Supporting Information

Miles et al. 10.1073/pnas.1109173109

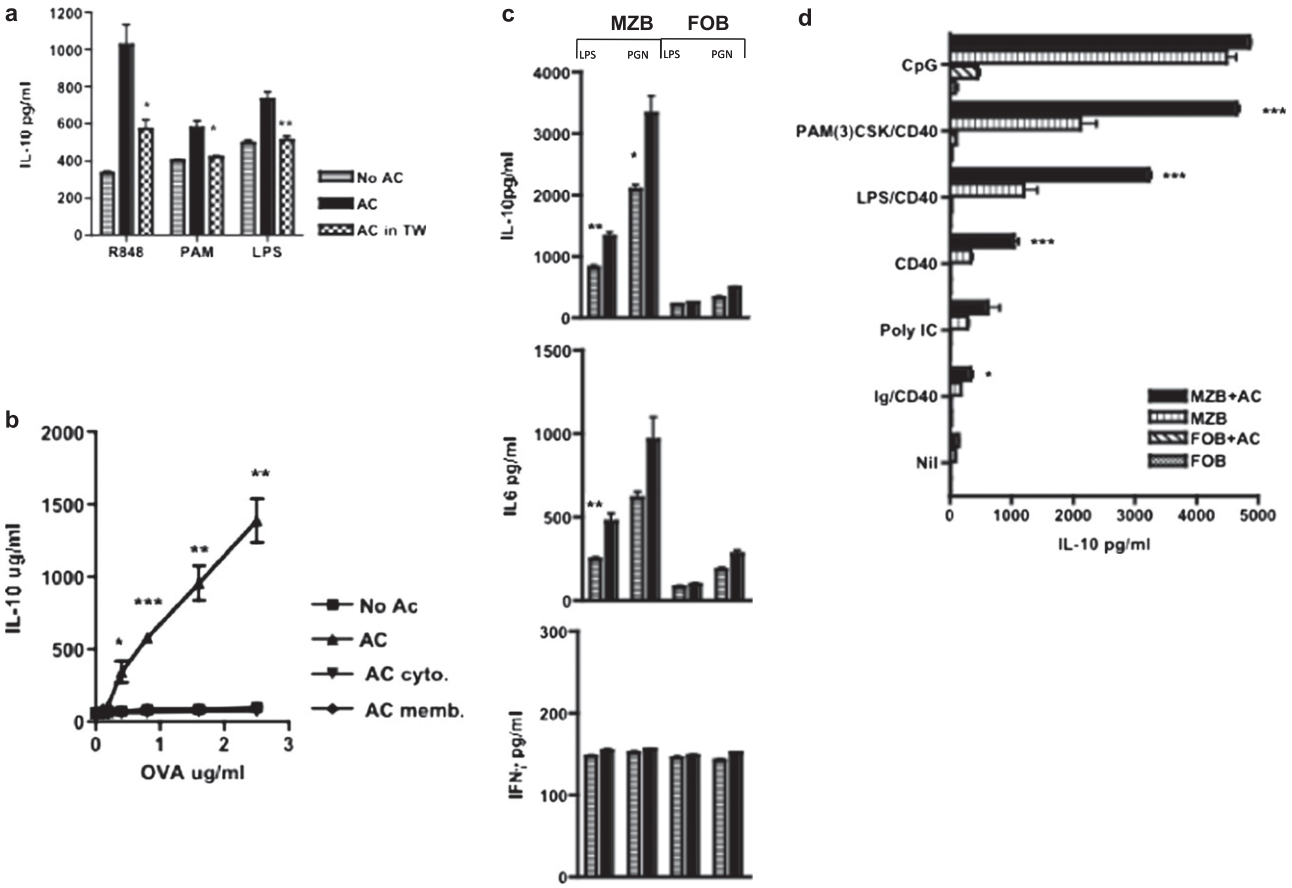
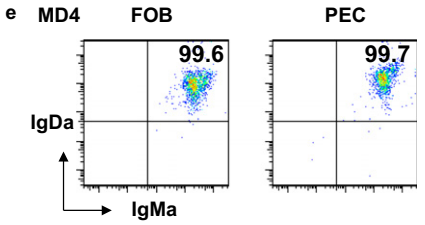
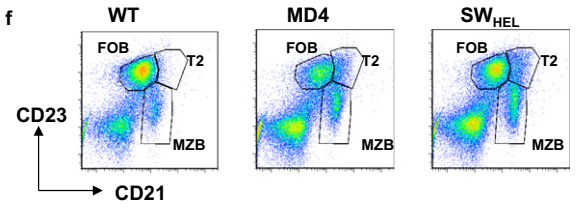


Fig. S1. (Continued)





Strain	FOB x10^6	MZB x10^6	T2 x10^6	Mouse number
WT	37.5±5.8	4.4±0.5	1.7±0.4	8
MD4	10.1± 2.1	4.8±0.9	3.2±0.7	8
SWHEL	14.1± 1.7	8.0±0.8	2.3±0.3	8

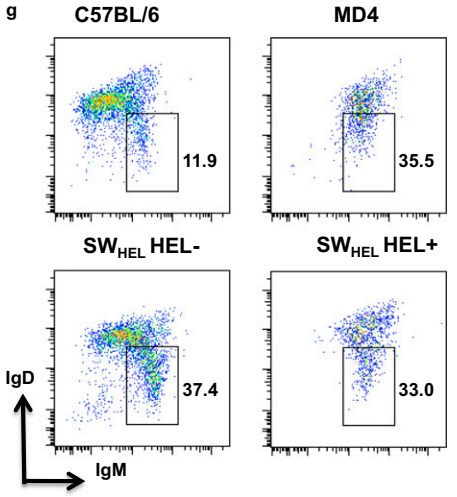


Fig. S1. (Continued)

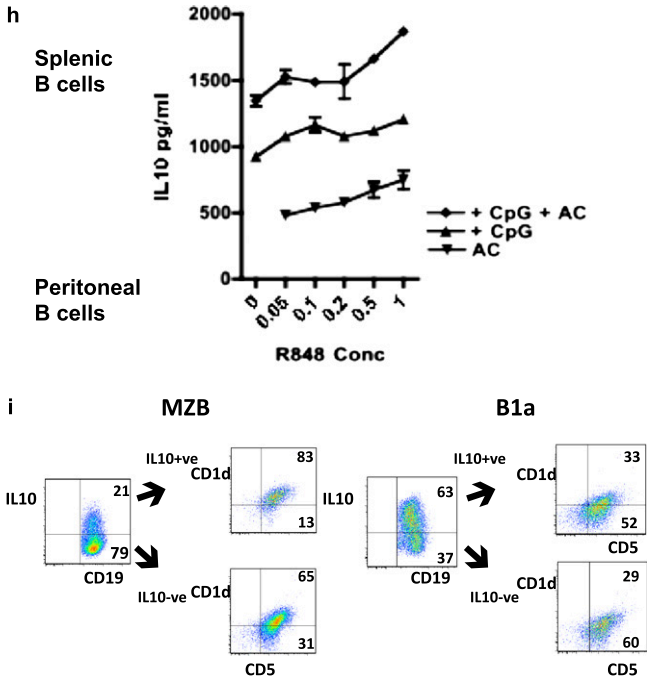


Fig. S1. (*A*) Splenic B cells were cocultured with the Toll-like receptors (TLRs) R848 (TLR7), PAM (TLR1/2), or LPS (TLR4) and apoptotic cells (ACs), which were either allowed to be in direct contact with B cells or separated by a Transwell (TW). (*B*) In cocultures similar to those shown in Fig. 1*A*. ACs were either left whole (AC) or made necrotic and then separated into membrane fractions (AC memb) or cytosolic fractions (AC cyto). After 72 h, IL-10 was measured in the supernatants. (*C*) Highly purified splenic B cells were sorted into follicular B cell (FOB) and marginal zone B cell (MZB) subsets and activated with the TLR ligands LPS (TLR4) and peptoglycan (PGN; TLR2). After 72 h of culture, IL-10, IL-6, and IFN-γ in the supernatants was measured by ELISA. (*D*) Highly purified splenic B cells were sorted into FOB and MZB subsets and activated with anti-CD40 and various TLR ligands, including TLR1/2 (PAM(3)CSK), TLR4 (LPS), TLR3 l(poly IC), and TLR9 (the latter at a higher CpG dose of 25 μg/mL). After 72 h of culture, IL-10 in the culture supernatants was measured by ELISA. The higher CpG dose elicited a maximal response from MZB cells such that they could no longer respond further to ACs by secreting IL-10, but had a minimal effect on FOB cells. (*E*) FACS analysis of viable $CD19^+$ cells from the spleen of WT mice and the peritoneum of MD4 mice showing the proportions of $IgDa^+$ and $IgMa^+$ cells. (*F*) FACS plots of splenic B cells from WT and hen egg lysozyme (HEL)-specific MD4 and SW_{HEL} mice showing the gating of B cells in each subset. Viable B cells were selected based on CD19 staining, and then subset analysis followed staining with CD21 and CD23 to show the proportions of MZB, T2, and FOB subsets. The table shows the mean ± SEM number of cells taken from splenic B cells of eight individual WT and HEL-specific MD4 and SW_{HEL} mice using the gates shown. (*G*) FACS analysis of viable $CD19^+$ cells from the spleen of WT, MD4, and SW_{HEL} mice showing the proportions of IgD^+ and IgM^+ cells. HEL^+ B cells were selected for their ability to bind to biotinylated HEL. The rectangular regions shown in the plots are $CD21^{high}CD23^{low}$ MZB cells, with the percentage of total B cells with this phenotype shown alongside. (*H*) MZB cells were stimulated with R848 alone or with CpG with and without ACs, and IL-10 was measured after 72 h. R848 synergizes with CpG and ACs to induce the greatest secretion of IL-10. (*I*) Splenic MZB cells and peritoneal-derived B1a resting B cells expressing GFP tagged to IL-10 were stimulated with R848, and IL-10 positivity was correlated with the CD5 and CD1d markers after 3 d.

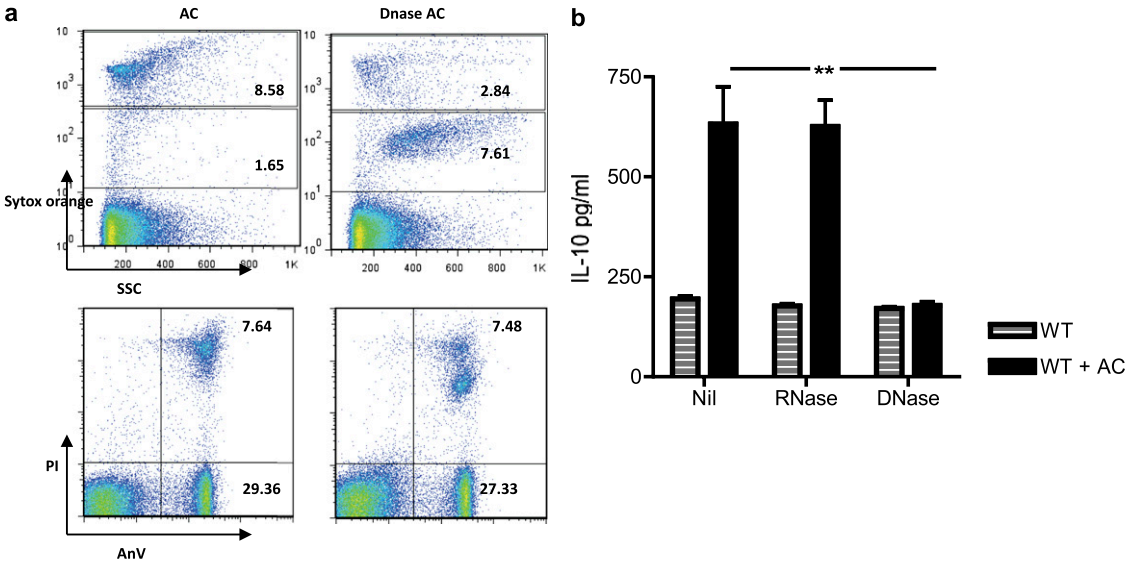


Fig. S2. (*A*) (*Upper*) ACs were stained with Sytox orange and DNase, resulting in decreased Sytox staining of DNA by a log order. (*Lower*) ACs treated with DNase were stained with annexin V and PI. Just slightly more than 7% of the cells were PI positive, accounting for the residual Sytox staining shown in the upper panel. (*B*) MZB cells were cocultured with ovalbumin (OVA)-specific T cells with or without ACs and treated with RNase or DNase for 72 h, after which IL-10 was measured.

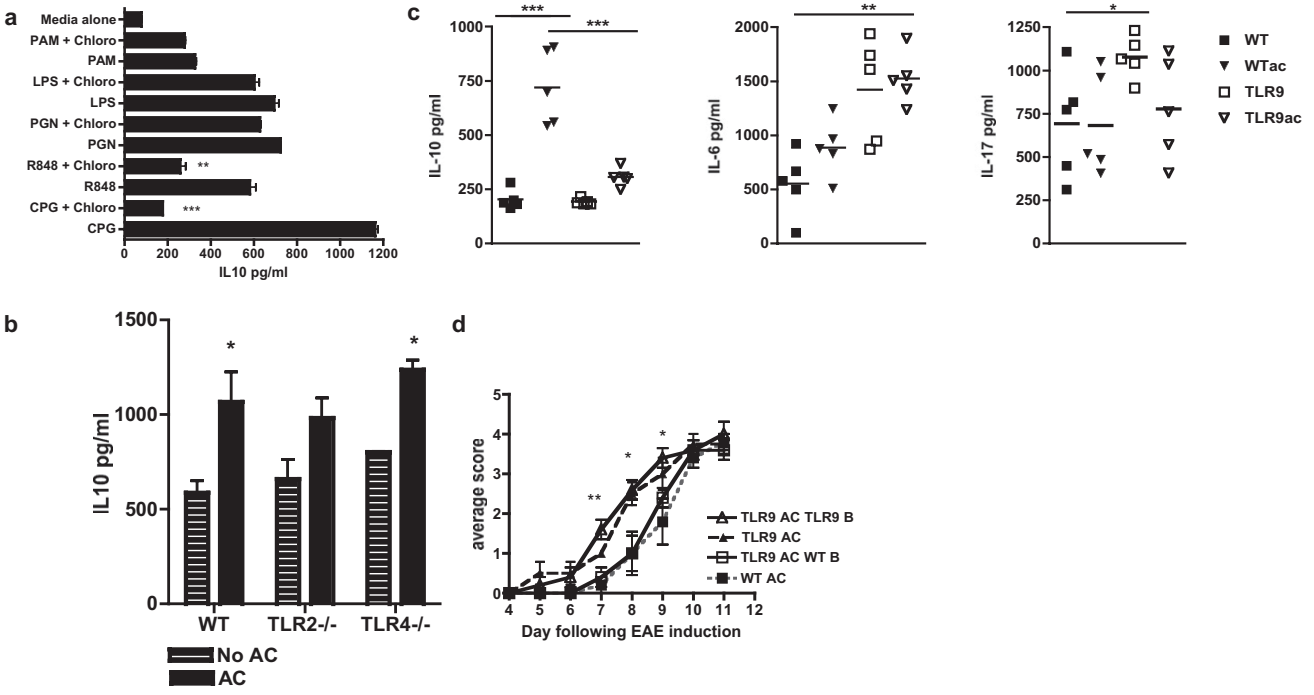


Fig. S3. (*A*) B cells were stimulated with various TLR ligands, including TLR1/2 with PAM(3)CSK, TLR2 (PGN), TLR4 (LPS), TLR7 (R848), and TLR9 (CpG), in the presence and absence of chloroquine. Secretion of IL-10 was inhibited only after TLR7 and TLR9 stimulation. (*B*) WT, TLR2-deficient, and TLR4-deficient B cells were cocultured with ACs, OVA-specific T cells, and OVA peptide at 2 μg/mL in vitro. After 72 h, IL-10 secretion was measured by ELISA. (*C*) At the end of the clinical experiment shown in Fig. 3*D*, splenocytes were isolated and restimulated with myelin oligodendrocyte glycoprotein peptide, and IL-10, IL-17, and IL-6 secretion were measured. Error bars represent SEM. ***$P \leq 0.0004$; **$P \leq 0.004$; *$P \leq 0.04$. (*D*) The same experiment as shown in Fig. 3*D* , but with the additional control of TLR9^{-} B cells added, which provided no additional protection. Error bars represent SEM. ***$P \leq 0.0004$; **$P \leq 0.004$; *$P \leq 0.04$.

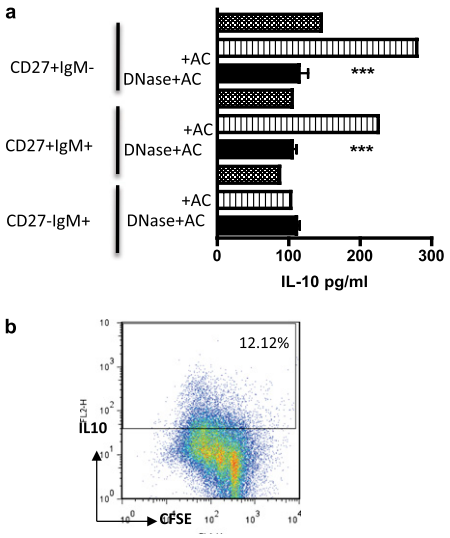


Fig. S4. (*A*) B cells were sorted into $CD27^{+}IgM^{-}$ memory B cells, $CD27^{+}IgM^{+}$ circulating MZB cells, and $CD27^{-}IgM^{+}$ naive B cells and then cultured with IL-4 with or without ACs for 3 d, after which IL-10 was measured. Some wells contained DNase. (*B*) Splenic MZB cells expressing GFP tagged to IL-10 were stimulated with R848, and IL-10 positivity was correlated with B cell proliferation after 3 d.