A developmental switch in microglial HDAC function

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Summary

The epigenetic mechanisms controlling microglia functions are largely unknown. In this issue of *Immunity*, Datta et al. (2018) uncover surprising and distinct effects of deleting the epigenetic regulators HDAC1 and HDAC2 during microglial development vs. during the course of neurodegeneration.

Main text

Microglia are tissue-resident macrophages in the brain and spinal cord that play important roles in development, health and diseases of the central nervous system (CNS) (Li & Barres 2017; Prinz & Priller 2014). In this issue of Immunity, Datta et al. (2018) show that the histone deacetylases HDAC1 and HDAC2 are critical regulators of microglia maturation and function. The authors find that deletion of *Hdac1* and *Hdac2* (henceforth referred to as *Hdac1-2)* in microglia prior to birth strongly impairs microglial numbers and morphological development, while deletion in adult microglia has no effect on either parameter. Strikingly however, the authors find that the loss of *Hdac1-2* reduces amyloid burden and improves cognitive function in an Alzheimer's disease mouse model (Figure 1). Thus, the authors have identified a critically important developmental switch in the requirement for HDAC1 and HDAC2 in microglial cells; loss of these proteins is detrimental during development, but beneficial during the process of neurodegeneration.

Microglia have been described as the damage sensors of the central nervous system (Fourgeaud et al. 2016). They constantly scan their environment with highly motile processes and react to nearly any perturbation of CNS homeostasis. They clear apoptotic cells but are also involved in eliminating synapses for proper neural circuit wiring (Aguzzi et al. 2013). Furthermore, a growing body of evidence suggests that microglial dysfunction can play a causal role in the pathogenesis of several neurological disorders, including Alzheimer's disease (Li & Barres 2017).

Microglia originate from erythro myeloid progenitor cells in the yolk sac and colonize the brain early during development where they are maintained by self-renewal with little contribution from bone marrow-derived immune cells (Salter & Stevens 2017). Microglia must fulfill different functions as they mature in synchrony with the neurons of the developing brain. Early during CNS development microglia

are involved in synaptic pruning and neurogenesis, whereas in the adult brain they regulate neuroinflammation and play homeostatic roles by clearing pathogens and cellular debris from the parenchyma. Indeed, development and maturation of microglia is accompanied by profound transcriptional and chromatin changes (Matcovitch-Natan et al. 2016). Datta et al. (2018) sought to identify the cascade of epigenetic events that orchestrate these changes and focused their analysis on the HDAC1 and HDAC2.

To investigate the roles of HDAC1-2 in microglia development, the authors generated mice that express the Cre-recombinase under the control of the CX3C chemokine receptor 1 (Cx3cr1) promoter in combination with conditional alleles of both *Hdac1* and *Hdac2* (*Cx3cr1Cre Hdac1*$^{fl/fl}$ *Hdac2*$^{fl/fl}$ mice). In these animals, the Cre-recombinase is expressed starting from mouse embryonic day 9 in primitive yolk sac macrophages that give rise to microglia. Consequently, microglia in these animals lack *Hdac1-2* starting from an early time point of development. To assess the effects of *Hdac1-2* deficiency on microglia, the authors performed immunostaining and quantified the number of cells expressing the microglia marker Iba1. Compared to control littermates that do not express the Cre recombinase (Cre^{-} mice), the number of cortical microglia was strongly reduced at embryonic day 16.5, postnatal day (P) 0 and at 2 weeks of age in mice expressing the Cre recombinase (Cre^{+}). Furthermore, microglia lacking *Hdac1-2* had fewer and shorter processes. Interestingly, the difference in microglia numbers normalized by 6 weeks of age due to increased microglia proliferation, while the morphological abnormalities persisted into adulthood.

Attempting to understand the molecular mechanisms underlying the reduced number and malformation of microglia lacking HDAC1-2, the authors performed transcriptomic analyses. Gene expression was most drastically altered at birth (P0) with 4338 differentially expressed genes comparing HDAC1-2 deficient microglia to microglia isolated from Cre^{-} mice. At 2 weeks and 6 weeks of age fewer differentially expressed genes were detected (2 weeks: 1820; 6 weeks: 692), consistent with the notion that HDAC1-2 are especially crucial for early microglia development. The biological functions associated with the differentially expressed genes included cell morphology, cell growth and proliferation, cell death and survival, consistent with the observed cellular phenotypes of microglia lacking HDAC1-2.

To investigate the effects of HDAC1-2 deficiency on adult microglia, the authors used a tamoxifen-dependent inducible Cre-recombinase under the control of the Cx3cr1 promoter (Cx3cr1CreERT2). Treatment of *Cx3cr1CreERT2 Hdac1*$^{fl/fl}$*Hdac2*$^{fl/fl}$ mice with tamoxifen significantly downregulated *Hdac1-2* expression compared to control animals. Surprisingly, the number and morphology of microglia was unchanged when *Hdac1-2* were deleted in adult mice. Thus, HDAC1-2 seem to be essential for proper microglia development but dispensable for the steady state maintenance of adult microglia.

It is well known that microglial morphology and function drastically change during neurodegeneration (Colonna & Butovsky 2017). In the diseased brain microglia exhibit a more amoeboid morphology, they actively migrate, and their proliferative activity is increased. Therefore, the authors next tested whether HDAC1-2 affect microglia effector functions in a neurodegenerative environment. To do so, the authors crossed *Cx3cr1CreERT2 Hdac1*$^{fl/fl}$*Hdac2*$^{fl/fl}$ mice with the 5XFAD model, a well-established mouse model harboring familial AD mutations. In the 5XFAD model altered amyloid processing results in the formation of amyloid plaques, a key pathological hallmark of Alzheimer's disease. To test whether the loss of HDAC1-2 in microglia is beneficial or detrimental to AD pathology, the authors injected 5XFAD *Cx3cr1CreERT2 Hdac1*$^{fl/fl}$*Hdac2*$^{fl/fl}$ (FAD:Hdac1-2-deficient) mice with tamoxifen before the onset of amyloid deposition and monitored the progression of pathology. Strikingly, the amyloid plaque burden was strongly reduced in the FAD:Hdac1-2-deficient mice compared to FAD mice. This was evidenced by

an immunohistochemical analysis which revealed a strong reduction of plaque area and intensity in both the cortex and hippocampus of the FAD:Hdac1-2-deficient mice compared to FAD mice. Similarly, enzyme-linked immunosorbent assay (ELISA) measurements showed reduced levels of both soluble and insoluble forms of the amyloid peptide in FAD:Hdac1-2-deficient mice. How does the loss of HDAC1-2 in microglia reduce the amyloid plaque burden in FAD mice? The authors hypothesized that the lack of HDAC1-2 might increase microglia phagocytosis of amyloid plaques. Consistent with this idea, FAD:Hdac1-2-deficient microglia exhibited increased Lamp2 staining and increased co-localization with amyloid beta signal via immunostaining. In addition, microglia lacking HDAC1-2 showed increased expression of several genes related to phagocytosis. While the authors did not directly demonstrate altered amyloid uptake and/or phagocytic capacity of microglia lacking HDAC1-2, the findings suggest that increased phagocytosis of amyloid plaques likely underlies the reduced amyloid burden in FAD:Hdac1-2-deficient mice.

Finally, the authors tested whether the loss of HDAC1-2 in microglia also rescues cognitive deficits apparent in the mouse model of AD. To test spatial learning and memory function, they performed Morris water maze tests, a navigation task during which the animal is required to find an invisible platform that allows it to escape the water. Once the animal has learned the position of the platform, the platform is removed and the time the animal spends in the target quadrant (where the platform used to be) is used as a measure of the animal's memory function. Indeed, FAD:Hdac1-2-deficient mice spent significantly more time in the target quadrant than FAD mice. FAD:Hdac1-2-deficient mice also performed better than FAD mice in a second behavioral paradigm that tests working and reference memory. Thus, deletion of *Hdac1-2* from adult microglia not only reduces amyloid plaque burden but also rescues spatial learning and memory deficits in FAD mice.

The contrasting effects of *Hdac1-2* deletion on developing microglia compared to mature microglia in AD model mice suggest numerous avenues for additional study. For instance, what factors govern the developmental changes in *Hdac1* and *Hdac2* expression? Considering the apparent increase in amyloid plaque phagocytosis in *Hdac1-2*-deficient microglia, is synaptic pruning also increased by *Hdac1-2* deletion? Are there morphological differences in microglia or alterations in neuroinflammation in FAD mice due to deletion of *Hdac1-2*? Are neuron and/or synapse integrity in FAD mice protected by microglial *Hdac1-2* deletion in parallel with the impressive reduction in amyloid plaque burden and improved cognitive function? A more in depth analysis of the transcriptional and epigenetic changes in microglia associated with loss of HDAC1-2 in FAD mice could also help to shed light on these and other questions.

Histone deacetylases play a crucial role during brain development, healthy brain function and neurological diseases (Gräff & Tsai 2013; Volmar & Wahlestedt 2014). Datta et al. (2018) provide the first genetic analysis of *Hdac1* and *Hdac2* functions in microglia. During development, *Hdac1-2* deletion disrupts microglial proliferation and morphology, effects that are not seen following deletion of *Hdac1/2* in adult microglia. Remarkably however, *Hdac1-2* deletion from adult microglia in AD model mice reduces amyloid plaque burden, increases amyloid co-localization with microglia and rescues cognitive deficits; thus HDAC1 and HDAC2 form an epigenetic blockade in microglia that prevents beneficial effects of these cells in the neurodegenerating brain. When this epigenetic blockade is released, microglia appear to become more phagocytic and can reduce AD pathology. Thus, the findings by Datta et al. indicate that microglia can be epigenetically reprogrammed towards a more beneficial state that has the capacity to ameliorate AD pathology. These are important insights as they might guide the development of therapeutic strategies for Alzheimer's disease.

Figure legend

Figure 1

HDACs 1 and 2 are essential for microglia development but their loss in adult microglia reduces pathology in a mouse model of Alzheimer's disease.

Genetic deletion of *Hdac1* and *Hdac2* in microglia prior to birth strongly impairs microglial development, resulting in reduced cell numbers and altered morphology (left). In contrast, deletion of *Hdac1* and *Hdac2* in adult microglia has no effect on cell number or morphology during homeostasis (middle). *Hdac1-2* deletion from adult microglia in Alzheimer's disease model mice reduces amyloid plaque burden, increases amyloid co-localization with microglia and rescues cognitive deficits (right).

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