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STAT5 Is Critical To Maintain Effector CD8+ T Cell Responses

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During an immune response, most effector T cells die, whereas some are maintained and become memory T cells. Factors controlling the survival of effector CD4+ and CD8+ T cells remain unclear. In this study, we assessed the role of IL-7, IL-15, and their common signal transducer, STAT5, in maintaining effector CD4+ and CD8+ T cell responses. Following viral infection, IL-15 was required to maintain a subpopulation of effector CD8+ T cells expressing high levels of killer cell lectin-like receptor subfamily G, member 1 (KLRG1), and lower levels of CD127, whereas IL-7 and IL-15 acted together to maintain KLRG1lowCD127high CD8+ effector T cells. In contrast, effector CD4+ T cell numbers were not affected by the individual or combined loss of IL-15 and IL-7. Both IL-7 and IL-15 drove phosphorylation of STAT5 within effector CD4+ and CD8+ T cells. When STAT5 was deleted during the course of infection, both KLRG1highCD127low and KLRG1lowCD127high CD8+ T cells were lost, although effector CD4+ T cell populations were maintained. Furthermore, STAT5 was required to maintain expression of Bcl-2 in effector CD8+, but not CD4+, T cells. Finally, IL-7 and IL-15 required STAT5 to induce Bcl-2 expression and to maintain effector CD8+ T cells. Together, these data demonstrate that IL-7 and IL-15 signaling converge on STAT5 to maintain effector CD8+ T cell responses. The Journal of Immunology, 2010, 185: 2116–2124.
protein (10). The generation of new conditional STAT5a/b-deficient mice has revealed a profound effect of the loss of STAT5a/b on thymocyte development, similar to Jak3−/−, IL-7R−/−, and IL-7R−/− mice (10). In addition, cell lineage-specific ablation of STAT5a/b by CD4Cre resulted in the dramatic loss of peripheral naive CD8+ and CD4+ T cells (11). However, the role of STAT5 in effector T cell survival remains unclear. In this work, we tested the role of IL-7, IL-15, and STAT5 in the maintenance of effector CD4+ and CD8+ T cells during viral infection.

Materials and Methods

Mice and viral infection
C57BL/6 were purchased from The Jackson Laboratory (West Grove, PA) or Taconic Farms (Germantown, NY). IL-15-deficient mice on a C57BL/6 background were purchased from Taconic Farms. STAT5a/ bfl/fl mice were a gift of L. Hennighausen (National Institutes of Health, Bethesda, MD) and were crossed to C57BL/6 mice, and then crossed to B6.Cg-Tg(M1Cre)1Cgn transgenic mice. All mice were used between 3 and 8 mo of age. Mice were infected i.p. with 2 × 10^7 PFU of the Armstrong strain of lymphocytic choriomeningitis virus (LCMV). LCMV was grown in BHK-21 cells, and viral titers from spleen and liver homogenates were determined by plaque assay on BHK-21 monolayers, as described (18). Animals were housed under specific pathogen-free conditions in the Division of Veterinary Services, and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the Cincinnati Children’s Hospital Research Foundation.

IL-7 and IL-15 manipulation in vivo
In vivo IL-7 blockade experiments, M25 was grown as ascites, purified by ammonium sulfate precipitation and ion exchange chromatography, and injected i.p. at a dose of 3 mg/mouse every other day. Effectiveness of IL-7 blockade was assessed by measuring the numbers of pre-B cells in the bone marrow of each mouse via flow cytometry, using Abs against IgM, B220, and CD24. For IL-7 and IL-15 delivery experiments, human rIL-7-anti–IL-7Rα immu

MHC tetramer staining and flow cytometry
A total of 1–2 million single spleen cell suspensions was stained with different combinations of the following cell surface Abs: anti-CD4, CD8, CD44, KLRG1, and CD127 (from either BD Biosciences, San Jose, CA, or eBioscience, San Diego, CA), and intracellularly with either anti-CD103 (clone 3F11, produced in house), anti-STAT5 (Santa Cruz Biosciences), or with Minitab for Windows Software (Release 14) (State College, PA).

Results

Reciprocal expression of CD122 and CD127 on effector CD8+ and CD4+ T cells
Whereas expression of cytokine receptors by effector CD8+ T cells is dynamic (22, 23), few studies have examined cytokine receptor expression on activated, non-TCR transgenic CD4+ T cells. In this study, we assessed the cell surface expression of CD122 (IL-2/15R β-chain) and CD127 on effector CD4+ and CD8+ T cells following infection with LCMV. On day 8 post infection, most LCMV-specific (sp.) CD4+ and CD8+ T cells had decreased expression of CD127, but the reciprocal was true of CD122 (Fig. 1A). Thus, whereas decreased CD127 expression limited the availability of IL-7 to most effector T cells, these same T cells had substantial ability to compete for IL-15 or IL-2, given their increased expression of CD122.

IL-15 is critical to maintain Bcl-2 in most effector CD8+, but not CD4+ T cells
As IL-15 is critical for survival of most effector CD8+ T cells (7, 8), and effector CD4+ T cells also increased CD122 expression, we next asked whether IL-15 contributed to effector CD4+ T cell survival. First, we assessed the role of IL-15 in maintaining levels of the anti-apoptotic molecule, Bcl-2, within LCMV-sp. CD4+ and CD8+ T cells from wild-type C57BL/6 (BL/6) versus IL-15−/− mice. By day 20 post infection, Bcl-2 levels were decreased in both LCMV-sp. CD8+ and CD4+ T cells, and the lack of IL-15 led to a significant decrease Bcl-2 levels in LCMV-sp. CD8+, but not CD4+ T cells (Fig. 1B–D). By day 20 post infection, levels of Bcl-2 were increased in effector CD8+ T cells in BL/6, but a large population of effector CD8+ T cells failed to increase Bcl-2 levels in IL-15−/− mice (Fig. 1B, 1D), as shown previously (8). In separate experiments, we found that the cells expressing low levels of Bcl-2 in IL-15−/− mice also expressed high levels of KLRG1 (data not shown). Interestingly, IL-15 was not required to maintain Bcl-2 expression in effector CD4+ T cells on day 20 post infection (Fig. 1C). Together, these data show that IL-15 is critical for normal Bcl-2 expression in effector CD8+, but not CD4+, T cells.

IL-7 and IL-15 contribute redundantly to effector CD8+, but not CD4+, T cell survival
After acute LCMV infection, most effector CD8+ T cells were composed of two major subpopulations (3), a population of...
KLRG1\textsuperscript{high}CD127\textsuperscript{low} cells and another population that is KLRG1\textsuperscript{low}CD127\textsuperscript{high} (Fig. 2A). Given that KLRG1\textsuperscript{high}CD127\textsuperscript{high} CD8\textsuperscript{T} cells expressed both CD127 and CD122, it was logical that IL-7 and IL-15 might be redundant for maintaining this effector subpopulation. To determine the relative redundancy of IL-7 and IL-15 on effector CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell survival, we infected groups of either BL/6 or IL-15\textsuperscript{−/−} mice and treated them with either isotype control Ab or anti-IL-7 neutralizing Ab between days 10 and 20 postinfection.

In BL/6 mice, we found that IL-7 was critical for survival of some KLRG1\textsuperscript{high}CD127\textsuperscript{low} and some KLRG1\textsuperscript{low}CD127\textsuperscript{high} CD8\textsuperscript{T} cells (Fig. 2B). In contrast, IL-15 was critical for survival of most KLRG1\textsuperscript{high}CD127\textsuperscript{low} and some KLRG1\textsuperscript{low}CD127\textsuperscript{high} effector CD8\textsuperscript{T} cells (Fig. 2B). Neutralization of IL-7 in IL-15\textsuperscript{−/−} mice further decreased the numbers of both subsets of CD8\textsuperscript{T} cells (Fig. 2B). Furthermore, Bcl-2 levels correlated with the cells’ loss as the combined effects of IL-7 and IL-15 were required to maintain high levels of Bcl-2 in both subsets of CD8\textsuperscript{T} cells (Supplemental Fig. 1A, 1B). At no time was KLRG1 expression observed on LCMV-sp. CD4\textsuperscript{T} cells, although CD127 expression was increased on most of these cells by day 20 postinfection (Fig. 2C). Thus, although KLRG1 marked subpopulations of LCMV-sp. effector CD8\textsuperscript{T} T cells, the lack of KLRG1 on effector CD4\textsuperscript{T} T cells left CD127 as a potential marker to identify effector CD4\textsuperscript{+} subpopulations.

Neither the individual nor combined loss of IL-7 and IL-15 had a significant effect on the total numbers of CD4\textsuperscript{+} gp61-sp. T cells, irrespective of their CD127 expression (Fig. 2C, 2D). To ensure the effectiveness of IL-7 neutralization, we assessed pre-B cells in the bone marrow, as described (6). In both BL/6 and IL-15\textsuperscript{−/−} mice, administration of M25 caused a 20-fold loss of IgM\textsuperscript{+}B220\textsuperscript{−} BM pre-B cells (Supplemental Fig. 1C). Thus, dynamic regulation of cytokine receptors controls cytokine availability and thereby contributes to effector CD8\textsuperscript{+}, but not CD4\textsuperscript{+}, T cell survival.

\textbf{STAT5 is critical to maintain effector CD8\textsuperscript{+} T cells during LCMV infection}

STAT5 is known to be a common downstream signaling molecule for both IL-7 and IL-15 (24). To determine whether IL-7 or IL-15 could activate STAT5, we cultured effector T cells with the cytokines and assessed pSTAT5 using a phospho-STAT5–specific mAb and intracellular flow cytometry. IL-7 drove STAT5 phosphorylation in KLRG1\textsuperscript{high}CD8\textsuperscript{T} T cells, and higher pSTAT5 in KLRG1\textsuperscript{low}CD8\textsuperscript{T} T cells, the lack of KLRG1 on effector CD4\textsuperscript{T} T cells left CD127 as a potential marker to identify effector CD4\textsuperscript{+} subpopulations.

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\begin{figure}
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\caption{Dynamic cytokine receptor expression in LCMV-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells. A, Groups of BL/6 mice were uninfluen (n = 2) or infected i.p. (n = 5) with LCMV (2 × 10\textsuperscript{5} PFU) and sacrificed at day 8 postinfection, and spleen cells were stained with MHC class I (left panels) and MHC class II tetramers (right panels). Results show the levels of CD127 (top panels) or CD122 (bottom panels) in naive total CD4\textsuperscript{+} or CD8\textsuperscript{+} (open histograms) versus D\textsuperscript{gp33}-sp. or I-A\textsuperscript{dp61}-sp. (gray histograms). B and C, Groups of BL/6 (n = 5) or IL-15\textsuperscript{−/−} (n = 5) mice were infected with LCMV and sacrificed on day 10 or 20, and Bcl-2 levels in T cells were assessed by intracellular flow cytometry. Results show the levels of Bcl-2 within D\textsuperscript{gp33}-sp. (B) versus I-A\textsuperscript{dp61}-sp. (C, gray histograms, compared with total naive CD8\textsuperscript{+} (B) or CD4\textsuperscript{+} (C, open histograms). Dashed line histograms in upper panels represent isotype control staining. D, Results show the mean fluorescence intensity of the Bcl-2 signal in CD8\textsuperscript{gp33}-sp. T cells in either BL/6 or IL-15\textsuperscript{−/−} mice on either day 10 or 20 postinfection. Data are representative of three independent experiments.
\end{figure}
a STAT5-specific Ab and intracellular flow cytometry, we found that by day 6 after LCMV infection, most CD4+ I-Abgp61–sp. T cells in Mx1Cre-STAT5fl/fl mice had significantly decreased expression of STAT5 (Fig. 3A). Similar decreases in STAT5 levels were observed using SDS-PAGE and Western blotting of purified CD4+ T cell lysates with LCMV-infected Mx1Cre-STAT5fl/fl versus STAT5fl/fl mice (Fig. 3B). From day 6 to day 8, the frequency of CD4+ I-A\(^b\) gp61–sp. T cells that were STAT5\(^\text{low}\) remained constant (Fig. 3C). Furthermore, the total numbers of CD4+ I-A\(^b\)gp61+ T cells were slightly increased in Mx1Cre-STAT5fl/fl mice on day 6 postinfection, but were unchanged on days 7 and 8 after LCMV infection (Fig. 3D). The slight increase in LCMV-sp. CD4+ T cells in Mx1Cre-STAT5fl/fl mice on day 6 was not reproducible (data not shown).

In contrast, the frequency of LCMV-sp. CD8+ T cells that were STAT5\(^\text{low}\) in Mx1Cre-STAT5fl/fl mice was increased on day 6, increased further on day 7, and then decreased by day 8 postinfection (Fig. 3E, 3F). SDS-PAGE and Western blotting again confirmed the loss of STAT5 in purified CD8+ T cells (Fig. 3E, 3F). Whereas the total numbers of LCMV-sp. CD8+ T cells were not different between Mx1Cre-STAT5fl/fl versus STAT5fl/fl mice on day 7 after LCMV infection, they were significantly lower in Mx1Cre-STAT5fl/fl mice by day 8 postinfection. The total number of CD8+ gp33-sp. T cells on day 8 postinfection was also not different

![FIGURE 2.](https://example.com/figure2.png)
between BL/6 and Mx1Cre transgenic mice, arguing against a non-specific effect of Cre on the T cell response (data not shown). As previous work showed that STAT5 was critical for perforin expression (27, 28), it was possible that the loss of STAT5 might result in persistent viral infection that could influence CD8+ T cell responses. However, no significant differences in viral loads were observed from either the livers or spleens of STAT5fl/fl nor Mx1Cre-STAT5fl/fl mice on days 6 or 7 postinfection (Supplemental Fig. 2B).

It was also possible that the diminution of T cell numbers in infected Mx1Cre-STAT5fl/fl mice reflected a STAT5 contribution to T cell proliferation. However, using in vivo BrdU labeling, the frequency of CD8+gp33-sp. T cells that were BrdU+ was actually increased in Mx1Cre-STAT5fl/fl compared with STAT5fl/fl mice on day 8 postinfection (Fig. 4A). In addition, there was no difference in BrdU uptake in cells that were STAT5low compared with those that were STAT5high at this time point (Fig. 4B). Together, these data show that STAT5 is critical to maintain CD8+, but not CD4+, effector T cells.

FIGURE 3. STAT5 is critical for maintaining effector CD8+, but not CD4+, T cells. Groups of STAT5fl/fl or Mx1Cre-STAT5fl/fl mice (n = 3–5/group) were infected with LCMV and sacrificed 6–8 d later. Spleen cells were surface stained with either 1-A^b gp61 or D^b gp33 tetramer along with Abs against total STAT5. A, C, E, and G, Frequencies of MHC tetramer+ cells that were STAT5low (C, G) and total overall numbers of tetramer+ T cells (D, H) are shown. Data from day 8 were pooled between two independent experiments. Shown are p values from a Student t test analysis. B and F, Groups of either STAT5fl/fl or Mx1Cre-STAT5fl/fl mice (n = 3–5/group) were infected with LCMV and sacrificed 8 d later. Total CD4+ (B) or CD8+ (F) were purified using a pan-T cell isolation kit (Miltenyi Biotec), and 1 × 10^6 cell equivalents were subjected to SDS-PAGE and Western blotting for either STAT5 or actin. Data in B and F were done in separate experiments. The bands displayed in B for actin and STAT5 were from separate lanes run on the same gel. Data are representative of two independent experiments.

STAT5 is critical to maintain both effector CD8+ T cell subpopulations

Because IL-15 was critical for KLRG1highCD127low cells, but IL-7 and IL-15 acted in concert to maintain KLRG1lowCD127high cells, we next tested the requirement for STAT5 on these two subpopulations. Importantly, deletion of STAT5 was similar in both CD8+ T cell subpopulations (Supplemental Fig. 3A). The frequency of KLRG1highCD127low and KLRG1lowCD127high CD8+ T cells was similar in both STAT5fl/fl and Mx1Cre-STAT5fl/fl mice (Fig. 5A). However, both subsets of effector CD8+ T cells were significantly reduced in Mx1Cre-STAT5fl/fl compared with STAT5fl/fl mice (Fig. 5A). As expected, the frequencies of CD127high and total numbers of LCMV-sp. CD4+ T cells were not different between STAT5fl/fl and Mx1Cre-STAT5fl/fl mice (Fig. 5B).

STAT5 is critical for IL-7– and IL-15–driven upregulation of Bcl-2

As IL-15 was critical for Bcl-2 expression (Fig. 1B) and STAT5 was critical for maintenance of effector CD8+ T cells (Fig. 3G, 3H), we next determined the role of STAT5 in promoting Bcl-2 expression. Even though fewer CD8+gp33-sp. cells were STAT5low by day 15 postinfection compared with day 8 (31.3 ± 4.8% versus 46.1 ± 8.1%, respectively; Supplemental Fig. 3B, 3C), Bcl-2 levels in LCMV-sp. CD8+ T cells from Mx1Cre-STAT5fl/fl mice were significantly reduced compared with cells from STAT5fl/fl mice (Fig. 5C). Furthermore, levels of Bcl-2 were significantly lower in STAT5low (Bcl-2 mean fluorescence intensity [MFI] = 2116 ± 101) compared with STAT5high (Bcl-2 MFI = 3356 ± 158) CD8+gp33-sp. T cells from Mx1Cre-STAT5fl/fl mice (p < 0.0001; Student t test).

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As expected, Bcl-2 levels in CD4+ gp61-sp. T cells were not affected by STAT5 deletion (Fig. 5C). Moreover, the frequencies of CD4+ gp61-sp. T cells that were STAT5low were similar between days 8 and 15 (56.5 ± 6.55% versus 50.6 ± 0.98%, respectively; Supplemental Fig. 3B).

To assess the requirement for STAT5 in upregulation of Bcl-2 by IL-7 and IL-15, we cultured spleen cells from day 7 LCMV-infected STAT5fl/fl or Mx1Cre-STAT5fl/fl mice with IL-7 or IL-15 and assessed levels of Bcl-2 within gp33-sp. CD8+ T cells the next day. Notably, both IL-7 and IL-15 increased expression of Bcl-2 in a dose-dependent fashion in T cells from STAT5fl/fl mice, whereas upregulation of Bcl-2 was significantly impaired in T cells from Mx1Cre-STAT5fl/fl mice (Fig. 6A, 6B). The slight induction that was observed in response to IL-7 and IL-15 in Mx1Cre-STAT5fl/fl T cells was most likely due to incomplete deletion of STAT5 within these cells, as only cells expressing high levels of STAT5 increased expression of Bcl-2 in response to IL-7 or IL-15 (Supplemental Fig. 3B). Combined, these data demonstrate that both IL-7 and IL-15 require STAT5 to induce Bcl-2 within effector CD8+ T cells.

The mechanism by which STAT5 controls Bcl-2 expression is controversial. One report suggested that the effects of STAT5 are indirect (i.e., STAT5 inducing expression of another factor that induces Bcl-2 transcription) (29). Other reports have suggested direct effects of STAT5 on Bcl-2 transcription (30, 31). To determine which of these mechanisms contributes to cytokine-driven Bcl-2 expression in T cells, we cultured purified CD8+ T cells from LCMV-infected BL/6 mice with IL-7 or IL-15 with or without cycloheximide for 3 h and then measured Bcl-2 mRNA levels by real-time RT-PCR. Interestingly, whereas both IL-7 and IL-15 drove significant induction of Bcl-2 within 3 h, the presence of cycloheximide did not reduce Bcl-2 mRNA levels in response to these cytokines (Fig. 6C, 6D), providing evidence that the effect of STAT5 on Bcl-2 expression is direct (i.e., it did not require new protein synthesis).

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IL-7 and IL-15 depend on STAT5 to maintain effector T cell responses

As both IL-7 and IL-15 required STAT5 to induce Bcl-2 expression in vitro, we next determined whether IL-7 or IL-15 required STAT5 to maintain effector T cell responses in vivo. To do this, we infected mice with LCMV, and starting on day 10 postinfection treated mice with long-acting forms of either IL-7 or IL-15 (6, 32) every other day until day 15 postinfection. On day 15, we found that both IL-7 and IL-15 significantly increased numbers of CD8+ gp33-sp. T cells in STAT5fl/fl mice (Fig. 6A, 6F). In contrast, IL-7 was unable to significantly increase gp33-sp. CD8+ T cells in Mx1Cre-STAT5fl/fl mice (Fig. 6F). Whereas IL-15 did significantly increase gp33-sp. T cells in Mx1Cre-STAT5fl/fl mice, the increase was less than in STAT5fl/fl mice (Fig. 6F) and the levels of STAT5 were enhanced in Mx1Cre-STAT5fl/fl compared with STAT5fl/fl controls (data not shown). Thus, IL-15 most likely enhanced selection of STAT5high T cells in vivo. Combined, these data show that IL-7 and IL-15 largely require STAT5 for maintaining effector CD8+ T cells in vivo.

Discussion

In this study, we demonstrate that STAT5 is critical to maintain effector CD8+ T cells. In this model, STAT5 is deleted in multiple tissues as the Mx1Cre transgene is expressed ubiquitously. It is possible that some of the effects we observe could be due to deletion of STAT5 in non-T cells. For instance, STAT5 signaling in dendritic cells (DCs) may influence survival of effector T cells, as it has recently been shown that IL-7 can act on DCs to regulate the size of the CD4 T cell pool under lymphopenic conditions (33). However, whether this axis operates under normal physiologic conditions or during viral infection remains unclear. If there were a non-T cell effect of STAT5 that was dominant, we would have expected to see similar effects on CD4+ and CD8+ T cell responses as expansion of both cells require DCs, and this was clearly not the case. Although we cannot completely rule out non-T cell effects of STAT5, our data clearly show that despite similar deletion of STAT5, CD8+ effector T cells rely on STAT5 considerably more than do effector CD4+ T cells. We were somewhat surprised to find that IL-15 was not required for maintaining effector CD4+ T cell responses, as a recent report showed that IL-15 contributes to survival of memory CD4+ T cells (34). It is possible that IL-15 becomes a more prominent survival factor for memory CD4+ T cells than for effector CD4+ T cells. Indeed, we found that expression of CD122 was quite transient on effector CD4+ T cells (data not shown), and previous work showed that increased expression of CD122 on memory CD4+ T cells (34) may facilitate their dependence on IL-15. We note that our data do not imply that IL-15 is not involved in effector CD4+ T cell survival, just that it is not strictly required. Thus, mechanisms maintaining effector CD4+ T cells remain unclear. Previous work has shown that Bcl-3, a NF-kB p50 family member, can promote survival of activated CD4+ T cells (35). Furthermore, activated CD4+ T cells have dramatically increased expression of A1 (1, 2), and A1 expression can be regulated by NF-kB signaling (36–38). A1 can also antagonize Bim, making A1 a logical candidate for promoting effector CD4+ T cell survival. Future experiments will evaluate the requirement for A1 in the survival of effector CD4+ T cells.

Our data in effector CD8+ T cells are consistent with previous reports showing that in cell lines, IL-7 and IL-15 control expression of Bcl-2 via STAT5 (29, 31, 39). However, other reports have suggested that STAT5 is not critical for Bcl-2 induction (40, 41). In one of these reports, mice were developed in which tyrosine 449 in the IL-7Rα gene was mutated to a phenylalanine (41). The Y449F mutation incapacitates both STAT5 and PI3K activation in response to IL-7 in lymphocytes and thymocytes (39, 41). Using T cells from these Y449F mice, the authors showed that although IL-7 was unable to induce detectable pSTAT5, it was able to increase expression of Bcl-2 (41). From these data it was concluded that Bcl-2 upregulation was independent of STAT5 activation. However, at baseline, Bcl-2 levels were significantly lower in peripheral CD4+ and CD8+ T cells directly isolated from Y449F mice (41), suggesting that, physiologically, IL-7RαY449 signaling controls expression of Bcl-2 in vivo. Consistent with this, another group showed, in a thymocyte cell line, that Bcl-2 upregulation and STAT5 activation in response to IL-7 signaling required Y449F of IL-7Rα (39). Furthermore, mice deficient in JAK3, which is required for IL-7 and IL-15 activation of STAT5, exhibited a profound loss of peripheral CD8+ T cells, but had nearly normal levels of CD4+ T cells (42–44). Moreover, JAK3 deficiency dramatically impaired Bcl-2 expression in CD8+CD4−, but not CD4+CD8+ thymocytes (45). Thus, the simplest explanation is that, under physiologic conditions, IL-7 and IL-15 require STAT5 to maintain Bcl-2 expression within CD8+ T cells.
Our data also suggest that STAT5 maintains Bcl-2 directly as cycloheximide failed to block Bcl-2 induction by IL-7 and IL-15. A previous paper showed in a pro-B cell line that cycloheximide blocked induction of Bcl-2 by IL-2, leading the authors to conclude that the effects of STAT5 on Bcl-2 induction by IL-2 were largely indirect (29). However, in this study, the effect of cycloheximide was only partial and these results were not reported to be significant. Our results in primary T cells suggest that the effects of IL-7 on Bcl-2 induction are direct and do not require new protein synthesis. These data are supported by a recent report showing that, in mast cells, STAT5 can bind to a site in intron 2 of the Bcl-2 gene (30). Further work will be required to determine whether cytokines promote STAT5 binding to this site in intron-2 in activated CD8+ T cells. These data have implications for the development of T cell memory that emerges from the effector pool. We and others have shown, common γ-chain cytokines can significantly enhance CD4+ and CD8+ T cell responses (6, 7, 48). Although these cytokines enhanced short-term T cell responses, their effects were transient once the cytokines were withdrawn. This makes sense given that survival of nearly all populations of T cells is achieved, at least in part, via competition for limiting amounts of cytokines. However, we note that, in general these studies were done in viral infections in which the T cell responses are extraordinarily robust. It remains unclear whether cytokine adjuvants, given short-term, may enhance long-term memory responses under conditions in which less robust T cell responses are generated. This approach could be beneficial for enhancing suboptimal vaccine responses that currently require several rounds of boosting.

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References


