Journal Club

Editor's Note: These short reviews of recent *JNeurosci* articles, written exclusively by students or postdoctoral fellows, summarize the important findings of the paper and provide additional insight and commentary. If the authors of the highlighted article have written a response to the Journal Club, the response can be found by viewing the Journal Club at www.jneurosci.org. For more information on the format, review process, and purpose of Journal Club articles, please see <http://www.jneurosci.org/content/jneurosci-journal-club>.

Adaptive Immune Cells Link Microbial Metabolites to Stroke Recovery

Kathleen Miller-Rhodes

Neuroscience Graduate Program, University of Rochester Medical Center, Rochester, New York 14620
Review of Sadler et al.

Ischemic stroke has pervasive effects outside the CNS, leading to a cascade of inflammatory events that drives multiorgan dysfunction and hampers long-term stroke recovery. Peripheral organs, such as the lung (Mai et al., 2017, 2019), heart (Bieber et al., 2017), and intestine (Singh et al., 2016; Stanley et al., 2016), are particularly susceptible to stroke-induced injury and inflammation. For example, stroke increases the permeability of intestinal barriers (Singh et al., 2016; Stanley et al., 2016), alters bacterial composition of the gut, causing gut dysbiosis (Houlden et al., 2016; Singh et al., 2016), and promotes seeding of gut bacteria within the lung, spleen, and liver (Stanley et al., 2016). Such peripheral effects might exert additional effects on the damaged brain, thus hindering recovery. Indeed, the gut microbiome has been shown to impact stroke recovery (Xia et al., 2019). It is unclear how the gut microbiota influences the brain following stroke, but one possibility is through the production of short chain fatty acids (SCFAs). Indeed, SCFAs are important immunomodulatory factors (Vinolo et al., 2011) that help maintain brain homeostasis and promote recovery in certain

neurologic conditions (e.g., Parkinson's disease) (Dalile et al., 2019). In a recent publication in *The Journal of Neuroscience*, Sadler et al. (2020) examined the role of SCFAs in stroke recovery and found that SCFAs improve recovery, in part, through effects on T cells.

To investigate the relationship between SCFAs and stroke, Sadler et al. (2020) modeled stroke in mice using the middle cerebral artery occlusion method. Stroke led to a reduction in circulating SCFAs. To determine whether this reduction in SCFAs affected stroke recovery, the authors turned to a photothrombotic stroke model, which produces small, cortical infarcts in a predefined area (in this study, the motor cortex), allowing detailed mechanistic analyses. Previous fMRI studies in human stroke patients have shown changes in transhemispheric connectivity between the lesioned (ipsilesional) and uninjured (contralesional) hemisphere. Disruptions in connectivity can impair long-term stroke recovery in humans (Rehme and Greffkes, 2013). With this in mind, Sadler et al. (2020) examined the effects of SCFAs on motor cortical plasticity after stroke using wide-field calcium imaging in mice expressing the calcium indicator GCaMP6S in neurons. Like fMRI, this system indirectly measures neuronal activity and provides insight into connectivity among brain regions (Sadler et al., 2020). The authors imaged the resting-state fluorescent calcium signal, then measured correlations between the signal in the lesioned motor cortex and the contralateral motor cortex as a readout

for cortical connectivity. Consistent with previous work, the area of the contralesional hemisphere with significant connectivity was increased at 3 and 6 weeks after stroke. Importantly, these effects were abrogated by SCFA supplementation (initiated 4 weeks before stroke induction and continued until endpoint analysis). SCFA supplementation also increased dendritic spine density within the contralesional hemisphere but simultaneously reduced the number of presynaptic synapses in the perilesional cortex. The changes in cortical connectivity and synapses might reflect an increase in inhibitory connections from the ipsilesional hemisphere to the contralateral hemisphere or increased expression of inhibitory GABA receptors (Huang et al., 2018). Importantly, SCFAs improved motor function 8 weeks after stroke.

To determine whether SCFAs act directly on neurons or affect neuronal activity and morphology indirectly through non-neuronal cells, Sadler et al. (2020) performed unbiased RNA sequencing on total RNA isolated from the perilesional cortex 2 weeks after stroke. A comparison between their data and existing RNA-seq data indicated that the genes differentially regulated between mice receiving SCFA supplementation and those receiving no supplementation were largely associated with microglia. Subsequent assessment of microglial morphology and density at the same time point revealed that SCFA supplementation resulted in more ramified (less activated) microglia and reduced microglia numbers in the ipsilesional hemisphere.

Received Mar. 17, 2020; revised May 15, 2020; accepted June 4, 2020.

This work was supported by American Heart Association Predoctoral Fellowship 18PRE33960172 to K.M.-R. I thank Patrick Miller-Rhodes for insightful discussion and initial review of this work.

The author declares no competing financial interests.

Correspondence should be addressed to Kathleen Miller-Rhodes at kathleen_millerrhodes@urmc.rochester.edu.

<https://doi.org/10.1523/JNEUROSCI.0634-20.2020>

Copyright © 2020 the authors

Based on the lack of SCFA receptor expression in microglia (Erny et al., 2015), existing evidence of T-cell infiltration in photothrombotic infarcts at this time point (Feng et al., 2017), as well as the fact that T cells tune the inflammatory status of microglia by secreting proinflammatory and anti-inflammatory cytokines (Wang et al., 2016), Sadler et al. (2020) asked whether altered T-cell infiltration might explain the effects of SCFAs on microglia. In support of this possibility, they observed a reduction in peripheral T-cell pools and a corresponding reduction of T-cell infiltration into the brain 2 weeks after stroke with SCFA supplementation. To test whether T cells are required for the effects of SCFAs on microglial activation following stroke, Sadler et al. (2020) evaluated microglial activation in mice depleted of T cells (i.e., Rag1^{−/−} mice). As hypothesized, SCFAs did not inhibit microglial activation after stroke in Rag1^{−/−} mice as they did in immunocompetent mice. However, the authors did not evaluate functional recovery in Rag1^{−/−} mice to determine whether the microglial effects are directly related to recovery (Sadler et al., 2020).

Overall, these findings demonstrate that T cells are required for the effects of SCFA on microglia during stroke recovery. Yet questions remain about how stroke alters the generation of SCFAs, whether SCFAs alter T-cell polarization, and whether the T-cell-dependent effects they observed on microglia are important for stroke recovery. Possible answers to these questions are discussed below.

What is the initial signal that reduces levels of circulating SCFAs after ischemic insult? Increases in intracranial pressure following stroke lead to hyperactivation of the sympathetic nervous system (Schmidt et al., 2018). Numerous studies have suggested that sympathetic nervous system activity drives intestinal gut leak and dysbiosis (Houlden et al., 2016; Singh et al., 2016; Liu et al., 2019). Gut leak occurs as early as 3 h after stroke (Stanley et al., 2016; Liu et al., 2019) with signs of dysbiosis by 24 h (Singh et al., 2016). These changes are a result of increased intestinal adrenergic signaling and elevated gut catecholamine levels (Houlden et al., 2016; Singh et al., 2016; Stanley et al., 2016). For example, studies that deplete peripheral adrenergic nerve endings demonstrate that a robust release of norepinephrine shifts microbial composition. When the nerve terminals regenerate, the gut bacterial composition returns to control levels (Lyte and Bailey, 1997). Because different microbial populations generate varying

amounts of SCFAs (Ratajczak et al., 2019), changes in the gut microbiome might explain the reduction in SCFAs seen by Sadler et al. (2020).

SCFAs could improve functional recovery by influencing T-cell polarization. There are numerous types of T cells, but they can be broken down into two general groups: proinflammatory and anti-inflammatory. The balance of proinflammatory and anti-inflammatory T-cell subsets often dictates the functional outcome of an immune response, although this depends on the context (i.e., anti-inflammatory responses are beneficial in some contexts but can be detrimental in others). While Sadler et al. (2020) did not evaluate distinct T-cell populations, a few studies show that SCFAs can influence T-cell polarization. For example, under physiological conditions, SCFAs facilitate differentiation of naive T cells into “helper” T cells (Park et al., 2015), which are important in modulating immune responses. Notably, dietary SCFAs increase the number and anti-inflammatory functions of regulatory helper T cells (Tregs) (Smith et al., 2013). In a recent publication, Lee et al. (2020) provided the first evidence demonstrating that SCFAs reduce proinflammatory, IL-17⁺ T-cell infiltration into the brain. Other studies looking at gut dysbiosis have also shown changes in T-cell polarization. For example, recolonizing the gut microbiome of germ-free mice increases the polarization of T cells into the Treg subset, resulting in reduced infarct volumes after stroke (Singh et al., 2016). In addition to influencing T-cell polarization, SCFAs may affect T-cell migration to the brain, as they alter T-cell migration from the gut to the eye during experimental autoimmune uveitis (Nakamura et al., 2017). While there is currently no evidence that SCFAs affect T-cell migration to the brain, previous studies have shown that intestinal T cells migrate to the brain after stroke (Benakis et al., 2016; Singh et al., 2016).

Although Sadler et al. (2020) show that the beneficial effects of SCFA on microglia are T cell-dependent, it is unclear whether these effects are important for stroke recovery. Indeed, their transcriptomic data showing differential expression of many microglial genes in the perilesional cortex suggest, but do not prove, the importance of microglia in the SCFA-mediated enhancement of recovery from stroke in mice. In fact the authors also identified transcriptional changes in neurons following SCFA supplementation, including increases in both the presynaptic vesicle

molecule synaptophysin and the BDNF receptor TrkB in the perilesional cortex. Thus, despite their conclusion that microglia are the main cellular target of SCFAs, neurons might be a more relevant target. This hypothesis is supported by the fact that neurons express the SCFA receptor (Dalile et al., 2019) unlike microglia (Erny et al., 2015) and T cells (Park et al., 2015). Overall, because the effects of SCFAs on stroke recovery could be mediated through direct effects on neurons, it remains possible that the microglial effects they observed are epiphenomenal and not important for functional recovery following SCFA supplementation, a point the authors allude to in their discussion. That said, SCFAs could exert effects on any of these cell types through mechanisms not involving the SCFA receptor. Indeed, SCFAs have been shown to alter T-cell polarization by inhibiting histone deacetylases independent of SCFA receptor (Park et al., 2015).

In conclusion, Sadler et al. (2020) identified a novel immunologic connection between microbial SCFAs and stroke recovery. Information about the specific T-cell subsets that mediate the effects of SCFA on stroke recovery can inform the development of immunomodulatory therapeutics that could be used to hasten or improve stroke recovery. In the meantime, dietary SCFA supplementation and/or fecal matter transplants (to restore the gut microbiome) could be explored in human stroke patients to determine whether the gut microbiome represents a viable therapeutic path for stroke recovery.

References

- Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, Sita G, Racchumi G, Ling L, Pamer EG, Iadecola C, Anrather J (2016) Commensal microbiota affects ischemic stroke outcome by regulating intestinal gammadelta T cells. *Nat Med* 22:516–523.
- Bieber M, Werner RA, Tanai E, Hofmann U, Higuchi T, Schuh K, Heuschmann PU, Frantz S, Ritter O, Kraft P, Kleinschnitz C (2017) Stroke-induced chronic systolic dysfunction driven by sympathetic overactivity. *Ann Neurol* 82:729–743.
- Dalile B, Van Oudenhove L, Vervliet B, Verbeke K (2019) The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol* 16:461–478.
- Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlaković T, Jakobshagen K, Buch T, Scherzweck V, Utermöhlen O, Chun E, Garret WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M (2015) Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* 18:965–977.

- Feng Y, Liao S, Wei C, Jia D, Wood K, Liu Q, Wang X, Shi FD, Jin WN (2017) Infiltration and persistence of lymphocytes during late-stage cerebral ischemia in middle cerebral artery occlusion and photothrombotic stroke models. *J Neuroinflammation* 14:248.
- Houlden A, Goldrick M, Brough D, Vizi ES, Lenart N, Martinecz B, Roberts IS, Denes A (2016) Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain Behav Immun* 57:10–20.
- Huang SY, Chang CH, Hung HY, Lin YW, Lee EJ (2018) Neuroanatomical and electrophysiological recovery in the contralateral intact cortex following transient focal cerebral ischemia in rats. *Neurol Res* 40:130–138.
- Lee J, d'Aigle J, Atadja L, Quaiocoe V, Honarpisheh P, Ganesh BP, Hassan A, Graf J, Petrosino JF, Putluri N, Zhu L, Durgan DJ, Bryan RM Jr, McCullough LD, Venna VR (2020) Gut microbiota-derived short-chain fatty acids promote post-stroke recovery in aged mice. *Circ Res*. Advance online publication. Retrieved May 1, 2020. doi: 10.1161/CIRCRESAHA.119.316448.
- Liu Q, Johnson EM, Lam RK, Wang Q, Bo Ye H, Wilson EN, Minhas PS, Liu L, Swarovski MS, Tran S, Wang J, Mehta SS, Yang X, Rabinowitz JD, Yang SS, Shamloo M, Mueller C, James ML, Andreasson KI (2019) Peripheral TREM1 responses to brain and intestinal immunogens amplify stroke severity. *Nat Immunol* 20:1023–1034.
- Lyte M, Bailey BS (1997) Neuroendocrine-bacterial interactions in a neurotoxin-induced model of trauma. *J Surg Res* 70:195–201.
- Mai N, Prifti L, Rininger A, Bazarian H, Halterman MW (2017) Endotoxemia induces lung-brain coupling and multi-organ injury following cerebral ischemia-reperfusion. *Exp Neurol* 297:82–91.
- Mai N, Miller-Rhodes K, Prifti V, Kim M, O'Reilly MA, Halterman MW (2019) Lung-derived SOD3 attenuates neurovascular injury after transient global cerebral ischemia. *J Am Heart Assoc* 8:e011801.
- Nakamura YK, Janowitz C, Metea C, Asquith M, Karstens L, Rosenbaum JT, Lin P (2017) Short chain fatty acids ameliorate immune-mediated uveitis partially by altering migration of lymphocytes from the intestine. *Sci Rep* 7:11745.
- Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, Kim CH (2015) Short-chain fatty acids induce both effector and regulatory T cells by suppression of the histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 8:80–93.
- Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M (2019) Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). *Acta Biochim Pol* 66:1–12.
- Rehme AK, Grefkes C (2013) Cerebral network disorders after stroke: evidence from imaging-based connectivity analyses of active and resting brain states in humans. *J Physiol* 591:17–31.
- Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurbier KR, Northoff BH, Heijink M, Goldberg MP, Plautz EJ, Roth S, Malik R, Dichgans M, Holdt LM, Benakis C, Giera M, Stowe AM, Liesz A (2020) Short-chain fatty acids improve poststroke recovery via immunological mechanisms. *J Neurosci* 40:1162–1173.
- Schmidt EA, Despas F, Pavy-Le Traon A, Czosnyka Z, Pickard JD, Rahmouni K, Pathak A, Senard JM (2018) Intracranial pressure is a determinant of sympathetic activity. *Front Physiol* 9:11.
- Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, Dichgans M, Liesz A (2016) Microbiota dysbiosis controls the neuroinflammatory response after stroke. *J Neurosci* 36:7428–7440.
- Smith PM, Howitt MR, Panikov N, Michaud M, Gallini C, Bohlooly-Y M, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic T cell homeostasis. *Science* 341:569–573.
- Stanley D, Mason LJ, Mackin KE, Srikantha YN, Lyras D, Prakash MD, Nurgali K, Venegas A, Hill MD, Moore RJ, Wong CH (2016) Translocation and dissemination of commensal bacteria in post-stroke infection. *Nat Med* 22:1277–1284.
- Vinolo MA, Rodrigues HG, Nachbar RT, Curi R (2011) Regulation of inflammation by short chain fatty acids. *Nutrients* 3:858–876.
- Wang S, Zhang H, Xu Y (2016) Crosstalk between microglia and T cells contributes to brain damage and recovery after ischemic stroke. *Neurol Res* 38:495–503.
- Xia GH, You C, Gao XX, Zeng XL, Zhu JJ, Xu KY, Tan CH, Xu RT, Wu QH, Zhou HW, He Y, Yin J (2019) Stroke Dysbiosis Index (SDI) in gut microbiome are associated with brain injury and prognosis of stroke. *Front Neurol* 10:397.