Supporting Information

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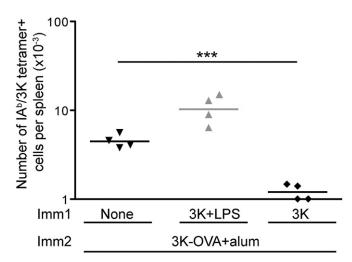


Fig. S1. Naive mice injected with soluble 3K peptide fail to respond to further reactivation. 40 d after naive B6 mice had been injected with 3K peptide or 3K peptide and lipoplysaccharide (LPS) i.v. these and naive mice were immunized with 3K-ovalbumin (OVA) and alum i.p. After an additional 6 d, the numbers of IA^b/3K tetramer⁺ CD44^{hi} in the spleens of these and naive age-matched mice were examined. Each symbol represents one mouse and the line shows the mean of the group. The *x* axis is set at the limit of detection as determined by the number of IA^b/3K tetramer⁺ CD44^{hi} cells in naive animals. These data are from one of two experiments with four mice per group, ***P < 0.001.

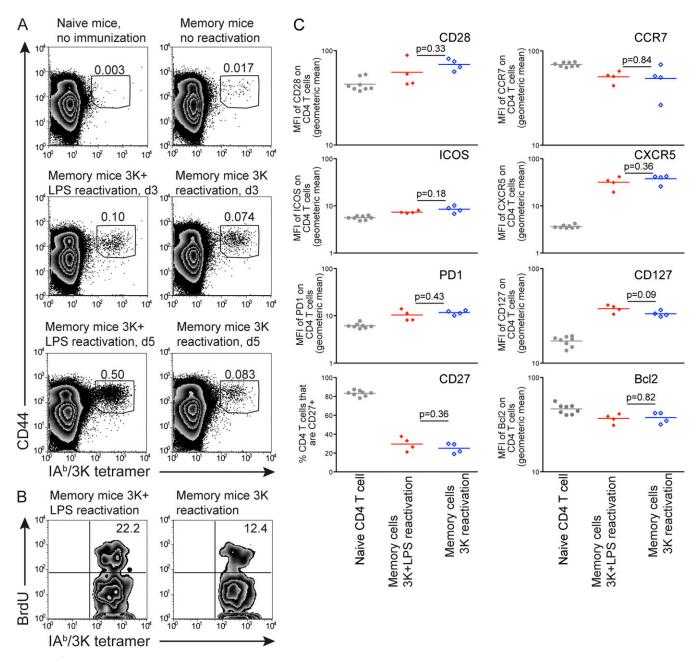


Fig. 52. IA^b/3K-specific memory CD4 T cells proliferate in response to 3K peptide delivered with or without LPS and return to the memory pool following reactivation. B6 mice immunized with 3K peptide and LPS i.v 40 d previously were injected with 3K + LPS or 3K without adjuvant. The percentages of CD4⁺ cells that were CD44^{hi} IA^b/3K tetramer⁺ were examined before or 3 and 5 d after the second immunization (A). These animals were injected with bromodeoxyuridine (BrdU) 15 h before the percentages of CD44^{hi} IA^b/3K tetramer⁺ that were BrdU⁺ were examined on day 3 after second immunization (Imm2) (B). In A, cells are gated on CD4⁺ dump negative live cells, and in B, on the CD44^{hi} IA^b/3K tetramer⁺ cells. The numbers in the plots show the percentages of the cells within the indicated gate or quadrant. These data are representative FACS plots from two experiments with three to four mice per group. Alternatively, these mice were rested for an additional 40 d following the second immunization and their expression of various cell-surface and intracellular molecules examined. Cells were either gated on naive CD4 T cells (CD4⁺ CD44^{low}, gray symbols) or on memory CD44^{hi} IA^b/3K tetramer⁺ cells from mice immunized twice with 3K + LPS (red symbols) or immunized once with 3K + LPS and reactivated with 3K alone (blue symbols) (C). In each graph, the symbols represent individual mice (naive CD4 T cells from the two immunized groups are combined as no differences were expected). The horizontal line in each group represents the mean for that group. The data are from one of two representative experiments with three to four mice per group.

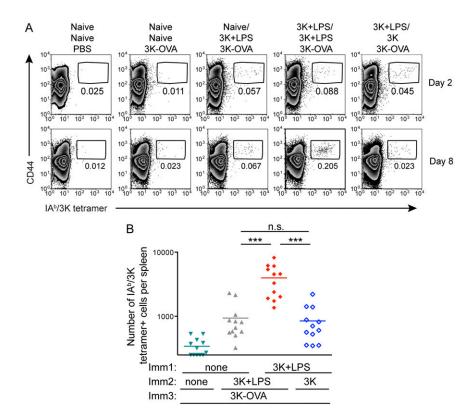


Fig. S3. Memory cells exposed to soluble peptide fail to induce a sustained delayed-type hypersensitivity response. (A) The percentages of IA^b/3K tetramer⁺ cells in the draining lymph nodes of B6 mice immunized as described were examined 2 or 8 d after footpad injection with 3K-OVA. The cells are gated on live CD4⁺ lymphocytes negative for CD8, B220, F4/80, and MHC II. The numbers in the plots show the percentages of CD4⁺ cells within the indicated IA^b/3K tetramer⁺ CD44^{hi} gate. (B) Alternatively, the numbers of IA^b/3K tetramer⁺ cells found in the spleens of these mice were examined on day 2 after 3K-OVA footpad injection. Each point represents a mouse and the horizontal line shows the mean of the group. The data are combined from three experiments with three to four mice per group. Statistical differences are indicated by ***P < 0.001. The x axis is set at the limit of detection as determined by staining spleen cells from naive mice with IA^b/3K tetramer.

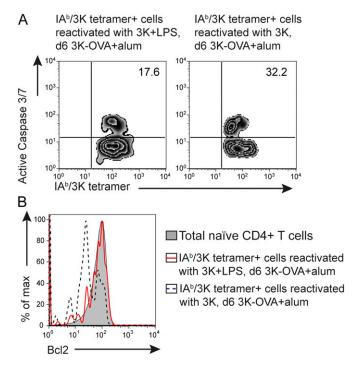


Fig. S4. Memory CD4 T cells activated with antigen alone express markers of apoptosis following reactivation in vivo. The levels of intracellular active caspase 3 and 7 (A) or B cell lymphoma 2 (Bcl2) (B) in IA^b/3K tetramer⁺ splenocytes from B6 mice immunized as described in Fig. 4 were determined 6 d after immunization with 3K-OVA and alum. The cells are gated on IA^b/3K tetramer⁺ cells and the FACS plots are representative samples from two independent experiments with four mice per group. The numbers in the plots show the percentages of cells within the indicated quadrant.

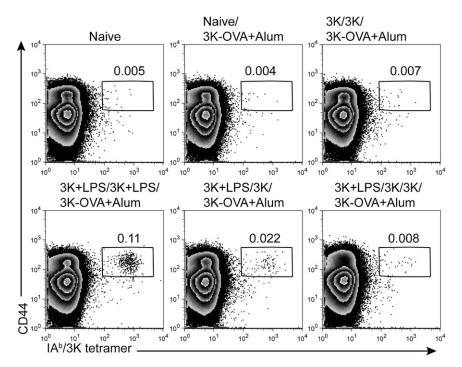


Fig. S5. No antigen-specific cells can be detected in memory mice injected twice with antigen alone then recalled with antigen and adjuvant. B6 mice were immunized with/without 3K peptide and LPS i.v. as indicated with immunizations given 40 d apart. Six days following immunization with 3K-OVA and alum i.p., the percentages of splenic CD4 T cells that were IA^b/3K tetramer⁺ CD44^{hi} were examined. The cells are gated on live CD4⁺ lymphocytes negative for B220, CD8, F4/80, and MHC II. The number indicates the percentages of CD4⁺ cells that are IA^b/3K tetramer⁺ CD44^{hi} as indicated by the gate. The data are representative FACS plots from two experiments with four mice per group.