Brain Research 1650 (2016) 172–177

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Interleukin-19 alleviates brain injury by anti-inflammatory effects in a mice model of focal cerebral ischemia

Weiying Xie [a,1], Lili Fang [a,1], Shuyuan Gan [c], Haojun Xuan [b,*]

[a] Department of Anesthesiology, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China
[b] Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou, China
[c] Department of Anesthesiology, First Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
1 September 2016
Accepted 4 September 2016
Available online 5 September 2016

Keywords:
Stroke
Cerebral ischemia
IL-19
Inflammation

ABSTRACT

Stroke causes brain injury with neuroinflammation which exacerbates the neuronal damage. Recent studies show that anti-inflammatory cytokine interleukin-19 (IL-19) plays a critical part in the inflammatory and ischemic vascular diseases, yet its potential role in ischemic stroke is unknown. Here, we tested the hypothesis that IL-19 exerts protective effects against brain ischemia by modulating inflammation after stroke. Mice were injected intraperitoneally with 10 ng/g per day recombinant mouse IL-19 starting pre-stroke, and were subjected to transient middle cerebral artery occlusion. Infarct volume was assessed by triphenyltetrazolium chloride and neurobehavioral outcome by neurological scores. Inflammation was measured using real-time quantitative PCR, immunochemistry, and fluorescence-activated cell sorting. Infarct volume at 72 h after stroke was significantly smaller in IL-19 treated group and focal neurological score was significantly better. IL-19 treatment markedly attenuated elevation of the expression of TNF-α and IL-6 mRNA, suppressed increases in the number of microglia, macrophages, $CD4^+$ T cells, $CD8^+$ T cells as well as B cells, and blocked activation of macrophages and neutrophils in the ischemic brain. In peripheral blood, IL-19 injection helped to robustly preserve the reduced immune cells, including macrophages, $CD4^+$ T cells, $CD8^+$ T cells and B cells, compared to control group. IL-19 reduced brain infarction and attenuated neurological deficits following stroke in mice, probably by inhibiting infiltration and activation of immune cells, and by suppressing increases in gene expression of proinflammatory cytokines. This may identify IL-19 as a new therapeutic to limit neuroinflammation after stroke.

1. Introduction

Stroke is a leading cause of death and disability worldwide, yet the only clinically effective treatment to date is thrombolysis within the first few hours (Blakeley and Llinas, 2007). As mentioned, ischemic stroke results in cerebral inflammatory response that exacerbates brain injury and triggers immunodepression, leading to an increased incidence of delayed mortality in stroke patients (Iadecola and Anrather, 2011; Vogelgesang and Dressel, 2011). Resident microglia in the ischemic brain are activated rapidly after stroke, and macrophages derived from microglia and/ or circulating monocytes also contribute to acute brain injury (del Zoppo et al., 2007; Liu et al., 2001). Recent studies demonstrate that other peripheral leukocytes, including T cells, B cells and neutrophils, also recruit into the ischemic brain in the early stage post-stroke and release proinflammatory mediators (Kleinschnitz et al., 2010; Chen et al., 2012). Moreover, clinical studies find that patients with ischemic stroke and metabolic syndrome had higher levels of inflammatory markers and arterial stiffness indexes (Tuttolomondo et al., 2014), and more and more data point to the involvement of $CD4^+CD28$ null T cells in the pathogenesis of acute brain ischemia (Nowik et al., 2007; Tuttolomondo et al., 2015). These immune cells and cytokines interact with each other and with brain cells, and play crucial roles in the tissue damage following cerebral ischemia, though the underlying mechanisms remain poorly understood.

IL-19 is a member of the IL-10 super-family cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29), sharing 20% amino acid similarity with IL-10 (Sabat et al., 2007). Previous findings indicated that IL-19 is primarily produced by activated monocytes, T and B lymphocytes and, to a lesser extent, by non-immune cells including keratinocytes, bronchial epithelial cells, and smooth muscle cells (England and Autieri, 2012). IL-19 signals through a heterodimeric receptor composed of IL-20Rα and IL-20Rβ that is also utilized by IL-20 and IL-24 (Dumoutier et al., 2001). Recent studies showed that IL-19 contributes to the pathogenesis of autoimmune diseases such as asthma, psoriasis (Huang et al., 2008;

* Corresponding author.
E-mail address: Haojunmed@163.com (H. Xuan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.brainres.2016.09.006
0006-8993/

Li et al., 2005), and IL-19 can modulate inflammation outside of the immune system, e.g., IL-19 levels increased in cardiac surgery patients with cardiopulmonary bypass (Hsing et al., 2006), IL-19 adenoviral gene transfer reduced proliferation and neointimal formation in balloon angioplasty-injured rat carotid arteries (Tian et al., 2008), and IL-19 attenuated experimental atherosclerosis in mice by promoting immune cell polarization and decreasing macrophage adhesion (Ellison et al., 2013).

Despite the importance of IL-19 signaling in the vascular diseases (England and Autieri, 2012), the importance of IL-19 in ischemic stroke is unknown. In this study, we observed the protective effects of IL-19 against brain injury in a mice model of middle cerebral artery occlusion (MCAO), and further investigated the effects of IL-19 on infiltration of inflammatory cells, and pro-/anti- inflammatory cytokines in ischemic brain and peripheral blood. These findings suggested that IL-19 may have translational potential in the stroke management.

2. Results

2.1. IL-19 treatment reduced infarction and attenuated neurological scores after stroke

Infarction volume at 72 h was significantly smaller in IL-19 treatment group ($36.9 \pm 2.6\%$), compared to control group ($49.8 \pm 3.5\%$; Fig. 1A, B). Focal neurological scores (FNS) were assessed from day 1 to day 3 after reperfusion. IL-19 injection statistically attenuated neurological deficit compared to ischemic controls at day 3, which was consistent with the protective effects on infarct volume (Fig. 1C).

2.2. IL-19 treatment inhibited brain inflammation and preserved immune cells in blood

We examined immune cell populations of $CD4^+$ T cells ($CD45^+CD4^+$), $CD8^+$ T cells ($CD45^+CD8^+$), B cells ($CD45^+CD19^+$) and macrophages ($CD45^+CD68^+$) in peripheral blood, and the numbers of these cells and microglia ($CD45^{inter}CD11b^+$) in ischemic hemisphere at 72 h post-stroke (Fig. 2). Inflammatory cell infiltration and microglial activation were significantly increased in the ischemic brains, while IL-19 treatment statistically attenuated their increases except $CD8^+$ T cells. The numbers of these immune cells in blood were sharply decreased after stroke, but IL-19 treatment significantly helped to maintain their populations.

Consistent with the fluorescence-activated cell sorting (FACS) results, morphometric analysis revealed that the total number of activated macrophages ($CD68^+$ cells) and neutrophils (MPO^+ cells) was robustly elevated in the ischemic area (< 1 mm from the infarct border), while IL-19 injection significantly suppressed their expression compared with control group (Fig. 3).

2.3. IL-19 treatment reduced TNF-α and IL-6 mRNA in ischemic brain

To evaluate the expression of proinflammatory cytokines in the early post-stroke stage, we collected ischemic hemispheres 12 h after reperfusion and measured mRNA levels of TNF-α, IL-1β, IL-6 and IL-10 using real-time quantitative PCR. They were all markedly increased after stroke compared with sham group, and IL-19 significantly suppressed expression of TNF-α and IL-6 mRNA, but not IL-1β or IL-10 (Table).

3. Discussion

The major finding of this study is that systemic administration of IL-19 starting pre-stroke attenuated ischemic brain injury, reduced infiltration of leukocytes, activation of microglia and gene expression of proinflammatory cytokines in the ischemic brain, and preserved immune cells in peripheral blood following stroke in mice.

Inflammation plays a pivotal role in the pathogenesis and progression of ischemia-induced brain injury (Iadecola and Anrather, 2011). Consistent with previous reports, our study showed that the numbers of resident microglia, migrating immune cells including macrophages, neutrophils, $CD4^+$ T cells, $CD8^+$ T cells as well as B cells, and the mRNA expression of TNF-α, IL-1β, IL-6 and IL-10, were all robustly increased in the brain after MCAO. The resident microglia are activated rapidly after occlusion of cerebral blood flow. Activated microglia not only synthesize neurotoxic mediators such as nitric oxide, glutamate, and reactive oxygen species (Nelson et al., 2002), but can also worsen neuroinflammation by releasing numerous proinflammatory cytokines and chemokines including TNF-α, IL-1β, IL-6, macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP) (Suzumura, 2013). Meanwhile, circulating

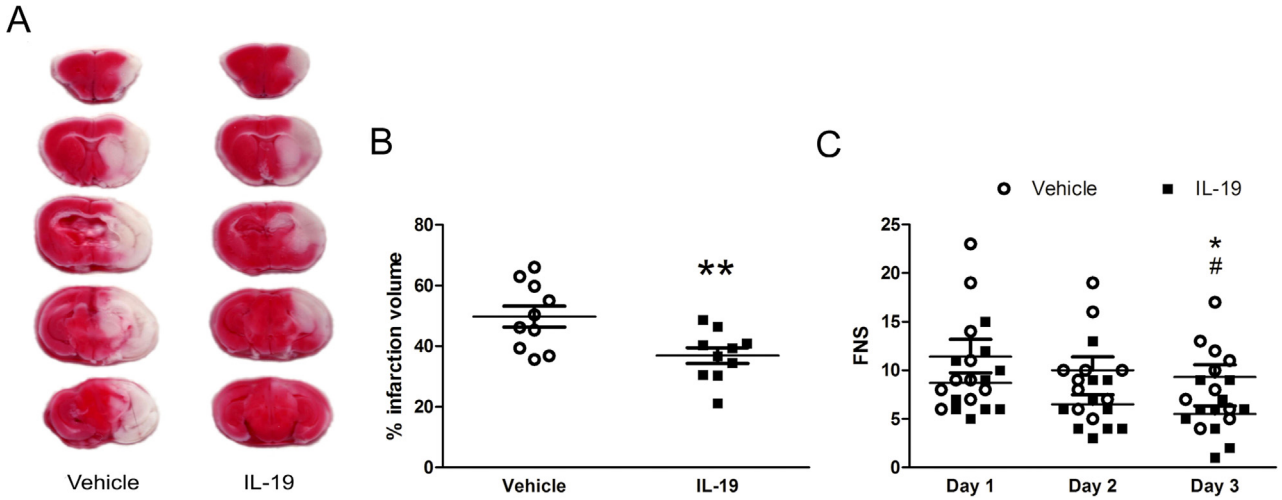

Fig. 1. IL-19 reduced infarct volume and neurological score. (A) Representative sections of TTC staining in the ischemic mouse brain. (B) Quantification of infarct volume expressed as a percent of hemispheric volume. Data are presented as mean ± SEM. $^{**}P < 0.01$ compared to control group. (C) The average focal neurological score (FNS) from day 1 to day 3 after reperfusion. Higher score indicates greater injury. $^{*}P < 0.05$ IL-19 group compared to control group, and $\#P < 0.05$ IL-19 group at Day 3 compared to the same group at Day 1. $n = 10$ mice/group.