Peyer's Patch B cells undergo cell death via neutrophil-released DNA after tissue injury

Ali A Tuz[1], Alexander Beer[1], Markus Gallert[1], Dimitris Ttoouli[4], Susmita Ghosh[3], Sai P Sata[3], Andreas Kraus[1], Sebastian Korste[4], Anja Hasenberg[1], Martin Stenzel[2], Benedikt Frank[2], Martin Köhrmann[2], Matthias Totzeck[4], Jianxu Chen[3], Dirk M Hermann[2], Matthias Gunzer[1,3], Vikramjeet Singh[1]

[1] Institute of Experimental Immunology and Imaging, University Duisburg-Essen, Medical Faculty, Essen
[2] Department of Neurology, University Hospital, University of Duisburg-Essen, DE-45147 Essen, Germany
[3] Leibniz-Institut für Analytische Wissenschaften – ISAS-e.V., Dortmund, Germany
[4] Department of Cardiology and Vascular Medicine, West German Heart and Vascular Center, University Hospital, University of Duisburg-Essen, DE-45147, Essen, Germany

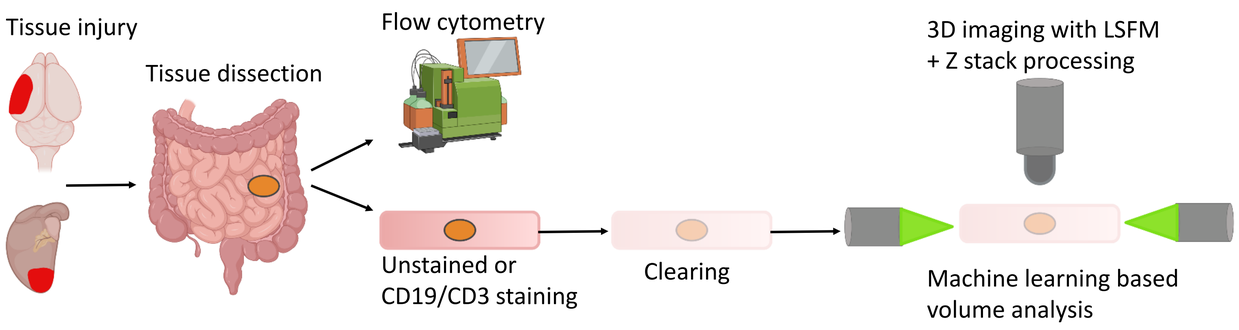
contact: ali.tuz@uni-due.de

Background

Sterile tissue injury influences immune responses that significantly deteriorate the disease outcomes. Mechanistically, massive loss of systemic lymphocytes after stroke increases susceptibility to bacterial infections, which promote neuroinflammation. Even though large number of lymphocytes reside in gastrointestinal tissues and protect mucosal barriers, the impact of lymphocyte loss in intestinal tissues and underlying mechanisms are not clear.

Methods

For modeling sterile tissue injury, we utilized clinically relevant mouse models of ischemic stroke and myocardial infarction. We studied immune cell populations in different lymphoid tissues using flow cytometry and light sheet fluorescence microscopy. Plasma soluble mediators are measured via ELISA and blocked via injection of respective medications.



Results

- Induction of brain and myocardial injury leads to a specific reduction of B lymphocytes in intestinal Peyer's patches (PP) that results in a massive shrinking of PP.
- Mechanistically, we revealed circulating DNA as a soluble mediator initiating apoptosis in B cells. Furthermore, using mouse models of neutrophil depletion, we confirmed neutrophil extracellular traps (NETs) as a major source of circulating DNA.
- Finally, the degradation of NETs with DNase-I inhibited the loss of B cells and shrinkages of PP in brain-injured mice.

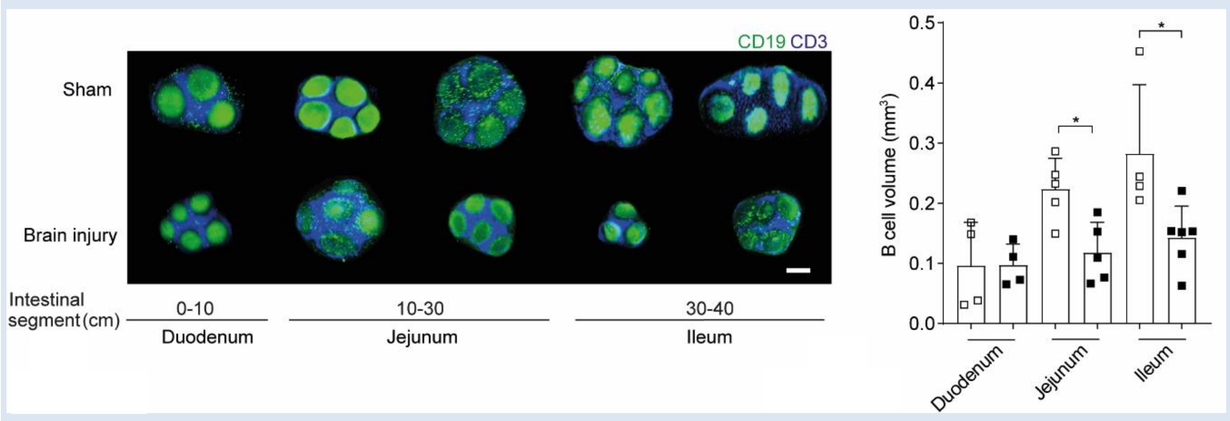


Figure 1 3D reconstruction of LSFM images of B cells and T cells in PP from duodenum, jejunum and ileum after one day of brain injury or sham controls reveals massive reduction via machine learning-based automated analysis of B cell follicles volume after one day of brain injury or sham controls (n=4-6 PP per group).

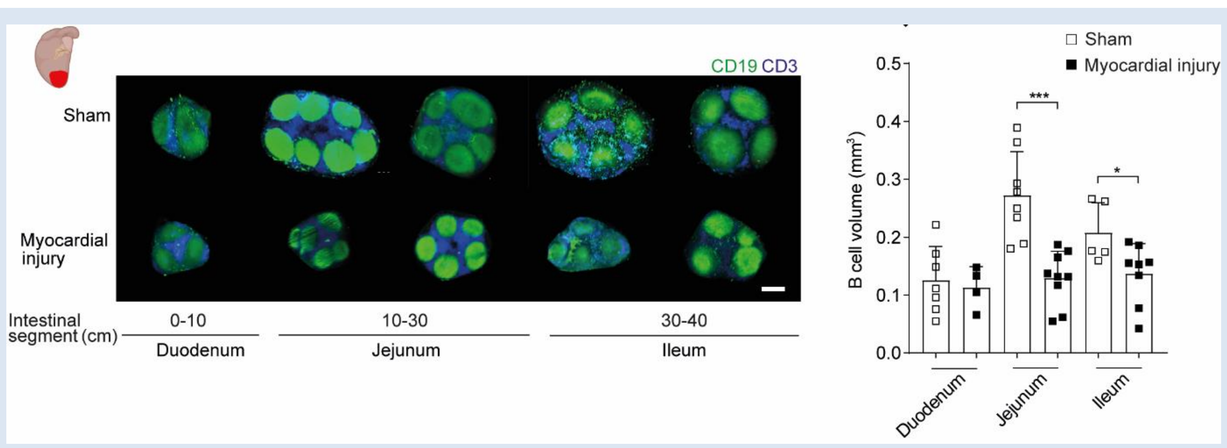


Figure 2 3D reconstruction of LSFM images of B cells and T cells in PP from duodenum, jejunum and ileum after one day of myocardial injury or sham controls reveals massive reduction via machine learning-based automated analysis of B cell follicles volume after one day of myocardial injury or sham controls (n=4-9 PP per group).

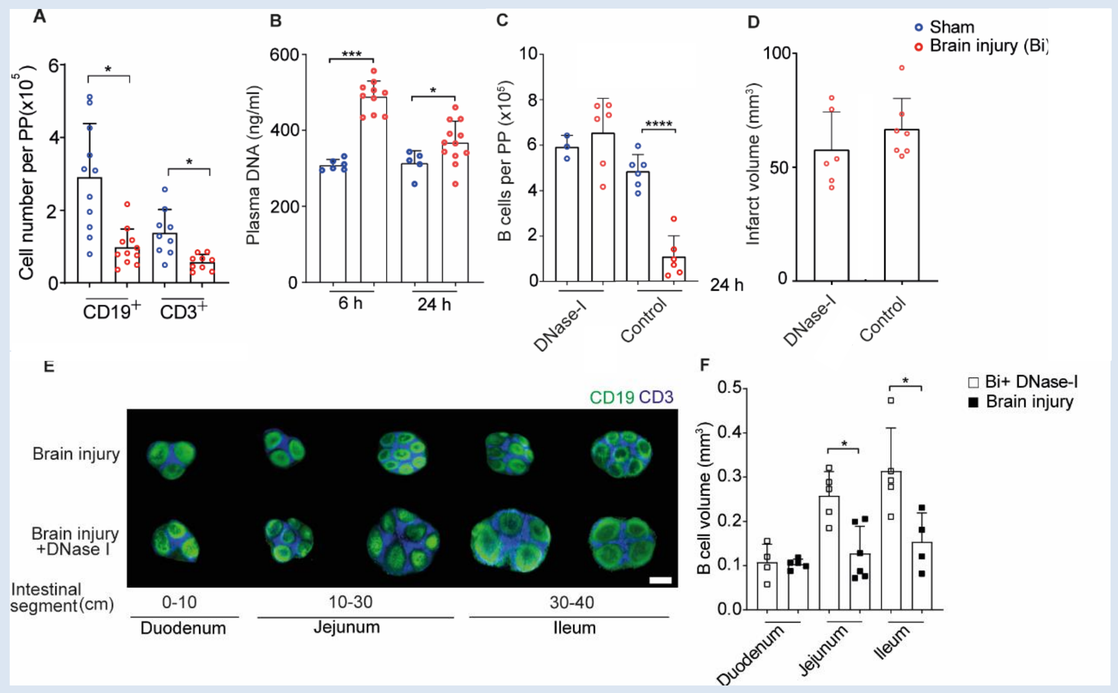


Figure 3 **(A)** CD19+ and CD3+ cell numbers 24h after brain injury and sham surgeries, **(B)** Plasma DNA levels at 6 h and 24 h after sham surgery or brain injury (n=5-12 mice per group). **(C)** Numbers of B cells in PP 24 h after sham-operation or brain injury in DNase-I and vehicle treated mice. **(D)** Brain infarct volumes in DNase-I treated and untreated mice at 24 h (n=6-7 mice per group). **(E)** 3D reconstruction images of CD19⁺ B cells and CD3⁺ T cells in PP after brain injury and DNase-I treatment. **(F)** The quantification of CD19⁺ B cell follicles volume in duodenum, jejunum and ileum 24 h after brain injury or brain injury + DNase-I treatment (n=4-6 PP per intestinal segment).

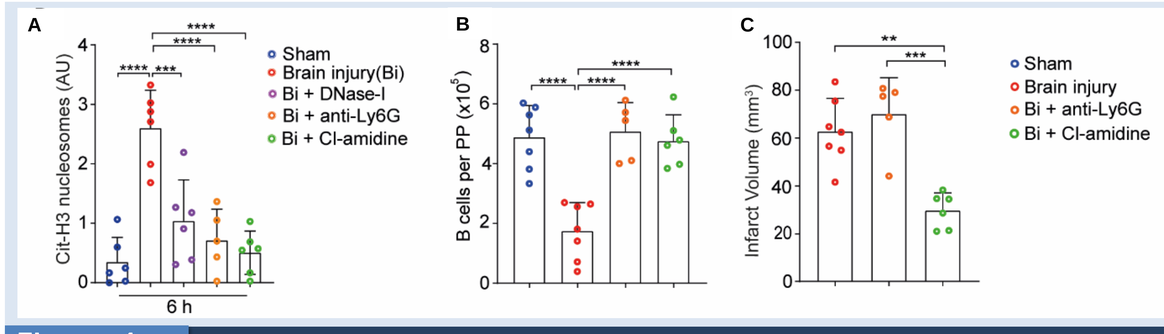


Figure 4 **(A)** Relative plasma levels of cit-H3 bound DNA after sham, brain injury, brain injury + DNase-I treatment or brain injury + anti-Ly6G antibody-treatment and brain injury + Cl-amidine. **(B)** Numbers of CD19⁺ B cells in intestinal PP in sham-operated, untreated brain injured and brain injured + anti-Ly6G antibody-treated and brain injury + Cl-amidine treated mice. **(C)** Brain infarct volumes in untreated brain injury, brain injury + anti-Ly6G treated and brain injury + Cl-amidine mice at 24 h (n=5-7 mice per group).

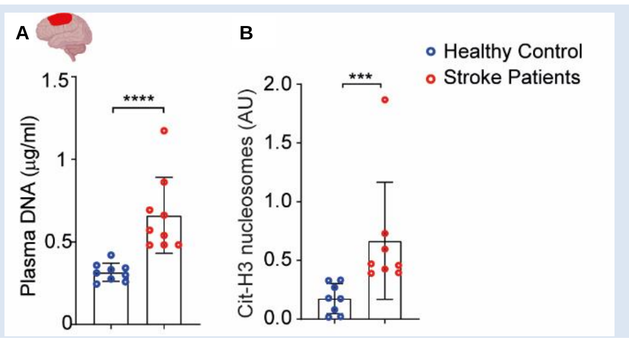
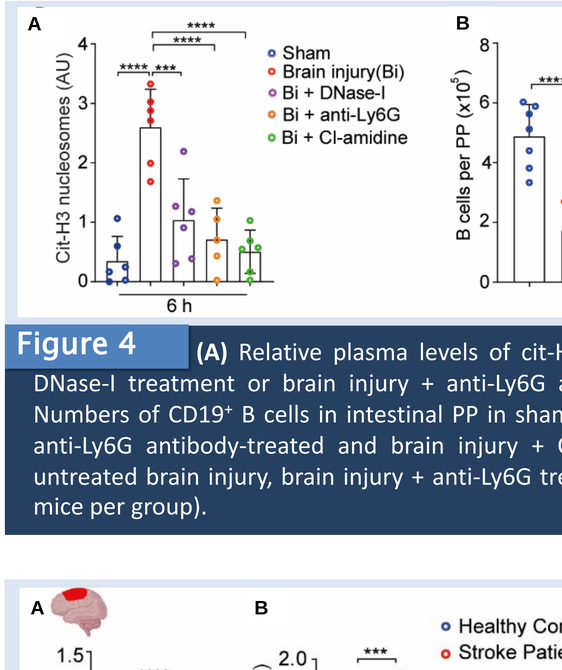


Figure 5 **(A)** Quantification of plasma DNA in ischemic stroke patients and healthy controls. **(B)** Relative plasma levels of cit-H3 bound DNA in stroke patients within three days of admission and healthy controls (n=8-9 per group).



Conclusion

Tissue injury of systemic organs can trigger lymphocyte contraction in intestinal immune cells via activated neutrophil-released NETs. Drugs targeting this pathway may help to maintain immune homeostasis at the mucosal gut barriers.

Universitätsklinikum Essen
UNIVERSITÄT DUISBURG ESSEN
Open-Minded
INSTITUTE FOR EXPERIMENTAL IMMUNOLOGY & IMAGING
ZMB ZENTRUM FÜR MEDIZINISCHE BIOTECHNOLOGIE
bioRxiv THE PREPRINT SERVER FOR BIOLOGY