Gut Microbiota as a Key Regulator of Systemic Immunity After Stroke

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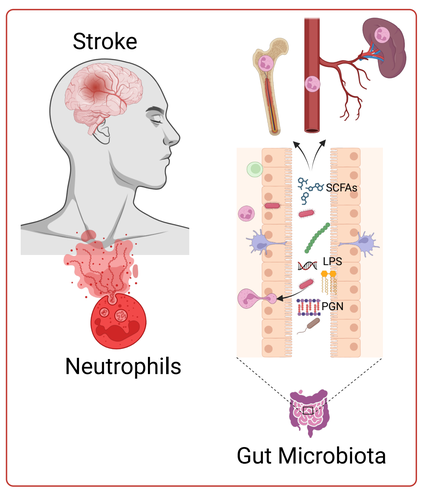
Background and Aims



Neuroinflammation triggered by systemic immune activation plays a crucial role in determining outcomes after ischemic stroke. Gut microbiota (GM) is a key player in immune function after stroke. In our current work, we show that depletion of GM —either germ-free conditions or through broad-spectrum antibiotics—attenuates neutrophil activation after stroke. This disarming of neutrophil responses was associated with decreased expression of inflammatory genes in the brain, reduced vascular thrombosis, smaller infarct volumes, and improved behavioral outcomes. In summary, our findings reveal that GM plays a decisive role in post-stroke systemic immune activation and directly influences stroke severity. Targeting GM composition may offer a promising therapeutic option for modulating neuroinflammation and improving stroke outcomes.

Methods

To investigate the effects of gut microbiota on neutrophil activation and stroke outcome after stroke, germ free (GF) female mice were colonized by housing with SPF mice for six weeks or SPF mice were treated with cocktail of broad-spectrum antibiotics. Afterward, stroke was induced by transient occlusion of the middle cerebral artery and mice were sacrificed three days after for analysis. The activation of neutrophils was analyzed using LC/MS based proteomics and multi-color flow cytometry. The amount of plasma neutrophil extracellular traps (NETs) was measured using citH3-DNA complex ELISA. Brain infarct volumes were quantified by analysis of cresyl violet stained histological sections. Brain Imaging was performed using light-sheet fluorescence microscopy (LSFM).

Results

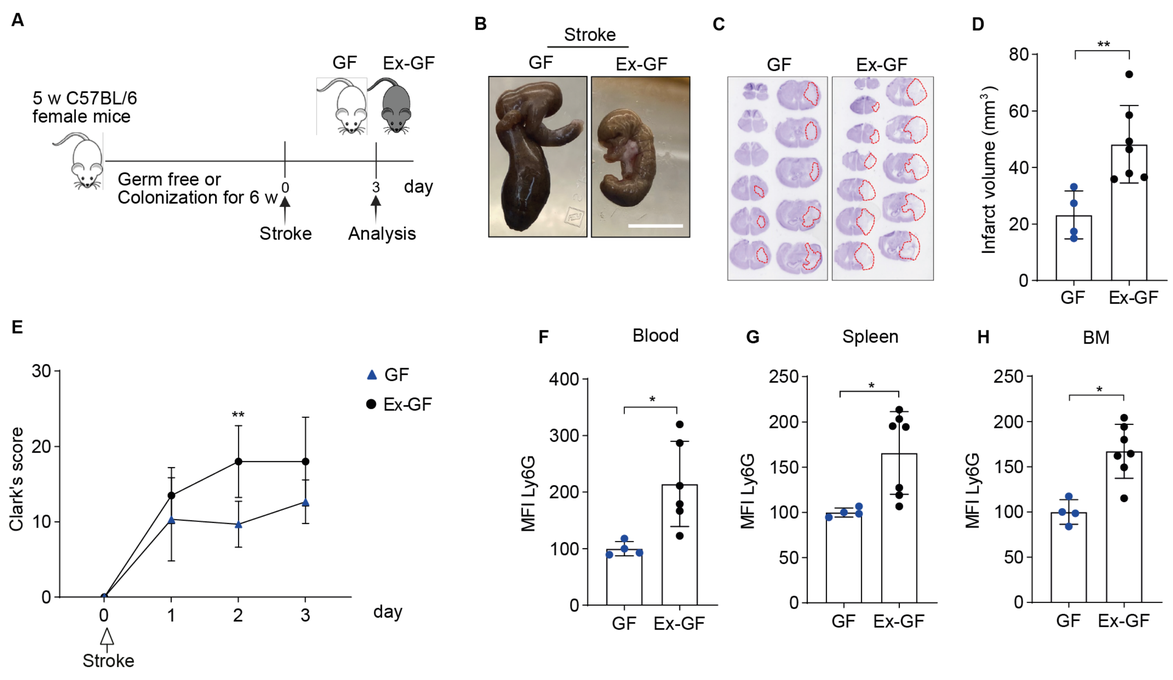


Figure 1. Gut microbiota trigger neutrophil activation, and increase brain infarcts and sensorimotor deficits. A. Scheme illustrating the experimental design. **B.** Images showing caecum size in GF and Ex-GF stroke mice. Scale bar = 1 cm. **C.** Representative images of cresyl violet stained brain sections from GF and Ex-GF stroke mice. The red outline marks the infarct regions. **D.** Brain infarct volumes (mm^3) of GF and Ex-GF mice after three days of stroke. **E.** Clark's score for GF and Ex-GF mice after one to three days of ischemic stroke. **F–H.** Mean fluorescence intensity (MFI) of Ly6G on neutrophils in blood, spleen and tibial BM in GF and Ex-GF stroke mice three days after surgery. Values are normalized to GF controls and presented as percentages relative to the 100% GF mean. Data are analyzed by the unpaired Mann–Whitney U test, *p < 0.05, **p < 0.01, n = 4–7 mice per group. GF = germ-free, BM = bone marrow.

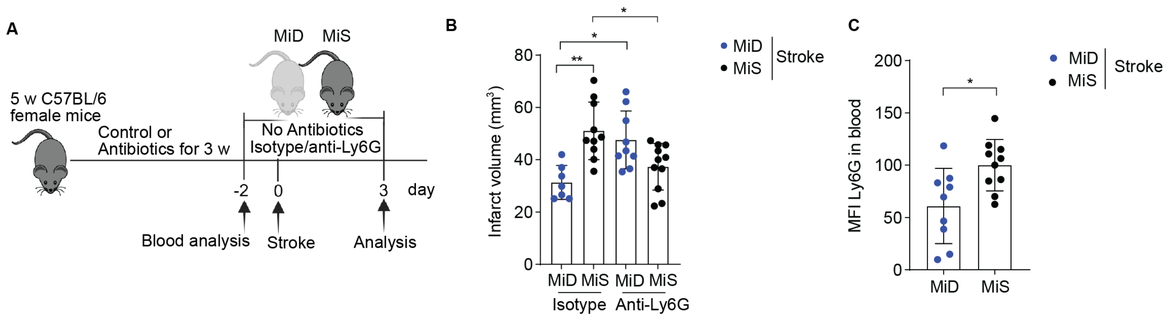


Figure 2. Gut microbiota deficiency reduces neutrophil activation and is protective to injured stroke brain. A. Scheme illustrating experimental design. **B.** Brain infarct volumes of MiD and MiS mice with or without neutrophil depletion after three days of stroke. **C.** MFI MFI of Ly6G in blood neutrophils of MiD and MiS stroke mice. Values are normalized to MiS controls and presented as a percent decrease. Data are analyzed by the unpaired Mann–Whitney U test for two groups and the Kruskal–Wallis test for multiple comparisons, *p < 0.05, **p < 0.01, n = 7–10 mice per group. MiD = microbiota-deficient, MiS = microbiota sufficient.

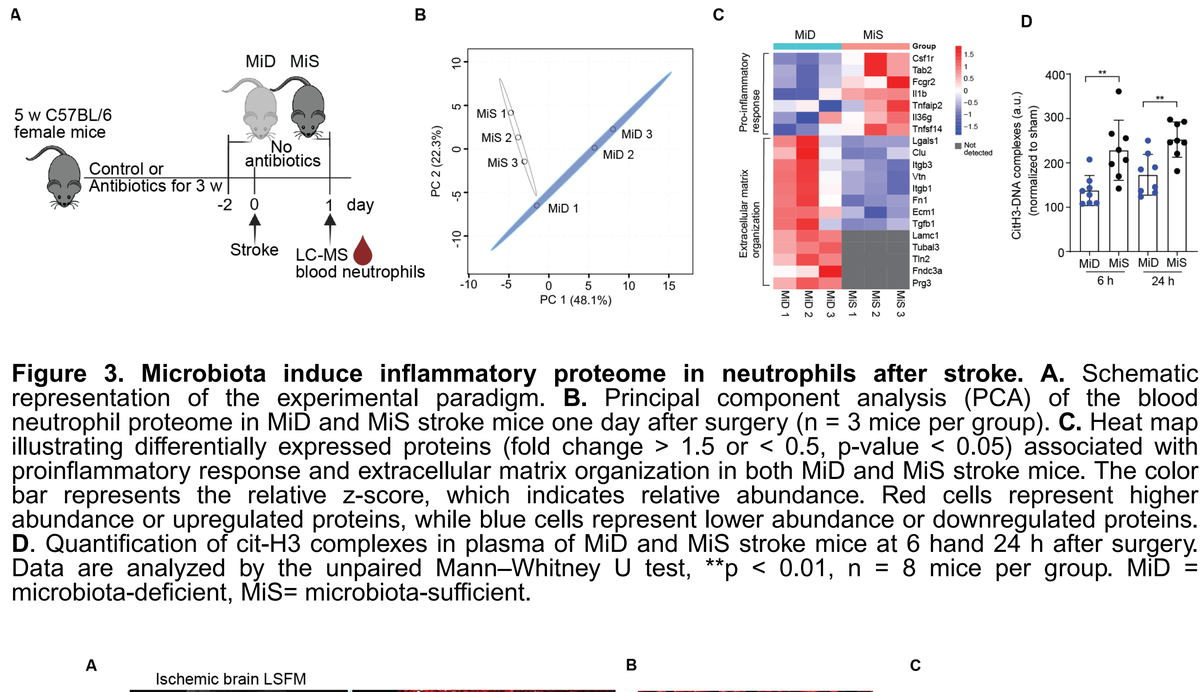


Figure 3. Microbiota induce inflammatory proteome in neutrophils after stroke. A. Schematic representation of the experimental paradigm. **B.** Principal component analysis (PCA) of the blood neutrophil proteome in MiD and MiS stroke mice one day after surgery (n = 3 mice per group). **C.** Heat map illustrating differentially expressed proteins (fold change > 1.5 or < 0.5, p-value < 0.05) associated with proinflammatory response and extracellular matrix organization in both MiD and MiS stroke mice. The color bar represents the relative z-score, which indicates relative abundance. Red cells represent higher abundance or upregulated proteins, while blue cells represent lower abundance or downregulated proteins. **D**. Quantification of cit-H3 complexes in plasma of MiD and MiS stroke mice at 6 hand 24 h after surgery. Data are analyzed by the unpaired Mann–Whitney U test, **p < 0.01, n = 8 mice per group. MiD = microbiota-deficient, MiS= microbiota-sufficient.

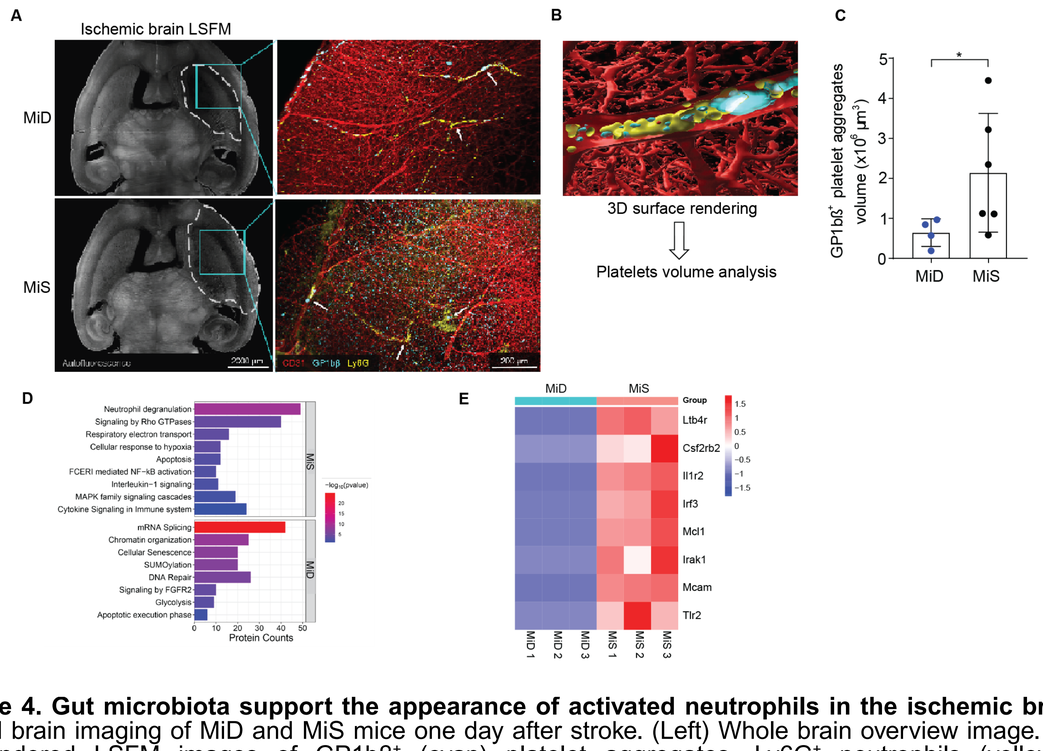


Figure 4. Gut microbiota support the appearance of activated neutrophils in the ischemic brain. A. LSFM brain imaging of MiD and MiS mice one day after stroke. (Left) Whole brain overview image. (Right) 3D-rendered LSFM images of GP1bβ$^+$ (cyan) platelet aggregates, Ly6G$^+$ neutrophils (yellow) and CD31+(red) brain vasculature of mice one day after stroke. **B.** High-resolution image showing 3D surface rendering for GP1bβ$^+$, Ly6G$^+$,and CD31$^+$ signals in ischemic brain vasculature. GP1b β$^+$ surfaces were used for quantification of platelet aggregate volumes. **C.** Quantification of total GP1b$^+$ aggregate volumes (×10^6 µm3) within CD31$^+$ microvasculature of 6 mm^3 ipsilateral brain region of MiD and MiS stroke mice. **D.** Functional annotation of differentially regulated proteins between two groups. **E.** Heat map illustrating the proteins uniquely detected in ischemic brain-infiltrated neutrophils in MiS stroke mice but not in MiD mice. Data are analyzed using the unpaired Mann–Whitney U test for two-group comparisons, *p < 0.05, n = 4–8 mice per group. MiD = microbiota-deficient, MiS = microbiota-sufficient.

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Conclusions and outlook

1. Gut microbiota act as a potential trigger of neutrophil activation responses after ischemic stroke.
2. Gut microbiota may also increase platelet-driven vascular thrombus formation and impact brain tissue reperfusion.
3. Future studies identifying underlying mechanisms of neutrophil activation through gut microbiota are needed to develop strategies to reduce inflammatory brain injury in stroke patients.