically compared. There is also no concrete evidence on potential differences in the overexpression of protease and NETs between the N1 and N2 phenotypes. The specific mechanism of neutrophil polarization in the brain, and how it is affected by the surrounding inflammatory environment and other factors, remains to be further characterized. Most importantly, it is necessary to find more accurate markers to define the N1 and N2 neutrophil subpopulations. At present, single-cell sequencing is the most accurate and promising method to reveal the subtypes of neutrophils after ischemic stroke. It is to be hoped that, with the application of this technology to the study of ischemic stroke in the future, the mystery surrounding neutrophil subtypes will eventually be solved.

LIST OF ABBREVIATIONS

NETs = Neutrophil Extracellular Traps

IVT = Intravenous Thrombolysis

rt-PA = Recombinant Tissue Plasminogen Activator

MT = Mechanical Thrombectomy

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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REFERENCES

[1] Johnson, C.O.; Nguyen, M.; Roth, G.A.; Nichols, E.; Alam, T.; Abate, D.; Abd-Allah, F.; Abdelalim, A.; Abraha, H.N.; Abu-Rmeileh, N.M.E.; Adebayo, O.M.; Adeoye, A.M.; Agarwal, G.; Agrawal, S.; Aichour, A.N.; Aichour, I.; Aichour, M.T.E.; Alahdab, F.; Ali, R.; Alvis-Guzman, N.; Anber, N.H.; Anjomshoa, M.; Arabloo, J.; Arauz, A.; Ärnlöv, J.; Arora, A.; Awasthi, A.; Banach, M.; Barboza, M.A.; Barker-Collo, S.L.; Bärnighausen, T.W.; Basu, S.; Belachew, A.B.; Belayneh, Y.M.; Bennett, D.A.; Bensenor, I.M.; Bhattacharyya, K.; Biadgo, B.; Bijani, A.; Bikbov, B.; Bin Sayeed, M.S.; Butt, Z.A.; Cahuana-Hurtado, L.; Carrero, J.J.; Carvalho, F.; Castañeda-Orjuela, C.A.; Castro, F.; Catalá-López, F.; Chaiah, Y.; Chiang, P.P-C.; Choi, J-Y.J.; Christensen, H.; Chu, D-T.; Cortinovis, M.; Damasceno, A.A.M.; Dandona, L.; Dandona, R.; Daryani, A.; Davletov, K.; de Courten, B.; De la Cruz-Góngora, V.; Degefa, M.G.; Dharmaratne, S.D.; Diaz, D.; Dubey, M.; Duken, E.E.; Edessa, D.; Endres, M.; Faraon, E.J.A.; Farzadfar, F.; Fernandes, E.; Fischer, F.; Flor, L.S.; Ganji, M.; Gebre, A.K.; Gebremichael, T.G.; Geta, B.; Gezae, K.E.; Gill, P.S.; Gnedovskaya, E.V.; Gómez-Dantés, H.; Goulart, A.C.; Grosso, G.; Guo, Y.; Gupta, R.; Haj-Mirzaian, A.; Haj-Mirzaian, A.; Hamidi, S.; Hankey, G.J.; Hassen, H.Y.; Hay, S.I.; Hegazy, M.I.; Heidari, B.; Herial, N.A.; Hosseini, M.A.; Hostiuc, S.; Irvani, S.S.N.; Islam, S.M.S.; Jahanmehr, N.; Javanbakht, M.; Jha, R.P.; Jonas, J.B.; Jozwiak, J.J.; Jürisson, M.; Kahsay, A.; Kalani, R.; Kalkonde, Y.; Kamil, T.A.; Kanchan, T.; Karch, A.; Karimi, N.; Karimi-Sari, H.; Kasaeian, A.; Kassa, T.D.; Kazemeini, H.; Kefale, A.T.; Khader, Y.S.; Khalil, I.A.; Khan, E.A.; Khang, Y-H.; Khubchandani, J.; Kim, D.; Kim, Y.J.; Kisa, A.; Kivimäki, M.; Koyanagi, A.; Krishnamurthi, R.K.; Kumar, G.A.; Lafranconi, A.; Lewington, S.; Li, S.; Lo, W.D.; Lopez, A.D.; Lorkowski, S.; Lotufo, P.A.; Mackay, M.T.; Majdan, M.; Majdzadeh, R.; Majeed, A.; Malekzadeh, R.; Manafi, N.; Mansournia, M.A.; Mehndiratta, M.M.; Mehta, V.; Mengistu, G.; Meretoja, A.; Meretoja, T.J.; Miazgowski, B.; Miazgowski, T.; Miller, T.R.; Mirrakhimov, E.M.; Mohajer, B.; Mohammad, Y.; Mohammadoo-khorasani, M.; Mohammed, S.; Mohebi, F.; Mokdad, A.H.; Mokhayeri, Y.; Moradi, G.; Morawska, L.; Moreno Velásquez, I.; Mousavi, S.M.; Muhammed, O.S.S.; Muruet, W.; Naderi, M.; Naghavi, M.; Naik, G.; Nascimento, B.R.; Negoi, R.I.; Nguyen, C.T.; Nguyen, L.H.; Nirayo, Y.L.; Norrving, B.; Noubiap, J.J.; Ofori-Asenso, R.; Ogbo, F.A.; Olagunju, A.T.; Olagunju, T.O.; Owolabi, M.O.; Pandian, J.D.; Patel, S.; Perico, N.; Piradov, M.A.; Polinder, S.; Postma, M.J.; Poustchi, H.; Prakash, V.; Qorbani, M.; Rafiei, A.; Rahim, F.; Rahimi, K.; Rahimi-Movaghar, V.; Rahman, M.; Rahman, M.A.; Reis, C.; Remuzzi, G.; Renzaho, A.M.N.; Ricci, S.; Roberts, N.L.S.; Robinson, S.R.; Roever, L.; Roshandel, G.; Sabbagh, P.; Safari, H.; Safari, S.; Safiri, S.; Sahebkar, A.; Salehi Zahabi, S.; Samy, A.M.; Santalucia, P.; Santos, I.S.; Santos, J.V.; Santric Milicevic, M.M.; Sartorius, B.; Sawant, A.R.; Schutte, A.E.; Sepanlou, S.G.; Shafieesabet, A.; Shaikh, M.A.; Shams-Beyranvand, M.; Sheikh, A.; Sheth, K.N.; Shibuya, K.; Shigematsu, M.; Shin, M-J.; Shiue, I.; Siabani, S.; Sobaih, B.H.; Sposato, L.A.; Sutradhar, I.; Sylaja, P.N.; Szoeke, C.E.I.; Te Ao, B.J.; Temsah, M-H.; Temsah, O.; Thrift, A.G.; Tonelli, M.; Topor-Madry, R.; Tran, B.X.; Tran, K.B.; Truelsen, T.C.; Tsadik, A.G.; Ullah, I.; Uthman, O.A.; Vaduganathan, M.; Valdez, P.R.; Vasankari, T.J.; Vasanthan, R.; Venketasubramanian, N.; Vosoughi, K.; Vu, G.T.; Waheed, Y.; Weiderpass, E.; Weldegwergs, K.G.; Westerman, R.; Wolfe, C.D.A.; Wondafrash, D.Z.; Xu, G.; Yadollahpour, A.; Yamada, T.; Yatsuya, H.; Yimer, E.M.; Yonemoto, N.; Yousefifard, M.; Yu, C.; Zaidi, Z.; Zamani, M.; Zarghi, A.; Zhang, Y.; Zodpey, S.; Feigin, V.L.; Vos, T.; Murray, C.J.L. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.,* **2019**, *18*(5), 439-458. http://dx.doi.org/10.1016/S1474-4422(19)30034-1 PMID: 30871944

[2] Goyal, M.; Menon, B.K.; van Zwam, W.H.; Dippel, D.W.J.; Mitchell, P.J.; Demchuk, A.M.; Dávalos, A.; Majoie, C.B.L.M.; van der Lugt, A.; de Miquel, M.A.; Donnan, G.A.; Roos, Y.B.W.E.M.; Bonafe, A.; Jahan, R.; Diener, H.C.; van den Berg, L.A.; Levy, E.I.; Berkhemer, O.A.; Pereira, V.M.; Rempel, J.; Millán, M.; Davis, S.M.; Roy, D.; Thornton, J.; Román, L.S.; Ribó, M.; Beumer, D.; Stouch, B.; Brown, S.; Campbell, B.C.V.; van Oostenbrugge, R.J.; Saver, J.L.; Hill, M.D.; Jovin, T.G. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet,* **2016**, *387*(10029), 1723-1731. http://dx.doi.org/10.1016/S0140-6736(16)00163-X PMID: 26898852

[3] Ao, L.; Yan, Y.Y.; Zhou, L.; Li, C.; Li, W.T.; Fang, W.; Li, Y. Immune Cells After Ischemic Stroke Onset: Roles, Migration, and Target Intervention. *J. Mol. Neurosci.,* **2018**, *66*(3), 342-355. http://dx.doi.org/10.1007/s12031-018-1173-4 PMID: 30276612

[4] Weisenburger-Lile, D.; Dong, Y.; Yger, M.; Weisenburger, G.; Polara, G.F.; Chaigneau, T.; Ochoa, R.Z.; Marro, B.; Lapergue, B.; Alamowitch, S.; Elbim, C. Harmful neutrophil subsets in patients with ischemic stroke. *Neurol. Neuroimmunol. Neuroinflamm.,* **2019**, *6*(4), e571. http://dx.doi.org/10.1212/NXI.0000000000000571 PMID: 31355307

[5] Use of anti-ICAM-1 therapy in ischemic stroke: Results of the enlimomab acute stroke trial. *Neurology,* **2001**, *57*(8), 1428-1434. http://dx.doi.org/10.1212/WNL.57.8.1428 PMID: 11673584

[6] Krams, M.; Lees, K.R.; Hacke, W.; Grieve, A.P.; Orgogozo, J.M.; Ford, G.A. Acute stroke therapy by inhibition of neutrophils (ASTIN): An adaptive dose-response study of UK-279,276 in acute ischemic stroke. *Stroke,* **2003**, *34*(11), 2543-2548. http://dx.doi.org/10.1161/01.STR.0000092527.33910.89 PMID: 14563972

[7] Ng, L.G.; Ostuni, R.; Hidalgo, A. Heterogeneity of neutrophils. *Nat. Rev. Immunol.,* **2019**, *19*(4), 255-265. http://dx.doi.org/10.1038/s41577-019-0141-8 PMID: 30816340

[8] Hua, X.; Hu, G.; Hu, Q.; Chang, Y.; Hu, Y.; Gao, L.; Chen, X.; Yang, P.C.; Zhang, Y.; Li, M.; Song, J. Single-cell RNA sequencing to dissect the immunological network of autoimmune myocarditis. *Circulation,* **2020**, *142*(4), 384-400. http://dx.doi.org/10.1161/CIRCULATIONAHA.119.043545 PMID: 32431172

[9] Szczerba, B.M.; Castro-Giner, F.; Vetter, M.; Krol, I.; Gkountela, S.; Landin, J.; Scheidmann, M.C.; Donato, C.; Scherrer, R.; Singer, J.; Beisel, C.; Kurzeder, C.; Heinzelmann-Schwarz, V.; Rochlitz, C.; Weber, W.P.; Beerenwinkel, N.; Aceto, N. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature,* **2019**, *566*(7745), 553-557. http://dx.doi.org/10.1038/s41586-019-0915-y PMID: 30728496

[10] Cuartero, M.I.; Ballesteros, I.; Moraga, A.; Nombela, F.; Vivancos, J.; Hamilton, J.A.; Corbí, Á.L.; Lizasoain, I.; Moro, M.A. N2 neutrophils, novel players in brain inflammation after stroke: modulation by the PPARγ agonist rosiglitazone. *Stroke,* **2013**, *44*(12), 3498-3508. http://dx.doi.org/10.1161/STROKEAHA.113.002470 PMID: 24135932

[11] Hou, Y.; Yang, D.; Xiang, R.; Wang, H.; Wang, X.; Zhang, H.; Wang, P.; Zhang, Z.; Che, X.; Liu, Y.; Gao, Y.; Yu, X.; Gao, X.; Zhang, W.; Yang, J.; Wu, C. N2 neutrophils may participate in spontaneous recovery after transient cerebral ischemia by inhibiting ischemic neuron injury in rats. *Int. Immunopharmacol.,* **2019**, *77*, 105970. http://dx.doi.org/10.1016/j.intimp.2019.105970 PMID: 31675618

[12] Basu, S.; Hodgson, G.; Katz, M.; Dunn, A.R. Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. *Blood,* **2002**, *100*(3), 854-861. http://dx.doi.org/10.1182/blood.V100.3.854 PMID: 12130495

[13] Borregaard, N. Neutrophils, from marrow to microbes. *Immunity,* **2010**, *33*(5), 657-670. http://dx.doi.org/10.1016/j.immuni.2010.11.011 PMID: 21094463

[14] Grassi, L.; Pourfarzad, F.; Ullrich, S.; Merkel, A.; Were, F.; Carrillo-de-Santa-Pau, E.; Yi, G.; Hiemstra, I.H.; Tool, A.T.J.; Mul, E.; Perner, J.; Janssen-Megens, E.; Berentsen, K.; Kerstens, H.; Habibi, E.; Gut, M.; Yaspo, M.L.; Linser, M.; Lowy, E.; Datta, A.; Clarke, L.; Flicek, P.; Vingron, M.; Roos, D.; van den Berg, T.K.; Heath, S.; Rico, D.; Frontini, M.; Kostadima, M.; Gut, I.; Valencia, A.; Ouwehand, W.H.; Stunnenberg, H.G.; Martens, J.H.A.; Kuijpers, T.W. Dynamics of transcription regulation in human bone marrow myeloid differentiation to mature blood neutrophils. *Cell Rep.,* **2018**, *24*(10), 2784-2794. http://dx.doi.org/10.1016/j.celrep.2018.08.018 PMID: 30184510

[15] Liew, P.X.; Kubes, P. The neutrophil's role during health and disease. *Physiol. Rev.,* **2019**, *99*(2), 1223-1248. http://dx.doi.org/10.1152/physrev.00012.2018 PMID: 30758246

[16] Lapidot, T.; Kollet, O. The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2mnull mice. *Leukemia,* **2002**, *16*(10), 1992-2003. http://dx.doi.org/10.1038/sj.leu.2402684 PMID: 12357350

[17] Eash, K.J.; Greenbaum, A.M.; Gopalan, P.K.; Link, D.C. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J. Clin. Invest.,* **2010**, *120*(7), 2423-2431. http://dx.doi.org/10.1172/JCI41649 PMID: 20516641

[18] Christopher, M.J.; Liu, F.; Hilton, M.J.; Long, F.; Link, D.C. Suppression of CXCL12 production by bone marrow osteoblasts is a common and critical pathway for cytokine-induced mobilization. *Blood,* **2009**, *114*(7), 1331-1339. http://dx.doi.org/10.1182/blood-2008-10-184754 PMID: 19141863

[19] Kolaczkowska, E.; Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.,* **2013**, *13*(3), 159-175. http://dx.doi.org/10.1038/nri3399 PMID: 23435331

[20] Németh, T.; Sperandio, M.; Mócsai, A. Neutrophils as emerging therapeutic targets. *Nat. Rev. Drug Discov.,* **2020**, *19*(4), 253-275. http://dx.doi.org/10.1038/s41573-019-0054-z PMID: 31969717

[21] Nahrendorf, M. Myeloid cell contributions to cardiovascular health and disease. *Nat. Med.,* **2018**, *24*(6), 711-720. http://dx.doi.org/10.1038/s41591-018-0064-0 PMID: 29867229

[22] Christoffersson, G.; Vågesjö, E.; Vandooren, J.; Lidén, M.; Massena, S.; Reinert, R.B.; Brissova, M.; Powers, A.C.; Opdenakker, G.; Phillipson, M. VEGF-A recruits a proangiogenic MMP-9–delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood,* **2012**, *120*(23), 4653-4662. http://dx.doi.org/10.1182/blood-2012-04-421040 PMID: 22966168

[23] Amulic, B.; Cazalet, C.; Hayes, G.L.; Metzler, K.D.; Zychlinsky, A. Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.,* **2012**, *30*(1), 459-489. http://dx.doi.org/10.1146/annurev-immunol-020711-074942 PMID: 22224774

[24] Meijer, M.; Rijkers, G.T.; van Overveld, F.J. Neutrophils and emerging targets for treatment in chronic obstructive pulmonary disease. *Expert Rev. Clin. Immunol.,* **2013**, *9*(11), 1055-1068. http://dx.doi.org/10.1586/1744666X.2013.851347 PMID: 24168412

[25] Silvestre-Roig, C.; Braster, Q.; Wichapong, K.; Lee, E.Y.; Teulon, J.M.; Berrebeh, N.; Winter, J.; Adrover, J.M.; Santos, G.S.; Froese, A.; Lemnitzer, P.; Ortega-Gómez, A.; Chevre, R.; Marschner, J.; Schumski, A.; Winter, C.; Perez-Olivares, L.; Pan, C.; Paulin, N.; Schoufour, T.; Hartwig, H.; González-Ramos, S.; Kamp, F.; Megens, R.T.A.; Mowen, K.A.; Gunzer, M.; Maegdefessel, L.; Hackeng, T.; Lutgens, E.; Daemen, M.; von Blume, J.; Anders, H.J.; Nikolaev, V.O.; Pellequer, J.L.; Weber, C.; Hidalgo, A.; Nicolaes, G.A.F.; Wong, G.C.L.; Soehnlein, O. Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. *Nature,* **2019**, *569*(7755), 236-240. http://dx.doi.org/10.1038/s41586-019-1167-6 PMID: 31043745

[26] Lande, R.; Ganguly, D.; Facchinetti, V.; Frasca, L.; Conrad, C.; Gregorio, J.; Meller, S.; Chamilos, G.; Sebasigari, R.; Riccieri, V.; Bassett, R.; Amuro, H.; Fukuhara, S.; Ito, T.; Liu, Y.J.; Gilliet, M. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci. Transl. Med.,* **2011**, *3*(73), 73ra19. http://dx.doi.org/10.1126/scitranslmed.3001180 PMID: 21389263

[27] Khandpur, R.; Carmona-Rivera, C.; Vivekanandan-Giri, A.; Gizinski, A.; Yalavarthi, S.; Knight, J.S.; Friday, S.; Li, S.; Patel, R.M.; Subramanian, V.; Thompson, P.; Chen, P.; Fox, D.A.; Pennathur, S.; Kaplan, M.J. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci. Transl. Med.,* **2013**, *5*(178), 178ra40. http://dx.doi.org/10.1126/scitranslmed.3005580 PMID: 23536012

[28] Giese, M.A.; Hind, L.E.; Huttenlocher, A. Neutrophil plasticity in the tumor microenvironment. *Blood,* **2019**, *133*(20), 2159-2167. http://dx.doi.org/10.1182/blood-2018-11-844548 PMID: 30898857

[29] Mantovani, A.; Cassatella, M.A.; Costantini, C.; Jaillon, S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.,* **2011**, *11*(8), 519-531. http://dx.doi.org/10.1038/nri3024 PMID: 21785456

[30] Mestas, J.; Hughes, C.C.W. Of mice and not men: differences between mouse and human immunology. *J. Immunol.,* **2004**, *172*(5), 2731-2738. http://dx.doi.org/10.4049/jimmunol.172.5.2731 PMID: 14978070

[31] Dancey, J.T.; Deubelbeiss, K.A.; Harker, L.A.; Finch, C.A. Neutrophil kinetics in man. *J. Clin. Invest.,* **1976**, *58*(3), 705-715. http://dx.doi.org/10.1172/JCI108517 PMID: 956397

[32] Hidalgo, A.; Chilvers, E.R.; Summers, C.; Koenderman, L. The Neutrophil Life Cycle. *Trends Immunol.,* **2019**, *40*(7), 584-597. http://dx.doi.org/10.1016/j.it.2019.04.013 PMID: 31153737

[33] Pillay, J.; den Braber, I.; Vrisekoop, N.; Kwast, L.M.; de Boer, R.J.; Borghans, J.A.M.; Tesselaar, K.; Koenderman, L. *In vivo* labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. *Blood,* **2010**, *116*(4), 625-627. http://dx.doi.org/10.1182/blood-2010-01-259028 PMID: 20410504

[34] Casanova-Acebes, M.; Pitaval, C.; Weiss, L.A.; Nombela-Arrieta, C.; Chèvre, R.; A-González, N.; Kunisaki, Y.; Zhang, D.; van Rooijen, N.; Silberstein, L.E.; Weber, C.; Nagasawa, T.; Frenette,

P.S.; Castrillo, A.; Hidalgo, A. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell,* **2013**, *153*(5), 1025-1035.
http://dx.doi.org/10.1016/j.cell.2013.04.040 PMID: 23706740

[35] Adrover, J.M.; del Fresno, C.; Crainiciuc, G.; Cuartero, M.I.; Casanova-Acebes, M.; Weiss, L.A.; Huerga-Encabo, H.; Silvestre-Roig, C.; Rossaint, J.; Cossío, I.; Lechuga-Vieco, A.V.; García-Prieto, J.; Gómez-Parrizas, M.; Quintana, J.A.; Ballesteros, I.; Martin-Salamanca, S.; Aroca-Crevillen, A.; Chong, S.Z.; Evrard, M.; Balabanian, K.; López, J.; Bidzhekov, K.; Bachelerie, F.; Abad-Santos, F.; Muñoz-Calleja, C.; Zarbock, A.; Soehnlein, O.; Weber, C.; Ng, L.G.; Lopez-Rodriguez, C.; Sancho, D.; Moro, M.A.; Ibáñez, B.; Hidalgo, A. A Neutrophil Timer Coordinates Immune Defense and Vascular Protection. *Immunity,* **2019**, *50*(2), 390-402.e10.
http://dx.doi.org/10.1016/j.immuni.2019.01.002 PMID: 30709741

[36] Eash, K.J.; Means, J.M.; White, D.W.; Link, D.C. CXCR4 is a key regulator of neutrophil release from the bone marrow under basal and stress granulopoiesis conditions. *Blood,* **2009**, *113*(19), 4711-4719.
http://dx.doi.org/10.1182/blood-2008-09-177287 PMID: 19264920

[37] Shi, J.; Gilbert, G.E.; Kokubo, Y.; Ohashi, T. Role of the liver in regulating numbers of circulating neutrophils. *Blood,* **2001**, *98*(4), 1226-1230.
http://dx.doi.org/10.1182/blood.V98.4.1226 PMID: 11493474

[38] Stark, M.A.; Huo, Y.; Burcin, T.L.; Morris, M.A.; Olson, T.S.; Ley, K. Phagocytosis of apoptotic neutrophils regulates granulopoiesis *via* IL-23 and IL-17. *Immunity,* **2005**, *22*(3), 285-294.
http://dx.doi.org/10.1016/j.immuni.2005.01.011 PMID: 15780986

[39] Bonaventura, A.; Liberale, L.; Carbone, F.; Vecchié, A.; Diaz-Cañestro, C.; Camici, G.; Montecucco, F.; Dallegri, F. The Pathophysiological Role of Neutrophil Extracellular Traps in Inflammatory Diseases. *Thromb. Haemost.,* **2018**, *118*(1), 006-027.
http://dx.doi.org/10.1160/TH17-09-0630 PMID: 29304522

[40] Silvestre-Roig, C.; Hidalgo, A.; Soehnlein, O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood,* **2016**, *127*(18), 2173-2181.
http://dx.doi.org/10.1182/blood-2016-01-688887 PMID: 27002116

[41] Garlichs, C.D.; Eskafi, S.; Cicha, I.; Schmeisser, A.; Walzog, B.; Raaz, D.; Stumpf, C.; Yilmaz, A.; Bremer, J.; Ludwig, J.; Daniel, W.G. Delay of neutrophil apoptosis in acute coronary syndromes. *J. Leukoc. Biol.,* **2004**, *75*(5), 828-835.
http://dx.doi.org/10.1189/jlb.0703358 PMID: 14742636

[42] Laval, J.; Touhami, J.; Herzenberg, L.A.; Conrad, C.; Taylor, N.; Battini, J.L.; Sitbon, M.; Tirouvanziam, R. Metabolic adaptation of neutrophils in cystic fibrosis airways involves distinct shifts in nutrient transporter expression. *J. Immunol.,* **2013**, *190*(12), 6043-6050.
http://dx.doi.org/10.4049/jimmunol.1201755 PMID: 23690474

[43] Colotta, F.; Re, F.; Polentarutti, N.; Sozzani, S.; Mantovani, A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood,* **1992**, *80*(8), 2012-2020.
http://dx.doi.org/10.1182/blood.V80.8.2012.2012 PMID: 1382715

[44] Ecker, S.; Chen, L.; Pancaldi, V.; Bagger, F.O.; Fernández, J.M.; Carrillo de Santa Pau, E.; Juan, D.; Mann, A.L.; Watt, S.; Casale, F.P.; Sidiropoulos, N.; Rapin, N.; Merkel, A.; Stunnenberg, H.G.; Stegle, O.; Frontini, M.; Downes, K.; Pastinen, T.; Kuijpers, T.W.; Rico, D.; Valencia, A.; Beck, S.; Soranzo, N.; Paul, D.S. Genome-wide analysis of differential transcriptional and epigenetic variability across human immune cell types. *Genome Biol.,* **2017**, *18*(1), 18.
http://dx.doi.org/10.1186/s13059-017-1156-8 PMID: 28126036

[45] Naranbhai, V.; Fairfax, B.P.; Makino, S.; Humburg, P.; Wong, D.; Ng, E.; Hill, A.V.S.; Knight, J.C. Genomic modulators of gene expression in human neutrophils. *Nat. Commun.,* **2015**, *6*(1), 7545.
http://dx.doi.org/10.1038/ncomms8545 PMID: 26151758

[46] Cowland, J.B.; Borregaard, N. Granulopoiesis and granules of human neutrophils. *Immunol. Rev.,* **2016**, *273*(1), 11-28.
http://dx.doi.org/10.1111/imr.12440 PMID: 27558325

[47] Silvestre-Roig, C.; Fridlender, Z.G.; Glogauer, M.; Scapini, P. Neutrophil Diversity in Health and Disease. *Trends Immunol.,* **2019**, *40*(7), 565-583.
http://dx.doi.org/10.1016/j.it.2019.04.012 PMID: 31160207

[48] Wang, X.; Qiu, L.; Li, Z.; Wang, X.Y.; Yi, H. Understanding the Multifaceted Role of Neutrophils in Cancer and Autoimmune Diseases. *Front. Immunol.,* **2018**, *9*, 2456.
http://dx.doi.org/10.3389/fimmu.2018.02456 PMID: 30473691

[49] Massena, S.; Christoffersson, G.; Vågesjö, E.; Seignez, C.; Gustafsson, K.; Binet, F.; Herrera, H.C.; Giraud, A.; Lomei, J.; Weström, S.; Shibuya, M.; Claesson-Welsh, L.; Gerwins, P.; Welsh, M.; Kreuger, J.; Phillipson, M. Identification and characterization of VEGF-A–responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. *Blood,* **2015**, *126*(17), 2016-2026.
http://dx.doi.org/10.1182/blood-2015-03-631572 PMID: 26286848

[50] Evrard, M.; Kwok, I.W.H.; Chong, S.Z.; Teng, K.W.W.; Becht, E.; Chen, J.; Sieow, J.L.; Penny, H.L.; Ching, G.C.; Devi, S.; Adrover, J.M.; Li, J.L.Y.; Liong, K.H.; Tan, L.; Poon, Z.; Foo, S.; Chua, J.W.; Su, I.H.; Balabanian, K.; Bachelerie, F.; Biswas, S.K.; Larbi, A.; Hwang, W.Y.K.; Madan, V.; Koeffler, H.P.; Wong, S.C.; Newell, E.W.; Hidalgo, A.; Ginhoux, F.; Ng, L.G. Developmental analysis of bone marrow neutrophils reveals populations specialized in expansion, trafficking, and effector functions. *Immunity,* **2018**, *48*(2), 364-379.e8.
http://dx.doi.org/10.1016/j.immuni.2018.02.002 PMID: 29466759

[51] Margraf, A.; Ley, K.; Zarbock, A. Neutrophil Recruitment: From Model Systems to Tissue-Specific Patterns. *Trends Immunol.,* **2019**, *40*(7), 613-634.
http://dx.doi.org/10.1016/j.it.2019.04.010 PMID: 31175062

[52] Briggs, J.A.; Weinreb, C.; Wagner, D.E.; Megason, S.; Peshkin, L.; Kirschner, M.W.; Klein, A.M. The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution. *Science,* **2018**, *360*(6392), eaar5780.
http://dx.doi.org/10.1126/science.aar5780 PMID: 29700227

[53] Grieshaber-Bouyer, R.; Radtke, F.A.; Cunin, P.; Stifano, G.; Levescot, A.; Vijaykumar, B.; Nelson-Maney, N.; Blaustein, R.B.; Monach, P.A.; Nigrovic, P.A. The neutrotime transcriptional signature defines a single continuum of neutrophils across biological compartments. *Nat. Commun.,* **2021**, *12*(1), 2856.
http://dx.doi.org/10.1038/s41467-021-22973-9 PMID: 34001893

[54] Xie, X.; Shi, Q.; Wu, P.; Zhang, X.; Kambara, H.; Su, J.; Yu, H.; Park, S.Y.; Guo, R.; Ren, Q.; Zhang, S.; Xu, Y.; Silberstein, L.E.; Cheng, T.; Ma, F.; Li, C.; Luo, H.R. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nat. Immunol.,* **2020**, *21*(9), 1119-1133.
http://dx.doi.org/10.1038/s41590-020-0736-z PMID: 32719519

[55] Zilionis, R.; Engblom, C.; Pfirschke, C.; Savova, V.; Zemmour, D.; Saatcioglu, H.D.; Krishnan, I.; Maroni, G.; Meyerovitz, C.V.; Kerwin, C.M.; Choi, S.; Richards, W.G.; De Rienzo, A.; Tenen, D.G.; Bueno, R.; Levantini, E.; Pittet, M.J.; Klein, A.M. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity,* **2019**, *50*(5), 1317-1334.e10.
http://dx.doi.org/10.1016/j.immuni.2019.03.009 PMID: 30979687

[56] Fridlender, Z.G.; Sun, J.; Kim, S.; Kapoor, V.; Cheng, G.; Ling, L.; Worthen, G.S.; Albelda, S.M. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" *versus* "N2" TAN. *Cancer Cell,* **2009**, *16*(3), 183-194.
http://dx.doi.org/10.1016/j.ccr.2009.06.017 PMID: 19732719

[57] Ma, Y.; Yabluchanskiy, A.; Iyer, R.P.; Cannon, P.L.; Flynn, E.R.; Jung, M.; Henry, J.; Cates, C.A.; Deleon-Pennell, K.Y.; Lindsey, M.L. Temporal neutrophil polarization following myocardial infarction. *Cardiovasc. Res.,* **2016**, *110*(1), 51-61.
http://dx.doi.org/10.1093/cvr/cvw024 PMID: 26825554

[58] Neely, C.J.; Kartchner, L.B.; Mendoza, A.E.; Linz, B.M.; Frelinger, J.A.; Wolfgang, M.C.; Maile, R.; Cairns, B.A. Flagellin treatment prevents increased susceptibility to systemic bacterial infection after injury by inhibiting anti-inflammatory IL-10+ IL-12-neutrophil polarization. *PLoS One,* **2014**, *9*(1), e85623.
http://dx.doi.org/10.1371/journal.pone.0085623 PMID: 24454904

[59] Hou, Y.; Yang, D.; Zhang, Q.; Wang, X.; Yang, J.; Wu, C. Pseudoginsenoside-F11 ameliorates ischemic neuron injury by regulating the polarization of neutrophils and macrophages *in vitro*. *Int. Immunopharmacol.,* **2020**, *85*, 106564.
http://dx.doi.org/10.1016/j.intimp.2020.106564 PMID: 32447220

[60] Buck, B.H.; Liebeskind, D.S.; Saver, J.L.; Bang, O.Y.; Yun, S.W.; Starkman, S.; Ali, L.K.; Kim, D.; Villablanca, J.P.; Salamon, N.;

Razinia, T.; Ovbiagele, B. Early neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke,* **2008**, *39*(2), 355-360. http://dx.doi.org/10.1161/STROKEAHA.107.490128 PMID: 18162626

[61] Denes, A.; McColl, B.W.; Leow-Dyke, S.F.; Chapman, K.Z.; Humphreys, N.E.; Grencis, R.K.; Allan, S.M.; Rothwell, N.J. Experimental stroke-induced changes in the bone marrow reveal complex regulation of leukocyte responses. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(4), 1036-1050. http://dx.doi.org/10.1038/jcbfm.2010.198 PMID: 21045863

[62] Cai, W.; Liu, S.; Hu, M.; Huang, F.; Zhu, Q.; Qiu, W.; Hu, X.; Colello, J.; Zheng, S.G.; Lu, Z. Functional dynamics of neutrophils after ischemic stroke. *Transl. Stroke Res.,* **2020**, *11*(1), 108-121. http://dx.doi.org/10.1007/s12975-019-00694-y PMID: 30847778

[63] Liu, Q.; Sorooshyari, S.K. Quantitative and correlational analysis of brain and spleen immune cellular responses following cerebral ischemia. *Front. Immunol.,* **2021**, *12*, 617032. http://dx.doi.org/10.3389/fimmu.2021.617032 PMID: 34194419

[64] Meisel, C.; Schwab, J.M.; Prass, K.; Meisel, A.; Dirnagl, U. Central nervous system injury-induced immune deficiency syndrome. *Nat. Rev. Neurosci.,* **2005**, *6*(10), 775-786. http://dx.doi.org/10.1038/nrn1765 PMID: 16163382

[65] Mracsko, E.; Liesz, A.; Karcher, S.; Zorn, M.; Bari, F.; Veltkamp, R. Differential effects of sympathetic nervous system and hypothalamic–pituitary–adrenal axis on systemic immune cells after severe experimental stroke. *Brain Behav. Immun.,* **2014**, *41*, 200-209. http://dx.doi.org/10.1016/j.bbi.2014.05.015 PMID: 24886966

[66] Zhang, J-H.; Zhang, J.H. Role of the sympathetic nervous system and spleen in experimental stroke-induced immunodepression. *Med. Sci. Monit.,* **2014**, *20*, 2489-2496. http://dx.doi.org/10.12659/MSM.890844 PMID: 25434807

[67] Courties, G.; Herisson, F.; Sager, H.B.; Heidt, T.; Ye, Y.; Wei, Y.; Sun, Y.; Severe, N.; Dutta, P.; Scharff, J.; Scadden, D.T.; Weissleder, R.; Swirski, F.K.; Moskowitz, M.A.; Nahrendorf, M. Ischemic stroke activates hematopoietic bone marrow stem cells. *Circ. Res.,* **2015**, *116*(3), 407-417. http://dx.doi.org/10.1161/CIRCRESAHA.116.305207 PMID: 25362208

[68] Pennypacker, K.R.; Offner, H. The role of the spleen in ischemic stroke. *J. Cereb. Blood Flow Metab.,* **2015**, *35*(2), 186-187. http://dx.doi.org/10.1038/jcbfm.2014.212 PMID: 25465042

[69] Sippel, T.R.; Shimizu, T.; Strnad, F.; Traystman, R.J.; Herson, P.S.; Waziri, A. Arginase I release from activated neutrophils induces peripheral immunosuppression in a murine model of stroke. *J. Cereb. Blood Flow Metab.,* **2015**, *35*(10), 1657-1663. http://dx.doi.org/10.1038/jcbfm.2015.103 PMID: 25966956

[70] Losy, J.; Zaremba, J.; Skrobański, P. CXCL1 (GRO-alpha) chemokine in acute ischaemic stroke patients. *Folia Neuropathol.,* **2005**, *43*(2), 97-102. PMID: 16012911

[71] Zaremba, J.; Skrobański, P.; Losy, J. The level of chemokine CXCL5 in the cerebrospinal fluid is increased during the first 24 hours of ischaemic stroke and correlates with the size of early brain damage. *Folia Morphol. (Warsz),* **2006**, *65*(1), 1-5. PMID: 16783727

[72] Zhang, Q.; Raoof, M.; Chen, Y.; Sumi, Y.; Sursal, T.; Junger, W.; Brohi, K.; Itagaki, K.; Hauser, C.J. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature,* **2010**, *464*(7285), 104-107. http://dx.doi.org/10.1038/nature08780 PMID: 20203610

[73] Shichita, T.; Hasegawa, E.; Kimura, A.; Morita, R.; Sakaguchi, R.; Takada, I.; Sekiya, T.; Ooboshi, H.; Kitazono, T.; Yanagawa, T.; Ishii, T.; Takahashi, H.; Mori, S.; Nishibori, M.; Kuroda, K.; Akira, S.; Miyake, K.; Yoshimura, A. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. *Nat. Med.,* **2012**, *18*(6), 911-917. http://dx.doi.org/10.1038/nm.2749 PMID: 22610280

[74] Kuboyama, K.; Harada, H.; Tozaki-Saitoh, H.; Tsuda, M.; Ushijima, K.; Inoue, K. Astrocytic $P2Y_1$ receptor is involved in the regulation of cytokine/chemokine transcription and cerebral damage in a rat model of cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(9), 1930-1941. http://dx.doi.org/10.1038/jcbfm.2011.49 PMID: 21487414

[75] Denes, A.; Vidyasagar, R.; Feng, J.; Narvainen, J.; McColl, B.W.; Kauppinen, R.A.; Allan, S.M. Proliferating resident microglia after focal cerebral ischaemia in mice. *J. Cereb. Blood Flow Metab.,* **2007**, *27*(12), 1941-1953. http://dx.doi.org/10.1038/sj.jcbfm.9600495 PMID: 17440490

[76] Kong, L.L.; Hu, J.F.; Zhang, W.; Yuan, Y.H.; Ma, K.L.; Han, N.; Chen, N.H. Expression of chemokine-like factor 1 after focal cerebral ischemia in the rat. *Neurosci. Lett.,* **2011**, *505*(1), 14-18. http://dx.doi.org/10.1016/j.neulet.2011.09.031 PMID: 21964493

[77] Scholz, M.; Cinatl, J.; Schädel-Höpfner, M.; Windolf, J. Neutrophils and the blood–brain barrier dysfunction after trauma. *Med. Res. Rev.,* **2007**, *27*(3), 401-416. http://dx.doi.org/10.1002/med.20064 PMID: 16758487

[78] Liu, Q.; Li, Z.; Gao, J.L.; Wan, W.; Ganesan, S.; McDermott, D.H.; Murphy, P.M. CXCR4 antagonist AMD3100 redistributes leukocytes from primary immune organs to secondary immune organs, lung, and blood in mice. *Eur. J. Immunol.,* **2015**, *45*(6), 1855-1867. http://dx.doi.org/10.1002/eji.201445245 PMID: 25801950

[79] Offner, H.; Subramanian, S.; Parker, S.M.; Wang, C.; Afentoulis, M.E.; Lewis, A.; Vandenbark, A.A.; Hurn, P.D. Splenic atrophy in experimental stroke is accompanied by increased regulatory T cells and circulating macrophages. *J. Immunol.,* **2006**, *176*(11), 6523-6531. http://dx.doi.org/10.4049/jimmunol.176.11.6523 PMID: 16709809

[80] Vahidy, F.S.; Parsha, K.N.; Rahbar, M.H.; Lee, M.; Bui, T.T.; Nguyen, C.; Barreto, A.D.; Bambhroliya, A.B.; Sahota, P.; Yang, B.; Aronowski, J.; Savitz, S.I. Acute splenic responses in patients with ischemic stroke and intracerebral hemorrhage. *J. Cereb. Blood Flow Metab.,* **2016**, *36*(6), 1012-1021. http://dx.doi.org/10.1177/0271678X15607880 PMID: 26661179

[81] Liu, Q.; Johnson, E.M.; Lam, R.K.; Wang, Q.; Bo Ye, H.; Wilson, E.N.; Minhas, P.S.; Liu, L.; Swarovski, M.S.; Tran, S.; Wang, J.; Mehta, S.S.; Yang, X.; Rabinowitz, J.D.; Yang, S.S.; Shamloo, M.; Mueller, C.; James, M.L.; Andreasson, K.I. Peripheral TREM1 responses to brain and intestinal immunogens amplify stroke severity. *Nat. Immunol.,* **2019**, *20*(8), 1023-1034. http://dx.doi.org/10.1038/s41590-019-0421-2 PMID: 31263278

[82] Cugurra, A.; Mamuladze, T.; Rustenhoven, J.; Dykstra, T.; Beroshvili, G.; Greenberg, Z.J.; Baker, W.; Papadopoulos, Z.; Drieu, A.; Blackburn, S.; Kanamori, M.; Brioschi, S.; Herz, J.; Schuettpelz, L.G.; Colonna, M.; Smirnov, I.; Kipnis, J. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. *Science.,* **2021**, *373*(6553), eabf7844. http://dx.doi.org/10.1126/science.abf7844 PMID: 34083447

[83] Cai, R.; Pan, C.; Ghasemigharagoz, A.; Todorov, M.I.; Förstera, B.; Zhao, S.; Bhatia, H.S.; Parra-Damas, A.; Mrowka, L.; Theodorou, D.; Rempfler, M.; Xavier, A.L.R.; Kress, B.T.; Benakis, C.; Steinke, H.; Liebscher, S.; Bechmann, I.; Liesz, A.; Menze, B.; Kerschensteiner, M.; Nedergaard, M.; Ertürk, A. Panoptic imaging of transparent mice reveals whole-body neuronal projections and skull–meninges connections. *Nat. Neurosci.,* **2019**, *22*(2), 317-327. http://dx.doi.org/10.1038/s41593-018-0301-3 PMID: 30598527

[84] Herisson, F.; Frodermann, V.; Courties, G.; Rohde, D.; Sun, Y.; Vandoorne, K.; Wojtkiewicz, G.R.; Masson, G.S.; Vinegoni, C.; Kim, J.; Kim, D.E.; Weissleder, R.; Swirski, F.K.; Moskowitz, M.A.; Nahrendorf, M. Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. *Nat. Neurosci.,* **2018**, *21*(9), 1209-1217. http://dx.doi.org/10.1038/s41593-018-0213-2 PMID: 30150661

[85] Jickling, G.C.; Liu, D.; Ander, B.P.; Stamova, B.; Zhan, X.; Sharp, F.R. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J. Cereb. Blood Flow Metab.,* **2015**, *35*(6), 888-901. http://dx.doi.org/10.1038/jcbfm.2015.45 PMID: 25806703

[86] Cui, L.; Zhang, Y.; Chen, Z.; Su, Y.; Liu, Y.; Boltze, J. Early neutrophil count relates to infarct size and fatal outcome after large hemispheric infarction. *CNS Neurosci. Ther.,* **2020**, *26*(8), 829-836. http://dx.doi.org/10.1111/cns.13381 PMID: 32374521

[87] Lin, S.K.; Chen, P.Y.; Chen, G.C.; Hsu, P.J.; Hsiao, C.L.; Yang, F.Y.; Liu, C.Y.; Tsou, A. Association of a high neutrophil-to-lymphocyte ratio with hyperdense artery sign and unfavorable short-term outcomes in patients with acute ischemic stroke. *J. Inflamm. Res.,* **2021**, *14*, 313-324. http://dx.doi.org/10.2147/JIR.S293825 PMID: 33574692

[88] Pektezel, M.Y.; Yilmaz, E.; Arsava, E.M.; Topcuoglu, M.A. Neutrophil-to-lymphocyte ratio and response to intravenous thrombolysis in patients with acute ischemic stroke. *J. Stroke Cerebrovasc. Dis.,* **2019**, *28*(7), 1853-1859. http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2019.04.014 PMID: 31072698

[89] Nam, K.W.; Kim, T.J.; Lee, J.S.; Kwon, H.M.; Lee, Y.S.; Ko, S.B.; Yoon, B.W. High neutrophil-to-lymphocyte ratio predicts stroke-associated pneumonia. *Stroke,* **2018**, *49*(8), 1886-1892. http://dx.doi.org/10.1161/STROKEAHA.118.021228 PMID: 29967014

[90] Kotfis, K.; Bott-Olejnik, M.; Szylińska, A.; Rotter, I. Could neutrophil-to-lymphocyte ratio (NLR) serve as a potential marker for delirium prediction in patients with acute ischemic stroke? A Prospective observational study. *J. Clin. Med.,* **2019**, *8*(7), 1075. http://dx.doi.org/10.3390/jcm8071075 PMID: 31336587

[91] Goyal, N.; Tsivgoulis, G.; Chang, J.J.; Malhotra, K.; Pandhi, A.; Ishfaq, M.F.; Alsbrook, D.; Arthur, A.S.; Elijovich, L.; Alexandrov, A.V. Admission neutrophil-to-lymphocyte ratio as a prognostic biomarker of outcomes in large vessel occlusion strokes. *Stroke,* **2018**, *49*(8), 1985-1987. http://dx.doi.org/10.1161/STROKEAHA.118.021477 PMID: 30002151

[92] Aly, M.; Abdalla, R.N.; Batra, A.; Shaibani, A.; Hurley, M.C.; Jahromi, B.S.; Potts, M.B.; Ansari, S.A. Follow-up neutrophil-lymphocyte ratio after stroke thrombectomy is an independent biomarker of clinical outcome. *J. Neurointerv. Surg.,* **2021**, *13*(7), 609-613. http://dx.doi.org/10.1136/neurintsurg-2020-016342 PMID: 32763917

[93] Mo, X.; Li, T.; Ji, G.; Lu, W.; Hu, Z. Peripheral polymorphonuclear leukocyte activation as a systemic inflammatory response in ischemic stroke. *Neurol. Sci.,* **2013**, *34*(9), 1509-1516. http://dx.doi.org/10.1007/s10072-013-1447-0 PMID: 23619532

[94] Dimasi, D.; Sun, W.Y.; Bonder, C.S. Neutrophil interactions with the vascular endothelium. *Int. Immunopharmacol.,* **2013**, *17*(4), 1167-1175. http://dx.doi.org/10.1016/j.intimp.2013.05.034 PMID: 23863858

[95] Lakhan, S.E.; Kirchgessner, A.; Hofer, M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *J. Transl. Med.,* **2009**, *7*(1), 97. http://dx.doi.org/10.1186/1479-5876-7-97 PMID: 19919699

[96] Kansas, G.S. Selectins and their ligands: current concepts and controversies. *Blood,* **1996**, *88*(9), 3259-3287. http://dx.doi.org/10.1182/blood.V88.9.3259.bloodjournal8893259 PMID: 8896391

[97] Neumann, J.; Riek-Burchardt, M.; Herz, J.; Doeppner, T.R.; König, R.; Hütten, H.; Etemire, E.; Männ, L.; Klingberg, A.; Fischer, T.; Görtler, M.W.; Heinze, H.J.; Reichardt, P.; Schraven, B.; Hermann, D.M.; Reymann, K.G.; Gunzer, M. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. *Acta Neuropathol.,* **2015**, *129*(2), 259-277. http://dx.doi.org/10.1007/s00401-014-1355-2 PMID: 25391494

[98] Anrather, J.; Iadecola, C. Inflammation and Stroke: An Overview. *Neurotherapeutics,* **2016**, *13*(4), 661-670. http://dx.doi.org/10.1007/s13311-016-0483-x PMID: 27730544

[99] Sreeramkumar, V.; Adrover, J.M.; Ballesteros, I.; Cuartero, M.I.; Rossaint, J.; Bilbao, I.; Nácher, M.; Pitaval, C.; Radovanovic, I.; Fukui, Y.; McEver, R.P.; Filippi, M.D.; Lizasoain, I.; Ruiz-Cabello, J.; Zarbock, A.; Moro, M.A.; Hidalgo, A. Neutrophils scan for activated platelets to initiate inflammation. *Science,* **2014**, *346*(6214), 1234-1238. http://dx.doi.org/10.1126/science.1256478 PMID: 25477463

[100] Schuhmann, M.K.; Guthmann, J.; Stoll, G.; Nieswandt, B.; Kraft, P.; Kleinschnitz, C. Blocking of platelet glycoprotein receptor Ib reduces "thrombo-inflammation" in mice with acute ischemic stroke. *J. Neuroinflammation,* **2017**, *14*(1), 18. http://dx.doi.org/10.1186/s12974-017-0792-y PMID: 28109273

[101] Simon, D.I.; Chen, Z.; Xu, H.; Li, C.Q.; Dong, J.; McIntire, L.V.; Ballantyne, C.M.; Zhang, L.; Furman, M.I.; Berndt, M.C.; López, J.A. Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J. Exp. Med.,* **2000**, *192*(2), 193-204. http://dx.doi.org/10.1084/jem.192.2.193 PMID: 10899906

[102] Stamatovic, S.M.; Phillips, C.M.; Keep, R.F.; Andjelkovic, A.V. A novel approach to treatment of thromboembolic stroke in mice: Redirecting neutrophils toward a peripherally implanted CXCL1-soaked sponge. *Exp. Neurol.,* **2020**, *330*, 113336. http://dx.doi.org/10.1016/j.expneurol.2020.113336 PMID: 32360283

[103] Hol, J.; Wilhelmsen, L.; Haraldsen, G. The murine IL-8 homologues KC, MIP-2, and LIX are found in endothelial cytoplasmic granules but not in Weibel-Palade bodies. *J. Leukoc. Biol.,* **2010**, *87*(3), 501-508. http://dx.doi.org/10.1189/jlb.0809532 PMID: 20007247

[104] Enzmann, G.; Mysiorek, C.; Gorina, R.; Cheng, Y.J.; Ghavampour, S.; Hannocks, M.J.; Prinz, V.; Dirnagl, U.; Endres, M.; Prinz, M.; Beschorner, R.; Harter, P.N.; Mittelbronn, M.; Engelhardt, B.; Sorokin, L. The neurovascular unit as a selective barrier to polymorphonuclear granulocyte (PMN) infiltration into the brain after ischemic injury. *Acta Neuropathol.,* **2013**, *125*(3), 395-412. http://dx.doi.org/10.1007/s00401-012-1076-3 PMID: 23269317

[105] Perez-de-Puig, I.; Miró-Mur, F.; Ferrer-Ferrer, M.; Gelpi, E.; Pedragosa, J.; Justicia, C.; Urra, X.; Chamorro, A.; Planas, A.M. Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol.,* **2015**, *129*(2), 239-257. http://dx.doi.org/10.1007/s00401-014-1381-0 PMID: 25548073

[106] Price, C.J.S.; Menon, D.K.; Peters, A.M.; Ballinger, J.R.; Barber, R.W.; Balan, K.K.; Lynch, A.; Xuereb, J.H.; Fryer, T.; Guadagno, J.V.; Warburton, E.A. Cerebral neutrophil recruitment, histology, and outcome in acute ischemic stroke: an imaging-based study. *Stroke,* **2004**, *35*(7), 1659-1664. http://dx.doi.org/10.1161/01.STR.0000130592.71028.92 PMID: 15155970

[107] Li, Y.; Zhong, W.; Jiang, Z.; Tang, X. New progress in the approaches for blood–brain barrier protection in acute ischemic stroke. *Brain Res. Bull.,* **2019**, *144*, 46-57. http://dx.doi.org/10.1016/j.brainresbull.2018.11.006 PMID: 30448453

[108] Shi, Y.; Zhang, L.; Pu, H.; Mao, L.; Hu, X.; Jiang, X.; Xu, N.; Stetler, R.A.; Zhang, F.; Liu, X.; Leak, R.K.; Keep, R.F.; Ji, X.; Chen, J. Rapid endothelial cytoskeletal reorganization enables early blood–brain barrier disruption and long-term ischaemic reperfusion brain injury. *Nat. Commun.,* **2016**, *7*(1), 10523. http://dx.doi.org/10.1038/ncomms10523 PMID: 26813496

[109] Jiang, X.; Andjelkovic, A.V.; Zhu, L.; Yang, T.; Bennett, M.V.L.; Chen, J.; Keep, R.F.; Shi, Y. Blood-brain barrier dysfunction and recovery after ischemic stroke. *Prog. Neurobiol.,* **2018**, *163-164*, 144-171. http://dx.doi.org/10.1016/j.pneurobio.2017.10.001 PMID: 28987927

[110] Khatri, R.; McKinney, A.M.; Swenson, B.; Janardhan, V. Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology,* **2012**, *79*(131) (Suppl. 1), S52-S57. http://dx.doi.org/10.1212/WNL.0b013e3182697e70 PMID: 23008413

[111] Abdullahi, W.; Tripathi, D.; Ronaldson, P.T. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. *Am. J. Physiol. Cell Physiol.,* **2018**, *315*(3), C343-C356. http://dx.doi.org/10.1152/ajpcell.00095.2018 PMID: 29949404

[112] Krizbai, I.A.; Bauer, H.; Bresgen, N.; Eckl, P.M.; Farkas, A.; Szatmári, E.; Traweger, A.; Wejksza, K.; Bauer, H.C. Effect of oxidative stress on the junctional proteins of cultured cerebral endothelial cells. *Cell. Mol. Neurobiol.,* **2005**, *25*(1), 129-139. http://dx.doi.org/10.1007/s10571-004-1378-7 PMID: 15962510

[113] Kang, L.; Yu, H.; Yang, X.; Zhu, Y.; Bai, X.; Wang, R.; Cao, Y.; Xu, H.; Luo, H.; Lu, L.; Shi, M.J.; Tian, Y.; Fan, W.; Zhao, B.Q. Neutrophil extracellular traps released by neutrophils impair revascularization and vascular remodeling after stroke. *Nat. Commun.,* **2020**, *11*(1), 2488. http://dx.doi.org/10.1038/s41467-020-16191-y PMID: 32427863

[114] Jolivel, V.; Bicker, F.; Binamé, F.; Ploen, R.; Keller, S.; Gollan, R.; Jurek, B.; Birkenstock, J.; Poisa-Beiro, L.; Bruttger, J.; Opitz, V.; Thal, S.C.; Waisman, A.; Bäuerle, T.; Schäfer, M.K.; Zipp, F.; Schmidt, M.H.H. Perivascular microglia promote blood vessel dis-

integration in the ischemic penumbra. *Acta Neuropathol.,* **2015**, *129*(2), 279-295.
http://dx.doi.org/10.1007/s00401-014-1372-1 PMID: 25500713

[115] Gülke, E.; Gelderblom, M.; Magnus, T. Danger signals in stroke and their role on microglia activation after ischemia. *Ther. Adv. Neurol. Disord.,* **2018**, *11*
http://dx.doi.org/10.1177/1756286418774254 PMID: 29854002

[116] Liddelow, S.A.; Guttenplan, K.A.; Clarke, L.E.; Bennett, F.C.; Bohlen, C.J.; Schirmer, L.; Bennett, M.L.; Münch, A.E.; Chung, W.S.; Peterson, T.C.; Wilton, D.K.; Frouin, A.; Napier, B.A.; Panicker, N.; Kumar, M.; Buckwalter, M.S.; Rowitch, D.H.; Dawson, V.L.; Dawson, T.M.; Stevens, B.; Barres, B.A. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature,* **2017**, *541*(7638), 481-487.
http://dx.doi.org/10.1038/nature21029 PMID: 28099414

[117] Kangwantas, K.; Pinteaux, E.; Penny, J. The extracellular matrix protein laminin-10 promotes blood–brain barrier repair after hypoxia and inflammation *in vitro*. *J. Neuroinflammation,* **2016**, *13*(1), 25.
http://dx.doi.org/10.1186/s12974-016-0495-9 PMID: 26832174

[118] Chen, A.Q.; Fang, Z.; Chen, X.L.; Yang, S.; Zhou, Y.F.; Mao, L.; Xia, Y.P.; Jin, H.J.; Li, Y.N.; You, M.F.; Wang, X.X.; Lei, H.; He, Q.W.; Hu, B. Microglia-derived TNF-α mediates endothelial necroptosis aggravating blood brain–barrier disruption after ischemic stroke. *Cell Death Dis.,* **2019**, *10*(7), 487.
http://dx.doi.org/10.1038/s41419-019-1716-9 PMID: 31221990

[119] Qiu, Y.; Zhang, C.; Chen, A.; Wang, H.; Zhou, Y.; Li, Y.; Hu, B. Immune cells in the BBB disruption after acute ischemic stroke: Targets for immune therapy? *Front. Immunol.,* **2021**, *12*, 678744.
http://dx.doi.org/10.3389/fimmu.2021.678744 PMID: 34248961

[120] Liddelow, S.A.; Barres, B.A. Reactive astrocytes: Production, function, and therapeutic potential. *Immunity,* **2017**, *46*(6), 957-967.
http://dx.doi.org/10.1016/j.immuni.2017.06.006 PMID: 28636962

[121] Wang, G.; Weng, Y.C.; Chiang, I.C.; Huang, Y.T.; Liao, Y.C.; Chen, Y.C.; Kao, C.Y.; Liu, Y.L.; Lee, T.H.; Chou, W.H. Neutralization of lipocalin-2 diminishes stroke-reperfusion injury. *Int. J. Mol. Sci.,* **2020**, *21*(17), 6253.
http://dx.doi.org/10.3390/ijms21176253 PMID: 32872405

[122] Patabendige, A.; Singh, A.; Jenkins, S.; Sen, J.; Chen, R. Astrocyte activation in neurovascular damage and repair following ischaemic stroke. *Int. J. Mol. Sci.,* **2021**, *22*(8), 4280.
http://dx.doi.org/10.3390/ijms22084280 PMID: 33924191

[123] Fernández-Klett, F.; Priller, J. Diverse functions of pericytes in cerebral blood flow regulation and ischemia. *J. Cereb. Blood Flow Metab.,* **2015**, *35*(6), 883-887.
http://dx.doi.org/10.1038/jcbfm.2015.60 PMID: 25853910

[124] Jickling, G.C.; Dziedzic, T. Neutrophil count is related to stroke outcome following endovascular therapy. *Neurology,* **2019**, *93*(5), 194-195.
http://dx.doi.org/10.1212/WNL.0000000000007851 PMID: 31239357

[125] Beuker, C.; Strecker, J.K.; Rawal, R.; Schmidt-Pogoda, A.; Ruck, T.; Wiendl, H.; Klotz, L.; Schäbitz, W.R.; Sommer, C.J.; Minnerup, H.; Meuth, S.G.; Minnerup, J. Immune cell infiltration into the brain after ischemic stroke in humans compared to mice and rats: A systematic review and meta-analysis. *Transl. Stroke Res.,* **2021**, *12*(6), 976-990.
http://dx.doi.org/10.1007/s12975-021-00887-4 PMID: 33496918

[126] Carbone, F.; Bonaventura, A.; Montecucco, F. Neutrophil-related oxidants drive heart and brain remodeling after ischemia/reperfusion injury. *Front. Physiol.,* **2020**, *10*, 1587.
http://dx.doi.org/10.3389/fphys.2019.01587 PMID: 32116732

[127] Ohms, M.; Möller, S.; Laskay, T. An Attempt to Polarize Human Neutrophils Toward N1 and N2 Phenotypes *in vitro*. *Front. Immunol.,* **2020**, *11*, 532.
http://dx.doi.org/10.3389/fimmu.2020.00532 PMID: 32411122

[128] Mihaila, A.C.; Ciortan, L.; Macarie, R.D.; Vadana, M.; Cecoltan, S.; Preda, M.B.; Hudita, A.; Gan, A.M.; Jakobsson, G.; Tucureanu, M.M.; Barbu, E.; Balanescu, S.; Simionescu, M.; Schiopu, A.; Butoi, E. Transcriptional profiling and functional analysis of N1/N2 neutrophils reveal an immunomodulatory effect of S100A9-blockade on the pro-inflammatory N1 subpopulation. *Front. Immunol.,* **2021**, *12*, 708770.
http://dx.doi.org/10.3389/fimmu.2021.708770 PMID: 34447377

[129] Granger, D.N.; Kvietys, P.R. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol.,* **2015**, *6*, 524-551.
http://dx.doi.org/10.1016/j.redox.2015.08.020 PMID: 26484802

[130] Chen, H.; He, Y.; Chen, S.; Qi, S.; Shen, J. Therapeutic targets of oxidative/nitrosative stress and neuroinflammation in ischemic stroke: Applications for natural product efficacy with omics and systemic biology. *Pharmacol. Res.,* **2020**, *158*, 104877.
http://dx.doi.org/10.1016/j.phrs.2020.104877 PMID: 32407958

[131] Garcia-Bonilla, L.; Moore, J.M.; Racchumi, G.; Zhou, P.; Butler, J.M.; Iadecola, C.; Anrather, J. Inducible nitric oxide synthase in neutrophils and endothelium contributes to ischemic brain injury in mice. *J. Immunol.,* **2014**, *193*(5), 2531-2537.
http://dx.doi.org/10.4049/jimmunol.1400918 PMID: 25038255

[132] Szabó, C.; Ischiropoulos, H.; Radi, R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat. Rev. Drug Discov.,* **2007**, *6*(8), 662-680.
http://dx.doi.org/10.1038/nrd2222 PMID: 17667957

[133] Chen, X.; Chen, H.; Xu, M.; Shen, J. Targeting reactive nitrogen species: a promising therapeutic strategy for cerebral ischemia-reperfusion injury. *Acta Pharmacol. Sin.,* **2013**, *34*(1), 67-77.
http://dx.doi.org/10.1038/aps.2012.82 PMID: 22842734

[134] Gu, Y.; Zheng, G.; Xu, M.; Li, Y.; Chen, X.; Zhu, W.; Tong, Y.; Chung, S.K.; Liu, K.J.; Shen, J. Caveolin-1 regulates nitric oxide-mediated matrix metalloproteinases activity and blood-brain barrier permeability in focal cerebral ischemia and reperfusion injury. *J. Neurochem.,* **2012**, *120*(1), 147-156.
http://dx.doi.org/10.1111/j.1471-4159.2011.07542.x PMID: 22007835

[135] Hakkim, A.; Fuchs, T.A.; Martinez, N.E.; Hess, S.; Prinz, H.; Zychlinsky, A.; Waldmann, H. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nat. Chem. Biol.,* **2011**, *7*(2), 75-77.
http://dx.doi.org/10.1038/nchembio.496 PMID: 21170021

[136] Shaul, M.E.; Levy, L.; Sun, J.; Mishalian, I.; Singhal, S.; Kapoor, V.; Horng, W.; Fridlender, G.; Albelda, S.M.; Fridlender, Z.G. Tumor-associated neutrophils display a distinct N1 profile following TGFβ modulation: A transcriptomics analysis of pro- *vs.* anti-tumor TANs. *OncoImmunology,* **2016**, *5*(11), e1232221.
http://dx.doi.org/10.1080/2162402X.2016.1232221 PMID: 27999744

[137] Prince, L.R.; Whyte, M.K.; Sabroe, I.; Parker, L.C. The role of TLRs in neutrophil activation. *Curr. Opin. Pharmacol.,* **2011**, *11*(4), 397-403.
http://dx.doi.org/10.1016/j.coph.2011.06.007 PMID: 21741310

[138] Bakele, M.; Joos, M.; Burdi, S.; Allgaier, N.; Pöschel, S.; Fehrenbacher, B.; Schaller, M.; Marcos, V.; Kümmerle-Deschner, J.; Rieber, N.; Borregaard, N.; Yazdi, A.; Hector, A.; Hartl, D. Localization and functionality of the inflammasome in neutrophils. *J. Biol. Chem.,* **2014**, *289*(8), 5320-5329.
http://dx.doi.org/10.1074/jbc.M113.505636 PMID: 24398679

[139] Cai, W.; Wang, J.; Hu, M.; Chen, X.; Lu, Z.; Bellanti, J.A.; Zheng, S.G. All trans-retinoic acid protects against acute ischemic stroke by modulating neutrophil functions through STAT1 signaling. *J. Neuroinflammation,* **2019**, *16*(1), 175.
http://dx.doi.org/10.1186/s12974-019-1557-6 PMID: 31472680

[140] Luheshi, N.M.; Kovács, K.J.; Lopez-Castejon, G.; Brough, D.; Denes, A. Interleukin-1α expression precedes IL-1β after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. *J. Neuroinflammation,* **2011**, *8*(1), 186.
http://dx.doi.org/10.1186/1742-2094-8-186 PMID: 22206506

[141] Wang, Q.; Tang, X.; Yenari, M. The inflammatory response in stroke. *J. Neuroimmunol.,* **2007**, *184*(1-2), 53-68.
http://dx.doi.org/10.1016/j.jneuroim.2006.11.014 PMID: 17188755

[142] Ceulemans, A.G.; Zgavc, T.; Kooijman, R.; Hachimi-Idrissi, S.; Sarre, S.; Michotte, Y. The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. *J. Neuroinflammation,* **2010**, *7*(1), 74.
http://dx.doi.org/10.1186/1742-2094-7-74 PMID: 21040547

[143] Thornton, P.; McColl, B.W.; Cooper, L.; Rothwell, N.J.; Allan, S.M. Interleukin-1 drives cerebrovascular inflammation *via* MAP kinase-independent pathways. *Curr. Neurovasc. Res.,* **2010**, *7*(4), 330-340.

http://dx.doi.org/10.2174/156720210793180800 PMID: 20854249

[144] Singh, N.; Hopkins, S.J.; Hulme, S.; Galea, J.P.; Hoadley, M.; Vail, A.; Hutchinson, P.J.; Grainger, S.; Rothwell, N.J.; King, A.T.; Tyrrell, P.J. The effect of intravenous interleukin-1 receptor antagonist on inflammatory mediators in cerebrospinal fluid after subarachnoid haemorrhage: a phase II randomised controlled trial. *J. Neuroinflammation,* **2014**, *11*(1), 1. http://dx.doi.org/10.1186/1742-2094-11-1 PMID: 24383930

[145] Orion, D.; Schwammenthal, Y.; Reshef, T.; Schwartz, R.; Tsabari, R.; Merzeliak, O.; Chapman, J.; Mekori, Y.A.; Tanne, D. Interleukin-6 and soluble intercellular adhesion molecule-1 in acute brain ischaemia. *Eur. J. Neurol.,* **2008**, *15*(4), 323-328. http://dx.doi.org/10.1111/j.1468-1331.2008.02066.x PMID: 18312408

[146] Bolton, S.J.; Anthony, D.C.; Perry, V.H. Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood–brain barrier breakdown *in vivo*. *Neuroscience,* **1998**, *86*(4), 1245-1257. http://dx.doi.org/10.1016/S0306-4522(98)00058-X PMID: 9697130

[147] Guo, Z.; Yu, S.; Chen, X.; Zheng, P.; Hu, T.; Duan, Z.; Liu, X.; Liu, Q.; Ye, R.; Zhu, W.; Liu, X. Suppression of NLRP3 attenuates hemorrhagic transformation after delayed rtPA treatment in thromboembolic stroke rats: Involvement of neutrophil recruitment. *Brain Res. Bull.,* **2018**, *137*, 229-240. http://dx.doi.org/10.1016/j.brainresbull.2017.12.009 PMID: 29258866

[148] Simi, A.; Tsakiri, N.; Wang, P.; Rothwell, N.J. Interleukin-1 and inflammatory neurodegeneration. *Biochem. Soc. Trans.,* **2007**, *35*(5), 1122-1126. http://dx.doi.org/10.1042/BST0351122 PMID: 17956293

[149] Zou, J.Y.; Crews, F.T. TNFα potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NFκB inhibition. *Brain Res.,* **2005**, *1034*(1-2), 11-24. http://dx.doi.org/10.1016/j.brainres.2004.11.014 PMID: 15713255

[150] Breckwoldt, M.O.; Chen, J.W.; Stangenberg, L.; Aikawa, E.; Rodriguez, E.; Qiu, S.; Moskowitz, M.A.; Weissleder, R. Tracking the inflammatory response in stroke *in vivo* by sensing the enzyme myeloperoxidase. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(47), 18584-18589. http://dx.doi.org/10.1073/pnas.0803945105 PMID: 19011099

[151] Matsuo, Y.; Onodera, H.; Shiga, Y.; Nakamura, M.; Ninomiya, M.; Kihara, T.; Kogure, K. Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. *Stroke,* **1994**, *25*(7), 1469-1475. http://dx.doi.org/10.1161/01.STR.25.7.1469 PMID: 8023364

[152] Chen, S.; Chen, H.; Du, Q.; Shen, J. Targeting Myeloperoxidase (MPO) Mediated Oxidative Stress and Inflammation for Reducing Brain Ischemia Injury: Potential Application of Natural Compounds. *Front. Physiol.,* **2020**, *11*, 433. http://dx.doi.org/10.3389/fphys.2020.00433 PMID: 32508671

[153] Üllen, A.; Singewald, E.; Konya, V.; Fauler, G.; Reicher, H.; Nusshold, C.; Hammer, A.; Kratky, D.; Heinemann, A.; Holzer, P.; Malle, E.; Sattler, W. Myeloperoxidase-derived oxidants induce blood-brain barrier dysfunction *in vitro* and *in vivo*. *PLoS One,* **2013**, *8*(5), e64034. http://dx.doi.org/10.1371/journal.pone.0064034 PMID: 23691142

[154] Xu, J.; Xie, Z.; Reece, R.; Pimental, D.; Zou, M.H. Uncoupling of endothelial nitric oxidase synthase by hypochlorous acid: role of NAD(P)H oxidase-derived superoxide and peroxynitrite. *Arterioscler. Thromb. Vasc. Biol.,* **2006**, *26*(12), 2688-2695. http://dx.doi.org/10.1161/01.ATV.0000249394.94588.82 PMID: 17023679

[155] Suzuki, M.; Asako, H.; Kubes, P.; Jennings, S.; Grisham, M.B.; Granger, D.N. Neutrophil-derived oxidants promote leukocyte adherence in postcapillary venules. *Microvasc. Res.,* **1991**, *42*(2), 125-138. http://dx.doi.org/10.1016/0026-2862(91)90081-L PMID: 1658575

[156] El Kebir, D.; József, L.; Pan, W.; Filep, J.G. Myeloperoxidase delays neutrophil apoptosis through CD11b/CD18 integrins and prolongs inflammation. *Circ. Res.,* **2008**, *103*(4), 352-359. http://dx.doi.org/10.1161/01.RES.0000326772.76822.7a PMID: 18617697

[157] Nussbaum, C.; Klinke, A.; Adam, M.; Baldus, S.; Sperandio, M. Myeloperoxidase: a leukocyte-derived protagonist of inflammation and cardiovascular disease. *Antioxid. Redox Signal.,* **2013**, *18*(6), 692-713. http://dx.doi.org/10.1089/ars.2012.4783 PMID: 22823200

[158] Stowe, A.M.; Adair-Kirk, T.L.; Gonzales, E.R.; Perez, R.S.; Shah, A.R.; Park, T.S.; Gidday, J.M. Neutrophil elastase and neurovascular injury following focal stroke and reperfusion. *Neurobiol. Dis.,* **2009**, *35*(1), 82-90. http://dx.doi.org/10.1016/j.nbd.2009.04.006 PMID: 19393318

[159] Yamaguchi, Y.; Matsumura, F.; Wang, F.S.; Akizuki, E.; Liang, J.; Matsuda, T.; Okabe, K.; Ohshiro, H.; Horiuchi, T.; Yamada, S.; Mori, K.; Ogawa, M. Neutrophil elastase enhances intercellular adhesion molecule-1 expression. *Transplantation,* **1998**, *65*(12), 1622-1628. http://dx.doi.org/10.1097/00007890-199806270-00014 PMID: 9665080

[160] Voisin, M.B.; Leoni, G.; Woodfin, A.; Loumagne, L.; Patel, N.S.A.; Di Paola, R.; Cuzzocrea, S.; Thiemermann, C.; Perretti, M.; Nourshargh, S. Neutrophil elastase plays a non redundant role in remodeling the venular basement membrane and neutrophil diapedesis post ischemia/reperfusion injury. *J. Pathol.,* **2019**, *248*(1), 88-102. http://dx.doi.org/10.1002/path.5234 PMID: 30632166

[161] Vandooren, J.; Van Damme, J.; Opdenakker, G. On the Structure and functions of gelatinase B/Matrix metalloproteinase-9 in neuroinflammation. *Prog. Brain Res.,* **2014**, *214*, 193-206. http://dx.doi.org/10.1016/B978-0-444-63486-3.00009-8 PMID: 25410359

[162] Rivera, S.; Khrestchatisky, M.; Kaczmarek, L.; Rosenberg, G.A.; Jaworski, D.M. Metzincin proteases and their inhibitors: foes or friends in nervous system physiology? *J. Neurosci.,* **2010**, *30*(46), 15337-15357. http://dx.doi.org/10.1523/JNEUROSCI.3467-10.2010 PMID: 21084591

[163] Rosell, A.; Lo, E. Multiphasic roles for matrix metalloproteinases after stroke. *Curr. Opin. Pharmacol.,* **2008**, *8*(1), 82-89. http://dx.doi.org/10.1016/j.coph.2007.12.001 PMID: 18226583

[164] Clark, A.W.; Krekoski, C.A.; Bou, S.S.; Chapman, K.R.; Edwards, D.R. Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. *Neurosci. Lett.,* **1997**, *238*(1-2), 53-56. http://dx.doi.org/10.1016/S0304-3940(97)00859-8 PMID: 9464653

[165] Park, K.P.; Rosell, A.; Foerch, C.; Xing, C.; Kim, W.J.; Lee, S.; Opdenakker, G.; Furie, K.L.; Lo, E.H. Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats. *Stroke,* **2009**, *40*(8), 2836-2842. http://dx.doi.org/10.1161/STROKEAHA.109.554824 PMID: 19556529

[166] Turner, R.J.; Sharp, F.R. Implications of MMP9 for Blood Brain Barrier Disruption and Hemorrhagic Transformation Following Ischemic Stroke. *Front. Cell. Neurosci.,* **2016**, *10*, 56. http://dx.doi.org/10.3389/fncel.2016.00056 PMID: 26973468

[167] Fujimoto, M.; Takagi, Y.; Aoki, T.; Hayase, M.; Marumo, T.; Gomi, M.; Nishimura, M.; Kataoka, H.; Hashimoto, N.; Nozaki, K. Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **2008**, *28*(10), 1674-1685. http://dx.doi.org/10.1038/jcbfm.2008.59 PMID: 18560439

[168] Jourquin, J.; Tremblay, E.; Décanis, N.; Charton, G.; Hanessian, S.; Chollet, A.M.; Le Diguardher, T.; Khrestchatisky, M.; Rivera, S. Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. *Eur. J. Neurosci.,* **2003**, *18*(6), 1507-1517. http://dx.doi.org/10.1046/j.1460-9568.2003.02876.x PMID: 14511330

[169] Pires, R.H.; Felix, S.B.; Delcea, M. The architecture of neutrophil extracellular traps investigated by atomic force microscopy. *Nanoscale,* **2016**, *8*(29), 14193-14202. http://dx.doi.org/10.1039/C6NR03416K PMID: 27387552

[170] Brinkmann, V.; Reichard, U.; Goosmann, C.; Fauler, B.; Uhlemann, Y.; Weiss, D.S.; Weinrauch, Y.; Zychlinsky, A. Neutrophil extracellular traps kill bacteria. *Science,* **2004**, *303*(5663), 1532-1535.

http://dx.doi.org/10.1126/science.1092385 PMID: 15001782

[171] Sorvillo, N.; Cherpokova, D.; Martinod, K.; Wagner, D.D. Extracellular DNA NET-works with dire consequences for health. *Circ. Res.,* **2019**, *125*(4), 470-488. http://dx.doi.org/10.1161/CIRCRESAHA.119.314581 PMID: 31518165

[172] Lood, C.; Blanco, L.P.; Purmalek, M.M.; Carmona-Rivera, C.; De Ravin, S.S.; Smith, C.K.; Malech, H.L.; Ledbetter, J.A.; Elkon, K.B.; Kaplan, M.J. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat. Med.,* **2016**, *22*(2), 146-153. http://dx.doi.org/10.1038/nm.4027 PMID: 26779811

[173] Remijsen, Q.; Berghe, T.V.; Wirawan, E.; Asselbergh, B.; Parthoens, E.; De Rycke, R.; Noppen, S.; Delforge, M.; Willems, J.; Vandenabeele, P. Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. *Cell Res.,* **2011**, *21*(2), 290-304. http://dx.doi.org/10.1038/cr.2010.150 PMID: 21060338

[174] Kim, S.W.; Davaanyam, D.; Seol, S.I.; Lee, H.K.; Lee, H.; Lee, J.K. Adenosine triphosphate accumulated following cerebral ischemia induces neutrophil extracellular trap formation. *Int. J. Mol. Sci.,* **2020**, *21*(20), 7668. http://dx.doi.org/10.3390/ijms21207668 PMID: 33081303

[175] Kim, S.W.; Lee, H.; Lee, H.K.; Kim, I.D.; Lee, J.K. Neutrophil extracellular trap induced by HMGB1 exacerbates damages in the ischemic brain. *Acta Neuropathol. Commun.,* **2019**, *7*(1), 94. http://dx.doi.org/10.1186/s40478-019-0747-x PMID: 31177989

[176] Liu, L.; Mao, Y.; Xu, B.; Zhang, X.; Fang, C.; Ma, Y.; Men, K.; Qi, X.; Yi, T.; Wei, Y.; Wei, X. Induction of neutrophil extracellular traps during tissue injury: Involvement of STING and Toll like receptor 9 pathways. *Cell Prolif.,* **2019**, *52*(3), e12579. http://dx.doi.org/10.1111/cpr.12579 PMID: 30851061

[177] Papayannopoulos, V.; Metzler, K.D.; Hakkim, A.; Zychlinsky, A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J. Cell Biol.,* **2010**, *191*(3), 677-691. http://dx.doi.org/10.1083/jcb.201006052 PMID: 20974816

[178] Wang, Y.; Li, M.; Stadler, S.; Correll, S.; Li, P.; Wang, D.; Hayama, R.; Leonelli, L.; Han, H.; Grigoryev, S.A.; Allis, C.D.; Coonrod, S.A. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J. Cell Biol.,* **2009**, *184*(2), 205-213. http://dx.doi.org/10.1083/jcb.200806072 PMID: 19153223

[179] Zheng, W.; Warner, R.; Ruggeri, R.; Su, C.; Cortes, C.; Skoura, A.; Ward, J.; Ahn, K.; Kalgutkar, A.; Sun, D.; Maurer, T.S.; Bonin, P.D.; Okerberg, C.; Bobrowski, W.; Kawabe, T.; Zhang, Y.; Coskran, T.; Bell, S.; Kapoor, B.; Johnson, K.; Buckbinder, L. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. *J. Pharmacol. Exp. Ther.,* **2015**, *353*(2), 288-298. http://dx.doi.org/10.1124/jpet.114.221788 PMID: 25698787

[180] Essig, F.; Kollikowski, A.M.; Pham, M.; Solymosi, L.; Stoll, G.; Haeusler, K.G.; Kraft, P.; Schuhmann, M.K. Immunohistological analysis of neutrophils and neutrophil extracellular traps in human thrombemboli causing acute ischemic stroke. *Int. J. Mol. Sci.,* **2020**, *21*(19), 7387. http://dx.doi.org/10.3390/ijms21197387 PMID: 33036337

[181] Ge, L.; Zhou, X.; Ji, W.J.; Lu, R.Y.; Zhang, Y.; Zhang, Y.D.; Ma, Y.Q.; Zhao, J.H.; Li, Y.M. Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: therapeutic potential of DNase-based reperfusion strategy. *Am. J. Physiol. Heart Circ. Physiol.,* **2015**, *308*(5), H500-H509. http://dx.doi.org/10.1152/ajpheart.00381.2014 PMID: 25527775

[182] Laridan, E.; Denorme, F.; Desender, L.; François, O.; Andersson, T.; Deckmyn, H.; Vanhoorelbeke, K.; De Meyer, S.F. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann. Neurol.,* **2017**, *82*(2), 223-232. http://dx.doi.org/10.1002/ana.24993 PMID: 28696508

[183] Xu, J.; Zhang, X.; Monestier, M.; Esmon, N.L.; Esmon, C.T. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. *J. Immunol.,* **2011**, *187*(5), 2626-2631. http://dx.doi.org/10.4049/jimmunol.1003930 PMID: 21784973

[184] Fuchs, T.A.; Bhandari, A.A.; Wagner, D.D. Histones induce rapid and profound thrombocytopenia in mice. *Blood,* **2011**, *118*(13), 3708-3714. http://dx.doi.org/10.1182/blood-2011-01-332676 PMID: 21700775

[185] von Brühl, M.L.; Stark, K.; Steinhart, A.; Chandraratne, S.; Konrad, I.; Lorenz, M.; Khandoga, A.; Tirniceriu, A.; Coletti, R.; Köllnberger, M.; Byrne, R.A.; Laitinen, I.; Walch, A.; Brill, A.; Pfeiler, S.; Manukyan, D.; Braun, S.; Lange, P.; Riegger, J.; Ware, J.; Eckart, A.; Haidari, S.; Rudelius, M.; Schulz, C.; Echtler, K.; Brinkmann, V.; Schwaiger, M.; Preissner, K.T.; Wagner, D.D.; Mackman, N.; Engelmann, B.; Massberg, S. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice *in vivo*. *J. Exp. Med.,* **2012**, *209*(4), 819-835. http://dx.doi.org/10.1084/jem.20112322 PMID: 22451716

[186] Ruhnau, J.; Schulze, J.; Dressel, A.; Vogelgesang, A. Thrombosis, Neuroinflammation, and Poststroke Infection: The Multifaceted Role of Neutrophils in Stroke. *J. Immunol. Res.,* **2017**, *2017*, 1-7. http://dx.doi.org/10.1155/2017/5140679 PMID: 28331857

[187] Maugeri, N.; Campana, L.; Gavina, M.; Covino, C.; De Metrio, M.; Panciroli, C.; Maiuri, L.; Maseri, A.; D'Angelo, A.; Bianchi, M.E.; Rovere-Querini, P.; Manfredi, A.A. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *J. Thromb. Haemost.,* **2014**, *12*(12), 2074-2088. http://dx.doi.org/10.1111/jth.12710 PMID: 25163512

[188] Yipp, B.G.; Petri, B.; Salina, D.; Jenne, C.N.; Scott, B.N.V.; Zbytnuik, L.D.; Pittman, K.; Asaduzzaman, M.; Wu, K.; Meijndert, H.C.; Malawista, S.E.; de Boisfleury Chevance, A.; Zhang, K.; Conly, J.; Kubes, P. Infection-induced NETosis is a dynamic process involving neutrophil multitasking *in vivo*. *Nat. Med.,* **2012**, *18*(9), 1386-1393. http://dx.doi.org/10.1038/nm.2847 PMID: 22922410

[189] Mishalian, I.; Bayuh, R.; Levy, L.; Zolotarov, L.; Michaeli, J.; Fridlender, Z.G. Tumor-associated neutrophils (TAN) develop protumorigenic properties during tumor progression. *Cancer Immunol. Immunother.,* **2013**, *62*(11), 1745-1756. http://dx.doi.org/10.1007/s00262-013-1476-9 PMID: 24092389

[190] García-Culebras, A.; Durán-Laforet, V.; Peña-Martínez, C.; Moraga, A.; Ballesteros, I.; Cuartero, M.I.; de la Parra, J.; Palma-Tortosa, S.; Hidalgo, A.; Corbí, A.L.; Moro, M.A.; Lizasoain, I. Role of TLR4 (toll-like receptor 4) in N1/N2 neutrophil programming after stroke. *Stroke,* **2019**, *50*(10), 2922-2932. http://dx.doi.org/10.1161/STROKEAHA.119.025085 PMID: 31451099

[191] Caldwell, R.W.; Rodriguez, P.C.; Toque, H.A.; Narayanan, S.P.; Caldwell, R.B. Arginase: A multifaceted enzyme important in health and disease. *Physiol. Rev.,* **2018**, *98*(2), 641-665. http://dx.doi.org/10.1152/physrev.00037.2016 PMID: 29412048

[192] Caldwell, R.B.; Toque, H.A.; Narayanan, S.P.; Caldwell, R.W. Arginase: an old enzyme with new tricks. *Trends Pharmacol. Sci.,* **2015**, *36*(6), 395-405. http://dx.doi.org/10.1016/j.tips.2015.03.006 PMID: 25930708

[193] Lee, J.; Ryu, H.; Ferrante, R.J.; Morris, S.M., Jr; Ratan, R.R. Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*(8), 4843-4848. http://dx.doi.org/10.1073/pnas.0735876100 PMID: 12655043

[194] Ma, T.C.; Campana, A.; Lange, P.S.; Lee, H.H.; Banerjee, K.; Bryson, J.B.; Mahishi, L.; Alam, S.; Giger, R.J.; Barnes, S.; Morris, S.M., Jr; Willis, D.E.; Twiss, J.L.; Filbin, M.T.; Ratan, R.R. A large-scale chemical screen for regulators of the arginase 1 promoter identifies the soy isoflavone daidzeinas a clinically approved small molecule that can promote neuronal protection or regeneration *via* a cAMP-independent pathway. *J. Neurosci.,* **2010**, *30*(2), 739-748. http://dx.doi.org/10.1523/JNEUROSCI.5266-09.2010 PMID: 20071539

[195] Fouda, A.Y.; Xu, Z.; Shosha, E.; Lemtalsi, T.; Chen, J.; Toque, H.A.; Tritz, R.; Cui, X.; Stansfield, B.K.; Huo, Y.; Rodriguez, P.C.; Smith, S.B.; Caldwell, R.W.; Narayanan, S.P.; Caldwell, R.B. Arginase 1 promotes retinal neurovascular protection from ischemia through suppression of macrophage inflammatory responses. *Cell Death Dis.,* **2018**, *9*(10), 1001. http://dx.doi.org/10.1038/s41419-018-1051-6 PMID: 30254218

[196] Garcia, J.M.; Stillings, S.A.; Leclerc, J.L.; Phillips, H.; Edwards, N.J.; Robicsek, S.A.; Hoh, B.L.; Blackburn, S.; Doré, S. Role of Interleukin-10 in Acute Brain Injuries. *Front. Neurol.,* **2017**, *8*, 244. http://dx.doi.org/10.3389/fneur.2017.00244 PMID: 28659854

[197] Grilli, M.; Barbieri, I.; Basudev, H.; Brusa, R.; Casati, C.; Lozza, G.; Ongini, E. Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage. *Eur. J. Neurosci.,* **2000**, *12*(7), 2265-2272. http://dx.doi.org/10.1046/j.1460-9568.2000.00090.x PMID: 10947805

[198] Sharma, S.; Yang, B.; Xi, X.; Grotta, J.C.; Aronowski, J.; Savitz, S.I. IL-10 directly protects cortical neurons by activating PI-3 kinase and STAT-3 pathways. *Brain Res.,* **2011**, *1373*, 189-194. http://dx.doi.org/10.1016/j.brainres.2010.11.096 PMID: 21138740

[199] Bazzoni, F.; Tamassia, N.; Rossato, M.; Cassatella, M.A. Understanding the molecular mechanisms of the multifaceted IL-10-mediated anti-inflammatory response: Lessons from neutrophils. *Eur. J. Immunol.,* **2010**, *40*(9), 2360-2368. http://dx.doi.org/10.1002/eji.200940294 PMID: 20549669

[200] Pérez-de Puig, I.; Miró, F.; Salas-Perdomo, A.; Bonfill-Teixidor, E.; Ferrer-Ferrer, M.; Márquez-Kisinousky, L.; Planas, A.M. IL-10 deficiency exacerbates the brain inflammatory response to permanent ischemia without preventing resolution of the lesion. *J. Cereb. Blood Flow Metab.,* **2013**, *33*(12), 1955-1966. http://dx.doi.org/10.1038/jcbfm.2013.155 PMID: 24022622

[201] Xie, G.; Myint, P.K.; Zaman, M.J.S.; Li, Y.; Zhao, L.; Shi, P.; Ren, F.; Wu, Y. Relationship of serum interleukin-10 and its genetic variations with ischemic stroke in a Chinese general population. *PLoS One,* **2013**, *8*(9), e74126. http://dx.doi.org/10.1371/journal.pone.0074126 PMID: 24040186

[202] Doll, D.; Barr, T.L.; Simpkins, J.W. Cytokines: their role in stroke and potential use as biomarkers and therapeutic targets. *Aging Dis.,* **2014**, *5*(5), 294-306. http://dx.doi.org/10.14336/ad.2014.0500294 PMID: 25276489

[203] Zhu, H.; Gui, Q.; Hui, X.; Wang, X.; Jiang, J.; Ding, L.; Sun, X.; Wang, Y.; Chen, H. TGF-β1/Smad3 signaling pathway suppresses cell apoptosis in cerebral ischemic stroke rats. *Med. Sci. Monit.,* **2017**, *23*, 366-376. http://dx.doi.org/10.12659/MSM.899195 PMID: 28110342

[204] Wang, S.; Yin, J.; Ge, M.; Dai, Z.; Li, Y.; Si, J.; Ma, K.; Li, L.; Yao, S. Transforming growth-beta 1 contributes to isoflurane postconditioning against cerebral ischemia–reperfusion injury by regulating the c-Jun N-terminal kinase signaling pathway. *Biomed. Pharmacother.,* **2016**, *78*, 280-290. http://dx.doi.org/10.1016/j.biopha.2016.01.030 PMID: 26898453

[205] Krieglstein, K.; Zheng, F.; Unsicker, K.; Alzheimer, C. More than being protective: functional roles for TGF-β/activin signaling pathways at central synapses. *Trends Neurosci.,* **2011**, *34*(8), 421-429. http://dx.doi.org/10.1016/j.tins.2011.06.002 PMID: 21742388

[206] Ma, M.; Ma, Y.; Yi, X.; Guo, R.; Zhu, W.; Fan, X.; Xu, G.; Frey, W.H., II; Liu, X. Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. *BMC Neurosci.,* **2008**, *9*(1), 117. http://dx.doi.org/10.1186/1471-2202-9-117 PMID: 19077183

[207] Zhang, Y.; Yu, P.; Liu, H.; Yao, H.; Yao, S.; Yuan, S.-Y.; Zhang, J.-C. Hyperforin improves post-stroke social isolation-induced exaggeration of PSD and PSA *via* TGF-β. *Int J Mol Med.,* **2019**, *43*(1), 413-425. http://dx.doi.org/10.3892/ijmm.2018.3971 PMID: 30387813

[208] Li, T.; Pang, S.; Yu, Y.; Wu, X.; Guo, J.; Zhang, S. Proliferation of parenchymal microglia is the main source of microgliosis after ischaemic stroke. *Brain,* **2013**, *136*(12), 3578-3588. http://dx.doi.org/10.1093/brain/awt287 PMID: 24154617

[209] Neumann, J.; Sauerzweig, S.; Rönicke, R.; Gunzer, F.; Dinkel, K.; Ullrich, O.; Gunzer, M.; Reymann, K.G. Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. *J. Neurosci.,* **2008**, *28*(23), 5965-5975. http://dx.doi.org/10.1523/JNEUROSCI.0060-08.2008 PMID: 18524901

[210] Steiger, S.; Harper, J.L. Neutrophil cannibalism triggers transforming growth factor β1 production and self regulation of neutrophil inflammatory function in monosodium urate monohydrate crystal-induced inflammation in mice. *Arthritis Rheum.,* **2013**, *65*(3), 815-823. http://dx.doi.org/10.1002/art.37822 PMID: 23280587

[211] Kanazawa, M.; Hatakeyama, M.; Ninomiya, I. Angiogenesis and neuronal remodeling after ischemic stroke. *Neural Regen. Res.,* **2020**, *15*(1), 16-19. http://dx.doi.org/10.4103/1673-5374.264442 PMID: 31535636

[212] Wang, X.; Xuan, W.; Zhu, Z.Y.; Li, Y.; Zhu, H.; Zhu, L.; Fu, D.Y.; Yang, L.Q.; Li, P.Y.; Yu, W.F. The evolving role of neuro-immune interaction in brain repair after cerebral ischemic stroke. *CNS Neurosci. Ther.,* **2018**, *24*(12), 1100-1114. http://dx.doi.org/10.1111/cns.13077 PMID: 30350341

[213] Balkaya, M.; Cho, S. Genetics of stroke recovery: BDNF val66met polymorphism in stroke recovery and its interaction with aging. *Neurobiol. Dis.,* **2019**, *126*, 36-46. http://dx.doi.org/10.1016/j.nbd.2018.08.009 PMID: 30118755

[214] Jin, K.; Mao, X.O.; Greenberg, D.A. Vascular endothelial growth factor stimulates neurite outgrowth from cerebral cortical neurons *via* Rho kinase signaling. *J. Neurobiol.,* **2006**, *66*(3), 236-242. http://dx.doi.org/10.1002/neu.20215 PMID: 16329123

[215] Wang, Y.; Jin, K.; Mao, X.O.; Xie, L.; Banwait, S.; Marti, H.H.; Greenberg, D.A. VEGF-overexpressing transgenic mice show enhanced post-ischemic neurogenesis and neuromigration. *J. Neurosci. Res.,* **2007**, *85*(4), 740-747. http://dx.doi.org/10.1002/jnr.21169 PMID: 17243175

[216] Teng, H.; Zhang, Z.G.; Wang, L.; Zhang, R.L.; Zhang, L.; Morris, D.; Gregg, S.R.; Wu, Z.; Jiang, A.; Lu, M.; Zlokovic, B.V.; Chopp, M. Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke. *J. Cereb. Blood Flow Metab.,* **2008**, *28*(4), 764-771. http://dx.doi.org/10.1038/sj.jcbfm.9600573 PMID: 17971789

[217] Kitagawa, K.; Matsumoto, M.; Mabuchi, T.; Yagita, Y.; Ohtsuki, T.; Hori, M.; Yanagihara, T. Deficiency of intercellular adhesion molecule 1 attenuates microcirculatory disturbance and infarction size in focal cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **1998**, *18*(12), 1336-1345. http://dx.doi.org/10.1097/00004647-199812000-00008 PMID: 9850146

[218] Jones, K.A.; Maltby, S.; Plank, M.W.; Kluge, M.; Nilsson, M.; Foster, P.S.; Walker, F.R. Peripheral immune cells infiltrate into sites of secondary neurodegeneration after ischemic stroke. *Brain Behav. Immun.,* **2018**, *67*, 299-307. http://dx.doi.org/10.1016/j.bbi.2017.09.006 PMID: 28911981

[219] El Amki, M.; Glück, C.; Binder, N.; Middleham, W.; Wyss, M.T.; Weiss, T.; Meister, H.; Luft, A.; Weller, M.; Weber, B.; Wegener, S. Neutrophils obstructing brain capillaries are a major cause of no-reflow in ischemic stroke. *Cell Rep.,* **2020**, *33*(2), 108260. http://dx.doi.org/10.1016/j.celrep.2020.108260 PMID: 33053341

[220] Shaw, S.K.; Owolabi, S.A.; Bagley, J.; Morin, N.; Cheng, E.; LeBlanc, B.W.; Kim, M.; Harty, P.; Waxman, S.G.; Saab, C.Y. Activated polymorphonuclear cells promote injury and excitability of dorsal root ganglia neurons. *Exp. Neurol.,* **2008**, *210*(2), 286-294. http://dx.doi.org/10.1016/j.expneurol.2007.11.024 PMID: 18201702

[221] Mai, N.; Prifti, V.; Kim, M.; Halterman, M.W. Characterization of neutrophil-neuronal co-cultures to investigate mechanisms of post-ischemic immune-mediated neurotoxicity. *J. Neurosci. Methods,* **2020**, *341*, 108782. http://dx.doi.org/10.1016/j.jneumeth.2020.108782 PMID: 32445795

[222] Allen, C.; Thornton, P.; Denes, A.; McColl, B.W.; Pierozynski, A.; Monestier, M.; Pinteaux, E.; Rothwell, N.J.; Allan, S.M. Neutrophil cerebrovascular transmigration triggers rapid neurotoxicity through release of proteases associated with decondensed DNA. *J. Immunol.,* **2012**, *189*(1), 381-392. http://dx.doi.org/10.4049/jimmunol.1200409 PMID: 22661091

[223] Nguyen, H.X.; O'Barr, T.J.; Anderson, A.J. Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-α. *J. Neurochem.,* **2007**, *102*(3), 900-912. http://dx.doi.org/10.1111/j.1471-4159.2007.04643.x PMID: 17561941

[224] Kwon, K.J.; Cho, K.S.; Kim, J.N.; Kim, M.K.; Lee, E.J.; Kim, S.Y.; Jeon, S.J.; Kim, K.C.; Han, J.E.; Kang, Y.S.; Kim, S.; Kim, H.Y.; Han, S.H.; Bahn, G.; Choi, J.; Shin, C.Y. Proteinase 3 induces oxidative stress-mediated neuronal death in rat primary cortical neuron. *Neurosci. Lett.,* **2013**, *548*, 67-72. http://dx.doi.org/10.1016/j.neulet.2013.05.060 PMID: 23748041

[225] Cao, J.; Dong, L.; Luo, J.; Zeng, F.; Hong, Z.; Liu, Y.; Zhao, Y.; Xia, Z.; Zuo, D.; Xu, L.; Tao, T. Supplemental N-3 polyunsaturated fatty acids limit A1-specific astrocyte polarization *via* attenuating mitochondrial dysfunction in ischemic stroke in mice. *Oxid. Med. Cell. Longev.,* **2021**, *2021*, 1-13. http://dx.doi.org/10.1155/2021/5524705 PMID: 34211624

[226] Wenzel, T.J.; Bajwa, E.; Klegeris, A. Cytochrome c can be released into extracellular space and modulate functions of human astrocytes in a toll-like receptor 4-dependent manner. *Biochim. Biophys. Acta, Gen. Subj.,* **2019**, *1863*(11), 129400. http://dx.doi.org/10.1016/j.bbagen.2019.07.009 PMID: 31344401

[227] Soto-Díaz, K.; Juda, M.B.; Blackmore, S.; Walsh, C.; Steelman, A.J. TAK1 inhibition in mouse astrocyte cultures ameliorates cytokine induced chemokine production and neutrophil migration. *J. Neurochem.,* **2020**, *152*(6), 697-709. http://dx.doi.org/10.1111/jnc.14930 PMID: 31782806

[228] Moreno-Flores, M.T.; Bovolenta, P.; Nieto-Sampedro, M. Polymorphonuclear leukocytes in brain parenchyma after injury and their interaction with purified astrocytes in culture. *Glia,* **1993**, *7*(2), 146-157. http://dx.doi.org/10.1002/glia.440070204 PMID: 7679369

[229] Xie, L.; Poteet, E.C.; Li, W.; Scott, A.E.; Liu, R.; Wen, Y.; Ghorpade, A.; Simpkins, J.W.; Yang, S.H. Modulation of polymorphonuclear neutrophil functions by astrocytes. *J. Neuroinflammation,* **2010**, *7*(1), 53. http://dx.doi.org/10.1186/1742-2094-7-53 PMID: 20828397

[230] Rakers, C.; Schleif, M.; Blank, N.; Matušková, H.; Ulas, T.; Händler, K.; Torres, S.V.; Schumacher, T.; Tai, K.; Schultze, J.L.; Jackson, W.S.; Petzold, G.C. Stroke target identification guided by astrocyte transcriptome analysis. *Glia,* **2019**, *67*(4), 619-633. http://dx.doi.org/10.1002/glia.23544 PMID: 30585358

[231] Morizawa, Y.M.; Hirayama, Y.; Ohno, N.; Shibata, S.; Shigetomi, E.; Sui, Y.; Nabekura, J.; Sato, K.; Okajima, F.; Takebayashi, H.; Okano, H.; Koizumi, S. Reactive astrocytes function as phagocytes after brain ischemia *via* ABCA1-mediated pathway. *Nat. Commun.,* **2017**, *8*(1), 28. http://dx.doi.org/10.1038/s41467-017-00037-1 PMID: 28642575

[232] Shinozaki, Y.; Shibata, K.; Yoshida, K.; Shigetomi, E.; Gachet, C.; Ikenaka, K.; Tanaka, K.F.; Koizumi, S. Transformation of astrocytes to a neuroprotective phenotype by microglia *via* $P2Y_1$ receptor downregulation. *Cell Rep.,* **2017**, *19*(6), 1151-1164. http://dx.doi.org/10.1016/j.celrep.2017.04.047 PMID: 28494865

[233] Xiong, X.Y.; Liu, L.; Yang, Q.W. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. *Prog. Neurobiol.,* **2016**, *142*, 23-44. http://dx.doi.org/10.1016/j.pneurobio.2016.05.001 PMID: 27166859

[234] Kim, Y.R.; Kim, Y.M.; Lee, J.; Park, J.; Lee, J.E.; Hyun, Y.M. Neutrophils return to bloodstream through the brain blood vessel after crosstalk with microglia during LPS-induced neuroinflammation. *Front. Cell Dev. Biol.,* **2020**, *8*, 613733. http://dx.doi.org/10.3389/fcell.2020.613733 PMID: 33364241

[235] Neumann, J.; Henneberg, S.; von Kenne, S.; Nolte, N.; Müller, A.J.; Schraven, B.; Görtler, M.W.; Reymann, K.G.; Gunzer, M.; Riek-Burchardt, M. Beware the intruder: Real time observation of infiltrated neutrophils and neutrophil—Microglia interaction during stroke *in vivo*. *PLoS One,* **2018**, *13*(3), e0193970. http://dx.doi.org/10.1371/journal.pone.0193970 PMID: 29543836

[236] Masuda, T.; Croom, D.; Hida, H.; Kirov, S.A. Capillary blood flow around microglial somata determines dynamics of microglial processes in ischemic conditions. *Glia,* **2011**, *59*(11), 1744-1753. http://dx.doi.org/10.1002/glia.21220 PMID: 21800362

[237] Otxoa-de-Amezaga, A.; Miró-Mur, F.; Pedragosa, J.; Gallizioli, M.; Justicia, C.; Gaja-Capdevila, N.; Ruíz-Jaen, F.; Salas-Perdomo, A.; Bosch, A.; Calvo, M.; Márquez-Kisinousky, L.; Denes, A.; Gunzer, M.; Planas, A.M. Microglial cell loss after ischemic stroke favors brain neutrophil accumulation. *Acta Neuropathol.,* **2019**, *137*(2), 321-341. http://dx.doi.org/10.1007/s00401-018-1954-4 PMID: 30580383

[238] Egashira, Y.; Suzuki, Y.; Azuma, Y.; Takagi, T.; Mishiro, K.; Sugitani, S.; Tsuruma, K.; Shimazawa, M.; Yoshimura, S.; Kashimata, M.; Iwama, T.; Hara, H. The growth factor progranulin attenuates neuronal injury induced by cerebral ischemia-reperfusion through the suppression of neutrophil recruitment. *J Neuroinflammation,* **2013**, *10*, 884. http://dx.doi.org/10.1186/1742-2094-10-105

[239] Horinokita, I.; Hayashi, H.; Oteki, R.; Mizumura, R.; Yamaguchi, T.; Usui, A.; Yuan, B.; Takagi, N. Involvement of progranulin and granulin expression in inflammatory responses after cerebral ischemia. *Int. J. Mol. Sci.,* **2019**, *20*(20), 5210. http://dx.doi.org/10.3390/ijms20205210 PMID: 31640144

[240] Kim, E.; Cho, S. Microglia and monocyte-derived macrophages in stroke. *Neurotherapeutics,* **2016**, *13*(4), 702-718. http://dx.doi.org/10.1007/s13311-016-0463-1 PMID: 27485238

[241] Hu, X.; Leak, R.K.; Shi, Y.; Suenaga, J.; Gao, Y.; Zheng, P.; Chen, J. Microglial and macrophage polarization—new prospects for brain repair. *Nat. Rev. Neurol.,* **2015**, *11*(1), 56-64. http://dx.doi.org/10.1038/nrneurol.2014.207 PMID: 25385337

[242] Esposito, E.; Hayakawa, K.; Ahn, B.J.; Chan, S.J.; Xing, C.; Liang, A.C.; Kim, K.W.; Arai, K.; Lo, E.H. Effects of ischemic postconditioning on neuronal VEGF regulation and microglial polarization in a rat model of focal cerebral ischemia. *J. Neurochem.,* **2018**, *146*(2), 160-172. http://dx.doi.org/10.1111/jnc.14337 PMID: 29570780

[243] Gelderblom, M.; Leypoldt, F.; Steinbach, K.; Behrens, D.; Choe, C.U.; Siler, D.A.; Arumugam, T.V.; Orthey, E.; Gerloff, C.; Tolosa, E.; Magnus, T. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke,* **2009**, *40*(5), 1849-1857. http://dx.doi.org/10.1161/STROKEAHA.108.534503 PMID: 19265055

[244] Viviani, G.L.; Dallegri, F.; Quercioli, A.; Veneselli, E.; Mach, F.; Mirabelli-Badenier, M.; Braunersreuther, V.; Montecucco, F. CC and CXC chemokines are pivotal mediators of cerebral injury in ischaemic stroke. *Thromb. Haemost.,* **2011**, *105*(3), 409-420. http://dx.doi.org/10.1160/TH10-10-0662 PMID: 21174009

[245] Taekema-Roelvink, M.J.; Kooten, C.V.; Kooij, S.V.D.; Heemskerk, E.; Daha, M.R. Proteinase 3 enhances endothelial monocyte chemoattractant protein-1 production and induces increased adhesion of neutrophils to endothelial cells by upregulating intercellular cell adhesion molecule-1. *J. Am. Soc. Nephrol.,* **2001**, *12*(5), 932-940. http://dx.doi.org/10.1681/ASN.V125932 PMID: 11316851

[246] Youn, J.I.; Kumar, V.; Collazo, M.; Nefedova, Y.; Condamine, T.; Cheng, P.; Villagra, A.; Antonia, S.; McCaffrey, J.C.; Fishman, M.; Sarnaik, A.; Horna, P.; Sotomayor, E.; Gabrilovich, D.I. Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer. *Nat. Immunol.,* **2013**, *14*(3), 211-220. http://dx.doi.org/10.1038/ni.2526 PMID: 23354483

[247] Mantovani, A.; Sica, A.; Sozzani, S.; Allavena, P.; Vecchi, A.; Locati, M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.,* **2004**, *25*(12), 677-686. http://dx.doi.org/10.1016/j.it.2004.09.015 PMID: 15530839

[248] Greenlee-Wacker, M.C. Clearance of apoptotic neutrophils and resolution of inflammation. *Immunol. Rev.,* **2016**, *273*(1), 357-370. http://dx.doi.org/10.1111/imr.12453 PMID: 27558346

[249] Jin, W.N.; Gonzales, R.; Feng, Y.; Wood, K.; Chai, Z.; Dong, J.F.; La Cava, A.; Shi, F.D.; Liu, Q. Brain ischemia induces diversified neuroantigen-specific T-cell responses that exacerbate brain injury. *Stroke,* **2018**, *49*(6), 1471-1478. http://dx.doi.org/10.1161/STROKEAHA.118.020203 PMID: 29695462

[250] Lei, T.Y.; Ye, Y.Z.; Zhu, X.Q.; Smerin, D.; Gu, L.J.; Xiong, X.X.; Zhang, H.F.; Jian, Z.H. The immune response of T cells and therapeutic targets related to regulating the levels of T helper cells after ischaemic stroke. *J. Neuroinflammation,* **2021**, *18*(1), 25. http://dx.doi.org/10.1186/s12974-020-02057-z PMID: 33461586

[251] Cramer, J.V.; Benakis, C.; Liesz, A. T cells in the post-ischemic brain: Troopers or paramedics? *J. Neuroimmunol.,* **2019**, *326*, 33-37. http://dx.doi.org/10.1016/j.jneuroim.2018.11.006 PMID: 30468953

[252] Pelletier, M.; Maggi, L.; Micheletti, A.; Lazzeri, E.; Tamassia, N.; Costantini, C.; Cosmi, L.; Lunardi, C.; Annunziato, F.; Romagnani, S.; Cassatella, M.A. Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood,* **2010**, *115*(2), 335-343. http://dx.doi.org/10.1182/blood-2009-04-216085 PMID: 19890092

[253] Barros, J.F.; Waclawiak, I.; Pecli, C.; Borges, P.A.; Georgii, J.L.; Ramos-Junior, E.S.; Canetti, C.; Courau, T.; Klatzmann, D.; Kunkel, S.L.; Penido, C.; Canto, F.B.; Benjamim, C.F. Role of chemokine receptor CCR4 and regulatory T cells in wound healing of diabetic mice. *J. Invest. Dermatol.,* **2019**, *139*(5), 1161-1170. http://dx.doi.org/10.1016/j.jid.2018.10.039 PMID: 30465800

[254] Scapini, P.; Cassatella, M.A. Social networking of human neutrophils within the immune system. *Blood,* **2014**, *124*(5), 710-719. http://dx.doi.org/10.1182/blood-2014-03-453217 PMID: 24923297

[255] Gelderblom, M.; Weymar, A.; Bernreuther, C.; Velden, J.; Arunachalam, P.; Steinbach, K.; Orthey, E.; Arumugam, T.V.; Leypoldt, F.; Simova, O.; Thom, V.; Friese, M.A.; Prinz, I.; Hölscher, C.; Glatzel, M.; Korn, T.; Gerloff, C.; Tolosa, E.; Magnus, T. Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood,* **2012**, *120*(18), 3793-3802. http://dx.doi.org/10.1182/blood-2012-02-412726 PMID: 22976954

[256] Pelletier, M.; Micheletti, A.; Cassatella, M.A. Modulation of human neutrophil survival and antigen expression by activated $CD4^+$ and $CD8^+$ T cells. *J. Leukoc. Biol.,* **2010**, *88*(6), 1163-1170. http://dx.doi.org/10.1189/jlb.0310172 PMID: 20686115

[257] Rosales, C. Neutrophils at the crossroads of innate and adaptive immunity. *J. Leukoc. Biol.,* **2020**, *108*(1), 377-396. http://dx.doi.org/10.1002/JLB.4MIR0220-574RR PMID: 32202340

[258] Rodriguez, P.C.; Quiceno, D.G.; Ochoa, A.C. l-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood,* **2007**, *109*(4), 1568-1573. http://dx.doi.org/10.1182/blood-2006-06-031856 PMID: 17023580

[259] Stubbe, T.; Ebner, F.; Richter, D.; Engel, O.R.; Klehmet, J.; Royl, G.; Meisel, A.; Nitsch, R.; Meisel, C.; Brandt, C. Regulatory T cells accumulate and proliferate in the ischemic hemisphere for up to 30 days after MCAO. *J. Cereb. Blood Flow Metab.,* **2013**, *33*(1), 37-47. http://dx.doi.org/10.1038/jcbfm.2012.128 PMID: 22968321

[260] Liesz, A.; Kleinschnitz, C. Regulatory T Cells in Post-stroke Immune Homeostasis. *Transl. Stroke Res.,* **2016**, *7*(4), 313-321. http://dx.doi.org/10.1007/s12975-016-0465-7 PMID: 27030356

[261] Li, P.; Wang, L.; Zhou, Y.; Gan, Y.; Zhu, W.; Xia, Y.; Jiang, X.; Watkins, S.; Vazquez, A.; Thomson, A.W.; Chen, J.; Yu, W.; Hu, X. C C chemokine receptor type 5 (CCR5) mediated docking of transferred tregs protects against early blood brain barrier disruption after stroke. *J. Am. Heart Assoc.,* **2017**, *6*(8), e006387. http://dx.doi.org/10.1161/JAHA.117.006387 PMID: 28768648

[262] Chen, C.; Ai, Q.D.; Chu, S.F.; Zhang, Z.; Chen, N.H. NK cells in cerebral ischemia. *Biomed. Pharmacother.,* **2019**, *109*, 547-554. http://dx.doi.org/10.1016/j.biopha.2018.10.103 PMID: 30399590

[263] Mócsai, A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J. Exp. Med.,* **2013**, *210*(7), 1283-1299. http://dx.doi.org/10.1084/jem.20122220 PMID: 23825232

[264] Jensen, K.N.; Omarsdottir, S.Y.; Reinhardsdottir, M.S.; Hardardottir, I.; Freysdottir, J. Docosahexaenoic acid modulates nk cell effects on neutrophils and their crosstalk. *Front. Immunol.,* **2020**, *11*, 570380. http://dx.doi.org/10.3389/fimmu.2020.570380 PMID: 33123143

[265] Costantini, C.; Cassatella, M.A. The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity. *J. Leukoc. Biol.,* **2011**, *89*(2), 221-233. http://dx.doi.org/10.1189/jlb.0510250 PMID: 20682626

[266] Bodhankar, S.; Chen, Y.; Vandenbark, A.A.; Murphy, S.J.; Offner, H. IL-10-producing B-cells limit CNS inflammation and infarct volume in experimental stroke. *Metab. Brain Dis.,* **2013**, *28*(3), 375-386. http://dx.doi.org/10.1007/s11011-013-9413-3 PMID: 23640015

[267] Puga, I.; Cols, M.; Barra, C.M.; He, B.; Cassis, L.; Gentile, M.; Comerma, L.; Chorny, A.; Shan, M.; Xu, W.; Magri, G.; Knowles, D.M.; Tam, W.; Chiu, A.; Bussel, J.B.; Serrano, S.; Lorente, J.A.; Bellosillo, B.; Lloreta, J.; Juanpere, N.; Alameda, F.; Baró, T.; de Heredia, C.D.; Torán, N.; Català, A.; Torrebadell, M.; Fortuny, C.; Cusí, V.; Carreras, C.; Diaz, G.A.; Blander, J.M.; Farber, C.M.; Silvestri, G.; Cunningham-Rundles, C.; Calvillo, M.; Dufour, C.; Notarangelo, L.D.; Lougaris, V.; Plebani, A.; Casanova, J.L.; Ganal, S.C.; Diefenbach, A.; Aróstegui, J.I.; Juan, M.; Yagüe, J.; Mahlaoui, N.; Donadieu, J.; Chen, K.; Cerutti, A. B cell–helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat. Immunol.,* **2012**, *13*(2), 170-180. http://dx.doi.org/10.1038/ni.2194 PMID: 22197976

[268] Costa, S.; Bevilacqua, D.; Cassatella, M.A.; Scapini, P. Recent advances on the crosstalk between neutrophils and B or T lymphocytes. *Immunology,* **2019**, *156*(1), 23-32. http://dx.doi.org/10.1111/imm.13005 PMID: 30259972

[269] Scapini, P.; Bazzoni, F.; Cassatella, M.A. Regulation of B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS) expression in human neutrophils. *Immunol. Lett.,* **2008**, *116*(1), 1-6. http://dx.doi.org/10.1016/j.imlet.2007.11.009 PMID: 18155301

[270] Felger, J.C.; Abe, T.; Kaunzner, U.W.; Gottfried-Blackmore, A.; Gal-Toth, J.; McEwen, B.S.; Iadecola, C.; Bulloch, K. Brain dendritic cells in ischemic stroke: Time course, activation state, and origin. *Brain Behav. Immun.,* **2010**, *24*(5), 724-737. http://dx.doi.org/10.1016/j.bbi.2009.11.002 PMID: 19914372

[271] Charmoy, M.; Brunner-Agten, S.; Aebischer, D.; Auderset, F.; Launois, P.; Milon, G.; Proudfoot, A.E.I.; Tacchini-Cottier, F. Neutrophil-derived CCL3 is essential for the rapid recruitment of dendritic cells to the site of Leishmania major inoculation in resistant mice. *PLoS Pathog.,* **2010**, *6*(2), e1000755. http://dx.doi.org/10.1371/journal.ppat.1000755 PMID: 20140197

[272] Bennouna, S.; Denkers, E.Y. Microbial antigen triggers rapid mobilization of TNF-alpha to the surface of mouse neutrophils transforming them into inducers of high-level dendritic cell TNF-alpha production. *J. Immunol.,* **2005**, *174*(8), 4845-4851. http://dx.doi.org/10.4049/jimmunol.174.8.4845 PMID: 15814711

[273] Odobasic, D.; Kitching, A.R.; Yang, Y.; O'Sullivan, K.M.; Muljadi, R.C.M.; Edgtton, K.L.; Tan, D.S.Y.; Summers, S.A.; Morand, E.F.; Holdsworth, S.R. Neutrophil myeloperoxidase regulates T-cell−driven tissue inflammation in mice by inhibiting dendritic cell function. *Blood,* **2013**, *121*(20), 4195-4204. http://dx.doi.org/10.1182/blood-2012-09-456483 PMID: 23509155

[274] Breedveld, A.; Groot Kormelink, T.; van Egmond, M.; de Jong, E.C. Granulocytes as modulators of dendritic cell function. *J. Leukoc. Biol.,* **2017**, *102*(4), 1003-1016. http://dx.doi.org/10.1189/jlb.4MR0217-048RR PMID: 28642280

[275] Parackova, Z.; Zentsova, I.; Vrabcova, P.; Klocperk, A.; Sumnik, Z.; Pruhova, S.; Petruzelkova, L.; Hasler, R.; Sediva, A. Neutrophil extracellular trap induced dendritic cell activation leads to Th1 polarization in type 1 diabetes. *Front. Immunol.,* **2020**, *11*, 661. http://dx.doi.org/10.3389/fimmu.2020.00661 PMID: 32346380

[276] Nicholls, A.J.; Wen, S.W.; Hall, P.; Hickey, M.J.; Wong, C.H.Y. Activation of the sympathetic nervous system modulates neutrophil function. *J. Leukoc. Biol.,* **2018**, *103*(2), 295-309. http://dx.doi.org/10.1002/JLB.3MA0517-194RR PMID: 29345350

[277] Tajalli-Nezhad, S.; Karimian, M.; Beyer, C.; Atlasi, M.A.; Azami Tameh, A. The regulatory role of Toll-like receptors after ischemic stroke: neurosteroids as TLR modulators with the focus on TLR2/4. *Cell. Mol. Life Sci.,* **2019**, *76*(3), 523-537. http://dx.doi.org/10.1007/s00018-018-2953-2 PMID: 30377701

[278] Fadakar, K.; Dadkhahfar, S.; Esmaeili, A.; Rezaei, N. The role of Toll-like receptors (TLRs) in stroke. *Rev. Neurosci.,* **2014**, *25*(5), 699-712. http://dx.doi.org/10.1515/revneuro-2013-0069 PMID: 24807166

[279] Wang, G.; Joel, M.D.M.; Yuan, J.; Wang, J.; Cai, X.; Ocansey, D.K.W.; Yan, Y.; Qian, H.; Zhang, X.; Xu, W.; Mao, F. Human umbilical cord mesenchymal stem cells alleviate inflammatory bowel disease by inhibiting ERK phosphorylation in neutrophils. *Inflammopharmacology,* **2020**, *28*(2), 603-616. http://dx.doi.org/10.1007/s10787-019-00683-5 PMID: 31938969

[280] Martinelli, S.; Urosevic, M.; Daryadel, A.; Oberholzer, P.A.; Baumann, C.; Fey, M.F.; Dummer, R.; Simon, H.U.; Yousefi, S. Induction of genes mediating interferon-dependent extracellular trap formation during neutrophil differentiation. *J. Biol. Chem.,* **2004**, *279*(42), 44123-44132. http://dx.doi.org/10.1074/jbc.M405883200 PMID: 15302890

[281] Liang, Y.B.; Tang, H.; Chen, Z.B.; Zeng, L.J.; Wu, J.G.; Yang, W.; Li, Z.Y.; Ma, Z.F. Downregulated SOCS1 expression activates the

JAK1/STAT1 pathway and promotes polarization of macrophages into M1 type. *Mol. Med. Rep.,* **2017**, *16*(5), 6405-6411. http://dx.doi.org/10.3892/mmr.2017.7384 PMID: 28901399

[282] Bouhlel, M.A.; Derudas, B.; Rigamonti, E.; Dièvart, R.; Brozek, J.; Haulon, S.; Zawadzki, C.; Jude, B.; Torpier, G.; Marx, N.; Staels, B.; Chinetti-Gbaguidi, G. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab.,* **2007**, *6*(2), 137-143. http://dx.doi.org/10.1016/j.cmet.2007.06.010 PMID: 17681149

[283] Na, S.Y.; Kang, B.Y.; Chung, S.W.; Han, S.J.; Ma, X.; Trinchieri, G.; Im, S.Y.; Lee, J.W.; Kim, T.S. Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFkappaB. *J. Biol. Chem.,* **1999**, *274*(12), 7674-7680. http://dx.doi.org/10.1074/jbc.274.12.7674 PMID: 10075655

[284] Certo, M.; Endo, Y.; Ohta, K.; Sakurada, S.; Bagetta, G.; Amantea, D. Activation of RXR/PPARγ underlies neuroprotection by bexarotene in ischemic stroke. *Pharmacol. Res.,* **2015**, *102*, 298-307. http://dx.doi.org/10.1016/j.phrs.2015.10.009 PMID: 26546745

[285] Zhao, H.; Li, G.; Wang, R.; Tao, Z.; Ma, Q.; Zhang, S.; Han, Z.; Yan, F.; Li, F.; Liu, P.; Ma, S.; Ji, X.; Luo, Y. Silencing of microRNA 494 inhibits the neurotoxic Th1 shift *via* regulating HDAC2 STAT4 cascade in ischaemic stroke. *Br. J. Pharmacol.,* **2020**, *177*(1), 128-144. http://dx.doi.org/10.1111/bph.14852 PMID: 31465536

[286] Li, F.; Zhao, H.; Li, G.; Zhang, S.; Wang, R.; Tao, Z.; Zheng, Y.; Han, Z.; Liu, P.; Ma, Q.; Luo, Y. Intravenous antagomiR 494 lessens brain infiltrating neutrophils by increasing HDAC2 mediated repression of multiple MMPs in experimental stroke. *FASEB J.,* **2020**, *34*(5), 6934-6949. http://dx.doi.org/10.1096/fj.201903127R PMID: 32239566

[287] Uhl, B.; Vadlau, Y.; Zuchtriegel, G.; Nekolla, K.; Sharaf, K.; Gaertner, F.; Massberg, S.; Krombach, F.; Reichel, C.A. Aged neutrophils contribute to the first line of defense in the acute inflammatory response. *Blood,* **2016**, *128*(19), 2327-2337. http://dx.doi.org/10.1182/blood-2016-05-718999 PMID: 27609642

[288] Moraga, A.; Pradillo, J.M.; García-Culebras, A.; Palma-Tortosa, S.; Ballesteros, I.; Hernández-Jiménez, M.; Moro, M.A.; Lizasoain, I. Aging increases microglial proliferation, delays cell migration, and decreases cortical neurogenesis after focal cerebral ischemia. *J. Neuroinflammation,* **2015**, *12*(1), 87. http://dx.doi.org/10.1186/s12974-015-0314-8 PMID: 25958332

[289] Zhang, D.; Chen, G.; Manwani, D.; Mortha, A.; Xu, C.; Faith, J.J.; Burk, R.D.; Kunisaki, Y.; Jang, J.E.; Scheiermann, C.; Merad, M.; Frenette, P.S. Neutrophil ageing is regulated by the microbiome. *Nature,* **2015**, *525*(7570), 528-532. http://dx.doi.org/10.1038/nature15367 PMID: 26374999

[290] Jayaraj, R.L.; Azimullah, S.; Beiram, R.; Jalal, F.Y.; Rosenberg, G.A. Neuroinflammation: friend and foe for ischemic stroke. *J. Neuroinflammation,* **2019**, *16*(1), 142. http://dx.doi.org/10.1186/s12974-019-1516-2 PMID: 31291966

[291] Easton, A.S. Neutrophils and stroke – Can neutrophils mitigate disease in the central nervous system? *Int. Immunopharmacol.,* **2013**, *17*(4), 1218-1225. http://dx.doi.org/10.1016/j.intimp.2013.06.015 PMID: 23827753