Supporting Information

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SI Text

Mice. BALB/c mice for DC immunizations were obtained from the National Cancer Institute and housed at the University of Iowa. All experiments were approved by the University of Iowa Institutional Animal Care and Use Committee. BALB/c mice for mosquito infections were obtained from Harlan Sprague Dawley and maintained in a laboratory animal suite at Iowa State University. All mice were treated and handled in accordance with guidelines established by the Institutional Animal Care and Use Committee at Iowa State University.

Dendritic Cells. Mice were primed with between 2.5×10^5 and 5×10^5 BMDC or splenic DC (sDC) coated with $CS_{252–260}$. Priming of mice with peptide-coated bone marrow dendritic cells (BMDC) followed by early boosting with *Listeria monocytogenes* has been described previously (1). Briefly, bone marrow cells were depleted of CD8+, CD4+, and B220+ cells by antibody-mediated complement lysis. Bone marrow cells were cultured in recombinant GM-CSF (1000 U/ml) and recombinant IL-4 (27 U/ml) for 6 days with the addition of LPS (100 ng/ml) for the last 14–18 h as a maturation stimulus. BMDC were pulsed with 2 μM $CS_{252–260}$, and epitope-coated BMDC were isolated using anti-CD11c microbeads (Miltyni Biotec) resuspended in saline and injected i.v.

sDC were isolated after i.p. injection of BALB/c mice with 5×10^6 B16 cells expressing Flt3L (provided by M. Prlic and M. Bevan, University of Washington). Two weeks later, mice were injected with 2 μg LPS i.v. to mature the DC. Spleens were harvested 16 h later and were digested with DNase and collagenase for 20 min at 37°C/7% CO_2 with shaking (120 RPM). Spleen pieces were forced through a nylon cell strainer (70 μm) to generate a single-cell suspension, RBC were lysed, and splenocytes were resuspended in 2 parts supplemented RPMI-1640 to 1 part B16-Flt3L-conditioned media + recombinant GM-CSF (1000 units/ml) + 2 μM $CS_{252–260}$ and incubated 2 h at 37°C/7%CO_2 with shaking (100 RPM). Spleen cells were washed, and CD11c+ cells were isolated using anti-CD11c microbeads (Miltyni Biotec). sDC were resuspended in saline and injected i.v. BMDC and sDC were analyzed by cell-surface staining for CD11c ($>$ 85%) before injection. CD11c+ cells also were analyzed for expression of CD86 ($>$ 85%) and MHC II ($>$ 90%).

Bacteria. *Listeria monocytogenes* expressing *P. berghei* $CS_{252–260}$ (LM-CS) was generated by ANZA Therapeutics. The LM-CS strain is an attenuated strain because of the in-frame deletion of both the *actA* and *inlB* genes (2). The $CS_{252–260}$ epitope is flanked by 3 native amino acids on either side and is secreted as a fusion peptide (2). Frozen aliquots of LM-CS were diluted in tryptic soy broth containing 50 μg/ml streptomycin (TSB-strep) and grown to an OD_{600} of $\approx$0.1, which equates to $\sim 10^8$ LM/ml. LM-CS cells were diluted in saline so that the target dose would be delivered in 0.2 ml. Mice were primed and boosted through the tail vein with 7×10^6 LM-CS or with 2×10^7 LM-CS. In some cases mice were boosted with titrating doses of LM-CS (2×10^4, 2×10^5, 2×10^6, or 2×10^7). The number of injected bacteria was verified by plating diluted aliquots onto tryptic soy agar containing 50 μg/ml streptomycin (TSA-Strep).

Quantification of Antigen-Specific T Cells. Single-cell suspensions from spleens were treated with Tris-ammonium chloride to lyse RBC and were washed with supplemented RPMI-1640 before stimulation. Livers were perfused with 10 ml cold HBSS through the hepatic portal vein. After removal of the gall bladder, the liver was excised and was forced through a wire mesh to make a single-cell suspension. Cells were filtered through a 70-μM filter and were rinsed with HBSS. Liver cells were spun at 400 *g* for 5 min at 4°C. Cells were resuspended in 15 ml of 35% Percoll/HBSS at room temperature and were spun at 500 *g* with no brakes for 10 min at room temperature. Pelleted cells were treated with Tris-ammonium chloride to lyse RBC, were washed with HBSS, and were centrifuged at 400 *g* for 5 min at 4°C. PBL were obtained by treating blood with Tris-ammonium chloride to lyse RBC followed by 2 washes.

Total spleen CS_{252}-specific CD8 T cells were determined by ICS for IFN-γ after 5 h of incubation in brefeldin A (Biolegend) in the presence or absence of 200 nM $CS_{252–260}$ as previously described (1). Total liver CS_{252}-specific CD8 T cells and the percent of PBL that were CS_{252}-specific CD8 T cells were determined by ICS for IFN-γ after 5 h of incubation in brefeldin A in the presence or absence of $CS_{252–260}$-coated P815 cells.

Antibodies. The following antibodies from Biolegend were used: IgG2A-PE (RTK2758), CD127-PE (SB/199), and IL-2-PE (JES6–5H4). The following antibodies from eBioscience were used: CD8-FITC (53–6.7), CD27-PE (LG.7F9), KLRG1-APC (2F1), and Golden Syrian Hamster IgG-APC. The following antibodies from BD PharMingen were used: IFN-γ-APC and CD62L-PE (MEL-14).

Passage of Blood-Stage *P. berghei* Through Mice. Modified *P. berghei* challenge protocols were followed (3, 4). Briefly, mice were infected with a *P. berghei* (ANKA strain clone 234) cryopreserved stock stabilate via i.p. injection. To visualize *P. berghei* infected erythrocytes, thin blood smears were prepared using the Hema 3 stain set (Fisher Diagnostics). Mice were anesthetized with a ketamine/xylazine mix at 80:8 mg/kg for blood collection and mosquito feeding. Parasitemic blood was collected via cardiac puncture into a heparinized needle and syringe, and 0.1 ml was subpassaged immediately to a new mouse. Mosquitoes were fed on anesthetized mice $\sim$ 3 days after subpassage or when parasitemia reached 5% to 20%.

Mosquitoes. *Anopheles stephensi* (Liston) strain STE2 (MR4–128, MR4, ATCC) were reared in controlled conditions (27°C ± 1°C and 80% ± 5% relative humidity) with a 16:8-hour photoperiod. Adult mosquitoes were housed in 0.5-L paper cartons and provided 10% sucrose (wt/vol) ad libitum. Sucrose was replaced with a water-soaked cotton ball 24 h before mosquito feeding. After exposure, engorged females were transferred immediately to holding cages and maintained at 20°C ± 1°C and 80% ± 5% relative humidity with a 16:8-hour photoperiod. To confirm infection before sporozoite collection, oocyst prevalence and intensity were monitored 7–14 days after exposure. *A. stephensi* midguts were dissected and stained using 0.5% aqueous Eosin Y (JT Baker) then viewed with bright field at 100× magnification.

Sporozoite Challenge. Salivary glands from *P. berghei*-infected *A. stephensi* were dissected using a stereomicroscope. Salivary glands were disrupted using a 0.1-ml dounce (Fisher Scientific) to release sporozoites. Sporozoites were quantified and injected i.v. into mice.

Identification of Infected and Protected Mice. Thin blood smears were performed 7 and 12 days after sporozoite challenge. Parasitized RBC were identified by Giemsa stain. Protected mice were

defined by the absence of blood-stage parasites and by the failure to succumb to infection.

1. Badovinac VP, Messingham KA, Jabbari A, Haring JS, Harty JT (2005) Accelerated CD8+ T-cell memory and prime-boost response after dendritic-cell vaccination. *Nat Med* 11:748–756.
2. Brockstedt DG, *et al.* (2004) Listeria-based cancer vaccines that segregate immunogenicity from toxicity. *Proc Natl Acad Sci USA* 101:13832–13837.
3. Sinden R (1997) Infection of mosquitoes with rodent malaria. *Molecular Biology of Insect Disease Vectors: A Methods Manual*. eds Crampton J, Beard C, Louis C, eds (Chapman and Hall, London).
4. Paskewitz SM, Shi L (2005) Bypassing the midgut results in development of *Plasmodium berghei* oocysts in a refractory strain of *Anopheles gambiae* (Diptera: Culicidae). *J Med Entomol* 42:712–715.

Fig. S1. Phenotype of memory CS252-specific CD8 T cells. BALB/c mice were primed with 5×10^5 DC coated with CS252–260 (DC-CS) or with 7×10^6 LM-CS_{252}. Seven days later DC-CS mice were boosted with 2.8×10^7 LM-CS_{252} (DC-CS+LM--CS). Phenotype of (*A*) spleen, (*B*) liver, and (*C*) blood CD8 T cells that are CS_{252} specific on day 61 as determined by ICS. One of 3 representative histograms is shown. Data are representative of 2 experiments.

Fig. S2. Similar phenotype of CS_{252}-specific CD8 T cells in mice given increasing doses of LM-CS_{252} boost. Mice were primed with 2.5×10^5 DC-CS and boosted with increasing doses of LM-CS_{252}. Peripheral blood CS252-specific CD8 T cells were identified by ICS and analyzed for the expression of CD27, CD62L, and CD127 and for the production of IL-2 on day 86. Shaded histograms are the isotype control, and open histograms are the indicated marker. Three representative examples from each group (2×10^4, 2×10^5, and 2×10^6, $n = 10$) and (2×10^7, $n = 7$) are shown.

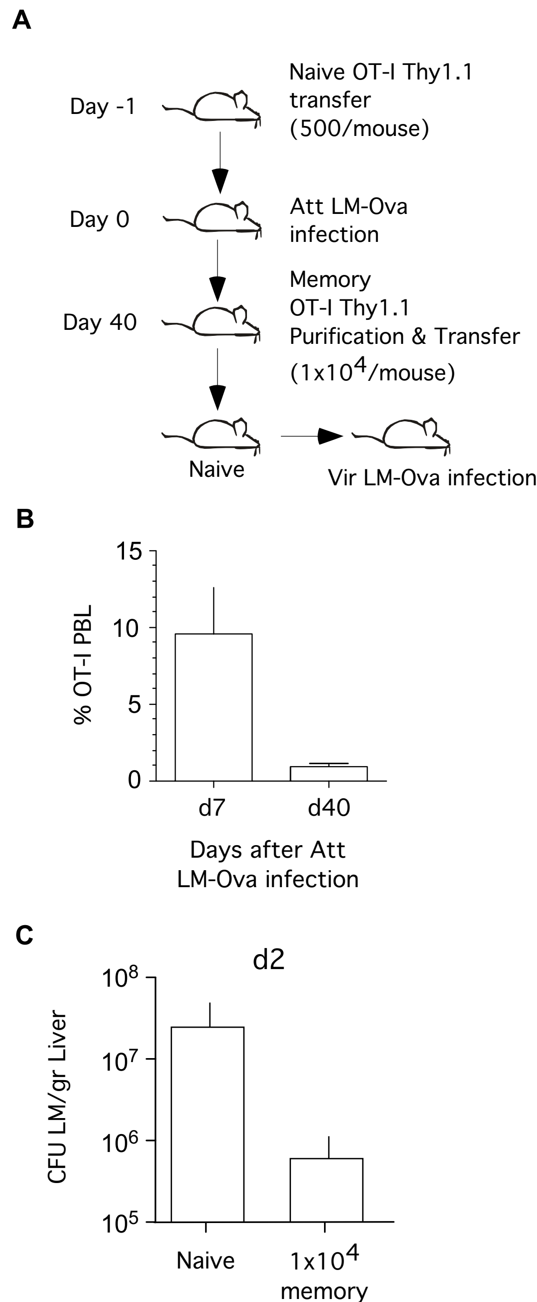

Fig. S3. Anti-listerial immunity provided by low number of memory CD8 T cells. (*A*) Experimental design. Purified naive OT-I Thy1.1 CD8 T cells (500/mouse) were transferred into naive B6 Thy1.2 mice, and 1 day later mice were immunized with Att LM-Ova (5×10^6 cfu/mouse). At day 40 after immunization, OT-I were purified from the spleens of immune mice by positive selection (Thy1.1) and were transferred (1×10^4/mouse) into naive B6 recipients 1 day before high-dose virulent LM-Ova infection (1×10^6 cfu/mouse). The group of naive mice (naïve) that did not receive memory OT-I cells served as controls. (*B*) Frequency of OT-I CD8 T cells in the blood at the indicated days after primary LM infection. (*C*) On day 2 after high-dose virulent LM-Ova infection, bacterial numbers were determined in the liver. Data are presented as mean + SD of 3 mice per group. One of 2 similar experiments is shown.