Infiltrating monocyte-derived macrophages (M-Mφ) influence stroke-induced brain injury. Although the inflammatory nature of M-Mφ in acute stroke has been well documented, their role during the resolution phase of stroke is less clear. With emerging evidence for the involvement of scavenger receptors in innate immunity, this study addresses an M-Mφ CD36 role in mediating phagocytosis during the recovery phase of stroke. Stroke increases CD36 and TSP-1/2 mRNA levels in the ipsilateral hemisphere at acute (3-day (d)) and recovery (7d) periods. Quantification of total, intracellular, and cell surface CD36 protein levels showed relatively unchanged expression at 3d post-ischemia. At 7d, there was a significant increase in cell surface CD36 (p < 0.05) with a concurrent reduction of intracellular CD36 (p < 0.05) in the ipsilateral hemisphere. Both cell surface and intracellular CD36 were found in whole brain lysates, whereas cell surface CD36 was predominantly detected in isolated brain mononuclear cells, blood monocytes, and peritoneal macrophages, suggesting that cell surface CD36 expressed in the post-ischemic brain originates from the periphery. The stroke-induced CD36 mRNA level correlated with increased expression of lysosomal acid lipase, an M2 macrophage marker. Functionally, higher CD36 expression in M-Mφ is correlated with higher phagocytic indices in post-ischemic brain immune cells. Moreover, pharmacological inhibition of CD36 attenuated phagocytosis in peritoneal macrophages and brain M-Mφ. These findings demonstrate that cell surface CD36 on M-Mφ mediates phagocytosis during the recovery phase in post-stroke brains and suggests that CD36 plays a reparative role during the resolution of inflammation in ischemic stroke.

Stroke elicits multiple pathological cascades involving inflammation and immunity (1, 2). These processes are tightly coupled with the activation of mononuclear phagocytes, including resident brain microglia and infiltrating peripheral immune cells. Over the past few decades, mechanistic and functional studies addressing stroke-induced inflammation and immunity have largely focused on the acute phase of stroke. Although the persistent presence of monocyte-derived macrophages (M-Mφ)2 in the post-ischemic brain has been linked to acute post-ischemic inflammation, the role of M-Mφ on the subsequent resolution phase of stroke is not clearly defined. The literature suggests that the ischemic environment changes from a toxic pro-inflammatory setting to a more permissive milieu in the resolution phase, allowing inflammation to resolve. Because the ischemic environment influences immune cell function (3), studies have reported conflicting protective and pathological functions of mononuclear phagocytes, suggesting that there are context-dependent roles of M-Mφ in the post-ischemic brain.

CD36 is a highly glycosylated class B scavenger receptor. The expression occurs in monocytes/macrophages and microglia as well as various other cells and tissues, including microvascular endothelial cells, platelets, adipocytes, and the heart (4–8). It has been reported that glycosylation of CD36 is required for trafficking of intracellular CD36 to the cell surface membrane (9). The extent of CD36 glycosylation varies in various steps of monocyte differentiation. Although intracellular CD36 with a lesser degree of glycosylated precursor resides in the subcellular compartment of the secretory pathway, heavily glycosylated CD36 is localized in the extracellular membrane (10). Because of the high affinity of CD36 toward many ligands, including apoptotic cells, thrombospondins (TSPs), fibrillar Amyloid β and oxidized lipids (11–14), the interactions between individual ligands and surface CD36 in specific cell types was shown to elicit diverse physiological and pathological functions. The interactions of CD36 with TSPs in endothelial cells or with fibrillar Amyloid β in microglia produce pro-inflammatory responses (5). As a pattern recognition receptor, CD36 is involved in clearance of cell debris and phagocytosis, which is an important function for tissue repair and inflammation resolution (12, 15).

Although CD36 expression is low in the normal brain, stroke up-regulates its expression, mainly in M-Mφ, in the brain (16–18). Genetic deletion and pharmacological intervention studies

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2 The abbreviations used are: M-Mφ, monocyte-derived macrophage(s); TSP, thrombospondin; MCAO, middle cerebral artery occlusion; d, days(s); LAL, lysosomal acid lipase; SAB, salvianolic acid B; CBF, cerebral blood flow; PE, phycoerythrin; ANOVA, analysis of variance; Contra, contralateral; Ipsi, ipsilateral; iNOS, inducible nitric oxide synthase; Cy7, cyanine 7; APC, allopurinol; ARRIVE, Animal Research: Reporting of in Vivo Experiments.
have shown that CD36 contributes to acute ischemic brain injury (18, 19), apparently mediated by CD36 expressed in endothelial cells (20). On the other hand, hemorrhagic and neonatal stroke studies showed a beneficial side of CD36 through enhancing phagocytic function (21–23). Therefore, these reports of detrimental and beneficial outcomes indicate a context-dependent role for CD36 involving inflammation and resolution. Thus, this study set out to determine the role of M-Mφ. CD36 during the recovery phase of stroke and its involvement in phagocytosis at the time of inflammation resolution. Here we report that cell surface CD36 protein expression in M-Mφ increases at the resolution phase of stroke and contributes to phagocytosis in the ischemic brain, demonstrating a post-stroke stage-specific role of CD36 in ischemic stroke.

Results

The Acute Pro-inflammatory Milieu Changes to a Less Inflammatory Milieu at 7 Days Post-stroke—Thirty-minute transient middle cerebral artery occlusion (MCAO) was performed in mice as described previously (19), with a slight modification in the use of monofilament suture. Compared with the heat-blunted monofilament surgical sutures used in the earlier study (19), Teflon-coated 6–0 monofilament sutures (Doccol, Redland, CA) resulted in an ~25% reduction in mean infarct size at 3 days (d) (~55 mm³ versus ~40 mm³, n = 8–10). To investigate changes in the ischemic milieu in the acute inflammatory and subsequent resolution phases of stroke, we first determined gene expression of inflammatory and macrophage phenotype markers in the ischemic brain at 3d post-stroke, when inflammation and infiltration of M-Mφ are greatest, and at 7d post-stroke, when post-stroke edema and inflammation are resolving (24). Expression of the pro-inflammatory (M1) markers Nos2 (inducible nitric oxide synthase, iNOS), C4d0, IL-1β, and CCR2 mRNA was increased at 3d post-stroke, and their expression was significantly attenuated at 7d post-stroke (Fig. 1, A–D). Expression of the anti-inflammatory (M2) markers arginase-1, lysosomal acid lipase (LAL), and TGFβ1 was also profoundly increased at 3d post-stroke (Fig. 1, E–G). CX3CR1 gene expression was slightly increased at 3d without statistical significance (Fig. 1H). With the exception of arginase-1 mRNA, which was reduced at 7d, LAL, TGFβ1, and CX3CR1 mRNA levels remained high at 7d. The results demonstrate overlapping expression of M1 and M2 macrophage markers at 3d with a change toward a less inflammatory ischemic milieus associated with M2 polarization at 7d.

Glycosylated Cell Surface CD36 Is Up-regulated in Brains 7 Days Post-stroke—CD36 transcription occurs in a feed-forward manner in the presence of ligands (25, 26). We first investigated expression of CD36 and the ligands thrombospondin 1 and 2 (TSP-1 and TSP-2) in the ischemic brain. CD36 and TSP-1/2 mRNA levels were low in sham animals and in the contralateral hemispheres of stroke mice. Stroke significantly increased CD36 and TSP-1/2 mRNA levels at 3d, intracellular and cell surface CD36 protein levels were relatively low in sham mice and the contralateral hemispheres of stroke mice. Stroke moderately increased cell surface CD36 expression at 3d without statistical significance. At 7d, stroke significantly increased cell surface CD36 protein levels with a concomitant reduction of intracellular CD36 protein levels (Fig. 2C). The findings indicate redistribution of intracellular CD36 to cell surface CD36 at 7d post-stroke.

Cell Surface CD36 Is Predominantly Expressed in [CD45]Hi Mononuclear Cells—Cell surface CD36 is thought to play a role in innate immunity by clearing debris and apoptotic bodies, which are critical processes for wound repair and remodeling. After observing elevated cell surface CD36 expression at 7d post-stroke, we investigated the source of cell surface CD36 in the ischemic brain during this resolution phase. There was no detectable cell surface CD36 protein in isolated immune cells from naïve brains and contralateral hemispheres; only the weak intracellular CD36 protein was observed (Fig. 3A). Compared with ischemic brain lysates containing both forms of CD36, mononuclear cells isolated from ischemic brains had only cell surface CD36 (Fig. 3A). Peripheral blood monocytes and peritoneal macrophages also expressed only cell surface CD36 (Fig. 3B), indicating that the source of cell surface CD36 expressed in the ischemic brain comes from infiltrating M-Mφ. To address which mononuclear subsets in the ischemic brain express cell surface CD36, the surface antigens CD45 and CD11b were used to distinguish [CD45]Hi/CD11b+ M-Mφ from [CD45]LO/CD11b−-resident microglia in the ischemic brain. We found that the CD36+ cells are a predominantly [CD45]Hi-expressing population in the stroke hemisphere at 7d (Fig. 3C), further supporting that the CD36+ cells in the ischemic brain are M-Mφ, and not resident microglia.

CD36 Expression Is Associated with M2 Polarization and Enhanced Phagocytosis—Phagocytosis is a salient feature of alternatively activated M2 macrophages (27, 28). CD36 and LAL are crucial for fatty acid oxidation, which is required for M2 macrophage polarization (29). There was increased CD36 and LAL mRNA expression at 7d, and the transcriptions were significantly correlated in the stroke hemisphere but not in the contralateral hemisphere (Fig. 4A). To address the function of the mature form of cell surface CD36, phagocytic activity was determined in brain immune cells. Compared with the contralateral hemisphere, phagocytic activity in immune cells from the ipsilateral hemisphere was significantly elevated (Fig. 4B). To address the involvement of CD36 in M-Mφ, in phagocytosis, brain immune cells isolated from stroke animals at 7d with small (Fig. 4C, red), medium (Fig. 4C, blue), and large (Fig. 4C, green) infarct (n = 2 each) were pooled and divided into two to...
assess CD36 protein expression and phagocytic activity in the same sample. Neither phagocytic activity nor CD36 protein was detected in the cells from the contralateral hemispheres. In the ipsilateral hemispheres, higher cell surface CD36 protein expression in brain immune cells correlated with higher phagocytic indices (Fig. 4C). The results showed that cell surface CD36 is associated with M2 polarization and phagocytosis in the brain during the post-stroke resolution phase.

Inhibition of CD36 Attenuates Phagocytosis in M-Mφ —To address the importance of CD36 in phagocytosis, phagocytic activity was determined in the presence of neutralizing anti-CD36 antibody or salvianolic acid B (SAB), a specific CD36 inhibitor (30). Treatment of peritoneal macrophages with neutralizing anti-CD36 antibody reduced macrophage phagocytic activity (Fig. 5A). Macrophages treated with SAB also reduced phagocytosis, and the effect was dose-dependent (Fig. 5B). There was a profound attenuation of intracellular fluorescent signals in macrophages treated with 200 μM SAB, indicative of reduced phagocytosis (Fig. 5C). Furthermore, SAB reduced phagocytosis in brain immune cells isolated from 7d post-stroke brain (Fig. 5D). The findings demonstrate that cell surface M-Mφ CD36 contributes to phagocytosis during the resolution phase of ischemic stroke.

Discussion

The literature indicates that infiltrating M-Mφ affect CNS inflammation and injury. In addition, there is increasing awareness that peripheral immunity regulates the resolution of
inflammation. With a limited current understanding of the events that underlie the transition from the post-stroke inflammation phase to the resolution phase, this study addresses the involvement of CD36 in the resolution of post-stroke inflammation. As an innate immune receptor expressed in peripheral monocytes/macrophages, key findings from this study include the presence of two distinct forms of CD36 protein, intracellular and cell surface CD36, in the post-ischemic brain and a specific increase of cell surface CD36 in M-M_{H9021} during the 7d resolution phase. Functionally, increased cell surface CD36 expression is associated with features of M2 macrophage polarization and increased phagocytosis in post-stroke brains during the resolution phase.

As a multifunctional receptor, various functions of CD36 have been observed, depending on the expressing cell type and the stroke milieu. Previous studies have implicated that the damaging/pro-inflammatory nature of CD36 is associated with the acute setting (≤3d post-stroke) (16, 17, 19). However, the oldest and most conserved function of CD36 is phagocytosis by mononuclear phagocytes that interact with phosphatidylserine moieties of apoptotic cells (11). The engulfment of apoptotic cells suppresses inflammation and polarizes macrophages to

![FIGURE 2. Stroke increases cell surface CD36 protein expression at 7 days post-stroke. A, gene expressions of CD36, TSP-1, and TSP-2 in the brain in sham and 3d and 7d after stroke. n = 6–8/group, Student’s t test, *, p < 0.05; **, p < 0.01; ***, p < 0.001; Contra versus Ipsi. B, identification of glycosylated CD36 in the presence of PNGase F (deglycosylation enzyme). Brain homogenates (2 µg) were obtained from naïve CD36 KO mice and 7d post-stroke wild-type (10× backcrossed with C57BL/6) mice. C, intracellular and cell surface CD36 protein levels in tissue homogenates (2 µg) obtained from 3d and 7d post-stroke or sham brains. n = 5, two-way ANOVA Bonferroni correction. *, p < 0.05; C, Contra; I, Ipsi.]

![FIGURE 3. Cell surface CD36 is predominantly expressed in [CD45]$^{\text{Hi}}$ mononuclear cells. A, CD36 protein expression in whole tissue homogenates (2 µg) and isolated immune cell lysates (2 µg) from 7d post-stroke brain. B, CD36 protein expression in peripheral monocytes and peritoneal macrophages in naïve WT mice. C, flow cytometry analysis of CD45$^{\text{hi}}$/CD11b$^{\text{low}}$ subsets in CD36$^{\text{hi}}$ populations in brain immune cells at 7d post-stroke. Immune cells from CD36 KO mice were used for negative control. n = 8, two-way ANOVA Bonferroni correction. #, p < 0.05, [CD45]$^{\text{Low}}$ versus [CD45]$^{\text{Hi}}$. **, p < 0.01, Contra versus Ipsi of [CD45]$^{\text{Hi}}$. Contra; I, Ipsi; N, naïve; M, protein marker.]

CD36-mediated Phagocytosis in Ischemic Stroke

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A. CD36 and LAL mRNA levels in brain homogenates and correlation between CD36 and LAL gene expression in the brain 7d post-stroke. B. Phagocytosis in isolated brain immune cells at 7d post-stroke. Immune cells incubated with fluorescent beads at 4 °C were used as a negative control. The phagocytic activity is expressed as a phagocytosis index calculated by (number of fluorescent cells) × (mean fluorescence intensity). C. Phagocytic assay performed in cells, and CD36 Western blotting was performed in immune cell lysate (1 μg). *p < 0.05. C, Contra; I, Ipsi.

Increased CD36 expression is associated with M2 polarization and enhanced phagocytosis. In our previous bone marrow transplantation study, in which wild-type mice received bone marrow from CD36 KO mice, we found an absence of stroke-induced CD36 expression in the ipsilateral hemisphere at acute phase (3d) (17), confirming that CD36 expressed in ischemic brains primarily comes from infiltrating M-Mφ. Conventionally, [CD45]Low/CD11b+ and [CD45]HI/CD11b+ cells in the post-stroke brain have been considered to represent resident microglia and M-Mφ, respectively. Because we observed that cell surface CD36+ immune cells were predominantly in the [CD45]HI/CD11b+ subset, our study further supports the peripheral origin of cell surface CD36 and demonstrates the importance of M-Mφ in mediating CD36 function in the post-stroke brain.

Macrophages polarize to either pro-inflammatory M1 or anti-inflammatory M2 phenotypes, depending on environmental stimuli. Although the expression of M1 and M2 markers may occur on a continuum, M2 polarization of macrophages is associated with enhanced phagocytic ability and a more permissive environment for tissue repair. We observed increased expression of pro-inflammatory mediators and M1 phenotype markers at 3d, whereas expression was reduced with increased and/or sustained expression of M2 markers at 7d (Fig. 1). Relevant to the M2 phenotype, this study addressed the importance of CD36 in M2 macrophage polarization. Considering the fact that M2 macrophages depend on fatty acid oxidation for energy, another study demonstrated that CD36 is critical for fatty acid uptake and that LAL is critical for subsequent lipolysis to sustain adequate metabolism for the M2 phenotype (29). In agreement, we found increased expression of CD36 in the brain 7d post-stroke, with a corresponding increase of LAL expression (Fig. 4A). A CD36 inhibitor reduced phagocytosis in peritoneal macrophages and 7d post-stroke brain immune cells.
In summary, this study reveals the presence of intracellular and cell surface CD36 in the ischemic brain. Importantly, there is increased cell surface CD36 expression in M-M/H9021 during the recovery phase in post-stroke brains, and CD36-expressing M-M/H9021 are crucial for mediating phagocytosis. Besides its inflammatory nature in acute ischemic stroke, CD36 has a reparative role through phagocytosis during the resolution phase of stroke, indicating that the role of CD36 in cerebral ischemia depends on the context and timescale of injury. Modulating CD36 as a therapeutic strategy should be carefully considered for specific post-stroke phases.

Experimental Procedures

Animals—The use of animals and procedures were approved by the Institutional Animal Care and Use Committee of Weill Medical College of Cornell University and in accordance with the Institutional Animal Care and Use Committee, National Institutes of Health, and Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines. The mice were bred at the institute’s animal facility, which maintained temperature, humidity, and a 12-h light/dark cycle. A maximum of five mice were housed in a cage with an individual ventilating system and irradiated bedding (1/8-inch Bed O’s Cobs, The Anderson, Maumee, OH). Sterilized food (PicoLab rodent diet 5053, LabDiet, St. Louis, MO) and water were freely accessible in their cages. Experiments were performed in 10- to 11-week-old male CD36WT and CD36 KO mice. Both genotypes were backcrossed 10 times with C57BL/6 (99.9% C57BL/6 background). Mice were subjected to 30-min MCAO and randomized for 3d or 7d post-stroke survival. Inclusion and exclusion criteria for mice were based on the severity of ischemia. Animals exhibiting cerebral blood flow (CBF) reduction greater than 80% during MCAO and CBF restoration greater than 80% by 10 min after reperfusion were included in the study.

Transient MCAO—Thirty-minute MCAO in mice was performed according to methods described previously (19) with the following modification. Heat-blunted monofilament surgical sutures used in the earlier study were replaced with 6–0 monofilament surgical sutures coated with Teflon (Doccol) (24, 34, 35). CBF was continuously monitored for 10 min prior to stroke, during 30 min MCAO, and for 10 min during reperfusion using laser-doppler flowmetry (Periflux System 5010, Perimed AB, Järífälla, Sweden). The core body temperature of the animals was maintained at 37 ± 0.5 °C during the entire procedure by a thermocouple-regulated temperature controller (Digi Sense R/S, Cole-Parmer, Vermon Hills, IL).

Tissue Section Strategy—Brains were excised, frozen, and sectioned using an unbiased stereological sampling strategy to reflect the MCA territory in both hemispheres as described previously (35). The brain tissue was sectioned and collected from each hemisphere to determine mRNA and protein levels.

Isolation of Brain Immune Cells, Peripheral Macrophages, and Blood Monocytes—Mice were anesthetized with isoflurane and pentobarbital and then perfused with ice-cold heparinized PBS to remove blood. Brains were removed, and the hemi-
spheres were separated and placed into ice-cold Hanks’ balanced salt solution (Life Technologies) without Ca\(^{2+}\) and Mg\(^{2+}\). The tissue was enzymatically and mechanically dissociated with MACS\textsuperscript{®} neural tissue dissociation kits with papain (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the instructions of the manufacturer. The dissociated tissue was used to obtain brain homogenates or isolated brain immune cells. Brain immune cells were isolated according to methods described previously (36). Cells were centrifuged by discontinuous Percoll PLUS (GE Healthcare) gradients and collected from 37–70% interphases. Peritoneal macrophages were prepared by peritoneal lavage as described previously (24). The adherent cells were used to obtain macrophage protein lysates. Blood monocytes were prepared according to a method published previously with minor modifications (35). After collecting total blood cells, red blood cells were lysed using RBC lysing buffer (Sigma-Aldrich, St. Louis, MO), and cells were sequentially applied to discontinuous Percoll (GE Healthcare) gradients and collected from 37–70% interphases.

**Flow Cytometry Analysis**—Isolated brain immune cells were counted by trypan blue staining. 0.5–1 × 10\(^6\) cells were preincubated with mouse BD Fc Block (anti-CD16/CD32, 1 μg/10\(^6\) cells in 100 μl, BD Biosciences) and then incubated with 10% FBS in PBS at 4 °C for 15 min. Cells were then stained with antibodies against CD11b (Phycoerythrin-Cyanine 7 (PE-Cy7) rat anti-mouse CD11b, 1:400, 552850), CD45 (Phycoerythrin (PE) rat anti-mouse CD45, 1:1,000, 553081), and CD36 (Allophycocyanin (APC) mouse anti-mouse CD36, 1:200, 562744) in 1% FBS in PBS at 4 °C for 1 h. After washing with PBS, cells were immediately analyzed using a flow cytometer (Accuri C6, BD Biosciences). Unstained cells and stained cells with the corresponding isotype controls (PE-Cy7 rat IgG2b, κ isotype control, 552849; PE rat IgG2b, κ isotype control, 553989; APC mouse IgA, κ isotype control, 562140) were used for negative controls. The specificity of CD36 signal in brain immune cells was confirmed using CD36 KO mice. All antibodies and isotype controls used for flow cytometry were purchased from BD Biosciences.

**Real-time RT-PCR Analysis**—Relative mRNA levels were quantified with real-time RT-PCR using fluorescent TaqMan technology as described previously (35). Total RNA from brain tissues or cell line cultures was reverse-transcribed using Quantitect reverse transcription kits (QiaGen, Valencia, CA). Gene-specific PCR primers and probes were obtained as TaqMan predesigned assay reagents for gene expression (Life Technologies), including iNOS (Mm00440485_m1), CD40 (Mm00441891_m1), IL-1β (Mm00434228_m1), Arginase-1 (Mm00475988_m1), TGFβ1 (Mm01178820_m1), CD36 (Mm00432398_m1), TSP-1 (Mm01335418_m1), TSP-2 (Mm01279240_m1), LAL (Mm00498820_m1), CCR2 (Mm00438270_m1) and β-actin (Mm00607939_s1). β-Actin was used as an internal control for sample normalization. The PCR reaction was performed using FastStart Universal Probe Master Mix (Roche) in an Applied Biosystems 7500 fast real-time PCR system (Life Technologies). Gene expression was presented as the β-actin normalized value according to the following formula: value = 2\(^{-}\Delta \Delta CT\), where \(\Delta CT\) = 

**Western Blotting Analysis**—Brain tissue was homogenized, and cells were lysed in lysis buffer (Cell Signaling Technology, Beverly, MA) supplemented with 1 mm PMSF and complete Mini protease inhibitor and Phospho Stop (Roche Applied Science). Tissue and cell lysates were centrifuged, and supernatants were used for Western blotting analyses. Protein concentration was determined, and the indicated amount of protein from brain tissue or cells was separated on NuPAGE 4–12% Bis-Tris gels (Thermo Fisher, Waltham, MA) and then transferred to polyvinylidene fluoride membranes (Bio-Rad). Membranes were incubated in blocking buffer (Li-Cor, Lincoln, NE) for 1 h, followed by anti-mouse goat CD36 (1:1,000, AF2519, lot VYQ0108041, R&D Systems, Minneapolis, MN) or anti-broad spectrum of species, including anti-mouse goat β-actin (1:1,000, sc-1615, lot B2206, Santa Cruz Biotechnology) antibody in blocking buffer at 4 °C. Membranes were washed with Tris-buffered saline containing 0.05% Tween 20 followed by incubation with appropriate secondary antibodies conjugated with Alexa Fluor 680 rabbit anti-goat (1:5,000, A21088, Thermo Fisher) or IRDye 800CW donkey anti-mouse (1:10,000, 926-32212, Li-Cor) in blocking buffer at room temperature for 1 h. Protein bands were visualized using the Odyssey imaging system (Li-Cor). Protein quantification was done using ImageJ, and CD36 protein levels were normalized by β-actin. The specificity of CD36 protein bands was confirmed using tissues from CD36 KO mice. To identify deglycosylated CD36 protein, protein lysates were incubated with denaturing buffer (New England Biolabs Inc., Ipswich, MA) at 100 °C for 10 min and then with PNGase F and 1% Nonidet P-40 (New England Biolabs Inc.) at 37 °C for 1 h. Western blotting was performed on the deglycosylated lysates to visualize naïve deglycosylated CD36.

**Phagocytosis Assay**—The phagocytic activity was determined according to a method described previously (37) with a minor modification in the number of cells and microspheres. Brain immune cells or peritoneal macrophages were plated on 24-well plates (1 or 2 × 10\(^5\) cells/well, respectively) and incubated at 37 °C with 5% CO\(_2\) for 1 h. After washing non-adherent cells, CD36 antibody (324205, Novus Biologicals, Littleton, CO) was added to peritoneal macrophages for 1 h. Different concentrations of SAB were added to cells for 1 h for brain immune cells or overnight for peritoneal macrophages. Fluorescent microspheres (1 μm, yellow-green (505/515), F-13081, Molecular Probes, Eugene, OR) were added to peritoneal macrophages (1 × 10\(^5\)) or isolated brain immune cells (1 × 10\(^6\)) and incubated at 37 °C with 5% CO\(_2\) for 3 h or 24 h, respectively. After discarding non-phagocytosed beads by washing multiple times with PBS, cells were harvested and resuspended in PBS and analyzed by flow cytometer. Cells incubated at 4 °C with beads were used as negative controls. For visualization of phagocytosis, cells were analyzed by fluorescent microscopy (Nikon Eclipse TS100, Nikon, Melville, NY).

**Statistical Analysis**—Statistical analyses were performed using Student’s t test for comparison between two groups. One-way or two-way ANOVA followed by post hoc Bonferroni’s multiple comparison tests were used for multiple group comparisons. Correlation analyses between gene expressions were made using linear regression. Differences were considered statistically significant at p < 0.05 in all analyses.
Author Contributions—M. S. W. performed experiments, analyzed the data, and wrote the manuscript. J. Y. performed experiments and wrote the manuscript. C. B. generated the animal model of stroke. S. C. designed the study and wrote the manuscript.

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