

SUPPLEMENTAL MATERIAL

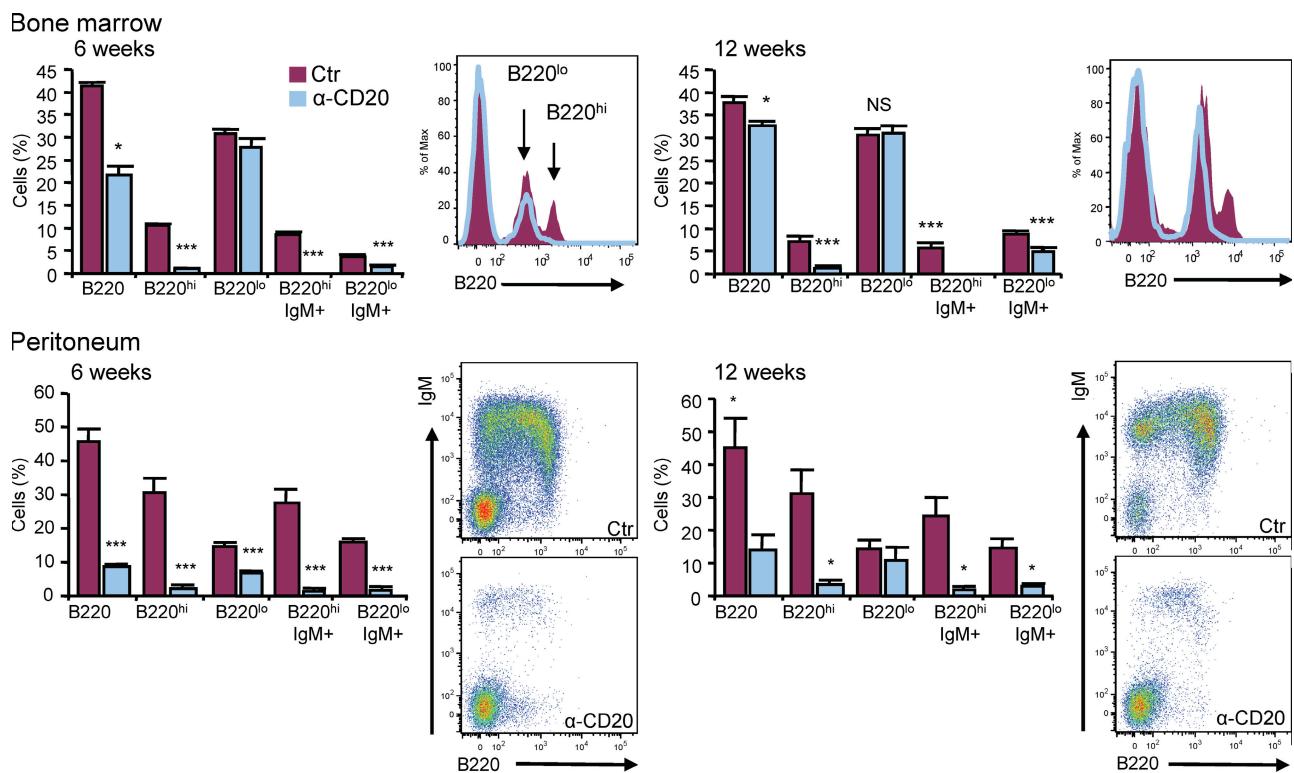
Ait-Oufella et al., <http://www.jem.org/cgi/content/full/jem.20100155/DC1>

Figure S1. CD20 mAb (α -CD20)-induced B cell depletion in bone marrow and peritoneum. Efficiency of B cell depletion in bone marrow (gated on lymphocytes, low forward scatter/low side scatter) and peritoneum (gated on total live cells) of $Apoe^{-/-}$ mice fed a Western diet for either 6 or 12 wk and treated with α -CD20 (blue) or a control antibody (magenta). 6-wk data are representative of five (peritoneum) and eight mice (bone marrow) per group and two separate experiments. 12-wk data are representative of eight mice per group and two separate experiments. Mean values \pm SEM are shown. *, P < 0.05; ***, P < 0.001.

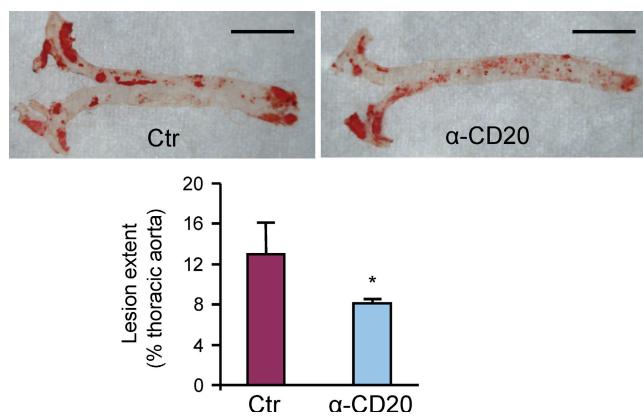


Figure S2. CD20 mAb (α -CD20) treatment reduces the development of atherosclerosis in the thoracic aorta. Representative photomicrographs and quantitative analysis of the extent of oil red O staining in the thoracic aortas of $Apoe^{-/-}$ mice fed a Western diet for 12 wk and treated with α -CD20 (blue) or a control antibody (magenta). Mean values \pm SEM are representative of 9 (Ctr) or 10 mice (α -CD20) per group. *, P < 0.05. Bars, 5 mm.

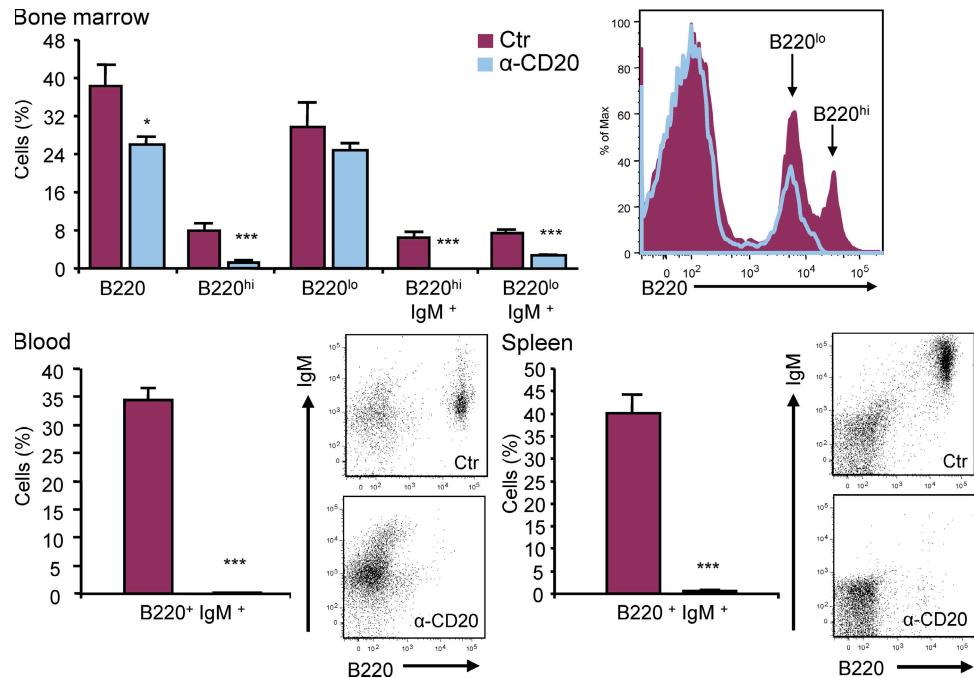


Figure S3. CD20 mAb (α -CD20)-induced B cell depletion in *Ldlr*^{-/-} mice. Efficiency of B cell depletion in bone marrow, blood (gated on lymphocytes, low forward scatter/low side scatter), and spleen (gated on total live cells) of *Ldlr*^{-/-} mice fed a Western diet for 6 wk and treated with α -CD20 (blue) or a control antibody (magenta). Data are representative of five (spleen, bone marrow), seven (Ctr, blood), and eight mice (α -CD20, blood) per group. Mean values \pm SEM are shown. *, P < 0.05; ***, P < 0.001.

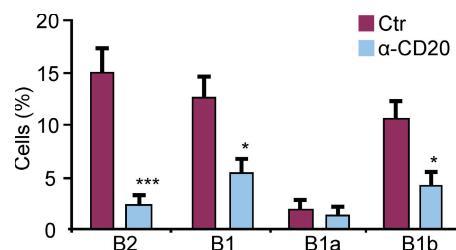


Figure S4. CD20 mAb (α -CD20)-induced B2 and B1 cell depletion in the peritoneum of *Apoe*^{-/-} mice. Efficiency of B2 (B220^{high} IgM^{low} CD5⁻), B1a (B220^{low} IgM⁺ CD11b⁺ CD5⁺), and B1b (B220^{low} IgM⁺ CD11b⁺ CD5⁻) cell depletion in the peritoneum (gated on total live cells) of *Apoe*^{-/-} mice treated with α -CD20 (blue) or a control antibody (magenta). Data are representative of at least nine mice per group and three separate experiments. Mean values \pm SEM are shown. *, P < 0.05; ***, P < 0.001.

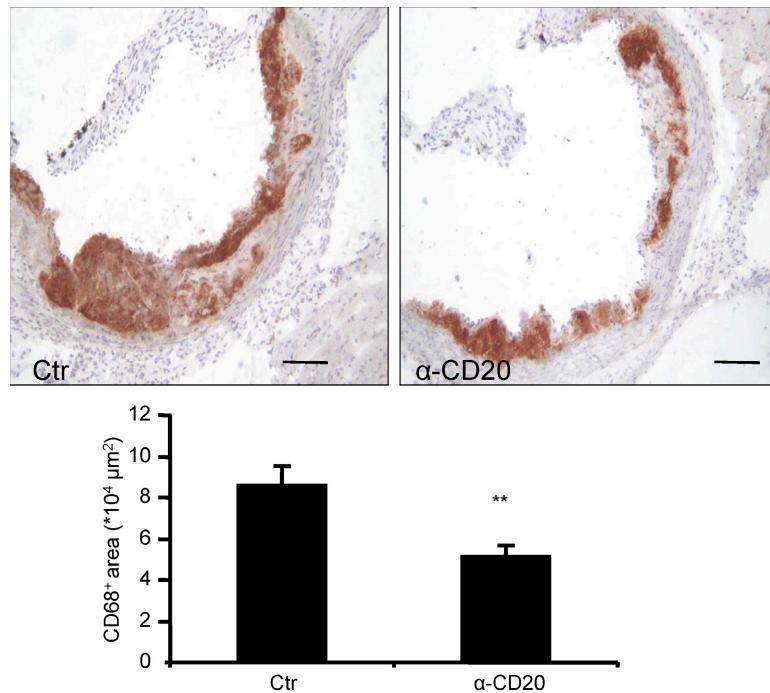


Figure S5. CD20 mAb (α -CD20) reduces macrophage accumulation within atherosclerotic lesions. Representative photomicrographs and quantitative analysis of CD68 (macrophage) staining in atherosclerotic lesions of *Apoe*^{-/-} mice fed a Western diet for 12 wk and treated with CD20 mAb (α -CD20) or control antibody (Ctr). Data are representative of 12–13 mice per group and two separate experiments. Mean values \pm SEM are represented. **, P < 0.01. Bars, 100 μ m.

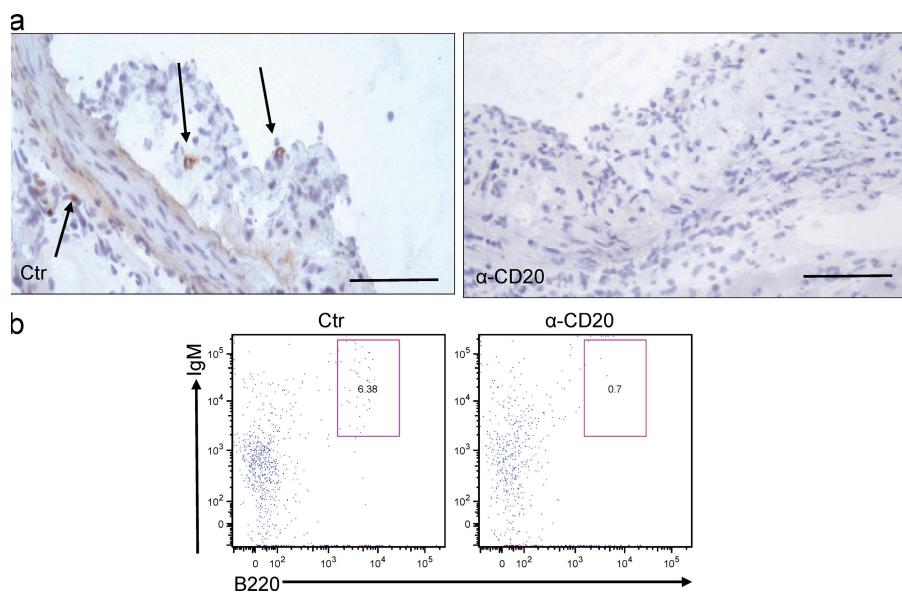


Figure S6. B cells are barely detectable within atherosclerotic lesions. (a) Examples (arrows) of B cell detection (B220⁺) within the intima or adventitia of atherosclerotic lesions from *Apoe*^{-/-} mice fed a Western diet for 12 wk. Data are derived from 12 mice and two separate experiments. Bars, 100 μ m. (b) An example of B cell detection (B220⁺IgM⁺) within atherosclerotic aortas of *Apoe*^{-/-} mice fed a Western diet for 12 wk and treated with CD20 mAb (α -CD20) or control antibody (Ctr). Data are representative of three mice per group.

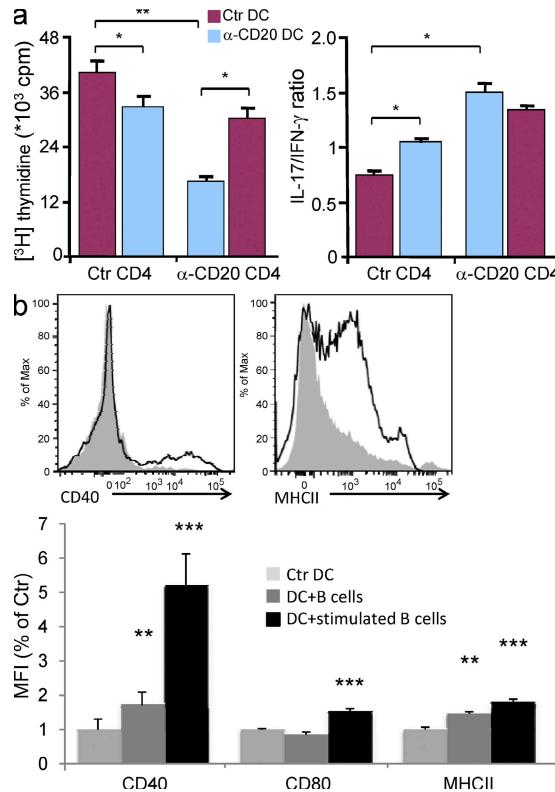


Figure S7. Modulation of DC and T cell functions through B cell activation or depletion. (a) Purified spleen-derived T cells from *Ldlr*^{-/-} mice fed a Western diet for 6 wk and treated with CD20 mAb (α -CD20) or control antibody (Ctr) were stimulated in vitro with anti-CD3 antibody in the presence of purified CD11c⁺ DCs, as described in Materials and methods. Data are representative of three mice per group and three separate experiments. Similar data were obtained using *Apoe*^{-/-} mice (not depicted). (b) DCs were isolated and co-cultured with unstimulated or prestimulated B cells, as described in Materials and methods. Examples of flow cytometry analysis and quantification of mean fluorescence intensity (MFI) are shown. Data are representative of six mice and two separate experiments. Values represent means \pm SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

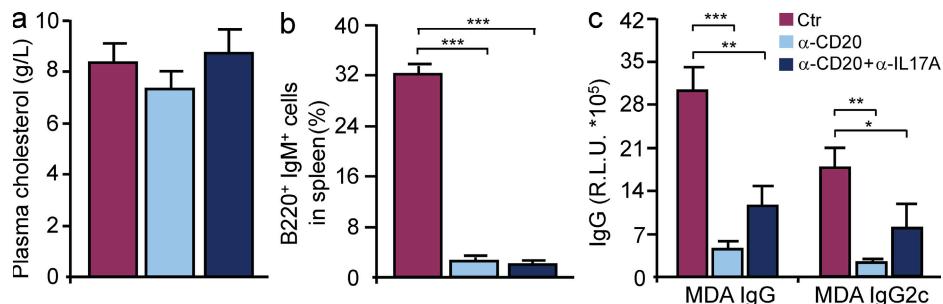


Figure S8. IL-17A neutralization does not affect plasma cholesterol levels and production of anti-oxLDL antibodies. (a–c) Plasma cholesterol levels (a), percentage of spleen B cells (b), and levels of circulating IgG antibodies against malondialdehyde (MDA)-modified LDL (c) in *Apoe*^{-/-} mice fed a Western diet for 6 wk and treated with CD20 mAb (α -CD20; blue) and/or neutralizing anti-L-17A mAb (α -IL-17A; dark blue) and/or control (Ctr; magenta) antibodies. Data are representative of five to seven mice per group. Mean values \pm SEM are shown. *, P < 0.05; **, P < 0.01; ***, P < 0.001. R.L.U., relative light units.

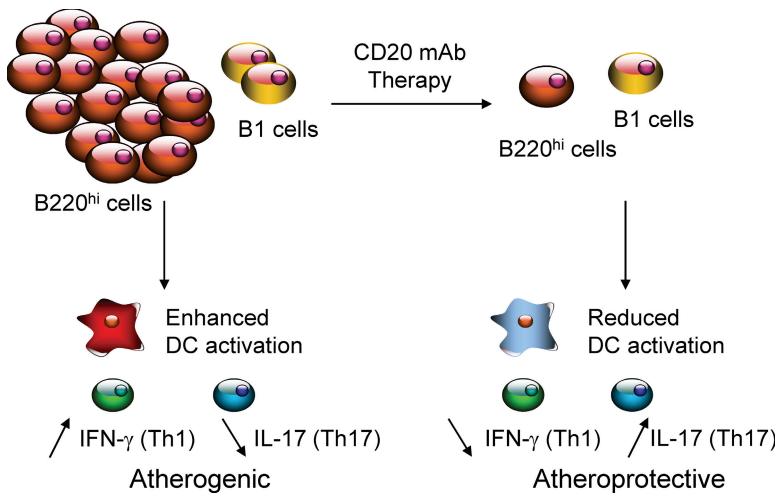


Figure S9. Proposed mechanisms for the atheroprotective effect of CD20 mAb. B1 cells have been shown to play atheroprotective roles in part through the production of IgM type anti-oxLDL antibodies. Our present study shows that B cells other than B1 cells play an important co-stimulatory role in atherosclerosis leading to enhanced DC activation and promotion of a Th1 response. Treatment with CD20 mAb almost eliminates B cells with co-stimulatory properties while preserving B1 cells. This leads to reduced DC activation, reduced T cell-dependent atherogenic IFN- γ production (Th1), and enhanced production of atheroprotective IL-17 (Th17).