The American Journal of Pathology, Vol. 184, No. 9, September 2014

The American Journal of
PATHOLOGY
ajp.amjpathol.org

CELL INJURY, REPAIR, AGING, AND APOPTOSIS

Evidence that Meningeal Mast Cells Can Worsen Stroke Pathology in Mice

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Accepted for publication June 4, 2014.

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Stroke is the leading cause of adult disability and the fourth most common cause of death in the United States. Inflammation is thought to play an important role in stroke pathology, but the factors that promote inflammation in this setting remain to be fully defined. An understudied but important factor is the role of meningeal-located immune cells in modulating brain pathology. Although different immune cells traffic through meningeal vessels en route to the brain, mature mast cells do not circulate but are resident in the meninges. With the use of genetic and cell transfer approaches in mice, we identified evidence that meningeal mast cells can importantly contribute to the key features of stroke pathology, including infiltration of granulocytes and activated macrophages, brain swelling, and infarct size. We also obtained evidence that two mast cell-derived products, interleukin-6 and, to a lesser extent, chemokine (C-C motif) ligand 7, can contribute to stroke pathology. These findings indicate a novel role for mast cells in the meninges, the membranes that envelop the brain, as potential gatekeepers for modulating brain inflammation and pathology after stroke. *(Am J Pathol 2014, 184: 2493—2504; http://dx.doi.org/10.1016/j.ajpath.2014.06.003)*

Stroke, the leading cause of adult disability and the fourth most common cause of death in the Unites States,[1,2] occurs when there is insufficient blood flow to the brain, and the resultant injury initiates a cascade of inflammatory events, including immune cell infiltration into the brain.[3—5] This post-stroke inflammation is a critical determinant of damage and recovery after stroke; understanding the interplay between the immune system and the brain after stroke holds much promise for therapeutic intervention.[4—7] However, successfully exploiting this therapeutic potential requires a detailed understanding of the interplay between the immune system and the brain after stroke.[4]

An understudied but important aspect of this interplay is the role of meningeal-located immune cells in modulating brain pathology. The meninges have long been recognized as an anatomical barrier that protects the central nervous system (CNS). However, accumulating evidence suggests that the meninges are important for communication between the CNS and immune system during health and disease.[8—10] All blood vessels pass through the meningeal subarachnoid space before entering the brain, and this vascular connection and the close proximity of the meninges to the underlying

Supported in part by NIH grants NS080062 and NS37520-08 (G.K.S.) and AI070813, AI023990, and CA072074 (S.J.G.); Russell and Elizabeth Siegelman (G.K.S.); Bernard and Ronni Lacroute (G.K.S.); William Randolph Hearst Foundation (G.K.S.); NHMRC Career Development Fellowship and NHMRC project grants (M.A.G.); and Stanford School of Medicine Dean's Fellowship (Neizer Funds) (A.A.).

T.M.B. and G.K.S. contributed equally to this work as senior authors.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2014.06.003

parenchymal nervous tissue make them ideally located to act as a gatekeeper to modulate immune cell trafficking to the CNS. To support this gatekeeper function is evidence that the meninges modulate brain infiltration of T cells, neutrophils, and monocytes during meningitis and autoimmune conditions,[11–14] with immune cells observed in some instances accumulating in the meninges before they infiltrate into the parenchyma.[11,13]

Emerging evidence suggests that the actions of immune cells resident in the meninges are important for this gatekeeper function.[11,12,15] Mast cells (MCs), best known as proinflammatory effector cells, can play critical roles in the development of inflammation in many disease settings.[16–18] MCs reside in high numbers within the meninges, but their function in this site has not been fully investigated in stroke pathology. Unlike most immune cells, mature MCs do not circulate in the blood but are long-term residents of tissues, often in perivascular locations, and can rapidly perform their functions *in situ*. CNS MCs are found in the brain parenchyma and the meninges of rodents and humans.[18] It has been proposed that brain parenchymal MCs can enhance brain neutrophil numbers after stroke and can exacerbate stroke pathology.[19–24] However, much of the evidence to support such conclusions is indirect. For example, some of the studies that implicate MCs in stroke pathology used pharmacologic approaches to interfere with MC activation,[19,20,22] but such drugs can have effects on other cell types.[25] Moreover, the role of the meningeal MCs in modulating post-stroke inflammation and pathology is unknown. Finally, little is understood about which among the many MC-derived mediators may be important in stroke pathology.[17,26]

To address these questions, we used genetic and cell transfer approaches to study the role of MCs in the pathology of ischemic stroke in mice. Specifically, we tested a c-*kit*–mutant mouse model (ie, WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice) which is profoundly MC deficient and can be repaired of this deficiency by engraftment of *in vitro*-derived MCs from wild-type (WT) mice. This MC knock-in approach enables the MC-dependent effects in the mutant mice to be separated from effects due to other abnormalities associated with their mutation,[11,17,26,27] because only the MC deficiency is repaired by MC engraftment. Furthermore, one can investigate the mechanisms by which MCs influence stroke pathology by engrafting MCs from transgenic mice that lack specific MC-associated products. We also tested our newly described *Cpa3-Cre; Mcl-1*$^{fl/fl}$ mice, in which MC (and basophil) numbers are reduced constitutively via Cre-mediated depletion of the anti-apoptotic factor, myeloid cell leukemia sequence 1 (Mcl-1), in the affected lineages.[28] *Cpa3-Cre; Mcl-1*$^{fl/fl}$ mice lack the other abnormalities associated with the c-*kit* mutations in WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice.[28]

With the use of these *in vivo* models, we identified meningeal MCs as important contributors to key features of stroke pathology, including increased numbers of brain granulocytes and activated macrophages, brain swelling, and infarct size. We also obtained evidence that two potentially proinflammatory MC-derived products, IL-6 and, to a lesser extent, chemokine (C-C motif) ligand 7 (CCL7), can contribute to pathology in this setting.

Materials and Methods

Mice

Male c-*kit*–mutant genetically MC-deficient (WB/Rej-*Kit*^{W}/J × C57BL/6J-*Kit*$^{W\text{-}v}$/J)F$_1$-*Kit*$^{W/Wv}$ (WBB6F$_1$-*Kit*$^{W/W\text{-}v}$) mice and their congenic WT (WBB6F$_1$-*Kit*$^{+/+}$) littermates were purchased from The Jackson Laboratory (Bar Harbor, ME). *Kit*$^{W/W\text{-}v}$ mice have a profound deficiency in MCs[29] and certain other hematological abnormalities; however, only the MC deficiency is repaired by MC engraftment.[17,26,30] *Kit*$^{W/W\text{-}v}$ mice have lower levels of neutrophils than the corresponding WT mice in the bone marrow (BM), blood, and spleen and have a mild anemia.[27] *W* is a null allele of *Kit* and *W*^{v} is a point mutation in the cytoplasmic tail of the receptor.[17,26] *Cpa3-Cre;Mcl-1*$^{fl/fl}$ mice are severely deficient in MCs and also have a marked deficiency in basophils.[28] In these mice, Cre recombinase is expressed under the control of carboxypeptidase A3 (*Cpa3*) promoter. Mcl-1 is an intracellular anti-apoptotic factor that is required for MC survival. C57BL/6-*Cpa3-Cre; Mcl-1*$^{+/+}$ mice were used as WT controls for *Cpa3-Cre;Mcl-1*$^{fl/fl}$ mice. IL6–knock-out (KO) mice (B6.129S2-*Il6*^{tm1Kopf}/J) were purchased from The Jackson Laboratory. CCL7-KO mice[31] on a C57BL/6 background were initially developed and were a kind gift from Israel F. Charo (University of California San Francisco, San Francisco, CA). All of the animal procedures were approved by Stanford University Administrative Panel on Laboratory Animal Care.

MC–Knock-In Mouse Model

The MC deficiency in WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice was selectively repaired by systemic (intravenously through retro-orbital injection under isoflurane anesthesia) or by meningeal administration of mouse BM-derived cultured MCs (BMCMCs) generated *in vitro*, as indicated. As described before,[32] the femoral and tibial BM cells from WBB6F1-*Kit*$^{+/+}$, C57BL/6-*Kit*$^{+/+}$, C57BL/6-IL6-KO, and C57BL/6-CCL7-KO mice were cultured in 20% medium conditioned by the growth of the WEHI-3 mouse myelomonocytic cell line (containing IL-3) for 4 to 5 weeks. Before engraftment, >95% of cultured cells were identified as BMCMCs by May-Grünwald-Giemsa stain. For systemic engraftment, 10^7 BMCMCs in 100 μL of phosphate-buffered saline were injected retro-orbitally into 9- to 11-week-old WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice (50 μL into each retro-orbital side). For meningeal engraftment, 10^6 BMCMCs or vehicle alone (as a control) were injected into 9- to 11-week-old WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mice, as described.[15] The mice were used for the experiments 8 to 10 weeks after either type (ie, i.v. or meningeal) of engraftment. In experiments that used such MC-engrafted mice, WT mice and MC-deficient mice