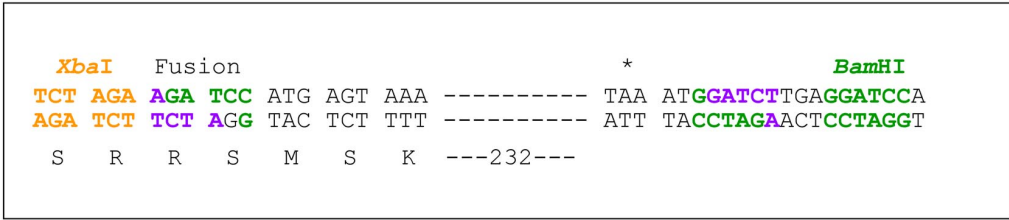
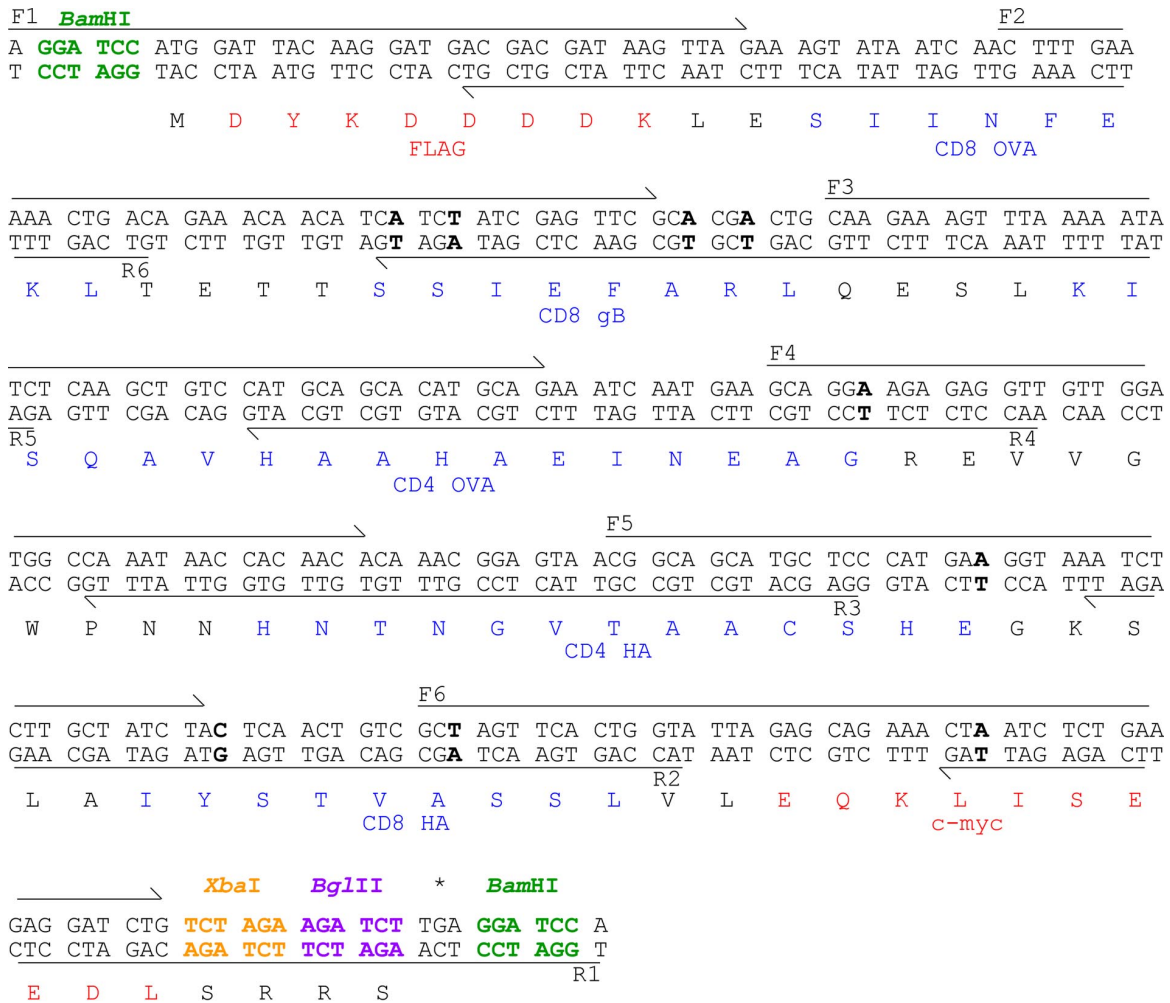


Supporting Information

Lundie *et al.* 10.1073/pnas.0806727105



Fusion of GFP to the 3' end of the polytope

Fig. S1. Design of the T cell polytope. Linearly arranged CD4⁺ and CD8⁺ T cell epitopes (blue) and FLAG and c-myc B cell epitopes (red) were flanked by at least two spacer amino acids that normally surround each of the epitopes in their native context (black) to minimize premature cleavage of the epitopes during processing and to promote correct tertiary structure and expression. Consistent with preferred codon usage in *Plasmodium*, G/C nucleotides were mutagenized (bold) where possible to increase the overall A/T content of the polytope. The polytope was generated by overlap extension PCR using oligonucleotides designed to overlap by ≈15 bp (F1–F6 and R1–R6). A BamHI site (green) was introduced at the 5' and 3' ends of the polytope to facilitate cloning into the *P. berghei* expression plasmid PbGFP_{CON} (17), and unique XbaI (orange) and BglII (purple) restriction sites were incorporated at the 3' end of the polytope, along with a stop codon (TGA). The unique palindromic BglII site (purple) and the 5' and 3' BamHI sites (green) of GFP were destroyed following fusion of the polytope to GFP (*Inset*). Note that the BamHI site at the 5' end of the polytope is still present, and that termination of translation is now regulated by GFP (stop codon TAA).

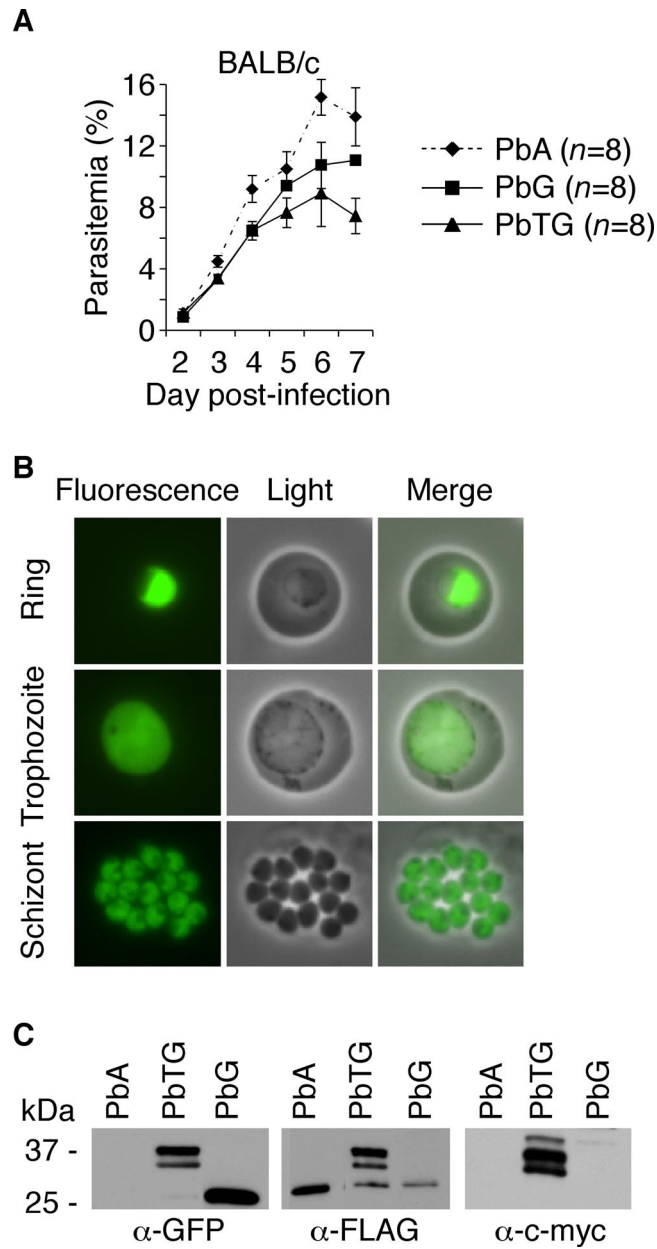


Fig. S2. Characterization of transgenic *P. berghei* parasites. (A) Parasitemia curves for *P. berghei* ANKA (PbA), PbG, and PbTG in BALB/c mice. Data pooled from two experiments; *n* represents total number of mice per group. Error bars represent SEM. (B) Detection of GFP expression by PbTG throughout the blood-stage lifecycle using live fluorescence microscopy. (C) Western blot analysis of mature trophozoite-stage parasite lysates using antibodies specific for GFP and the B cell epitopes FLAG and c-myc.

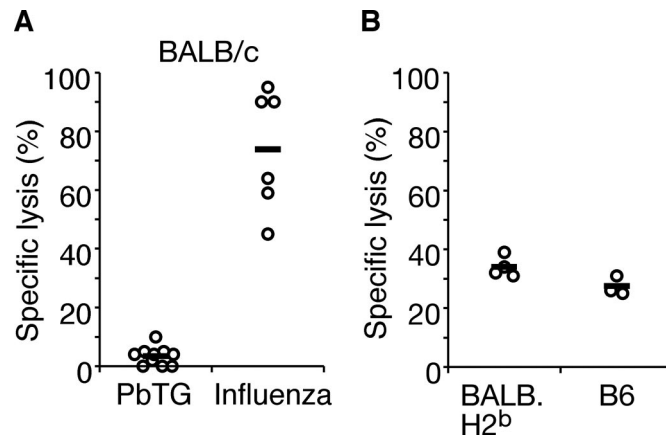


Fig. S3. Functional CTL are not induced in BALB/c mice infected with transgenic *P. berghei*. (A) Percentage specific lysis of HA_{518–526} peptide-pulsed target cells by endogenous CTL generated in the spleens of BALB/c mice infected with PbTG, or in the popliteal lymph nodes of mice infected with influenza virus PR8, for 6 days. (B) Percentage specific lysis of OVA_{257–264} peptide-pulsed target cells by endogenous CTL generated in the spleens of BALB.H-2^b and B6 mice infected with PbTG for 6 days. Data represent one experiment (similar results were obtained in two experiments with mice infected for 4 days). Open circles represent values for individual mice; horizontal bars represent values of the mean. All values are relative to naive mice, which were designated as 0% lysis.

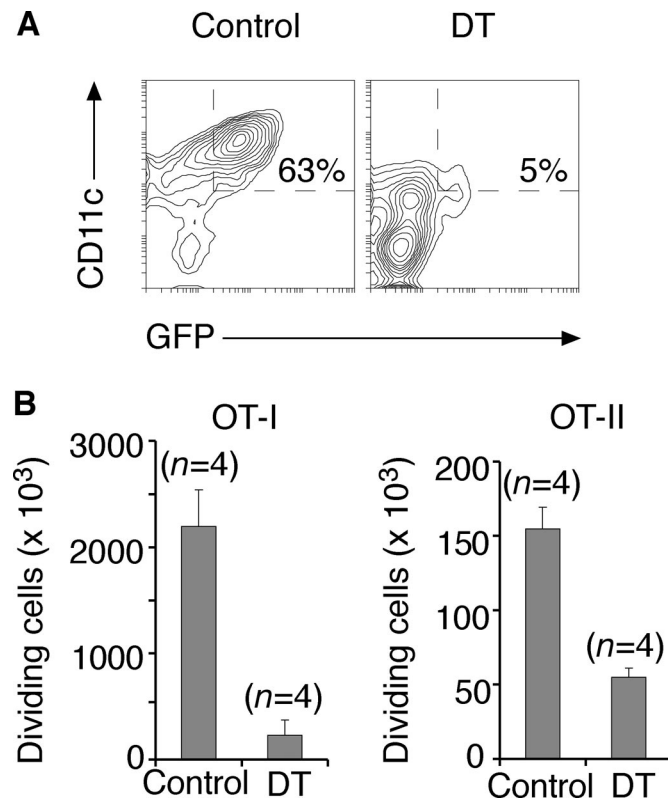


Fig. 54. CD11c⁺ DC are required for initiation of T cell responses to antigens expressed during blood-stage infection. (A) Representative flow cytometry profiles of DC purified from the spleens of CD11c-DTR chimeric mice left untreated (control) or treated with diphtheria toxin (DT). (B) Proliferation of OT-I and OT-II OVA-specific T cells 3 days after transfer into CD11c-DTR chimeric mice left untreated (control) or treated with DT, and infected with PbTG. Data pooled from two experiments; *n* represents total number of mice per group. Error bars represent SEM.

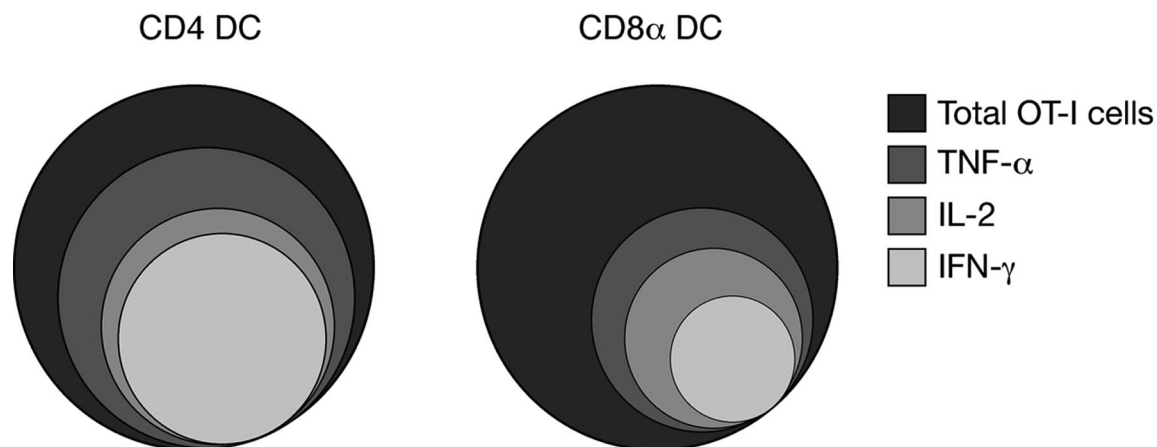
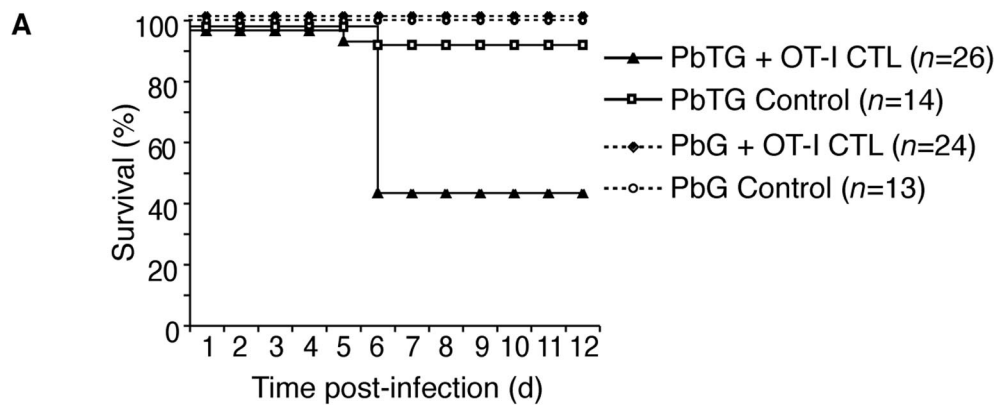


Fig. S5. Diagrammatic representation of the proportion of OT-I T cells producing TNF- α , IL-2, and IFN- γ following stimulation by CD4 or CD8 α DC subtypes isolated from the spleens of B6 mice on day 3 postinfection with PbTG. Of the total OT-I T cell population, 68% produced TNF- α after stimulation by CD4 DC, compared with 38% after stimulation by CD8 α DC; 42% produced IL-2 (and TNF- α) in the presence of CD4 DC, compared with 26% in the presence of CD8 α DC; 34% produced IFN- γ (and TNF- α and IL-2) in the presence of CD4 DC and 12% in the presence of CD8 α DC. One representative experiment of two performed using 100×10^3 DC/well.



B

Table 1. Number of RAG-1-deficient mice that survived until day 12 post-infection

	PbTG + OT-I CTL	PbTG Control	PbG + OT-I CTL	PbG Control
Exp. 1	0/2	2/2	2/2	2/2
Exp. 2	0/7	3/4	5/5	4/4
Exp. 3	0/6	6/6	6/6	5/5
Exp. 4	6/6	n.d.	6/6	n.d.
Exp. 5	2/2	2/2	2/2	2/2
Exp. 6	3/3	n.d.	3/3	n.d.

Fig. S6. CTL specific for parasite-expressed antigens are capable of causing lethal disease in RAG-1-deficient mice. (A) Survival curves for RAG-1-deficient mice following adoptive transfer of OT-I CTL on day 2 postinfection with PbTG or PbG. Control mice did not receive OT-I CTL. Data pooled from four to six experiments; n represents total number of mice per group. (B) The number of RAG-1-deficient mice that survived until day 12 postinfection from six individual experiments. Note that lethal disease has been inconsistent, with 100% lethality in three experiments and no lethality in another three experiments. We are currently unable to explain the variability. Also note that in some of these experiments, some experimental groups were not determined (n.d.) as they were not included.