Interleukin-2 enhances CD4+ T cell memory by promoting the generation of IL-7Rα-expressing cells

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The common γ chain cytokines interleukin (IL)-2 and IL-7 are important regulators of T cell homeostasis. Although IL-2 is implicated in the acute phase of the T cell response, IL-7 is important for memory T cell survival. We asked whether regulated responsiveness to these growth factors is determined by temporal expression of the cytokine-specific IL-2 receptor (R) α and IL-7Rα chains. We demonstrate that IL-2Rα is expressed early after priming in T cell receptor–transgenic CD4+ T cells, whereas IL-7Rα expression is lost. In the later stage of the response, IL-7Rα is reexpressed while IL-2Rα expression is silenced. This reciprocal pattern of IL-2Rα/IL-7Rα expression is disturbed when CD4+ T cells are primed in the absence of IL-2 signals. Primed IL-2−/− or CD25−/− (IL-2Rα−/−) CD4+ T cells, despite showing normal induction of activation markers and cell division, fail to reexpress IL-7Rα late in the response. Because the generation of CD4+ memory T cells is dependent on IL-7–IL-7Rα interactions, primed IL-2−/− or CD25−/− CD4+ T cells develop poorly into long-lived memory cells. Retrovirus-mediated expression of IL-7Rα in IL-2−/− T cells restores their capacity for long-term survival. These results identify IL-2 as a factor regulating IL-7Rα expression and, consequently, memory T cell homeostasis in vivo.

The generation of a population of memory cells providing long-term protective immunity is an important outcome of a primary T cell response and the goal of vaccination. Substantial progress has been made in identifying the factors that contribute to the development and survival of CD8+ memory T cells, whereas less is known about CD4+ memory T cells. It has been postulated that brief stimulation of naive CD8+ T cells with antigen is sufficient to initiate the program leading to effector and memory cell development (1, 2). Long-term persistence of the generated memory CD8+ T cells is independent of continued TCR stimulation (3, 4) but depends on the presence of CD4+ helper T cells (5–9). Besides this newly emerging role of CD4+ T cells in CD8+ T cell memory maintenance, the cytokines IL-7 and IL-15, members of the common γ chain (γc) family, play an important role in the homeostasis of CD8+ memory T cells (10–12). Just as for CD8+ T cells, survival of CD4+ memory T cells does not require continued contact with MHC molecules once the antigen has been cleared (13). However, the functional activity of the surviving memory cells seems to be positively influenced by contact with MHC (14). Also, antigen dose and the maturation state of the APC during priming seem to influence the subsequent capacity of T cells to acquire long-term survival potential (15).

Studies using in vitro priming followed by adoptive transfer into immunocompetent recipient mice have shown that proper effector T cell differentiation and exposure to IL-2 are required for the successful long-term survival of the primed CD4+ T cells in vivo (16, 17). However, there are very few studies addressing the problem of CD4+ memory T cell generation using in vivo priming systems. Moreover, most of these studies use lymphopenic animals, wherein the true requirements for memory cell survival may not surface because of the absence of competition with endogenous lymphocytes for survival factors (17). Thus, it has been postulated that γc family cytokines are not required for the generation and maintenance of CD4+ memory T cells in γc-deficient, lymphopenic mice (18). Recent evidence to the contrary suggests that IL-7 is an important survival factor for resting CD4+ memory T cells residing in an intact lymphoid compartment (19–21), whereas IL-2 contributes to the generation of memory cells during priming (17).
The phenotypes of mice deficient in IL-2, IL-7, or their receptors suggest an important role for these cytokines in the maintenance of T cell homeostasis (22). However, because both cytokines have a broad range of activities at different stages of T cell development, as well as on a variety of T cell subsets, it is difficult to define their specific roles in the generation of memory cells in intact, lymphocyte-sufficient animals. Because IL-2 and IL-7 share the γc for signaling, specificity of responsiveness and function will likely depend on temporally regulated expression of their unique, high affinity α chain receptor component. The factors regulating expression of CD25 are fairly well described: TCR engagement or IL-2 itself induces CD25 during T cell priming (23). However, the expression pattern and factors regulating IL-7Rα expression on mature CD4+ T cells are not well defined. Recent data from in vitro experiments suggest that IL-7 and IL-2 are negative regulators of IL-7Rα expression (24, 25). Given the importance of IL-7 signaling for the maintenance of naive and memory T cell populations, the mechanisms that control responsiveness to this cytokine in vivo are clearly crucial for understanding T cell homeostasis and memory.

In this study, we have followed IL-7Rα and IL-2Rα expression on CD4+ T cells during the priming and memory phases of an immune response. Using both in vitro and in vivo systems, we found that IL-2Rα and IL-7Rα show a reciprocal expression pattern after priming. Surprisingly, early up-regulation of IL-2Rα, allowing IL-2 signaling, promoted the generation of activated T cells reexpressing IL-7Rα. IL-7Rα reexpression correlated with the development of a long-lived memory cell population, consistent with the function of IL-7 to promote long-term survival of CD4+ memory T cells (19–21). Thus, IL-2 and IL-7 serve unique functions in the generation of CD4+ memory T cells.

RESULTS

Reciprocal regulation of IL-2Rα and IL-7Rα expression during a T cell response

The ability of CD4+ T cells to respond effectively to the cytokines IL-2 and IL-7 depends on the expression of their high affinity IL-2Rα and IL-7Rα receptor chains on the cell surface. To follow expression of IL-2Rα and IL-7Rα during a CD4+ T cell response, naive OVA-specific T cells from DO.11 TCR transgenic mice were primed with 1 μg/ml OVA peptide and APCs in vitro and analyzed for the presence of the receptors by antibody staining and flow cytometry (Fig. 1 A). As expected, naive T cells were negative for IL-2Rα and expressed IL-7Rα. Antigen stimulation of DO.11 T cells led to a strong induction of IL-2Rα by day 2, whereas IL-7Rα was down-regulated in response to TCR triggering. By day 5, however, IL-7Rα expression was restored on activated T cells, whereas IL-2Rα was diminishing (Fig. 1 A).

Because conditions of antigen presentation and cytokine exposure during in vitro priming of T cells do not necessarily mimic the in vivo situation, we developed a system to follow IL-2Rα and IL-7Rα on DO.11 T cells primed in vivo. 5 × 10^6 naive, carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled DO.11 T cells were adoptively transferred into BALB/c mice and primed in vivo 24 h later with 3 × 10^6 OVA-pulsed DCs. IL-2Rα and IL-7Rα expression on the CD4+ T cell population. (D) Spleens of the primed mice from B were harvested on the indicated days, and IL-2Rα expression on CD4+ T cells was determined as in A. Data are representative of three separate experiments.

Figure 1. Reciprocal regulation of IL-2Rα and IL-7Rα after T cell activation. [A] 2.5 × 10^6 CD4+ T cells from DO.11 mice were primed in vitro with 1 μg/ml OVA peptide and 2.5 × 10^6 APCs. Naive T cells and cells harvested on the indicated days, and IL-2Rα expression levels were analyzed by flow cytometry. Data are representative of two independent experiments. (B) 5 × 10^6 naive DO.11 T cells were adoptively transferred into BALB/c mice and primed in vivo 24 h later with 3 × 10^6 OVA-pulsed DCs. Histograms show expression of CD62L and CD44 on naive and primed (day 3) KJ1-26+CD4+ T cells in the spleen. (C) CFSE-labeled DO.11 T cells were primed as in B (left) or with control DCs (no OVA; right). On day 3 after priming, spleen cell suspensions were stained with KJ1-26 and anti-CD4. Dot plots show CFSE dilution versus KJ1-26 staining gated on the CD4+ T cell population. Numbers indicate percentages of KJ1-26+ and KJ1-26+ T cells within the total CD4+ T cell population. (D) Spleens of the primed mice from B were harvested on the indicated days, and IL-2Rα and IL-7Rα expression on KJ1-26+CD4+ T cells was determined as in A. Data are representative of three separate experiments.
of IL-2Rα and IL-7Rα. To ensure that the DO.11 cells had been properly stimulated, we measured expression of the activation markers CD44 and CD62L and CFSE dilution (Fig. 1, B and C). 3 d after priming with OVA-pulsed DCs, all DO.11 cells showed increased CD44 and reduced CD62L levels and had lost the CFSE dye, whereas no CFSE dilution was observed when control DCs, which did not present OVA, were injected (Fig. 1, B and C). These data demonstrated that the DO.11 cells had been activated and cycled strictly in response to antigen. 1 d after priming with DCs, a substantial fraction of the DO.11 T cells expressed IL-2Rα on the cell surface (Fig. 1 D). In contrast, expression of IL-7Rα was lost early after priming. By day 3, IL-2Rα expression had decreased to the level of naive cells, whereas IL-7Rα was reexpressed. Furthermore, IL-7Rα was further up-regulated by day 10. Thus, priming of DO.11 T cells in vitro and in vivo results in reciprocal expression of IL-2Rα and IL-7Rα, suggesting that regulation of receptor expression determines the temporal responsiveness of T cells to the respective cytokines.

IL-2–IL-2Rα promotes the generation of activated T cells expressing IL-7Rα

We have previously shown that IL-2 signals are required for the generation of memory cells (17). Because expression of IL-7Rα appears to be a cardinal property of memory T cells, we postulated that one key function of IL-2 may be to promote sustained expression of the IL-7Rα chain. To address this possibility, IL-2−/− DO.11 T cells were primed in vitro in the absence or presence of recombinant IL-2. Under IL-2–free priming conditions, the T cells show normal cell division and up-regulation of activation markers but fail to survive as well as WT T cells (17). Fig. 2 A demonstrates that IL-2−/− T cells lost IL-7Rα expression early after activation, independent of the presence of IL-2 and similar to WT T cells. However, IL-2−/− T cells failed to reexpress IL-7Rα at the later stages of activation unless IL-2 was added back to the cultures. Thus, IL-2 is required during priming to induce the reexpression of IL-7Rα on late-activated T cells. Analysis of IL-7Rα mRNA by quantitative PCR confirmed the flow cytometry data (unpublished data).

Next, we asked if IL-2 was also required for IL-7Rα expression during in vivo priming of T cells. Initially, we compared IL-7Rα expression on WT and IL-2−/− DO.11 T cells primed in vivo with OVA-pulsed DCs and could not detect any differences between these cell populations. However, it is possible that IL-2 produced by host T cells could compensate for the lack of autocrine IL-2 production by the transferred IL-2−/− DO.11 T cells. Therefore, we analyzed IL-7Rα expression on DO.11 T cells deficient in CD25, the high affinity IL-2Rα. Because CD25−/− T cells are unable to respond to IL-2 present in the environment, they could be primed under exactly the same conditions as WT cells yet would be deprived of IL-2 signals. We found that CD25−/− T cells failed to restore IL-7Rα expression to the level of naive or activated WT cells on day 4 after in vivo priming (Fig. 2 B). Thus, one of the functions of the IL-2–IL-2Rα system during the T cell response is to provide signals for the reexpression of IL-7Rα on late-activated T cells.

IL-7–IL-7Rα is required for memory cell survival

IL-7 is an important survival factor for naive T cells (26). More recently, evidence has been presented that IL-7 is also important for the long-term survival of CD4+ memory T cells (20, 21). Therefore, reexpression of the IL-7Rα at the later stages of T cell activation could be necessary for the long-term survival of activated T cells once the antigen has been cleared. To formally test whether activated DO.11 T cells are dependent on IL-7 for their survival, we primed DO.11 T cells in vitro for 4 d and adaptively transferred 30 × 10^6 activated T cells into unmanipulated BALB/c mice. Recipient mice were given either a blocking anti–IL-7Rα antibody or an isotype control antibody every other day for 10 d. The number of DO.11 cells surviving in lymph nodes and spleen was determined after 12 d. Treatment with
anti–IL-7Rα reduced the number of DO.11 cells present in lymphoid tissues (Fig. 3 A). The recovered cells all showed a resting memory phenotype (Fig. 3 B). Total CD4+ T cell numbers were also reduced, confirming that IL-7 is required for naive T cell survival (unpublished data).

**IL-2 during priming is required for both central and effector memory T cells**

Our previous experiments demonstrating a role of IL-2 in the generation of memory CD4+ T cells have focused on memory cells in lymphoid organs (presumably corresponding to central memory cells) (17). To ask if the same requirement would be seen for memory cells in nonlymphoid peripheral tissues, we primed IL-2−/− DO.11 T cells in vitro for 4 d in the absence or presence of IL-2, adoptively transferred equal numbers of cells to BALB/c mice, and evaluated T cell survival in different organs 4 wk later. In agreement with previous findings (17), DO.11 cells primed in the absence of IL-2 lacked the capacity to survive long-term in the secondary lymphoid organs (Fig. 4 A). We also found that IL-2−/− DO.11 cells failed to survive in nonlymphoid tissues such as the lung, whereas a population of DO.11 was recovered from this organ if the T cells had been primed in the presence of IL-2 (Fig. 4 A). This result shows that the absence of IL-2−/− memory cells in lymphoid organs is not caused by skewed homing of T cells to nonlymphoid organs or preferential development of effector memory cells, which are known to reside in nonlymphoid tissues.

It has been debated what the precise role of IL-2 is during CD4+ T cell responses and whether this cytokine is absolutely required as a T cell growth factor in vivo (27). To evaluate the role of IL-2 during an in vivo T cell response, naive, CFSE-labeled CD25−/− DO.11 T cells were adoptively transferred into BALB/c mice and primed with OVA-pulsed DCs. T cell numbers and cell division were followed over time in different organs. We have found that antigen-presenting DCs do not survive in lymphoid organs beyond 3 d after transfer, because freshly transferred DO.11 T cells cannot be primed after this time (28). Early evaluation of the spleen (day 2) revealed that equal numbers of DO.11 cells had settled there (Fig. 4 B), and no CFSE dilution had taken place by this time (not depicted). On day 4, WT and CD25−/− DO.11 T cells had expanded markedly in the spleen, and both had cycled equivalently (Fig. 4 C). Recovery of CD25−/− cells was significantly less than that of WT cells.

During an in vivo T cell response, the expansion phase is followed by deletion of the activated T cells to restore homeostasis. By days 18–21, ~60–90% of the primed T cells had been lost (Fig. 4 B). However, a significant population of WT DO.11 cells remained in the spleen and in nonlymphoid tissues, and these cells exhibited a memory phenotype (unpublished data). Approximately 50–60% fewer memory cells were recovered from hosts that had received CD25−/− DO.11 T cells, both in the spleen and in nonlymphoid tissues (lung and liver; Fig. 4 B). These data indicate that IL-2 is not required to drive primed T cells through initial replication but is required for initial survival and for the subsequent development of a memory cell population.

**IL-7Rα–reexpressing T cells show an enhanced capacity to survive as memory cells**

From the previous experiments, it was not clear if the diminished recovery of memory cells from CD25−/− T cells was the consequence of deficient IL-7Rα reexpression or the result of reduced clonal expansion in response to antigenic stimulation. To distinguish between these possibilities, we separated the expansion and memory phases of the response by priming WT and CD25−/− DO.11 T cells in a first recipient, harvesting the primed cells on day 3, and retransferring the recovered cells to a second recipient. Phenotypic analysis of the primed cells showed that at day 3 CD44 and CD62L were up- and down-regulated, respectively, similarly in activated WT and CD25−/− T cells, whereas CD25 was already mostly lost (Fig. 5 A). Confirming the data shown in Fig. 2 B, primed CD25−/− cells showed ~50% reduction in IL-7Rα expression level compared with WT cells or naive
CD25<sup>−/−</sup> cells (Fig. 5 B). The primed DO.11 WT and CD25<sup>−/−</sup> cells were subsequently purified from lymph nodes and spleen, equal numbers were transferred to new recipients, and survival followed. In all tissues examined (lymph nodes, spleen, and lung), CD25<sup>−/−</sup> cells survived poorly compared with WT cells, as predicted from reduced IL-7R<sub>α</sub> expression (Fig. 5 C). Next, we asked whether the surviving CD25<sup>−/−</sup> T cells were bona fide memory cells. The surviving cells, whether WT or CD25<sup>−/−</sup>, all exhibited a resting, memory phenotype, with high CD44 and low CD62L (Fig. 6 A). Interestingly, the surviving CD25<sup>−/−</sup> cells all expressed high levels of IL-7R<sub>α</sub>, further suggesting that cells expressing high levels of IL-7R<sub>α</sub> are preferentially retained. However, the recall responses of the surviving CD25<sup>−/−</sup> memory T cells, as measured by effector cytokine production upon antigenic challenge, were markedly reduced when the T cells were primed in the absence of IL-2 signals (Fig. 6 B). Thus, it is possible that IL-2, analogous to what recently has been shown for CD8<sup>+</sup> memory cells (29), is required for programming secondary responses in CD4<sup>+</sup> memory T cells.

**Retrovirus-mediated expression of IL-7R<sub>α</sub> in IL-2<sup>−/−</sup> T cells restores memory cell survival**

To formally demonstrate a causal relationship between diminished IL-7R<sub>α</sub> expression and the failure of primed IL-2<sup>−/−</sup> T cells to generate a memory population, we restored IL-7R<sub>α</sub> expression in DO.11 IL-2<sup>−/−</sup> T cells and followed the generation of memory cells. To do this, a bicistronic retroviral vector, containing an IL-7R<sub>α</sub> cDNA and Thy1.1 reporter gene, was introduced into IL-2<sup>−/−</sup> DO.11 T cells during in vitro priming, resulting in enhanced expression of IL-7R<sub>α</sub> in the Thy1.1<sup>+</sup> cell population (Fig. 7 A). 3 × 10<sup>6</sup>
IL-2\(^{-/-}\) DO.11 T cells, transduced with either a control vector (48% of transferred cells being Thy1.1\(^+\)) or an IL-7R\(\alpha\)-containing vector (24% Thy1.1\(^+\)), were adoptively transferred into BALB/c recipients, and the number of DO.11 cells positive for Thy1.1 was determined 3 wk later. DO.11 IL-2\(^{-/-}\) cells that were transduced with IL-7R\(\alpha\) survived markedly better than cells that received the control vector (Fig. 7 B). The difference was even more striking after normalizing cell numbers to equal input cell numbers (Fig. 7 C). In an additional experiment, Thy1.1\(^+\) IL-2\(^{-/-}\) DO.11 T cells were purified from the transfection cultures by cell sorting, and equal numbers of cells expressing IL-7R\(\alpha\) or control vector were transferred into BALB/c recipients. 2 wk later, markedly higher numbers of DO.11 T cells were present in the lymph nodes and spleen of recipients that received cells expressing IL-7R\(\alpha\) versus control vector (Fig. 7, D and E). Transfer of sorted DO.11 T cells resulted in lower cell recoveries than transfer of unsorted cells (Fig. 7, C and E), perhaps because of the loss of antibody-coated cells. Thus, these data demonstrate that overexpression of IL-7R\(\alpha\) in IL-2\(^{-/-}\) cells is sufficient to restore their capacity for long-term survival by enhancing their competitiveness to receive IL-7 survival signals.

**DISCUSSION**

The cytokines IL-2 and IL-7 both use the \(\gamma\)c for transducing survival- and growth-promoting signals in T cells. Specificity of the response to IL-2 and IL-7 is assured by integrating a cytokine-specific high affinity receptor chain in the receptor complex, the IL-2R\(\alpha\) or IL-7R\(\alpha\), respectively. In this paper, we demonstrate that the expression of IL-2R\(\alpha\) and IL-7R\(\alpha\) is reciprocally regulated during a T cell response to antigen. This expression pattern provides a mechanism for the cell to respond to each cytokine at the appropriate time during the response, avoiding competition between the cytokines for \(\gamma\)c usage. Naive T cells, which are dependent on IL-7 for their survival (26), express IL-7R\(\alpha\) on the cell surface. Because naive T cells do not produce IL-2, they cannot use IL-2 as an autocrine survival factor and, predictably, they do not express high levels of IL-2R\(\alpha\). Upon TCR stimulation, IL-7R\(\alpha\) is down-regulated, whereas IL-2R\(\alpha\) expression is induced. TCR stimulation also induces production of IL-2, which T cells use in an autocrine fashion for clonal expansion and differentiation. Furthermore, the decline of IL-7R\(\alpha\) expression means that the T cells cannot be maintained by the constitutively produced survival cytokine, IL-7, and become dependent on the newly secreted growth factor, IL-2. Such a pattern of receptor expression would promote preferential clonal expansion of the antigen-stimulated (and, therefore, antigen-specific) T cells, the goal of the adaptive immune response.
response. At the end of the acute phase of the response, IL-2 production ceases, IL-2Rα expression wanes, and IL-7Rα is reexpressed. Because it has recently been demonstrated that IL-7 is required for the survival of CD4+ memory T cells, it can be postulated that activated T cells reexpressing IL-7Rα will preferentially enter the memory T cell pool. Similarly, it has been demonstrated that the subpopulation of CD8+ effector T cells expressing IL-7Rα at the peak of an antiviral response was most likely to develop into long-lived memory cells (30). Our data suggest that, also for CD4+ T cells, high levels of IL-7Rα at the peak of the response predict enhanced potential for memory cell generation (Figs. 4 B and 5 C).

Importantly, high IL-7Rα expression, although required, was not the sole determinant of memory cell generation, because the majority of primed, IL-7Rαhigh cells still died massively (8 × 10^6 on day 4 vs. 6 × 10^5 on day 18 in the spleen; Fig. 4 B). Thus, either a subset of committed memory cells exists within the IL-7Rαhigh primed CD4+ T cells, or the ensuing memory cell population has, in a stochastic manner, escaped deletion mechanisms operating during the contraction phase of the response.

Because the IL-7–IL-7Rα system is essential for CD4+ and CD8+ memory T cell homeostasis, it is important to identify the factors that regulate IL-7Rα expression during a T cell response. Based on our previous data showing that IL-2 is needed for memory responses, as well as the results of a microarray study cataloging IL-2–dependent gene expression in primed T cells (unpublished data), we hypothesized that IL-2 was required for IL-7Rα expression on primed T cells.

We found that DO.11 T cells primed with antigen and APCs in vitro and in vivo in the absence of IL-2 signals failed to reexpress IL-7Rα with the same kinetics as WT cells after initial TCR-triggered down-regulation of the receptor (Fig. 2). IL-7Rα reexpression was more dramatically dependent on IL-2 in T cells primed in vitro than in vivo, suggesting that additional factors controlling IL-7Rα expression may be present in vivo. IL-15, sharing the IL-2/15Rβ for signaling with IL-2, is a possible candidate for this function. Our results contrast with those from an earlier report indicating that IL-2 negatively regulates IL-7Rα expression on activated T cells (25). However, whereas we used a physiologically relevant T cell priming system with antigen and APCs in vitro and in vivo in the absence of IL-2 signals failed to reexpress IL-7Rα with the same kinetics as WT cells after initial TCR-triggered down-regulation of the receptor (Fig. 2).

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We have previously shown that T cells primed with IL-2 acquire a competitive survival advantage over cells primed in the absence of IL-2 (17). Thus, IL-2−/− DO.11 cells primed in vitro and subsequently adoptively transferred to BALB/c mice survived poorly in comparison with WT DO.11 cells. However, primed IL-2−/− DO.11 T cells persisted efficiently after adoptive transfer into lymphopenic Rag−/− mice, where competition with other lymphocytes was absent (17). The differential long-term survival capacity of primed IL-2−/−
T cells in intact versus lymphopenic mice can be explained by deficient IL-7Rα expression: CD4+ memory T cells are dependent on IL-7 to persist in intact hosts (20, 21), whereas survival and homeostatic proliferation of memory CD4+ T cells in lymphopenic animals is IL-7 independent (31). Now we also show that if DO.11 T cells are unable to receive IL-2 signals caused by deletion of the IL-2Rα gene, the cells develop poorly into long-lived memory cells after priming with antigen and APCs in vivo.

There has been ongoing discussion about the role of IL-2 in T cell responses. Although initially described as the prototypic T cell growth factor (32), analysis of mice deficient in IL-2 or IL-2Rα/β surprisingly showed a lymphoproliferative phenotype leading to autoimmune disease (33–35). This generated considerable interest in the function of IL-2 as a negative regulator of the immune system. The dependence of T regulatory cells on IL-2 for development and maintenance (36–39) and the capacity of IL-2 to sensitize repeatedly stimulated T cells for cell death by activating the Fas/FasL death pathway (40–42) have both been proposed as explanations for the negative regulatory role of IL-2. On the other hand, priming of IL-2−/− (43) or CD25−/− (44) T cells in vivo showed only a limited impact of IL-2 deficiency on initial T cell clonal expansion. These studies have raised uncertainties about the obligatory role of IL-2 as a T cell growth factor in vivo. By using CD25−/− T cells to exclude the effects of bystander IL-2, priming with antigen and APCs, and separating the acute and memory phases of the T cell response, we have

Figure 7. Retrovirus-mediated expression of IL-7Rα in IL-2−/− T cells restores memory cell generation. (A) IL-2−/− DO.11 T cells were retrovirally transduced with IL-7Rα or empty vector, and IL-7Rα and Thy1.1 expression were evaluated on day 4 by antibody staining and flow cytometry. MFI of IL-7Rα staining on the gated (Thy1.1+) population is indicated. (B) 3 × 10^6 IL-2−/− DO.11 T cells, of which 48% (empty vector) and 24% (IL-7Rα) expressed the Thy1.1 reporter gene, were adoptively transferred to BALB/c recipients, and the presence of Thy1.1+ DO.11 T cells was analyzed 3 wk after transfer. (right) Dot plots show the percentage of KJ1-26+Thy1.1+ cells within the CD4+ population in the spleen of one representative recipient. (C) To normalize for the difference in the number of Thy1.1+ cells transferred between both groups, the number of KJ1-26+Thy1.1+ memory cells recovered from the lymph nodes and spleen per 10^6 KJ1-26+Thy1.1+ cells transferred was calculated. Data points represent individual mice. (D) IL-2−/− DO.11 T cells were retrovirally transduced as in A, and Thy1.1+KJ1-26+CD4+ cells were purified from the cultures on day 4 by cell sorting. 2 × 10^6 Thy1.1+KJ1-26+CD4+ cells expressing either IL-7Rα or control vector were adoptively transferred into BALB/c recipients, and the presence of Thy1.1+ DO.11 T cells was analyzed 2 wk after transfer. Dot plots show the percentage of KJ1-26+Thy1.1+ cells within the CD4+ population in the spleen of one representative recipient. (E) Total number of KJ1-26+Thy1.1+CD4+ cells recovered from the lymph nodes and spleen per 2 × 10^6 purified cells transferred. Data points represent individual mice.
further elucidated the role of IL-2 during an in vivo CD4⁺ T cell response. Our data demonstrate in a definitive way that IL-2 signals during priming are not required for initial cell proliferation but are more important to endow primed T cells with long-term survival potential once the antigen has been cleared. Confirming our previous studies with in vitro primed IL-2⁻/⁻ T cells (17), IL-2 is not required to drive T cells through the cell cycle in vivo (Fig. 4 C). Although CD25⁻/⁻ T cells expanded considerably after antigenic stimulation, we generally recovered less primed CD25⁻/⁻ T cells than WT cells at days 3–4, in accordance with another study (44). Whether this diminished clonal expansion of primed T cells is a direct effect of the lack of IL-2 growth factor activity or is caused by a failure to receive IL-7 signals remains an open question. However, the most striking consequence of IL-2 deprivation during priming is the subsequent diminished capacity to generate a memory T cell population. This defect is likely caused by failed reexpression of IL-7Rα on late-activated T cells, because we (Fig. 3) and others have shown that survival of CD4⁺ memory T cells is dependent on IL-7Rα (19–21) and that ectopic expression of IL-7Rα in primed IL-2⁻/⁻ T cells restores memory cell generation (Fig. 7). Identification of the IL-2–induced signaling pathway that leads to IL-7Rα reexpression will be an important goal of future research. Also of interest is to investigate whether GA binding protein, a transcription factor recently implicated in IL-7Rα expression on T cells, is induced by IL-2 (45).

Finally, these results demonstrate that optimal generation of memory T cell responses will require strategies for maximizing the expression of IL-7Rα on primed cells. It is also likely that analyzing IL-7Rα expression on antigen-specific T cells after vaccination may predict the efficacy of the vaccine. Such prospective studies may help improve the success of prophylactic vaccination, based on experimental results.

**MATERIALS AND METHODS**

**Mice.** BALB/c mice were purchased from Charles River Laboratories and used as recipients for adoptive transfer experiments at 6–8 wk of age. DO.11.10 transgenic mice, expressing a TCR specific for the hen egg albumin peptide OVA257-264 presented by MHC class II molecule I-A², were a gift from K. Murphy (Washington University, St. Louis, MO). DO.11.10 × IL-2⁻/⁻ mice were generated in our laboratory by crossing DO.11.10 mice with IL-2⁻/⁻ mice (backcrossed for >10 generations onto the BALB/c background; The Jackson Laboratory). Generation of DO.11.10 × CD25⁻/⁻ mice has been previously described (46). Transgenic mice were genotyped using FCR and flow cytometry. All mice were bred and maintained in accordance with the guidelines of the Laboratory Animal Resource Center of the University of California, San Francisco.

**Antibodies, CFSE labeling, and flow cytometry.** DO.11.10 T cells were detected by staining with allophycocyanin– or PE-conjugated KJ1-26 TCR clonotypic antibody, generated in our lab from the hybridoma cell line (a gift from P. Marrack, National Jewish Research and Medical Center, Denver, CO), and peridinin chlorophyll-protein–conjugated anti-CD4 mAb after blocking Fc receptors with anti-CD16/CD32 (both purchased from BD Biosciences). The following antibodies coupled to the indicated fluorochromes were used for detection of activation markers and cytokine receptors: anti-CD44–PE (IM7), anti-CD62L–PE or –FITC (MEL-14/L-selectin), anti-CD25–allophycocyanin or –FITC (PC61/IL-2Rα; all obtained from BD Biosciences), and anti-CD127–biotin/streptavidin–PE (ATR34/IL-7Rα; Chemicon International). Fluorescence intensities were measured with a flow cytometer (FACSCalibur; Becton Dickinson), and data were analyzed with CellQuest software (Becton Dickinson). For in vivo blocking experiments, mAbs specific for IL-7Rα (clone A7R34) produced by the hybridoma cell line were purified with Protein G Sepharose 4 Fast Flow (GE Healthcare). Rat IgG (Jackson Immunoresearch Laboratories) was used as a control. To follow cell division during priming, naive CD4⁺ T cells were labeled with 1 μM CFSE (Invitrogen) for 10 min at room temperature in serum-free medium. Excess CFSE was quenched with 50% FCS, followed by three washes with PBS. On days 3–4 after adoptive transfer and priming, spleens from the recipient mice were harvested, and CFSE content of KJ1-26 CD4⁺ T cells was determined by flow cytometry. For intracellular cytokine staining, splenocytes were restimulated ex vivo for 4 h with 1 μg/ml OVA peptide in the presence of 10 μg/ml BrefeldinA (Epicentre Biotechnologies) for the last 3 h and stained with anti-IL-2, anti–IL-4, and anti–IFN-γ antibodies (BD Biosciences) using a Cytofix/Cytoperm Kit (BD Biosciences), according to the manufacturer's instructions.

**T cell priming and adoptive transfer.** In vitro T cell priming and adoptive transfer of WT and IL-2⁻/⁻ DO.11 T cells were performed as previously described (17). In brief, 2.5–5 × 10⁶ CD4⁺ T cells from WT and IL-2⁻/⁻ DO.11 mice were primed with 2.5 × 10⁵ mitomycin C–treated BALB/c splenocytes and 1 μg/ml OVA257-264 in 24-well plates for 4–5 d. IL-2⁻/⁻ splenocytes were used for cultures with IL-2⁻/⁻ DO.11 cells to avoid the effects of bystander IL-2. In some experiments, 10 ng/ml recombinant mIL-2 (R&D Systems) was added to the cultures. Primed T cells were harvested on day 4, and dead cells were removed by centrifugation over a density gradient (Lympholyte-M; Cedarlane Laboratories). Equal numbers of viable KJ1-26 CD4⁺ T cells were adoptively transferred into unmanipulated BALB/c mice by tail vein injection. For in vivo priming, 1 × 10⁵ naive WT or CD25⁻/⁻ DO.11 T cells were adoptively transferred to sex-matched BALB/c mice and primed the next day with 1–3 × 10⁵ bone marrow–derived DCs. DCs were generated by culturing 5–10 × 10⁶ bone marrow cells in 100-mm suspension culture dishes (Corning) for 8–11 d with 20 ng/ml GM-CSF in Iscove's modified Dulbecco's medium (Sigma-Aldrich) supplemented with 1 mM l-glutamine, penicillin, streptomycin (all obtained from Life Technologies), 5 × 10⁻³ M 2-ME, and 10% fetal bovine serum (Sigma-Aldrich). On days 5 and 8, nonadherent and loosely adherent cells were harvested by treatment with 3 mM EDTA in PBS and transferred to new plates. 1 ng/ml IL-4 was added for the last 3 d of culture. DCs were matured with 1 μg/ml LPS (Escherichia coli O26:B6; Sigma-Aldrich) and pulsed with 1 μg/ml OVA peptide 24 h before injection. For the experiments described in Fig. 5, lymph nodes and spleen were harvested on days 3–4 after in vivo priming, and CD4⁺ T cells were magnetically purified with anti-mouse CD4-coated Dynabeads, according to the instructions of the manufacturer (DynaL). Equal numbers of viable KJ1-26 CD4⁺ T cells were transferred to a second set of BALB/c recipients by tail vein injection.

**Analysis of memory T cells in lymphoid and nonlymphoid tissues.** DO.11 memory T cells in spleen, pooled peripheral lymph nodes (submandibular, axillar, brachial, inguinal, and popliteal), liver, and lung were quantified 2–4 wk after priming. To recover lymphocytes from nonlymphoid tissues, mice were perfused with PBS, and lungs and liver were excised. Lung and liver tissue was mechanically dissociated and treated with 400 U/ml and 100 U/ml, respectively, collagenase VIII (C2139; Sigma-Aldrich) for 30 min at 37°C, regularly pipetting the suspension up and down. The resulting suspension was passed through a 70-μM nylon screen, and lymphocytes were isolated by density gradient centrifugation using Lympholyte-M. The percentage of KJ1-26 CD4⁺ memory T cells in the cell suspensions was determined by flow cytometry. Absolute numbers of DO.11 T cells in lymph nodes and spleen were calculated from this percentage and counts of total cell numbers in a hemocytometer (Improved Neubauer; Reichert) using trypan blue dye exclusion. Recall responses of WT and CD25⁻/⁻ memory
T cells were evaluated by rechallenging previously primed recipients of WT and CD8\(^+\) DO.11 T cells with 3 × 10\(^6\) OVA-pulsed DCs and staining splenocytes for cytokine production 3 d later using intracellular cytokine staining and flow cytometry.

**Cloning and retrovirus-mediated transfection of IL-7R.** A full-length cDNA clone of the mouse IL-7R\(\alpha\) (clone 675848; I.M.A.G.E. Consortium) (47) was obtained from Open Biosystems. The IL-7R\(\alpha\) coding sequence was isolated by restriction digest with the enzyme BsrG I (New England Biolabs, Inc.) and ligated into the BsrG I site of the retroviral vector sequence was isolated by restriction digest with the enzyme BsrG I (New England Biolabs, Inc.) and ligated into the BsrG I site of the retroviral vector.

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