SUPPLEMENTAL MATERIAL

Budhu et al., http://www.jem.org/cgi/content/full/jem.20091279/DC1

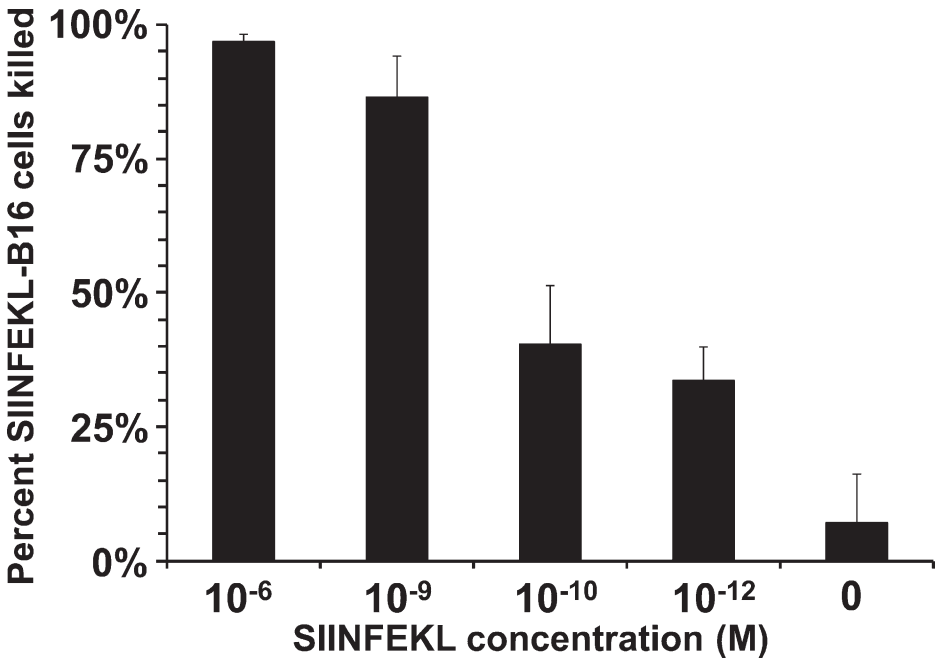

Figure S1. SIINFEKL peptide concentration required for optimal killing of B16 cells in collagen–fibrin gels. B16 cells were pulsed with the indicated concentrations of SIINFEKL peptide and coincubated at a concentration of 10^5/ml collagen-fibrin gel without or with 10^7 OT-1 cells/ml of gel at 37°C for 24 h. The gels were lysed and assayed for viable B16 cells. Data shown represent mean ± SEM of $n = 3$ experiments performed in duplicate.

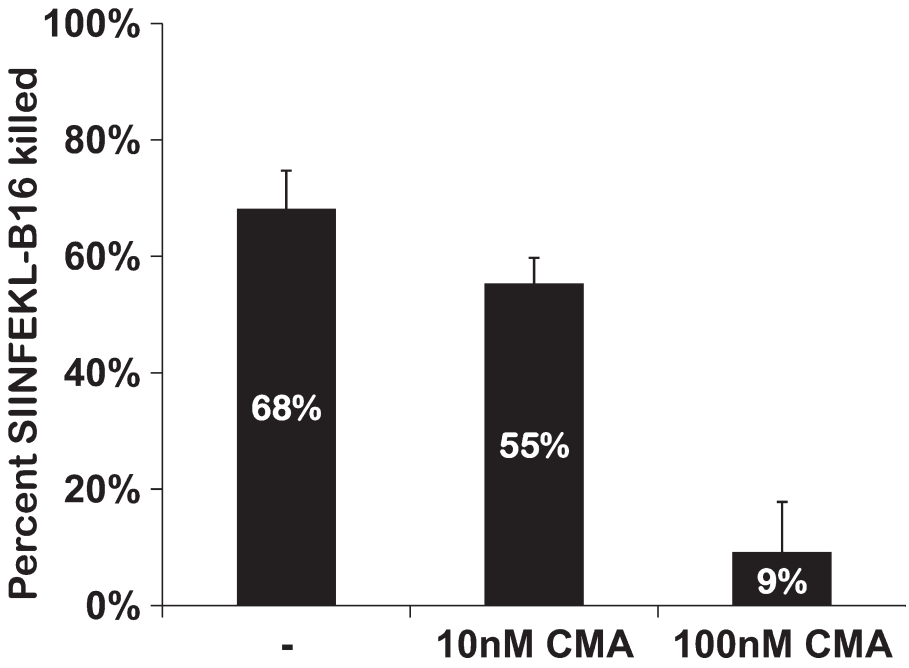

Figure S2. CMA inhibits OT-1 cell killing of SIINFEKL–B16 cells. Collagen-fibrin gels containing 10^5 SIINFEKL-B16 cells and 10^6 OT-1 cells/ml of gel were overlaid with 0.5 ml RPMI 1640 containing 10% FBS, 5×10^{-5} M β-ME, and the indicated concentration of CMA and incubated at 37°C for 24 h. The gels then were lysed and assayed for viable B16 cells as described in Materials and methods. Data shown represent mean ± SEM of $n = 3$ experiments performed in duplicate.

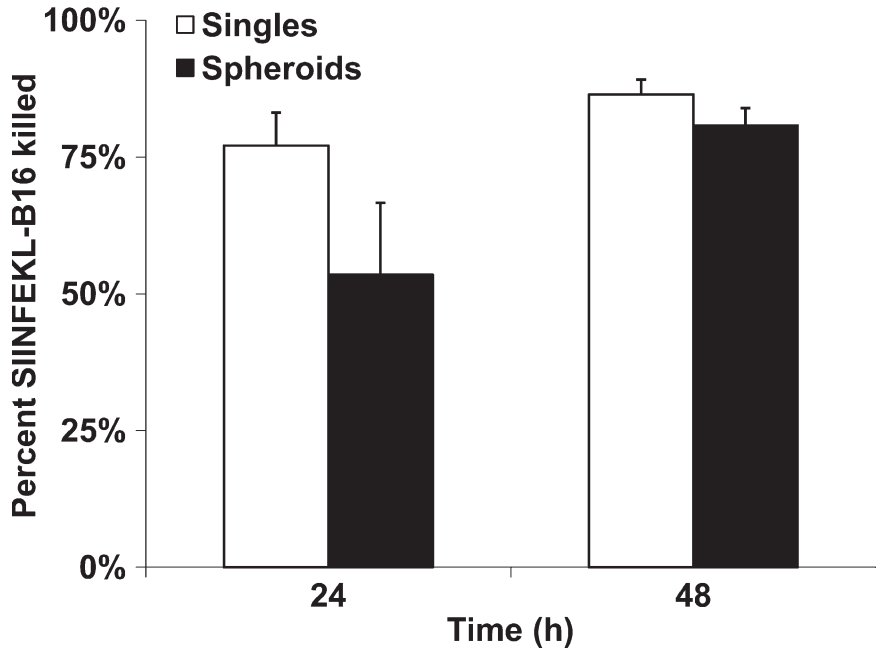

Figure S3. OT-1 cell killing of SIINFEKL–B16 cells in spheroids versus single SIINFEKL–B16 cells dissociated from these spheroids. Collagen-fibrin gels containing 10^6 OT-1 cells and 10^3 SIINFEKL peptide-pulsed B16 spheroids or 10^5 SIINFEKL-B16 cells dissociated from SIINFEKL peptide-pulsed spheroids were incubated in OT-1 growth medium at 37°C. At 24 and 48 h, gels were lysed and surviving B16 cells were assessed by colony formation as described in Fig. 1. Shown is the mean percentage of B16 cells killed ± SEM for three experiments, each performed in duplicate.

Table S1. Activated OT-1 cells kill growing and nongrowing SIINFEKL-pulsed B16 cells with approximately equal efficiency

OT-1 cells	10^4 B16 cells/ml		10^5 B16 cells/ml		10^6 B16 cells/ml		Mean
	Growing	Nongrowing	Growing	Nongrowing	Growing	Nongrowing	
	%	%	%	%	%	%	%
10^4	9 ± 3	11 ± 3	14 ± 4	10 ± 4	14 ± 1	14 ± 1	12 ± 2
10^5	27 ± 7	26 ± 4	31 ± 5	30 ± 3	34 ± 4	31 ± 4	30 ± 3
10^6	65 ± 6	64 ± 6	65 ± 1	61 ± 7	66 ± 6	61 ± 6	62 ± 2
10^7	98 ± 2	97 ± 1	99 ± 1	98 ± 1	98 ± 1	97 ± 1	98 ± 1

Killing efficiencies from Fig. 2 and unpublished data. Values represent the mean percentage of B16 killed ± SEM at *t = 24* for three experiments performed in duplicate.

Table S2. Addition of naïve spleen cells had no effect on killing efficiency of OT-1 cells in collagen-fibrin gels

OT-1 cells/ml	Naive spleen cells/ml	Total lymphocytes/ml	B16 killed (± SEM)
			%
0	0	0	0
0	10^7	10^7	0
10^4	0	10^4	18 ± 5
10^4	9.99×10^6	10^7	19 ± 3
10^5	0	10^5	35 ± 6
10^5	9.9×10^6	10^7	33 ± 2
10^6	0	10^6	75 ± 6
10^6	9×10^6	10^7	71 ± 3
10^7	0	10^7	97 ± 2

Collagen-fibrin gels contained 10^6/ml of gel SIINFEKL-B16 cells, 10^4, 10^5, 10^6, or 10^7/ml of gel in vitro–activated OT-1 cells, and, where indicated, a sufficient concentration of naive splenocytes from wild-type C57BL/6 mice to produce a final concentration of 10^7 lymphocytes/ml of gel. Gels were incubated at 37°C for 24 h, digested, and the number of clonogenic B16 cells remaining was assessed as described in Materials and methods. Data shown represent the mean percentage of B16 cells killed ± SEM at 24 h for three experiments, each performed in duplicate.

Table S3. OT-1 cell concentration determines the efficiency of killing of SIINFEKL-B16 cells

B16 cells added	Packed volume B16 cells	OT-1 cells added	Packed volume OT-1 cells	Spleno- cytes added	Packed volume spleno- cytes	Packed volume all cells	OT-1/ B16 cell ratio	OT-1 cell concen- tration	B16 cells killed
	nl		*nl*		*nl*				%
2×10^4	49	10^5	16	8.9×10^5	198	263 nl	5:1	2.4×10^9	∼18
4×10^4	99	10^5	16	6.7×10^5	148	263 nl	2.5:1	2.4×10^9	∼18
10^5	247	10^5	16	0	0	263 nl	1:1	2.4×10^9	∼18

B16 cells were pulsed with 1 µM SIINFEKL as described in Materials and methods.

Table S4. OT-1 cell killing of ova-B16 cells in 8-d-old tumors in vivo

Days after inoculation[a]	ova-B16 tumor volume (control mice)[b]	ova-B16 cells per tumor (control mice)[b,c]	Intratumoral OT-1 cells/g of tumor[b]	ova-B16 tumor volume (OT-1 cell–inoculated mice)[b]	ova-B16 cells per tumor (OT-1–inoculated mice)[b,c]	ova-B16 cells in tumors of OT-1 cell–inoculated mice on the day indicated/ova-B16 cells in tumors on day 0	*g*/min[d]	*k* ml/OT-1 cell/min[e]	CTC (OT-1 cells/ml) = *g*/*k*
	mm^3			mm^3					
0	90.5	2.71×10^7	0	90.5	2.71×10^7	day 0 = 1			
3	615.4	1.84×10^8	days 0–3 = 3×10^6, mean days 0–3 = 1.5×10^6	299.6	8.99×10^7	day 3 = 3.32	days 0–3 = 4.4×10^{-4}	days 0–3 = 1.1×10^{-10}	days 0–3 = 4×10^6
5	1,267	3.8×10^8	day 5 = 5×10^6, mean days 3–5 = 4×10^6	212.7	6.38×10^7	day 5 = 2.35	days 3–5 = 2.5×10^{-4}	days 3–5 = 0.92×10^{-10}	days 3–5 = 2.7×10^6
7	∼1,900	5.7×10^8	day 7 = 3×10^6, mean days 5–7 = 4×10^6	149.5	4.48×10^7	day 7 = 1.65	days 5–7 = 1.4×10^{-4}	days 5–7 = 0.66×10^{-10}	days 5–7 = 2.1×10^6

[a]Days after i.p. inoculation of in vitro–activated OT-1 cells into mice bearing 8-d-old ova-B16 tumors.
[b]Data from Petersen et al. (2006. *J. Immunother.* doi:10.1097/01.cji.0000203078.97493.c3). See also Agger et al. (2007. *J. Immunother.* doi:10.1097/01.cji.0000211326.38149.7e).
[c]B16 cells/mm^3 or /mg of wet weight of tumor = 3×10^5 (Stephens, T.C., and J.H. Peacock. 1978. *Br. J. Cancer.* 38:591–598.).
[d]*g* = growth rate of ova-B16 cells calculated as in Li et al. (2004. *J. Exp. Med.* doi:10.1084/jem.20040725) and in Materials and methods for days 0–3, 3–5, and 5–7, assuming 3×10^5 ova-B16 cells/mm^3 tumor and tumor volume as reported in Fig. 4 of Petersen et al. (2006. *J. Immunother.* doi:10.1097/01.cji.0000203078.97493.c3).
[e]Killing constant (*k*) calculated using Eq. 1 from Li et al. (2004. *J. Exp. Med.* doi:10.1084/jem.20040725) as described in Materials and methods.

Table S5. Polyoma virus antigen-specific $CD8^+$ T-cell killing of polyoma virus-infected splenocytes in mouse spleen in vivo.

Days after polyoma virus infection	Plaque forming units polyoma virus per mg spleen[a]	Number of polyoma antigen–specific $CD8^+$ T cells per spleen[a]	Intrasplenic concentration of polyoma antigen–specific $CD8^+$ T cells[b]	PFU polyoma virus/ mg spleen on the day indicated/ PFU on day 3[c]	*k* (ml/polyoma virus antigen–specific $CD8^+$ T cell/min)[d]	CTC/ml[e]
3	4.2×10^3	1.7×10^4	1.7×10^5	1		
4	5.5×10^3	Mean days 3–5 = 2.8×10^5	Mean days 3–5 = 2.8×10^6	1.31		
5	6.1×10^3	5.4×10^5	5.4×10^6	1.45	days 4–5 = 2.13×10^{-11}	8.63×10^6
6	1.2×10^3	Mean days 5–7 = 1.46×10^6	Mean days 5–7 = 1.46×10^7	0.29	days 5–6 = 8.98×10^{-11}	2.05×10^6
7	4.5×10^2	2.39×10^6	2.39×10^7	0.11	days 6–7 = 3.63×10^{-11}	5.07×10^6
8	1.2×10^1	Mean days 7–9 = 2.46×10^6	Mean days 7–9 = 2.46×10^7	0.003	days 7–8 = 1.11×10^{-11}	1.66×10^6
9	5.8	2.49×10^6	2.49×10^7	0.001	days 8–9 = 2.78×10^{-11}	6.62×10^6

For all calculations, b_0 is 4.2×10^3, the number of polyoma virus PFU/mg of spleen on day 3.
[a]Data in this table is from Lukacher et al. (1999. *J. Immunol.* 163:3369–3378). $g = \ln b_t/b_0$ (5,500/4,200) divided by 1.44×10^3 min/d = 1.87×10^{-4}/min.
[b]Calculated assuming spleen vol = 0.1 ml.
[c]Calculated from Fig. 2. in Lukacher et al. (1999. *J. Immunol.* 163:3369–3378)
[d]Calculated as in Li et al. (2002. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.122244799) and Li et al. (2004. *J. Exp. Med.* doi:10.1084/jem.20040725), $k = \ln b_t/b_0$ divided by the sum of the intrasplenic concentration of polyoma virus antigen–specific $CD8^+$ T cells/ml × time in minutes + g × time in minutes. *k* (mean) = 3.7×10^{-11} ml/polyoma virus antigen–specific $CD8^+$ T cell/min.
[e]CTC = *g*/*k* as in Li et al. (2002. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.122244799) and Li et al. (2004. *J. Exp. Med.* doi:10.1084/jem.20040725). CTC (mean) = 4.8×10^6 polyoma antigen–specific $CD8^+$ T cells/ml.