PD-1+ Monocytes Mediate Cerebral Vasospasm Following Subarachnoid Hemorrhage

Christopher M. Jackson, MD *
John Choi, BS*
Denis Routkevitch, BS*
Ayush Pant, BS‡
Laura Saleh, BS*
Xiaobu Ye, MD*
Justin M. Caplan, MD*
Judy Huang, MD *
Cameron G. McDougall, MD*
Drew M. Pardoll, MD, PhD‡
Henry Brem, MD*§
Rafael J. Tamargo, MD*
Michael Lim, MD*

*Department of Neurosurgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland; ‡The Bloomberg~Kimmel Institute for Immunotherapy, The Sidney Kimmel Comprehensive Cancer Center ; §Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Correspondence:
Michael Lim, MD,
Department of Neurosurgery,
The Johns Hopkins University School of Medicine,
600 N Wolfe St,
Phipps 123,
Baltimore, MD 21287, USA.
Email: mlim3@jhmi.edu

Received, July 5, 2020.
Accepted, September 9, 2020.
Published Online, December 28, 2020.

BACKGROUND: Cerebral vasospasm is a major source of morbidity and mortality following aneurysm rupture and has limited treatment options.
OBJECTIVE: To evaluate the role of programmed death-1 (PD-1) in cerebral vasospasm.
METHODS: Endovascular internal carotid artery perforation (ICAp) was used to induce cerebral vasospasm in mice. To evaluate the therapeutic potential of targeting PD-1, programmed death ligand-1 (PD-L1) was administered 1 h after ICAp and vasospasm was measured histologically at the level of the ICA bifurcation bilaterally. PD-1 expressing immune cell populations were evaluated by flow cytometry. To correlate these findings to patients and evaluate the potential of PD-1 as a biomarker, monocytes were isolated from the peripheral blood and analyzed by flow cytometry in a cohort of patients with ruptured cerebral aneurysms. The daily frequency of PD-1+ monocytes in the peripheral blood was correlated to transcranial Doppler velocities as well as clinical and radiographic vasospasm.
RESULTS: We found that PD-L1 administration prevented cerebral vasospasm by inhibiting ingress of activated Ly6c+ and CCR2+ monocytes into the brain. Human correlative studies confirmed the presence of PD-1+ monocytes in the peripheral blood of patients with ruptured aneurysms and the frequency of these cells corresponded with cerebral blood flow velocities and clinical vasospasm.
CONCLUSION: Our results identify PD-1+ monocytes as mediators of cerebral vasospasm and support PD-1 agonism as a novel therapeutic strategy.

KEY WORDS: Subarachnoid hemorrhage, Cerebral vasospasm, Programmed death-1, Programmed death ligand-1, Monocyte

Neurosurgery 88:855–863, 2021 DOI:10.1093/neuros/nyaa495 www.neurosurgery-online.com

Cerebral aneurysms have an estimated incidence of 3% and an annual rupture rate of 1%.[1,2] Aneurysmal subarachnoid hemorrhage (aSAH) accounts for 5% of strokes and up to 25% of stroke-related mortalities.[3] Diffuse narrowing of cerebral arteries following aSAH, known as cerebral vasospasm, is radiographically detected in 70% of patients for up to 2 wk after aneurysm rupture and 20% of patients develop delayed cerebral ischemia resulting in neurologic deficits.[4] Cerebral vasospasm is thus a significant source of morbidity and mortality for aSAH patients, yet the pathophysiology of this process is unknown and there are limited treatment options.[5]

The frequency and severity of cerebral vasospasm have been linked to inflammation[6] and correlate with hemorrhage volume.[7] Alterations in cytokine levels including IL-1, IL-6, and IL-8 have been reported in the blood and cerebrospinal fluid of patients following aSAH,[8] as well as upregulation of the leukocyte adhesion molecules ICAM-1, VCAM-1, and LFA-1.[9] Although nonsteroidal anti-inflammatory drugs (NSAIDs) and statins partially ameliorate vasospasm in animal models,[10,11] the specific underlying inflammatory pathways have not yet been defined, thus far precluding a targeted approach.

ABBREVIATIONS: aSAH, aneurysmal subarachnoid hemorrhage; **CI,** confidence interval; **CM,** cisterna magna; **CNS,** central nervous system; **ICAp,** internal carotid artery perforation; **NK,** natural killer; **NSAIDs,** nonsteroidal anti-inflammatory drugs; **PD-1,** programmed death-1; **PD-L1,** programmed death ligand-1; **PD-L2,** programmed death ligand-2; **SEM,** standard error of the mean; **TCD,** transcranial Doppler; **VLA-4,** very late antigen-4

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Programmed death-1 (PD-1) is an inhibitory immune checkpoint expressed on activated immune cells.[12] PD-1 binding its ligands, programmed death ligand-1 (PD-L1) or programmed death ligand-2 (PD-L2), limits collateral damage in the setting of chronic infection and protects against autoimmunity.[13-16] PD-L1 is constitutively expressed in the tumor microenvironment, where it facilitates immunologic escape by tumor cells.[17] Monoclonal antibodies against PD-1 and PD-L1 are a major breakthrough in clinical oncology and have become a cornerstone of treatment for many advanced cancers.[18] Recent animal data indicate that PD-1 agonism may be an effective anti-inflammatory strategy in some contexts. PD-L1 fusion immunoglobulins show activity in models of some autoimmune diseases including lupus,[19] colitis,[20] and collagen-induced arthritis.[21] Administration of PD-L1 fusion proteins was recently shown to reduce hemorrhage volume and edema as well as ameliorate neurologic deficits in a murine model of intracerebral hemorrhage,[22] demonstrating a potential role for PD-1 signaling in acute cerebral inflammation. Based on these findings, we hypothesized that the PD-1 pathway mediates the inflammatory response underlying cerebral vasospasm and can be leveraged as a novel therapeutic strategy.

METHODS

See **Text, Supplemental Digital Content 1** and **Tables, Supplemental Digital Contents 2 and 3**.

RESULTS

Administration of Soluble PD-L1 Prevents Luminal Wall Thickness

Subarachnoid hemorrhage was induced by endovascular internal carotid artery perforation (ICAp) in C57BL/6 mice as previously described[23] (Figure 1A). We found that ICAp produced severe SAH (**Figure**, **Supplemental Digital Content 4A**). We standardized our histologic evaluation of the ICA termination based on surrounding brain structures as shown in **Figure**, **Supplemental Digital Content 4B** and found that ICAp reliably produced severe vasospasm (**Figure**, **Supplemental Digital Content 4B**). To evaluate PD-1 as a therapeutic target for cerebral vasospasm, we administered soluble PD-L1 via intraperitoneal injection 1 h after ICAp and measured the arterial wall thickness and lumen diameter of the terminal ICA at 48 h (Figure 1B). Administration of PD-L1 prevented vasospasm in this model ($P < .0001$, ANOVA with post hoc Tukey test), whereas pretreatment with PD-1 blocking antibodies 1 h prior to ICAp abrogated the therapeutic effect of PD-L1 ($P < .0001$) (Figure 1C).

ICA Perforation Induces PD-1 Expression on Brain Myeloid Cells

We compared 2 models of SAH to identify the PD-1+ cell populations mediating cerebral vasospasm. Although the ICAp model produced severe vasospasm, injecting blood into the cisterna magna (CM) resulted in minimal narrowing of the intracranial ICA (Figure 2A). Diverse immune cell populations have been posited as mediators of cerebral vasospasm, including macrophages (CD11b+ and CD45-bright),[24] microglia (CD11b+ and CD45-dim),[25] lymphocytes (CD45+, CD11b, and CD4+/CD8+),[26] granulocytes (CD45+, CD11b+, Ly6g+, and Ly6c–),[27] and natural killer (NK) cells (CD45+ and CD49b+).[28] We found that only CD45+, CD11b+ myeloid cells exhibited an increase in PD-1 expression in the spasmogenic ICAp model compared with the CM model (Figure 2B; **Figure**, **Supplemental Digital Content 5**) ($P = .037$, *t*-test). Based on these data, we chose to focus on myeloid cells in subsequent experiments exploring the role of the PD-1 pathway in cerebral vasospasm.

PD-1+ Monocytes Traffic Into the Brain From the Periphery

To determine the origin of PD-1+ myeloid cells, we harvested brains, peripheral blood, and bone marrow from mice 6 and 24 h after ICAp. At both time points, CD45-dim microglia expressed stable levels of PD-1. Conversely, the frequency of PD-1+ macrophages increased dramatically from 6 to 24 h ($P = .001$, *t*-test) (Figure 2C). The timing of PD-1 expression on peripheral monocytes corresponded with that of brain-infiltrating monocytes/macrophages as the frequency of PD-1+ monocytes in the blood and bone marrow increased 24 h after SAH ($P = .0063$ and $P = .0059$, respectively, *t*-test). These data are consistent with the hypothesis that SAH stimulated release of PD-1+ monocytes from the bone marrow and these cells subsequently trafficked to brain in a time course concordant with histologic vasospasm.

PD-L1 Administration Prevents Cerebral Vasospasm by Inhibiting Trafficking of PD-1+ Inflammatory Monocytes Into the Central Nervous System

We measured Ly6c and CCR2 expression (**Figure**, **Supplemental Digital Content 6**) on monocytes in the blood and bone marrow 24 and 48 h after ICAp and found a higher frequency of activated monocytes in the blood ($P = .049$ at 24 h, $P = .003$ at 48 h, *t*-test) and a lower frequency of PD-1+ monocytes in the bone marrow ($P > .05$ at 24 h, $P = .036$ at 48 h, *T*-test) with PD-L1 treatment (Figure 3A). Analysis of brain-infiltrating macrophages at these time points showed a lower frequency of Ly6c+ and CCR2+ monocytes in PD-L1-treated animals (Figure 3A). In addition, PD-L1-treated animals had a higher frequency of blood monocytes expressing the integrin very late antigen-4 (VLA-4) ($P = .032$, *t*-test) (Figure 3B).

Catecholamine Signaling Is Required for PD-1+ Monocytes to Migrate Into the Central Nervous System Following SAH

We hypothesized that the peripheral nervous system mediated release of activated PD-1+ monocytes from the bone marrow

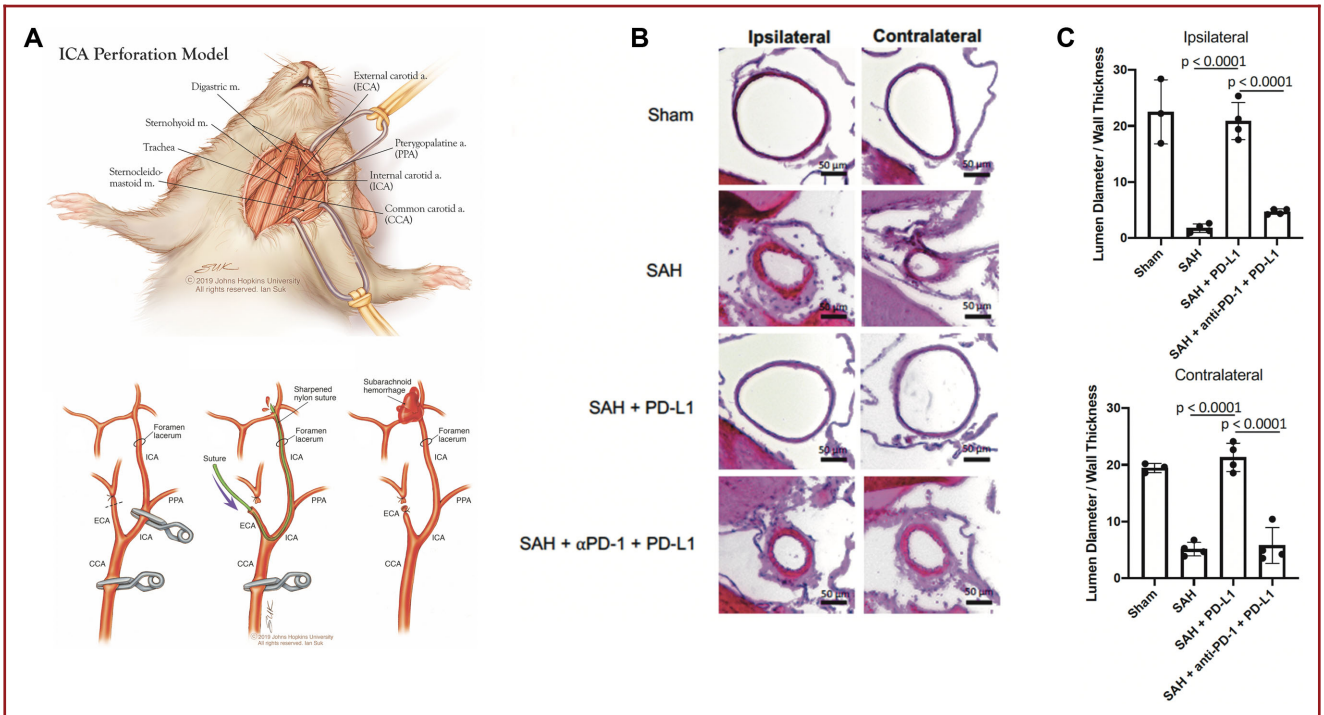

FIGURE 1. *Administration of PD-L1 prevents cerebral vasospasm by inhibiting migration of activated monocytes into the CNS.* **A**, *Illustration of the ICA perforation model technique. ©2019 Department of Neurosurgery, Johns Hopkins University. Used with permission.* **B** *and* **C**, *intraperitoneal injection of PD-L1 (50 μg) 1 h after ICAp prevents vasospasm at 48 h (P < .0001), whereas administration of PD-1 blocking antibodies 1 h before ICAp abrogates the effect of PD-L1 on vasospasm (P < .0001, ipsilateral; P = .0001 contralateral). N = 4 mice per group. Data were analyzed using a one-way ANOVA with post hoc Tukey test. Error bars represent +/– standard error of the mean (SEM).*

following SAH. Consistent with this hypothesis, we found that administering propranolol 1 h before ICAp decreased the frequency of PD-1+ myeloid cells in the brain (Figure 3C) ($P = .0009$, *t*-test). To evaluate the potential for using catecholamine blockade to prevent vasospasm, we measured functional outcomes following ICAp and found that administration of propranolol resulted in significantly worse behavioral outcomes as measured by the Garcia Stroke Scale ($P = .006$) (Figure 3D). Of note, we did not observe behavioral differences in mice receiving PD-L1 following ICAp (**Figure**, **Supplemental Digital Content** 7).

PD-1+ Monocytes Predict Cerebral Blood Flow Velocities in Patients With aSAH

To determine whether PD-1+ monocyte frequency in the peripheral blood correlates with cerebral artery constriction, we studied6 consecutive patients admitted to our institution with aSAH. The PD-1+ monocyte gating strategy is shown in **Figure**, **Supplemental Digital Content 8**. Patient and aneurysm characteristics are summarized in **Table**, **Supplemental Digital Content 9**. In patients who developed radiographic vasospasm (patients 1 and 5), vasospasm was preceded by an increase in PD-1+ monocytes in the peripheral blood. Patient 5 had an elevated percentage of circulating PD-1+ monocytes beginning on day 6, which peaked on day 7 at 9.31% (Figure 4A). On day 8, this patient developed aphasia and computed tomography angiography confirmed vasospasm in the left middle cerebral artery.

Twenty-two percent of patient 1's peripheral blood monocytes expressed PD-1 at presentation (**Figure**, **Supplemental Digital Content 10**). On day 4, there was an increase in transcranial Doppler (TCD) velocities that reached a maximum on day 7 (>120 cm/s) (Figure 4A). Vasospasm was detected by magnetic resonance angiography on day 5 and confirmed by catheter-based angiography on day 10. In both patients, there was an early abundance of CD14++ and CD16– (classical) monocytes, followed by an increase in CD14++ and CD16+ (intermediate) monocytes (Figure 4A; **Figure**, **Supplemental Digital Content 10**). PD-1+ monocytes were of the CD14++ and CD16+ (intermediate) subtype.

We hypothesized that an increase in PD-1+ monocytes in the peripheral blood would precede elevations in TCD velocities. We paired daily changes in PD-1+ monocyte frequency with changes in maximum TCD velocities the following day. An inter-rater agreement between the daily change in PD-1+

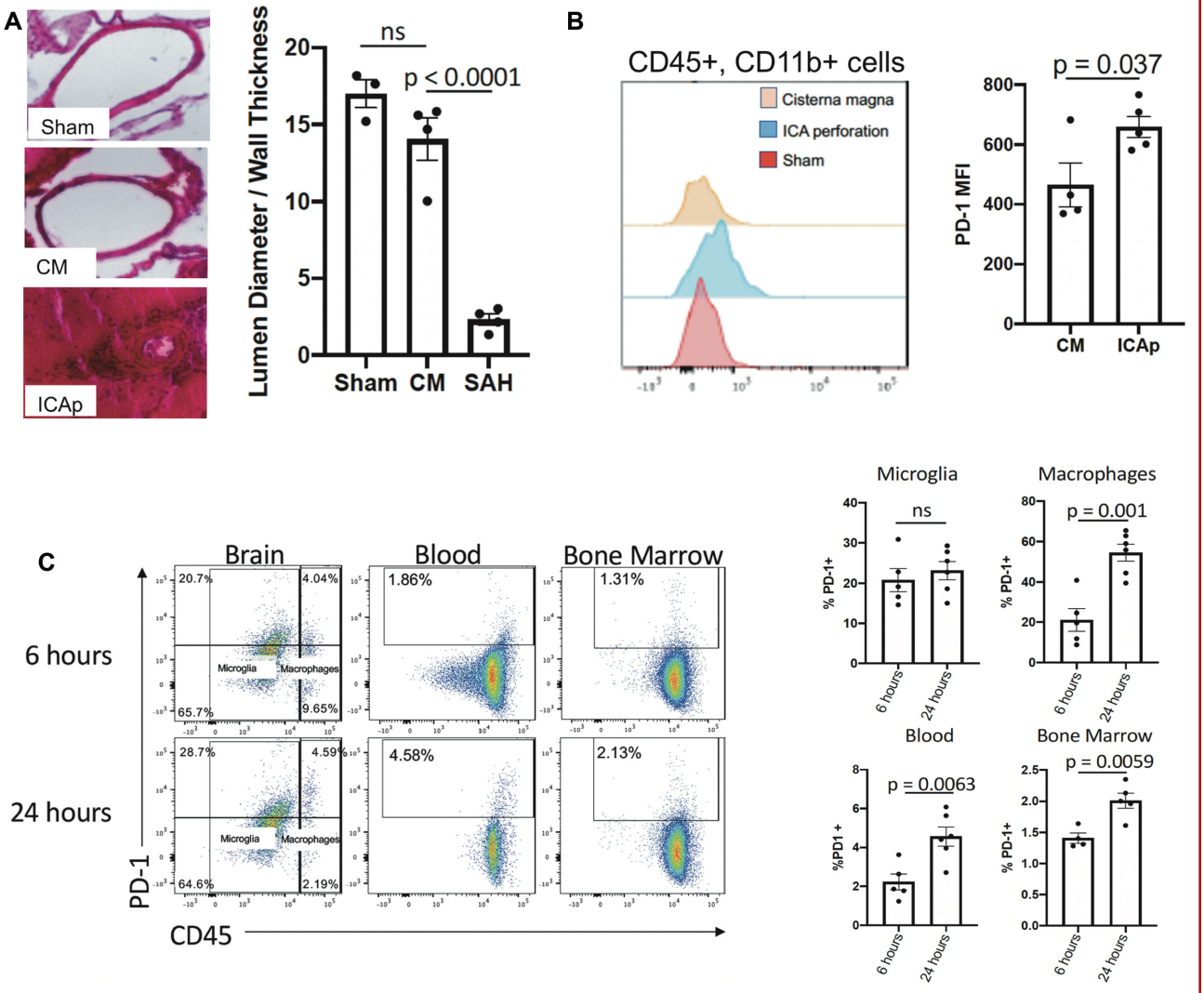

FIGURE 2. *Vasospasm is associated with an increased frequency of PD-1+ myeloid cells.* **A**, *CM injection results in minimal change in the caliber of the ipsilateral terminal ICA, whereas ICAp causes severe vasospasm (P < .0001, ANOVA).* **B**, *Flow cytometric analysis of brain myeloid cells (CD3-, CD45+, CD11b+) showed an increase in PD-1 mean fluorescence intensity on myeloid cells in ICAp mice compared with CM (P = .037).* **C**, *PD-1 expression is increased following ICAp on CD45-high brain macrophages (P = .001) as well as blood monocytes (P = .0063) and bone marrow (P = .0059), but not on CD45-dim microglia. N = 4 to 6 mice per experimental group. Error bars represent +/– SEM.*

monocyte frequency and changes in TCD velocities the following day was assessed by Cohen's kappa coefficient, $\kappa = 0.48$ (95% CI: 0.2-0.76; $P = .0018$) (Figure 4B). A possible correlation was estimated using Pearson correlation coefficient at r = 0.33 (95% CI: −0.01 to 0.60; $P = .06$) (**Figure**, **Supplemental Digital Content 11**). We performed a sensitivity analysis excluding the day 3 outlier for patient 1 and found r = 0.486 (95% CI: 0.17-0.71; $P = .0037$) (Figure 4C). Using a threshold of >5% PD-1+ monocyte frequency showed that >5% PD-1+ monocytes were associated with higher TCD velocities above baseline the following day compared with <5% PD-1+ monocytes ($P = .0012$, *t*-test) (Figure 4D).

DISCUSSION

A systemic inflammatory response is observed in aSAH patients and correlates with cerebral vasospasm as well as hydrocephalus and other complications of aneurysm rupture.[29] The specific immune pathways mediating this inflammatory response, however, have not previously been identified. Here, we show that

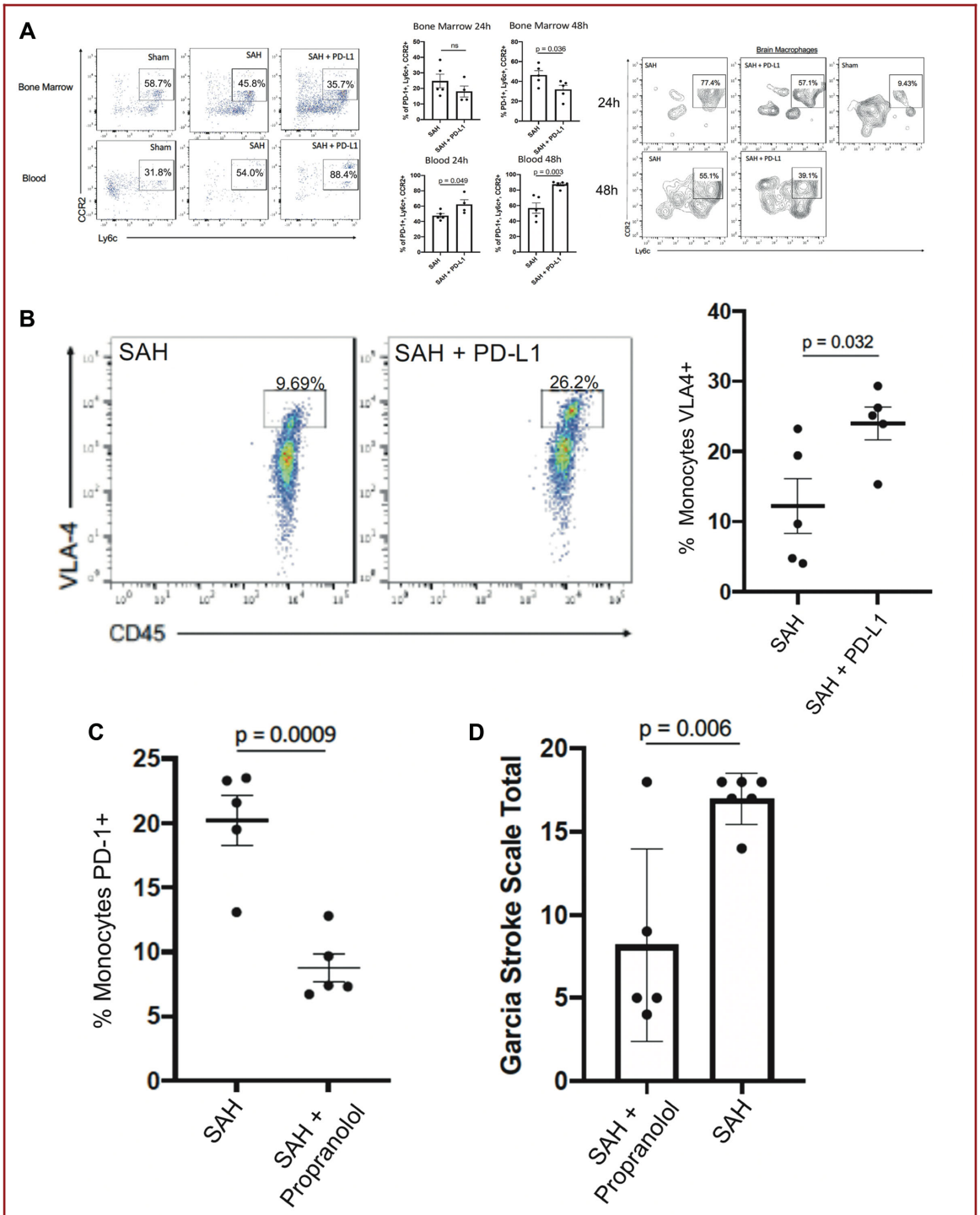

FIGURE 3. *PD-L1 administration prevents ingress of activated PD-1+ monocytes into the brain.* **A**, *ICAp mice receiving PD-L1 have a higher frequency of PD1+, Ly6c+, CCR2+ monocytes in the blood at 24 and 48 h compared with untreated mice (P = .049 and P = .003, respectively) and a decreased frequency of PD-1+, Ly6c+, CCR2+ cells in the bone marrow at 48 h (P = .036). Concatenated data show fewer PD-1+, Ly6c+, CCR2+ cells in the brains of mice receiving PD-L1 at 24 and 48 h.* **B**, *PD-L1-treated mice have a higher frequency of VLA-4+ monocytes in the blood at 48 h compared with untreated mice (P = .032).* **C**, *Administration of propranolol 1 h prior to ICAp decreases the frequency of PD-1+ monocytes in the blood at 24 h (P = .0009), but* **D** *results in a more severe neurologic deficits based on the Garcia Stroke Scale (P = .006). N = 4 to 6 mice per experimental group. Error bars represent +/– SEM.*

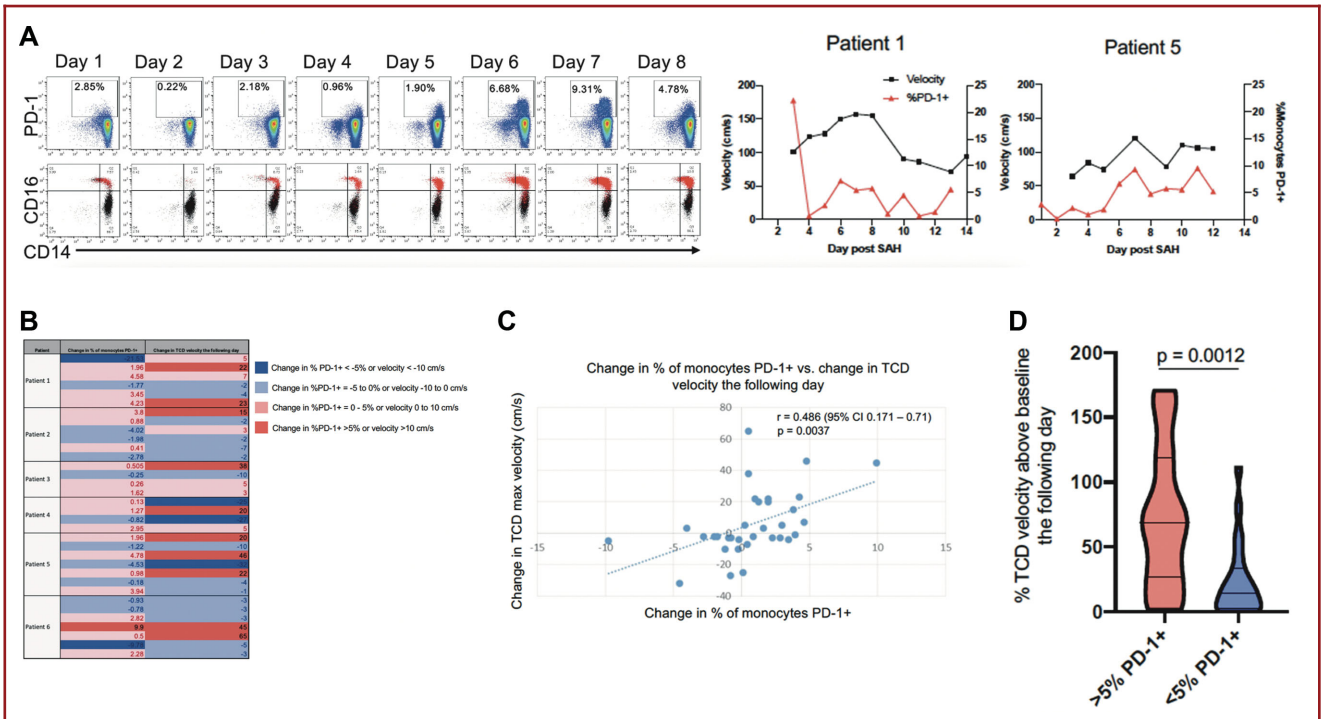

FIGURE 4. *PD-1+ monocyte frequency in the peripheral blood of aSAH patients correlates with changes in cerebral blood flow velocities the following day.* **A**, *In the CD 14 vs CD16 plots for patient 5, red dots represent PD-1+ cells. Maximum TCD velocity and %PD-1+ monocytes correlated over time for patient 1 and patient 5.* **B**, *Heatmap of change in %PD-1+ monocytes (vs previous day) paired with change in maximum TCD velocity the following day.* **C**, *Linear regression analysis of change in %PD-1+ monocytes vs change in maximum TCD velocity the following day, excluding the day 3 timepoint outlier (>4x higher than the SD) from patient 1.* **D**, *Violin plots of maximum TCD values the following day for PD-1+ monocytes > 5% vs <5% (P = .0012). Correlation coefficient (r) = 0.486 (95% CI 0.171-0.71) with P = .0037. N = 6 patients.*

PD-1+ monocytes are critical mediators of cerebral vasospasm and PD-1 agonism may represent a novel therapeutic strategy.

We found that PD-L1 administration prevented vasospasm and that pretreating mice with PD-1 blocking antibodies abrogated this effect. Measuring the frequency of PD-1+, Ly6c+, and CCR2+ monocytes in the blood, bone marrow, and brain at 24 and 48 h after SAH revealed that activated monocytes in the blood of PD-L1-treated mice were increased compared with untreated animals. Correspondingly, analysis of brain-infiltrating monocytes/macrophages at these timepoints revealed a lower frequency of Ly6c+ and CCR2+ cells in PD-L1-treated animals. These experiments indicate that agonism of the PD-1 signaling pathway prevents vasospasm by inhibiting ingress of activated monocytes into the brain (Figure 5).

No mechanistic link has been established between activation of the PD-1 pathway and NSAIDs, calcium channel blockers, or statins, which have shown some activity in preventing vasospasm. In fact, evidence from the oncology literature indicates that inhibition of cyclooxygenase activity and prostaglandin synthesis may increase inflammation in the setting of PD-1 blockade.[30,31] However, these studies focused on PD-1 regulation of cytotoxic lymphocytes, and future studies are needed to evaluate interplay of these pathways in myeloid cells in order to determine if PD-1 agonism works through an entirely independent mechanism in this setting.

In order to delineate the inflammatory mechanisms underlying the treatment effect observed with PD-L1 administration, we employed 2 mouse SAH models, ICAp, and CM injection. It is worth noting that the ICAp model closely mirrored our human data regarding vessel reactivity and inflammatory infiltrate, whereas the CM model did not. We believe this finding has implications for future implementation of these models in vasospasm research, particularly for studies evaluating the underlying inflammatory response.

PD-1+, Ly6c+, and CCR2+ monocytes were released from the bone marrow following ICAp in response to catecholamines. Of note, although beta-adrenergic signaling stimulates a pro-inflammatory response in trauma and sepsis[31] and has been associated with cerebral vasospasm,[33,34] the clinical benefit of beta blockade in aSAH is equivocal.[35] Our mouse experiments mirrored this clinical observation as mice receiving propranolol developed neurologic deficits consistent with brain

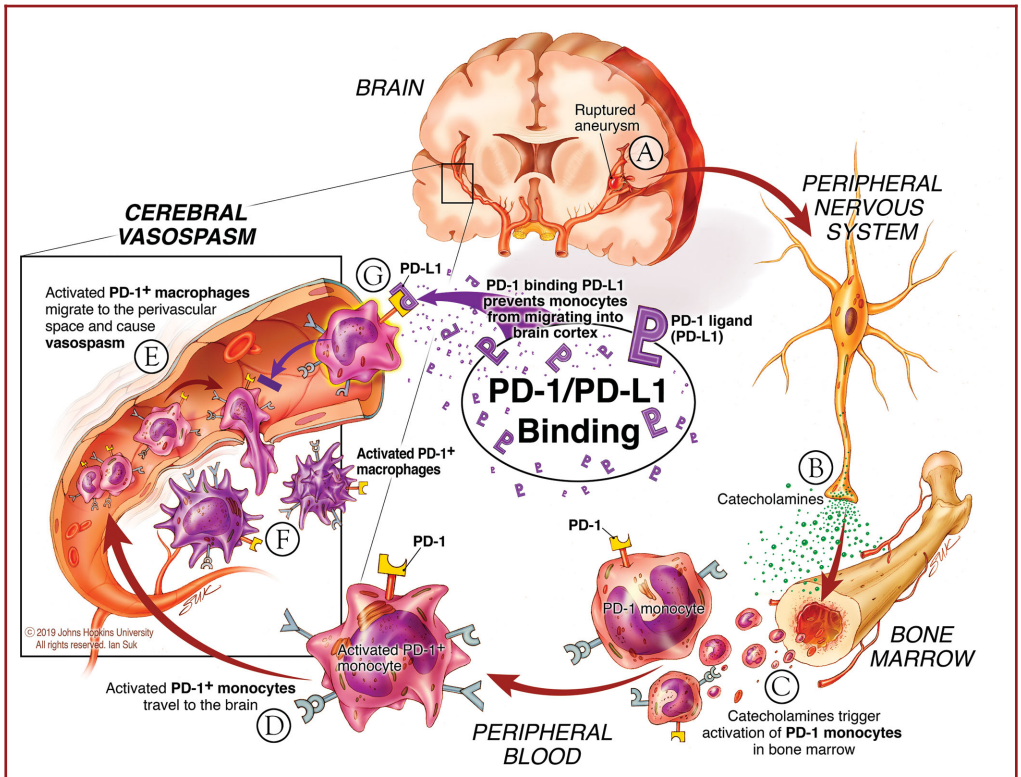

FIGURE 5. *Mechanism of PD-1+ monocyte-induced vasospasm following aneurysm rupture. (A) is the rupture of an intracranial aneurysm results in arterial bleeding in the subarachnoid space. (B) is the activation of the peripheral nervous system in response to subarachnoid hemorrhage causes (C), release of activated, PD-1+ monocytes from the bone marrow. (D-F) are the activated PD-1+ monocytes extravasate from the cerebral vasculature into the perivascular space. (G) is the binding of PD-1 to its ligand PD-L1 prevents extravasation of activated monocytes and prevents cerebral vasospasm. ©2019 Department of Neurosurgery, Johns Hopkins University. Used with permission.*

ischemia. These data support the notion that although beta blockade reduces pathologic inflammation, impaired autoregulation precludes a clinical benefit. Furthermore, many aSAH patients receive catecholamines to augment blood pressure in the setting of vasospasm. Future work will be required to determine if exogenous catecholamines exacerbate pathologic cerebral inflammation.

Circulating PD-1+ monocytes expressed the activation markers Ly6c and CCR2 as well as the homing molecule VLA-4. Ly6c+ monocytes are activated in the bone marrow and licensed to migrate into tissue.[36] CC chemokine receptor 2 (CCR2)-expressing monocytes have been implicated in inflammatory central nervous system (CNS) pathologies,[37] and binding of VLA-4 on immune cells to VCAM-1 on vascular endothelium is necessary for transmigration into perivascular spaces in the CNS.[38] Taken together, these data indicate that PD-1+ monocytes are licensed to migrate into the CNS.

In humans, the frequency of PD-1+ monocytes in the peripheral blood corresponded with cerebral blood flow velocities (TCD). Furthermore, PD-1 was expressed on inflammatory CD14++ and CD16+ (intermediate) blood monocytes, which have been identified as negative prognostic indicators in other inflammatory vascular pathologies, including acute myocardial infarction and ischemic stroke.[39] Thus, PD-1 agonism may be an effective strategy not only for treating cerebral vasospasm, but should also be explored for other monocyte-mediated inflammatory diseases.

Patients are currently monitored for vasospasm by TCD velocities and serial clinical exams. Biomarkers that predict the development of cerebral vasospasm, rather than detect vessel constriction and ischemia once they have occurred, could afford an opportunity to treat aSAH proactively. Several such biomarkers have been studied, including biomarkers of endothelial activation, heme degradation, calcium metabolism, nitric oxide activity, and components of the coagulation cascade[40]; however, none of these biomarkers has yet been successfully translated into clinical practice. Furthermore, an ideal biomarker would directly monitor the underlying pathogenic inflammatory process rather than secondary vessel reactivity or tissue ischemia. Based on the mechanistic data from our murine studies and the observation that

PD-1+ monocytes correlated with cerebral blood flow velocities in humans, we hypothesized that measuring PD-1+ monocyte frequency could serve as a blood-based biomarker for vasospasm. To this end, we found that high PD-1 expression (>5%) predicted elevated cerebral blood flow velocities the following day for the duration of the time period these patients were at risk for developing cerebral vasospasm. We expect that confirmative studies in larger patient cohorts will refine the parameters of a PD-1+ monocyte-based assay as a clinical biomarker and inform administration of PD-1 agonists.

Limitations

This study has important limitations. Although we present a bench-to-bedside examination of the role of PD-1 in cerebral vasospasm, our conclusions regarding the underlying immune mechanisms and potential efficacy of PD-1 agonists are based primarily on data from animal models. Furthermore, we administered soluble, monomeric PD-L1; however, other PD-1 agonists, such as monoclonal antibodies and PD-L1 dimers, are in various stages of development and may prove more effective for clinical translation. Finally, our series of 6 patients is too small to validate PD-1+ monocytes as a biomarker and only 2/6 patients developed clinically significant vasospasm. Thus, the threshold of 5% PD-1+ was exploratory based and on our data distribution. A formal sensitivity/specificity analysis in larger studies will be required to validate PD-1 as a biomarker and determine the optimal threshold value.

CONCLUSION

Our data indicate that PD-1 is a previously unidentified mediator of cerebral vasospasm that may have utility as a therapeutic target and biomarker. Larger prospective studies are required to develop a receiver operating curve for PD-1+ monocytes in predicting vasospasm. Furthermore, future mechanistic studies will determine how PD-1+ monocytes mediate cerebral vasospasm, leading the way for development PD-1 targeted therapeutics.

Funding

This work was funded through the generosity of private donors to Dr Lim's laboratory.

Disclosures

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article. Dr Jackson, Mr Choi, Dr Tamargo, and Dr Lim are inventors on a patent filed by Johns Hopkins for using PD-1 agonists to treat cerebral vasospasm and other monocyte-mediated inflammatory diseases as well as a diagnostic assay for cerebral vasospasm based on PD-1+ monocyte frequency in the peripheral blood. Drs Jackson and Lim are co-founders of Egret Therapeutics and have equity interests in the company. Dr Lim receives research support from Aegenus, Accuray, Bristol-Myers Squibb, and DNAtrix and is a consultant for Tocagen, SQZ Technologies, Stryker, and Baxter. Dr Huang is a stockholder in Longeviti. Dr Brem receives research funding from NIH, Johns Hopkins University, Arbor Pharmaceuticals, Bristol-Myers Squibb, Acuity Bio Corp∗, and philanthropy and is a consultant for AsclepiX Therapeutics, StemGen, InSightec, Accelerating Combination Therapies∗, Camden Partners∗, LikeMinds Inc∗, Galen Robotics Inc∗, and Nurami Medical∗ (∗includes equity or options).

REFERENCES

1. Vlak MH, Algra A, Brandenburg R, Rinkel GJ. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. *Lancet Neurol*. 2011;10(7):626-636.
2. Juvela S, Poussa K, Lehto H, Porras M. Natural history of unruptured intracranial aneurysms. *Stroke*. 2013;44(9):2414-2421.
3. Ferro JM, Canhão P, Peralta R. Update on subarachnoid haemorrhage. *J Neurol*. 2008;255(4):465-479.
4. Charpentier C, Audibert G, Guillemin F, Stroke TC. Multivariate analysis of predictors of cerebral vasospasm occurrence after aneurysmal subarachnoid hemorrhage. *Stroke*. 1999;30(7):1402-1408.
5. Lucke-Wold B, Logsdon A, Manoranjan B, et al. Aneurysmal subarachnoid hemorrhage and neuroinflammation: a comprehensive review. *Int J Mol Sci*. 2016;17(4):497-493.
6. Chaichana KL, Levy AP, Miller-Lotan R, Shakur S, Tamargo RJ. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. *Stroke*. 2007;38(12):3266-3271.
7. Hijdra A, Van Gijn J, Nagelkerke NJ, Stroke MV. Prediction of delayed cerebral ischemia, rebleeding, and outcome after aneurysmal subarachnoid hemorrhage. *Stroke*. 1988;19(10):1250-1256.
8. Al-Tamimi YZ, Bhargava D, Orsi NM, et al. Compartmentalisation of the inflammatory response following aneurysmal subarachnoid haemorrhage. *Cytokine*. 2019;123:154778-2.
9. Xie X, Wu X, Cui J, Li H, Yan X. Increase ICAM-1 and LFA-1 expression by cerebrospinal fluid of subarachnoid hemorrhage patients: involvement of TNF-α. *Brain Res*. 2013;1512:89-96.
10. Pradilla G, Thai Q-A, Legnani FG, et al. Local delivery of ibuprofen via controlled-release polymers prevents angiographic vasospasm in a monkey model of subarachnoid hemorrhage. *Oper Neurosurg*. 2005;57(Suppl_1):184-190.
11. Shen J, Shen J, Zhu K, Zhou H, Tian H, Yu G. Efficacy of statins in cerebral vasospasm, mortality, and delayed cerebral ischemia in patients with aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis of randomized controlled trials. *World Neurosurg*. 2019;131:e65-e73.
12. Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. *Trends Immunol*. 2001;22(5):265-268.
13. Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol*. 2001;2(3):261-268.
14. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439(7077):682-687.
15. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*. 1999;11(2):141-151.
16. Nishimura H. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 2001;291(5502):319-322.
17. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8(8):793-800.
18. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015;27(4):450-461.
19. Zhou H, Xiong L, Wang Y, et al. Treatment of murine lupus with PD-LIg. *Clin Immunol*. 2016;162:1-8.
20. Song M-Y, Hong C-P, Park SJ, et al. Protective effects of Fc-fused PD-L1 on two different animal models of colitis. *Gut*. 2015;64(2):260-271.
21. Wang G, Hu P, Yang J, Shen G, Wu X. The effects of PDL-Ig on collagen-induced arthritis. *Rheumatol Int*. 2011;31(4):513-519.
22. Han R, Luo J, Shi Y, Yao Y, Hao J. PD-L1 (programmed death ligand 1) protects against experimental intracerebral hemorrhage–induced brain injury. *Stroke*. 2017;48(8):2255-2262.
23. Fanizzi C, Sauerbeck AD, Gangolli M, Zipfel GJ, Brody DL, Kummer TT. Minimal long-term neurobehavioral impairments after endovascular perforation subarachnoid hemorrhage in mice. *Sci Rep*. 2017;7(1):306-302.

24. Froehler MT, Kooshkabadi A, Miller-Lotan R, et al. Vasospasm after subarachnoid hemorrhage in haptoglobin 2-2 mice can be prevented with a glutathione peroxidase mimetic. *J Clin Neurosci*. 2010;17(9):1169-1172.
25. Schallner N, Pandit R, LeBlanc R III, et al. Microglia regulate blood clearance in subarachnoid hemorrhage by heme oxygenase-1. *J Clin Invest*. 2015;125(7):2609-2625.
26. Kubota T, Handa Y, Tsuchida A, Stroke MK. The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. *Am Heart Assoc*. 1993;24(12):1993-2000.
27. Provencio JJ, Fu X, Siu A, Rasmussen PA, Hazen SL, Ransohoff RM. CSF neutrophils are implicated in the development of vasospasm in subarachnoid hemorrhage. *Neurocrit Care*. 2010;12(2):244-251.
28. Spitzer D, Spitzer NJ, Deininger M, et al. Activation of cytotoxic natural killer cells after aneurysmal subarachnoid hemorrhage. *World Neurosurg*. 2017;101:666-676.e1.
29. Wessell AP, Kole MJ, Cannarsa G, et al. A sustained systemic inflammatory response syndrome is associated with shunt-dependent hydrocephalus after aneurysmal subarachnoid hemorrhage. *J Neurosurg*. 2019;130(6):1984-1991.
30. Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell*. 2015;162(6):1257-1270.
31. Hamada T, Cao Y, Qian ZR, et al. Aspirin use and colorectal cancer survival according to tumor CD274 (programmed cell death 1 ligand 1) expression status. *J Clin Oncol*. 2017;35(16):1836-1844.
32. Loftus TJ, Efron PA, Moldawer LL, Mohr AM. β-Blockade use for traumatic injuries and immunomodulation. *Shock*. 2016;46(4):341-351.
33. Bunc G, Kovačič S, Strnad S. The influence of noradrenergic blockade on vasospasm and the quantity of cerebral dopamine β-hydroxylase following subarachnoid haemorrhage in rabbits. *Wien Klin Wochenschr*. 2003;115(17-18):652-659.
34. Chalouhi N, Daou B, Okabe T, et al. Beta-blocker therapy and impact on outcome after aneurysmal subarachnoid hemorrhage: a cohort study. *J Neurosurg*. 2019;125(3):730-736.
35. Chang MM, Raval RN, Southerland JJ, et al. Beta blockade and clinical outcomes in aneurysmal subarachnoid hemorrhage. *Open Neurol J*. 2016;10(1):155-163.
36. Mildner A, Schönheit J, Giladi A, et al. Genomic characterization of murine monocytes reveals C/EBPβ transcription factor dependence of Ly6C$^-$ cells. *Immunity*. 2017;46(5):849-862 e7.
37. Prinz M, Priller J. Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J Neuroimmunol*. 2010;224(1-2):80-84.
38. Mastorakos P, McGavern D. The anatomy and immunology of vasculature in the central nervous system. *Sci Immunol*. 2019;4(37):eaav0492.
39. Stansfield BK, Ingram DA. Clinical significance of monocyte heterogeneity. *Clin Transl Med*. 2015;4(1):2527-2522.
40. Jordan JD, Nyquist P. Biomarkers and vasospasm after aneurysmal subarachnoid hemorrhage. *Neurosurg Clin N Am*. 2010;21(2):381-391.

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Supplemental Digital Content 1. Text. A detailed description of the methodological details for this study.

Supplemental Digital Content 2. Table. Antibodies used for immunophenotyping murine monocytes.

Supplemental Digital Content 3. Table. Antibodies used for immunophenotyping human monocytes.

Supplemental Digital Content 4. Figure. A, ICAp produces diffuse SAH. **B**, Histologic localization of the ICA bifurcation.

Supplemental Digital Content 5. Figure. CD45+, CD11b+ myeloid cells upregulate PD-1 in the ICA perforation model. PD-1 expression in CD4 lymphocytes (CD3+, CD4+, CD8-), CD8 lymphocytes (CD3+, CD4-, CD8+), granulocytes (CD45+, CD11b+, Ly6g+), and NK cells (CD45+, CD3-, CD49b+).

Supplemental Digital Content 6. Figure. Mouse monocyte gating strategy.

Supplemental Digital Content 7. Figure. Garcia scores 24 and 48 h after ICA perforation show no difference in PD-L1-treated and -untreated animals.

Supplemental Digital Content 8. Figure. Human blood monocyte gating strategy.

Supplemental Digital Content 9. Table. Patient demographics and clinical course.

Supplemental Digital Content 10. Figure. CD14, CD16, and PD-1 expression on blood monocytes from patient 1.

Supplemental Digital Content 11. Figure. Linear regression analysis of change in %PD-1+ monocytes vs change in maximum TCD velocity the following day, including the day 3 timepoint outlier.