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α4 Integrin is a Regulator of Leukocyte Recruitment Following Experimental Intracerebral Hemorrhage

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Abstract

Background and Purpose—Intracerebral hemorrhage (ICH) is swiftly followed by an inflammatory response. A key component of this response is the recruitment of leukocytes into the brain, which promote neurological injury in rodent models. However, the mechanisms by which leukocytes transmigrate across the endothelium into the injured brain are unclear. The present study examines leukocyte adhesion molecules (α4 integrin, L-selectin, and αLβ2 integrin) on four leukocyte subtypes to determine which are important for leukocyte recruitment after ICH.

Methods—We used the blood injection mouse model of ICH, whereby 25 μl of blood were injected into the striatum. Flow cytometry was used to quantify leukocyte populations and adhesion molecule expression in brain and blood. An α4 integrin blocking antibody was administered to evaluate the contribution of α4 in leukocyte migration and neurological injury.

Results—α4 integrin was elevated on all leukocyte populations in brain after ICH, whereas L-selectin was unchanged and αLβ2 was increased only on T cells. Antagonism of α4 resulted in decreased leukocyte transmigration and lessened neurobehavioral disability.

Conclusions—α4 integrin is an important cell adhesion molecule involved in neuroinflammation following ICH.

Keywords

intracerebral hemorrhage; monocytes; inflammation; adhesion molecules; integrins

Introduction

Intracerebral hemorrhage (ICH) initiates an inflammatory response that is characterized by leukocyte recruitment and elevated cytokine levels[1]. Specific leukocyte populations,

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including neutrophils, T cells, and inflammatory monocytes, promote secondary injury in models of ICH[2–4]. It is thought that these cells principally inflict damage through the release of reactive oxygen species, pro-inflammatory cytokines, and proteases[3, 5], but the mechanisms used for migration into the CNS after ICH are unclear. While several studies have shown the importance of endothelial cell adhesion molecules, namely VAP-1 and ICAM-1, for leukocyte recruitment after ICH[6, 7], no study has examined adhesion molecules on the surface of leukocytes. In the present study we examined changes in the levels of adhesion molecules on leukocytes in blood and brain. We also blocked α4 integrin function, which resulted in decreased leukocyte recruitment and improved motor function after ICH.

Methods

Protocols were approved by the UConn Health IACUC and were performed in accordance with NIH's Guide for the Care and Use of Laboratory Animals. ICH was modeled[8] using 25 μl autologous blood. Cells were analyzed using an LSRII cytometer (BD). For α4 integrin blocking, mice were injected with isotype control or anti-α4 (clone R1-2; 300 μg/mouse) 2–6 hours before ICH. Analysis was performed blinded to treatment. Detailed methods are in the Online Supplement.

Results

To determine how ICH affects leukocyte adhesion molecule expression, we performed flow cytometry on blood and brain 2 days following ICH. A mean of 11,128 ± 10,879 leukocytes were isolated from ICH brains versus 4,079 ± 305 cells in shams (n=4). The α4 integrin chain was elevated on all leukocyte populations in the ICH brain compared to blood (Figure 1A; Figure I Online Supplement). Inflammatory monocytes, which had the highest baseline α4, represented the largest population recruited to the ICH brain at day 2 (Figure II Online Supplement). Conversely, L-selectin was decreased on all leukocyte populations examined in the brain except for CD4 T cells, which were unchanged (Figure 1B). αLβ2 was higher on T cells in brain, while myeloid cells were unaffected (Figure 1C). Uniformly elevated α4 on all leukocyte populations suggests that it may mediate leukocyte recruitment after ICH.

To determine if α4 is required for entry into the brain, we treated mice with an anti-α4 blocking antibody before ICH. Brain and blood samples were examined using flow cytometry 2 or 7 days later. Concentrations of T cells, neutrophils, and inflammatory monocytes were unchanged in blood by treatment (Figure 2A), as were physiological variables (Table I Online Supplement). However, recruitment of T cells and inflammatory monocytes was significantly diminished in day 2 anti-α4-treated brains, suggesting α4 integrin function is a fundamental mechanism by which leukocytes migrate into the hemorrhagic brain (Figure 2B). Leukocyte quantities isolated from isotype control-treated brains were similar to the untreated ICH brains in Figure 1. Importantly, anti-α4-treated mice displayed significantly improved left forelimb use by the cylinder test up to day 2 (Figure 2C). Together, these data demonstrate that α4 is an important cell adhesion molecule involved in acute leukocyte recruitment following ICH.

Discussion

The present study aimed to understand how adhesion molecules on leukocytes are involved in cell recruitment following ICH. All leukocyte populations examined displayed increased α4 integrin, whereas only T cells showed elevated αLβ2, and no population displayed increased L-selectin in brain. Interestingly, inflammatory monocytes, which were recently shown to worsen ICH injury[3], represented the largest leukocyte population in brain and had the highest baseline α4 in blood. However, increases in adhesion molecules may not necessarily correlate with the influence of a particular molecule, as conformational changes influence ligand affinities[9] and molecules may be downregulated after tissue entry. We therefore confirmed the role of α4 with an antagonist. Treatment with the α4 blocking antibody decreased leukocyte recruitment and reduced early motor deficits, indicating its importance in ICH. α4 heterodimerizes with β1 or β7 integrins. α4β1 is expressed on leukocytes and microglia, whereas α4β7 is found on gut-homing T cells and some vascular endothelium. Because the antibody recognizes the α4 subunit, we cannot attribute the observed benefit to a specific α4 heterodimer. Similarly, we cannot rule out the possibility that the antibody crosses a weakened blood brain barrier and binds microglial α4 in addition to that on leukocytes, or has systemic effects. Nonetheless, these results identify α4 integrin as an important cell adhesion molecule during acute sterile neuroinflammation.

Previous studies using α4 blocking antibodies in ischemic stroke models have shown benefits, both by reduced infarct volumes and improved neurobehavioral functions[10–12]. While these studies mainly attributed improvements to reduced T cell recruitment, they also showed decreased myeloperoxidase and Gr1[10, 11], markers common to inflammatory monocytes and neutrophils[13, 14], indicating myeloid cells were also decreased by treatment. Using flow cytometry, the present study discriminates between inflammatory monocytes, neutrophils, and microglia and identifies monocytes as having the largest decrease during α4 blockade. Interestingly, many leukocytes entered the brain despite α4 blockade, yet there was little disability up to day 2 in treated mice. This suggests that treatment may also inhibit integrin signaling, contributing to altered phenotypes once in tissue. This requires further study.

The study was not intended to evaluate α4 integrin as a therapeutic target. However, our findings suggest that blocking α4 function may provide neurological benefits by reducing acute inflammation. The absence of differences at day 7 is likely due to the single treatment. *While the half-life of the antibody is unknown, it is likely similar to the half-life of the isotype control (4–6 days)*[15]. *In addition, redundant migration mechanisms may compensate for blockade*. Additional studies are needed to better characterize α4-mediated leukocyte recruitment using translationally-relevant dosing paradigms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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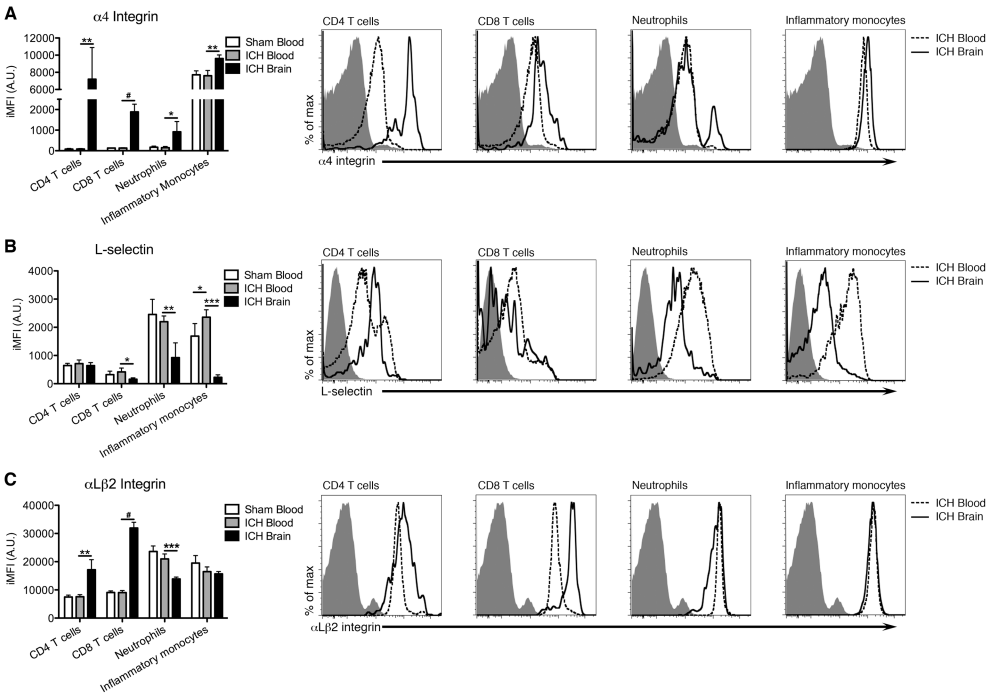

Figure 1.

Adhesion molecule modulation following ICH. **A**, α4 integrin integrated mean fluorescence intensities (iMFIs) were elevated on leukocytes in brains at day 2. **B**, L-selectin iMFIs were not increased relative to blood. **C**, αLβ2 staining was increased on T cells, but decreased on neutrophils and unchanged on inflammatory monocytes. Shaded traces depict negative controls. N=4; **p*<0.05, ***p*<0.01, ****p*<0.001 by *t* test; #*p*<0.05 by *U* test; bars indicate mean ± SD.

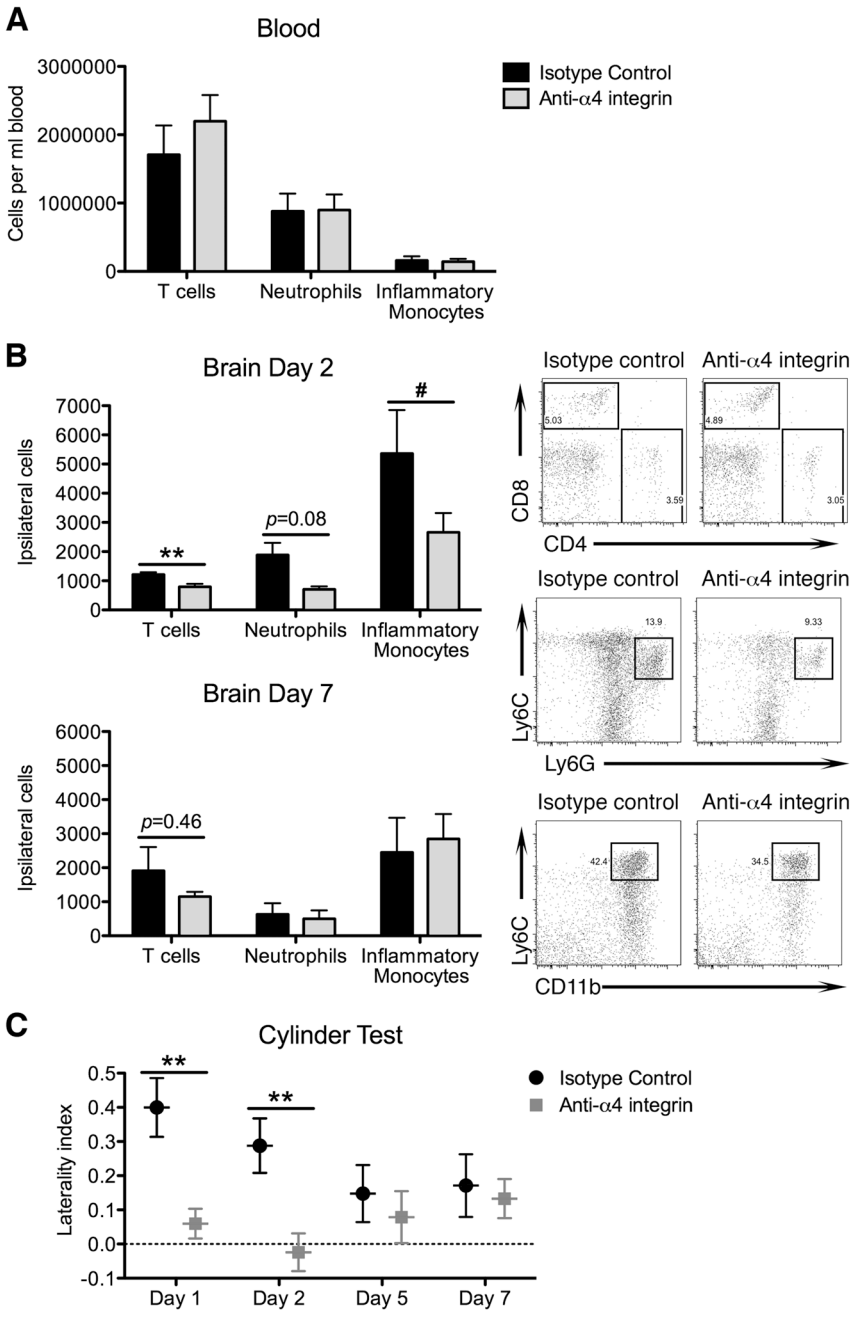

Figure 2.

α4 integrin blocking diminishes acute neuroinflammation. **A**, Concentrations of T cells, neutrophils, and inflammatory monocytes were unchanged in blood 2 days after antibody treatment. N=8–9. **B**, α4 blockade decreased T cell and inflammatory monocyte recruitment at day 2, but not day 7. Representative flow cytometry plots depict CD4 and CD8 T cells; $Ly6G^{+}$ neutrophils; and $Ly6C^{hi}$ inflammatory monocytes at day 2. N=5–9. **C**, Anti-α4-treated mice displayed improved left forelimb use in the cylinder test. N=7–9. $^{**}p<0.01$ by *t* test; $\#p<0.05$ by *U* test; bars indicate mean ± SEM.