

CUTTING EDGE

Cutting Edge: IL-7-Independent Regulation of IL-7 Receptor α Expression and Memory CD8 T Cell Development¹

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Expression of IL-7R α on a subset of Ag-specific effector CD8 T cells is believed to identify memory cell precursors. However, whether IL-7 regulates IL-7R α expression in vivo and is responsible for selective survival of IL-7R α ⁺ effector cells is unknown. Our results show that in the absence of IL-7, IL-7R α expression was extinguished on the majority of CD8 T cells responding to virus infection, sustained on a subset of effector cells transitioning to memory, and expressed at high levels by memory cells. Additionally, an IL-7-deficient environment was capable of supporting bcl-2 up-regulation and memory cell development in response to virus infection. Thus, IL-7R α regulation occurs independently of IL-7 in responding CD8 T cells, indicating that CD8 memory T cell precursors are not selected by IL-7/IL-7R α interactions. The Journal of Immunology, 2006, 177: 4247–4251.

The cytokine IL-7 is central to development and survival of T lymphocytes as deficiencies in either IL-7 or IL-7R α block the development of the T cell lineage (1) and IL-7 signaling is required for the survival of naive T cells and their homeostatic proliferation in immunodeficient hosts (2–4). The regulation of survival of CD8 T cells is due in part to bcl-2 induction through IL-7R signaling (5, 6). Although IL-7R α is highly expressed by naive and memory CD8 T cells, most responding effector cells transiently down-regulate IL-7R α (2). Also, a small population of effector CD8 T cells retain or re-express IL-7R α (7), and this population is thought to represent memory T cell precursors. In support of this concept are studies showing that when early inflammation is blocked following infection, the contraction phase of the immune response is bypassed by IL-7R α -expressing cells (8). However, immunization with peptides in adjuvant or peptide-pulsed dendritic cells (DCs)⁴ induces increased numbers of IL-7R α ⁺ effector cells that do not transit to the memory compartment. Thus,

IL-7R expression does not necessarily identify CD8 memory T cell precursors.

The potential survival advantage for IL-7R α ⁺ memory CD8 T cell precursors implies that IL-7 binding to IL-7R α modulates the receptor. Although the inclusion of IL-7 as an adjuvant in vaccination protocols has been proposed to selectively expand memory cells (9), this notion implies that IL-7 selects IL-7R α ⁺ cells into the memory pool. Indeed, IL-7 has been shown to regulate IL-7R α expression in vitro (10, 11). However, whether IL-7 regulates IL-7R α expression following CD8 T cell activation in vivo is unknown. We now show that regulation of IL-7R α expression is IL-7 independent, calling into question the notion that IL-7 selects IL-7R α ⁺ CD8 T cells into the memory population.

Materials and Methods

Mice, adoptive transfers, and infections

B6.129P-IL-7^{tm1} mice (IL-7^{-/-}) were obtained from DNAX and backcrossed to C57BL/6J (*Ptprc*^b = CD45.2). Wild-type controls were generated from IL-7^{+/+} littermates. CD8 T cell responses were monitored following i.v. infection with 10⁵ PFU of vesicular stomatitis virus (VSV). For adoptive transfers, 0.5–1 × 10⁵ B6.SJL-*Ptprc*^b *Peprc*^b/BoyJ-Tg(TcraTcrb)1100Mjb/J-B6.129S7-*Rag1*^{tm1Mom} (CD45.1 OT-I-RAG^{-/-}) CD8 lymph node (LN) T cells were injected into IL7^{+/+}, IL-7^{-/-}, or B6.129S7-*Il7*^{tm1bms}/J mice (IL-7R α ^{-/-}) and infected with 10⁵ PFU rVSV expressing OVA (VSV-OVA).

Tissue isolation and flow cytometry

Lymphocytes were isolated from tissues as previously described (12). VSV-specific CD8 T cells were detected by staining with an H-2K^b tetramer containing the VSV N protein derived peptide for 1 h at room temperature with anti-CD8, CD11a (BD Pharmingen) and IL-7R α mAbs (eBioscience). For adoptive transfers, cells were stained with Abs specific for CD45.1 (BD Pharmingen), CD8, CD11a, and IL-7R α , fixed overnight, and stained with an anti-bcl-2 or isotype control mAb (BD Pharmingen). Relative fluorescence intensities were measured using a FACSCalibur (BD Biosciences).

Results and Discussion

IL-7R α expression following VSV infection

Previous studies demonstrate that following infection with VSV or lymphocytic choriomeningitis virus (LCMV), the majority of Ag-specific effector CD8 T cells down-regulate IL-7R α (2, 7). In the case of LCMV-specific CD8 T cells, the total

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⁴ Abbreviations used in this paper: DC, dendritic cell; VSV, vesicular stomatitis virus; VSV-OVA, VSV expressing OVA; LCMV, lymphocytic choriomeningitis virus; TSLP, thymic stromal lymphopoietin.

number of effector cells which either maintain or rapidly re-express IL-7R α correlates with the number of memory cells generated (7). To address whether a similar phenomenon occurred following VSV infection, IL-7R α expression was monitored on responding Ag-specific CD8 T cells (Fig. 1A). Five days postinfection, 21% of the Ag-specific CD8 T cells expressed IL-7R α and by day 8, 31% of the cells expressed IL-7R α . Through the contraction phase, the percentage of IL-7R α ⁺ Ag-specific cells gradually increased, with 91% of the cells IL-7R α ^{high} 17 days postinfection. Moreover, a steady-state number of IL-7R α ⁺ Ag-specific cells was maintained throughout the response (Fig. 1B), suggesting that this population of IL-7R α ⁺ cells was selected to seed the memory cell pool.

Studies using mouse lymphocytes and both human and non-human primate PBMC demonstrate that culture with IL-7 dramatically reduces IL-7R α expression on responding cells, presumably due to the interaction of IL-7 with its cognate receptor (10, 11, 13). However, it is difficult to compare these studies with in vivo analyses because local IL-7 concentrations cannot be accurately measured because serum levels of IL-7 are low (14) and the primary reservoir of IL-7 in vivo is thought to be bound to extracellular matrices (15). To determine whether IL-7 modulated IL-7R α expression on effector CD8 T cells during a virus infection, we infected IL-7^{-/-} and control IL-7^{+/-} littermates with VSV and monitored IL-7R α expression on the Ag-specific CD8 T cells in the spleen and lung (Fig. 2). Seven days postinfection, 44 and 20% of CD8 T cells in normal or IL-7^{-/-} mice, respectively, were tetramer⁺ (Fig. 2A). The splenic response at this time was blunted in the IL-7^{-/-} mice as compared with controls. Interestingly, IL-7R α down-regulation had occurred on Ag-specific CD8 T cells from both IL-7^{+/-} or IL-7^{-/-} mice (Fig. 2A), with IL-7^{-/-} CD8 T cells expressing somewhat more IL-7R α than controls. On day 14 after infection, the CD8 T cell response had increased in the IL-7^{-/-} mice from day 7 values, while the response in the normal mice had declined (Fig. 2B). At this time, ~70–90% of the Ag-specific CD8 T cells expressed high levels of IL-7R α in both control and IL-7^{-/-} mice (Fig. 2B). Forty-four days postinfection,

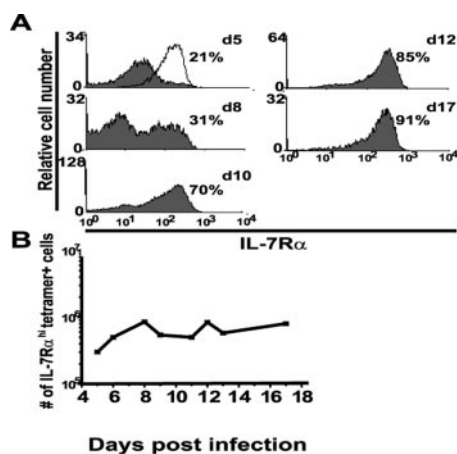


FIGURE 1. The kinetics of IL-7R α expression on effector CD8 T cells. *A*, Mice were infected with VSV and the level of IL-7R α expression on splenic tetramer⁺ CD8 T cells (solid histogram) was monitored. Values in each panel represent the percentage of Ag-specific cells expressing IL-7R α compared with naive CD8 T cells (open histogram). *B*, The total number of tetramer⁺ cells expressing IL-7R α . Both panels represent an experiment with two to four mice per group.

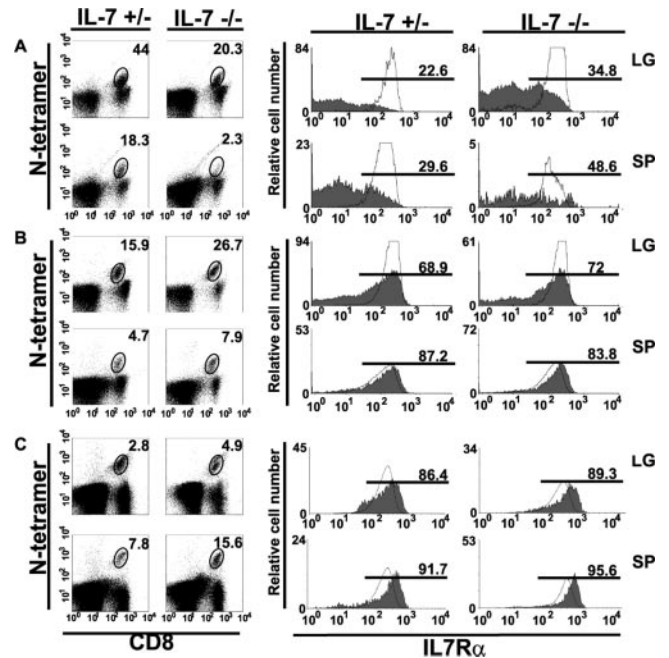


FIGURE 2. IL-7 deficiency fails to impact the proliferation and kinetics of IL-7R α expression by Ag-specific CD8 T cells. IL-7^{+/-} and IL-7^{-/-} mice were infected with VSV and the frequency of tetramer⁺ CD8 T cells (*left*) and their respective level of IL-7R α expression (*right*) in the spleen and lung 7 (*A*), 14 (*B*), and 44 days (*C*) postinfection was determined. Values in the dot plots are the percentage of tetramer⁺ cells in the CD8 T cell pool. The solid histograms (*right*) represent the tetramer⁺ cells compared with naive CD8 T cells (open histogram) and values are the percentage of Ag-specific cells expressing IL-7R α . The data depicted represents two experiments with two to four mice per group.

tion, memory cells were present in both normal and IL-7^{-/-} mice (Fig. 2C) and similar levels of IL-7R α were maintained whether or not IL-7 was present (Fig. 2C). Indeed, as compared with naive CD8 T cells, an increased level of IL-7R α was noted on memory CD8 T cells even in the absence of IL-7. These results indicated that IL-7 was dispensable not only for down-regulation of IL-7R but that IL-7 was also not required for IL-7R α re-expression or for selection of IL-7R α ⁺ CD8 T cells into the memory population. Although TSLP also uses IL-7R α as a component of its receptor, treatment with an anti-TSLP-R mAb failed to impact IL-7R α expression kinetics on Ag-specific CD8 T cells in both wild-type and IL-7^{-/-} mice, although the efficacy of such treatment has not been determined (data not shown).

Memory CD8 T cells are generated in IL-7^{-/-} mice

Because the expression pattern of IL-7R α on Ag-specific CD8 T cells was not altered in IL-7^{-/-} mice, we wished to determine whether memory cells could be generated in these animals. To this end, the total number of IL-7R α ⁺ effector and memory cells generated in the spleen and lung of IL-7^{-/-} and IL-7^{+/-} mice was calculated (Fig. 3). Although the frequency of Ag-specific CD8 T cells expressing IL-7R α at the peak of the response was greater in IL-7^{-/-} mice (Fig. 2A), the total number of cells expressing IL-7R α in IL-7^{-/-} mice was less than in control mice (Fig. 3), perhaps due to the lymphopenic environment of IL-7^{-/-} mice, resulting in a delayed proliferative peak of the CD8 T cell response. Nonetheless, 14 days after infection, the number of Ag-specific CD8 T cells in the lung and spleen was

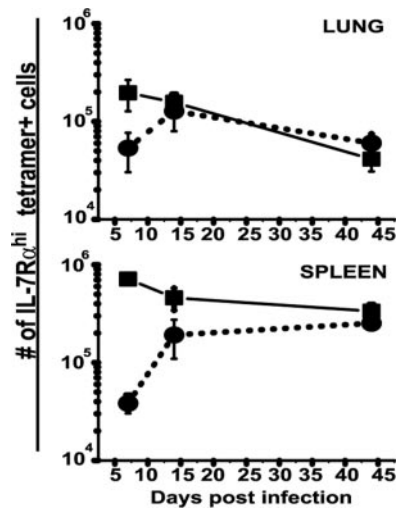


FIGURE 3. CD8 T cell memory is generated in IL-7^{-/-} mice. IL-7^{+/+} (solid line) and IL-7^{-/-} (dotted line) mice were infected with VSV and the total number of tetramer⁺IL-7R α ^{high} CD8 T cells was quantitated. Each data point represents the average of two to four mice per group.

equivalent in both groups and remained similar into the memory phase of the response (day 44). Therefore, both the regulation of IL-7R α expression on effector CD8 T cells and the generation of the resultant memory cell pool occurred independently of IL-7 signaling. These findings suggested that regulation of IL-7R α expression was controlled by factors other than IL-7, and that IL-7 did not provide a selective survival advantage to cells expressing IL-7R α .

IL-7-deficient environment does not alter IL-7R α expression kinetics and memory cell generation

Because endogenous responding T cells in IL-7^{-/-} animals may be defective in terms of their long-term stability due to a deficiency in homeostatic proliferation (2), we adoptively transferred OT-I TCR CD8 T cells from a normal host into an IL-7 or IL-7R α null environment. Following infection with VSV-OVA, the kinetics of the proliferative response of the donor OT-I cells in the blood of all recipients was assessed. Although the expansion of the OT-I T cells in the IL-7^{+/+} and IL-7^{-/-} mice was parallel until day 7, from day 11 onward the percentage of OT-I cells was greater in both the IL-7^{-/-} and IL-7R α ^{-/-} mice than in control mice (Fig. 4A). This increased frequency may be due to the lymphopenic environments of IL-7^{-/-} and IL-7R α ^{-/-} mice, although other factors such as increased availability of other growth factors may also play a role. However, when the mice were sacrificed 38 days postinfection, the total number of OT-I memory cells isolated from the spleen of all recipients was similar (Fig. 4B).

We also examined the expression of IL-7R α by responding OT-I cells (Fig. 4C). Early after infection (day 3), nearly all OT-I cells in either the IL-7^{+/+}, IL-7^{-/-}, or IL-7R α ^{-/-} mice had down-regulated IL-7R α . By day 5, the peak of the OT-I CD8 T cell response to VSV-OVA, 35–40% of the OT-I cells expressed IL-7R α irrespective of host type (Fig. 4C). The percentage of IL-7R α ⁺ OT-I cells gradually increased over time in both groups and by day 38, the OT-I memory cells transferred into both the IL-7^{+/+} and IL-7^{-/-} mice expressed levels of IL-7R α slightly greater than the level expressed by naive CD8 T cells (Fig. 4C). IL-7 is produced primarily by stromal cells, in-

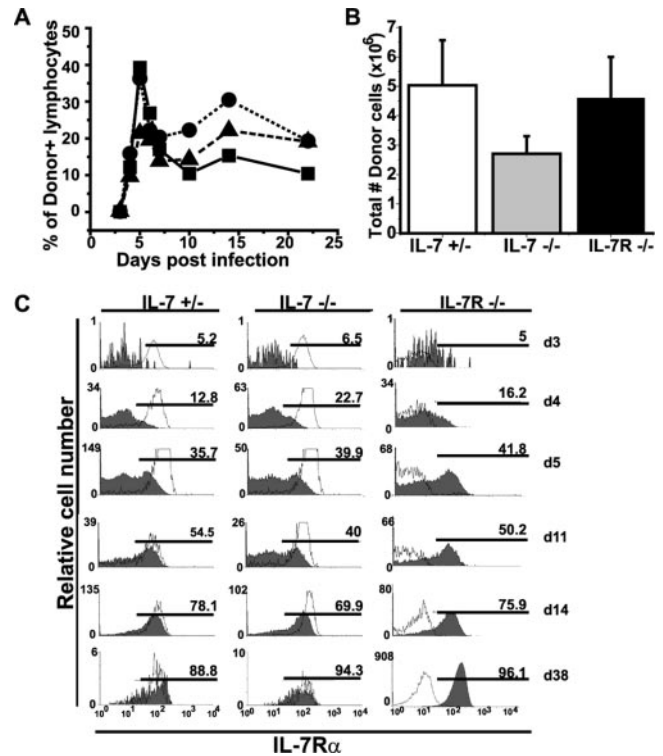


FIGURE 4. Regulation of IL-7R α expression and memory cell generation is IL-7 independent. *A*, Naive OT-I T cells were transferred into IL-7^{+/+} (■), IL-7^{-/-} (●), or IL-7R α ^{-/-} (▲) mice, infected with VSV-OVA, and the frequency of donor⁺ cells was assessed in the blood. *B*, Thirty-eight days postinfection, the total number of OT-I memory CD8 T cells was determined in IL-7^{+/+} (□), IL-7^{-/-} (▤), and IL-7R α ^{-/-} (■) recipients. *C*, The kinetics of IL-7R α expression on OT-I T cells in the blood following activation in IL-7^{+/+}, IL-7^{-/-}, or IL-7R α ^{-/-} mice (shaded histograms). Open histograms represent the level of IL-7R α expression on the endogenous naive CD8 T cells. All data represent two experiments with two to three mice per group.

testinal epithelial cells, and some subsets of human dendritic cells, it is not produced by T cells (16–18), thus, autonomous IL-7 produced by the transferred cells is not responsible for the generation of memory in IL-7^{-/-} mice. Taken together, these data further demonstrated that regulation of IL-7R α expression and memory CD8 T cell generation occurred in the absence of IL-7.

The proposed selective survival advantage imposed on those effector CD8 T cells maintaining IL-7R α expression has been linked to the IL-7-mediated induction of the antiapoptotic molecule bcl-2. Indeed, work from our laboratory previously demonstrated that activated OT-I IL-7R α ^{-/-} T cells survived poorly in recipient mice, presumably due to the inability of these cells to express bcl-2 (2). Thus, we decided to assess the status of bcl-2 expression by transferred OT-I T cells in normal and IL-7-deficient environments following VSV-OVA infection. Although naive OT-I T cells expressed bcl-2 directly ex vivo (Fig. 5A), after activation the responding effector cells in both the spleen and lung down-regulated bcl-2 and sustained this low level expression through the contraction phase (Fig. 5B). By day 30 postinfection, although levels had not yet returned to that of the starting population, 25–35% of the responding T cells expressed bcl-2 in both tissues of either the IL-7^{+/+} or IL-7^{-/-} recipients. Thus, because the kinetics of bcl-2 expression in IL-7^{+/+} and IL-7^{-/-} recipients was similar

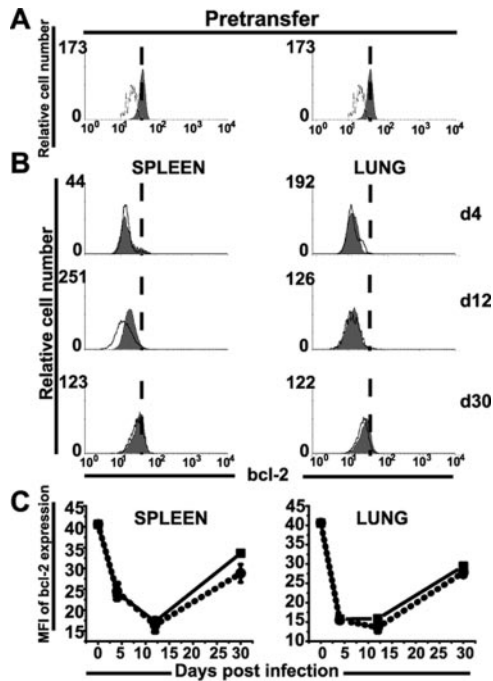


FIGURE 5. bcl-2 regulation in OT-I CD8 T cells responding to virus infection is not mediated by IL-7. *A*, Pretransfer, the levels of intracellular bcl-2 (solid histogram) and isotype control mAb staining (open histogram) was assessed on LN-derived OT-I donors. *B*, Four, 12, and 30 days post-VSV-OVA infection, the level of bcl-2 staining was determined in IL-7^{+/-} (shaded histogram) and IL-7^{-/-} (open histogram) recipients. *C*, The mean fluorescent intensity (MFI) of bcl-2 staining on OT-I T cells activated in IL-7^{+/-} (solid line) and IL-7^{-/-} (dotted line) recipients. Each data point represents the average of two to three mice.

(Fig. 5C), the parallel down-modulation and reacquisition of IL-7R α /bcl-2 before and during the transition to memory was IL-7 independent.

Thus, is IL-7R α a bona-fide identifier of effector cells destined to enter the memory cell pool? And if so, what molecule(s) other than IL-7 regulate the expression of IL-7R α by responding effector CD8 T cells? Several studies have analyzed the relationship between IL-7R α expression and memory cell development with conflicting results. Acute infections with VSV and LCMV induce robust immune responses, preceded by concomitant TCR and IL-7R α down-regulation (2, 7). Stimulation using anti-CD3 and anti-CD28 in vitro also results in IL-7R α repression (19). Following acute infection, IL-7R α is maintained or re-expressed by a subset of CD8 T cells. However, in models of persistent viral infection, Ag-specific CD8 T cells fail to re-express IL-7R α , likely due to their continual stimulation via the TCR, and remain as IL-7R α ^{low} "pseudo-effector" T cells (20–23). At the other end of the spectrum, weaker Ag stimuli such as peptide-pulsed DCs do not efficiently induce IL-7R α down-regulation (24). Thus, there appears to be a link between the strength of signal through the TCR, IL-7R α suppression, and memory development, as opposed to an IL-7-directed survival advantage. It has been shown that as effector cells transition to memory, a distinct program is initiated altering gene expression (25). Perhaps strong TCR stimulation induces this program for memory cell generation and alters IL-7R α expression, whereas those effectors receiving suboptimal TCR signaling from a weak stimulus or brief Ag encounter fail or inefficiently initiate memory development. An alternate hypothesis

is that the level of inflammation affects IL-7R α expression. This possibility is supported by the findings that either under non-inflammatory priming conditions using either peptide-pulsed DCs (24) or when animals were pretreated with antibiotics before *Listeria monocytogenes* infection (8), IL-7R α down-regulation is limited. However, in both cases memory development proceeds normally while in acute inflammatory infections, IL-7R α is down-regulated and memory is generated (Ref. 7, Fig. 2).

Our data suggested that IL-7 expression was irrelevant for delivery of the appropriate survival signals to effector cells transitioning to memory cells. In addition, dying Ag-specific CD8 T cells express more IL-7R α than their viable counterparts during the contraction phase of the immune response (24). To account for this discrepancy, it has been suggested that the limited supply of IL-7 in vivo is tightly regulated such that those cells which have received a signal via IL-7R α down-regulate IL-7R α , thereby increasing the bioavailability of IL-7 (11). However, our data indicated that engagement of IL-7R α by IL-7 was not required for receptor down-regulation (Figs. 2 and 3). Additionally, compensatory molecules involved in long-term survival may exist which are capable of signaling via IL-7R α or alternate receptors after TCR triggering. Indeed this may be likely because IL-7R α -deficient CD8 T cells express less bcl-2 following activation and fail to survive long-term, presumably due to intrinsic defects in IL-7R α signaling (2). However, our results suggested that this was an IL-7-independent phenomenon (Fig. 5). Mice deficient in another γ_c cytokine, IL-15, generate memory cells, albeit at a lower frequency (26), but with similar IL-7R α expression kinetics (data not shown). Considering both IL-7 and IL-15 are required for the homeostatic proliferation of memory and memory-phenotype CD8 T cells in lymphopenic environments (3, 4), it is possible that IL-15/IL-15R α signaling may provide compensatory survival signals in the absence of IL-7. Along similar lines, IL-7^{-/-} mice are lymphopenic, thus perhaps resulting in increased basal levels of IL-15 which may affect memory CD8 T cell development. The lymphopenic environment could also increase the bioavailability of other cytokines such as IL-2 which have been shown to regulate IL-7R α expression in vitro (19). Alternatively, the absence of a large portion of the lymphocyte pool could also alter the stoichiometry of APCs available to present Ag to the responding CD8 T cells, thus enhancing memory generation in the IL-7^{-/-} mice. Finally, IL-7R α can form heterodimers with the thymic stromal lymphopoietin receptor (TSLPR) that specifically binds TSLP (27, 28), a cytokine important for CD4 development and homeostasis (29, 30). Signaling through the TSLPR, however, does not appear to influence IL-7R α expression as in vivo blocking studies using an anti-TSLP-R mAb failed to impact IL-7R α expression kinetics on Ag-specific CD8 T cells in both wild-type and IL-7^{-/-} mice following infection (data not shown).

Together, our data suggested that stimulation of IL-7R α with IL-7 is dispensable for the selection and maintenance of memory CD8 T cells and that perhaps stimulation of other receptors, such as the TCR, initially regulates IL-7R α expression. Therefore, the current paradigm that implicates IL-7 signaling via IL-7R α as a master regulator of both IL-7R α expression and memory cell generation requires re-evaluation.

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Disclosures

The authors have no financial conflict of interest.

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