The Lymphopenic Environment of CD132 (Common γ-Chain)-Deficient Hosts Elicits Rapid Homeostatic Proliferation of Naive T Cells via IL-15[1]

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Homeostatic proliferation for naive T cells is observed readily only under lymphopenic conditions in response to elevated levels of IL-7 and contact with self-MHC/peptide ligands. Homeostatic proliferation occurs at a slow pace and gradually induces the dividing cells to acquire characteristics of memory cells. We describe a novel type of homeostatic proliferation whereby naive T cells proliferate at a significantly faster rate, resembling the proliferation speed induced by foreign Ags, and the expanding cells rapidly differentiate into central memory cells. Remarkably, such rapid homeostatic proliferation is driven by a combination of IL-2 and IL-15, with IL-15 playing a bigger role, and applies for a wide repertoire of $CD8^+$ naive T cells, including many TCR-transgenic lines, even those that fail to undergo IL-7-driven homeostatic proliferation. Thus, naive T cells can be induced to undergo homeostatic proliferation of variable speed with a few members of the common γ-chain (CD132) family of cytokines, the speed of proliferation depending on the levels of the particular cytokine involved. ***The Journal of Immunology,*** **2008, 180: 5320–5326.**

As with most types of cells in the body, homeostatic mechanisms govern the overall size and composition of the mature T cell pool (1, 2). Homeostatic signals for T cells reflect contact with self-MHC/peptide ligands and two members of the common γ-chain (γ_c)[3] receptor family of cytokines, IL-7 and IL-15 (2, 3). Naive $CD4^+$ and $CD8^+$ T cells require joint contact with self-MHC/peptide ligands and IL-7 for survival whereas most memory $CD4^+$ and $CD8^+$ T cells are MHC independent and are maintained by IL-7 and IL-15, both for survival and intermittent cell division. In addition to supporting cell survival, homeostatic signals can drive mature T cells to undergo acute homeostatic proliferation in response to T cell lymphopenia (2, 3). Thus, naive T cells, which normally reside in interphase, undergo spontaneous homeostatic proliferation upon adoptive transfer into syngeneic lymphopenic hosts in response to increased availability of IL-7 and self-MHC/peptide ligands. Memory T cells also have the capacity to undergo acute homeostatic proliferation in lymphopenic hosts in response to elevated IL-7 and IL-15 levels (3, 4).

IL-7-dependent homeostatic proliferation of naive T cells in lymphopenic hosts is driven characteristically slow, each division requiring 24–36 h, and leads to only gradual and partial restoration of the overall size of the T cell pool. The expanding naive T cells slowly acquire the phenotypic characteristics of memory cells but do not transition through an effector cell stage. In contrast to this typical lymphopenia-induced slow homeostatic proliferation, we now describe a novel form of rapid homeostatic expansion of naive T cells in the lymphopenic environment of CD132 $(\gamma_c)^-$ hosts. Donor T cell proliferation in these hosts is intense but, in contrast to responses to foreign Ags, the expanding cells display the characteristics of central memory cells rather than effector cells.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from the breeding colony at The Scripps Research Institute (TSRI). B6.PL*Thy1a*/Cy, IL-2^-, RAG^-, β_2m^-, $CD25^-$, $CD122^-$, and $CD132^-$ mice, all in a B6 background, were purchased from The Jackson Laboratory. B6.HY^- TCR-transgenic were purchased from Taconic Farms. IL-7^- (5), IL-15^- (6), OT-I (7) Smarta (8) mice were obtained from DNAX, Immunex, and Drs. F. Carbone (University of Melbourne Parkville, Victoria, Australia) and H. Hengartner (University Hospital Zurich, Zurich, Switzerland), respectively.

Adoptive transfer and immunization

Unseparated lymph node (LN) cells or T cells purified from LN, by killing non-T cells using a mixture of anti-CD24 and anti-MHC class II (MHC-II) mAbs plus complement, were labeled with CFSE (Invitrogen Life Technologies) and injected i.v. into host mice as described previously (9). Irradiated hosts were exposed to 600 cGy of whole body irradiation 1 day before donor cell transfer. Mice were immunized by i.v. injecting 1×10^4 PFU of a recombinant strain of *Listeria monocytogenes* expressing OVA (LM-OVA) (10).

Abs and flow cytometry

The CFSE profiles and recoveries of donor T cells in host spleen were analyzed by flow cytometry after staining for Thy-1.1, CD8, and/or CD4. Cell surface phenotype of donor T cells was analyzed after staining for Thy-1.1, CD8, and one of the phenotypic markers. Cell suspensions were

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Received for publication October 3, 2007. Accepted for publication February 18, 2008.

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[1] This work was supported by U.S. Public Health Service Grants AI045809, AI064586, and AG020186 (to C.D.S.) and AI046710, AI007244, and AG001743 (to J.S.). C.R. was supported by a National Institutes of Health Training Grant T32 AI07244.

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[3] Abbreviations used in this paper: γ_c, common γ-chain; LN, lymph node; LM, *Listeria monocytogenes*; TG, transgenic; MHC-I, MHC class I; MHC-II, MHC class II; Treg, regulatory T cell.

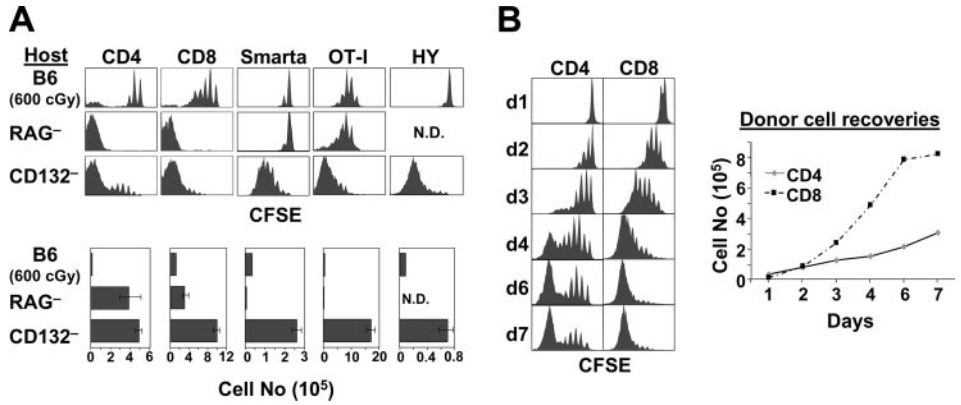

FIGURE 1. Naive T cells undergo strong expansion in CD132$^-$ hosts. *A*, Rapid proliferation applies to both polyclonal and TCR-TG T cells. Purified naive T cells from LN of B6.PL (Thy-1.1$^+$) and the indicated TG mice on a Thy-1.1$^+$ RAG$^-$ background were CFSE labeled and transferred at low doses (1×10^6/mouse except for 3×10^5 for HY) by i.v. injection into irradiated (600 cGy) B6, and unirradiated RAG$^-$ and CD132$^-$ hosts. CFSE profiles of donor cells in host spleen were analyzed 7 days later by flow cytometry after staining for Thy-1.1, CD8, and CD4. The histograms display the CFSE profiles on gated donor T cells, and the bar graphs show the total donor T cell recovery from each types of host analyzed individually. These results are representative of at least two to four independent experiments. *B*, Strong proliferation applies to a diverse repertoire of T cells. Purified LN T cells from B6.PL mice were CFSE-labeled and transferred at 1×10^6/mouse into a group of CD132$^-$ hosts. CFSE profiles on donor T cells in host spleen were analyzed on sequential days for 7 days as described in *A*. The graph shows the total recoveries of donor T cells.

prepared and stained for donor cells according to standard protocols as described previously (9). Staining for IL-15Rα and reagents was previously described (11). The following mAbs conjugated with various fluorochromes were used: anti-CD8α (53-6.7) was purchased from BD Biosciences; anti-CD4 (L3T4), anti-CD25 (PC61.5), anti-CD62L (MEL-14), anti-CD122 (TM-β1), anti-CD127 (A7R34), anti-CD44 (IM7.4), anti-Thy-1.1 (HIS51), and anti-Thy-1.2 were purchased from eBioscience. Flow cytometry data were analyzed by using FlowJo software (Tree Star).

RT-PCR

Total RNA from minced spleens from B6 and CD132$^-$ mice was extracted using an RNeasy mini kit (Qiagen) according to the manufacturer's recommendations. From each sample, 100 ηg of total RNA was used in a RT-PCR using the TaqMan One-Step RT-PCR kit (Applied Biosystems). Specific murine primer-probe sequences for IL-2, IL-7, and IL-15 were generated as previously described (12). Each RNA sample was assayed in triplicate in appropriate 94-well plates. Comparative cycle threshold analysis (user bulletin 2; Applied Biosystems) was performed to each data set that was retrieved from the ABI Prism 7700 instrument (Applied Biosystems).

Intracellular IFN-γ staining

Spleen cells plated at 1×10^6 cells/well in 96-well plates with or without 0.1 μM OVA peptide (323–339) were treated with GolgiStop (BD Biosciences) during the 5 h of culture. The activated cells were then stained with cell surface markers, then fixed/permeabilized using Cytofix/Cytoperm (BD Biosciences) buffer, and stained with PE-conjugated anti-IFN-γ mAb (XMG1.2; eBioscience) using Perm/Wash (BD Biosciences) buffer, and analyzed by flow cytometry as described (9).

ELISA

Sera from 8- to 12-wk-old B6 and CD132$^-$ mice were collected and then tested in IL-2, IL-7, and IL-15 ELISA kits purchased from eBioscience and R&D Systems according to the manufacturer's recommendations.

Results

Proliferation of naive T cells transferred into CD132$^-$ hosts

In the course of analyzing the ability of various strains of lymphopenic mutant mice to support homeostatic proliferation of adoptively transferred syngeneic naive T cells, it became apparent that the proliferation induced in CD132$^-$ mice was unique. As shown previously (13), CFSE-labeled donor B6.PL LN T cells proliferated slowly in control irradiated B6 hosts as analyzed 7 days later (Fig. 1*A*); as discussed earlier, slow proliferation in these lymphopenic hosts is driven by elevated levels of IL-7 plus TCR contact with low-affinity self-MHC/peptide ligands (13, 14). In control RAG$^-$ hosts, which are also lymphopenic, CFSE-labeled donor B6.PL T cells proliferated much more rapidly during the same period, but the bulk of this proliferation is due to the response of a small fraction of cells, mainly CD4$^+$ cells, to non-self Ags which appear to be derived from commensal bacteria (15). Notably, the component of fast donor T cell proliferation is not observed when TCR-transgenic (TG) CD4$^+$ (Smarta) or CD8$^+$ (OT-1) cells are used (Fig. 1*A*) or if the immunodeficient hosts are raised under germfree conditions (15).

The results were quite different with CD132$^-$ hosts. Young CD132$^-$ mice are severely lymphopenic because their lymphoid cells lack expression of γ_c, a shared component of the receptors for IL-2, -4, -7, -9, -15, and -21 (16). As in RAG$^-$ mice, proliferation of donor B6.PL LN T cells in young CD132$^-$ hosts was very rapid; however, the donor cell recoveries were greater in CD132$^-$ hosts, especially for CD8 cells, where recoveries were 3- to 4-fold higher in CD132$^-$ hosts than in RAG$^-$ hosts (Fig. 1*A*). Daily analysis of the kinetics of donor B6 LN T cell expansion in CD132$^-$ hosts revealed that strong proliferation applied to nearly all donor T cells, although a minor subset of donor CD4$^+$ cells appeared to expand at a faster rate than the majority of CD4$^+$ cells (Fig. 1*B*). These findings clearly contrasted with RAG$^-$ hosts where only a small fraction of polyclonal CD4$^+$ and CD8$^+$ cells underwent rapid proliferation (15). The fast proliferation of naive T cells in CD132$^-$ hosts applied to 4- to 8 wk-old mice, which contained $<10 \times 10^6$ T cells in their spleens, most of which were activated CD4$^+$ cells, as previously described (17). Similar findings applied to older (3–4 mo) CD132$^-$ mice, which have a much larger pool of activated CD4$^+$ cells (data not shown). Most of the experiments discussed below were performed using young ($\geq$2 mo age) CD132$^-$ mice.

As mentioned above and confirming previous findings (15), proliferation of TCR-TG Smarta and OT-I cells was as slow in RAG$^-$ hosts as in irradiated B6 hosts, presumably reflecting that these T cells do not display any cross-reactivity to the non-self Ags present in RAG$^-$ hosts (Fig. 1*A*). In marked contrast, naive Smarta and OT-I cells proliferated very rapidly in CD132$^-$ hosts during the same period and cell yields after 7 days were $\sim$10-fold higher than in RAG$^-$ and irradiated B6 hosts (Fig. 1*A*). Similar findings applied to 2C CD8$^+$ TCR-TG cells (data not shown). Like most

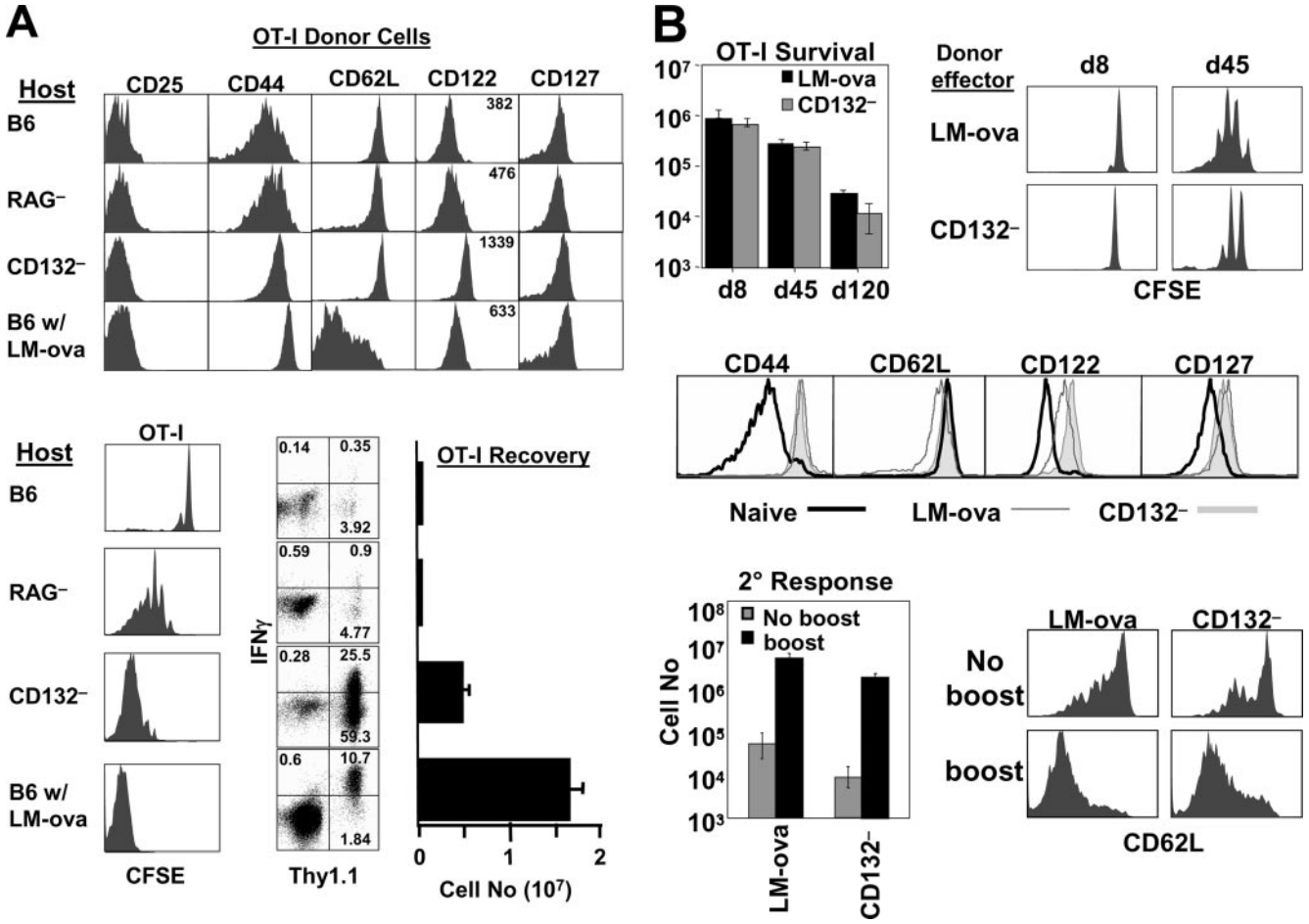

FIGURE 2. Naive OT-I cells expanding in CD132$^-$ hosts display characteristics of central memory T cells. *A*, Phenotype and functional characteristics of expanded OT-I cells. Purified Thy-1.1$^+$RAG$^-$ OT-I cells (1×10^6/mouse) were transferred into CD132$^-$ hosts and the expanded OT-I cells were analyzed 7 days later (*top*). As controls, OT-I cells were analyzed after transfer to normal B6 hosts, RAG$^-$ hosts, and B6 hosts were injected with LM-OVA. Donor OT-I cells in host spleen were analyzed by flow cytometry after staining for Thy-1.1, CD8, and either CD25, CD43, CD44, CD62L, CD122, or CD127. The histograms (*top*) show the relative expression levels of the indicated markers on gated donor OT-I cells. The numbers in the histogram boxes represent the mean fluorescent intensity (MFI) of expression. Using the same approach, a separate experiment was performed, but with CFSE-labeled OT-I cells (*bottom*), and the ability of donor OT-I cells to proliferate and to synthesize IFN-γ was analyzed; intracellular IFN-γ production was assessed by stimulating host spleen cells in vitro with OVA peptide for 5 h and staining for Thy-1.1, CD8, and IFN-γ as described in *Materials and Methods*. *Bottom left histograms*, The CFSE profiles of donor OT-I cells in the indicated hosts after 7 days. The dot plots display expression of intracellular IFN-γ by donor OT-I cells (*middle*). Total recoveries of donor OT-I cells from the host spleen are shown in bar graph (*right*). These experiments were repeated at least two to three times. *B*, OT-I cells expanded in CD132$^-$ hosts maintain characteristics of central memory cells upon adoptive transfer into normal B6 hosts. Naive Thy-1.1$^+$RAG$^-$ OT-I cells were transferred CD132$^-$ hosts and the donor cells harvested 9 days later, CFSE-labeled, and then adoptively transferred into a group of normal B6 hosts. As controls, a group of B6 mice was also injected with CFSE-labeled OT-I effector cells generated in response to LM-OVA as describe in *A*. At 8, 45, and 120 days later, the CFSE profiles and the phenotype of donor OT-I cells were analyzed as described above. Shown are donor cell recoveries on all days (*top left*), CFSE profiles on days 8 and 45 (*top right*) and the surface marker phenotypes on days 45 (*middle*). Some of the hosts were challenged with LM-OVA on days 115 and the recoveries and phenotype of donor OT-I cells analyzed 5 days later as described above. The bar graphs (*bottom left*) show the recoveries of OT-I cells with or without LM-OVA infection (boost) from hosts that were initially injected with LM-OVA-induced effector OT-I cells or CD132$^-$ host-expanded OT-I cells. The histograms (*bottom right*) show the expression of CD62L on OT-I cells with or without LM-OVA infection (boost) from hosts that were initially injected with LM-OVA-induced effector OT-I cells or CD132$^-$ host-expanded OT-I cells.

polyclonal B6 T cells, OT-I and 2C CD8$^+$ TG cells are thought to have relatively high TCR affinity for self-MHC/peptide ligands (18). The self-MHC affinity of Smarta CD4$^+$ cells is presumed to be "below average" because proliferation of these cells in RAG$^-$ and irradiated B6 hosts is very limited (8). Thus, in the experiment shown (Fig. 1*A*), division of the transferred cells was minimal and involved only a single division of a small proportion of the cells. This finding contrasted markedly with the intense division of Smarta cells in CD132$^-$ hosts. Similar rapid division in CD132$^-$ hosts also applied to HY TCR-TG CD8$^+$ cells (Fig. 1*A*). This latter finding is striking because, confirming previous findings (18), proliferation of HY TCR-TG cells in control irradiated B6 mice was almost undetectable (Fig. 1*A*). The failure of HY CD8$^+$ cells to undergo typical homeostatic expansion is thought to reflect that these cells have only very low self-MHC reactivity (18). It should be noted that the above results with the four lines of TG T cells applied whether the cells were in RAG$^+$ or on RAG$^-$ background, indicating that expression of endogenous TCR was not relevant. In subsequent experiments, we focused on OT-I CD8$^+$ cells.

Characteristics of OT-I cells expanded in CD132$^-$ hosts

To characterize the expanding naive T cells, Thy-1.1$^+$RAG$^-$OT-I cells were transferred into CD132$^-$ hosts and the phenotype of the donor OT-I cells analyzed 7 days later; as controls, we used OT-I

cells parked in normal B6 hosts, OT-I cells undergoing homeostatic proliferation in RAG$^-$ hosts, and effector OT-I cells generated in B6 hosts 7 days after immunization with LM-OVA. As in normal B6 and RAG$^-$ hosts, the OT-I cells that expanded in CD132$^-$ hosts uniformly expressed the phenotype of central memory CD8$^+$ T cells: CD44^{high}, CD62L^{high}, CD43^{low}, CD122^{high}, and CD127^{high} (Fig. 2*A* and data not shown). When these expanded OT-I cells were stimulated in vitro with OVA, only ~30% of the cells produced IFN-γ (Fig. 2*A*). By contrast, OT-I cells stimulated with LM-OVA displayed the typical CD62L^{low} phenotype of early effector memory cells and nearly all of these cells were IFN-γ producers (Fig. 2*A*). Hence, the naive CD8$^+$ cells that expanded in CD132$^-$ host resembled central memory cells rather than recently activated effector cells. It should be noted that the magnitude of OT-I cell expansion in CD132$^-$ hosts, while ~10-fold higher than in RAG$^-$ hosts, was nevertheless considerably less than the expansion induced by LM-OVA. Hence, the rate of OT-I cell division in CD132$^-$ hosts, albeit much faster than IL-7-driven homeostatic proliferation in irradiated B6 and RAG$^-$ hosts, was not as fast as the cells stimulated with cognate foreign Ags.

We next determined whether the memory-like OT-I cells generated in CD132$^-$ hosts could differentiate into functional memory cells upon adoptive transfer into normal B6 hosts. To address this question, naive Thy-1.1$^+$RAG$^-$OT-I cells were first expanded in CD132$^-$ hosts for 9 days, retrieved from the host spleen, CFSE labeled, and then adoptively transferred into a group of normal B6 hosts. As a control, OT-I effector cells expanded by LM-OVA in vivo for 9 days were similarly transferred into a separate group of B6 mice. Analysis of the donor OT-I cells that expanded in CD132$^-$ hosts generally engrafted and persisted at a similar efficiency as effector OT-I cells generated to LM-OVA, but only at early time points, 8–45 days. Thereafter, their survival declined and led to a 3-fold decrease in cell recoveries by 120 days, relative to LM-OVA-stimulated cells (Fig. 2*B*, *top left*). This reduction in survival correlated with a slight decrease in the background rate of proliferation of the transferred cells (Fig. 2*B*, *top right*). CFSE dilution was almost undetectable at day 8, indicating that the cells immediately entered interphase after transfer to normal hosts. By day 45, most of the cells had divided one or more times, presumably in response to endogenous IL-15, but proliferation was less for the cells expanded in CD132$^-$ mice. For the latter cells, their slower rate of basal homeostatic turnover was not due to a defect in expression of CD122 or CD127, which were expressed at normal levels (Fig. 2*B*, *bottom*). One possible reason for their poor long-term survival is that the OT-I cells proliferating in CD132$^-$ hosts failed to receive CD4$^+$"help" for the generation of functionally intact memory-like CD8$^+$ cells on subsequent transfer (19). Nonetheless, immunization of the secondary B6 hosts with LM-OVA revealed that the progeny of OT-I memory-like cells expanded in CD132$^-$ primary hosts were as responsive as bona fide memory OT-I cells primed with LM-OVA. Thus, both populations of parked cells proliferated vigorously after restimulation and down-regulated CD62L like typical effector cells (Fig. 2*B*, *bottom*). Collectively, these data showed that the naive CD8$^+$ T cells that expanded in CD132$^-$ hosts acquired the typical phenotypic and functional characteristics of central memory cells, though long-term survival of these cells was somewhat reduced relative to bona fide Ag-primed memory cells.

Role of MHC/peptide ligands in expansion of naive CD8$^+$ cells in CD132$^-$ hosts

Although expansion of naive TCR-TG CD8$^+$ cells in CD132$^-$ hosts applied to HY as well as OT-I and 2C cells, total yields of the proliferating cells were much lower for HY cells (Fig. 1*A*). This finding suggested that proliferation was controlled by TCR recognition of self-MHC/peptide ligands, i.e., as for slow homeostatic proliferation in irradiated B6 mice (9, 20). To assess this possibility, CFSE-labeled naive Thy-1.1$^+$ RAG$^-$ OT-I cells were transferred into CD132$^-$ mice crossed to a β_2m$^-$ background. Because OT-I cells express MHC class I (MHC-I), a very low dose of OT-I cells (1×10^5/mouse) was injected to minimize the donor cells from providing self-MHC/peptide ligands to each other. Whereas homeostatic proliferation of OT-I cells was very limited in control irradiated β_2m$^-$ hosts, strong OT-I cell proliferation occurred in CD132$^-\beta_2$m$^-$ hosts, though cell yields were 2-fold lower than in CD132$^-\beta_2$m$^+$ hosts (Fig. 3). These results suggest that OT-I cell proliferation in CD132$^-$ hosts is partly dependent on TCR contact with self-MHC/peptide ligands. The residual proliferation could be directed to the low level of MHC-I expressed by β_2m$^-$ cells (21). Experiments with CD132$^-$MHC-I$^-$ hosts will be needed to assess this idea.

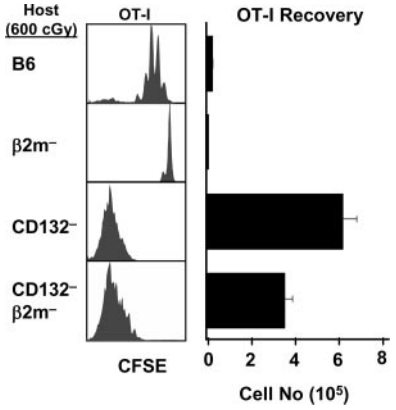

FIGURE 3. Role of self-MHC I on naive OT-I cell expansion in CD132$^-$ hosts. A very small number (1×10^5) of purified CFSE-labeled Thy-1.1$^+$ RAG$^-$ OT-I cells was transferred into irradiated B6, β_2m$^-$, CD132$^-$ and CD132$^-$ β_2m$^-$ hosts. On days 5, CFSE profiles on donor OT-I cells in host spleen were analyzed by flow cytometry after staining for Thy-1.1 and CD8. The histograms show the CFSE profiles and the bar graph shows the donor OT-I recoveries. The result is a representative of three independent experiments.

CD132$^-$ mice possess elevated basal levels of IL-2 and IL-15

The most likely factors that drive strong donor T cell proliferation in CD132$^-$ hosts are cytokines, especially IL-2 from residual activated CD4$^+$ T cells as well as IL-7 and IL-15; these cytokines are synthesized but cannot be consumed by host cells. Comparison of mRNA levels of these three cytokines in the spleens from CD132$^-$ and B6 mice by RT-PCR revealed that while IL-2 and IL-7 levels were comparable, IL-15 mRNA levels were ~30% higher in CD132$^-$ mice (Fig. 4*A*). The levels of cytokine proteins in serum were measured using commercially available kits, but the sensitivity of these kits was not sufficient to assay IL-7 or IL-15 (data not shown). Significantly, serum IL-2 levels were ~5-fold higher in CD132$^-$ mice than in normal B6 mice (Fig. 4*B*). For IL-15, this cytokine is known to be expressed largely in cell-associated form bound to IL-15Rα (22, 23). In line with the RT-PCR data, IL-15Rα levels on DC from CD132$^-$ spleen were noticeably higher than that on splenic DC from B6 mice (Fig. 4*C*). However, direct measurement of IL-15 protein on the cells was not possible.

Naive CD8$^+$ cell expansion in CD132$^-$ hosts is driven primarily by IL-15 and partly by IL-2

To seek direct evidence of the role of cytokines in proliferation, we bred CD132$^-$ mice to be deficient in expression of IL-7, IL-2, or

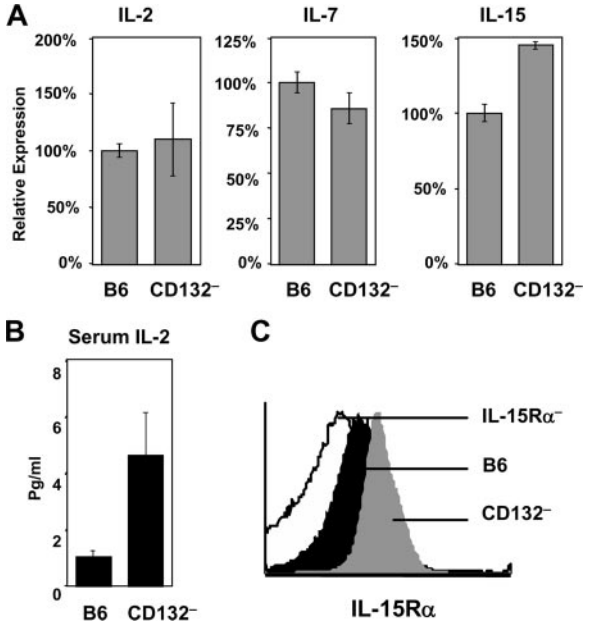

FIGURE 4. CD132$^-$ mice express elevated levels of IL-2 and IL-15. *A*, Expression levels of cytokine mRNA. Total RNA was extracted from spleens of B6 and CD132$^-$ mice and subjected to real-time RT-PCR for IL-7, IL-2, and IL-15 as described in *Materials and Methods*. Five mice were used per group and each sample was analyzed in triplicates for each cytokine and presented as expression relative to B6. *B*, Serum levels of IL-2 protein. Sera from five B6 and CD132$^-$ mice were analyzed for IL-2 protein using ELISA as described in *Materials and Methods*. *C*, Expression of IL-15Rα as an indicator of IL-15 protein expression. Purified splenic DC from B6, CD132$^-$, and IL-15Rα^- mice were analyzed for IL-15Rα by flow cytometry after staining for IL-15Rα and CD11c. The results are representative of three independent experiments.

IL-15, and used these mice as hosts for CFSE-labeled Thy-1.1$^+$OT-I cells. Expansion of OT-I cells transferred into IL-7$^-$CD132$^-$ hosts was just as strong as in CD132$^-$ hosts, indicating little or no role for IL-7 (Fig. 5*A*, *left*). In IL-2$^-$CD132$^-$ hosts, proliferation of OT-I cells was somewhat slower than in CD132$^-$ hosts and correlated with a consistent ~30% lower recovery of OT-I cells (Fig. 5*A*, *middle*). The results with IL-15$^-$CD132$^-$ hosts were more dramatic. Here, yields of OT-I cells were 4- to 5-fold lower than in control CD132$^-$ mice (Fig. 5*A*, *right*). These findings suggest that rapid proliferation of donor OT-I cells in CD132$^-$ hosts does not involve IL-7 and is driven primarily by IL-15, and to a lesser extent by IL-2. These cytokines are also likely to influence the overall magnitude of the donor cell expansion by affecting their viability.

To complement these studies, we tried to generate CD132$^-$ mice deficient in both IL-2 and IL-15, but this approach was unsuccessful. Instead, we bred OT-I mice deficient in expression of CD122, thus generating cells that were unable to respond to either IL-2 or IL-15. The striking finding was that, unlike control OT-I cells, most CFSE-labeled CD122$^-$ OT-I cells transferred into CD132$^-$ mice underwent slow homeostatic proliferation, very similar to the pattern observed in irradiated B6 hosts (Fig. 5*B*, *left*). This finding clearly indicates that the strong proliferation of OT-I cells in CD132$^-$ mice is driven primarily by a combination of IL-2 and IL-15. When binding to these two cytokines is abrogated, the OT-I cells revert to undergoing slow lymphopenia-driven homeostatic proliferation driven by IL-7.

We also bred OT-I mice deficient in the expression of CD25, which selectively impairs responsiveness to IL-2 but not IL-15. In line with the above data with IL-2$^-$ CD132$^-$ hosts, CD25$^-$ OT-I cells proliferated well in CD132$^-$ hosts, total cell yields in these mice being only 30% less than with transfer of WT OT-I cells (Fig. 5*B*, *right*). These findings confirm that IL-2 does contribute to proliferation in CD132$^-$ hosts but to only a minor extent relative to IL-15.

Strong expansion of donor naive CD8$^+$ cells in RAG-1$^-$CD132$^-$ hosts

Despite being severely lymphopenic CD132$^-$ mice possess a small population of CD4$^+$ cells with activated phenotype that

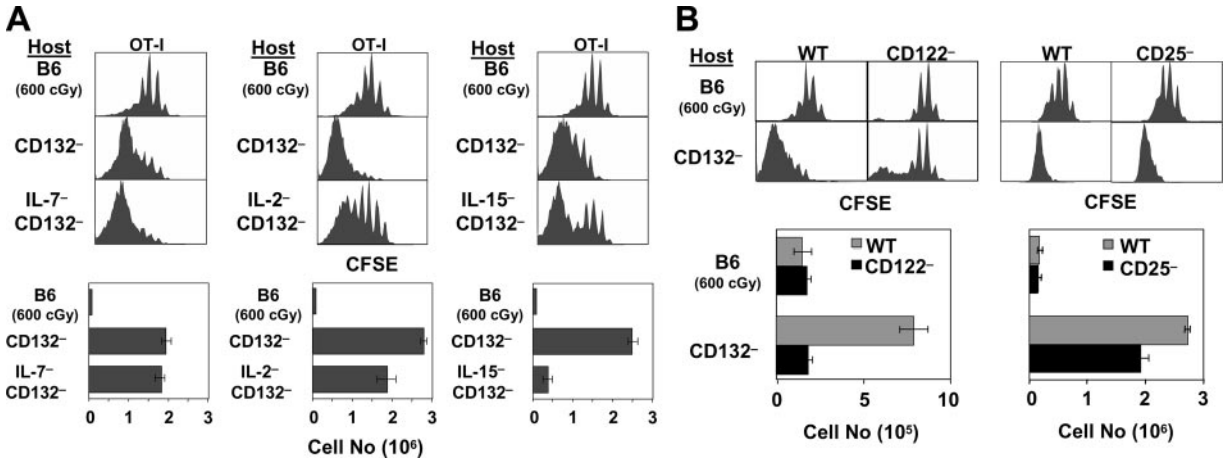

FIGURE 5. IL-2 and IL-15 synergistically induce strong proliferation of OT-I cells in CD132$^-$ mice. *A*, Deficiency in IL-2 or IL-15, but not IL-7, diminishes OT-I cell expansion in CD132$^-$ hosts. Purified CFSE-labeled Thy-1.1$^+$RAG$^-$ OT-I cells (1×10^6/mouse) were transferred into irradiated B6, CD132$^-$, and either IL-2$^-$ CD132$^-$, IL-7$^-$CD132$^-$, or IL-15$^-$CD132$^-$ hosts. The CFSE profiles of the donor cells in host spleen were then analyzed on day 7 by flow cytometry after staining for Thy1.1 and CD8. The histograms show the CFSE profile of donor cells gated on donor OT-I cells and the bar graphs show the recoveries of OT-I cells. The ages of host mice at the time of experiment were 6–8 wk for B6 and IL-7$^-$CD132$^-$ mice and 7–8 wk for IL-2$^-$CD132$^-$ and IL-15$^-$CD132$^-$ mice. The data are representative of two to four experiments. *B*, Inability to bind to either IL-2 or IL-15 abrogates strong OT-I cell proliferation in CD132$^-$ hosts. Purified Thy-1.1$^+$ OT-I cells were mixed at a 1:1 ratio with either Thy-1.1/1.2$^+$CD122$^-$ OT-I cells (0.3×10^6/mouse) or Thy-1.1/1.2$^+$CD25$^-$ OT-I cells (1×10^6/mouse), CFSE-labeled, and injected into irradiated B6 and CD132$^-$ hosts. The ages of B6 and CD132$^-$ mice at the time of experiment were 6–8 wk. The CFSE profiles of donor cells in host spleen were analyzed by flow cytometry after staining for Thy-1.1, Thy-1.2, and CD8. The histograms display the CFSE profiles of donor OT-I cells gated on either Thy-1.1$^+$ OT-I wild type or Thy-1.1/1.2$^+$CD122$^-$ OT-I or CD25$^-$ OT-I cells. The bar graph shows the total donor T cells recovery. The results are representative of three experiments.

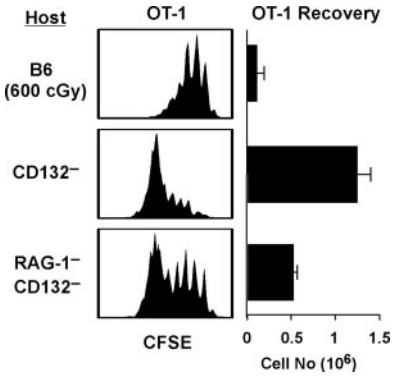

FIGURE 6. Naive OT-I cells undergo strong expansion in RAG$^-$CD132$^-$ hosts. A small number (0.75×10^6) of purified CFSE-labeled Thy-1.1$^+$RAG$^-$ OT-I cells were transferred into irradiated B6, CD132$^-$, and RAG-1$^-$CD132$^-$ hosts. On day 7, CFSE profiles on donor OT-I cells in host spleen were analyzed by flow cytometry after staining for Thy-1.1 and CD8. The histograms show the CFSE profiles and the bar graph shows the donor OT-I recoveries. The data were obtained from two to three mice per group analyzed individually.

expands with age (17). Because CD132$^-$ mice are devoid of regulatory T cells (Tregs) (24) these residual CD4$^+$ cells may be in a state of chronic activation and produce large amounts of cytokines, especially IL-2, and may also induce activation of APC to produce IL-15. To determine the role of host CD4$^+$ T cells in eliciting the high cytokine levels in CD132$^-$ mice, we generated CD132$^-$ mice in a RAG-1$^-$ background to compare the proliferation of donor OT-I cells in these hosts with control CD132$^-$ hosts. Expansion of donor CFSE-labeled naive OT-I cells was moderately lower in RAG-1$^-$CD132$^-$ mice compared with that observed in control CD132$^-$ hosts, but it was still much greater than that observed in control irradiated B6 host (Fig. 6). This finding suggests that host T cells do play a partial role in establishing the high cytokine levels in CD132$^-$ mice.

Discussion

Slow homeostatic proliferation of naive T cells in lymphopenic hosts is well-documented and occurs whenever total T cell numbers are reduced to low levels (2, 25). Under these conditions of "extra space" in the lymphoid tissues, elevated concentrations of IL-7 augment TCR signaling and initiate the typical pattern of slow proliferation and gradual expansion of T cell numbers that has become the hallmark of homeostatic proliferation. Because this pattern of proliferation is seen after T cell transfer to a number of T cell-deficient hosts, e.g., RAG$^-$, SCID, and nude mice, similar findings would be expected in CD132$^-$ hosts. As shown here, however, this is not the case. Thus, naive T cells, especially CD8$^+$ cells, proliferated very rapidly in CD132$^-$ hosts despite their paucity of total T cell numbers. The data were especially dramatic for TCR-TG cells where total recoveries of the proliferating donor cells after 1 wk were far higher than in other immunodeficient hosts. Hence, the data provide a notable exception to the rule that homeostatic proliferation of T cells in lymphopenic hosts is characteristically slow.

With regard to the stimuli for proliferation for naive CD8$^+$ T cells, the data with cytokine-deficient CD132$^-$ hosts and CD122$^-$ donor CD8$^+$ T cells indicated that proliferation was driven largely by IL-15 with a lesser contribution from IL-2. Evidence of elevated levels of IL-15 in sera from CD132$^-$ mice, however, could not be obtained either by an ELISA or by the ability of sera to support survival of CD122^{high} CD8$^+$ cells under in vitro conditions (data not shown). Hence, the manifestation of the increased levels of IL-15 appears to be limited mostly to the IL-15/IL-15Rα complexes on the surface of APC and stromal cells, the naturally presented form of IL-15 (22, 23). Accordingly, while injection of a large amount of IL-15 alone is unlikely to induce strong proliferation of donor naive CD8$^+$ cells in lymphopenic B6 hosts, it is possible that such a feat could be accomplished by injecting IL-15/IL-15Rα complexes, which display a much stronger activity than free IL-15 (11, 26). This idea is currently under investigation. In contrast to IL-15, serum levels of IL-2 were ~4-fold higher in CD132$^-$ than B6 mice, reflecting increased synthesis of IL-2 by residual activated CD4$^+$ cells, presumably responding to self and/or environmental Ags in the absence of Tregs, these cells being γ_c dependent (24). Though significant, the stimulatory effect of IL-2 in CD132$^-$ mice was quite minor, relative to IL-15, and contrasts with the intense IL-2- driven proliferation of donor T cells documented recently in CD122$^-$ mice (27). These latter mice display prominent lymphoid hyperplasia and their IL-2 levels are ~50-fold higher than in CD132$^-$ mice.

Although it is possible that the absence of Tregs is a major contributing factor allowing the strong proliferation of donor naive T cells in CD132$^-$ hosts, we believe this to be unlikely for two reasons. First, in contrast to CD132$^-$ hosts, excessively strong proliferation of donor naive T cells, especially TCR-TG T cells, was not observed in other strains of T cell-deficient mice, such as in RAG$^-$ hosts, which are also deficient in Tregs. Second, strong proliferation of naive T cells was observed even when whole LN cells, including Tregs, were adoptively transferred into CD132$^-$ hosts. Nonetheless, it is possible that the absence of Tregs could indirectly contribute by inducing overproduction of IL-2 and or IL-15. One distinct possibility is that lack of consumption of IL-2 by Tregs could have further raised IL-2 levels in CD132$^-$ hosts.

The most likely reason that IL-2 and IL-15 levels are elevated in CD132$^-$ mice is from lack of consumption. IL-2 appears to be mostly produced by hosts CD4$^+$ cells, which are present in very low numbers in young mice but become increasingly prominent with age (17). Moreover, because these T cells also cannot express IL-2R, IL-2 production is likely to be sustained for longer than usual by lack of feedback inhibition by IL-2 (28). Hence, precluding development of CD4$^+$ cells in RAG-1$^-$CD132$^-$ mice caused a notable reduction in the ability of CD132$^-$ mice to support expansion of donor OT-I cells. In addition, the absence of host T cells caused a more profound reduction in expansion of donor OT-I cells than in IL-2$^-$CD132$^-$ mice, suggesting that host T cells also augment production of IL-15 by APC. Such a scenario is also possible for CD132$^-$ mice bred to be deficient in IL-2, IL-7, or IL-15. The exact role of host T cells in enhancing production of IL-2 and IL-15 is currently under investigation.

In contrast to IL-2, there appears to be more than just the lack of consumption for why IL-15 levels are elevated in CD132$^-$ mice. This is because even when RAG$^-$ mice were irradiated or treated with mAbs to deplete NK cells, to remove all cells that can consume IL-15, the proliferation of OT-I cells was no stronger than in untreated RAG$^-$ hosts (data not shown). In addition, the relative basal levels of IL-15, which could be raised significantly by the microbial products that induce type I IFN, did not change significantly, as measured by expansion of OT-I cells, whether CD132$^-$ and IL-2$^-$CD132$^-$ mice were raised in conventional conditions or in much cleaner conditions after treatment with a mixture of antibiotics (data not shown). Hence, an additional mechanism appears to be involved in inducing high IL-15 levels in CD132$^-$ mice. The identity of this is currently obscure and significant portion of it appears to be independent of T cells, as IL-15

appears to be prominent even in RAG$^-$CD132$^-$ hosts. One possibility we favor is that a feedback mechanism exists whereby lymphocytes, upon binding of IL-15/IL-15Rα on APC, cause down-regulation of IL-15 production by APC. This scenario may apply to other situations where IL-15 recognition by lymphocytes is abrogated, such as in CD122$^-$ mice, which indeed, do possess elevated IL-15 levels (27).

In terms of physiological relevance of the present finding, it is notable that an immune response against many microbes is associated with huge amounts of type 1 IFN production by APC, which in turn induces synthesis of IL-15 by APC and stromal cells and IL-2 by T cells. Hence, it is possible that supranormal levels of IL-15 and IL-2 can be transiently achieved during an immune response, leading to some proliferation of nearby naive T cells in an Ag-independent manner. This mechanism may contribute to homeostatic proliferation of very low fraction of naive phenotype cells T cells that is apparent in normal mice, and to the transition of these cells to memory phenotype cells. Moreover, such a scenario repeated multiple times over one's life could gradually lead to an accumulation of memory phenotype cells evident with advanced age. Although evidence for such a mechanism is yet to be obtained, one study has found that weak proliferation of bystander T cells can be induced upon transfer into syngeneic mice harboring a bacterial infection (29).

Finally, in the light of the fact that CD132$^-$ or CD132$^-$RAG$^-$ mice are becoming increasingly used as recipients for transplantation of various cells types, one should be aware of the potential complications that may arise from the elevated IL-2 and/or IL-15 in these mice. As recipients of human hematopoietic stem cells (30), there could be minimal complications, as human cells generally do not recognize mouse cytokines, even though the reverse is often the case. However, as recipients for mouse lymphocytes, the elevated cytokines will definitely affect the survival and function of donor cells. Hence, conclusions from previous studies on T cell memory and homeostasis that have used CD132$^-$RAG$^-$ mice as hosts (31, 32) may need to be re-evaluated in the context of current findings.

Acknowledgments

We thank the Flow Cytometry laboratory for their support. This is manuscript 18952 from The Scripps Research Institute.

Disclosures

The authors have no financial conflict of interest.

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