Supporting Information

Carrington et al. 10.1073/pnas.1417620112

SI Materials and Methods

Mice. C57BL/6 (B6, WT), B6.Ly5.1, B6.Ly5.1/Ly5.2 (F1), 129(12):2285 T Cells. 10^6 spleen cells were determined by OVA-coated Splenocytes were enriched for DCs using a Bcl-2 analyzed by ELISA. The capture Ab was RMMA-1 (PBL (for-

10^6(3):1054 (7) mice were generated on, or backcrossed to, The experimental pro-

145(1):145 TREA1 (15), Mcl-1 (clone 19C4-15), rabbit anti

1o f7 to measure RNA expression using a CFX384 real-time PCR primer assay (QuantiTect Mm_Mcl1_1_SG) was

10 145(1):145 -Tet transactivator (1), Mcl-1 fl/fl


3. Ottina E, et al. (2012) Targeting antiapoptotic A1/Bfl-1 by in vivo RNAi reveals mul-


8. Vandenberg CJ, Cory S (2013) ABT-199, a new Bcl-2-specific BH3 mimetic, has in vivo efficacy against aggressive Myc-driven mouse lymphomas without provoking throm-

9. Carrington et al.

www.pnas.org/cgi/content/short/1417620112

Carrington et al. 10.1073/pnas.1417620112

1 of 7

Fig. S1. (A) Detection of BCL-W. Sorted pDCs and cDCs were either uncultured or cultured for 5 h with or without 5 nM CpG. Cell lysates were then used for detection of BCL-W by Western blot. No BCL-W was detected from all DC preparation. Anti–BCL-W Ab (clone 13F9) detects BCL-W in positive control (testis). A1 was included as a control. (B) Expression of A1 and BCL-2 by LN DCs. DCs were isolated from skin-draining LNs. Three main DC populations within LN were sorted and assessed for expression of BCL-2 and A1. Proteins (Left) and mRNAs (Right). (C) Expression of antiapoptotic molecules after activation. WT mice were injected i.p. with either PBS or 40 nmol CpG for 18 h. Spleen DCs were then purified for detection of proteins (Left) and mRNAs (Right).
Fig. S2. (A–E) In vitro killing of activated pDCs and cDCs. Purified pDCs and cDCs were incubated with 100 nM CpG for 16 h and then exposed to ABT-199 or ABT-737 for 24 h. Viable cells were then counted. (A) DC sorting purity, (B) CD86 up-regulation, (C) survival enhancement by CpG activation, and (D and E) sensitivity of activated pDCs and cDCs to ABT-199 or ABT-737. (D) Data show percentage of cell survival relative to corresponding cultures without drug treatment. (E) Data show numbers of viable cells with or without drug treatment. **P < 0.01, compared with cultures without CpG. (F) In vitro sensitivity of DCs and lymphocytes. Spleen cells were incubated with indicated concentration of ABT-199 for 24 h. After surface staining, viable DC subsets, T cells and B cells were determined. Data are shown as the proportion (percentage) of cell subsets isolated relative to the average number isolated from untreated cultures with means ± SEM (Left) and numbers of recovered live DCs (Right). (G) In vitro killing of pDCs and cDCs. DC-enriched spleen cells were incubated with the indicated drug for 24 h. Data show percentage of cell recovery relative to untreated. ABT-199 antagonizes BCL-2; ABT-737 antagonizes BCL-2, BCL-XL, and BCL-W; and WEHI-539 antagonizes BCL-XL. (H) BCL-2 antagonism does not impede cDC function. DC-enriched spleen cells (2 × 10⁵ per well) were incubated with 100 nM CpG with or without ABT-199 or ABT-737 for 24 h. Culture supernatants were measured for cytokine levels by Bio-plex.
Mice with microRNA-based shRNA targeting of an irrelevant gene, Renilla luciferase (shluc), contain comparable proportions of cDCs and pDCs to control animals. Mice containing a luc-targeted shRNA sequence and GFP reporter under control of the tetracycline responsive CMVmin promoter (TRE) (TRELuc) were intercrossed to mice expressing the tet transactivator (tTA) under control of the Vav-2 promoter to generate double transgenic (Tg) animals. DCs were assessed in single cell suspensions by flow cytometry with GFP reporting shluc expression. (A) Transgenic expression of GFP in cells isolated from spleen and LNs from TRELuc single transgenic (control) or double Tg mice. Percentages of GFP low and GFP high cells are shown. (B) Percentages of cDCs and pDCs from representative DC-enriched spleen and LNs. (C) Percentages of cDCs or pDCs from total DCs isolated from spleen and LNs from TRELuc (control) or GFP high cells from double Tg mice. Data represent \( n = 1 \) (TRELuc) and \( n = 5 \) (double Tg) animals ± SEM.
Fig. S4. (A) Both pDCs and cDCs express Mcl-1. Spleens from WT mice, CD11c-Cre+/Mcl-1fl/+, Mcl-1 fl/fl, and CD11c-Cre+ mice were analyzed for DC composition. CD11c-Cre+/Mcl-1fl/+ but not the other three types of mice show human CD4 expression, as indication of Mcl-1 transcription. (B) Impact of pDC deficiency on viral-induced IFN-α production. LCMV infections were performed via injection of 2 × 10^6 pfu LCMV docile into the tail vein of mice (five WT and four CD11c-Cre+/Mcl-1fl/fl). Serum IFN-α levels after 1 and 3 d infection was determined using an IFN-α assay kit from PBL Assay Science. **P < 0.01, compared with WT.
Fig. S5. Nonspleen DCs in WT vs. CD11c-Cre+ Mcl-1f/f mice. Thymi and LNs were harvested from individual mice. Single cell suspensions were stained for surface DC markers. Representative FACS plots show cell gating of different DC subsets and relative proportion. Bar graphs show the absolute cell numbers of DCs from each organ and proportion of DC subsets. (A) Thymus, (B) skin-draining peripheral LNs, and (C) gut-draining mesenteric LNs. ***P < 0.001 compared with WT.
Fig. S6. pDC and cDC subsets are not disproportionally affected in mice deficient for proapoptotic molecules BIM, PUMA, or NOXA. Individual mice of each genotype were used for evaluation of spleen DC composition. Comparisons were made between (A) WT mice and Bim−/− mice, (B) WT mice and Puma−/− mice, and (C) WT mice and Noxa−/− mice.