Stroke

ADVANCES IN STROKE

Translational Interdisciplinary Science—Immune Cell Niches: Possible Targets for Stroke Therapy?

Louise D. McCullough, MD, PhD; María A. Moro, PhD

Stroke is the second most common cause of death and disability worldwide. The global burden of stroke remains high and is expected to increase due to population growth and aging. Acute ischemic stroke accounts for over 80% of strokes.[1] Following the disruption of cerebral blood flow, ischemic neural cells rapidly release damage-associated molecular patterns leading to inflammation in the ischemic region. In the brain parenchyma, an inflammatory activation of microglial cells, resident immune cells of myeloid lineage that derive from embryonic yolk sac precursors, is an early event in the tissue response to stroke injury. Resident glial activation, secretion of inflammatory mediators, and infiltration of peripheral immune cells through the breached blood-brain barrier ensue. Circulating neutrophils and inflammatory monocytes infiltrate the ischemic brain and increase postischemic neuroinflammation.[2] Importantly, the response is not limited to the brain tissue, as an important systemic response is also elicited by the ischemic injury.[3] Peripheral immune cells participate in both the acute injury and in later lesion resolution.

Increased neutrophils and monocytes are consistently observed in human patients[4–8] and in rodents[4,9] after acute ischemic stroke. Circulating hyperactivated neutrophils are induced within 6 hours after stroke onset.[7] Monocyte subtypes can predict clinical outcomes after acute ischemic stroke,[10] which are largely a consequence of de novo hematopoiesis.[11] Experimental research has suggested that the primary mechanisms mediating neutrophilia and monocytosis involve cellular mobilization from the spleen[9] and the bone marrow (BM), which responds with enhanced myelopoiesis.[11] Increased sympathetic innervation is responsible for the myelopoiesis bias via activating β_3-adrenergic receptors on hematopoietic niche cells.[5,11]

THE ORIGIN AND ROUTES OF BRAIN ACCESS OF IMMUNE CELLS IN STROKE

Recruited immune cells have short life spans in the brain and can originate from several sources, including the blood, spleen, and BM. The contribution of BM cells is becoming increasingly evident, especially those coming from the skull, given its close proximity to the brain parenchyma, meninges, and lymphatics. BM, located in both flat and long bones, is a complex tissue enclosed in vascularized and innervated bone. Hematopoietic stem cells reside in the marrow and generate the hematopoietic progenitor cells required to replenish both the blood and immune system. Hematopoietic stem cells are mainly located contiguous to sinusoids, where endothelial cells and mesenchymal stromal cells promote their maintenance by producing different factors.[12]

THE ROUTES FOR ACCESS TO THE ISCHEMIC LESION MAY DEPEND ON THE ORIGIN OF THE INFILTRATING IMMUNE CELL SUBSETS

Several routes have been proposed for the entry of leukocytes into the CNS. For example, peripheral leukocytes have been traditionally proposed to infiltrate

Key Words: blood-brain barrier ■ bone marrow ■ hematopoiesis ■ immune system ■ monocytes ■ neutrophils

Correspondence to: Louise D. McCullough, MD, PhD, Department of Neurology, McGovern Medical School, The University of Texas Health Science Center, Memorial Hermann Hospital-TMC, 6431 Fannin St, Houston, TX 77030, Email louise.d.mccullough@uth.tmc.edu or María A. Moro, PhD, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain, Email mamoro@cnic.es

For Sources of Funding and Disclosures, see page 3694.

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Nonstandard Abbreviations and Acronyms

BM	bone marrow
CNS	central nervous system
CSF	cerebrospinal fluid

the injured brain parenchyma by transendothelial migration through the blood-brain barrier; however, additional pathways may be playing an important role in the access of leukocytes into the brain parenchyma, including the choroid plexus, meninges,[13] cerebrospinal fluid (CSF), and lymphatics (reviewed in Croese et al[14]). Recently discovered skull channels connecting the cranial BM to the meninges, in mice and humans, constitute a novel leukocyte portal into the CNS.[15–17] Dural lymphatic vessels that allow CSF outflow appears to facilitate neuroimmune communication.[18] In preclinical models, the skull's hematopoietic niche appears to respond more quickly than more remote marrow niches such as the tibia via transport of CSF to the cranial BM via paravascular routes.[15]

RESIDENT IMMUNE CELLS SUBSETS IN NEUROIMMUNE INTERFACES

Although once regarded as an immune-privileged organ, the CNS is immune competent and interacts actively with the peripheral immune system.[14] Together with areas such as the choroid plexus and the circumventricular organs, structures such as meninges, perivascular spaces (reviewed in Mastorakos and McGavern[19]) and dural venous sinuses[20] are sites of immune cell surveillance that have a diverse immune repertoire. CNS-associated macrophages, in steady-state conditions, reside in the choroid plexus, perivascular spaces, and meningeal spaces.[21,22] In addition to resident macrophages (dural and leptomeningeal macrophages), different immune cell populations inhabit the meninges, including dendritic cells, innate lymphoid cells, mast cells, neutrophils, B cells, and T cells. Both perivascular and meningeal macrophages act as strategically positioned sentinels to sense damage as well as respond to and sequester pathogens before they reach the parenchyma. Likewise, T cells appear to regulate meningeal lymphatics homeostasis and to influence CNS functions such as cognition and behavior (reviewed in Mastorakos and McGavern[19]), suggesting that the meningeal lymphatics participate in the trafficking of immune cells out of the CNS meninges and the CSF in the steady state. In addition, the venous dural sinuses have also been identified as a neuroimmune interface in which patrolling T cells survey brain- and CSF-derived antigens to enable CNS immune surveillance.[20]

Of note, this equilibrium is disturbed in pathological situations, where these cell subsets may contribute deleteriously to the disease process. For instance, after stroke, perivascular macrophages proliferate, promote vascular leakage, migrate in brain parenchyma, and are subsequently replaced by peripheral monocytic cells.[23,24] In addition, activation and immune infiltration of the meninges take place in stroke and other neuroinflammatory conditions, with the participation of several cell subsets including mast cells, T cells, macrophages, neutrophils, etc that contribute to pathology (reviewed in Mastorakos and McGavern[19]). These data suggest that neuroimmune interfaces could serve as targets for intervention in stroke. Moreover, they could be perturbed by comorbidities and in aging; whether this could affect the fate of these cell subsets and impact of disease outcome remains to be studied.

PERIPHERAL HEMATOPOIETIC NICHES AND POTENTIAL FOR INTERVENTION

Mobilization of BM cells has been described in numerous studies,[25,26] and the timing of entry and the composition of these cells may differ based on several factors. Based on preclinical work, age is an important but understudied factor in the immune response to stroke. There are marked differences in the composition of circulating and infiltrating leukocytes recruited to the ischemic brain of old male mice compared with young male mice. Blood neutrophilia and neutrophil invasion into the brain are increased in aged animals and may contribute to secondary hemorrhage. Higher numbers of neutrophils were found in postmortem human brain samples of old (>71 years) acute ischemic stroke subjects compared with nonischemic controls. Many of these neutrophils were found in the brain parenchyma, expressed matrix metalloproteinase-9, and were positively correlated with areas of hemorrhage and hyperemia. Therefore, the BM response to stroke is altered with aging. Heterochronic BM chimeras were generated from green fluorescent protein-expressing hosts (10 weeks or 18 months of age) to determine the contribution of peripheral immune senescence to age- and stroke-induced inflammation. Old hosts that received young BM had attenuation of age-related reductions in growth factors at baseline and had improved locomotor activity compared with isochronic controls. Microglia in young heterochronic mice (that received old BM) developed a senescent-like phenotype. After stroke, aged animals reconstituted with young BM had reduced behavioral deficits compared with isochronic controls and had significantly fewer brain-infiltrating neutrophils. Increased rates of hemorrhagic transformation were seen in young mice reconstituted with aged BM, suggesting that age-related changes can be reversed by manipulation of the peripheral immune cells in the BM.[27]

However, the origin of the donor immune cells (skull or more distant sites) was not investigated.

CNS-ASSOCIATED HEMATOPOIETIC NICHES

Recent evidence indicates that vascular beds present in the skull and meninges play an active role in the communication between the immune system and the CNS, both in homeostasis and in pathological situations. The CNS is located within bony structures equipped with 2 local hematopoietic niches, the skull and the vertebral BMs, that generate immune cells with the ability to infiltrate brain and spinal tissues in situations of damage and inflammation. Several meningeal cell subsets originate from skull BM and migrate through microscopic vascular channels crossing the skull-dura interface directly to the brain; remarkably the skull BM contributed significantly more neutrophils and myeloid cells, which arrived more quickly than those located in the tibia.[15]

In agreement with these findings, elegant work from Kipnis and Colonna's labs has recently demonstrated that, under homeostatic conditions, skull and vertebral BM are able to provide monocytes and neutrophil populations to the meninges, which show specific transcriptional signatures, different from peripheral blood-borne subsets.[17] The very novel piece of evidence indicating that cranial hematopoiesis is modulated by CSF outflow from the dura into skull BM through skull channels places CSF as a major contributor to neuroinflammation, opening new avenues of investigation in several neurological disorders including stroke.[18]

Additional work from the Colonna and Kipnis' labs has also identified the presence of a lymphopoietic niche in the calvaria BM that gives rise to B cells able to reach the meninges through specific vascular connections that mature in the dura and recognize and tolerate CNS antigens.[16] In aging mice, the meninges become populated with antigen-experienced, aged B cells derived from the peripheral circulation that have the potential to disrupt the balance of the distinct CNS immune milieu. An age-associated B cells phenotype is a relatively recent discovery that may play an important role in both neurodegenerative and vascular disease (reviewed in Engler-Chiurazzi[28]). Functionally, these cells are largely anergic and proinflammatory. Given the role of B cells in cognitive impairment,[29] whether this process plays any role in chronic stroke and in post-stroke dementia remains to be investigated.

PARTICIPATION OF PERIPHERAL TISSUES

Although neutrophils have shown a heterogeneous behavior in the context of stroke,[4,30,31] it was not until recently when it was reported that neutrophils, in homeostasis, possess the ability to adapt to tissues where they acquire distinct phenotypic and functional properties to support organ homeostasis.[32] As the predominant source of meningeal neutrophils is the skull BM in experimental stroke models, this could be a novel nonhematogenous access route to the brain. Aging is also associated with augmented neutrophil pathogenicity in ischemic stroke, and modulation of neutrophil phenotype could be a future therapeutic goal.[33]

FINAL CONSIDERATIONS

These recent studies clearly question the traditional anti-inflammatory approaches targeting transendothelial infiltration that have been tested thus far in stroke, and strongly support the design of novel therapies that consider these neuroimmune interfaces and CNS-associated hematopoietic niches, as well as its modulation by the CSF. The occurrence of different transcriptional signatures of CNS-associated niche subsets versus blood-borne ones, by which the former would favor a protective setting whereas the latter could be more proinflammatory, point to the importance of the effect of phenotypic cell heterogeneity on stroke outcome. Importantly, investigation of potential modulation of these niches for therapeutic management may lead to novel treatments for stroke.

ARTICLE INFORMATION

Affiliations

Department of Neurology, McGovern Medical School, The University of Texas Health Science Center, Memorial Hermann Hospital, Houston (L.D.M.). Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain (M.A.M.). Unidad de Investigación Neurovascular, Departamento de Farmacología y Toxicología, Universidad Complutense de Madrid (UCM), Madrid, Spain (M.A.M.). Instituto Universitario de Investigación en Neuroquímica (IUIN), UCM, Madrid, Spain (M.A.M.). Instituto de Investigación Hospital 12 de Octubre (i+12), Madrid, Spain (M.A.M.).

Sources of Funding

This work was supported by grants from the NIH R01NS103592, R37NS096493, and RFIAG069466 (Dr McCullough), Spanish Ministry of Science and Innovation PID2019-106581RB-I00 (Dr Moro), Leducq Foundation for Cardiovascular Research TNE-19CVD01 (Dr Moro), and Fundación La Caixa HR17_00527 (Dr Mora). Centro Nacional de Investigaciones Cardiovasculares (CNIC) is supported by Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación and Pro-CNIC Foundation.

Disclosures

None.

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