MolecularMedicine

Supplemental Data

Neural Signaling in the Spleen Controls B-Cell Responses to Blood-Borne Antigen

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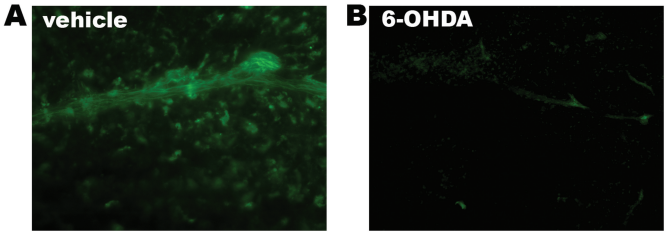
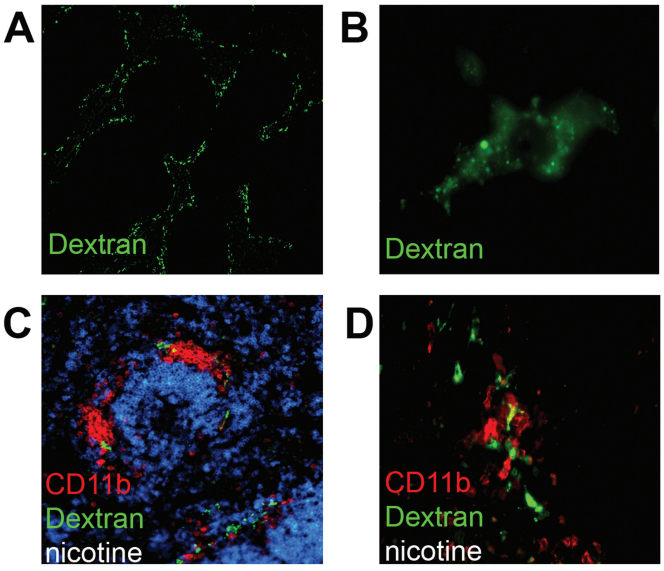
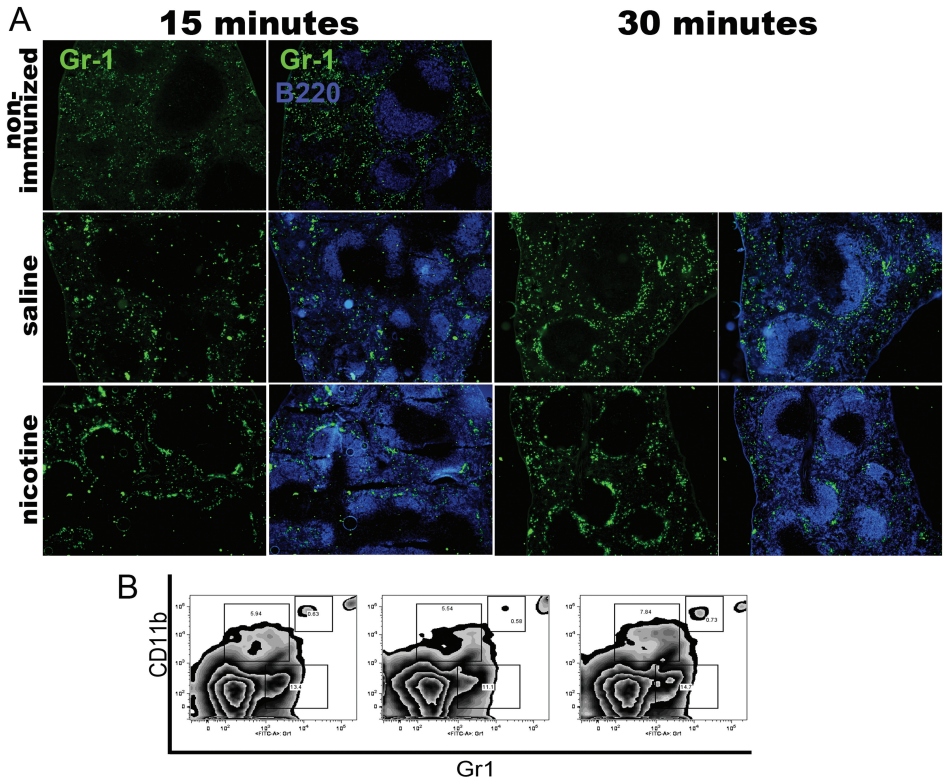


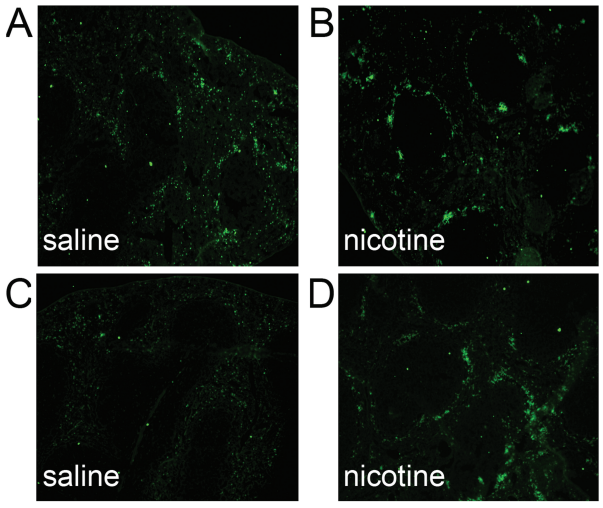
Supplementary Figure 1. Chemical sympathectomy 7 d after 6-hydroxydopamine injection was evaluated by histofluorescence using the sucrose-phosphate-glycoxylic acid method in snap frozen spleens from untreated mice **(A)** or mice that had been treated with three doses of 6-hydroxydomapine (6-OHDA) on d -7, -5 and -3 before immunization **(B)**.



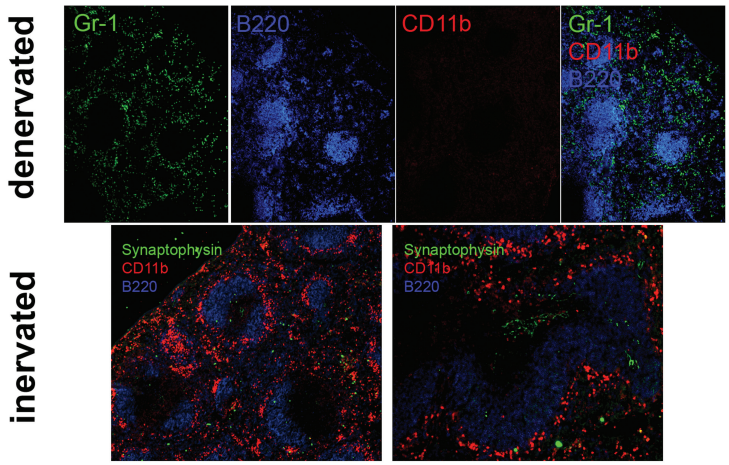
Supplementary Figure 2. Mice were intravenously injected with FITC-labeled dextran one h before nicotine or saline injection and intravenous antigen challenge with heat-killed *Streptococcus pneumoniae*. They were sacrificed 15 min post-immunization Sections were stained for B220 and CD11b. **A.** Distribution of fluoresceinated dextran in the MZ. **B.** High magnification of a cell from (A) demonstrated the internalized dextran. **C.** Sections from dextran injected and nicotine-treated animals were stained for CD11b. **D**. Higher magnification image to demonstrate the close contacts between dextran+ macrophages (MZM) and CD11b+ cells. A superimposed image of all three channels or the red and green channels only, is shown in c and d, respectively.



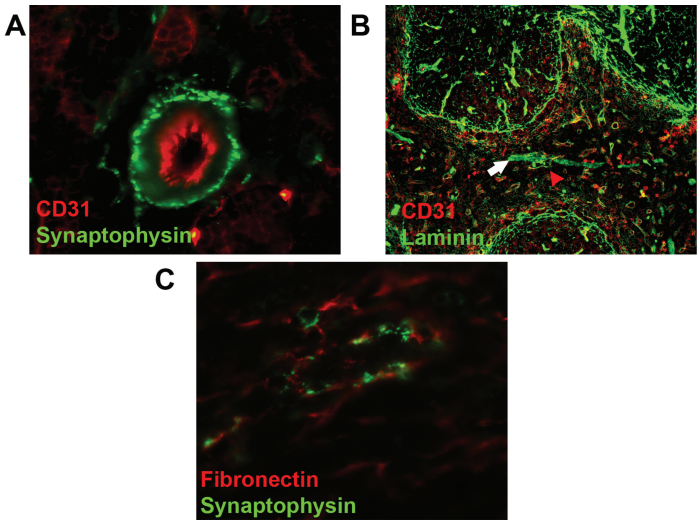
Supplementary Figure 3. A. Mice were euthanized 15 or 30 min post-immunization and injection with saline or nicotine as indicated, and the spleens stained for Gr-1 and B220. The distribution of Gr-1 and B220 in sections from non-immunized animals is shown for comparison. **B.** Total splenocytes isolated 15 min post-immunization were stained for CD11b and Gr-1 and analyzed by flow cytometry. Plots represent one of two similar independent experiments with two three animals per group each.



Supplementary Figure 4. Mice were euthanized 15 min after intravenous injection with ~1 x 105 CFU/mouse of live *Streptococcus pneumoniae* virulent strain ATCC 6303 five min after i.p. administration of saline (**A,C**) or nicotine (**B,D**), and the spleens stained for Gr-1. Sections from two different animals representative of 10 mice per group in two independent experiments are shown.



Supplementary Figure 5. Spleen sections from mice that underwent splenic neurectomy (denervated) or from sham controls stained for CD11b, Gr1, B220, and synaptophysin to show that CD11b+, but not Gr-1+ cells are absent from denervated areas. Images representative of 10 animals per group.



Supplementary Figure 6. Spleen sections stained for endothelial cells (CD31) and synaptophysin **(A)** or CD31 and laminin **(B)** to illustrate the fact that nerves had a perivascular localization and that vascular structures were not always associated with the laminin-containing conduits of the red pulp. The red arrow indicates a CD31+ blood vessel traversing a laminin fibrous structure marked with a white arrow (B). Other sections were stained for synaptophysin and fibronectin **(C)** to show that as opposed to their localization surrounding blood vessels, nerves are localized in close apposition with the fibronectin of the reticular fibers.