Supplementary information

Human cells

Peripheral blood was obtained after signed, informed consent from adult normal donors using a protocol approved by the institutional review board of the University of Pennsylvania and processes. Serum was obtained from fresh whole blood drawn by peripheral venipuncture and collected after centrifiguation in BD Vacutainer Serum Separator Tubes. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll centrifugation (Amersham Pharmacia Biotech). CD56+CD3^{neg}CD14^{neg}CD21^{neg} natural killer cells (>90%) were isolated from PBMC using magnetic bead purification columns using a kit (Miltenyi Biotech) according to the manufacturer's instructions.

Flow cytometry reagents

Flow cytometry reagents for mouse studies were CD3-PerCP or -APC-Cy7, F4/80-PerCP, CD45-V500, CD19-APC, CD86-FITC, CD80-PE, CD70-FITC, H2-D^{b}-PE (MHC class I), and I-A,I-E-PE (MHC class II) (BD Pharmingen). The agonist CD40 mAb FGK45 (rat IgG2a) and purified rat IgG2a (clone 2A3) were purchased from BioXCell and certified to contain <2 endotoxin units/mg; the agonist CD40 mAb 1C10 and 3/23 were purchased from Abcam. Recombinant murine soluble CD40L was purchased from Peprotech. F(ab)'$_2$ fragments were generated by enzymatic digestion with a pepsin kit (G Biosciences) according to the manufacturer's instructions. FGK45-PE was purchased from Miltenyi Biotec. Flow cytometry in all experiments was performed with a FACS Canto (BD Biosciences) and data analyzed using FlowJo software (Treestar, Inc.).

Flow cytometry reagents for human studies were CD3-PerCP, CD14-PerCP, CD19-APC, CD86-FITC, CD70-FITC, HLA-ABC-FITC, HLA-DR-FITC, and 7-AAD (BD Biosciences), CD40-FITC (clone LOB7/6 from Serotec, and clone HB14 from Caltag Medsystems), and FITC-

conjugated goat anti-human IgG Fc fragment and FITC-conjugated goat anti-human IgG F(ab)'$_2$ fragment (Jackson ImmunoResearch). HB14 competes with CP-870,893 for CD40 binding, but LOB7/6 does not.

Antibody-dependent cellular cytotoxicity assay

Lymphoblastoid cell lines (CD40^{neg} CD20^{neg} SR, CD40^{high}CD20^{high} Ramos, and CD40^{high}CD20^{high} Daudi) were harvested from culture, washed, and labeled with carboxyfluorescein succinimidyl ester (CFSE) by incubation in PBS at 50 nM for 10 minutes and then washed with RPMI complete media. Isolated NK cells were added to CFSE-labeled tumor cells at an NK cell-to-target ratio of 10:1 in the presence of 1 μg/ml CP-870,893, human IgG2, rituximab, human IgG1 (Sigma-Aldrich) or equimolar concentrations of CP-870,893 F(ab)'$_2$ or rituximab F(ab)'$_2$. After incubation at 37C/5% CO_2 for 4 hr, cells were washed with PBS/0.5% BSA and stained with 7-AAD for viability. Before flow cytometric analysis, equal quantities of fluorescently labeled beads were added to each sample for calibration. After gating on tumor cells by forward and side scatter, viable target cells were identified as 7-AAD^{lo} and CFSE^{hi} events. Percent cytotoxicity was calculated by comparing the calibrated number of live cells in experimental conditions to controls without NK cells.

Complement-mediated cytotoxicity assay

SR, Ramos, and Daudi lymphoblastoid cell lines were harvested from culture, washed, and labeled with CFSE as in the ADCC assay and incubated in either 3% fresh human serum or serum that had been heat-inactivated (by incubation at 56C for 30 min) for 4 hr at 37C/5% CO_2. Cells were then analyzed by flow cytometry and the percent cytoxicity calculated as described for the ADCC assay.