

REVIEW

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Neutrophils in traumatic brain injury (TBI): friend or foe?

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Abstract

Our knowledge of the pathophysiology about traumatic brain injury (TBI) is still limited. Neutrophils, as the most abundant leukocytes in circulation and the first-line transmigrated immune cells at the sites of injury, are highly involved in the initiation, development, and recovery of TBI. Nonetheless, our understanding about neutrophils in TBI is obsolete, and mounting evidences from recent studies have challenged the conventional views. This review summarizes what is known about the relationships between neutrophils and pathophysiology of TBI. In addition, discussions are made on the complex roles as well as the controversial views of neutrophils in TBI.

Keywords: Neutrophils, Polymorphonuclear cell, Traumatic brain injury, Edema, Neuroinflammation, Blood-brain barrier, Neurodegeneration, Nerve recovery

Background

Traumatic brain injury (TBI) is a complex injury that causes the marked brain pathology, disruption of the normal function of the brain, and death. It is an important public health issue considering not only its high mortality rate but also the numerous complications in the patients who survive the injury [1, 2], such as hospital-acquired infection, injury-related dementia, hemiplegia, and depression. These complications lead to the economic burden of society and impact the live quality of the individuals and their families [3]. Several risk factors are associated with the development and recovery of TBI, including the age, race, and gender, most of them are unchangeable factors [2]. Even though the risk factors and primary injury cannot be controlled, secondary brain damage can be reduced. Secondary injury after primary insult is an important therapeutic window that determines the development and recovery of TBI. The alteration of immune system post-TBI has been shown to play important roles in the initiation and development of secondary injury after TBI [1, 4]. Neutrophils, as the major component of the innate immune system, are

regarded as the short-lived players in acute inflammation that fight against the pathogens and cause indiscriminate damage to the tissue. However, mounting evidence indicates that neutrophils are non-negligible cells that connect the innate and adaptive immune systems, promote tissue recovery, and play critical roles in anti-inflammation and chronic inflammation responses [5, 6]. In addition, neutrophils can either contribute to repair mechanisms or exacerbate the pathophysiology of trauma dependent on the stage of injury [7]. In this review, we will summarize what is known about the function of neutrophils in CNS with a focus on the discussion of their pivotal roles in the pathogenesis of TBI.

Neutrophils: origin and physiological functions in the healthy brain

Neutrophils are the most abundant circulating granulocytes in mammals. Neutrophils, basophils, and eosinophils are also called polymorphonuclear cells (PMNs) [8]. The PMNs are generated and matured in the bone marrow, involving multiple stages including myeloid precursors, promyelocyte, myelocyte, metamyelocyte, band cell, and finally, polymorphonuclear cells [9]. Physiologically, mature neutrophils are located in the bone marrow, spleen, liver, and lung, where they are also cleared up from the circulation. In the central nervous system (CNS), neutrophils are rarely found in the brain parenchyma due to the existence of blood-brain barrier (BBB) [6]. In some specific

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compartments like cerebrospinal fluid (CSF), meninges and pia membrane, there are a small number of neutrophils and other immune cells that provide the immune surveillance. However, under pathological conditions like infections, trauma, ischemia, and hemorrhage, increased numbers of neutrophils enter into brain tissue [10].

The differentiation and maturation of neutrophils are tightly connected with neutrophils' functions. They can be modulated by various endogenous factors, such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF) [11], or external stimuli including stress, trauma, drugs and radiation [12, 13]. With the differentiation and maturation, there are changes in the expression levels of various membrane proteins, which are important for neutrophils to sense the danger or infection signals, and transmigrate to the targeted tissues, as well as phagocytose tissue debris. For instance, in matured and activated neutrophils, integrin $\beta 1$, as well as C-X-C chemokine receptor 4 (CXCR4), is downregulated, while CXCR2, Toll-like receptor 4, *N*-formyl-leucyl-phenylalanine receptor, CD11b, and CD35 are upregulated [14, 15]. Besides the membrane receptors, various granule proteins undergo significant changes in their expression levels, including matrix metalloproteinases (MMPs), myeloperoxidase, neutrophils elastase (NE), neutrophils gelatinase-associated lipocalin, and SGP28. These are important degranulated molecules for neutrophils to fight against pathogens [14, 16, 17]. In addition to the degranulation and phagocytosis, neutrophils extracellular trap (NET) is another defensive mechanism for neutrophils to eliminate pathogens, which is composed of the histones, enzymes, and granules. The accumulated neutrophils eliminate pathogens through phagocytosis, degranulation, and NETs at target tissues, forming the first line defender of the innate immune system. Actually, neutrophils themselves could amplify their activation in an autocrine dependent manner (platelet-activating factor, leukotriene B4 and IL-18), rendering it difficult to stop immediately after the danger signal has already disappeared [18–20]. This process would lead to tissue damage indiscriminately by neutrophils. So the neutrophils are often phagocytosed or inhibited by macrophages or lymphocytes after digestion of pathogens to minimize the tissue damage.

The neutrophil-derived cytokines are quite complicated and vary depending on the underlying stimuli and tissues involved [6]. For example, brain trauma is associated with a specific alteration of surface phenotype, chemotaxis, and phagocytosis, which is quite different with that of a typical inflammation [4, 21, 22]. In addition to the common inflammation-related cytokines (Tumor necrosis factor (TNF) family; pro-inflammatory cytokines; CXC- chemokines; CC- chemokines), other anti-inflammatory cytokines, immunoregulatory cytokines, angiogenic, and neurotrophic

factors are detected in neutrophils as well [23–29]. Table 1 summarizes the neutrophil-derived chemokines and molecules and their functions in brain injury. Recently, neutrophils are also regarded as an important player in cancer, autoimmune diseases, and chronic inflammation, challenging our traditional views about neutrophils [6, 30]. Thus, neutrophils need to be re-evaluated for their roles in several diseases, including TBI.

Traumatic brain injury (TBI)

TBI is an uncontrollable event that alters the brain structure and functions dramatically. It is caused by external forces like mechanical forces and acceleration or deceleration forces [31]. The brain is an immune-privileged organ under normal condition due to following three reasons: the existence of BBB, the lack of obvious lymphatic vessels, and limited numbers of antigen-presenting cells [10]. However, trauma could cause a direct damage to BBB, resulting in infusion of a large number of peripheral antigen-presenting cells, as well as instant activation of microglia in situ [2, 32]. Recent research shows the existence of lymphatic vasculature in meninges in the central nervous system, suggesting another new route for peripheral immune cells to enter and exit brain [33]. All of these disrupt the well-balanced immune-privileged environment of CNS, forcing the communication between CNS and peripheral immune system under TBI [1].

TBI is also a complicated pathological process that is caused by primary and secondary brain damage focally or diffusely [2, 31, 32]. The primary damage of TBI is the result of the kinetic forces on the brain tissue, leading to the deformations of axons, vessels, and brain cells. The interrupted axons would trigger swelling by accumulating transported materials at the moment of injury [34]. The destroyed blood vessels pour out the blood content indiscriminately, impairing the blood supply and integrity of BBB. The damaged neuronal and glial cells could release various inflammatory factors and neurotransmitters to induce a cascade inflammatory response. All the processes in primary damage are tightly associated with the secondary damage, including alteration in blood flow (hemorrhage, ischemia), continuous breakdown of BBB, dysregulation of CSF, dysfunction of brain tissue metabolism (hypoxia, edema), neuroinflammation, and cell damage (excitotoxicity, oxidative stress, free radical production, neuronal apoptosis/necrosis) (Table 2).

Roles of neutrophils in TBI

Neutrophils and blood flow

Previous studies in animals and humans indicated that focal or global cerebral hypoperfusion frequently occurred at the early stage of TBI [35, 36]. Cerebral hypoperfusion is also associated with poor neurological outcome after severe TBI [37, 38]. The hypoperfusion of blood flow

Table 1 Summary of the neutrophil-derived mediators and their functions in brain injury

Name	Effects	References
IL-1 α	#IL-1 α expression is closely associated with areas of BBB breakdown and neuronal death at stage of injury &Induces angiogenesis in brain endothelial cells	[182, 212]
IL-1 β	#Localizes with the area of neurons loss and might induce neurons death directly <Induces astrocytes to secrete hemolymphopoietic cytokines (IL-6, IL-8)	[23, 77, 212]
IL-3	&Suppresses secondary degeneration caused by TBI	[213]
IL-4	&Induces M2-polarization of microglia or macrophages &Promotes white matter integrity and long-term neurological recovery	[26, 214]
IL-6	&Triggers nerve growth factor production in astrocytes <Might be a prognosis marker of TBI patient	[66, 215]
IL-7	<Contributes to injury-induced reactive gliosis	[216]
IL-9	#Exacerbates excitotoxic brain damage	[217]
IL-10	&IL-10 in traumatic brain and CSF both increases significantly during the first days and may downregulate pro-inflammatory cytokines following traumatic brain damage.	[25]
IL-12	#Highly increased in nonsurvival TBI patients	[215]
IL-16	<Activates microglia and lymphocytes <Promotes activated microglia and lymphocytes accumulated in microvessels	[218]
IL-17	<Promotes neutrophils invasion	[219]
IL-18	<Peaks at 7 to 14 days after injury and might participate in delayed neuroinflammation #Induces brain injury in caspase-1-dependent manner <Induces neutrophils themselves to secrete inflammatory related cytokines	[18, 220, 221]
IL-23	#Leads to brain damage and neurological deficits	[222]
CXCL1	<Recruits circulating-neutrophils into injured brain	[24, 61, 223]
CXCL2	<Peaks at 4 h after TBI, chemotactic for polymorph nuclear leukocytes	[61, 224]
CXCL3	<Promotes neutrophils to migrate across epithelial barriers	[61]
CXCL4	<Induces macrophage to differentiate into a unique phenotype	[225, 226]
CXCL5	#Increases microglia activation as well as BBB damage #Jeopardizes myelination and promotes astrogliosis	[227]
CXCL8	<Promotes neutrophils to infiltrate into brain parenchyma	[228]
CXCL9	<Promotes lymphocytes to collaborate with mesenchymal stem cells to inhibit T cells' functions	[229]
CXCL10	<Promotes blood-derived monocytes to accumulate around perivascular vessels	[230, 231]
CXCL11	&Promotes regenerative processes	[230]
CCL2	<Promotes macrophage to infiltrate into parenchyma <Peaks at 8–12 h after TBI <Induces transmigration of monocytes and macrophages across BBB <Activates and induces chemotaxis of T cells and monocytes	[224, 228, 230, 232]
CCL3	<Peaks at 4 h after injury <Activates and induces chemotaxis of T cells and monocytes <Recruits CCR2-positive leukocytes to injured brain	[232, 233]
CCL4	<Activates and induces chemotaxis of T cells and monocytes	[232]
CCL17	<Participates in leukocytes recruitment	[234]
CCL22	<Participates in leukocytes recruitment	[234]
G-CSF	&Anti-inflammatory <Promotes myeloid differentiation and M-CSF secretion <Reduces T cells' infiltration &Prolongs neuronal survival	[175, 235, 236]
M-CSF	#Promotes microglia activation	[236, 237]
GM-CSF	&Suppresses secondary degeneration caused by TBI	[213]
HGF	&Promotes survival reconstruction of specific neurons in response to cerebral injury	[176]
TGF- α	&Induces proliferation, migration, and differentiation of neural stem cells after neurons damage	[238]

Table 1 Summary of the neutrophil-derived mediators and their functions in brain injury (Continued)

Name	Effects	References
TGF- β	^{&} Down-modulates cellular activation ^{&} Blocks inflammatory responses ^{&} Plays a role in nerve regeneration by stimulating nerve growth factor production as well as controlling the astrocytosis and scar formation after injury	[25, 173, 186]
VEGF	^{&} Promotes angiogenesis as well as brain plasticity ^{&} Increases the expression of BDNF in brain endothelial cells [#] Enhance the leakage of BBB	[27, 180, 185]
Prokineticin 2	^{&} Participates in constitutive and injury-induced neurogenesis [#] Might promote over-inflammation	[239, 240]
TNF- α	[#] Induces astrocytes to secrete hemolymphopoietic cytokines (IL-6, IL-8) [#] Mediates PMN-driven neurotoxicity directly ^{<} Early pro-apoptotic effect in neutrophils	[23, 241, 242]
Arginase	^{&} Anti-inflammation ^{&} Augments neurite growth	[26]
BDNF	^{&} Increases cell proliferation ^{&} Upregulates expression of growth factors and induces neurogenesis	[27, 243, 244]
Midkine	^{&} Inhibits apoptosis of neurons ^{&} Promotes neurite extension	[179, 245]
Oncostatin M	[#] Induces the expression of IL-6 and MMPs ^{&} Protects neurons from excitotoxic injury	[246, 247]
NGF	^{&} Supports neurons survival and nerve growth	[25, 177]
NT4	^{&} Prevents neuronal cell death after TBI	[28, 178]
ROS	[#] Enhances BBB dysfunction [#] Participates in brain energy perturbation (glucose, lactate glycerol) [#] Leads to neurons cell death [#] Induces microglia activation	[97, 119, 131, 241, 248]
iNOS	^{<} Leads to vasodilatation of blood vessel and improve microcirculation after TBI ^{&} Works as an endogenous antioxidant ^{<} Contributes to protein nitrosylation and nitration	[249, 250]
MMP9	[#] Breaks down the integrity of BBB ^{<} Promotes infiltration of neutrophils ^{&} Participates in vessel remodeling and angiogenesis [#] Mediates PMN-driven neurotoxicity directly	[57, 165, 184, 241, 251]
MPO	^{<} Reflects the infiltration of neutrophils in brain tissue	[189]
Cathepsins	[#] Contributes to TBI-induced cell death through the programmed cell necrosis and mitochondria-mediated apoptotic pathways	[252]
Defensins	[#] Penetrates a considerable distance to disrupt the BBB sites	[120]
Cathelicidin	^{<} Attracts peripheral blood neutrophils, monocytes, and T cells ^{<} Promotes IL-1 β processing and release	[121, 253]
NE	[#] Causes cellular stress (astrocytes and microglia) in the injured brain [#] Induces acute neurons death	[111, 166, 254]

[#]Detrimental to the brain tissue and recovery

[&]Beneficial to the brain tissue and recovery

[<]Neutral or hard to judge

enhances the interactions of neutrophils with blood vessels [39] and promotes neutrophils to tumble and adhere by inducing the expression of L-selectin and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells [40, 41]. Neutrophils influence microcirculation rheology at sites where vessel diameters are around 4–100 μm . To some extent, the quiescent and activated neutrophils are important to sustain pressure of microvessels [42]. This may partially explain why the neutropenic rats exhibit reduced blood flow pressure in injured

hemisphere compared with normal rats within 24 h after TBI [43]. Furthermore, the activated neutrophils could form pseudopods and bind to endothelium and platelets, hindering blood flow through the microvasculature [44]. This will lead to a vicious cycle of hypoperfusion and neutrophils adherence, promoting the development of ischemia or early coagulopathy [45, 46]. Palmer et al. pointed out that neutrophils contribute to vascular dysfunction either during the insult or early hours (<4–8 h) instead of 24 h in the hypoxia-ischemia model [47]. The exact time frame during

Table 2 Therapeutic approaches of neutrophil-dependent processes in brain injury

Neutrophil's processes	Approaches	Protective	References
Maturation differentiation and elimination	Ly6G/Gr-1 antibody	Not clear	[113, 255]
	G-CSF	Not clear	[193, 256]
	Progesteron	Yes	[257]
Activation	TLR antagonist	Yes	[196, 258]
	P-selectin blockage	Yes	[82]
	VLA-4 blockage	Yes	[259–261]
	IL-1 receptor inhibitor	Not clear	[262–264]
	IL-8 receptor inhibitor	Yes	[265]
	TNF inhibitor	Yes	[266, 267]
	IL-10	Yes	[268]
Migration	C5a or C3 antagonist	Yes	[269, 270]
	CXCR2 or CXCR4 inhibitor	Not clear	[85, 86, 271]
	TGF- β 1	Yes	[272]
	Mac-1 antibody	Yes	[81, 273]
	ICAM-1 antibody	Yes	[79, 80]
Cell killing	MMP inhibitor	Yes	[93, 94]
	ROS inhibitor	Yes	[98]
	NO inhibitor	Not clear	[99, 249, 250, 274]
	NE inhibitor	Yes	[111, 166, 254]

which neutrophils participate in hypoperfusion needs to be further investigated.

Hyperemia/hyperperfusion (cerebral blood flow (CBF) > 55 ml/100 g/min) may occur immediately in some animal models of moderate TBI [2, 38, 48], which could convert to ischemia subsequently. While some study indicated that the mean CBF of patients could increase from 24 h to 6 days after TBI as well [38, 49]. It seems that phasic elevation in CBF after injury is a protective mechanism for achieving functional recovery [49], and elevated CBF does not cause an increase in intracranial pressure if the auto-regulatory mechanism in the brain is well retained [50], and elevated CBF does not cause an increase in intracranial pressure if the auto-regulatory mechanism in the brain is well retained [50]. The point is that hyperemia is tightly associated with the peak rate of neutrophils influx and the degree of hyper permeability [51, 52], which is undeniably involved in enhanced neutrophils infiltration, cerebral edema, or intracerebral hemorrhage in acute phase [42, 53]. Of note, reoccurrence of hemorrhage could localize at the initial injury lesion, expand or even develop new non-contiguous hemorrhagic lesions, which make it the most life-threatening complication in TBI patients [54]. Some studies showed that neutrophils are strongly associated with hemorrhagic areas with local high activities of MMP-9 as well as degradation of basal lamina collagen IV [55–57], which deteriorate the integrity of blood

vessels and extend the hemorrhagic lesions. Neutrophils could also express inducible nitric oxide synthases (iNOS) within 24 and 48 h, contributing to cerebrovasodilation and hemorrhage [58]. It seems that neutrophils act as an effector in the conversion between hyperperfusion and hypoperfusion during the early stage of TBI, although the underlying mechanism remains unclear.

Neutrophils and cerebrospinal fluid (CSF)

Elevated intracranial pressure after TBI injury is mainly attributed to fluid retention in the brain and CSF cavity. The circulation and communication between cerebral capillary and CSF cavity could be significantly distorted even though the physical site of injury is remote from the ventricular system [59]. The choroid plexus epitheliums, which are also called Kolmer cells, could sense the danger/pro-inflammatory signals like interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) released by injured brain tissue [60], and synchronize neutrophils' trafficking as well. Neutrophils could infiltrate the choroid plexus and circulate with CSF close to the sites of injury [60, 61], which means the breakdown of blood–CSF barrier is another mechanism for neutrophils to intrude brain parenchyma. Carlos et al. reported that neutrophils were predominantly migrated from blood vessels and located at ipsilateral leptomeninges and choroid plexus within 4 h after TBI. Neutrophils were then infiltrated into the injured parenchyma and peaked within 24 to 48 h mainly through the vessels of

leptomeninges and choroid plexus [62]. ICAM-1 is constitutively expressed in peripheral vessels, while platelet-endothelial adhesion molecule (PECAM-1) is exclusively expressed by choroid plexus [63], which indicates that neutrophils migrate choroid plexus endothelium with separate molecules involved. The following study also confirmed that blocking ICAM-1 or PECAM-1 could inhibit infiltration of neutrophils from peripheral vessels and choroid plexus respectively.

The components of CSF change significantly after TBI and can serve as biomarkers for the diagnosis and prognosis of TBI patients (reviewed in [64]). Several cytokines and amino acids increase dramatically in CSF, such as TNF- α , IL-6, IL-10, transforming growth factor β (TGF- β), C-X-C motif ligand chemokine 1-3 (CXCL1-3), and glutamate [25, 61, 65, 66]. Notably, these cytokines could modulate neutrophils' function significantly. For instance, TNF- α and IL-6 promote transmigration of neutrophils; CXCL1-3 enhances chemotaxis of neutrophils; glutamate induces neutrophils' migration by activating class I metabotropic glutamate receptors [67, 68]; TGF- β could convert neutrophils from pro-inflammatory type to anti-inflammatory type [69]. Using in vivo two-photon microscopy, Szmydynger-Chodobska et al. found that neutrophils could migrate into choroidal stroma paracellularly and reach to intercellular space between choroidal epithelial cells in rat TBI mode, jeopardizing the integrity of choroid plexus epithelium [61]. These neutrophils were accumulated at the velum interpositum within 24 h after injury and secreted vascular endothelial growth factor (VEGF) into extracellular matrix directly [70]. The secreted VEGF could circulate with CSF, exacerbating brain edema through enhancing angiogenesis and microvessel permeability. It is apparent that the CSF system could transport neutrophil-related molecules and educate neutrophils during TBI.

Neutrophils and blood-brain barrier (BBB)

The BBB is composed of three structures: specialized endothelial cells, underlying basal membrane, and astrocytic pseudopodia. Brain endothelial cells express both endothelial and brain markers (like acetylated low density lipoprotein and gamma glutamyl transpeptidase), which make it unique compared with peripheral endothelium [71]. Of note, the expression profile of adhesion molecules in brain venules is different from peripheral tissues like post capillary venules and pulmonary capillary. For post capillary venules and pulmonary capillary, P-selectins, ICAM-1, and ICAM-2 on endothelium could be upregulated instantly at the sites of infection or injury. While in brain venules, it seems that longer time is needed for upregulation of these molecules, and this upregulation is not restricted to the damaged vessels [72]. This might determine the unique rolling, adhering and diapedesis of neutrophils in CNS during TBI compared with peripheral

inflammation [71, 73]. Furthermore, the tight junction in basal membrane and pericytes around the endothelial cells also prevent specific factors and molecules from entering into parenchyma [74]. Leukocytes are usually excluded from brain by the BBB, but under pathological conditions, leukocytes can adhere to activated post-capillary venules and infiltrate the brain parenchyma across the BBB. There are two main routes for leukocytes to extricate through BBB: paracellular and transcellular. For neutrophils, transcellular pathway is the primary mechanism to migrate into brain tissue [75]. Further study indicate that neutrophils are observed only in regions exhibiting BBB damage and are initially found in injured cortex, and areas with damaged BBB within 12 h post-trauma, lining the vasculature and filling subarachnoid/subdural spaces [76]. Neutrophils then migrate from the damaged vasculature into traumatized cortical and hippocampal parenchyma by 24 h. During this process, neutrophils' transmigration is tightly connected with activated endothelial cells, the interaction between activated neutrophils and endothelium plays an important role in secondary injury after TBI [2]. Neutrophils and endothelium could be activated by several cytokines like TNF- α , IL-1 β CXCL1, CXCL2, and CXCL5 released by injured parenchyma [23, 24, 77]. Activation of endothelial cells is a decisive step in this process and can occur in vascular endothelium within minutes [78]. However, there are some differences between brain endothelium and peripheral vascular endothelium. Brain endothelium will take longer time to upregulate expression of adhesion-related molecules (ICAM-1 and VCAM-1) [72]. In addition, endothelial cell leukocytes adhesion molecule-1 (ECAM-1) is highly induced by TNF- α only in peripheral vascular endothelium but not brain endothelium [71]. The delayed activation of brain endothelium might lead to delayed neutrophils infiltration. For neutrophils migrating across the BBB from blood vessels, there are four steps involved in this process between endothelium and neutrophils: rolling, adhesion, crawling, and transmigration. In endothelium, P-selectin and E-selectin mediate the tethering and rolling of neutrophils; ICAM-1, ICAM-2, and PECAM1 participate in the adhesion and transmigration of neutrophils [62]. Inhibition of this process with ICAM1 antibody can provide benefits after injury [79, 80]. In peripheral tissues, expression of P-selectin, glycoprotein ligand 1 and CD11a/CD18 on neutrophils are vital for rolling [81] while CXCR1 and CXCR2 are indispensable for the binding to the endothelium [30]. Expression of CXCR2 ligands, such as CXCL1, CXCL2, and CXCL5, can force invasion of neutrophils into the CNS parenchyma. There are some benefits for TBI with P-selectin blocking [82-84] as well as CXCR2 and CXCR4 inhibitors [85, 86]. Other than transmigration, neutrophils also damage the tight junction and permeability of BBB simultaneously. For example, leukocytes recruitment leads to degradation and redistribution of occludin, zonula

occludens-1, β -catenin, and vinculin, which are vital for the integrity of BBB. Neutrophil-released NE significantly disrupts the cadherin–cadherin binding, causing hyperpermeability of BBB [87, 88]. Neutrophil-derived MMPs also play vital roles in dysregulation of blood–brain barrier after TBI, including MMP2, MMP3, and MMP9 [89, 90]. These MMPs can be significantly induced by IL-1 β within 24 h and degrade structures of the neurovascular unit after injury. Blocking the activity of MMP9 by related drugs [91–94], or knocking down expression of the MMP9, shows some protections for BBB in acute phase [95]. Activated neutrophils are also the pivotal source of free radicals like reactive oxygen species (ROS), nitrous oxide (NOS), and NADPH oxidase. These free radicals induce direct oxidative damage, rearrangement of claudin-5 and occludin in endothelium via PI3K-AKT pathways, contributing to the break-up of the integrity of BBB [96, 97]. ROS or NO inhibitor could improve the integrity of BBB after injury [98, 99]. In addition to the effect from the above neutrophil-derived cytokines, the formation of NETs could directly lead to epithelial and endothelial cell death [100]. Obviously, breakdown of BBB by the neutrophils shall help to recruit more immune cells to fight against pathogens; this also leads to indiscriminate tissue damage at the same time. Strategies to inhibit activated neutrophils or improve the stability of BBB shall prove to be beneficial for the inflammatory resolution and the following recovery.

Neutrophils and edema

Cerebral edema is the result of excess accumulated fluid in the intracellular or extracellular spaces of the brain, leading to expansion of brain tissue in limited skull cavity. It is a vital pathogenic impact since it increases intracranial pressure, impairs cerebral perfusion and oxygenation, and contributes to additional ischemic injuries after TBI [101]. There are three major types of edema after TBI [102]: vasogenic edema, cytotoxic edema, and osmotic edema. Vasogenic edema is due to a breakdown of BBB, which allows intravascular proteins and fluid to penetrate into the parenchyma extracellular space. Cytotoxic edema is the result of abnormal water collection in the cells. Osmotic edema is caused by osmotic imbalances between blood and tissue. Neutrophils and edema are tightly connected after trauma and several studies show that depletion of neutrophils significantly decreases tissue edema [43, 82, 92, 103, 104]. Since the vasogenic edema is tightly connected with vascular integrity of BBB, the interaction between neutrophils and BBB could influence vasogenic edema as well. As discussed above (see the “Neutrophils and blood–brain barrier (BBB)” section), neutrophils are involved in the breakdown of BBB through the abnormal interactions between endothelium and neutrophils. Despite the chemokines, proteases, and free

radicals released by injured tissue or neutrophils, various mediators could enhance vasogenic and cytotoxic edema, such as glutamate, lactate, H⁺, K⁺, Ca²⁺, histamine, and kinins, and could also promote extensive neutrophils infiltration and neuronal–astroglial loss as well [105].

Cytotoxic edema is usually accompanied by the accumulation of Na⁺ and other cations intracellularly. Extracellular Na⁺, Cl⁻ and water enter into cells and create a new gradient for these molecules to move across the capillary of BBB [106]. Na⁺/H⁺ exchanger is an important channel that modulates this process [107]. Over engulfment of Na⁺ by the cells leads to the H⁺ accumulation extracellularly, facilitating the activation of neutrophils in a leukotriene B₄ dependent manner [108]. Suzuki et al. showed that inhibition of Na⁺/H⁺ exchanger with SM-2022 could significantly attenuate cerebral infarct volume, water content, and the neutrophils accumulation in ischemia model [107]. Thus, apart from traditional cytokines, both the acidity and cations in the injured brain tissue could modulate activation of neutrophils and cytotoxic edema. It is noteworthy that BBB breakdown and cell swelling are not the only reason for brain edema after injury. The osmotic imbalance between blood and tissue is another mechanism for brain edema. For instance, hyponatremia, which is a common feature of clinical TBI, could cause osmotic edema. Hyperperfusion or hypoperfusion is also crucial for the osmotic edema as well, whose relationships with neutrophils have been discussed in the “Neutrophils and blood flow” section. The permeability of blood vessel could also modulate osmotic edema. The neutrophil-released granules or particle-like elastase, azurocidin, and lipoxin are all tightly connected with the vascular permeability in the peripheral tissues [109–111]. The connection between neutrophil infiltration and brain edema seems to be a time-dependent issue. Depletion of neutrophils decreases the tissue edema from injury after 24–48 h or longer time [47, 112, 113]. On the other hand, depletion of neutrophils within 4 h after TBI showed no impact on edema [104]. Some people also claimed that there is no direct relationship between brain edema and leukocytes accumulation [82]. More work needs to be done to better understand when and how neutrophils influence edema after TBI.

Neutrophils and hypoxia

The imbalance between cerebral oxygen delivery and consumption is another characteristic of TBI [2, 32]. The changes in the volume and flow rate of blood and arterial oxygen content after injury could instantly compromise the oxygen delivery, leaving the injured area under a hypoxic condition for both brain resident cells as well as the infiltrated neutrophils. The duration and extent of hypoxia are correlated with the outcomes of TBI patients [114]. The deprivation of oxygen after TBI

may also occur even though the patients have the normal cerebral perfusion pressure or blood oxygen saturation [115]. A previous study showed that low levels of oxygen and glucose induce the expression of hypoxia-inducible factor -1α and NF κ B, leading to prolongation of neutrophils' survival time and their activation simultaneously [116]. Activated neutrophils could consume more oxygen to generate and release superoxide hydrogen peroxide to maintain its phagocytic functions [117, 118], accompanied with overt release of NADPH oxygenase-related molecules (ROS) [97, 119] and antibacterial proteins (cathelicidin and defensins) [120, 121]. Meanwhile, neutrophils could induce over-production of ROS themselves in an IL-17 autocrine manner, aggravating ROS related injury [122]. It is possible that neutrophils compete with brain cells to consume more oxygen to maintain their activated status and lead to uncontrolled damage. Several experiments reported that hypoxia could induce microglia to release nitric oxide (NO) and a series of cytokines to maintain the activation of neutrophils, leading to persistent hypoxia and neuronal death [123, 124]. In the clinic, patients with head trauma are frequently associated with respiratory problems, like lung injury, pneumonia, or respiratory distress syndrome, resulting in secondary hypoxic insult as well as overwhelming activation of neutrophils [125]. Hypoxia is an important factor that promotes and maintains the over production of free radicals from neutrophils after TBI, blockade of degranulation, and oxidative burst of neutrophils has shown benefits for brain injury [98].

Neutrophils and neuroinflammation

Neuroinflammation is the inflammatory responses in CNS, where brain resident cells and peripheral immune cells participate together. Although the response is initiated to protect the CNS from infection and damage, it is also an important mechanism to induce secondary injury after TBI. TBI-related neuroinflammation is characterized by activated glia, recruited leucocytes, and upregulated inflammatory cytokines in the brain [126].

Glia in CNS consists of microglia, oligodendrocyte, and astrocytes, all of which are indispensable to maintain homeostasis in the brain and support normal neuronal functions [127]. Microglia is innate immune cell of the brain, which is analogous to macrophage in peripheral tissue. It functions in scavenging debris, surveying circumambient microenvironment and transferring inflammatory signals. There are two states of microglia: quiescent or priming. The quiescent microglia can be induced to priming microglia by stroke, aging, brain injury, and neurodegenerative disease. In addition to the two states, there are two subpopulations in microglia based upon their functions. The subtype associated with phagocytosis and capacity to kill pathogens is called M1; the one that is

involved in tissue repair and growth stimulation is called M2. So far, there is no direct evidence to indicate that neutrophils influence the subpopulation (M1 and M2) of microglia in TBI model. However Moxon-Emre and Schlichter showed that depletion of neutrophils could reduce microglia/macrophage populations after intracerebral hemorrhage, as well as decrease the activation marker CD68 on microglia/macrophage, indicating that neutrophils participate in modulating microglia state [128]. The resident microglia senses the change of damage-associated molecular patterns as well as pathogen-associated molecular patterns. The primed microglia could initiate the activation of endothelial cells as well as the recruitment of peripheral leukocytes into the CNS [129]. The activated microglia rapidly produces large amounts of inflammatory cytokines and chemokines (IL- 1β , TNF- α , IL-6, CXCL1–5, CXCL8–10), as well as rearranges patterns of receptor expressions (major histocompatibility complex II and complement receptor 3) [130]. These inflammatory mediators are strong chemokines to recruit and activate neutrophils. In addition to the above microglia-derived cytokines, neutrophils release some other molecules to activate microglia reciprocally, such as ROS [131], lipocalin 2 [132], and MMP9 [133]. The activation of microglia and neutrophils is a cascade amplification. Interestingly, the activated microglia behaves like a double-edged sword: on the one hand, it promotes neutrophils to secrete more pro-inflammatory cytokines to form the cytokine storm; on the other hand, the activated microglia is also correlated with the reduction of neuronal damage, releasing microglia neuroprotective factors [134]. Some of microglia secreted cytokines are also beneficial for nerve recovery (listed in the Table 1). Total ablation of microglial cells results in a significant increase in the infarcted size and the number of apoptotic neurons [135]. The interactions between quiescent microglia and neutrophils are quite different. The inactivated microglia generally engulf and scavenge the motile and robust neutrophils in an $\alpha_v\beta_3$ -integrin and lectin-like receptor-dependent manner, protecting the neurons from damage [136]. It is unclear if neutrophils could influence microglia M1/M2 polarization in the settings of severity, location, and period of injury.

Mature oligodendrocytes wrap around axons or blood vessels, forming the myelin sheath of nerve insulation structure and maintaining the normal functions of neurons. They are classified into three sorts based upon the distribution: interfascicular oligodendrocytes, perineuronal oligodendrocytes, and perivascular oligodendrocytes. The interfascicular oligodendrocytes are distributed along the white matters and nerve fibers, which is rapidly reduced within the phase of myelin formation. The perineuronal oligodendrocytes, also called perineuronal satellite cells, can be proliferated from oligodendrocyte precursor cells (OPCs) as a response to injury or

disease. They are thought to maintain homeostasis and participate in post-injury repair. OPCs are known to constitute the majority of MMP9-expressing cells in the acute phase of brain injury, and standard MMP inhibitor GM6001 reduced the early BBB leakage and neutrophils infiltration [137]. Oligodendrocytes also secrete significant amounts of CXC chemokines, such as CXCL1–5 and CCL21 to induce neutrophils invasion and astrogliosis [24, 138, 139], which indicates that oligodendrocytes might collaborate with neutrophils to aggravate inflammation. Interestingly, neutrophils could damage oligodendrocytes as well. Mice that are deficient in CXCR2, the predominant receptor for recruiting neutrophils, are more resistant to demyelination after injury. Specifically, CXCR2⁺ neutrophils are the main driving force to cause oligodendrocytes loss and demyelination, influencing the myelination of damage axon and long-term recovery after TBI [140]. There are limited studies about the relationship between perivascular oligodendrocytes and neutrophils. Whether perivascular oligodendrocytes interact with endothelium to facilitate neutrophil's transmigration is an interesting research direction.

Astrocytes are the amplest glia cell in CNS, which indispensably maintain the CNS homeostasis and contribute to the integrity of BBB with perivascular cells. Astrocytes usually exhibit a pathological process termed as “reactive astrogliosis” in response to CNS injury, contributing to CNS neuropathology [141]. However, the role of astrogliosis in injury recovery remains equivocal. Under injury conditions, neutrophils and astrocytes are tightly connected each other and respond to shared cytokines. Astrocytes are vital cytokine source that provides several cytokines and proteinases, like IL-6, CCL2, CXCL1, CXCL2, GM-CSF, glutamate-leucine-arginine motif containing chemokines, MMP2 and MMP9 [142–144]. Some cytokines promote BBB disruption, leukocytes recruitment, and inflammation initiation such as CXCL1, CXCL2, and GM-CSF [141, 145–148], while others are essential for the astrocytes and neurons' protection like IL-6 and CCL2 [66, 149]. These cytokines are also important for modulating glutamate uptake by astrocytes, which is considered as a protective mechanism during neuroinflammation. IL-1 β and TNF- α inhibit astrocytes glutamate uptake in a dose-dependent manner, whereas interferon- γ alone stimulates this activity [150]. Meanwhile, the reactive astrocytes could form perivascular scars as well that restricts the spread of neutrophils from the damaged tissue into healthy parenchyma during the acute inflammatory responses [151]. In an ex vivo experiment, Xie et al. showed dependent on the way (direct vs indirect) neutrophils interact with astrocytes, the outcome can be different [152]. Under direct cell-cell contact, astrocytes could decrease the apoptosis, respiratory burst, and degranulation of

neutrophils, enhancing neutrophils phagocytic capability and pro-inflammatory cytokine expression. Under indirect interaction, astrocytes attenuate apoptosis and enhance necrosis in neutrophils, augment neutrophils phagocytosis and respiratory burst, and inhibit neutrophils degranulation [152]. Reciprocally neutrophils can influence the evolution of astrocytes reactivity as well. Treatment of mice with anti-Ly6G antibody dampened the astrogliosis and worsened the behavioral outcomes in spinal cord injury [113]. In another in vitro study, Hooshmand and Nguyen showed that neutrophils could induce astrogliogenesis via generating C1q and C3a [153]. All of these data suggest that neutrophils and astrocytes work as the main source of cytokines during neuroinflammation, boosting the inflammation cascade with mutual stimulation. Neutrophils are definitely an important driver to promote reactive astrogliosis during injury. Whether astrogliosis is good or bad in brain injury remains an open question.

Neutrophils and neurodegeneration

A retrospective study showed that the neuroinflammation and white matter degeneration would persist for several years after TBI [154]. The TBI patients are more vulnerable to develop dementia, Alzheimer's disease (AD), and Parkinson's disease (PD) [154, 155]. In the animal model, neutrophils seem to be involved in white matter inflammation and degeneration [5, 156]. Neutrophils could also drive initiation of the AD-like symptoms [157] and participate in the formation of A β plaques [158]. These results highlight the fact that neutrophils function beyond the acute phase and work with other innate immune cells to contribute to the pathogenesis of degenerative diseases after TBI. Several acute inflammatory cytokines, like IL-1 β , ROS, and complement 5a (C5a), are all shown to be tightly related to neurodegeneration [159–161]. There is an elevated level of oxidative stress in neutrophils isolated from AD and PD patients [160]. The author speculated that the overwhelmed reactive species might influence the mitochondrial electron membrane of CNS cells. In return, the degenerative substances like beta-amyloid could also stimulate neutrophils to produce more oxidative substances. However, some research suggest that there is no direct relationship between neutrophils and degeneration [162]. In that study, the authors found that neutrophils were observed in the parenchyma 1 year after the induction, indicating that neutrophils might survive for a long-term period in CNS environment [162]. But there is no evidence of neuronal degeneration in the tissue. Neutrophils might modulate the degeneration indirectly with other immune cells like Treg cells, Th17 cells, and $\gamma\delta$ T cells [6]. Intriguingly, a recent investigation indicated that the accumulation of amyloid-beta peptide might be an antimicrobial peptide to fight against inherent brain

infection [163], which could be interpreted that AD might be an infectious disease. It is well-known that brain trauma usually causes open injury in the tissue, leaving the possibility of concomitant infection for a lifelong time. Whether neutrophils would collaborate with the amyloid-beta peptide to fight against infection is another interesting and promising direction for the future.

Neutrophils and nerve recovery

It is clear that infiltrated neutrophils cause brain tissue damage, especially to neurons. Neutrophils were shown to affect the survival of neurons directly in a neutrophils-neurons coculture study: the neutrophils caused the neurons' death through direct contact and released proteases, like MMP9 and NE [57, 164–166]. However, it is unclear whether the infiltrated neutrophils have preference for neurons. In other words, is it possible that damaged neurons positively attract neutrophils? Chung-ha et al. and Hayakawa et al. confirmed that neurons could exchange signalings with astrocytes by releasing mitochondria [167, 168]. Leow-Dyke et al. showed that neurons could release a cluster of cytokines (CXCL1, TNF- α , and IL-6) to promote neutrophils' infiltration across the endothelial monolayer [169]. These neuron-released substances might also be potential "helping signal" in the CNS. Neutrophils are actively recruited to the injured brain tissue, where they clean up debris, protect against potential infection of the exposed parenchyma, and promote tissue regeneration [170]. With visualization by two-photon microscopy in a TBI model, Roth et al. revealed that infiltrated neutrophils were primarily swarmed in the damaged meningeal area and interacted with dead cells and debris. Blocking this process led to increased amounts of cell death in the meninges, suggesting that at least in meningeal space, neutrophils play a protective role [171]. Kurimoto et al. confirmed that neutrophils promoted retinal ganglion cells to regenerate lengthy axons in injured optic nerve. The influx of neutrophils started to appear in the mouse eyes within hours after injury as well as the elevated expression levels of atypical growth factor oncomodulin (Ocm). Depletion of neutrophils diminished the Ocm and abolished the pro-regenerative effects of inflammation at the same time [172]. TGF- β is another factor that neutrophils could secrete after stimulation [173]. It could promote nerve regeneration by stimulating nerve growth factor production as well as controlling the astrogliosis and scar formation after injury [174]. Neutrophils could also secrete several other molecules that are beneficial for promoting neuron cell survival, like G-CSF, hepatocyte growth factor (HGF), nerve growth factor (NGF), and neurotrophin-4 (NT4) [28, 175–178]. Several cytokines that modulate neutrophils' function also show the effects of augmenting neurite growth as well as preventing neuronal cell death, such as arginase and midkine [26, 179]. Angiogenesis is an

essential process for damaged brain tissue to restore perfusion after TBI. Of note, neutrophils themselves are a source of VEGF to modulate their migration and contribute to angiogenesis in an autocrine manner [180]. The newborn blood vessels could provide mediators that are critical for neurogenesis at injury sites such as brain-derived neurotrophic factor (BDNF) and VEGF [27]. In general, chemokines that promote neutrophil infiltration (some of the chemokines are also produced by themselves) also promote angiogenesis, such as CXCL1, CXCL8, and granulocyte chemotactic protein-2 [181, 182], MMP8 and MMP9 [183], all of which are tightly connected with vessel growth and remodeling [184, 185]. Finally, increasing evidences suggest that neutrophils could also disseminate anti-inflammatory microparticles and participate in inflammation resolution [186]. Several cytokines produced by neutrophils can also promote resolution of inflammation, including IL-4, IL-10, and TGF- β [187, 188]. It is still unknown whether neutrophils' action contributes to nerve repair or exacerbate inflammation.

Discussions

Neutrophils have long been believed to be short-lived immune cells and contribute to brain damage during the acute phase of brain injury. Elimination of neutrophils or inhibition of neutrophils recruitment show some protective effects in brain injury [83, 84, 104, 189]. However, inhibition of neutrophils in the clinic might carry a significant risk of severe hospitalized infections and immunologic dissonance [190–192]. Furthermore, existing evidence does not fully support the notion that more neutrophils' recruitment leads to the more severe brain injury [46, 162, 165, 171, 172, 193]. Recently, the development and application of advanced neurotechnologies have helped researchers to gain deep insight into the roles of neutrophils in CNS injury and other diseases, which challenges our conventional views on neutrophils and call for more studies on many new questions in the future. Listed below are four important questions that deserve attention in the near future:

1. *Can neutrophils be divided into two subpopulations?* Several studies suggest the presence of two subpopulations of neutrophils with opposite roles, similar to microglia and macrophage (M1 and M2). In tumor models, neutrophils can be converted from anti-tumor phenotype (N1: release ROS and TNF- α) to pro-tumor phenotype (N2: increased Arginase, CCL2, and CCL5) by the TGF- β stimulation [194]. In infection models, there are also two distinct subpopulations of neutrophils: pro-inflammatory neutrophils (N1: IL-12 and macrophage inflammatory proteins producing) and anti-inflammatory neutrophils (N2: IL-10 and CCL2 producing) [195]. In a stroke

model, it was shown that rosiglitazone, one of the agonists for peroxisome proliferator-activated receptor- γ , could promote the infiltration of N2-like neutrophils into the ischemic core and protect neuron damage concomitantly through facilitating the dissolution of inflammatory responses [196]. This study proves that N2 phenotype of neutrophils is beneficial for the brain injury. Despite the compelling evidence for the separate subsets of neutrophils, there are no specific markers to identify and distinguish them, which warrants more investigation in the future. Their roles in the CNS diseases may depend on pro/anti-inflammatory (N1, N2) phenotypes that are regulated by specific environmental cues in the brain after injury. Further understanding of these cues and the outcomes associated with particular phenotypes may allow neutrophils to serve as disease-modifying factors in the CNS.

2. *How long will neutrophils survive?* Latest studies raise another important question about the average lifespan of neutrophils under normal and disease conditions. It has long been regarded that the average half lifespan of neutrophils in circulation is around 1.5 and 8 h in mice and human, respectively. The exact half-life of human neutrophils varies from 7 to 22 h in different disease conditions and labeling methods (reviewed in [197]). Recent studies indicated that the average lifespan of human neutrophils in circulation could be as long as 5.4 days [198]. The time window that neutrophils might play in TBI may be significantly longer than previously thought since specific conditions in the brain could prolong the survival time of neutrophils like hypoxia [116], glutamate [199], ATP, and adenosine [200, 201]. In addition, due to the damage of BBB after TBI, circulating neutrophils still can continuously transmigrate into brain tissue even though the damaged tissue start to gradually recover from TBI. Data from previous studies of other groups and our recent work (unpublished) show that neutrophils can be detected in damaged brain tissue after 14 days or even 1 year later [162]. Thus the real function of neutrophils in TBI is likely to go beyond fighting against pathogens in the acute phase. A role of neutrophils in modulating the activation of T cells and B cells in chronic phase should also be taken into consideration [202, 203].
3. *Is the recruitment cascade of neutrophils in the brain similar to that in peripheral tissues?* The recruitment of neutrophils involves several steps including tethering, rolling, adhesion, crawling, and transmigration [30]. Previous studies show that

P-selectin and E-selectin mediate the tethering and rolling of neutrophils; ICAM-1, ICAM-2, and PECAM1 participate in the adhesion and transmigration of neutrophils [62]. However, it appears that rolling and migration of neutrophils in the brain venule are governed by a different mechanism, as brain endothelium (BE) differs from peripheral endothelium (PE) at least three aspects: (1) adhesion molecules are different (BE: ICAM-1^{high}, VCAM-1^{high}, ECAM-1^{low}; PE: ICAM-1^{high}, VCAM-1^{high}, ECAM-1^{high}) [71]; (2) activation times are different (BE: 2–48 h; PE: within minutes) [72]; and (3) existence of pericytes and astrocytes around BE [204, 205]. The specific molecules involved in adhesion and transmigration of neutrophils in brain venules are currently unknown and require more studies in the future [30].

4. *Is it possible that the infiltrated neutrophils could be reprogrammed and return to peripheral in TBI?* The infiltrated neutrophils could be reprogrammed by the brain resident cells or molecules. Glutamate, as the essential neurotransmitter in the brain, could enhance migration, promote immune responses, and prolong survival time of neutrophils [67, 199, 206]. Astrocytes, as the amplest glia in the brain, could also modulate neutrophils' functions directly and indirectly [152]. Previous studies have also shown that neutrophils could be reprogrammed into specific phenotype with augmented phagocytic index [196, 207]. Since the reprogrammed neutrophils have enhanced capacity of cell killing and degranulation, they might induce more severe tissue damage if they move back to peripheral circulation. Recently, some studies showed that neutrophils might migrate back to vessel from infiltrated tissue, which is also called reverse migration [208, 209]. This phenomenon not only provides a novel mechanism of inflammation resolution but also provides a possibility of inducing secondary damage in peripheral organs when they migrate back to circulation. In severe TBI patients, the acute respiratory injury and multiple organ failures are quite common, which is tightly connected with neutrophils' function [210, 211]. Thus, we speculate that injured brain tissue might re-educate neutrophils to become a pro-inflammatory and long-survival subtype. Upon return back to circulation, these re-educated neutrophils can cause damages to the distant peripheral organs/tissues.

Conclusions

Neutrophils are a crucial component of the innate immune system, whose inappropriate or excessive activation could lead to tissue damage. TBI-induced changes in immune system play a decisive role in its development and prognosis. The phenotype, function, and survival time of neutrophils are tightly connected with every pathological process of TBI, including changes in blood flow and CSF component, breakdown of BBB, alterations in brain metabolism, neuroinflammation, neurodegeneration, and nerve recovery. The plasticity of neutrophils and crosstalk with other cells also complicate their functions in TBI. Thus, it is hasty to conclude that neutrophils function in a well-defined and limited manner since the roles of neutrophils of being protective or harmful depend on the phase and type of insult, and the type of cells neutrophils are interacting with. Literature indicates that the different microenvironments contribute to the different features of neutrophils in the brain compared with those in peripheral tissues under inflammatory conditions. However, the molecular mechanisms of how the brain environment regulates neutrophils' functions and how the neutrophils' functions affect brain injury and repair have not been well elucidated. More studies are needed to better define the complex roles of neutrophils at different stages of TBI as well as the underlying mechanisms. It is possible that a new and improved therapeutic strategy will be developed in the future that will selectively eliminate the harmful effect of neutrophils while keeping their beneficial effect in TBI.

Abbreviations

AD: Alzheimer's disease; BBB: Blood-brain barrier; BDNF: Brain-derived neurotrophic factor; BE: Brain endothelium; CBF: Cerebral blood flow; CCL: C-C motif ligand chemokine; CNS: Central nervous system; CSF: Cerebrospinal fluid; CXCL: C-X-C motif ligand chemokine; CXCR: C-X-C chemokine receptor; ECAM: Endothelial cell adhesion molecule; G-CSF: Granulocyte-colony stimulating factor; GM-CSF: Granulocyte-macrophage-colony stimulating factor; HGF: Hepatocyte growth factor; ICAM: Intercellular adhesion molecule; IL: Interleukin; iNOS: Inducible nitric oxide synthases; MMPs: Matrix metalloproteinases; NE: Neutrophils elastase; NETs: Neutrophils extracellular traps; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NGF: Nerve growth factor; NOS: Nitrous oxide; NT4: Neurotrophin-4; Ocm: Oncomodulin; OPCs: Oligodendrocyte precursor cells; PD: Parkinson's disease; PE: Peripheral endothelium; PECAM: Platelet-endothelial adhesion molecules; PMNs: Polymorph nuclear cells; ROS: Reactive oxygen species; TBI: Traumatic brain injury; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VCAM: Vascular cell adhesion molecule; VEGF: Vascular endothelial growth factor

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Availability of data and materials

References for this Review were identified by searches of PubMed and Google Scholar until December, 2017. Selected articles were also obtained from the reference lists of papers identified by previous searches. The search terms "Neutrophils," "Polymorph nuclear cell," "Traumatic brain injury," "Neuroinflammation," "Blood-brain barrier," "Neurodegeneration," "Nerve recovery," "Edema," "Hypoxia," "Hypoperfusion," and "Hyperemia/hyperperfusion" were used. Only reports published in English were included.

The final reference list was generated on the basis of relevance to the topics covered in this review.

Authors' contributions

YWL wrote the primary draft; SL revised the draft and gave some linguistic suggestions. SSD organized the frame of this review. All authors read and approved the final manuscript.

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