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CUTTING EDGE

Cutting Edge: IL-7-Dependent Homeostatic Proliferation of CD8$^+$ T Cells in Neonatal Mice Allows the Generation of Long-Lived Natural Memory T Cells[1]

Thomas Schüler,[2] Günter J. Hämmerling, and Bernd Arnold

***Healthy, nonimmunized C57BL/6 (B6) mice contain memory phenotype CD8$^+$ T cells, which are assumed to be generated in response to environmental Ags. Since neonatal mice are functionally lymphopenic within the first days after birth, we investigated the alternative possibility that the memory CD8$^+$ T cells of untreated B6 mice are the result of lymphopenia-induced proliferation during neonatal life. We show here that adoptively transferred CD8$^+$ T cells proliferate in neonatal B6 mice, rapidly produce IFN-γ, and develop into memory cells which are maintained until adulthood. In contrast to CD4$^+$ T cells, neonatal lymphopenia-induced proliferation of CD8$^+$ T cells was IL-7 dependent. Thus, neonatal lymphopenia seems to allow CD8$^+$ thymic emigrants to undergo lymphopenia-induced proliferation during early neonatal life to equip the immune system with a set of preactivated CD8$^+$ T cells before any infection, which might contribute to the rapid initiation of immune responses in the adult. The Journal of Immunology,* 2004, 172: 15–19.**

Upon primary encounter with foreign Ags, naive CD8$^+$ T cells start to proliferate and differentiate into CD8$^+$ effector T cells which migrate to peripheral tissues to kill infected target cells and secrete antiviral cytokines such as IFN-γ. After pathogen clearance, most effector CD8$^+$ T cells die and only those that have further differentiated into memory CD8$^+$ T cells survive (1). Memory phenotype CD8$^+$ T cells from healthy, nonimmunized C57BL/6 (B6) mice were used in several experimental systems to study memory CD8$^+$ T cell biology, assuming that they had been generated in response to foreign Ags (2–4). However, naturally activated lymphocytes have been detected in germfree mice, indicating that foreign Ags do not necessarily have to be involved in lymphocyte activation (5). Indeed, complexes of self-peptides associated with MHC molecules (self-peptide-MHC) are sufficient to induce the proliferation of naive T cells that are transferred to mice rendered lymphopenic by irradiation or gene deletion (6–9). During this homeostatic or lymphopenia-induced proliferation (LIP),[3] the naive CD8$^+$ T cells acquire a memory phenotype as shown by the up-regulation of CD44, the ability to secrete cytokines, kill target cells in vitro, and confer increased protection against viral infections and tumors (10–15).

LIP of T cells can be blocked if high numbers of competitor T cells are cotransferred into the same host (16). Therefore, LIP does not play a major role in the generation of the natural memory CD8$^+$ T cells in untreated adult, lymphocyte-competent mice. However, newborn mice provide a lymphopenic environment supporting the proliferation of recent thymic emigrants (17, 18) and adoptively transferred T cells (19, 20). The factors required for CD4$^+$ T cells to undergo LIP in neonates were studied in detail only recently. It was shown that self-MHC and costimulatory molecules were required for their response while IL-7 was dispensable (20). The last point was in apparent contrast to previous reports showing that IL-7 is critical for LIP of naive CD4$^+$ T cells and CD8$^+$ T cells in adult lymphopenic hosts (4, 21–23) and therefore suggested that the factors governing LIP in neonates and adult lymphopenic mice might differ (20).

However, it remained unknown whether and how CD8$^+$ T cells undergoing LIP in neonatal mice survive and maintain their memory phenotype until adulthood. To address these issues, neonatal B6 mice were reconstituted with purified CD8$^+$ T cells from naive adult congenic mice. After transfer, a large proportion of CD8$^+$ T cells proliferated and up-regulated CD44, indicating their conversion into memory CD8$^+$ T cells. IL-7 blockade in B6 neonates prevented LIP, which was not impaired in IL-15$^{-/-}$ neonates. Thus, during the first days of life, neonatal B6 mice provide a lymphopenic environment, supporting IL-7-dependent LIP of naive CD8$^+$ T cells. This allows their differentiation into long-lived, IFN-γ-producing memory cells, thereby creating a pool of natural memory CD8$^+$ T cells before any infection.

Materials and Methods

Mice

C57BL/6J mice (B6; Thy 1.2$^+$) and congenic B6.PL-Thy1a/Cy mice (Thy 1.1$^+$) were purchased from Charles River Breeding Laboratories (Sulzfeld, Germany). RAGE-deficient (RAGE$^{-/-}$) mice (24) constitutively expressing the

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[3] Abbreviations used in this paper: LIP, lymphopenia-induced proliferation; eGFP, enhanced green fluorescent protein; RAG, recombination-activating gene; RAGE, receptor for advanced glycation end product.

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enhanced green fluorescent protein (eGFP), recombination-activating gene (RAG) 2-deficient (RAG2$^{-/-}$), and IL-15$^{-/-}$ mice (25), all on the C57BL/6 background, were bred in our animal facility.

Adoptive T cell transfer

Single-cell suspensions of spleens and lymph nodes were prepared from male mice of the indicated strains. Total CD8$^+$ T cells were purified using CD8α-specific microbeads and autoMACS (both Miltenyi Biotec, Bergisch Gladbach, Germany). The purity ranged from 85 to 95%. For the purification of naive CD8$^+$ T cells expressing low levels of CD44 (CD44^{low}), cell suspensions were incubated with a FITC-labeled mAb specific for CD44 (IM7; BD PharMingen, Hamburg, Germany). Subsequently, anti-FITC microbeads and the CD8$^+$ T cell isolation kit were used to deplete CD44$^{int/high}$ and CD8$^-$ cells by autoMACS (all Miltenyi Biotec). The depletion of CD44$^{int/high}$ cells ranged from 98 to 99.8%. For CFSE, 1×10^7/ml purified T cells were incubated with 5 μM CFSE (Molecular Probes, Eugene, OR) in PBS for 15 min at 37°C. Cells were washed twice with ice-cold PBS and were finally resuspended in PBS before injection. As indicated, 1.5×10^6–4.0×10^6 CD8$^+$ T cells were injected i.p. into neonatal mice or i.v. into adult male mice. At the indicated time points, recipient spleens were removed and single-cell suspensions were stained with mAbs for CD8α (53-6.7), CD44 (IM7), Thy1.1 (OX-7), or CD122 (TM-β1; all BD PharMingen) and analyzed with a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA).

Intracellular detection of IFN-γ

For the polyclonal restimulation of T cells, tissue culture plates were coated with 4 μg/ml anti-CD3ϵ mAb (145-2C11; BD PharMingen) or were left untreated. Spleen cells were cultured in the respective plates for 6 h in RPMI 1640 plus 10% FCS, penicillin/streptomycin, MEM, and 2-ME (50 μM) containing brefeldin A. For intracellular detection of IFN-γ, the intracellular staining kit from BD PharMingen and mAbs for CD8α, Thy1.1, and IFN-γ (XMG1.2; BD PharMingen) were used.

Blockade of IL-7 function in neonates

A mixture of mAbs specific for the IL-7Rα chain (A7R34; 40 μg/g body weight) and IL-7 (M25; 20 μg/g body weight) were injected at the day of birth and subsequently every 2–3 days until the day of analysis. A control group received irrelevant rat and mouse Igs (both from Dianova, Hamburg, Germany) at identical concentrations. CD8$^+$ T cells were transferred within 24 h after the first Ab injection.

Results and Discussion

CD8$^+$ T cells undergo homeostatic proliferation in neonatal B6 mice

Despite any previous immunizations, the CD8$^+$ T cell pool of naive B6 mice contains a population of CD44^{high}CD122^{high} memory cells (Fig, 1*A*). Since neonatal mice are lymphopenic within the first days after birth (26) and LIP of CD8$^+$ T cells allows their differentiation into memory cells (10–12), we asked whether neonatal lymphopenia was sufficient to cause the generation of the naturally occurring memory CD8$^+$ T cell pool of adult B6 mice. To test this, Thy1.1$^+$CD8$^+$ T cells were purified from spleens and lymph nodes of B6.PL-Thy1.1 mice, labeled with CFSE, and injected into neonatal B6 mice (Thy1.2$^+$) within 24 h after birth (day 1) or at days 4, 7, or 14 after birth. Age-matched neonatal lymphopenic RAG2$^{-/-}$ mice were treated identically and served as positive controls for maximum LIP. Thy1.1$^+$CD8$^+$ T cells were also transferred to adult RAG2$^{-/-}$ and B6 mice, the latter to determine the degree of background proliferation. Recipient spleens were harvested and the transferred Thy1.1$^+$CD8$^+$ T cells were analyzed for their CFSE content and expression levels of CD44 (Fig. 2*A*). Nearly all Thy1.1$^+$CD8$^+$ T cells recovered from RAG2$^{-/-}$ mice, irrespective of their age, were CFSE^{low}CD44^{high}, showing that they had undergone proliferation and memory differentiation, as expected. In contrast, in adult B6 mice only very few Thy1.1$^+$CD8$^+$ T cells were CFSE^{low}CD44^{high}. However, when Thy1.1$^+$CD8$^+$ T cells were recovered from neonates injected at day 1 after birth (day 1), 67% of Thy1.1$^+$CD8$^+$ T cells were CFSE^{low}CD44^{high} (Fig. 2*B*). The percentage of CFSE^{low}CD44^{high}Thy1.1$^+$CD8$^+$ T cells was similar after transfer into day 4 neonates (64%) and decreased to 41% in day 7 neonates. After transfer into day 14 neonates, 27% of the Thy1.1$^+$CD8$^+$ T cells were CFSE^{low}. Comparable numbers were found in adult B6 mice (26%). A contribution of exogenous Ags to the CD8$^+$ T cell proliferation observed in day 1 to day 7 B6 neonates and RAG2$^{-/-}$ mice is highly unlikely because the proliferative response was at background levels in day 14 B6 neonates and adult B6 mice despite identical housing conditions and thus a similar antigenic environment. As mentioned above, high numbers of T cells transferred into adult lymphopenic mice can prevent the proliferation of a second cohort of T cells transferred to the same host (16). Adult T cell numbers are reached in the spleen of neonatal mice within 16 days after birth (26), suggesting that the age-dependent decrease of LIP in B6 mice resulted from increasing numbers of host T cells. We conclude that day 1 to day 4 neonates provided a functionally lymphopenic environment that allowed LIP of Thy1.1$^+$CD8$^+$ T cells.

As shown in Fig. 2*A*, all CFSE^{low}Thy1.1$^+$CD8$^+$ T cells derived from B6 neonates were CD44^{high}, indicating their memory phenotype. To test whether 1) this memory phenotype was stable until adulthood and 2) whether the injection of CD8$^+$ T cells at the day of birth altered the composition of the endogenous CD8$^+$ T cell pool, one group of day 1 B6 neonates was injected with CD8$^+$ T cells expressing the eGFP, whereas a second group was left untreated. Eight weeks after transfer, the expression patterns of CD44 and CD122 by eGFP$^+$CD8$^+$ T cells were compared with those of CD8$^+$ T cells from neonatally treated (Fig. 2*D*) and untreated mice (Fig. 2*E*). Similar to the data presented in Fig. 2, *A* and *B*, the majority of the neonatally transferred eGFP$^+$CD8$^+$ T cells showed a CD44^{high} memory phenotype (Fig. 2*C*) and represented around 3% of the adult memory CD8$^+$ T cell pool. Thirty percent of the eGFP$^+$CD8$^+$ T cells had remained naive and were CD44^{low} (Fig. 2*C*). In contrast to that, 65% of the endogenous CD8$^+$ T cells were CD44^{low} (Fig. 2*D*). Since similar percentages of naive and memory CD8$^+$ T cells were found in age-matched control mice that had not received CD8$^+$ T cells (Fig. 2*E*), this suggested that 1) the adoptive transfer of CD8$^+$ T cells at the day of birth did not alter the composition of the recipients' CD8$^+$ T cell pool and 2) LIP-induced memory CD8$^+$ T cells generated in neonates maintained their phenotype until adulthood and contributed to the hosts' natural memory CD8$^+$ T cell pool (Fig. 1*A*).

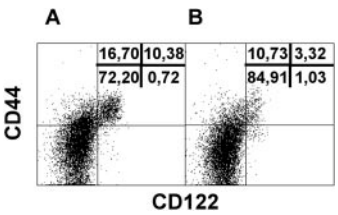

FIGURE 1. Adult untreated B6 mice contain a population of CD44^{high}CD122^{high} memory CD8$^+$ T cells. Spleen cells of untreated B6 mice (*A*, $n = 10$) and IL-15$^{-/-}$ mice (*B*, $n = 10$) were analyzed by flow cytometry. *A* and *B,* Shown is the log fluorescence intensity for CD44 and CD122 after gating on CD8$^+$ T cells. Specificity of staining was verified using isotype-matched control Abs.

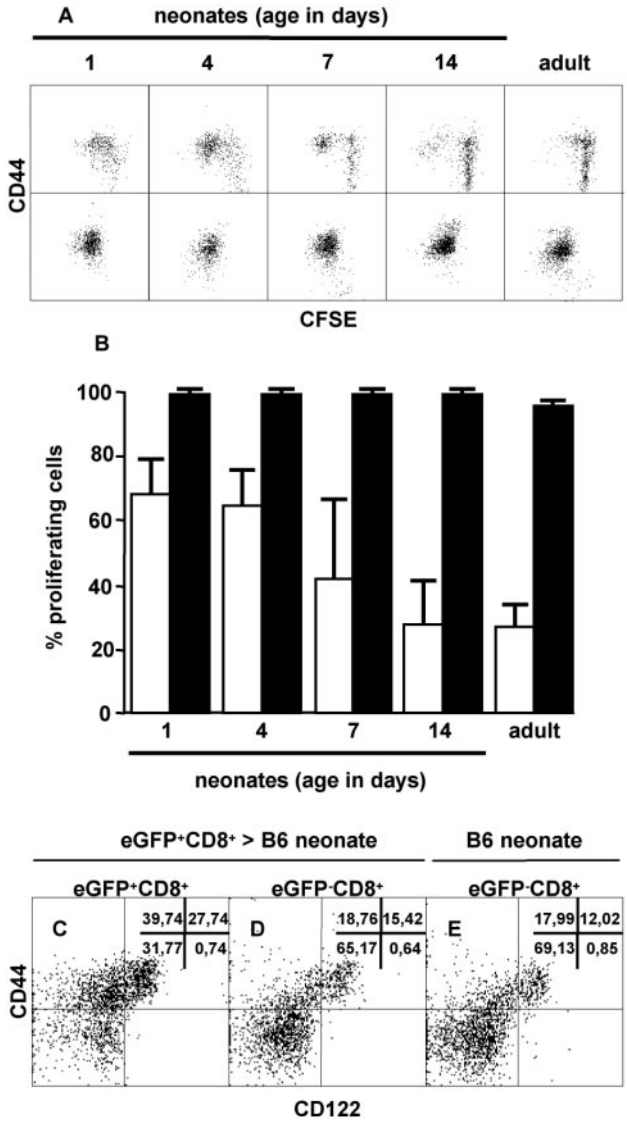

FIGURE 2. $CD8^+$ T cells proliferate homeostatically in neonatal B6 mice and acquire a memory phenotype that is maintained until adulthood. *A* and *B*, Briefly, 1.5×10^6 $CFSE^+Thy1.1^+CD8^+$ T cells were injected i.p. into B6 (*upper row*) or $RAG2^{-/-}$ mice (*lower row*) within 24 h after birth (day 1: B6, $n = 9$; $RAG2^{-/-}$ $n = 8$) or at day 4 (B6, $n = 7$; $RAG2^{-/-}$, $n = 7$), day 7 (B6, $n = 9$; $RAG2^{-/-}$, $n = 6$), and day 14 (B6, $n = 9$; $RAG2^{-/-}$, $n = 7$). Adult B6 ($n = 9$) and $RAG2^{-/-}$ mice ($n = 7$) were injected i.v. Sixteen to 28 days after transfer, spleen cells were analyzed by flow cytometry. *A,* The log fluorescence intensities for CFSE and CD44 are shown for one representative mouse per group after gating on $Thy1.1^+CD8^+$ T cells. *B,* The results are summarized and presented as the percentages of proliferating cells ($CFSE^{low}Thy1.1^+CD8^+$ T cells) ± SDs in B6 (□) and $RAG2^{-/-}$ neonates (■). *C* and *D,* Neonatal B6 mice (day 1, $n = 5$) were injected with 4×10^6 $CD8^+$ T cells from eGFP-transgenic mice or were left untreated (*E*, $n = 5$). Fifty-five days after transfer, spleen cells were analyzed by flow cytometry. Shown are the log fluorescence intensities for CD44 and CD122 after gating on $eGFP^+$ (*C,* transferred) or $eGFP^-$ (*D* and *E*, endogenous) $CD8^+$ T cells. The numbers indicate the percentages of cells in the respective quadrants. Specificity of staining was verified using isotype-matched control Abs.

LIP of naive $CD8^+$ T cells in neonatal mice requires IL-7 but not IL-15

The experiments described in Fig. 2 were done with total $CD8^+$ T cells containing naive $CD44^{low}$ and memory $CD44^{high}CD8^+$ T cells. Since $CD44^{high}CD8^+$ T cells have a higher proliferative capacity than $CD44^{low}CD8^+$ T cells (2), we asked next whether only $CD44^{high}CD8^+$ T cells had expanded in day 1 to day 7 B6 neonates or whether the neonatal environment also promoted the proliferation of naive $CD44^{low}Thy1.1^+CD8^+$ T cells and their differentiation into memory $CD8^+$ T cells. For this purpose, CFSE-labeled $CD44^{low}Thy1.1^+CD8^+$ T cells were transferred to day 1 B6 neonates. Adult B6 and $RAG2^{-/-}$ mice were injected in parallel. As shown in Fig. 3*A*, 29% of $Thy1.1^+CD8^+$ T cells were $CFSE^{low}CD44^{high}$ after transfer into day 1 B6 neonates. In adult B6 mice, ~12% were $CFSE^{low}CD44^{high}$ (Fig. 3*D*) and in $RAG2^{-/-}$ mice 96% (Fig. 3*E*). It is important to note that the numbers of $CFSE^{low}CD44^{high}$ cells obtained after transfer of naive $CD8^+$ T cells (Fig. 3*A*) were clearly lower than after transfer of total $CD8^+$ T cell (Fig. 2). This suggested that $CD44^{high}$ as well as $CD44^{low}$ cells had contributed to the generation of $CFSE^{low}CD44^{high}$ cells (Fig. 2). Nevertheless, the data presented in Fig. 3*A* showed that the lymphopenic environment provided by neonatal B6 mice was sufficient to induce the proliferation of naive $CD44^{low}CD8^+$ T cells and their differentiation into memory $CD44^{high}CD8^+$ T cells.

Next, we wanted to determine the factors that were required for LIP of naive $CD8^+$ T cells in neonates. Since adult IL-$15^{-/-}$ mice largely lack $CD44^{high}CD122^{high}$ natural memory $CD8^+$ T cells (Fig. 1*B* and Ref. 25), we asked whether neonates had to provide IL-15 for the induction of LIP and subsequent memory differentiation of naive $CD8^+$ T cells. $CFSE^+CD44^{low}Thy1.1^+CD8^+$ T cells were injected into day 1 IL-$15^{-/-}$ neonates and their proliferation and differentiation was measured. Twenty-eight percent of the $Thy1.1^+CD8^+$ T cells were found to be $CFSE^{low}CD44^{high}$ (Fig. 3*B*), a percentage that was nearly identical to that observed in B6 neonates (Fig. 3*A*). This demonstrated that host IL-15 was not required for the induction of LIP in neonates and suggested that the reduction of natural memory $CD8^+$ T cells in adult IL-$15^{-/-}$ mice (Fig. 1*B* and Ref. 25) did not result from their impaired generation in the neonatal phase but rather from the lack of IL-15-dependent survival signals (3).

IL-7 was shown to be critical for LIP of T cells in adult lymphopenic mice (4, 21–23). However, a recent study demonstrated that IL-7 was not required for LIP of $CD4^+$ T cells in neonatal B6 mice (20). To test whether IL-7 was required for LIP of naive $CD8^+$ T cells in neonates, $CFSE^+CD44^{low}Thy1.1^+CD8^+$ T cells were transferred to day 1 B6 neonates that were treated with a mixture of mAbs against IL-7 and the IL-7Rα chain. As shown in Fig. 3*C*, 10% of $Thy1.1^+CD8^+$ T cells were $CFSE^{low}CD44^{high}$, a percentage that was similar to the background values detected in adult B6 mice (Fig. 3*C*). LIP-induced memory $CD8^+$ T cell generation in control Ig-treated neonates was similar to that in untreated B6 (Fig. 3*A*) and IL-$15^{-/-}$ neonates (Fig. 3*B* and data not shown), suggesting that the prevention of LIP after injection of anti-IL-7/anti-IL-7Rα chain Abs (Fig. 3*C*) was due to the specific blockade of IL-7 function rather than due to unspecific effects. Thus, the blockade of IL-7 function in neonates prevented LIP of naive $Thy1.1^+CD8^+$ T cells.

The observation that IL-7 in neonates was critical for LIP of $CD8^+$ T cells (Fig. 2*C*) but not for $CD4^+$ T cells (20) demonstrates an important difference between both T cell subsets that has not yet been observed in adult lymphopenic mice (4, 21–23).

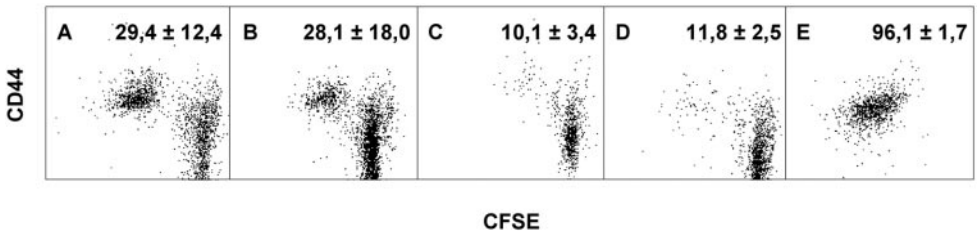

FIGURE 3. Homeostatic proliferation of naive CD8$^+$ T cells in B6 neonates requires IL-7 but not IL-15. 1.5–2.5 × 10^6 CFSE$^+$CD44^{low}Thy1.1$^+$CD8$^+$ T cells were transferred to B6 day 1 neonates (*A*, $n = 19$), IL-15$^{-/-}$ day 1 neonates (*B*, $n = 19$), B6 day 1 neonates treated with anti-IL-7/anti-IL-7R mAbs (*C*, $n = 10$), adult B6 (*D*, $n = 10$), and adult RAG2$^{-/-}$ mice (*E*, $n = 10$). *A–E,* Twelve to 15 days after transfer, spleen cells were analyzed by flow cytometry. Shown are the log fluorescence intensities for CFSE and CD44 after gating on Thy1.1$^+$CD8$^+$ cells. The numbers indicate the mean percentages of CFSE^{low}CD44^{high} cells ± SDs.

LIP-induced memory CD8$^+$ T cells produce IFN-γ

As a result of LIP in adult lymphopenic mice, CD8$^+$ T cells acquire effector functions such as the ability to kill target cells and to produce IFN-γ (10–12, 14, 15). To test whether this was also the case in neonates, CFSE$^+$CD44^{low}Thy1.1$^+$CD8$^+$ T cells were transferred to day 1 B6 neonates, adult B6, and adult RAG2$^{-/-}$ mice and splenocytes were analyzed 2 wk later. As shown in Fig. 4*A*, ~19% of the CFSE^{low}Thy1.1$^+$CD8$^+$ T cells from B6 neonates produced IFN-γ while CFSE^{high}CD8$^+$ T cells did not. In RAG2$^{-/-}$ mice, 40% of the CFSE^{low} Thy1.1$^+$CD8$^+$ T cells produced IFN-γ (Fig. 4*B*) and in adult B6 mice only background levels of ~2% could be detected (Fig. 4*C*). Without stimulation, IFN-γ was not detectable (Fig. 4, *D–F*).

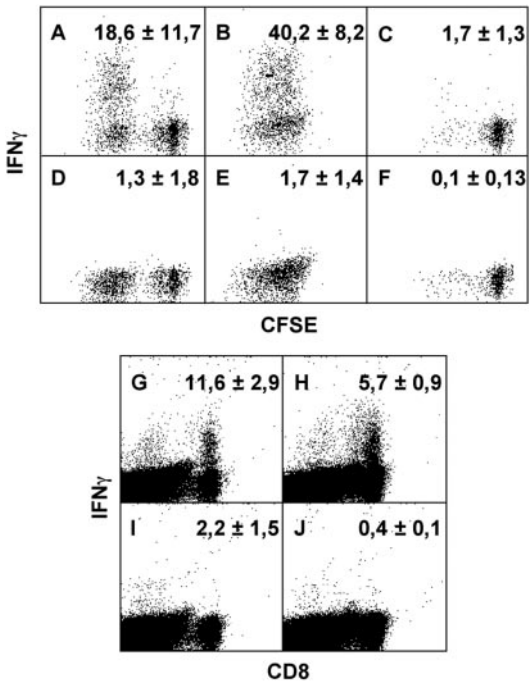

FIGURE 4. CD8$^+$ T cells that have proliferated in B6 neonates produce IFN-γ. Spleen cells recovered from B6 neonates (*A*, *D*, *G*, and *I*), adult RAG2$^{-/-}$ (*B* and *E*), and adult B6 mice (*C*, *F*, *H*, and *J*) that had been reconstituted 12–15 days before with CD44^{low}Thy1.1$^+$CD8$^+$ T cells (Fig. 3) were cultured for 6 h in anti-CD3-coated (*A–C*, *G*, and *H*) or untreated tissue culture plates (*D–F*, *I*, and *J*). IFN-γ was measured by intracellular cytokine staining. *A–F,* Shown are the log fluorescence intensities for CFSE and IFN-γ after gating on Thy1.1$^+$CD8$^+$cells. The numbers indicate the mean percentages of IFN-γ$^+$CFSE^{low} cells ± SDs. *G–J,* The log fluorescence intensities for IFN-γ and CD8α after gating on recipient (Thy1.1$^-$) spleen cells from neonatal (*G* and *I*) and adult B6 recipients (*H* and *J*). The numbers indicate the mean percentages of IFN-γ$^+$CD8$^+$ cells ± SDs. Specificity of staining was verified using isotype-matched control Abs.

The results presented so far demonstrated that neonatal B6 mice provided a lymphopenic environment that allowed the proliferation and differentiation of naive CD8$^+$ T cells into long-lived, IFN-γ-producing memory CD8$^+$ T cells. However, it remained unclear whether the endogenous CD8$^+$ T cells underwent a similar differentiation process. To answer this question, the spleen cell cultures derived from neonatal (Fig. 4, *A* and *D*) and adult B6 mice (Fig. 4, *C* and *F*) were reanalyzed by gating out Thy1.1$^+$ cells to determine the IFN-γ production of recipient CD8$^+$ T cells (Thy1.1$^-$). Approximately 12% of the neonatal (Fig. 4*G*) and 6% of the adult CD8$^+$ T cells (Fig. 4*H*) produced IFN-γ after TCR stimulation but not in its absence (Fig. 4, *I* and *J*). Since IFN-γ could be produced only by CD8$^+$ T cells that had previously divided (Fig. 4, *A–F*), Fig. 4*G* indicated that also endogenous CD8$^+$ T cells acquired effector functions as a result of LIP. However, it remains to be established how many of the IFN-γ$^+$CD8$^+$ T cells derived from the adult were generated as a result of LIP in the neonatal phase or in response to environmental Ags encountered later in life.

It was shown that LIP can induce inflammatory bowel disease in adult lymphopenic mice (27). An important question arising from our results is how autoimmunity is prevented in neonates although CD8$^+$ T cells undergoing LIP acquire effector functions (Fig. 4). The fact that all adoptively transferred CD8$^+$ T cells underwent LIP in T cell-deficient RAG2$^{-/-}$ mice (Fig. 2, *A* and *B*) but not in B6 neonates (Figs. 2, *A* and *B*, and 3) suggests that neonatal recipient T cells contributed to the regulation of LIP. Whether this required particular T cell subsets or resulted from clonal competition remains to be shown.

Thus, under lymphopenic conditions, there appears to be a hierarchy that favors the generation of a memory CD8$^+$ T cell pool before the constitution of the naive T cell repertoire. Due to their low activation thresholds, long life span, and stable phenotype, the natural memory CD8$^+$ T cells generated in early neonatal life may contribute to immune responses against foreign Ags and the regulation of T cell homeostasis in the adult.

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References

1. Kaech, S. M., E. J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev. Immunol. 2:251*.
2. Tough, D. F., and J. Sprent. 1994. Turnover of naive- and memory-phenotype T cells. *J. Exp. Med. 179:1127*.
3. Ku, C. C., M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack. 2000. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. *Science 288:675*.
4. Tan, J. T., B. Ernst, W. C. Kieper, E. LeRoy, J. Sprent, and C. D. Surh. 2002. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. *J. Exp. Med. 195:1523*.
5. Pereira, P., L. Forni, E. L. Larsson, M. Cooper, C. Heusser, and A. Coutinho. 1986. Autonomous activation of B and T cells in antigen-free mice. *Eur. J. Immunol. 16:685*.
6. Viret, C., F. S. Wong, and C. A. Janeway, Jr. 1999. Designing and maintaining the mature TCR repertoire: the continuum of self-peptide:self-MHC complex recognition. *Immunity 10:559*.
7. Bender, J., T. Mitchell, J. Kappler, and P. Marrack. 1999. CD4+ T cell division in irradiated mice requires peptides distinct from those responsible for thymic selection. *J. Exp. Med. 190:367*.
8. Ernst, B., D. S. Lee, J. M. Chang, J. Sprent, and C. D. Surh. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity 11:173*.
9. Goldrath, A. W., and M. J. Bevan. 1999. Low-affinity ligands for the TCR drive proliferation of mature $CD8^+$ T cells in lymphopenic hosts. *Immunity 11:183*.
10. Cho, B. K., V. P. Rao, Q. Ge, H. N. Eisen, and J. Chen. 2000. Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. *J. Exp. Med. 192:549*.
11. Goldrath, A. W., L. Y. Bogatzki, and M. J. Bevan. 2000. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J. Exp. Med. 192:557*.
12. Murali-Krishna, K., and R. Ahmed. 2000. Cutting edge: naive T cells masquerading as memory cells. *J. Immunol. 165:1733*.
13. Bachmann, M. F., T. M. Kundig, H. Hengartner, and R. M. Zinkernagel. 1997. Protection against immunopathological consequences of a viral infection by activated but not resting cytotoxic T cells: T cell memory without "memory T cells"? *Proc. Natl. Acad. Sci. USA 94:640*.
14. Oehen, S., and K. Brduscha-Riem. 1999. Naive cytotoxic T lymphocytes spontaneously acquire effector function in lymphocytopenic recipients: a pitfall for T cell memory studies? *Eur. J. Immunol. 29:608*.
15. Dummer, W., A. G. Niethammer, R. Baccala, B. R. Lawson, N. Wagner, R. A. Reisfeld, and A. N. Theofilopoulos. 2002. T cell homeostatic proliferation elicits effective antitumor autoimmunity. *J. Clin. Invest. 110:185*.
16. Dummer, W., B. Ernst, E. LeRoy, D. Lee, and C. Surh. 2001. Autologous regulation of naive T cell homeostasis within the T cell compartment. *J. Immunol. 166:2460*.
17. Piguet, P. F., C. Irle, E. Kollatte, and P. Vassalli. 1981. Post-thymic T lymphocyte maturation during ontogenesis. *J. Exp. Med. 154:581*.
18. Le Campion, A., C. Bourgeois, F. Lambolez, B. Martin, S. Leaument, N. Dautigny, C. Tanchot, C. Penit, and B. Lucas. 2002. Naive T cells proliferate strongly in neonatal mice in response to self-peptide/self-MHC complexes. *Proc. Natl. Acad. Sci. USA 99:4538*.
19. Ichii, H., A. Sakamoto, M. Hatano, S. Okada, H. Toyama, S. Taki, M. Arima, Y. Kuroda, and T. Tokuhisa. 2002. Role for Bcl-6 in the generation and maintenance of memory $CD8^+$ T cells. *Nat. Immunol. 3:558*.
20. Min, B., R. McHugh, G. D. Sempowski, C. Mackall, G. Foucras, and W. E. Paul. 2003. Neonates support lymphopenia-induced proliferation. *Immunity 18:131*.
21. Schluns, K. S., W. C. Kieper, S. C. Jameson, and L. Lefrancois. 2000. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. *Nat. Immunol. 1:426*.
22. Tan, J. T., E. Dudl, E. LeRoy, R. Murray, J. Sprent, K. I. Weinberg, and C. D. Surh. 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl. Acad. Sci. USA 98:8732*.
23. Goldrath, A. W., P. V. Sivakumar, M. Glaccum, M. K. Kennedy, M. J. Bevan, C. Benoist, D. Mathis, and E. A. Butz. 2002. Cytokine requirements for acute and basal homeostatic proliferation of naive and memory $CD8^+$ T cells. *J. Exp. Med. 195:1515*.
24. Constien, R., A. Forde, B. Liliensiek, H. J. Grone, P. Nawroth, G. Hämmerling, and B. Arnold. 2001. Characterization of a novel EGFP reporter mouse to monitor Cre recombination as demonstrated by a Tie2 Cre mouse line. *Genesis 30:36*.
25. Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med. 191:771*.
26. Garcia, A. M., S. A. Fadel, S. Cao, and M. Sarzotti. 2000. T cell immunity in neonates. *Immunol. Res. 22:177*.
27. Barthlott, T., G. Kassiotis, and B. Stockinger. 2003. T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med. 197:451*.