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Alternative activation-skewed microglia/macrophages promote hematoma resolution in experimental intracerebral hemorrhage

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ABSTRACT

Microglia/macrophages (MMΦ) are highly plastic phagocytes that can promote both injury and repair in diseased brain through the distinct function of classically activated and alternatively activated subsets. The role of MMΦ polarization in intracerebral hemorrhage (ICH) is unknown. Herein, we comprehensively characterized MMΦ dynamics after ICH in mice and evaluated the relevance of MMΦ polarity to hematoma resolution. MMΦ accumulated within the hematoma territory until at least 14 days after ICH induction. Microglia rapidly reacted to the hemorrhagic insult as early as 1–1.5 h after ICH and specifically presented a "protective" alternatively activated phenotype. Substantial numbers of activated microglia and newly recruited monocytes also assumed an early alternatively activated phenotype, but the phenotype gradually shifted to a mixed spectrum over time. Ultimately, markers of MMΦ classic activation dominated at the chronic stage of ICH. We enhanced MMΦ alternative activation by administering intraperitoneal injections of rosiglitazone, and subsequently observed elevations in CD206 expression on brain-isolated $CD11b^+$ cells and increases in IL-10 levels in serum and perihematomal tissue. Enhancement of MMΦ alternative activation correlated with hematoma volume reduction and improvement in neurologic deficits. Intraventricular injection of alternative activation signature cytokine IL-10 accelerated hematoma resolution, whereas microglial phagocytic ability was abolished by IL-10 receptor neutralization. Our results suggest that MMΦ respond dynamically to brain hemorrhage by exhibiting diverse phenotypic changes at different stages of ICH. Alternative activation-skewed MMΦ aid in hematoma resolution, and IL-10 signaling might contribute to regulation of MMΦ phagocytosis and hematoma clearance in ICH.

1. Introduction

Intracerebral hemorrhage (ICH) accounts for the highest mortality among all strokes (van Asch et al., 2010), and no therapeutic strategy has been approved for its treatment (Keep et al., 2012). After an intraparenchymal bleed, primary injury develops within the first few hours as a result of hematoma formation, expansion, and the mass effect. Subsequently, secondary damage activates cytotoxic and inflammatory cascades (Wang, 2010; Zhou et al., 2014) that contribute substantially to neurologic deterioration in patients with ICH (Wu et al., 2010; Ziai, 2013). Although preclinical studies have tried to identify potential neuroprotectants that target secondary injury (Kellner and Connolly, 2010), mortality and morbidity of ICH have not declined (Xi et al., 2014). As targeting secondary injury does not appear to provide adequate intervention for ICH patients (Toyoda and Grotta, 2015), an alternative strategy might be to remove the hematoma before it can release the toxic hemoglobin degradation products that elicit secondary injury cascades (Ni et al., 2016; Sonni et al., 2014). Clinical evidence shows that hematoma size and expansion are the major determinants of ICH outcomes (Brott et al., 1997; LoPresti et al., 2014). Until now, however, little preclinical work has evaluated potential strategies to limit hematoma expansion or accelerate hematoma resolution after ICH (Flores et al., 2016; King et al., 2011; Wu et al., 2016; Zhao et al., 2015b; Zhao et al., 2007b).

Microglia/macrophages (MMΦ) are the major phagocytes in the innate immune system. Under in vitro conditions, MMΦ can be polarized into distinct classically and alternatively activated phenotypes; however, in response to microenvironmental signals in vivo, MMΦ dynamically switch their phenotype, both temporally during an immune response and based on the local stimuli (Hu et al., 2015; Mosser and Edwards, 2008). Accumulating evidence indicates that classically activated and alternatively activated MMΦ within the lesion area can individually

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contribute to tissue damage or repair in the brain under conditions of spinal cord injury (Kigerl et al., 2009; Kroner et al., 2014), traumatic brain injury (Kumar et al., 2013; Wang et al., 2013), ischemic stroke (Hu et al., 2012), multiple sclerosis (Mikita et al., 2011), and Parkinson's disease (Pisanu et al., 2014). To date, no study has comprehensively characterized MMΦ dynamics or delineated the functional significance of MMΦ phenotype on hematoma resolution in vivo (Wan et al., 2016; Zhang et al., 2016; Zhao et al., 2015a).

Under physiologic conditions, macrophages have the ability to clear senescent erythrocytes and regulate iron homeostasis by metabolizing hemoglobin and its metabolic byproducts (Mosser and Edwards, 2008). Microglia, the brain's resident macrophages, are capable of clearing erythrocytes after ICH (Egashira et al., 2015), and pharmacologic enhancement of microglial phagocytosis has been shown to reduce hematoma volume in a mouse blood-injection model of ICH (Zhao et al., 2007b). Modulation of MMΦ phenotype might offer a therapeutic strategy for treating ICH by limiting hematoma expansion (Wang et al., 2003; Zhao et al., 2015a; Zhao et al., 2009). However, the relationship between MMΦ phenotype, phagocytosis, and hematoma resolution after ICH is still unknown (Zhao et al., 2015a). One subset of alternatively activated MMΦ that might potentially be beneficial for the process of hematoma clearance is interleukin (IL)-10-regulated MMΦ (Mosser and Edwards, 2008; Naito et al., 2014). IL-10/heme oxygenase (HO)-1 signaling not only regulates microglial erythrocyte clearance in subarachnoid hemorrhage (Schallner et al., 2015), but also promotes resolution of inflammation by skewing alternative activation of macrophages in a mouse model of peripheral nerve injury (Siqueira Mietto et al., 2015).

In the present study, we sought to gain an understanding of the kinetics of MMΦ polarization after ICH and how MMΦ polarization affects hematoma clearance.

2. Materials and methods

2.1. Animals

All experimental protocols were conducted in accordance with the National Institutes of Health guidelines and were approved by the Johns Hopkins University and Yale University Animal Care and Use Committee. The experiments were conducted in C57BL/6 male mice (Charles River Laboratories; Frederick, MD) and Cx3cr1$^{GFP/+}$ mice (C57BL/6 background; kind gift from Dr. Jonathan Bromberg, University of Maryland, Baltimore, MD). The sham experiments for Figs. 1, 2, 4, and 6, and the Rosiglitazone verses GW9662 experiments for Fig. 8E and F and Supplementary Fig. 3 were conducted in C57BL/6 male mice (The Jackson Laboratory) at Yale University. A total of two hundred and sixty-three mice were subjected to this study. Group size calculation was based on our previous experience of the variability, reproducibility and statistical analyses of the outcome measures in this model. To enhance the clinical relevance of the study, we used mice that were 10–12 months old because ICH occurs more often in middle-aged and elderly individuals. All efforts were made to minimize the numbers of animals used and ensure minimal suffering.

2.2. ICH model

After anesthetizing mice with 1–3% isoflurane inhalation and ventilating them with oxygen-enriched air (20%:80%), we injected a total of 0.5 μL of 0.1 U collagenase VII-S (Sigma, St. Louis, MO) at 0.1 μL/min into the left basal ganglion at the following coordinates relative to bregma: 0.8 mm anterior, 2 mm lateral, and 2.8 mm deep, as described previously (Chang et al., 2014). The craniotomy was sealed with bone wax, and the scalp was closed with 4–0 silk sutures. Rectal temperature was maintained at 37.0 ± 0.5 °C

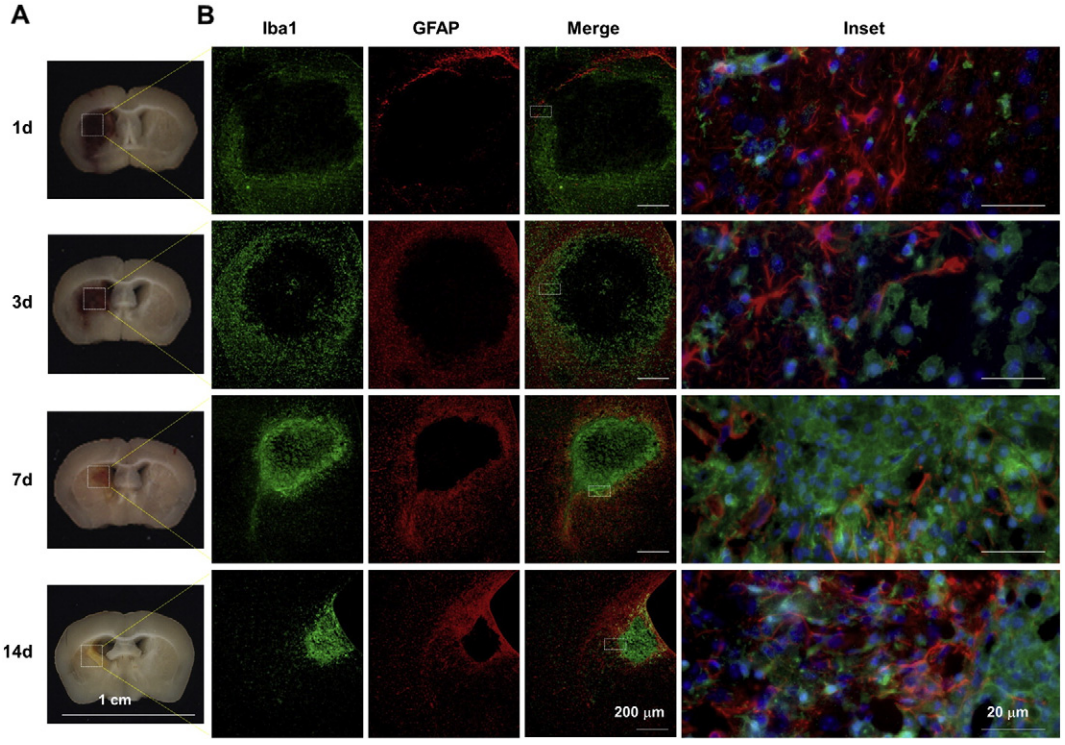

Fig. 1. Microglia/macrophages (MMΦ) accumulate within the hematoma territory until 14 days after ICH. (**A**) Representative coronal brain sections show the temporal changes in hematoma resolution after ICH. The boxed area indicates the location of representative fluorescence images in the hemorrhagic brain. Scale bar: 1 cm. $n = 3$ mice per time point. (**B**) Identification of Iba1- and GFAP-positive cells in brain sections at 1, 3, 7, and 14 days post-ICH. Immunoreactivity of Iba1 (ionized calcium binding adaptor molecule 1; MMΦ marker) and GFAP (glial fibrillary acidic protein; astrocyte marker) are shown in green and red, respectively. The inset column represents higher magnification of the boxed area in the corresponding merged images. Nuclei were stained with DAPI (blue). Scale bars: 200 μm (merged column); 20 μm (inset column). $n = 3$ mice per time point.