Mucosal Bacterial Infections and Immunodeficiency after Stroke caused by Neutrophil Extracellular Traps

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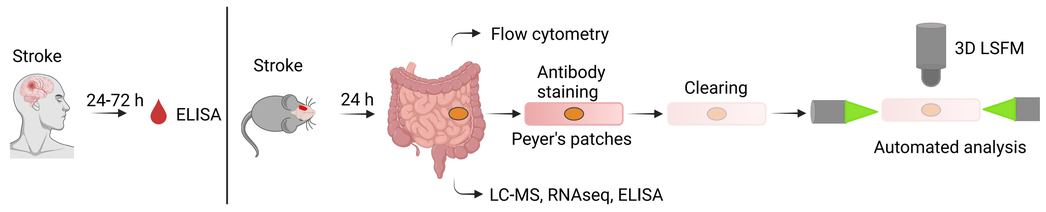
Background and Aims

Imbalance of humoral immunity after sterile brain injury is responsible for bacterial infections and poor disease outcomes. 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 patients and pre-clinical animal models, we dissected the causes of post-injury immune defects and increased susceptibility to lung infections.

Methods



Results

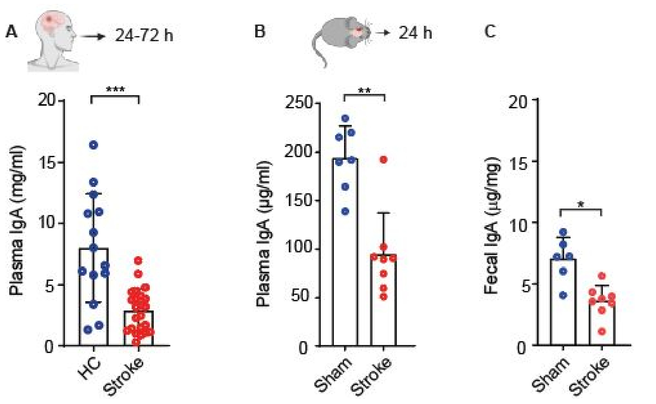


Figure 1. Stroke reduces plasma and fecal IgA levels. A, The amounts of plasma IgA in stroke patients and healthy subjects (HC). **B,** The levels of plasma IgA in stroke mice compared to sham controls. **C,** The levels of fecal IgA in stroke mice compared to sham controls. **D,** Macroscopic overview of the mouse gastrointestinal tract with the demarcation of PP one day after sham surgery or stroke. Data are mean ± s.d., statistical analyses were performed by two-tailed Mann-Whitney U test, *p<0.05, **p<0.01, ***p<0.001.

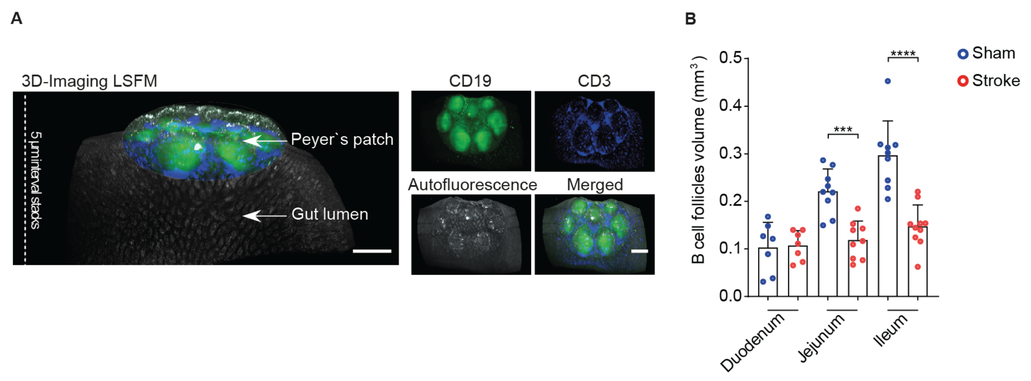


Figure 2. Stroke decreases B cell follicles volume in PP. A, 3D reconstruction of LSFM images of CD19⁺ B cells (green) and CD3⁺ T cells (blue) in PP isolated from duodenum, jejunum and ileum 24 h after stroke or sham surgery. **B,** Deep learning based automated analysis of B cell follicles volume in PP from duodenum, jejunum and ileum one day after stroke or sham surgery. Data are mean ± s.d., statistical analyses were performed by two-tailed Mann-Whitney U test, ***p<0.001, ****p<0.0001, LSFM=light-sheet fluorescence imaging.

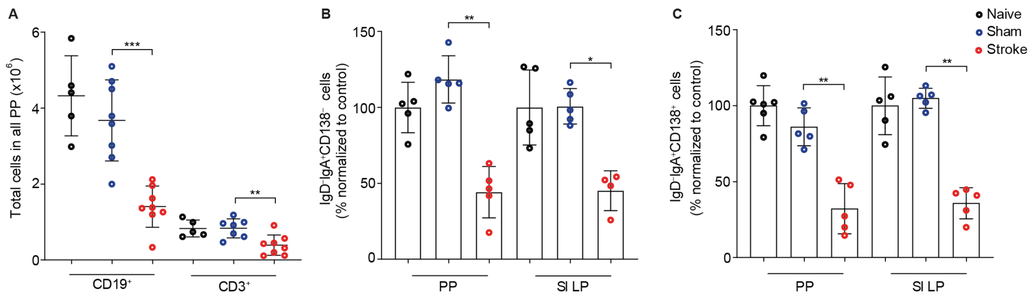


Figure 3. Stroke induces B cell loss in PP and lamina propria. A, Flow cytometry-based quantification of the number of CD19⁺ B cells and CD3⁺ T cells in all intestinal PP 24 h after sham surgery or stroke and unoperated naïve mice (n=5-8 per group)**. B,** Quantification of IgA⁺ IgD⁻ CD138⁻ plasma cell precursors in all PP and SI LP after 24 h of sham surgery or stroke and naïve mice (n=5 per group). **C,** Quantification of IgA⁺ IgD⁻CD138⁺ plasma cells in all PP and SI LP (n=5 per group). Data are mean ± s.d., statistical analyses were performed by two-tailed Mann-Whitney U test, *p<0.05, **p<0.01, ***p<0.001. PP=Peyer's patches, SI LP= small intestine lamina propria.

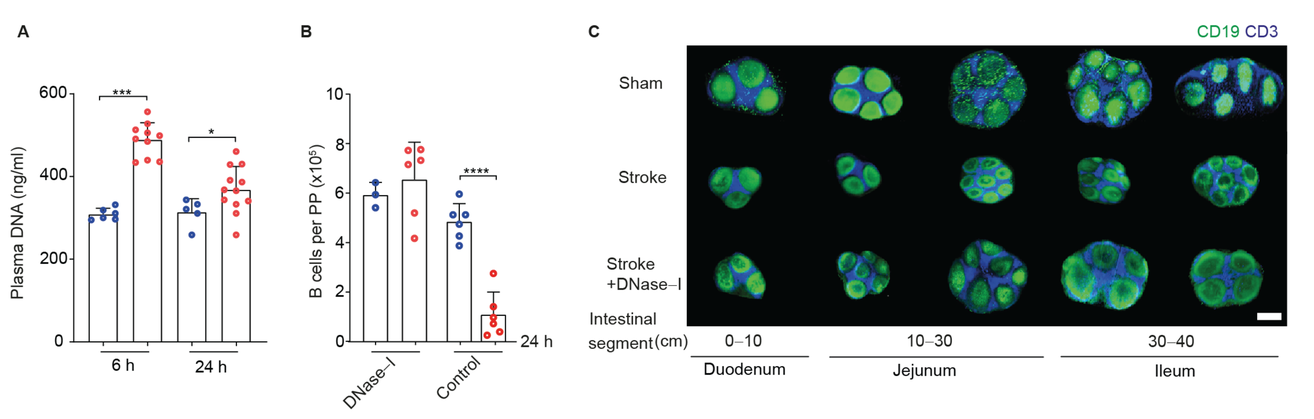


Figure 4. Stroke induced B cell loss in PP is dependent on the circulating DNA. A, Quantification of plasma DNA 6 h and 24 h after stroke or sham surgery using Qubit assays. **B,** Numbers of B cells in PP 24 h after stroke or sham surgery in DNase-I and vehicle-treated mice analyzed by flow cytometry. **C,** 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.

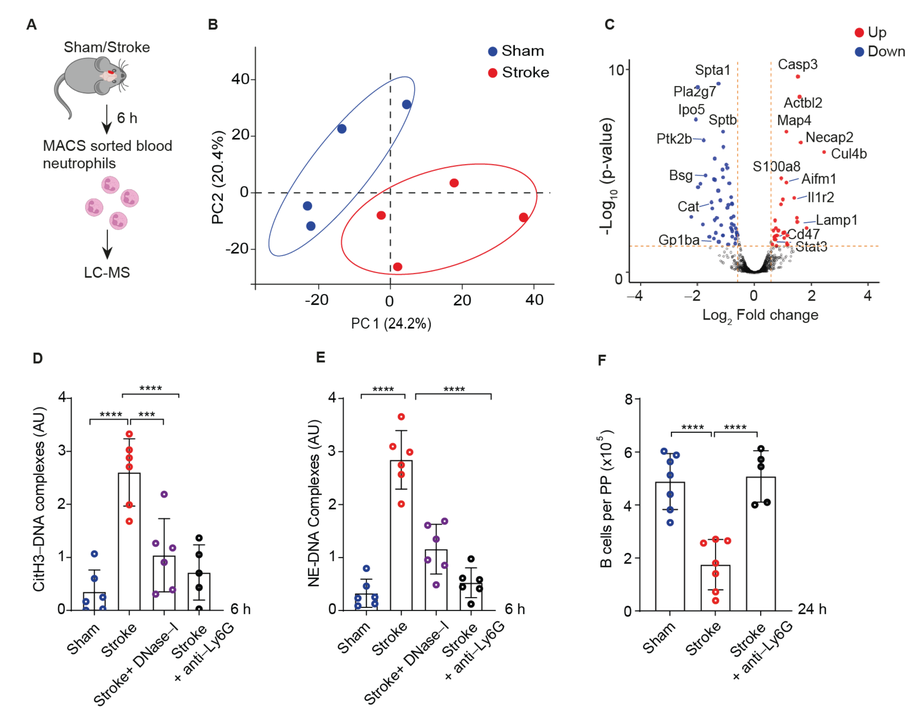


Figure 5. Stroke activated neutrophils release NETs and mediate B cell loss in PP. A, Schematic of the experimental paradigm for neutrophil mass-spectrometry and proteomics analysis. **B,** Principal component analysis of neutrophil proteomics after sham or stroke. **C,** Volcano plot comparing the normalized 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.

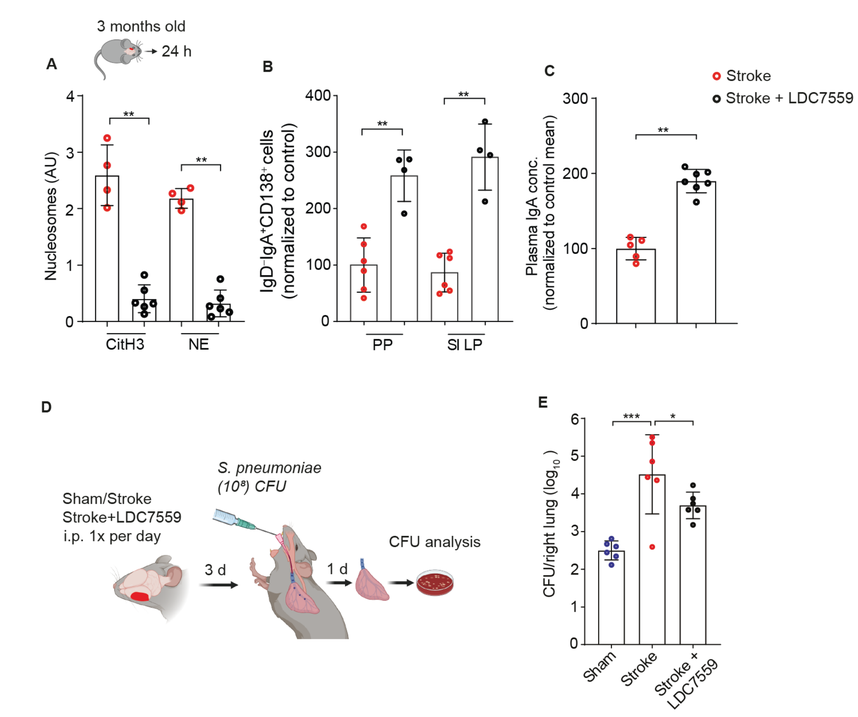


Figure 6. Inhibition of NETs with a Gasdermin D blocker protects immunity and reduce lung bacterial burden after stroke. A, Relative plasma levels of citH3-DNA and NE-DNA complexes in stroke + vehicle and stroke + LDC7559 treated mice. **B,** Numbers of IgD⁻IgA⁺CD138⁺ plasma cells in all PP and SI LP in stroke and stroke + LDC7559 treated mice. **C,** Relative concentrations of plasma IgA in stroke and stroke + LDC7559 treated mice. **D,** Sham or stroke mice were treated with vehicle or LDC7559 every day and after 3 d, all mice were intratracheally inoculated with *S. pneumoniae* (10^8 CFU). Mice were sacrificed 1 d after infection to analyze bacterial burden in the lungs. **E,** CFU in the lungs of infected sham + vehicle, stroke + vehicle and stroke + LDC7559 mice (n=6 mice per group). Data represent mean ±s.d., two-tailed Mann-Whitney U test or Kruskal-Wallis test *p<0.05, **p<0.01, ***p<0.001. NE=neutrophil elastase, PP=Peyer's patches, SI LP=small intestine lamina propria.

Summary

Funding

DFG Deutsche Forschungsgemeinschaft
Universitätsmedizin Essen Universitätsklinikum
Institute for Experimental Immunology and Imaging