

Table S1. Total Splenic T Cell Subsets at Different Times after HSCT

		Number of Cells (x 10 ⁵)			
Day after		Treatment Group ^a			
Transplant	T Cell Subset	Unmanipulated T	CD25-depleted T	BM Only	Normal Controls
5	CD8	5.0 ± 1.1	6.4 ± 1.8	0.4 ± 0.3 ‡‡	60.0 ± 6.1
	CD4	20.6 ± 8.5	17.0 ± 8.5	4.0 ± 0.2 ‡‡	130.1 ± 16.0
	Treg	2.5 ± 0.8	1.4 ± 0.6 †	0.5 ± 0.2 ‡‡	13.3 ± 2.1
8	CD8	4.4 ± 1.4	5.8 ± 2.6	0.3 ± 0.1 ‡‡	
	CD4	18.0 ± 5.0	17.7 ± 3.7	5.9 ± 3.6 ‡‡	
	Treg	4.0 ± 0.8	2.5 ± 0.6 †	0.5 ± 0.2 ‡‡	
11	CD8	12.7 ± 6	16.8 ± 5.4	1.0 ± 0.1 ‡‡	
	CD4	25.1 ± 4.6	24.3 ± 1.5	2.0 ± 0.1 ‡‡	
	Treg	7.4 ± 1.7	5.4 ± 0.8 †	0.7 ± 0.1 ‡‡	
18	CD8	15.5 ± 4.1	28.0 ± 10.0	1.6 ± 0.1 ‡‡	
	CD4	25.5 ± 6.8	32.0 ± 7.6	5.4 ± 0.3 ‡‡	
	Treg	8.5 ± 2.3	8.5 ± 0.9	2.2 ± 0.2 ‡‡	

^a A/J mice were transplanted with 5x10⁷ syngeneic BM cells plus 3x10⁶ unmanipulated T cells or CD25-depleted T cells. Some mice were transplanted with BM cells only, and normal non-transplanted mice were included as a control group. Spleens were collected at different times after HSCT. The data for each group represents the mean absolute numbers of CD8, CD4 and Treg (CD4⁺CD25⁺Foxp3⁺) cells ± SEM. 6-10 mice were analyzed for each group (combined from 2-3 independent experiments).

† p<0.05 for comparisons between Unmanipulated T and CD25-depleted T.

‡‡ p<0.01 for comparisons between Unmanipulated T and BMT only.

Table S2. Percentages of CD4 T Cells after HSCT

Day after Transplant	Percentages of CD4 Cells			
	Treatment Group ^a			
	Unmanipulated T	<i>In vivo</i> CD4-depleted	CD25-depleted T	CD4-depleted T
11	5.0 ± 1.1	0.01 ± 0.002 ††	4.6 ± 0.1	1.0 ± 0.3 ‡‡
18	5.3 ± 1.6	0.31 ± 0.02 ††	4.9 ± 0.8	1.7 ± 0.2 ‡
25	7.6 ± 0.9	3.1 ± 0.5 ††	7.3 ± 1.0	6.2 ± 0.8
50	11.4 ± 0.7	6.5 ± 1.0 †	10.8 ± 1.2	9.7 ± 1.0

^a A/J mice were transplanted with BM cells plus T cells presensitized to tumor antigens that were either unmanipulated (Unmanipulated T), depleted of CD25⁺ cells (CD25-depleted T), or CD4⁺ cells (CD4-depleted T) cells *ex vivo* using immunomagnetic sorting. Some mice given unmanipulated T cells and treated with *in vivo*-depleting anti-CD4 mAb (250 mg intraperitoneally) on days -1, 2, 5, and 8 after transplantation (*In vivo* CD4-depleted). Spleens were collected at the indicated times after transplant and the percentages of CD4 T cells were assessed. The data for each group represents the mean percentage ± SEM from 6 total mice (combined from two independent experiments).

† p<0.05; †† p<0.01 for comparisons between Unmanipulated T and *In vivo* CD4-depleted groups.

‡ p<0.05; ‡‡ p<0.01 for comparisons between Unmanipulated T and CD4-depleted T groups.

Figure S1. Tumor-bearing recipients given HSC grafts consisting of 5×10^6 bone marrow cells plus 2×10^6 of unmanipulated or CD25-depleted T cells from tumor-bearing **nonvaccinated** donors (A) or plus 0.67×10^6 unmanipulated T cells or CD25-depleted T cells, from tumor-bearing **vaccinated (presensitized)** donors (B). The recipients were vaccinated as in Figure 1. Tumor burdens were monitored by bioluminescent imaging at days 1 and 14 after transplantation. The results are depicted as photon flux per minute and imaging data was compared using the Student's *t*-test.

Figure S2. Tumor-bearing recipients given HSC grafts consisting of bone marrow cells plus 2×10^6 unmanipulated T cells, CD25-depleted T cells, or T cells plus added CD25⁺ cells (at a 1:2 CD25⁺ cell-to-T cell ratio; CD25-supplemented) from tumor-bearing **nonvaccinated** donors or plus 0.67×10^6 unmanipulated T cells, CD25-depleted T cells, or T cells plus added CD25⁺ cells (at a 1:2 CD25⁺ cell-to-T cell ratio; CD25-supplemented) from tumor-bearing **vaccinated (presensitized)** donors. The recipients were vaccinated as in Figure 1. Tumor burdens in mice from panels were monitored by bioluminescent imaging at days 1 and 14 after transplantation. The results are depicted as photon flux per minute and imaging data was compared using the Student's *t*-test. The data represents the combined results of 2 independent experiments with a combined 11-12 mice per group.

Figure S2

