Intestinal immune defects in cerebrovascular disease-How neutrophils do this?

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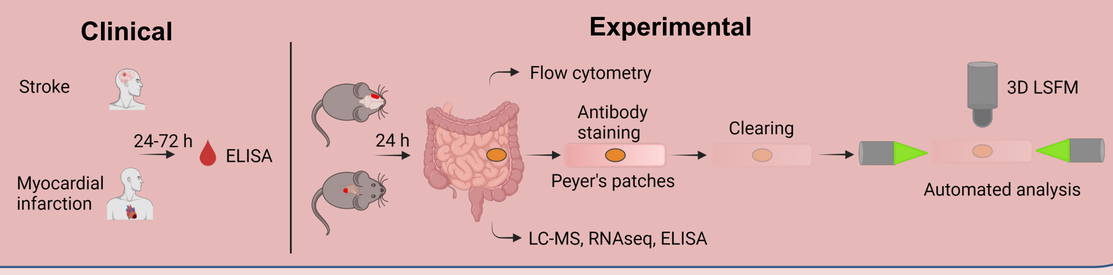
INTRODUCTION and METHODS

Dysfunction of humoral immunity after tissue injury is responsible for infections and poor outcomes in cardiovascular disease. However, the mechanisms underlying these life-threatening changes are not well known. Among immunoglobulins (Ig), IgA, the most frequent mucosal antibody, is produced by plasma B cells in Peyer's patches (PP) and lamina propria. Using blood samples from different clinical cohort of ischemic stroke and myocardial ischemia patients and pre-clinical animal models, we dissected the causes of post-injury immune defects i.e. IgA and lymphocyte loss.



RESULTS

Stroke and heart attack patients have low IgA

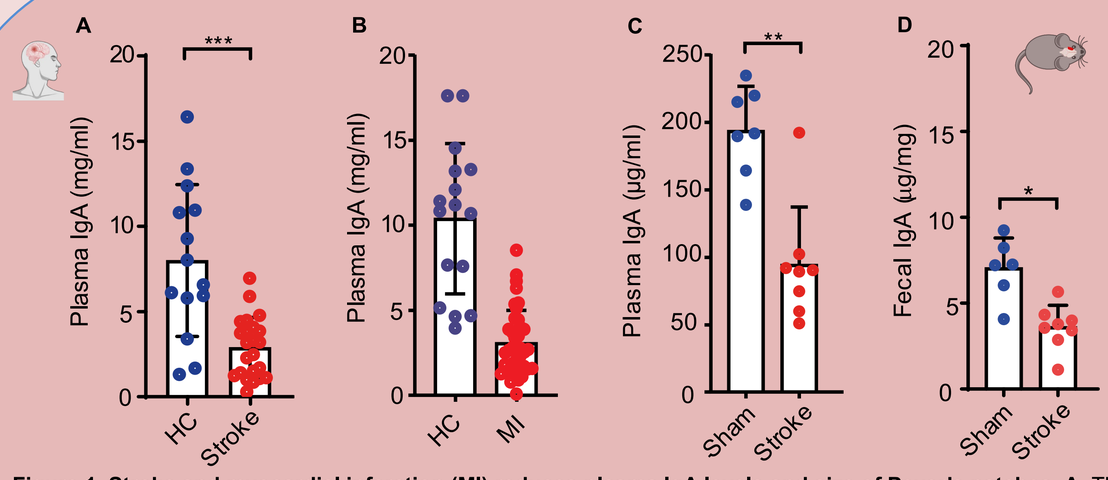


Figure 1. Stroke and myocardial infarction (MI) reduces plasma IgA levels and size of Peyer's patches. A, The amounts of plasma IgA in stroke patients and healthy subjects. **B,** The amounts of plasma IgA in MI patients and healthy subjects. **C,** The levels of plasma IgA in stroke mice compared to sham controls. **D,** The levels of fecal IgA in stroke mice compared to sham controls. MI=myocardial infarction, HC=healthy controls.

B cell follicles shrink after stroke and heart attack

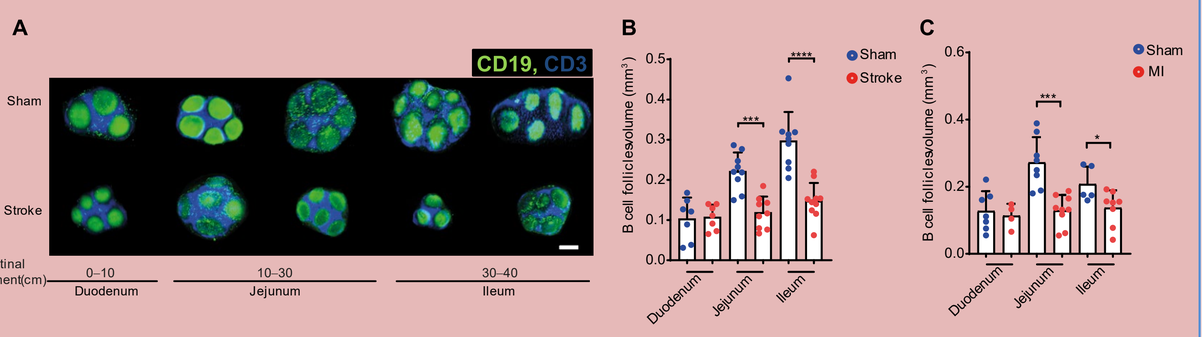


Figure 2. Stroke and myocardial infarction (MI) decreases B cell follicles volume in PP. A, 3D reconstruction of LSFM images of CD19⁺ B cells (green) and CD3⁺ T cells (blue) in PP isolated from duodenum, jejunum and ileum 24 h after stroke or sham surgery. **B,** Deep learning based automated analysis of B cell follicles volume in PP from duodenum, jejunum and ileum one day after stroke or sham surgery. **C,** Analysis of B cell follicles volume in PP one day after MI or sham surgery. MI=myocardial infarction, LSFM=light-sheet fluorescence imaging.

IgA producing B cells are lost under side-effect of circulating DNA

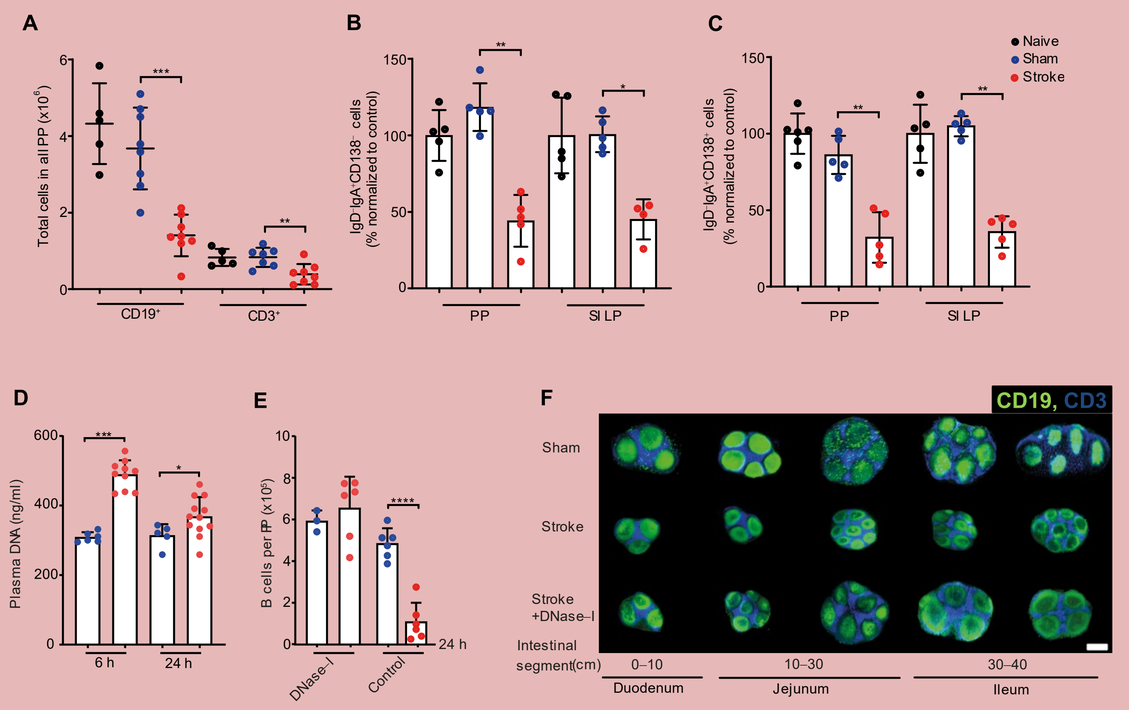


Figure 3. Stroke induced B cell loss in PP is dependent on the circulating DNA. A, Flow cytometry-based quantification of the number of CD19⁺ B cells and CD3⁺ T cells in all intestinal PP 24 h after stroke, sham surgery and unoperated naïve mice. **B,** Quantification of IgD⁻IgA⁺CD138⁻ plasma cell precursors in all PP and SI LP after 24 h of sham surgery or stroke and naïve mice. **C,** Quantification of IgD⁻IgA⁺CD138⁺ plasma cells in all PP and SI LP. **D,** Quantification of plasma DNA 6 h and 24 h after stroke or sham surgery using Qubit assays. **E,** Numbers of B cells in PP 24 h after stroke or sham surgery in DNase-I and vehicle-treated mice analyzed by flow cytometry. **F,** 3D reconstruction of LSFM images of CD19⁺ B cells (green) and CD3⁺ T cells (blue) in PP isolated from duodenum, jejunum and ileum 24 h after sham, stroke and stroke+DNase-I treated mice. PP=Peyer's patches, SI LP= small intestine lamina propria.

Post-injury activated neutrophils release toxic NETs

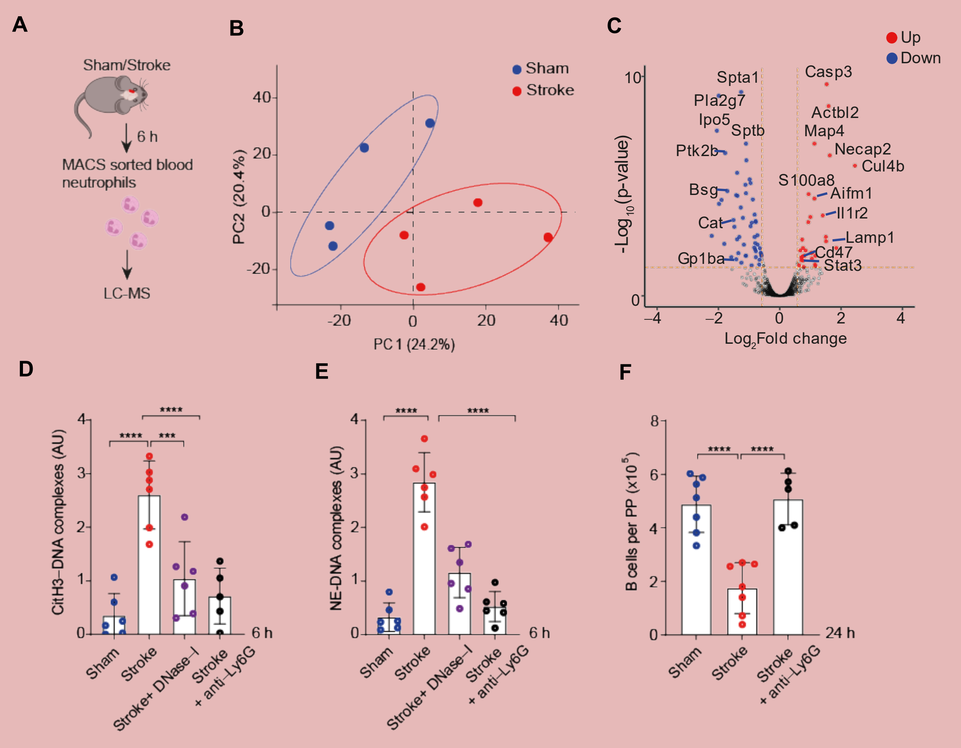


Figure 4. Stroke activated neutrophils release NETs and mediate B cell loss in PP. A, Schematic of the experimental paradigm for neutrophil mass-spectrometry and proteomics analysis. **B,** Principal component analysis of neutrophil proteomics after sham or stroke. **C,** Volcano plot comparing the normalized protein abundance in blood neutrophils of stroke mice vs sham-operated mice. **D,** Relative plasma levels of citH3-DNA or **E,** NE-DNA complexes after sham + isotype antibody, stroke + isotype antibody, stroke + DNase-I treatment or stroke + anti-Ly6G antibody treatment. **F,** Numbers of CD19⁺ B cells in intestinal PP in sham-operated + isotype antibody, stroke + isotype antibody and stroke + anti-Ly6G antibody-treated mice.

NET blockage protects IgA producing plasma cells

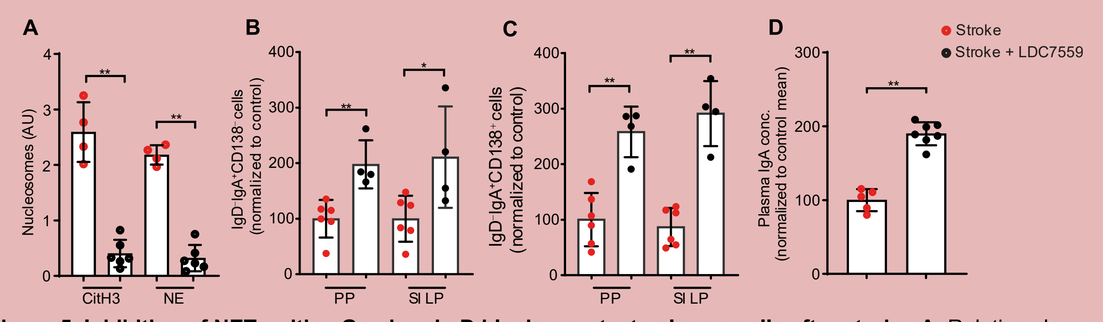


Figure 5. Inhibition of NETs with a Gasdermin D blocker protects plasma cells after stroke. A, Relative plasma levels of citH3-DNA and NE-DNA complexes in stroke + vehicle and stroke + LDC7559 treated mice. **B,** Numbers of IgD⁻IgA⁺CD138⁻ plasma cell precursors in all PP and SI LP in stroke and stroke + LDC7559 treated mice. **C,** Numbers of IgD⁻IgA⁺CD138⁺ plasma cells in all PP and SI LP in stroke and stroke + LDC7559 treated mice. **D,** Relative concentrations of plasma IgA in stroke and stroke + LDC7559 treated mice. NE=neutrophil elastase, PP=Peyer's patches, SI LP=small intestine lamina propria.

NETs in patients can be targeted to correct immune defects

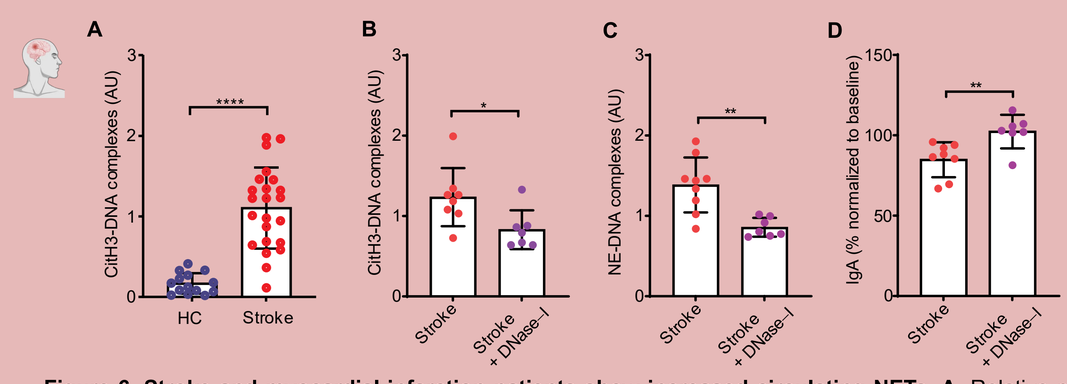
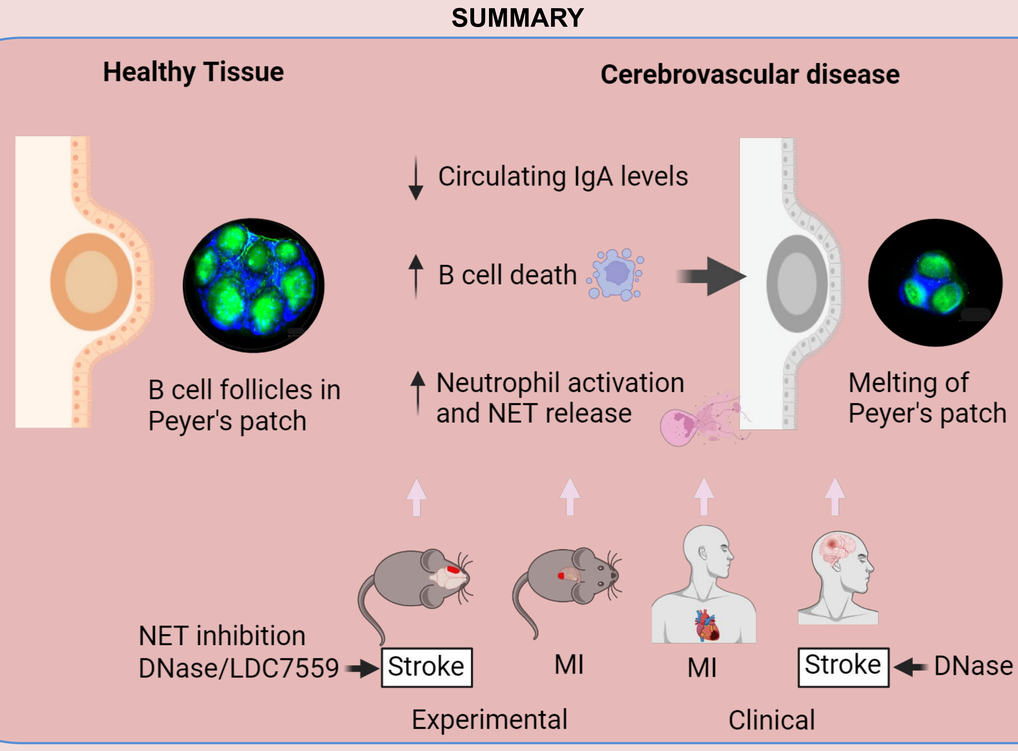


Figure 6. Stroke and myocardial infarction patients show increased circulating NETs. A, Relative plasma levels of citH3-DNA complexes in stroke patients and healthy subjects. **B,** CitH3-DNA complexes in stroke patients with and without DNase-I treatment. **C,** NE-DNA complexes in stroke patients with and without DNase-I treatment. **D,** The baseline normalized levels of circulating IgA in stroke patients with and without DNase-I treatment. HC=Healthy controls.

SUMMARY



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