Neutrophil granulocytes in cerebral ischemia – Evolution from killers to key players

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Neutrophil granulocytes (or polymorphonuclear cells, PMNs) have long been considered as crude killing machines, particularly trained to attack bacterial or fungal pathogens in infected tissues. That perspective has fundamentally changed over the last decades, as PMNs have been shown to exert a livery exchange between other cells of the innate and adaptive immune system. PMNs do provide major immunomodulatory contribution during acute inflammation and subsequent clearance. Following sterile inflammation like cerebral ischemia, PMNs are among the first hematogenous cells attracted to the ischemic tissue. As inflammation is a crucial component within stroke pathophysiology, several studies regarding the role of PMNs following cerebral ischemia have been carried out. And indeed, recent research suggests a direct connection between PMNs’ influx and brain damage severity. This review highlights the latest research regarding the close interconnection between PMNs and co-working cells following cerebral ischemia. We describe how PMNs are attracted to the site of injury and their tasks within the infarcted brain tissue and the periphery. We further report of new findings regarding the interaction of PMNs with resident microglia, immigrating macrophages and T cells after stroke. Finally, we discuss recent research results from experimental studies in the context with current clinical trials and point out potential new therapeutic applications that could emerge from this new knowledge on the action and interaction of PMNs following cerebral ischemia.

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Abbreviations: BBB, Blood-brain barrier; CCL-2-4, CC-chemokine ligand-2-4; CCR-1-4, C-C chemokine receptor type-1-4; CRFL1, Chemokine-like factor 1; CRAMP, Cathelin-related antimicrobial peptide; CXCL-1-8, Chemokine (C-X-C motif) ligand-1-8; CXCR-1-2, C-X-C chemokine receptor-1-2; DAMPS, Damage-associated molecular patterns; ESL-1, E-selectin-ligand-1; FoxP3, Forkhead box P3; G-CSF, Granulocyte-colony stimulating factor; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HMGB-1, High-Mobility-Group-Protein B1; ICAM-1, Intercellular Adhesion Molecule-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-17A, Interleukin-17A; IL-17R, Interleukin-17 receptor; IC; IL-23, Interleukin-23; IFNγ, Interferon gamma; LFA-1, Lymphocyte function-associated antigen-1; MAPK, Mitogen-activated protein kinase; MCAO, Middle cerebral artery occlusion; MMP-8/9, Matrix metallopeptidase-8/9; MPO, Myeloperoxidase; NET, Neutrophil extracellular traps; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, Natural killer cells; PD-1, Programmed cell death protein-1; PMN, Polymorphonuclear neutrophil; PR3, Proteinase-3; PSGL-1, P-selectin glycoprotein ligand-1; RNS, reactive nitrogen species; ROS, Reactive oxygen species; TNF-α, Tumor necrosis factor-α; VCAM-1, Vascular cell adhesion protein-1; VLA-4, Very Late Antigen-4.

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1. Introduction

For a long time, neutrophil granulocytes or polymorphonuclear neutrophils (PMN) were widely seen as innate immune systems’ crude and aggressive hitmen, less sophisticated than monocytes, macrophages or T cells. However, current research suggests that this assumption takes only a narrow bandwidth of PMNs skills into account. Actually, neutrophil granulocytes are, in their function as first defence wave, the first cell population to arrive at the site of microbial invasion with the task to eliminate the intruders. To accomplish this, PMNs are equipped with impressive and various armouries of biological weapons. These include reactive oxygen species (ROS; Westen et al., 2006), protease stuffed vesicles, antibacterial biomolecules (Fauraux and Borregaard, 2003) and the capacity to release net-formed chromatin as so called neutrophil extracellular traps (NETs) for the capture of extracellular pathogens (Ruhna et al., 2014). However, all of these armaments work efficiently on most live material including the host’s structures, making the PMN a dangerous cell. Therefore, PMNs’ action has to be precisely adjusted in order to avoid severe collateral damage during inflammation initiation, maintenance and clearance back to tissue homeostasis. Therefore and unsurprisingly, PMNs action is embedded within a finely orchestrated cellular collaboration, be it during the clearance of an infected wound or fungal infestation. Besides of the classic “wound care”, the need for an outbalanced immune cell reaction also applies to any sterile inflammation like ischemic stroke. Following cerebral ischemia, PMNs are the first hematogenous cells to arrive at the site of inflammation and are said to contribute to further blood-brain barrier (BBB) damage by the release of proteolytic enzymes and oxygen radicals (Matsuo et al., 1994; Pelus et al., 2004). However, recent research indicates a significant influence of PMNs on the regulation and orchestration of the post ischemic inflammatory response and that PMNs have a prominent role as effector cells in both, innate and adaptive immune regulation after stroke.

2. How to enter the ischemic brain and what to do as neutrophil granulocyte

Cerebral oxygen and nutrient deprivation results in neuronal damage, release of cytokines, chemokines and danger molecules, endothelial presentation of adhesion molecules, subsequent breakdown of the blood-brain barrier, glial activation and influx of a variety of hematogenous immune cells (Schilling et al., 2003, 2009; Gelderblom et al., 2009; Schuette-Nuetgen et al., 2012). These include, next to PMNs, monocytes, macrophages, T-cells and dendritic cells. The main task of this cell collaboration is to clear the debris and finally support the tissue restoring its pre-inflammatory state. Particularly the release of damage-associated molecular pattern molecules (DAMPS) like DNA, RNA, HMGB1, ATP, peroxiredoxin proteins (Shchita et al., 2012) by indigent and dying cells lead to a prominent inflammatory reaction via activation of e.g. toll-like receptors. This inflammation-like process, involving a plethora of cell types, is orchestrated by the release of chemokines like TNF-α, IL-6, IL-1β and the chemokine-like factor 1 (CKLF1, Kong et al., 2014) with subsequent activation of brain glia, platelets and vascular endothelial cells within the affected tissue (Fig. 1a and b).

Endothelial activation and presentation of adhesion molecules on the luminal vessel wall results in the start of a chemokine-driven infiltration of hematogenous cells. The immigration of attracted PMNs is divided into four subsequent phases namely chemotraction, rolling adhesion, tight adhesion and diapedesis/migration, making the PMN finally crossing the blood-brain barrier into the inflamed tissue (Fig. 1c). Known proteins involved in PMN attraction are Selectins, ICAM-1, VCAM-1, LFA-1 and Mac-1 (reviewed in Frijns and Kappelle, 2002). Neumann et al. (2014) showed in their recent work, that the very late antigen-4 (VLA-4 or integrin αβ1) is furthermore essential for the PMN infiltration through the BBB after cerebral ischemia. They could verify their finding by antibody-mediated blocking of VLA-4, which resulted in reduced vessel-attached PMNs and altered PMN/endothel interaction. PMNs stayed in slow rolling-status and diapedesis and migration was limited. It is worth mentioning that the temporospatial location of PMNs during and after immigration has been controversially discussed lately. In a recent study, Enzmann et al. (2013) report that after ischemia/reperfusion almost no PMNs enter the actual brain parenchyma but rather stay attached to luminal surfaces or within the perivascular space. Thus, Enzmann and colleagues conclude to generally question the clinical relevance of PMNs in stroke and that previous studies could be flawed due to e.g. antibodies not specific enough or non-confocal analysis of (too) thin brain sections. This was surprising, as several other studies report intra-parenchymal PMNs beyond the perivascular space after transient vessel occlusion (Ulrich et al., 2014; Neumann et al., 2015) or even after permanent cerebral ischemia (Perez-de-Puig et al., 2015; Fig. 1d). These publications demonstrate, using confocal or two-photon intravital microscopy, that PMNs indeed enter the brain parenchyma and can be found beyond vascular structures like basement membrane layers. Next to the plain neuronal loss, a further cause for post-ischemic damage is the breakdown of the blood-brain barriers’ integrity. It has been proposed that cerebral ischemia induced loss of BBB-integrity is actually proceeding in several phases (Shi et al., 2016). The early phase, characterized by endothelial actin-stress fibre formation and endothelial cell contraction is accompanied by disassembling of tight- and adherens junction proteins. This in turn results in enhanced susceptibility of basement membrane and BBB—components to degradation by MMP-9, particularly secreted by PMNs, leading to irreversible damage to the neurovascular unit (Fig. 1e). Next to their capability to release proteinases and reactive oxygen, PMNs are also known to trap e.g. bacteria in projected extracellular decondensed chromatin. These structures, consisting of DNA, histones and enzymes have been termed neutrophil extracellular traps (NETs) (Wang et al., 2009). This formation of NETs (or NETosis) is accompanied by pronounced nuclear histone citrullination, which has been recently used as marker to demonstrate the presence of NETosis even after cerebral ischemia (Perez-de-Puig et al., 2015). Following permanent cerebral occlusion, intracerebral PMNs show sporadically citrullinated histones and NETosis could be seen within the perivascular space, the vessel lumen and the parenchyma. As some NETs were also seen surrounding pericytes, the authors speculate that DNA, histones and enzymes expelled by PMNs could further damage BBB-related structures (Fig. 1f). Additionally Perez-de-Puig and colleagues suggest that NETosis could be...
a reason for secondary microthrombosis as intra-luminal NETs promote blood coagulation and thrombosis. Investigations regarding the activation state of PMNs within patient blood after stroke, however, showed contrary results. Matching the hypothesis of a stroke induced overall immune-suppression, circulating PMNs likewise displayed reduced oxidative burst and NETosis (Ruhnau et al., 2014).

3. Where do they come from?

The inflammatory response following cerebral ischemia is not, as one might think, locally restricted to the brain parenchyma. Several clinical trials showed that stroke induces a pronounced inflammatory response during the acute phase within the periphery, leading to increased PMN counts and pro-inflammatory chemokine levels within the circulation (Smith et al., 2004). Recently, it has been shown that elevated counts of activated, peripheral PMNs positively correlate with the risk of poorer outcome and higher NIHSS score (reviewed in Mo et al., 2013). Studies in rodent stroke models demonstrate a peripheral inflammatory response (with regards to plasma IL-6 and CXCL-1 levels) peaking at 4 h after stroke onset and thereby preceding the brain inflammation peak which takes place approximately 24 h after onset of ischemia (Chapman et al., 2009; Fig. 2a). It is assumed that this temporospatial separated chemokine gradient could indeed channel PMNs and other leukocytes from their peripheral origins, particularly bone-marrow and spleen, to the site of inflammation. Cerebral ischemia leads to rapid (4 h after ischemia/reperfusion) phosphorylation of NFκB p65 and p38 MAPK in PMNs undergoing NETosis close to the endothelial wall and perivascular macrophages. Abbreviations: BBB: Blood-brain barrier; CCL-2: CC-chemokine ligand-2; CXCL-8: Chemokine (C-X-C motif) ligand-8; DAMPS: Damage-associated molecular patterns; ESL-1: E-selectin-ligand-1; HMGB-1: High-Mobility-Group-Protein B1; ICAM-1: Intercellular Adhesion Molecule-1; IL-1β: Interleukin-1β; IL-6: Interleukin-6; LFA-1: Lymphocyte function-associated antigen-1; MMP-8/9: Matrix metallopeptidase-8/9; MPO: Myeloperoxidase; PSGL-1: P-selectin glycoprotein ligand-1; RNS: reactive nitrogen species; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor-α; VCAM-1: Vascular cell adhesion protein-1; VLA-4: Very Late Antigen-4.

Fig. 1. Stroke induced recruitment and action of polymorphonuclear neutrophils. a: Oxygen and nutrient deprived neurons release DAMPs which activate particularly glial toll-like receptors leading to the activation of local micro- and astroglia. b: Glial chemokines trigger endothelial activation, which in turn present selectins and adhesion molecules on the luminal vessel wall. c: Circulating PMNs are captured by selectins recognizing the ligand counterparts expressed by the PMNs, which are subsequently tethered to the endothelial wall. Further contact with adhesion molecules leads to firm adhesion and rolling of the PMNs following the built up chemokine gradients. Finally, the PMNs migrate through the endothelial wall and arrive at the site of inflammation within the parenchyma or perivascular space. d: Once arrived at the site of infarction, PMNs release a plethora of anti-microbial enzymes, chemokines and reactive oxygen/nitrogen species resulting in further inflammation and chemotaxis/activation of adjacent immune cells. e+f: The release of proteases like MMP-9 is said to contribute to further damage of the blood-brain barrier, as well as PMNs undergoing NETosis close to the endothelial wall and perivascular macrophages.
Belinga et al., 2016). Splenectomized rats show reduced immigration of T cells, macrophages and PMNs accompanied with diminished IL-1β and TNF-α (Zhang et al., 2013). A further interesting observation is that the spleen, as a consequence of cerebral ischemia, shrinks. This loss of spleen mass could be particularly assigned to immune cell deployment but also splenocyte apoptosis (Offner et al., 2006; Bao et al., 2010, Fig. 2c and d), accompanied by increased regulatory T cell counts and reduced TNF-α, IFN-γ and IL-6 expression, suggesting peripheral immunosuppression (Fig. 2e). A recent clinical study confirmed that splenic atrophy also occurs in patients suffering stroke (Sahota et al., 2013).

4. Of invaders and sentinels: polymorphonuclear neutrophils and microglia

Microglia, the CNS’ resident immunocompetent cell population with its primary duty of brain homeostasis surveillance and maintenance, scans its environment constantly for defect neurons, plaques and infectious agents. Following cerebral ischemia, toll-like receptor expressing microglia becomes activated particularly by DAMPs, changes its morphology from ramified to motile amoeboid shape, proliferates and migrates towards the ischemic tissue to perform its emergency task: phagocytosis of debris and modulation of the inflammatory response (Schilling et al., 2005; Fig. 3a and b). During ischemia, microglia particularly secretes next to IL-1β, IL-6 and TNF-α, CCL-2, which in turn attracts and activates e.g. CCR-2-expressing leukocytes like Ly6C+ monocytes, macrophages, PMNs and brain resident macrophages (reviewed in Taylor and Sansing, 2013; Benakis et al., 2015, Fig. 3d). A recent study found that immigrated PMNs release Proteinase-3 (PR3) within the inflamed tissue, resulting in further microglial activation and additional neuronal death (Cho et al., 2015; Fig. 3e). It has also been proposed that PR3, secreted by PMNs, leads to an enhanced endothelial production of ICAM-1 and CCL-2 which in turn leads to further monocyte and macrophage attraction (Taekema-Roelvink et al., 2001). However, microglia role during acute ischemia and subsequent clearance seems not strictly limited to phagocytosis and immune cell conducting and whether microglia exerts mainly beneficial or detrimental role remains under debate to the present day (reviewed in Huang and Feng, 2013). Current research suggests several additional sophisticated microglial skills during inflammation, clearance and repair. For instance, it has been shown that PMNs, upon crossing the blood-brain barrier are engulfed and phagocytosed by activated microglia (Neumann et al., 2008; 2015).
This observation has been suggested to be potentially neuroprotective, conceiving a microglial thwarting of PMN’s action (Denes et al., 2007; Weston et al., 2007; Fig. 3f). Furthermore, in a model of oxygen-glucose deprived hippocampal slices, microglial interception of PMNs resulted in a strongly reduced neuronal damage (Neumann et al., 2008), giving further indication of a detrimental role of PMNs during post-stroke inflammation. The finding of an ischemia-induced microglia-PMN interaction has also been shown recently using a paradigm of permanent cortical experimental stroke (Neumann et al., 2014). By using two-photon intravital microscopy, Neumann and colleagues showed that PMNs are, upon entering the brain parenchyma, chased and trapped by microglial protrusions forming net-like structures. However, whether captured PMNs were also phagocytosed could not be clarified within this experimental setting. Interestingly, the researchers were able to see microglia forming cytoplasmatic processes shielding endothelial leaks, indicating a further beneficial microglial task within strokes pathology (Fig. 3i). Another intriguing, even though hypothetical explanation for microglial uptake of PMNs could originate from observations following bacterial infections where macrophages have been observed phagocytosing both apoptotic and viable PMNs. The authors conclude, that by ingesting PMN granules, microglia/macrophages could enhance their otherwise limited anti-microbial skills resulting in a more efficient clearance (reviewed in Silva, 2010). Nevertheless, whether these mechanisms could apply to post-stroke microglia-PMN interaction has to be investigated in future studies.

5. Polymorphonuclear neutrophils and macrophages: a close cooperation

Regarding the relationship between PMNs and macrophages one can say that these cells have, next to their similar bone marrow precursor (Akashi et al., 2000), several traits in common. Alongside their main task, the professional phagocytosis of pathogenic or other unwanted material, both show similar, albeit time-shifted kinetics towards the inflamed tissue and carry out various immunomodulatory effects (Gelderblom et al., 2009). Additionally, both present similar pattern recognition receptors, and thus become activated by common danger and pathogenic signals (Akira et al., 2001). Despite of these similarities, PMNs and macrophages have
distinct features complementing and not able to replace each other, constituting a powerful defence for the host. In consequence of sterile inflammation like cerebral ischemia, monocytes and macrophages enter the brain parenchyma over a time-course of several days following a chemokine/cytokine gradient released by the affected brain cells (Schilling et al., 2003). Once arrived microglia, monocytes, macrophages and PMNs collaborate in subsequent immunomodulatory activities and start to secrete a complex network of inflammation-related proteins. Macrophages and PMNs share a plethora of chemokines (CXCL-1–3, CXCL-8, CCL-2, CCL-3/4) and receptors (CXCR-2, CXCR-1, CCR-2, CCR-1/4) (reviewed by Silva, 2010; Fig. 3d), modulating each other’s activity as well as maintaining the local inflammation by creating signal feedback loops. This is especially important for a constant replacement of required PMNs within the infarmed area, as activated PMNs show a short life-span of only 24–48 h within the infarcted parenchyma. Besides, this PMN-life-span is even enhanced by adjacent anti-apoptotic macromolecule signalling, as rodent PMNs usually die after 6–12 h (Yamashiro et al., 2001; Kobayashi et al., 2005). Human PMNs have been shown to survive up to five days within circulation (Maeda et al., 2006), but whether this time-span also applies to activated and immigrated cells after stroke still needs to be clarified. Anti-apoptotic factors prolonging PMNs life-span are partic-ularly IL-1β, TNF-α, C-CSF and GM-CSF which are secreted partially by macrophages (reviewed by Silva, 2010; Fig. 3g). Furthermore, activated PMNs produce IL-1β and TNF-α which in turn attracts additional macrophages and PMNs to the affected tissue. Note-worthy, CRAMP (cathelin-related antimicrobial peptide), a neutrophil-derived cathelicidin released by immigrated PMNs, also attracts pro-inflammatory monocytes across the endothelium into the infarmed tissue (Wantha et al., 2013). However, this specific insight has been gathered from experiments within cremaster post capillary venules. Whether this applies to the CNS’ blood-brain barrier has yet to be tested. When it comes to terms to restoring tissue homeostasis, senescent PMNs have to be removed fast and before cell lysis, as latter could be harmful for the surrounding tissue due to possible release of proteases etc. This task is executed by scavenging macrophages and monocytes, which clear the battleground of apoptotic PMNs (Savill et al., 1989; Mikolajczyk et al., 2009; Fig. 3h).

6. Neutrophil and T cell crosstalk

Following cerebral ischemia, T cells lag slightly behind PMNs arriving at the inflammatory site. Nevertheless, as both cell types share similar temporo-spacial niches adjacent to cerebral vessels, T cell-PMN cross-talk seems likely. In a recent in-vivo study, it has been shown that T cells and PMN interact even under non-inflammatory conditions (Thewissen et al., 2011). Mice lacking conventional T cells (so called Rag-deficient mice) and “unconventional” T cells (Tcrd-deficient; devoid of γδ T cells) develop smaller infarcts and show improved functional outcome following ischemic stroke. By using a vast variety of genetically altered mice, Kleinschnitz et al. (2010) that conventional CD3+ T cells contribute to damage evolvement particularly during the early phase of brain infarction. Interestingly, T cell-mediated aggravation was still seen in mice lacking proteins essential for classical adaptive immunity like antigen recognition (signal 1) or co-stimulation (signal 2) (Gelderblom et al., 2012). The authors therefore conclude that post-ischemic inflammation, injury development and even long-term regeneration are partially T cell but not antigen mediated. Further studies have shown CD4+ , CD8+ and γδ T cell action to be detrimental in ischemic stroke, whereas B-cells seem to be neuroprotective (Yilmaz et al., 2006; Shichita et al., 2005a,b; Kleinschnitz et al., 2010; Gelderblom et al., 2012). Whether regulatory T cell (Treg) action is particularly beneficial or detrimental is still a matter of debate as current research draws contradictory conclusions. Whereas one recent study concludes that Treg absence results in undamped activation of pro-inflammatory cells and exacerbated brain damage (Liesz et al., 2009), another study suggests harmful effects exerted by Tregs. Here, DEREg mice (devoid of Tregs) showed dramatically reduced infarcts which could be reversed by adoptive transfer of the missing cells (Kleinschnitz et al., 2013). The results of another recent study report beneficial effects of thera-peutic Treg application after experimental stroke: mice subjected to intravenous injection of regulatory T cells developed smaller infarcts and showed reduced BBB-damage due to PD-1-mediated suppression of PMN-derived MMP-9 (Li et al., 2013; Li et al., 2014; Fig. 4a). When it comes to PMN/T cell interplay, recent experiments have shown that upon brain ischemia, IL-17A secreted by γδ T cells triggers local astroglia to produce CXCL-1, an essential PMN-chemoattractant. And indeed, Rag-1-deficient or IL-17R-deficient mice show markedly reduced numbers of immigrated PMNs after cerebral ischemia when compared to wildtype animals. A clear indication for a crucial role for γδ T cells in PMN attraction (Gelderblom et al., 2012, Fig. 4b). Furthermore, it has been shown that T cell derived cytokines like Interferon-γ (IFN-γ) exert anti-inflammatory effects on PMNs (Fig. 4c) and further induce IL-23 secretion in macrophages, which in turn induces IL-17A in γδ T cells (Klebanoff et al., 1992; Shichita et al., 2005a,b; Fig. 4d). Vice versa, chemokines like IL-2, IL-4 or IL-12 secreted by PMNs have been shown to modulate T cell differentiation (signal three) (Tateda et al., 2001). Furthermore, NETs released by PMNs prime T cells via reduction of their activation threshold in vitro. NET-primed T cells in turn secreted increased IFN-γ and IL-17A, indicating T cell differentiation towards pro-inflammatory phenotypes by NETosis (Tillack et al., 2012, Fig. 4e). However, whether NET-priming of T cells takes place in cerebral ischemia still needs to be determined. Arginase-1, when released by (dying) PMNs depletes extracellular L-arginine by enzymatic degradation. This in turn results in a profound T cell suppression, particularly by down-regulation of the CD3ε chain prompting the T cell to stay in the G0-G1 cell cycle phase (Munder et al., 2006; Rodriguez et al., 2007). Interestingly, this particular PMN-triggered T cell suppression has recently even been demonstrated in a model of experimental ischemia. The results of this study show that stroke can be accompanied by a markedly reduced proliferation capacity of splenic T cells and impaired IFN-γ production (Fig. 4f). As these effects sustain over 10 days post stroke, the authors suggest a peripheral immunosuppression and T cell hypofunction induced by MCAO (Sippel et al., 2015). Despite of the already known splenic shrinkage during the early phase, Sippel and colleagues observed a significant accumulation of PMNs within the spleen during the sub-acute phase on day 4 following MCAO. Besides, this splenic PMN-enrichment was time-synchronized with the organ returning to its normal size (Seifert et al., 2012; Fig. 4f). Leukocyte aggregation within the spleen after cerebral ischemia has recently been addressed in two studies, which suggest splenic overload by leukocytes after therapeutically administration of bone marrow cells in combination with growth factor G-CSF (Posel et al., 2014; Strecker et al., 2016).

7. From pole to pole: neutrophil phenotype switching

As the results in many different studies obtained inconclusive results regarding the potential detrimental or beneficial role of neutrophil granulocytes in strokes pathology, it has been proposed that PMNs are subjected to some sort of threshold effect respond-ing to the respective inflammatory milieu (Easton, 2013). Recent evidence suggests that human as well as rodent neutrophils are, similarly to the concept of classically activated M1 and alternatively...
activated M2 macrophages, adaptable cells which in turn react to extracellular stimuli and can as well adopt a pro-inflammatory so-called N1 and an anti-inflammatory N2 phenotype (Fridlender et al., 2009). This neutrophil plasticity could be demonstrated just recently in a rodent stroke model (Cuartero et al., 2013). Here the authors report that modulation of the peroxisome proliferator-activated receptor-γ (PPARγ) with its ligand Rosiglitazone results in a PMN-shift towards a more pronounced M2-like N2-phenotype after stroke. Notably, N2-polarized PMNs were preferentially phagocytosed by microglia/macrophages during the 48 h survey indicating a further crucial aspect of PMNs plasticity during wound clearance. PMNs are even able to transdifferentiate into cell hybrids, expressing classical PMN-marker like Ly6G, CXCR-2, 7/4 but also marker specific for dendritic cells (DCs) namely CD11c, MHC-class-II, CD80 and CD86 and have been shown to acquire antigen-presenting cell properties (Geng et al., 2013). Particularly the expression of MHC-class-II by PMN/DC-hybrids suggests an even more intense cross-talk of PMNs and T cells. However, until the present day, PMN/DC-hybrids have yet to be characterized in the context of cerebral ischemia.

8. What can be done about it?

 Plenty of preclinical studies characterize the role of PMNs within strokes pathology, thereby offering many possibilities of beneficial therapeutic intervention. Plain depletion of circulating PMNs has been shown to reduce infarct volume, reduce BBB-disruption and ameliorate functional outcome (Dawson et al., 1996; Kitagawa et al., 1998; Gautier et al., 2009; Herz et al., 2015; Neumann et al., 2015). Therapeutic interfering with endothelial cell adhesion molecules represents another promising strategy to limit the immigration of potential detrimental cells. Indeed, antibody-mediated blocking of P-selectin prior to transient focal cerebral ischemia leads to reduced infarct volume and less petechial hemorrhage in rats (Goussev et al., 1998). Blocking of CD47, an integrin crucial for the transmigration of PMNs across the BBB results in reduced PMN influx combined with reduced infarct size, less brain edema, decreased MMP-9 levels and reduced BBB-integrity loss (Jin et al., 2009). Blocking of several other adhesion molecules like ICAM-1, CD11b or CD18 has been shown to reduce infarct size and to improve functional outcome (reviewed in jickling et al., 2015). Inducing transient occlusion in mice deficient for CD18 results in reduced infarct size, improved cerebral blood-flow and decreased mortality after transient occlusion of the middle cerebral artery (Prestigiacomo et al., 1999). However, loss of CD18 did not show any cerebroprotective effects in a model of permanent ischemia, prompting the authors to suggest a more pronounced impact of PMNs within reperfusion injury. Furthermore, gene silencing of the very-late-antigen-4 (VLA-4)/vascular adhesion molecule-1 (VCAM-1) axis by in vivo small interfering RNA injection inhibits the immigration of PMNs, T cells and upregulation of VCAM-1 in combination with reduced post ischemic infarct volume and neuroinflammation (Liesz et al., 2011). However, blocking with anti-CD49d antibody (α4 integrin) showed no effects regarding infarct volume, functional outcome and post-ischemia survival (Langhauser et al., 2014). Modulation of the circulating and infiltrating PMNs could also be a promising target in stroke therapy. Several approaches to reduce immigrating PMN numbers showed promising results such as reduced infarct volume and improved functional outcome (Hein et al., 1994; Matsuo et al., 1994; Dawson et al., 1996). Blocking accumulation of PMNs within the ischemic cerebral cortex also resulted in reduced infarct size, neuron degeneration and improved functional outcome (Chu et al., 2006). Considering the convincing amount of evidence regarding the adverse role of PMNs, it is somewhat surprising that potential strategies withholding PMNs from entering the ischemic brain parenchyma showed no beneficial effects in human stroke so far (Krams et al., 2003). Indeed, clinical studies found a relation between PMN numbers and stroke outcome (Akopov et al., 1996). Patients with high PMN count (>8.2 x 109 PMNs/L) have a higher risk for stroke and furthermore, neutrophilia indicates increased probability of poor outcome after cerebral ischemia (Grau et al., 2004). By using diffusion-weighted MR imaging it has been shown that high PMN, but not lymphocyte counts, result in increased infarct size early after cerebral ischemia (Buck et al., 2008). Beyond that, a recent clinical trial demonstrated an association between high neutrophil counts on admission and increased incidence of thrombolysis associated intracerebral hemorrhage (Maestrini et al., 2015). However, past clinical trials on
interfering with adhesion-molecule-mediated PMN-trafficking have basically failed (Enlimomab Acute Stroke Trial Investigators, 2001; Becker, 2002; Krams et al., 2003).

9. Conclusion

Considering the fact that only a limited number of patients are eligible for thrombolysis or thrombectomy, new and innovative strategies for stroke treatment are urgently needed. Taking into account that both, circulating and peripheral PMNs do release harmful enzymes, reactive oxygen and nitrogen species, modulate the immune response, attract and activate further cells of the innate and adaptive immune system, makes it obvious that modulating or reducing the circulating and infiltrating PMNs after cerebral ischemia bears viable therapeutic strategies and promising possibilities. However, most results and insights into the role of PMNs within stroke pathways are obtained from experimental stroke models in mouse and rat. In particular, immune cell interplay and potential ways to manipulate latter have to be certainly validated within humans’ stroke pathology with the possibility to develop more sophisticated strategies for aimed and effective intervention.

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References


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