A Biodegradable Nanoparticle Platform for the Induction of Antigen-Specific Immune Tolerance for Treatment of Autoimmune Disease

Zoe Hunter,[*,^] Derrick P. McCarthy,[*,^] Woon Teck Yap,[*,#] Christopher T. Harp,[^] Daniel R. Getts,[^] Lonnie D. Shea,[#,†] and Stephen D. Miller[^,†]

*Co-first authors contributed equally to this work

[†]Co-corresponding authors

^Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, 6-713 Tarry Building, 303 E. Chicago Avenue, Chicago, IL 60611; [#]Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Rd, Evanston, IL 60208

Running Title: Amelioration of EAE by Antigen-Coupled Nanoparticle Tolerance

[†]Corresponding Author Address:

Stephen D. Miller, Ph.D.
Department of Microbiology-Immunology
Feinberg School of Medicine, Northwestern University
303 E. Chicago Avenue
Chicago, IL 60611
Phone: 312-503-7674
FAX: 312-503-1154
Email: s-d-miller@northwestern.edu

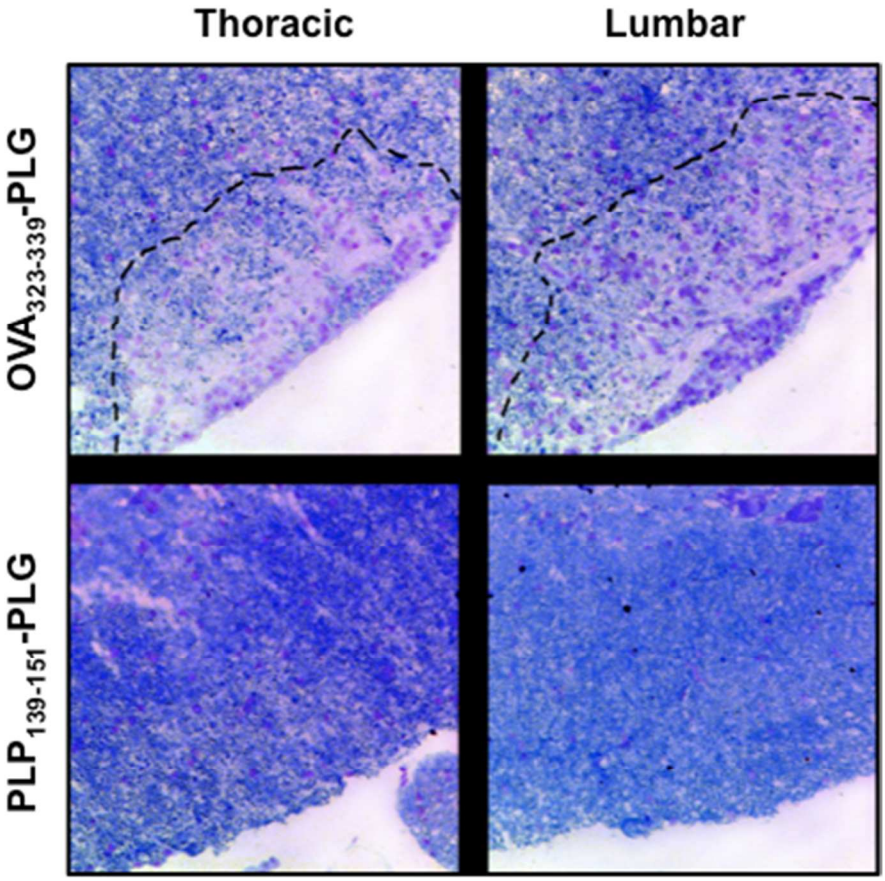

Figure S1. Induction of therapeutic tolerance with myelin Ag-coupled PLG-PEMA nanoparticles reduces the number of demyelinating lesions and CNS-infiltrating cells. EAE was induced in 6-8 week old female SJL/J mice by adoptive transfer of $2.5x10^6$ $PLP_{139-151}$-specific blasts. Mice were injected iv with either 1.25 mg of $OVA_{323-339}$- or $PLP_{139-151}$-PLG-PEMA nanoparticles on day +14 relative to EAE induction. Thoracic (left) and lumbar (right) spinal cord sections from mice tolerized with $OVA_{323-339}$ -PLG-PEMA (top panel) or $PLP_{139-151}$-PLG-PEMA (bottom panel) nanoparticles were stained with Luxol Fast Blue on day 42**.** Areas of interest are outlined; demyelination is indicated by a clear decoloration in the area of the lesion, whereas cellular infiltration is indicated by purple, punctate staining cells.

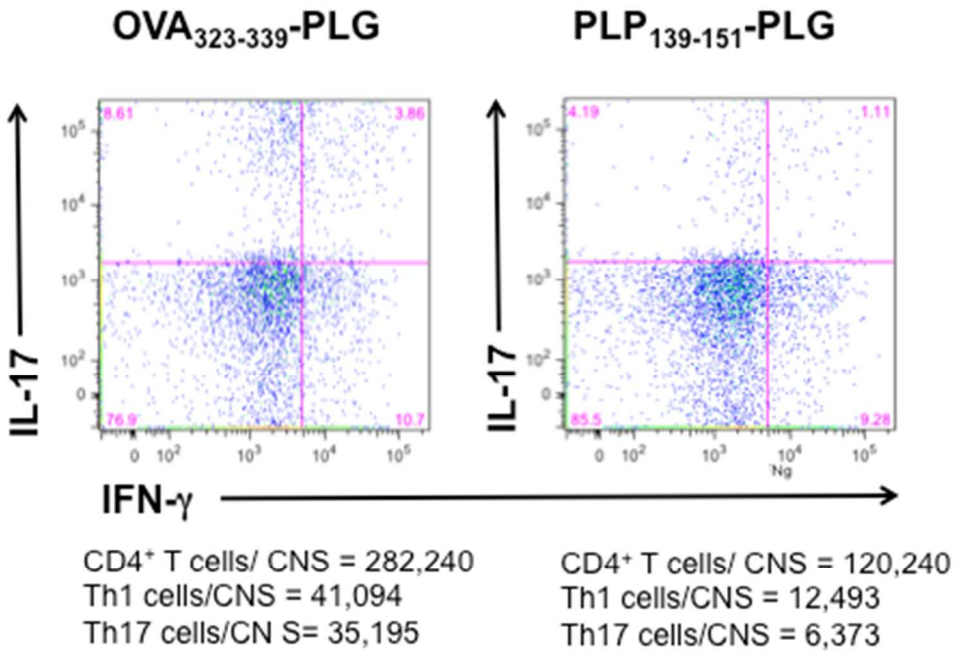

Figure S2. Induction of therapeutic tolerance with myelin Ag-coupled PLG-PEMA nanoparticles reduces cytokine-secreting Th1 & Th17 T cells in the CNS. SJL/J mice were injected i.v. with PLG-PEMA nanoparticles coupled with $OVA_{323-339}$ or $PLP_{139-151}$ 2 days following EAE induced by adoptive transfer of activated $PLP_{139-151}$-specfic T cell blasts. At the peak of disease in the $OVA_{323-339}$-PLG treated controls (d+14), brains and spinal cords were removed, processed into single cell suspensions for analysis by flow cytometry. CNS cell preparations from the two treated groups were stimulated with PMA and ionomycin for 5 h prior to intracellular staining for IFN-γ (x-axis) and IL-17A (y-axis). Dot plots from representative animals tolerized with $OVA_{323-339}$ -PLG-PEMA (left) or $PLP_{139-151}$-PLG-PEMA (right) nanoparticles are shown. Populations are gated on $CD3^+CD4^+$ cells. The percentages generated in the dot plots were used to calculate total Th1/Th17 cell number.