Evaluation of CD45-positive cells in the brain and liver of NPC1$^{-/-}$ mice

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BACKGROUND

Niemann-Pick type C (NPC) disease is an autosomal recessive neurodegenerative disorder associated with mutations in NPC1 and NPC2 genes and characterized by an accumulation of unesterified cholesterol and glycosphingolipids in lysosomes. Deficiency of the corresponding NPC intracellular cholesterol transporter 1 protein thus leads to an abnormal cellular lipid and cholesterol composition. The most widely used NPC1 mouse model is the NPC1$^{-/-}$ mouse that is characterized to display a strong neuroinflammation as shown by astrocytosis and increased levels of CD45-positive immune cells in the cerebellum and hippocampus.

MATERIALS and METHODS

To further evaluate levels and morphology of CD45-positive immune cells in NPC1$^{-/-}$ mice, neuronal and visceral tissues were evaluated in comparison to wild type (WT) littermates. To this end, 10 µm thick cryo sections of the liver of 4 weeks old NPC1$^{-/-}$ mice and the brain of 8 weeks old NPC1$^{-/-}$ mice were assessed for CD45 expression by immunofluorescent labeling. In a next step the immunoreactive area was quantified in both the liver and brain tissue. Additionally, the numerical density, object intensity and object size of CD45-positive objects were evaluated in the thalamus and substantia nigra. Quantitative image analysis was performed with Image Pro 10 (Media Cybernetics) software and measured automatically so results are operator-independent and fully reproducible.

RESULTS - Liver

Immunofluorescent labeling of the liver showed a prominent enlargement of CD45-positive immune cells. Evaluating the overall immunoreactive (IR) area revealed that the signal was significantly increased when compared to WT littermates (Figure 1).

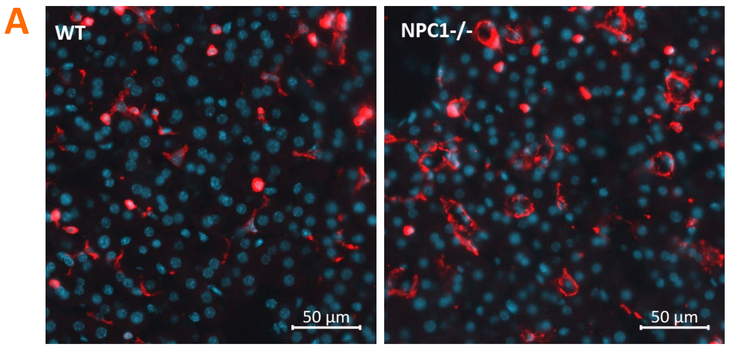

Figure 1. CD45-positive leucocytes in the liver of NPC1$^{-/-}$ mice. **A**: CD45 immunofluorescent signal (red) in the liver of 4 weeks old WT and NPC1$^{-/-}$ mice. Note enlarged CD45-positive objects indicating leucocytes. Nuclei are labeled with DAPI (blue).
B: The liver of NPC1$^{-/-}$ mice was analyzed for leucocytes (CD45). Immunoreactive area in percent at the age of 4 weeks compared to WT littermates. Unpaired Student's t-test. n = 3 per group. Mean + SEM. *p<0.05.

RESULTS - Brain

NPC1$^{-/-}$ mice presented a highly increased CD45-positive immunoreactive area, numerical density and object size but no changes in the object intensity in the thalamus and substantia nigra (Figure 2).

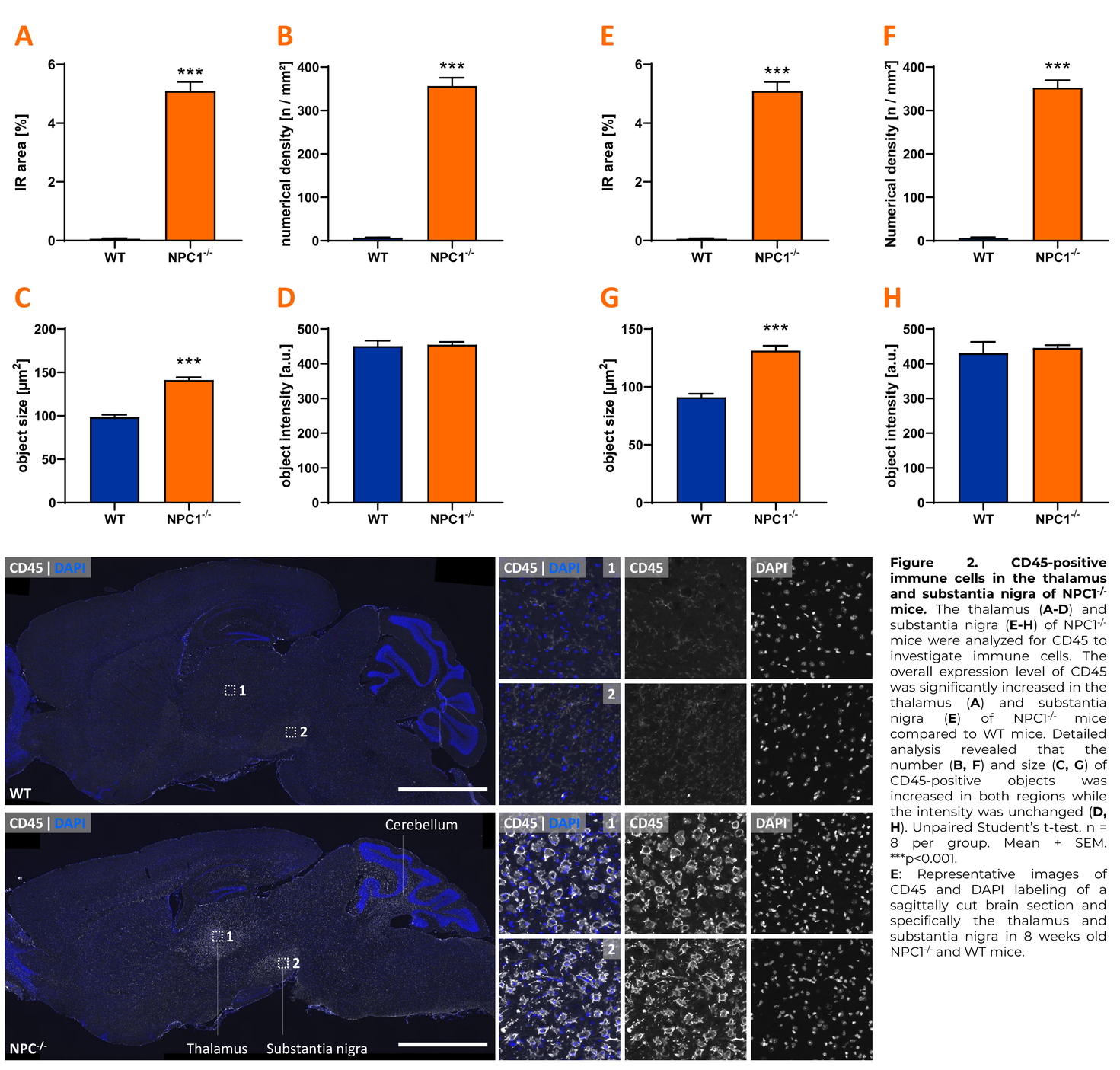

Figure 2. CD45-positive immune cells in the thalamus and substantia nigra of NPC1$^{-/-}$ mice. The thalamus (**A-D**) and substantia nigra (**E-H**) of NPC1$^{-/-}$ mice were analyzed for CD45 to investigate immune cells. The overall expression level of CD45 was significantly increased in the thalamus (**A**) and substantia nigra (**E**) of NPC1$^{-/-}$ mice compared to WT mice. Detailed analysis revealed that the number (**B, F**) and size (**C, G**) of CD45-positive objects was increased in both regions while the intensity was unchanged (**D, H**). Unpaired Student's t-test. n = 8 per group. Mean + SEM. ***p<0.001.
E: Representative images of CD45 and DAPI labeling of a sagittally cut brain section and specifically the thalamus and substantia nigra in 8 weeks old NPC1$^{-/-}$ and WT mice.

SUMMARY and CONCLUSION

Our data suggest that NPC1 deficiency causes a severe neuroinflammation in the thalamus and substantia nigra as well as a severe change of hepatic leukocyte representation in the NPC1$^{-/-}$ mouse model that perfectly mimics the pathology of the human lysosomal storage disease Niemann-Pick C1. These pathological hallmarks of NPC1$^{-/-}$ mice will be especially valuable as readout in efficacy studies for the development of new treatments against Niemann-Pick disease.

REFERENCE

Santiago-Mujica E, Flunkert S, Rabl R, Neddens J, Loeffler T, Hutter-Paier B. Hepatic and neuronal phenotype of NPC1$^{-/-}$ mice. Heliyon. 2019 Mar 14;5(3):e01293. PMID: 30923761.

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