Neutrophils in Stroke

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Neutrophils play an important role in an innate immune system and are recruited to the site of brain damage after stroke. Three recent studies reported new aspects of neutrophil involvement in stroke pathology.

Frieler et al (**Frieler RA, Chung Y, Ahlers CG, et al. Genetic neutrophil deficiency ameliorates cerebral ischemia-reperfusion injury. *Experimental Neurology*. 2017;298:104–111. doi: 10.1016/j.expneurol.2017.08.016**) used myeloid Mcl1 knockout mice as a model of genetic neutrophil deficiency to examine the roles of neutrophils in acute stroke pathology. Mcl1 is a member of the antiapoptotic Bcl-2 family, and previous reports showed that Mcl1 regulates apoptosis in immune cells, and genetic ablation of Mcl1 in myeloid cells induces neutrophil deficiency but does not change macrophage or myeloid survival. The authors subjected wild-type and the myeloid Mcl1 knockout mice to transient middle cerebral artery occlusion (MCAO; 90 minutes occlusion by filament insertion followed by reperfusion) as a stroke model, and 24 hours later, acute stroke pathology such as infarction volume and neurological function was assessed. As expected, the myeloid Mcl1 knockout mice exhibited a nearly complete absence of neutrophils in the ischemic hemisphere, which was accompanied with smaller infarction volume. However, there were no significant differences in stroke-induced neurological deficits and mRNA levels of inflammatory genes between wild-type and knockout mice. When neutrophil infiltration was partially inhibited by a CXCR2 pepducin in wild-type mice, the drug-treated stroke mice showed no change in infarction volume compared with vehicle-treated stroke mice. Therefore, the authors proposed a scheme that neutrophils contribute to infarct development in acute stroke phase, and complete deficiency, but not partial inhibition, in neutrophil infiltration may be necessary to prevent neutrophil-mediated injury during stroke.

To date, many molecules are identified to be involved in neutrophil infiltration after stroke. Lee et al (**Lee HK, Kim ID, Lee H, Luo L, Kim SW, Lee JK. Neuroprotective and anti-inflammatory effects of a dodecamer peptide harvoring Ninjurin 1 cell adhesion motif in the postischemic brain [published online ahead of print November 14, 2017]. *Molecular Neurobiology*. doi: 10.1007/s12035-017-0810-1**) revealed that Ninj1 (Ninjurin 1, nerve injury-induced protein 1) is also a key player for neutrophil infiltration in stroke brain. Ninj1 is a cell adhesion molecule, which was originally found to be upregulated in neurons and Schwann cells after injury. However, Ninj1 is now known to be widely expressed in various cell types, especially under pathological conditions. The authors first examined the Ninj1 expression pattern after stroke using a rat stroke model (transient MCAO model by filament insertion; 60-minute followed by reperfusion). Double staining with cell specific marker antibodies showed that in the acute phase stroke (≈ 1 day after MCAO), Ninj1 was predominantly induced in neutrophils and endothelial cells. When Ninj1 was inhibited by Ninj1 siRNA (administered intranasally at 3 hour after MCAO) or injection of a dodecamer peptide harboring Ninj1 N-terminal adhesion motif (administered immediately after MCAO), stroke rats with Ninj1 inhibition exhibited reduction of infarct volume and less neutrophil infiltration at day 2 along with decreased neurological/motor deficits up to 2 weeks. In addition, in vitro cell culture experiments using human umbilical vein endothelial cells and differentiated human promyelocytic leukemia cells neutrophils showed that Ninj1 regulates both neutrophil adhesion to endothelial cells and neutrophil infiltration via phosphoinositide 3-kinase-Rac1 signaling. These data suggest that Ninj1 may contribute to acute stroke pathology by regulating neutrophil infiltration.

Although neutrophil infiltration after brain injury is considered detrimental, neutrophils may also possess beneficial roles in stroke brain. A recent study by Zhao et al (**Zhao X, Ting SM, Liu CH, et al. Neutrophil polarization by IL-27 as a therapeutic target for intracerebral hemorrhage. *Nature Communications*. 2017;8:602. doi: 10.1038/s41467-017-00770-7**) focused on the beneficial property of neutrophils after intracerebral hemorrhage (ICH). After ICH, several soluble factors such as cytokines and chemokins are released from microglia, which then enhances the recruitment of polymorphonuclear neutrophils (PMN). In bone marrow (BM), developing PMNs (BM-PMNs) are maturated and released into circulation, and in the case of ICH, released PMNs from BM carry their prepared granule content to the site of damaged region in ICH brain. In this study, using rodent models of ICH (intrastriatal injection of autologous blood), the authors first showed that the level of an immunoregulatory cytokine IL (interleukin)-27 increased within 1 hour of ICH onset, peaked around 3 hours, and then declined over the next 2 days. In vitro cell culture

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experiments using cultured BM-PMNs from mice 24 hour after ICH demonstrated that recombinant IL-27 treatment downregulated proinflammatory genes but increased anti-inflammatory ones in BM-PMNs. Also, in vivo gain- or loss-of-function experiments confirmed that endogenous IL-27 is a critical factor for modifying PMN phenotypes in BM. And finally, the authors provided in vivo data for proof-of-concept that IL-27 administration is effective for ICH pathology via increasing production of beneficial molecules from PMNs. These data support a new idea that shifting neutrophils toward a beneficial phenotype can be an attractive therapeutic approach for ICH.

These 3 papers describe novel mechanisms for neutrophils in stroke pathology. Further studies are warranted to continue to pursue neutrophil targets for stroke therapy.

Stroke Literature Synopses: Basic Science

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