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Materials and Methods
SOM Text
Fig. S1
Tables S1 and S2
References and Notes

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Naïve and Memory CD4$^+$ T Cell Survival Controlled by Clonal Abundance

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Immunity to a plethora of microbes depends on a diverse repertoire of naïve lymphocytes and the production of long-lived memory cells. We present evidence here that low clonal abundance in a polyclonal repertoire favors the survival and activation of naïve CD4$^+$ T cells as well as the survival of their memory cell progeny. The inverse relation between clonal frequency and survival suggests that intraclonal competition could help maintain an optimally diverse repertoire of T cells and an optimal environment for the generation of long-lived memory cells.

Protective immunity against infectious disease depends on antigen-specific memory T cells that survive for many years following initial exposure to antigen (*1*). One major paradigm, based largely on studies of CD8$^+$ T cells, suggests that memory results from the conversion of naïve cells to long-lived memory cells that self-renew through the actions of the cytokine interleukin-15 (IL-15) (*1*). However, this mechanism may not apply to memory CD4$^+$ T cells because they are less dependent on IL-15 and may be derived from naïve precursors that are themselves long-lived (*1*). Furthermore, polyclonal virus–specific memory CD4$^+$ T cells have been seen to decline for almost a year after infection, indicating that not all memory CD4$^+$ T cells are stably maintained (*2*). Such discrepancies prompted us to study the stability of naïve and memory CD4$^+$ T cell populations.

To assess the in vivo survival of polyclonal naïve CD4$^+$ T cells, CD44^{low} CD4$^+$ cells from mice positive for the CD90.1 marker were tracked using antibodies to CD90.1 after adoptive transfer into congenic CD90.2$^+$ recipients (*3*). During the first 2 months, the transferred naïve CD4$^+$ T cells declined in the secondary lymphoid organs or blood of recipient mice with an estimated 50-day half-life (Fig. 1) (*4*), as reported by others (*5*, *6*). However, a longer observation period revealed an overall half-life estimate of 124 days, with 10% of the cells still remaining 1 year after transfer.

The survival time of transferred polyclonal naïve CD4$^+$ T cells was probably underestimated with this approach because of the loss of some CD44^{low} cells as a result of participation in immune responses to unknown foreign antigens. It was therefore necessary to test the survival of monoclonal naïve CD4$^+$ T cells that could not be unintentionally activated by foreign antigen. Monoclonal populations of CD4$^+$ T cells from ovalbumin peptide-I-A^d–specific DO11.10 (*7*) or flagellin peptide-I-A^b–specific SM1 (*8*) T cell antigen receptor (TCR) transgenic mice were used for this purpose. One million naïve DO11.10 or 4 × 10^6 SM1 CD4$^+$ T cells were transferred intravenously into histocompatible BALB/c or C57BL/6 mice, respectively, resulting in the seeding of about 10^5 cells in the spleen and lymph nodes (Fig. 2A and Fig. 3, A and B). The monoclonal cells then declined with half-lives of 12 and 7 days, respectively (Fig. 3A, triangles, and Fig. 3B, diamonds), revealing them to be short-lived as compared with their polyclonal counterparts. The poor survival of transferred naïve TCR transgenic cells was not related to rejection because potential sensitization of recipient mice by one injection of transgenic cells did not cause a second inoculum of transgenic cells to disappear more quickly (fig. S1).

The increased survival of polyclonal compared with monoclonal naïve CD4$^+$ T cells could have been related to clone size. The polyclonal repertoire of naïve T cells in a normal adult mouse is composed of about 2 × 10^6 unique clones of 50 cells each (*9*, *10*). Thus, 10^5 transferred monoclonal cells represents a clone size of ~2000 times that of the typical naïve clonal population. However, the conventional approach of sampling a small fraction of secondary lymphoid tissue to enumerate adoptively transferred T cells was inadequate to detect the 100 cells seeded by a 1000-cell transfer (Fig. 2B) because the background was too high (Fig. 2C).

The conventional method was improved using a magnetic bead–based enrichment step that concentrated all of the transferred cells from the spleen and lymph nodes of individual mice (compare Fig. 2, A and D) into a small volume and reduced the number of contaminating recipient cells by a factor of several hundred. This enrichment method enabled detection of

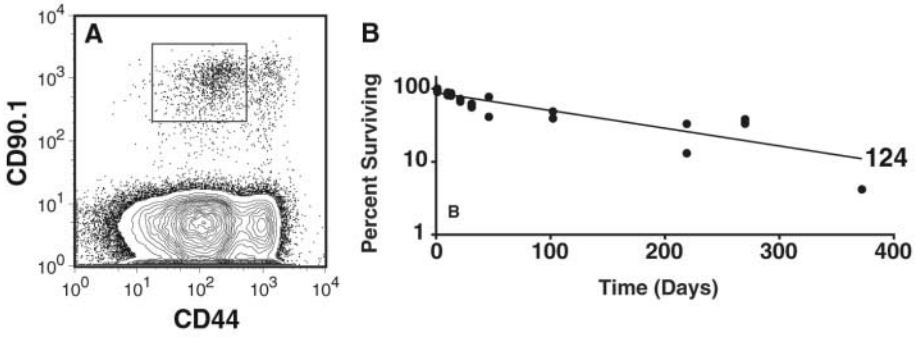

Fig. 1. Polyclonal naïve CD4$^+$ T cells persist in normal recipients for more than 1 year. Two million polyclonal CD90.1$^+$ CD4$^+$ T cells were injected into CD90.2$^+$ C57BL/6 mice. (**A**) The naïve transferred T cells were then identified as CD4$^+$, B220$^-$ (not shown), CD90.1$^+$, CD44^{low} cells. (**B**) The percentage of these cells remaining in each recipient (pooled from two independent experiments) over time, fit to an exponential decay curve. The estimated half-life of the population (in days) is shown.

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about 100 cells seeded by a 1000-cell transfer (Fig. 2E) and reduced the background level to fewer than 20 cells (Fig. 2F). The background was reduced to 0 by excluding cells that bound antibody to CD4 nonspecifically (Fig. 2G) and by transferring cells that expressed CD90.1 as an additional marker. This low background rate allowed detection of only 10 transferred cells (compare Fig. 2, H and I).

Using the enrichment method described here, we found that 10 or 80 naïve DO11.10 cells per mouse survived with half-lives of 50 and 104 days, respectively. Both half-lives represent a significant increase over the 12-day half-life of 10^5 cells ($P < 0.01$) (Fig. 3A) and were similar to the survival time seen for polyclonal naïve CD4$^+$ T cells. Likewise, 50 or 200 naïve SM1 cells per mouse survived with half-lives of greater than 100 days, which again were significantly increased compared with the 7-day half-life of 3×10^5 cells ($P < 0.01$) (Fig. 3B). Not only were naïve monoclonal T cells longer lived at low frequency, but they also underwent transient cell division reminiscent of homeostatic proliferation (Fig. 4), which is typically observed in lymphopenic hosts (*1*). Therefore, naïve monoclonal CD4$^+$ T cells proliferated more and survived longer when present at physiologically appropriate numbers than at a much higher frequency.

Clonal abundance was also an important factor in the activation of naïve cells by foreign antigen. Starting from 10^5 per recipient, naïve DO11.10 cells divided fewer than eight times (fig. S2) and increased by a factor of 20 to a peak 3 days after intravenous injection of the relevant foreign antigen, ovalbumin peptide (Fig. 3C, triangles). The cells then declined rapidly to 10^5 per recipient on day 9, converted to the CD45RB^{low} phenotype (*11*), and survived (Fig. 3C, triangles) with a short 11-day half-life that was not significantly different ($P = 0.29$) than that of 10^5 naïve DO11.10 cells (Fig. 3A, triangles). In contrast, an initial cohort of about 100 naïve DO11.10 cells underwent more efficient activation, dividing at least eight times (fig. S2) and increasing by a factor of more than 200 to a later peak on day 8 (Fig. 3C, diamonds). The cells then declined rapidly until day 21 to 1500 cells and survived during the memory phase with a half-life of 46 days (Fig. 3C, diamonds). In a similar experiment starting from 500 naïve DO11.10 cells, 5000 cells entered the memory phase and survived with a half-life of 40 days (Fig. 3C, circles). The 46-day and 40-day half-lives of 1500 and 5000 memory cells, respectively, were each significantly longer than the 11-day half-life of 10^5 memory cells ($P < 0.01$) (Fig. 3C, triangles). Therefore, low naïve CD4$^+$ T cell clonal abundance accentuated antigen-driven proliferation and resulted in the generation of memory cells with increased survival.

Notably, monoclonal memory CD4$^+$ T cells that were generated from a large number of naïve cells and declined in their original recipients with a 10-day half-life survived with a significantly longer half-life of 28 days after transfer into new naïve recipients at a level of 40 cells per mouse ($P < 0.01$) (Fig. 3D). Thus, like naïve cells, memory cells benefited from a low clonal abundance. However, clonal abundance was not the only determinant of survival, because memory cells generated from a large number of naïve cells did not survive as well at low frequency (Fig. 3D, open triangles) as naïve cells at the same frequency (Fig. 3B, squares) ($P = 0.01$). Therefore, the greater potency of stimulation that rare naïve cells experience during the primary response likely promotes the formation of inherently long-lived memory cells. This possibility is supported by the recent finding that low naïve cell frequency is important for the formation of stable effector memory CD8$^+$ T cells (*12*).

Previous studies showed that CD4$^+$ T cell survival in lymphopenic hosts is not controlled by the TCR or MHC II (major histocompatibility complex II) molecules (*5*, *13*), perhaps because of compensation by a cytokine such as IL-7 (*14*). In contrast, our results suggest that monoclonal CD4$^+$ T cells in full hosts compete for a clone-specific survival signal that is most easily explained by TCR recognition of a specific and limiting self peptide-MHC II ligand. This model is supported by the finding that induced ablation of the TCR or MHC II shortens the life span of polyclonal CD4$^+$ T cells (*15*–*17*). In addition, the observations that monoclonal CD4$^+$ T cells do not compete with other clones (*18*–*21*), and that most polyclonal CD4$^+$ T cells do not survive well when transferred into hosts containing a single self peptide-MHC II ligand (*22*), suggest that the relevant TCR ligands are clone specific. Intraclonal competition might also explain the finding that virus antigen–specific memory CD4$^+$ T cells decline after infection is cleared and then stabilize as they become less abundant (*2*). The tendency identified here for

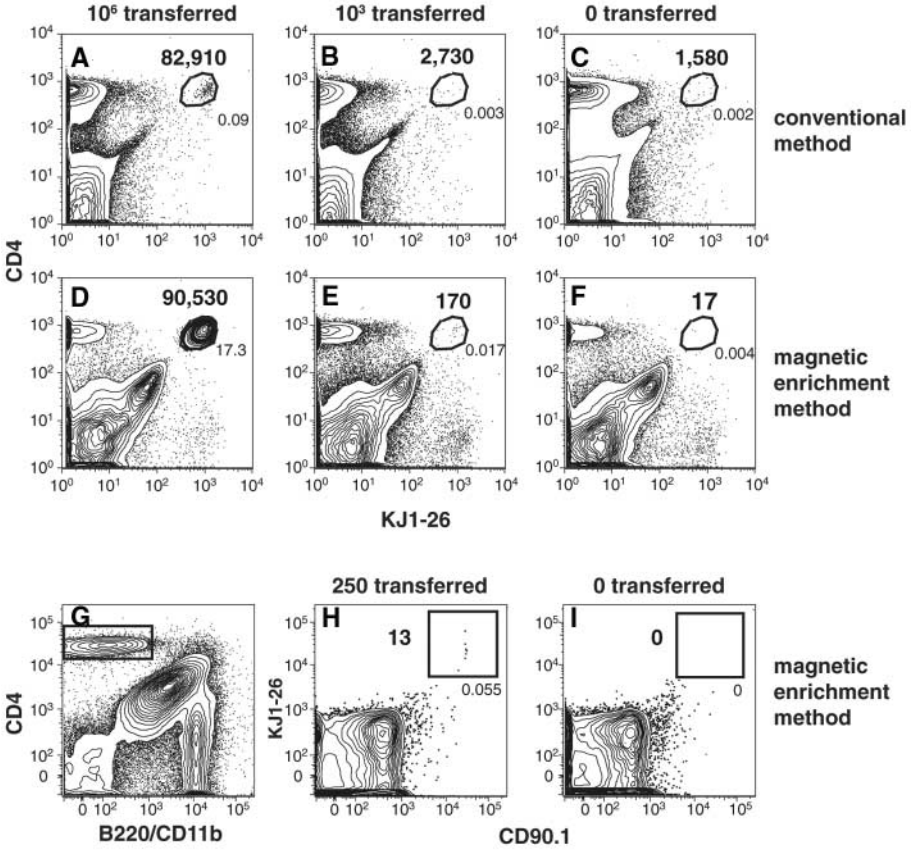

Fig. 2. Rare monoclonal T cells can be detected after transfer by an enrichment method. CD4$^+$, KJ1-26$^+$ cells detected by the conventional flow cytometry method (**A** to **C**) (*24*) or the enrichment method (**D** to **F**) in the spleen and mesenteric lymph nodes of BALB/c mice that received 10^6 [(A) and (D)], 10^3 [(B) and (E)], or no [(C) and (F)] DO11.10 T cells. The percentage and total number of DO11.10 cells detected in each sample are shown on each plot to the right and above the CD4$^+$, KJ1-26$^+$ gate, respectively. To test the sensitivity of the system, 250 CD90.1$^+$ DO11.10 T cells were transferred into CD90.2$^+$ recipients and detected as B220$^-$, CD11b$^-$, CD4$^+$ (**G**), KJ1-26$^+$, CD90.1$^+$ (**H**) cells. Inclusion of the CD90.1 marker and exclusion of B220$^+$ and CD11b$^+$ cells reduced the background to zero in four mice that did not receive DO11.10 cells (**I**).

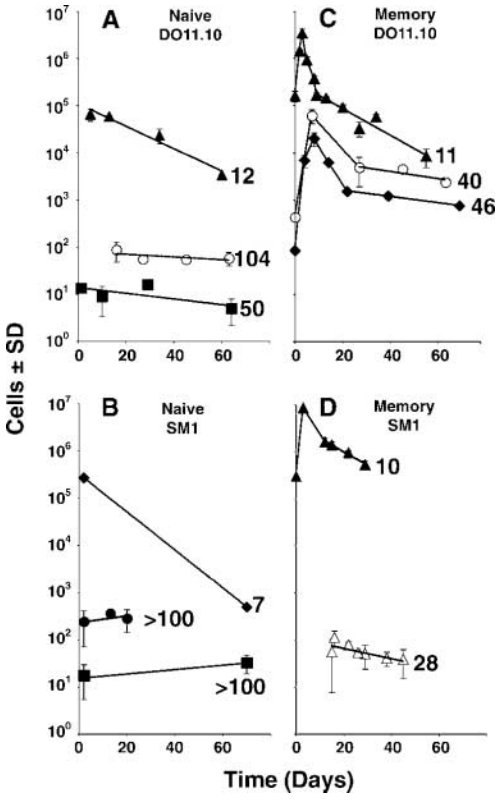

Fig. 3. Monoclonal CD4$^+$ T cells quickly decline at high frequency but persist at low frequency. (**A**) Naïve DO11.10 cells remaining in the spleen and lymph nodes of recipient BALB/c mice after initial parking of 10^5 (triangles), 80 (circles), or 13 (squares) cells. (**B**) Naïve SM1 cells remaining in C57BL/6 mice after seeding of 3×10^5 (diamonds), 200 (circles), or 50 (squares) cells. (**C**) DO11.10 cells in BALB/c mice after initial seeding of 10^5 (triangles), 500 (circles), or 80 (diamonds) cells and intravenous injection of ovalbumin peptide plus lipopolysaccharide (LPS) on day 0. (**D**) SM1 cells in C57BL/6 mice after initial seeding of 3×10^5 cells and intravenous injection of FliC peptide plus LPS (filled triangles), or memory SM1 cells that were generated in C57BL/6 recipients from 3×10^5 naïve cells by intravenous injection of FliC peptide plus LPS and then seeded into new naïve recipients on day 12 at 40 cells per mouse (open triangles, pooled from two experiments). All of the individual points shown are greater than the limit of detection defined as 1 SD above the average number of events detected in at least four untransferred mice assayed on different days over the time course. The individual values at each time point for the naïve groups (A and B) and the values for the memory groups at day 10 [(C) and (D), filled triangles)] or day 20 [(C), open circle and filled diamonds)] were fit to exponential curves. The estimated half-lives (in days) are shown near each curve.

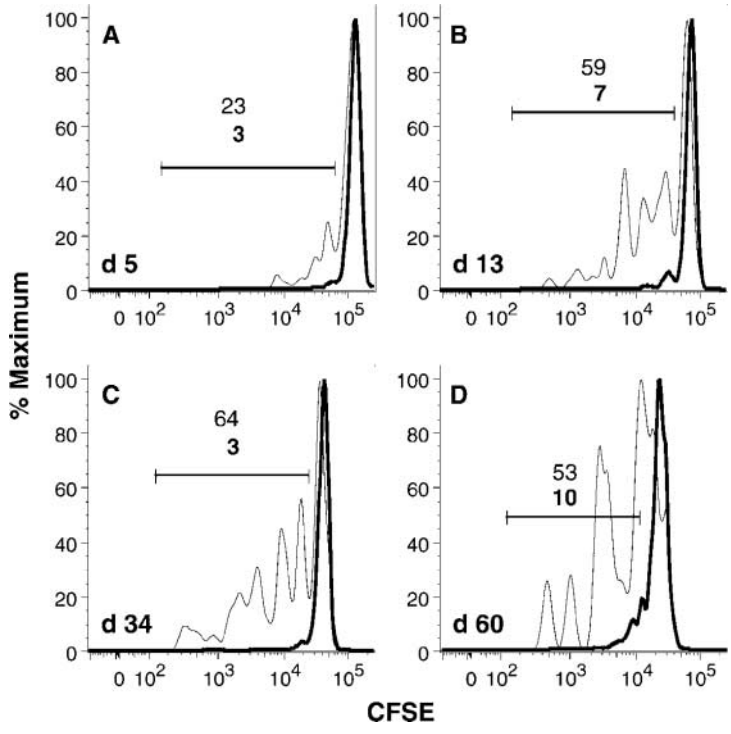

Fig. 4. Naïve CD4$^+$ T cells undergo a short burst of cell division after transfer at low frequency, but not high frequency. Carboxyfluorescein diacetate succinimidyl ester (CFSE) histograms for naïve DO11.10 T cells (identified as B220$^-$, CD11b$^-$, CFSE$^+$, CD4$^+$, KJ1-26$^+$ cells) initially seeded at 10^5 (thick line) or 500 (thin line) cells in the spleen and lymph nodes of BALB/c recipients 5 (**A**), 13 (**B**), 34 (**C**), or 60 (**D**) days after transfer. The percentages of cells with one or more cell divisions in the high (bold text) or low (regular text) transfer groups are indicated.

individual clones to be maintained in small numbers may explain how millions of different naïve clones can coexist stably and how single memory cell populations are prevented from squeezing out others (*23*).

Together these results suggest that the stability of individual CD4$^+$ T cell clones is due to their independent regulation in separate niches. This permits the simultaneous long-term survival of millions of unique clones, thereby maximizing the chance that a few will be specific for a given pathogen. In the event of infection, the low abundance of the relevant clones will also ensure minimal competition for foreign antigen, maximizing activation and the formation of long-lived memory cells.

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Materials and Methods
Figs. S1 and S2
Table S1
References

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