Inhibition of CD147 (Cluster of Differentiation 147) Ameliorates Acute Ischemic Stroke in Mice by Reducing Thromboinflammation

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Background and Purpose—Inflammation and thrombosis currently are recognized as critical contributors to the pathogenesis of ischemic stroke. CD147 (cluster of differentiation 147), also known as extracellular matrix metalloproteinase inducer, can function as a key mediator of inflammatory and immune responses. CD147 expression is increased in the brain after cerebral ischemia, but its role in the pathogenesis of ischemic stroke remains unknown. In this study, we show that CD147 acts as a key player in ischemic stroke by driving thrombotic and inflammatory responses.

Methods—Focal cerebral ischemia was induced in C57BL/6 mice by a 60-minute transient middle cerebral artery occlusion. Animals were treated with anti-CD147 function–blocking antibody (αCD147) or isotype control antibody. Blood–brain barrier permeability, thrombus formation, and microvascular patency were assessed 24 hours after ischemia. Infarct size, neurological deficits, and inflammatory cells invaded in the brain were assessed 72 hours after ischemia.

Results—CD147 expression was rapidly increased in ischemic brain endothelium after transient middle cerebral artery occlusion. Inhibition of CD147 reduced infarct size and improved functional outcome on day 3 after transient middle cerebral artery occlusion. The neuroprotective effects were associated with (1) prevented blood–brain barrier damage, (2) decreased intravascular fibrin and platelet deposition, which in turn reduced thrombosis and increased cerebral perfusion, and (3) reduced brain inflammatory cell infiltration. The underlying mechanism may include reduced NF-κB (nuclear factor κB) activation, MMP-9 (matrix metalloproteinase-9) activity, and PAI-1 (plasminogen activator inhibitor-1) expression in brain microvascular endothelial cells.

Conclusions—Inhibition of CD147 ameliorates acute ischemic stroke by reducing thromboinflammation. CD147 might represent a novel and promising therapeutic target for ischemic stroke and possibly other thromboinflammatory disorders.

Visual Overview—An online visual overview is available for this article. **(*Stroke*. 2017;48:3356-3365. DOI: 10.1161/STROKEAHA.117.018839.)**

Key Words: blood–brain barrier ■ CD147 ■ inflammation ■ ischemic stroke ■ microvascular dysfunction ■ middle cerebral artery occlusion ■ thrombosis

Stroke is a leading cause of death and permanent disability worldwide. Reperfusion therapy with intravenous tPA (tissue-type plasminogen activator) initiated within 3 to 4.5 hours of stroke onset remains the only approved and validated therapy for acute ischemic stroke.[1] However, a subset of patients still exhibit progressive neurological deterioration despite successful thrombolysis. Although the underlying mechanisms remain poorly understood, thrombotic events occurring in downstream cerebral microvessels may be of particular relevance for brain injury progression after stroke.[2,3] Recent studies have suggested that thrombosis and inflammation are 2 closely intertwined processes that crucially contribute to ischemic brain injury and orchestrate stroke progression.[4–7] These findings have given rise to the novel concept of thromboinflammation in which ischemic stroke is considered to be a thromboinflammatory disease.[8,9] Accordingly, it has been recently proposed that simultaneous targeting of both thrombotic and inflammatory processes could represent a novel therapeutic strategy for acute ischemic stroke.[9]

CD147 (cluster of differentiation 147), a type I transmembrane glycoprotein of the immunoglobulin superfamily, is broadly expressed on the surface of various cell types, including 3 major cell types (ie, leukocytes, platelets, and endothelial cells) that are integrally involved in stroke-induced inflammation and thrombosis.[10] Increased expression of CD147 has been implicated in many human

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diseases such as cancer, cardiovascular diseases, and neurological disorders. Therapeutic targeting of CD147 has yielded encouraging results in experimental models of human diseases, such as rheumatoid arthritis, asthmatic lung inflammation, myocardial ischemia/reperfusion injury, multiple sclerosis, and experimental autoimmune encephalomyelitis.[11–15] Although it has been reported that CD147 expression was increased in the brain after focal cerebral ischemia,[16,17] whether increased CD147 expression simply serves as an associative marker or substantially contributes to ischemic brain injury remains unknown. In this study, we tested the hypothesis that CD147 acts as a key player in ischemic stroke by driving thrombotic and inflammatory responses. We examined the therapeutic potential and mechanisms of neuroprotection by pharmacological inhibition of CD147 in mice after focal cerebral ischemia/reperfusion injury.

Methods

Details of materials and experimental procedures are available from the online-only Data Supplement. This article adheres to the AHA Journals' implementation of the Transparency and Openness Promotion Guidelines.

Stroke Model and Antibody Treatment

Focal cerebral ischemia was induced in C57BL/6 mice by a 60-minute transient middle cerebral artery occlusion (tMCAO) as described previously.[18,19] Two hours after tMCAO, the mice were randomly assigned to the following treatment groups: a rat anti–mouse CD147 monoclonal antibody (RL73.2, eBbioscience, named αCD147 mAb throughout this article) or isotype control antibody (rat IgG2a) administered via tail vein injection in 100 μL volume of PBS. This anti-CD147 antibody has been well characterized to block CD147 function in various mouse models.[11–15] In the 24-hour experiments, a single dose of antibody was given at 4 hours after onset of ischemia. In the 72-hour experiments, antibody treatment was initiated at 4 hours and repeated at 24 and 48 hours after onset of ischemia.

Infarct Volume and Neurological Deficits

Infarct volume was measured in 2,3,5-triphenyltetrazolium chloride-stained coronal sections on day 3 after tMCAO.[19] The modified Bederson score (global neurological function)[20] and the grip strength test (motor function and coordination)[21] were performed by a blinded investigator.

Blood–Brain Barrier Permeability and Microvascular Perfusion

Blood–brain barrier (BBB) permeability was determined by the extravasation of Evans blue (961 Da) and sodium fluorescein (376 Da) in the brain 24 hours after tMCAO.[22] Cerebral microvascular perfusion was assessed by the fluorescein isothiocyanate-dextran–labeled vessels.[23]

Western Blot and Gelatin Zymography

Protein extracts were obtained from the cerebral cortices (bregma +2 to −3 mm) and the isolated cerebral microvessels. Two assays were performed as described previously.[19,24]

Brain Cell Isolation and Flow Cytometry

Brain cell isolation and flow cytometric analysis was performed as described previously.[25,26] Flow cytometry was performed on a Becton Dickinson FACSCalibur, and data were analyzed with CellQuest Pro software.

Immunohistochemistry

Double immunofluorescence staining was performed as described previously.[19,27] The number of vessels positively stained with fibrin/fibrinogen and thrombocytes and the number of occluded microvessels were counted in the ischemic boundary zone (both cortex and striatum). All immunostaining data were analyzed by a blinded investigator, and data are presented as the density of immunoreactive vessels relative to the imaged area (square millimeter).[27,28]

Statistical Analysis

All results were expressed as mean±SEM. GraphPad Prism 5 software package was used for statistical analysis. Unless otherwise indicated, multiple comparisons were made using a 1-way ANOVA followed by the Bonferroni post hoc test. If only 2 groups were compared, unpaired, 2-tailed Student *t* test was applied. $P<0.05$ was considered statistically significant.

Results

CD147 Expression Is Rapidly Increased in Brain Microvessels After tMCAO

Immunofluoresence staining showed abundant expression of CD147 in the ischemic hemisphere at 24 hours after tMCAO, with the most prominent expression seen in the peri-infarct cortex, but only minimal expression seen in the contralateral hemisphere (Figure 1A). Double staining further showed that CD147 colocalized extensively with CD31-positive microvascular endothelial cells in the peri-infarct cortex (Figure 1B) but rarely colocalized with other brain cells (astrocytes, microglia, and neurons; Figure 1B; Figure IA and IB in the online-only Data Supplement) at 24 hours after tMCAO. Interestingly, we observed that CD147 staining colocalized extensively with GFAP-positive (glial fibrillary acidic protein-positive) astrocytes (Figure 1B), partially with ionized calcium-binding adapter molecule 1-positive microglia, but rarely with neuronal nuclear antigen-positive neurons at 72 hours after tMCAO (Figure IA in the online-only Data Supplement). Furthermore, Western blotting was used to assess CD147 protein levels in the ischemic cerebral cortex and specifically in brain endothelial cells using isolated microvessels. The data showed that CD147 protein levels were significantly increased as early as 4 hours and remained elevated 24 hours after tMCAO in the ischemic cortex (Figure 1C) and also in the microvascular endothelial cells (Figure 1D). Collectively, these data indicate that brain microvascular endothelial cells are the major cellular source of increased CD147 expression in the ischemic brain during the first 24 hours after tMCAO.

Inhibition of CD147 Improves Acute Stroke Outcome

Acute stroke outcome was assessed on day 3 after tMCAO. Infarct volumes were significantly reduced in mice treated with αCD147 at a dose of 1 to 2 mg/kg ($P<0.001$), whereas a lower dose of 0.1 mg/kg αCD147 had no significant effect ($P>0.05$) compared with isotype control–treated mice (Figure 2A and 2B). Importantly, the smaller infarct volumes translated into better neurological outcome. Mice treated with αCD147 (1 or 2 mg/kg) showed significant improvement in overall neurological function (Bederson score, Figure 2C) and motor function and coordination (grip test, Figure 2D) compared with isotype controls. On the basis of these data, the dose 1.0 mg/kg of αCD147

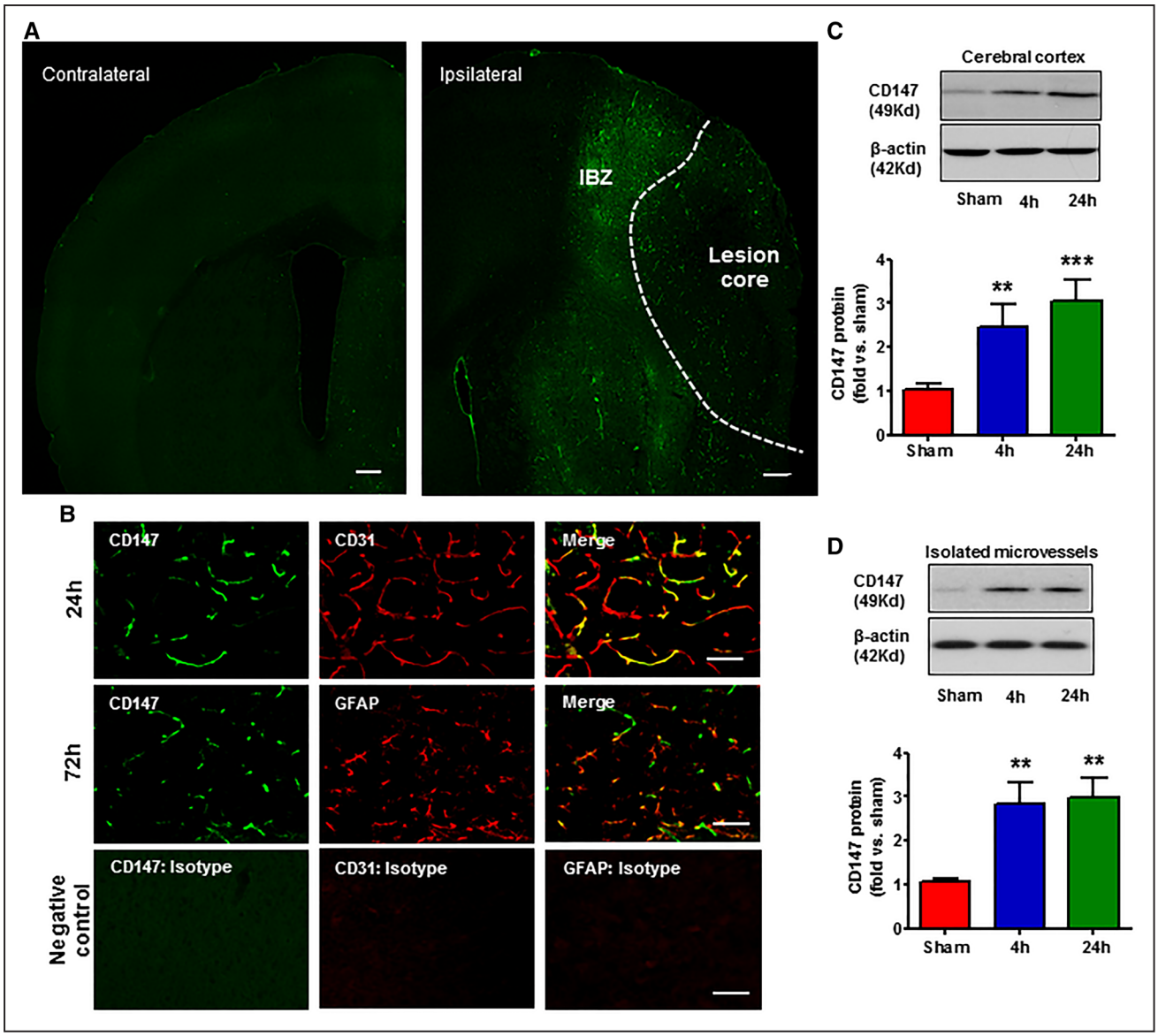

Figure 1. CD147 (cluster of differentiation 147) upregulation in the ischemic brain. **A**, Representative microphotograph of an intact coronal brain section (≈ +0.5 mm to bregma) showing the distribution of transient middle cerebral artery occlusion (tMCAO)–induced CD147 expression in the ipsilateral (ischemic) and contralateral hemisphere 24 hours after onset of ischemia. CD147 was robustly induced in the ischemic hemisphere, prominently shown in microvessel-like structures. Bar=100 μm. **B**, Representative images of double immunostaining showing the colocalization of CD147 (green) with CD31 (endothelial marker, red) or GFAP (astrocytic marker, red) at 24 and 72 hours after tMCAO. n=5 per group. Images were acquired from the peri-infarct cortex. Bar=50 μm. Negative control staining with isotype-matched control antibody did not show detectable labeling. **C** and **D**, Representative images of Western blots showing CD147 protein levels using protein extracts from ipsilateral cortices (**C**) or isolated brain microvessels (a pool of 5 mice per group; **D**) at 4 and 24 hours after tMCAO. Semi-quantification of immunoblots was analyzed by densitometry. Data are expressed as mean±SEM from 3 independent experiments. $^{**}P<0.01$, $^{***}P<0.001$ vs sham control. GFAP indicates glial fibrillary acidic protein-positive; and IBZ, ischemia boundary zone.

or isotype antibody was used for further experiments. Physiological parameters remained within normal range in all experimental groups (Table I in the online-only Data Supplement).

Inhibition of CD147 Reduces BBB Permeability Through Inhibition of Microvascular Matrix Metalloproteinase-9 Activity

The BBB permeability was assessed by measuring extravasation of Evans blue and sodium fluorescein dyes. Isolated cerebral microvessels were analyzed by gelatin zymography for MMP (matrix metalloproteinase)-2/-9 MMP enzymatic activity. The amount of extravasated Evans blue (Figure 3A) and sodium fluorescein (Figure 3B) dyes in the ipsilateral hemispheres and MMP-9 activity (Figure 3C and 3D) in isolated microvessels were significantly increased at 24 hours after tMCAO compared with sham controls. All of these measures were markedly reduced in the αCD147-treated mice compared with isotype-treated mice. Although tMCAO induced a mild but statistically significant increase in MMP-2 activity, there was no significant difference between the αCD147-treated and isotype-treated mice.

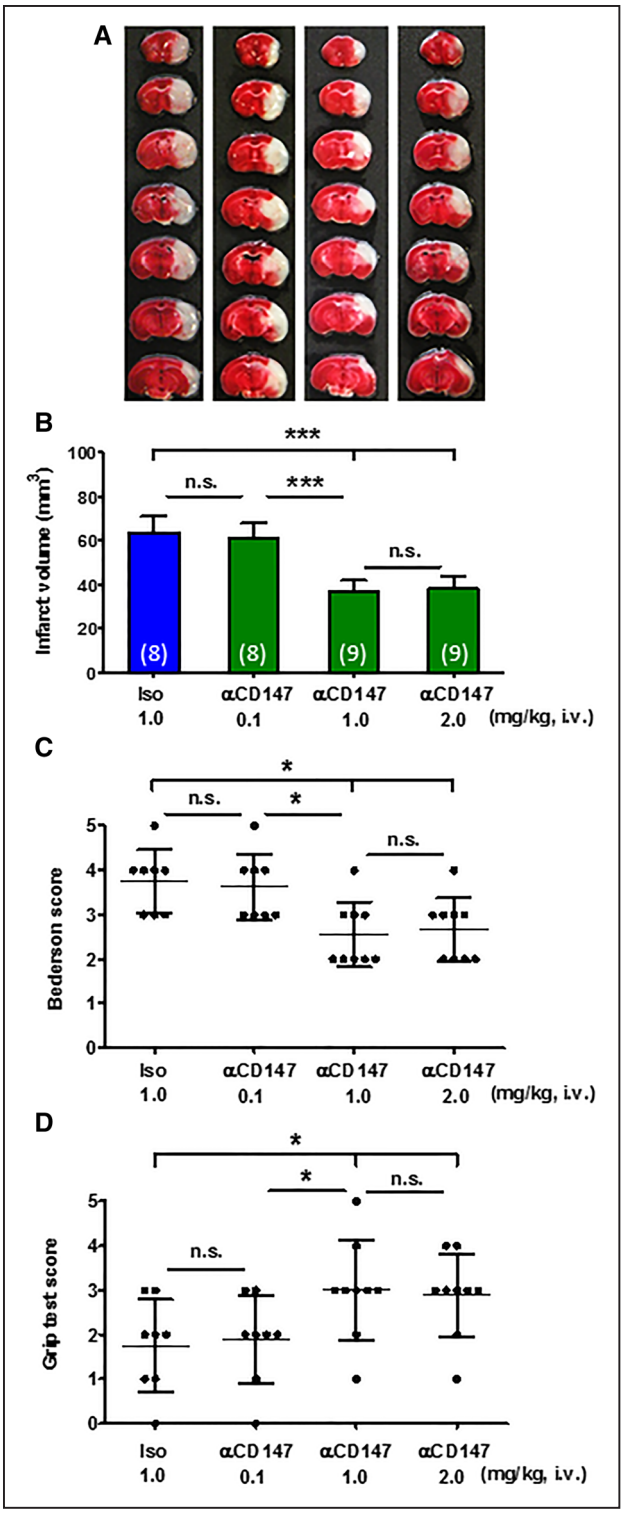

Figure 2. Inhibition of CD147 (cluster of differentiation 147) reduces infarct volumes and neurological deficits 72 hours after transient middle cerebral artery occlusion. **A**, Representative images of 2,3,5-triphenyltetrazolium chloride-stained brain coronal sections of mice (**left** to **right**): receiving different doses (0.1, 1.0, and 2.0 mg/kg) of the anti-CD147 function–blocking antibody (αCD147) or 1.0 mg/kg of isotype control antibody (Iso). **B**, Quantitative analysis of infarct volumes. **C** and **D**, Neurological deficits were assessed using the Bederson test (**C**) and the grip test (**D**). n=8 to 9 per group. n.s. indicates not statistically significant. **P*<0.05, ****P*<0.001.

Inhibition of CD147 Reduces Microvascular Thrombosis and Improves Microvascular Patency

Fibrin and platelet deposition crucially contribute to secondary microvascular thrombosis after ischemic stroke.[2,3] Double immunofluorescence staining showed that tMCAO induced extensive deposition of both fibrin/fibrinogen (Figure 4A) and platelets (Figure 4B) in brain microvessels, which was accompanied by increased microvascular occlusion (Figure 4C) and reduced microvascular patency (Figure 4D) at 24 hours after tMCAO. Importantly, inhibition of CD147 with αCD147 treatment (given at 4 hours after the onset of ischemia) profoundly reduced tMCAO-induced microvascular thrombosis, thereby improving microvascular patency (Figure 4A through 4D).

Activation of NF-κB (nuclear factor κB) and upregulation of PAI-1 (plasminogen activator inhibitor-1) modulate inflammatory and thrombotic responses after ischemic stroke.[29,30] Isolated cerebral microvessels were analyzed by Western blot to evaluate changes in protein levels. Data showed that tMCAO robustly increased the phosphorylation of p65 (ser536) and its upstream signal IκB kinase (IKK)α (ser176) and IKKβ (ser177/181), as well as the expression of PAI-1 protein in brain microvascular endothelial cells. Importantly, all these effects were almost completely blocked with the αCD147 treatment (Figure 5A and 5B).

Inhibition of CD147 Reduces Inflammatory Infiltrate in the Postischemic Brain

Flow cytometry was used to assess inflammatory infiltrate in the mouse brain 72 hours after tMCAO. Figure 6A showed the flow cytometry strategy for gating and identifying immune cells. The number of infiltrating immune cells including neutrophils (Gr-1+), total T cells (CD3+), T helper cells (CD3+CD4+), T cytotoxic (CD3+CD8+) cells, macrophages (CD11b+CD45^{high}), and brain resident microglia (CD11b+CD45^{low}) were markedly increased in the ischemic hemispheres compared with their respective sham controls (Figure 6B). These data are generally consistent with the results of previous studies.[31,32] Importantly, we found that inhibition of CD147 with αCD147 treatment (initiated at 4 hours and repeated at 24 and 48 hours after onset of ischemia) profoundly reduced the accumulation of these inflammatory cells in the ischemic brain, compared with isotype control treatment (Figure 6B).

Discussion

The present study, for the first time, demonstrates that CD147 acts detrimentally in ischemic stroke by driving brain inflammation and microvascular thrombosis. Inhibition of CD147 with αCD147 treatment initiated 4 hours after stroke onset substantially reduced infarct volume and neurological deficits. The observed beneficial effects are likely attributed to reduced BBB damage, inflammation, thrombosis, and improved microvascular patency.

Endothelial cells represent important targets for therapeutic intervention in many cardiovascular and neurological diseases. After ischemic stroke, brain microvascular endothelial cells are rapidly converted into a proinflammatory

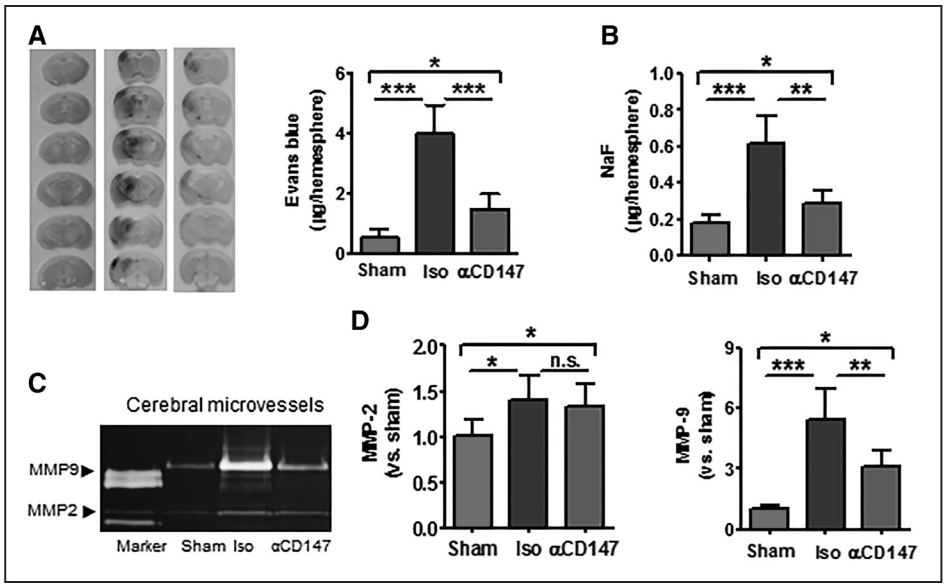

Figure 3. Inhibition of CD147 (cluster of differentiation 147) reduces blood–brain barrier permeability through inhibition of MMP-9 (matrix metalloproteinase-9) in brain microvascular endothelial cells 24 hours after transient middle cerebral artery occlusion (tMCAO). **A** and **B**, Representative pictures of Evans blue leakage in coronal sections from a sham control and tMCAO mice receiving 1.0 mg/kg of isotype control antibody (Iso) or αCD147 treatment (**A**, **left**). Quantitative analysis of Evans blue (**A**, **right**) and sodium fluoride (NaF; **B**) extravasation in the brain parenchyma. n=6 per group. **C**, Representative images of gelatin zymography for MMP-2 and -9 enzymatic activity using protein extracts from brain microvessels (a pool of 5 mice per group) isolated at 24 hours after tMCAO. **D**, Semi-quantification of MMP bands was analyzed by densitometry. Data are expressed as mean±SEM from 3 independent experiments. n.s. indicates not statistically significant. **P*<0.05, ***P*<0.01, ****P*<0.001.

and prothrombotic state, which potentiate microvascular damage and infarct progression.[33] Therefore, targeting brain microvascular endothelial cells represents a promising therapeutic approach for ischemic stroke. In the present study, we provided the first evidence that targeting CD147 may have therapeutic potential for endothelial dysfunction in ischemic stroke. This is supported by the following findings: (1) CD147 expression was rapidly increased in the ischemic brain microvascular endothelial cells after tMCAO and (2) inhibition of CD147 with αCD147 treatment (given at 4 hours after ischemia onset) reduces MMP-9 activity and PAI-1 expression in brain microvascular endothelial cells by analyzing brain microvessels isolated at 24 hours after tMCAO, which is likely associated with profound inhibition of tMCAO-induced activation of NF-κB signaling (p65 and its upstream IKKα). Activation of NF-κB signaling is required for the transcriptional induction of many proinflammatory and prothrombotic mediators involved in ischemic stroke.[29] Considering that significant increase in expression of CD147 in other brain cells (reactive astrocytes and activated microglia) occurred relatively late (72 hours) after tMCAO and antibodies are large molecules that cannot easily cross the BBB, we speculate that the observed anti-inflammatory and antithrombotic actions by αCD147 treatment are mediated mainly through direct targeting of endothelial CD147 in the brain microvessels at least in the early phase (within 24 hours) after tMCAO.

We demonstrated that αCD147 treatment protects against BBB disruption after acute ischemic stroke through inhibition of MMP-9 in ischemic brain microvascular endothelial cells. MMP-9 is known to promote BBB disruption in acute ischemic stroke.[34,35] Notably, although well known as an inducer of extracellular MMPs, CD147 may not always necessarily be associated with induction of MMPs under certain pathological conditions. It has been reported that antibody blockade of CD147 does not influence MMP activity in several mouse models.[11–13] For instance, Seizer et al[13] have reported that inhibition of CD147 with αCD147 treatment had no impact on MMP-2 and MMP-9 activity in the myocardium after ischemia/reperfusion injury. In both mouse and rat tMCAO stroke models, we and others have previously demonstrated that MMP-9 expression was markedly increased in ischemic brain microvessels during the first 24 hours after stroke, whereas infiltrating leukocytes (especially neutrophils) seem to be the major cellular source of brain MMP-9 at later time points (24–72 hours) after stroke.[19,36] In the present study, we further demonstrate that CD147 is highly expressed in ischemic brain microvessels and inhibition of CD147 with αCD147 treatment (given at 4 hours after onset of ischemia) profoundly suppressed MMP-9 activity detected in isolated brain microvessels that was markedly increased 24 hours after tMCAO. These data provide strong, albeit indirect, evidence that CD147 acts as an inducer of MMP-9 in brain microvascular endothelial cells after ischemic stroke. Nevertheless, further research is needed to investigate whether and how CD147 blockade directly inhibits MMP-9 enzymatic activity in brain endothelial cells using in vitro brain endothelial cell culture models. In addition, αCD147 treatment also reduced brain MMP-9 levels at 72 hours after tMCAO (Figure II in the online-only Data Supplement), which could be attributed by reduced leukocyte (mainly neutrophils) infiltration.

Despite successful thrombolysis, ≈25% of patients with acute ischemic stroke do not show any clinical improvement because of incomplete reperfusion.[37,38] Downstream microvascular thrombosis is recognized as a key contributing

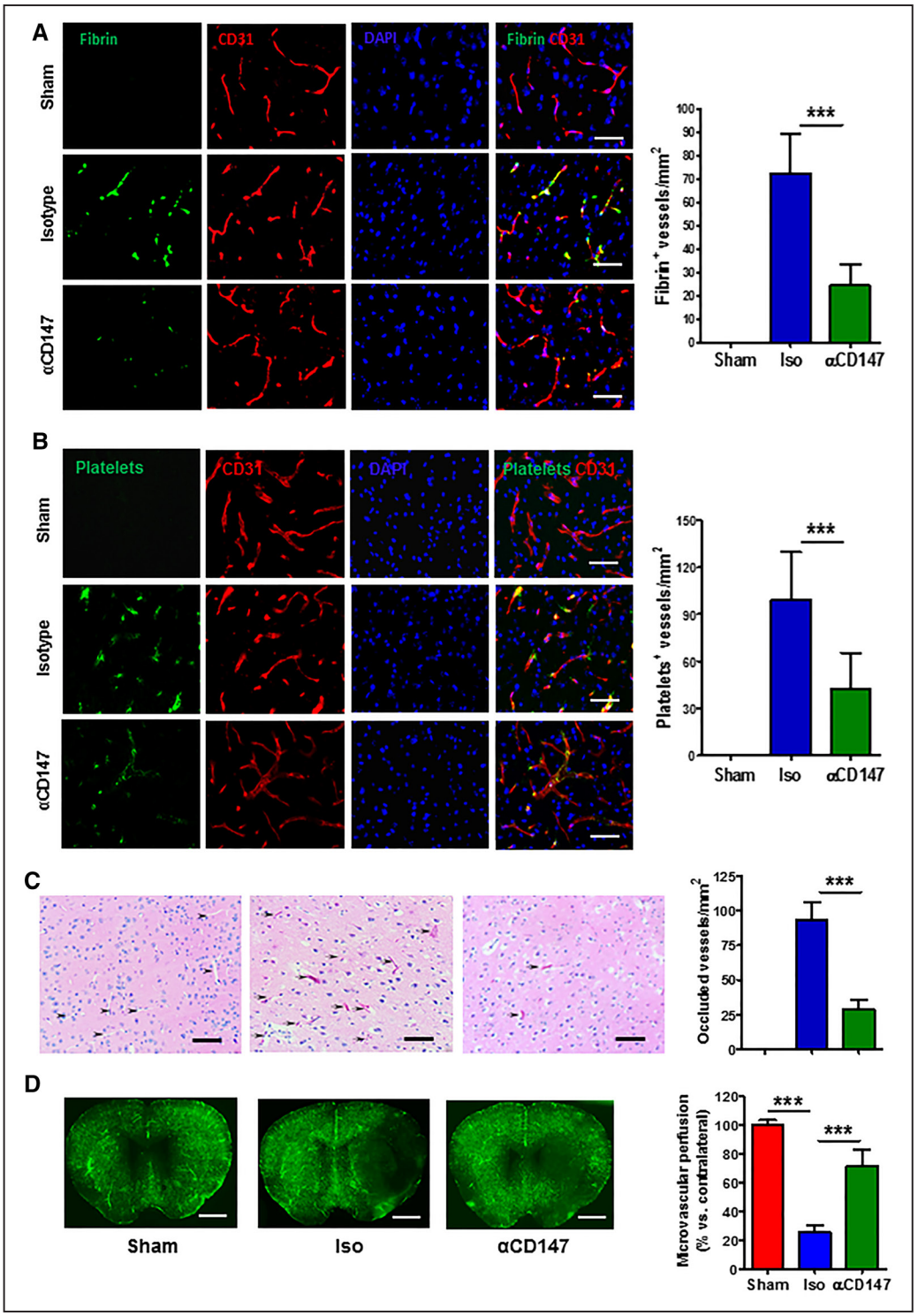

Figure 4. Inhibition of CD147 (cluster of differentiation 147) reduces intravascular fibrin and platelet deposition correlated with reduced microvascular thrombosis and improved microvascular perfusion 24 hours after transient middle cerebral artery occlusion. **A** and **B**, Representative images of double immunostaining showing the staining positive for fibrin/fibrinogen (green, in [**A**]) or thrombocytes (green, in [**B**]) in the cerebral microvessels (marked by CD31 staining, red) in the peri-infarct cortex. **C**, Hematoxylin and eosin staining showing occluded microvessels (indicated by arrows) and patent vessels (indicated by arrowheads) in the peri-infarct cortex. Data are presented as the number of fibrin/fibrinogen-positive (**A**, **left**) or thrombocyte/platelet-positive (**B**, **left**) vessels or occluded vessels (**C**, **left**) relative to the imaged area (square millimeter), as described in the Methods section. Bars=50 μm. n=5 per group. ***P<0.001. **D**, Representative images show microvascular FITC–dextran perfusion from the 3 groups of mice. Quantitative data are expressed as the percentage of the microvascular area perfused with FITC–dextran between the ipsilateral vs contralateral hemisphere. Bars=2 mm. n=5 per group. ***P<0.001. DAPI indicates 4,6-diamidino-2-phenylindole; and Iso, isotype control antibody.

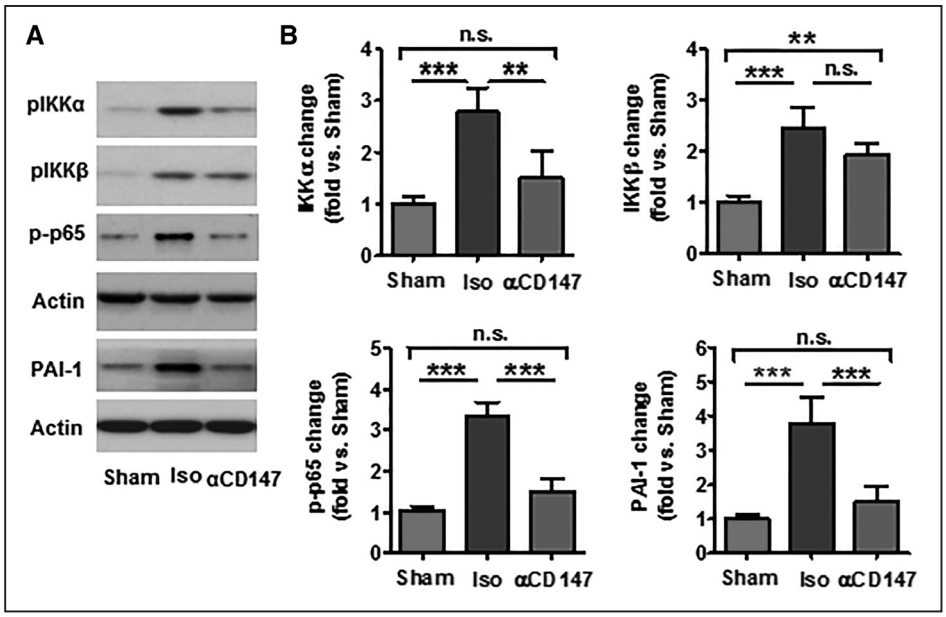

Figure 5. Inhibition of CD147 (cluster of differentiation 147) reduces NF-κB (nuclear factor κB) activation and PAI-1 (plasminogen activator inhibitor-1) expression in cerebral microvascular endothelial cells 24 hours after transient middle cerebral artery occlusion (tMCAO). Protein extracts were prepared from isolated brain microvessels (a pool of 5 mice per group) at 24 hours after tMCAO. **A**, Representative images of Western blots showing the phosphorylated proteins (IKKα, IKKβ, and NF-κB p65) and PAI-1 protein levels. **B**, Semi-quantification of immunoblots was analyzed by densitometry. Data are expressed as mean±SEM from 3 independent experiments. n.s. indicates not statistically significant. ***P*<0.01, *** *P*<0.001.

factor to incomplete reperfusion after ischemic stroke.[37,38] Corroborating these clinical findings, experimental studies have shown that even if the site of major arterial occlusion is reopened, microvascular thrombosis continues to occur at distal sites, which in turn reduces microvascular potency and contributes to neurological progression after ischemic stroke.[23,39,40] Considering that microvascular thrombosis occurs immediately after onset of ischemia[23,39] and that a salvageable penumbra may exist for at least 4.5 hours in some stroke patients,[40] preventing microvascular thrombosis is considered to be essential for successful neuroprotection in treatment of ischemic stroke.[41] It has been reported that intravascular fibrin and platelet deposition critically contribute to downstream microvascular obstruction after acute ischemic stroke.[42,43] Upregulation of PAI-1 expression in brain endothelial cells after ischemic stroke foster intravascular fibrin and platelet deposition, contributing to microvascular obstruction.[28,30] In the present study, we demonstrate that inhibition of CD147 with αCD147 treatment exerts potent antithrombotic effects by reducing intravascular fibrin and platelet deposition, which in turn reduces downstream microvascular thrombosis and improves microvascular patency 24 hours after tMCAO. This finding suggests that CD147-targeted therapy represents a promising approach to prevent microvascular thrombosis, thus conferring microvascular protection in acute ischemic stroke.

Brain inflammation has been implicated as a secondary injury mechanism after ischemic stroke, where the main cellular players are immune cells including activated resident microglial cells and infiltrating leukocytes, mainly neutrophils, monocytes/macrophages, and T cells.[44] These inflammatory cells are involved in stroke progression by producing reactive oxygen species, proinflammatory cytokines and chemokines, MMPs, and other neurotoxic factors.[44] Moreover, neutrophil infiltration can impair microvascular perfusion by occluding cerebral capillaries, a phenomenon commonly referred to as no reflow.[45,46] It has been reported that inhibition of CD147 with αCD147 treatment via interrupting CD147–cyclophilin interaction reduces leukocyte infiltration into inflamed tissues in several mouse models.[11–13] In the present study, we demonstrate that inhibition of CD147 with αCD147 treatment (initiated at 4 hours and repeated at 24 and 48 hours after the onset of ischemia) profoundly reduced inflammatory cell infiltration, including neutrophils, T cells, and macrophages/activated microglia invaded in the ischemic brain 72 hours after tMCAO. Interestingly, intravital microscopy showed that αCD147 treatment almost completely abrogated leukocyte adhesion in the brain microvasculature at 24 hours tMCAO (Figure III in the online-only Data Supplement). Taken together, our findings suggest that targeting CD147 may represent an effective approach to reduce inflammatory cell infiltration in acute ischemic stroke.

It should be noted, however, that the mechanisms whereby anti-CD147 treatment protects against brain injury after ischemic stroke should be much more complex than those we examined in this study. Although the findings of this study suggest that brain endothelial cells may be a primary target of anti-CD147 treatment, we cannot exclude the possibility that CD147 blockade also works importantly by targeting other cells, such as leukocytes or even platelets. Leukocytes and platelets are central to both inflammation and thrombosis, 2 important pathological pathways of ischemic stroke. Further research is needed to answer the following questions: (1) the time-dependent expression profiles of CD147 in blood

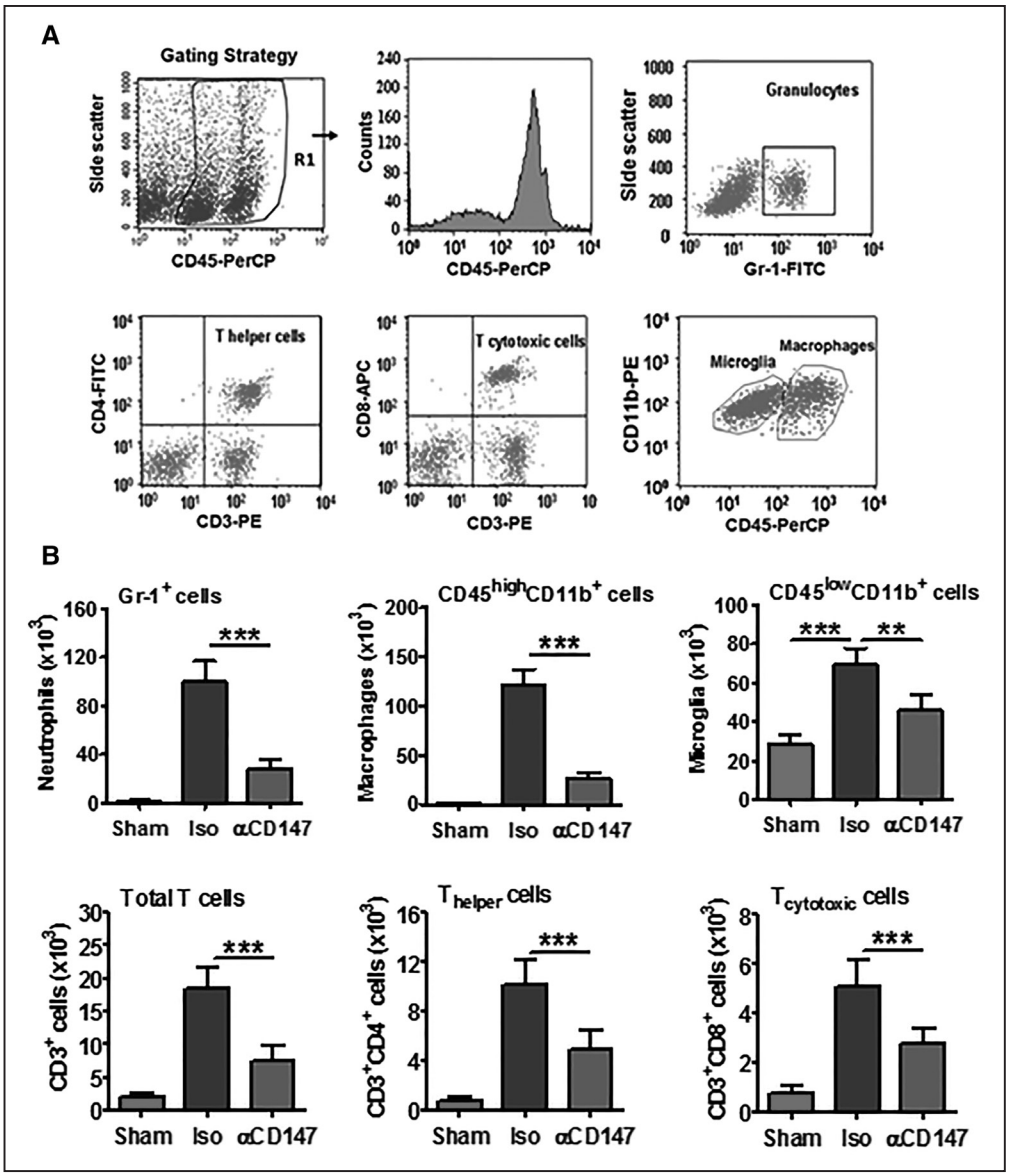

Figure 6. Inhibition of CD147 (cluster of differentiation 147) reduces inflammatory cell infiltration in the ischemic brain 72 hours after transient middle cerebral artery occlusion (tMCAO). Immune cells were isolated from the ipsilateral hemispheres (a pool of 3 mice per group) at 72 hours after tMCAO. Single-cell suspensions were analyzed by flow cytometry. **A**, Gating strategy for identifying immune cell populations: granulocytes/neutrophils (Gr-1+), total T cells (CD3+), T helper cells (CD3+CD4+), and T cytotoxic cells (CD3+CD8+), microglia (CD11b+CD45low), and macrophages (CD11b+CD45high). CD45+ cells were gated for analysis of viable immune populations. **B**, Flow cytometric quantification of cell subpopulations in the 3 groups studied. Data are expressed mean±SEM from 3 independent experiments. $^{**}P<0.01$, $^{***}P<0.001$.

leukocyte subtypes and platelets after ischemic stroke; (2) the effects of CD147 blockade on circulating platelet and leukocyte activation and their interaction, as well as their interaction with brain endothelial cells and subsequent leukocyte trafficking into the ischemic brain; and (3) the relative importance of CD147 expressed in the brain cells versus blood cells by selective targeting of blood leukocytes (or platelets) versus brain endothelial cells.

In summary, pharmacological inhibition of CD147 improves stroke outcome by a combined antithrombotic and anti-inflammatory mechanism and thereby represents a novel and promising therapeutic strategy for ischemic stroke and possibly other thromboinflammatory disorders.

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Disclosures

None.

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