

European Journal of Immunology

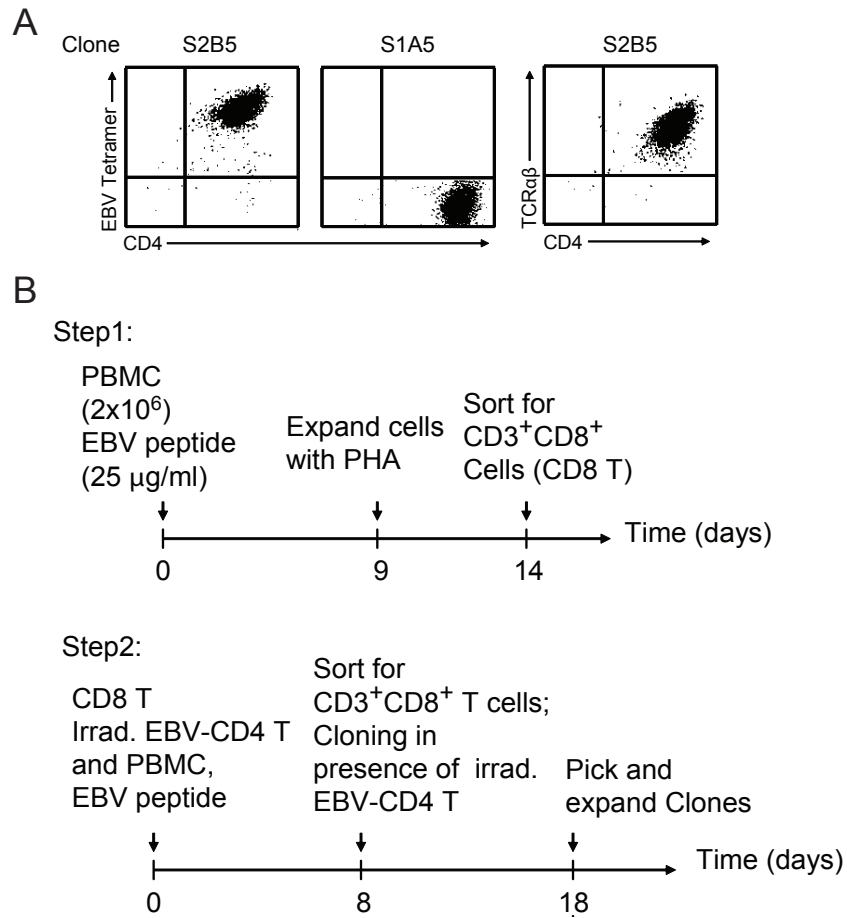
Supporting Information

for

DOI 10.1002/eji.201141618

A clonal model for human CD8⁺ regulatory T cells: Unrestricted contact-dependent killing of activated CD4⁺ T cells

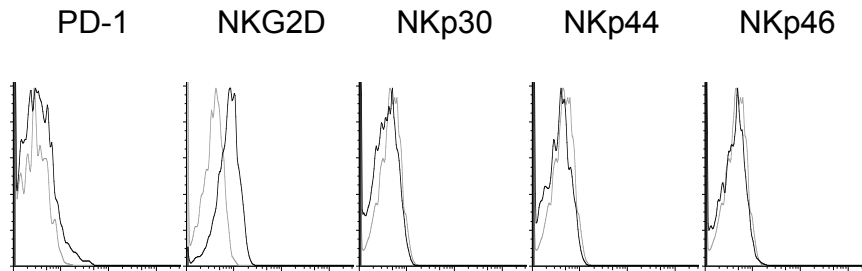
Dan Hu, Xia Liu, Wanyong Zeng, Howard L. Weiner and Jerome Ritz



Supporting Information Figure 1. EBV-specific CD4⁺ T cell clones and strategy of isolating CD8⁺ Treg cell clones.

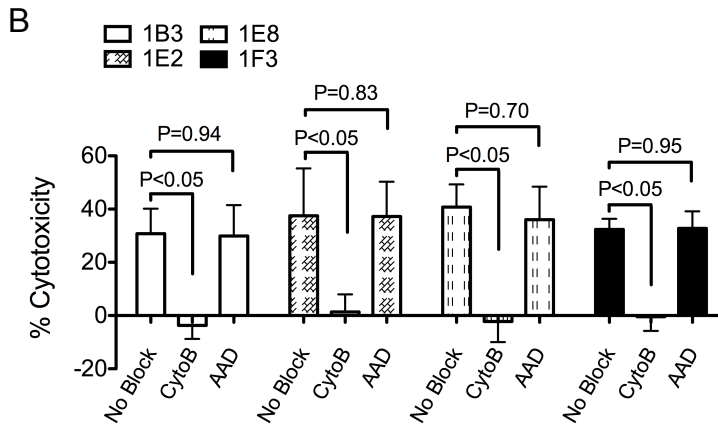
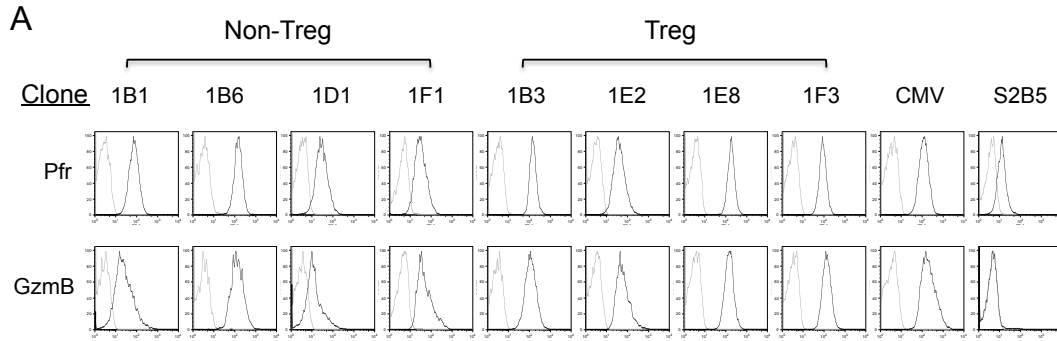
(A) EBV-specific CD4⁺ T cell clones. CD4⁺ T cell clones isolated from pEBV peptide-stimulated HLA-DR1⁺ normal human PBMCs were analyzed for CD4 expression and binding to pEBV-presenting HLA-DR1 tetramer (EBV-Tetra) (y-axis). Data are from one experiment representative of four.

(B) Strategy and timeline for isolation of CD8⁺ Treg cell clones. DR1-positive PBMCs were stimulated with pEBV (25 μg/ml). Peptide stimulated cells were expanded in the presence of PHA and feeder cells on day 9 followed by isolation of CD8⁺ T cells on day 14. Isolated CD8⁺ T cells were further stimulated with irradiated EBV-specific CD4⁺ T cells in the presence of irradiated autologous PBMCs and pEBV (9 μg/ml). On day 8 of step 2, CD8⁺ T cells were isolated and subcloned. Clones were expanded for further analysis.



Supporting Information Figure 2. Additional cell surface antigen expression analysis in CD8⁺ Treg cell clones.

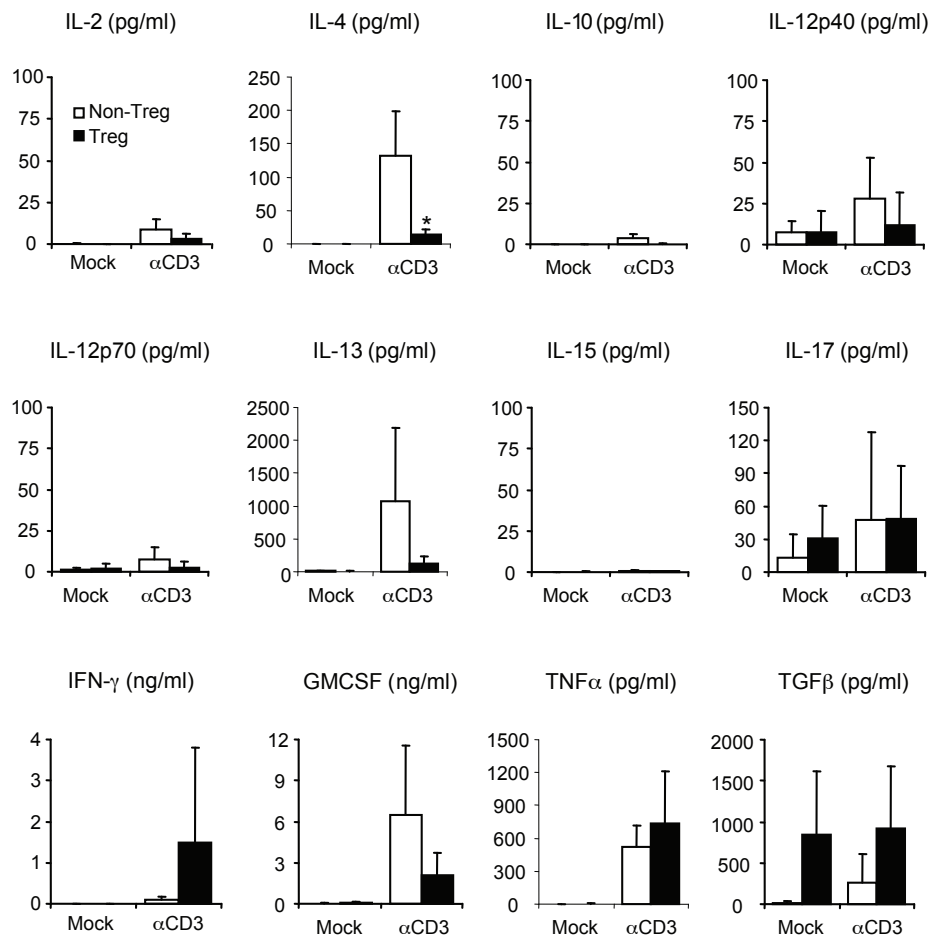
CD8⁺ Treg cell clones (1B3, 1E2, 1E8 and 1F3) were expanded via PHA stimulation for 8 and 12 days and harvested for flow cytometry analysis. Data are from one experiment representative of three. Black line indicates specific antibody staining and gray line indicates control.



Supporting Information Figure 3. CD8⁺ Treg cell clones express granzyme B and perforin but its cytotoxicity is not blocked granzyme B inhibitor.

(A) Intracellular staining of granzyme B (GzmB) and perforin (Pfr) in T cell clones. CMV: CMV specific CD8⁺ CTL clone CMV66; S2B5, an EBV-specific CD4⁺ T cell clone. Data are from one experiment representative of three. Black line indicates specific antibody staining and gray line indicates isotype control staining.

(B) Cytotoxicity mediated by CD8⁺ Treg cells is resistant to granzyme B inhibitor. CytoTx assays were performed to assess the blocking effect of Cytochalasin B (CytoB, 10 µg/ml) and AAD-cmk (AAD, 50 µM), which were added to CD8⁺ Treg cells first and then co-incubated with CD4⁺ target cells for 4 hr. Results are shown as mean ± SD of data pooled from three independent experiments. P values were obtained via unpaired, two-tailed *t*-test.



Supporting Information Figure 4. Cytokine production of CD8⁺ Treg cell clones.

Cytokine secretion by CD8⁺ T cell clones (1×10^5 cells per well) after over night culturing in the presence or absence of anti-CD3 IgE mAb (1 μ g/ml) was quantitated with Multiplex ELISA array. CD8⁺ Treg cell clones, n=3; autologous non-Treg CD8⁺ T cell clones, n=3. Results are shown as mean \pm SD. *, P value < 0.05; without indications, P value > 0.05. P values were obtained via unpaired, two-tailed *t*-test.