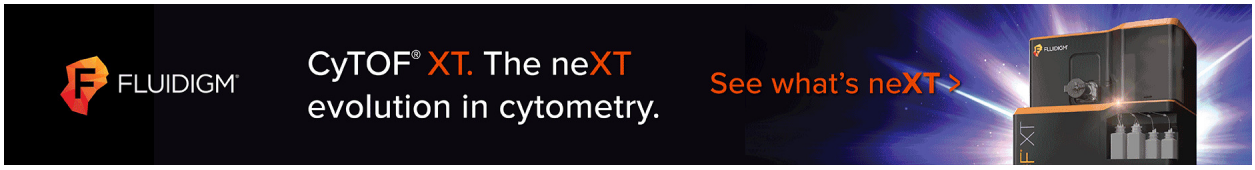

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CUTTING EDGE

Cutting Edge: Limiting Amounts of IL-7 Do Not Control Contraction of CD4$^+$ T Cell Responses[1]

Pulak Tripathi, Thomas C. Mitchell,† Fred Finkelman,* and David A. Hildeman[2]**

***During the acute T cell response most effector T cells die while some survive and become memory T cells. Selective expression of CD127 (IL-7Rα) on effector T cells has been proposed to engender their survival into the memory pool. We assessed the role of IL-7 in effector T cell survival using MHC class II tetramers to track a CD4$^+$ T cell response following infection with a recombinant vaccinia virus (rVV-2W1S). Exogenous IL-7 prevented the contraction of the 2W1S-specific CD4$^+$ T cell response after rVV-2W1S infection. IL-7 increased proliferation of, and Bcl-2 expression within, 2W1S-specific T cells; the latter was required for IL-7-driven prevention of contraction. Conversely, in vivo neutralization of IL-7 or Bcl-2 did not exacerbate the contraction of 2W1S-specific CD4$^+$ T cells. These data suggest that IL-7 administration may enhance the survival of effector T cells but that IL-7 is not the limiting factor during normal contraction of the response. The Journal of Immunology,* 2007, 178: 4027–4031.**

Following T cell clonal expansion, homeostasis is re-established via the induction of apoptosis in the majority of activated T cells while some survive and become memory T cells. This decision between death and survival is likely crucial for promoting immunological memory and protective immunity. However, the factors that control this cell death/survival decision remain unclear.

Selective expression of CD127 on a subpopulation of effector CD8$^+$ T cells has been proposed to "mark" long-lived memory T cells or precursors thereof (1, 2). Because IL-7 is a survival factor for naive and memory T cells (3–7), perhaps the few CD127^{high} effector T cells compete for limiting amounts of IL-7 and are, therefore, selected to become memory T cells. However, we recently showed that significant numbers of lymphocytic choriomeningitis virus (LCMV)[3]-specific CD127^{low} T cells also survive contraction (8). Furthermore, others have reported that a substantial number of Ag-specific CD127^{high} T cells die during the contraction of the T cell response to peptide immunization (9). Moreover, during chronic LCMV infection a substantial number of CD127^{low} T cells persist and can re-express CD127 once the virus is cleared (10). Thus, it remains unclear whether competition for IL-7 is the mechanism that regulates contraction of the T cell response.

In this study, we determined whether the manipulation of IL-7 levels in vivo could affect the contraction of the Ag-specific CD4$^+$ T cell response to a recombinant vaccinia virus (rVV) infection. IL-7 prevented the contraction of the response through the induction of the prosurvival molecule Bcl-2. Interestingly, neutralization of either Bcl-2 or IL-7 failed to exacerbate contraction of the response. Taken together, these data suggest that IL-7 is not the limiting factor governing the survival of effector CD4$^+$ T cells during the contraction of the response.

Materials and Methods

Mice and injections

C57BL/6 mice were purchased from either The Jackson Laboratory or Taconic Farms. Mice were used between 8 and 11 wk of age and were housed under specific pathogen-free conditions in the Animal Facility at the Children's Hospital Research Foundation (Cincinnati, OH). Mice were injected with rVV (4×10^6 pfu/mouse) via the i.p. route. Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the Children's Hospital Research Foundation. ABT-737 (11) was dissolved and diluted in 30% polyethylene glycol, 5% Tween 80, and 65% of a 5% dextrose in water solution. Mice were injected i.p. once a day with 75 mg/kg in 0.2 ml.

Cytokines

Recombinant human IL-7 was obtained through the National Institute of Allergy and Infectious Diseases (Bethesda, MD) reagents program. IL-7 immune complexes (ICs) were generated by incubating IL-7 with anti-IL-7 (M25) in a 2:1 molar ratio for 2 min at room temperature in PBS. Complexes were diluted in balanced salt solution (BSS) with 5% normal mouse serum and injected i.p. For 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 per mouse every other day.

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[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; BM, bone marrow; BSS, balanced salt solution; IC, immune complex; int, intermediate; rVV, recombinant vaccinia virus.

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Generation of recombinant virus and MHC tetrameric staining reagents

rVV expressing I-A^b with the covalently bound I-Eα mutant peptide EAWGA LANWAVDSA, referred to as "rVV-2W1S" (12, 13) was generated by cloning cDNA encoding a I-A$^b\beta$-chain-2W1S peptide-GFP fusion protein into the pSC11 vector. Homologous recombination was performed by transfecting 143B cells with pSC11 and then infecting them with the vaccinia virus. Viral stocks were purified by infecting 143B cells and sorting for GFP$^+$ cells 24 h after infection. Initial viral stocks were purified by three rounds of sorting and stocks of virus were grown from the initial seed stock. Class II MHC tetrameric staining reagents were created as described (8, 14).

Flow cytometric staining

To detect 2W1S-specific CD4$^+$ T cells, 2×10^6 lymph node or spleen cells per well were stained with I-A^{b}2W1S tetrameric staining reagent for 2 h at 37°C. During the last 45 min of incubation cells were stained with various combinations of cell surface marker Abs (e.g., anti-CD4, -CD8, -CD16/32, -CD44, -CD62L, or -CD127 from either BD Pharmingen or eBioscience or produced in house) and then washed and fixed with 2% paraformaldehyde. Intracellular staining for Bcl-2 was as described (15). Effectiveness of IL-7 blockade was assessed by measuring the numbers of immature B cells in the bone marrow (BM) via flow cytometry using fluorescent Abs against IgM, B220, and CD24. T cell proliferation in vivo was determined by i.p. injection of BrdU (1 mg per mouse per day) between days 7–14 after the infection and incorporation of BrdU was assessed using a kit (BD Biosciences). Data were acquired using a FACSCalibur flow cytometer and analyzed using CellQuest software.

Statistical analyses

Statistical analyses were performed using an unstacked one-way ANOVA with Minitab for Windows software (release 14). Results show the mean ± SEM.

Results and Discussion

Characterization of Ag-specific CD4$^+$ T cells during recombinant vaccinia virus infection

We generated a rVV that expresses an I-A^b peptide molecule, previously referred to as 2W1S (8, 12), along with I-A^b tetrameric staining reagents containing the 2W1S peptide (8, 12, 13). To assess the surrogate anti-VV CD4$^+$ T cell response, we infected groups of C57BL/6 mice with rVV-2W1S, sacrificed them at various times after infection, and tracked 2W1S-specific T cells using the I-A^b-2W1S tetramer. After i.p. infection, the kinetics of the response were nearly identical between the lymph nodes and the spleen of infected animals, although >90% of the 2W1S-specific T cells were observed in the spleen (data not shown). The 2W1S-specific CD4$^+$ T cell response peaked on days 7–8 after infection, rapidly declined between days 9 and 15, and then slowly declined between days 15 and 120 (Fig. 1*A*).

We next characterized the cell surface markers on 2W1S-specific CD4$^+$ T cells following rVV-2W1S infection. At the peak of the response roughly 0.5% of CD4$^+$ T cells were 2W1S-specific, an amount that declined roughly 5-fold by day 14 after infection (Fig. 1*A*). As expected, nearly all of the 2W1S-specific T cells expressed the activation/memory marker CD44 (Fig. 1*A*). Similar to our previous data on LCMV-specific CD4$^+$ T cells (8), the expression of CD127 was dynamic on 2W1S-specific CD4$^+$ T cells after rVV-2W1S infection. On day 7 after infection only 23% of 2W1S-specific T cells were CD127^{high} (Fig. 1*B*). By day 14 after infection nearly all of the 2W1S-specific T cells were CD127^{high} and remained CD127^{high} through at least day 120 (Fig. 1*B*). Thus, the proportion of 2W1S-specific T cells that are CD127^{high} increased during the contraction phase of the response, as has been previously described in other models (1, 2, 8).

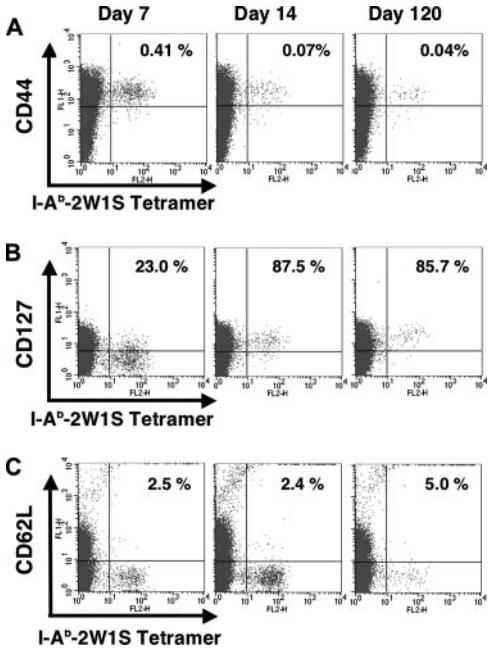

FIGURE 1. Characterization of the 2W1S-specific CD4$^+$ T cell response. Groups of C57BL/6 mice (n = 3–6 per time point) were infected with rVV-2W1S, sacrificed at 7, 14, and 120 days after infection, and stained with class II MHC tetramers and Abs to CD44, CD62L, and CD127. Numbers in the *upper right quadrants* indicate the percentages of CD4$^+$ T cells that were I-A^b-2W1S$^+$ (*A*) or the percentages of I-A^b-2W1S$^+$ T cells that were either CD127^{high} (*B*) or CD62L$^+$ (*C*) at the indicated days after infection. Data are pooled from independent experiments and are representative of at least two experiments with similar results.

We next examined levels of CD62L to distinguish between "central" memory cells (i.e., CD62L^{high}) and "effector" memory (i.e., CD62L^{low}) on CD44^{high} 2W1S-specific T cells. Interestingly, at all time points examined essentially all of the 2W1S-specific T cells were CD62L^{low} (Fig. 1*C*). These data suggest a relative lack of central memory T cell development within 2W1S-specific T cell population. Thus, following rVV-2W1S infection, Ag-specific CD4$^+$ T cells first expand and develop into an effector memory population.

Administration of IL-7 IC prevents contraction of the I-A^b-2W1S+ T cell response

Observations that CD127^{high} T cells become enriched during the contraction of the T cell response (1, 2, 8) suggest that T cells expressing high levels of CD127 may better compete for IL-7, survive, and become memory cells. To test whether IL-7 is a limiting factor for effector T cell survival, we treated rVV-2W1S-infected mice with IL-7. To prolong cytokine availability in vivo we injected mice with IL-7 ICs because complexing the cytokine with Ab significantly increases IL-7 half-life (16). In a dose-dependent fashion, the administration of IL-7 IC increased numbers of 2W1S-specific CD4$^+$ T cells on day 15 (Fig. 2*A*). We next assessed whether IL-7 administration affected the proliferation of 2W1S-specific CD4$^+$ T cells by performing in vivo BrdU labeling. IL-7 IC significantly increased the percentage of 2W1S-specific T cells that were BrdU$^+$ (Fig. 2*B*), suggesting that IL-7 either increased proliferation or rescued proliferating cells that normally would have died.

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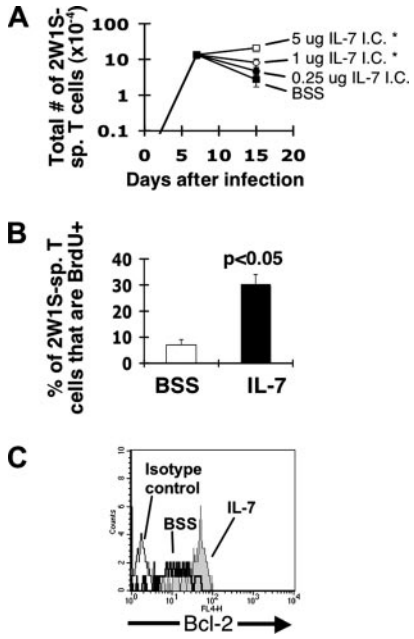

FIGURE 2. Administration of IL-7 IC blocks the contraction of the 2W1S-specific T cell response. *A*, Groups of C57BL/6 mice ($n = 3$ mice/group) were infected with rVV-2W1S. One group was sacrificed on day 7 after infection while other groups received either 0, 250 ng, 1 μg, or 5 μg of IL-7 IC on days 7, 9, and 11 after infection and were sacrificed on day 15. Results show the number of I-A^b-2W1S-specific T cells ± SEM. *, A significant increase in the numbers of 2W1S-specific T cells compared with BSS-treated mice ($p \leq 0.04$). *B* and *C*, Groups of C57BL/6 mice ($n = 3$–5 mice per group) were infected with rVV-2W1S and were injected with either the IL-7 IC (2.5 μg/mouse) or BSS on days 7, 9, 11, and 13 after infection and sacrificed at day 15. Mice in both groups received BrdU. *B*, Results show the percentage of 2W1S-specific T cells that were $BrdU^+$ ± SEM. A significant increase in $BrdU^+$ T cells was observed in mice treated with IL-7 IC ($p < 0.05$). *C*, IL-7 increases Bcl-2 expression in 2W1S-specific $CD4^+$ T cells. Histogram shows Bcl-2 staining in 2W1S-gated T cells from mice treated with BSS (dark line, open histogram) or mice treated with IL-7 (light line, gray-shaded histogram). Data are representative of two experiments with similar results.

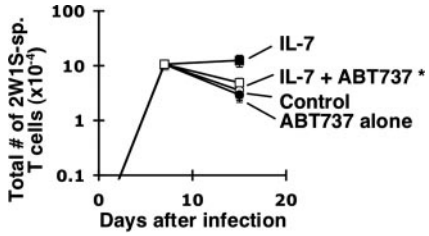

FIGURE 3. ABT-737 prevents the antiapoptotic effects of IL-7 but does not exacerbate normal contraction of the response. Groups of C57BL/6 mice ($n = 4$–6) were infected with rVV-2W1S. One group of mice was sacrificed on day 7 after infection. The other groups were injected with vehicle alone on days 6–14 (control; ○), ABT-737 (75 mg/kg) alone on days 7–14 (ABT737 alone; ●), IL-7 IC on days 7, 9, 11, and 13 and vehicle on days 7–14 (IL-7; ■), or IL-7 IC on days 7, 9, 11, and 13 and ABT-737 (75 mg/kg) on days 7–14 (IL-7 + ABT-737; □). On day 15 after infection, mice were sacrificed and the numbers of $CD4^+$ 2W1S-specific T cells were assessed in the spleen. *, Significant difference in the numbers of 2W1S-specific T cells between IL-7 treated and IL-7 plus ABT-737 treated mice ($p \leq 0.05$). No significant difference was observed between control mice and mice receiving ABT-737 alone. Data are representative of two experiments with similar results.

We next assessed the effect of IL-7 on survival by measuring levels of the anti-apoptotic molecule Bcl-2 in 2W1S-specific $CD4^+$ T cells by intracellular flow cytometry. As expected, IL-7 substantially increased levels of Bcl-2 within 2W1S-specific T cells compared with PBS-injected controls (Fig. 2*C*). Taken together, these data suggest that IL-7 maintains effector $CD4^+$ T cell number by promoting proliferation and/or enhancing survival through increasing Bcl-2 expression.

A synthetic Bcl-2/Bcl-x_L/Bcl-w inhibitor significantly prevents IL-7-driven rescue of effector T cells but does not exacerbate the normal contraction of the response

We next tested whether a Bcl-2 family member was required for IL-7-driven survival effects in vivo by using a synthetic inhibitor of Bcl-2, ABT-737 (11). ABT-737 has high affinity for Bcl-2, Bcl-x_L, and Bcl-w but has very low affinity for Mcl-1 and A1 (11). We administered IL-7 ICs with and without ABT-737 to groups of rVV-2W1S-infected mice and assessed the numbers of 2W1S-specific $CD4^+$ T cells. ABT-737 significantly reduced the numbers of 2W1S-specific T cells when coadministered with IL-7, thereby attenuating the prosurvival effects of IL-7 (Fig. 3). Thus, IL-7-driven induction of anti-apoptotic Bcl-2 family members is critical to its survival effects in vivo, although the identity of the particularly critical Bcl-2 family member is unclear.

We also administered ABT-737 to rVV-2W1S infected mice that had not received IL-7 to control for the effects of ABT-737 on the 2W1S-specific T cell response. When given between days 7–14 after rVV-2W1S infection, ABT-737 essentially had no effect on the contraction of the 2W1S-specific T cell response (Fig. 3). If certain Bcl-2 family members were critical for the IL-7 driven survival and IL-7 was the limiting factor for T cells surviving contraction under normal circumstances, then ABT-737 should have significantly exacerbated the contraction of the response. Thus, Bcl-2, Bcl-x_L, or Bcl-w is not required for the survival of effector $CD4^+$ T cells in vivo. Further, because ABT-737 largely prevented IL-7-dependent survival in vivo but did not exacerbate the contraction of the response, this result brings into question the role of IL-7 in the selective survival of effector T cells.

IL-7 levels do not dictate the contraction of the 2W1S-specific $CD4^+$ T cell response

To determine whether IL-7 is the limiting factor that promotes in vivo survival of effector T cells, we neutralized IL-7 in rVV-2W1S-infected mice. In contrast to the small amounts of anti-IL-7 used to deliver IL-7, large doses of anti-IL-7 neutralizes IL-7 activity by outcompeting endogenous receptors for IL-7. It is unlikely that these large doses of anti-IL-7 will have a stimulatory effect because when bound to M25 IL-7 cannot simultaneously bind to CD127 and when IL-7 dissociates from M25 it will be readily bound by another anti-IL-7 Ab as M25 is in vast excess. Interestingly, IL-7 neutralization had little effect on the percentage of 2W1S-specific T cells (Fig. 4*A*). Although the numbers of 2W1S-specific T cells were slightly decreased with anti-IL-7 treatment, the difference was not significant (Fig. 4*C*). Further, the effect was not specific for 2W1S-specific $CD4^+$ T cells because naive ($CD44^{low}$) $CD4^+$ T cells were decreased similarly (64.4 ±15.5 vs 74 ± 4.7% remaining, respectively, $p < 0.526$; one-way ANOVA). Because it was possible

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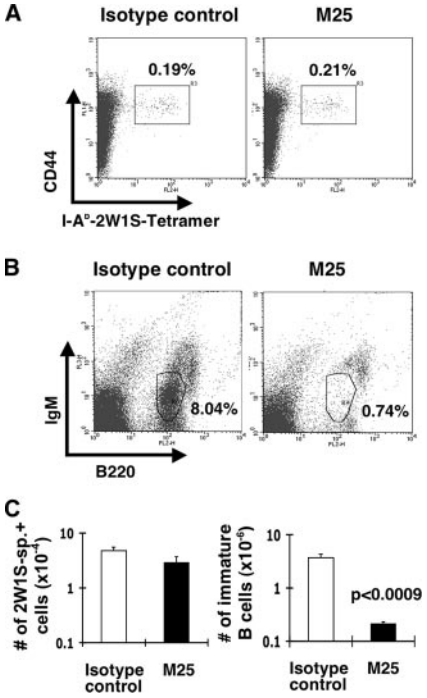

FIGURE 4. IL-7 neutralization does not enhance contraction of CD4$^+$ T cell responses. Groups of C57BL/6 mice ($n = 4$–5 mice/group) were injected i.p. with rVV-2W1S and, on days 6, 8, 10, and 12, were injected i.p. with 3 mg of either isotype control or anti-IL-7 (M25) Ab. Mice were sacrificed on day 15 and the numbers of I-A^b-2W1S-specific T cells were quantified in the spleen. As an internal control, BM from the individual mice was harvested and stained for B220, IgM, and HSA. *A* and *B*, Percentages of splenic I-A^b-2W1S$^+$ T cells (*A*) and BM immature B cells (IgM$^+$B220^{int}) in isotype control vs M25 treated mice. *C*, Total numbers of I-A^b-2W1S$^+$ T cells and immature B cells in isotype control vs M25-treated mice. Numbers of 2W1S-specific (2W1S-sp.) T cells between isotype control and M25-treated mice were not significantly different ($p < 0.117$). Numbers of immature B cells were significantly decreased in M25-treated mice ($p < 0.0009$).

that we had not neutralized all of the IL-7 in vivo and pre-B cells require IL-7 for survival and differentiation (17), we assessed the effects of IL-7 neutralization on BM pre-B (IgM$^-$B220^{int}) and immature B cells (IgM^{dull}B220^{int}, where int is intermediate). Anti-IL-7 treatment nearly completely ablated the population of IgM$^{-\text{ to dull}}$B220^{int} cells, demonstrating the effectiveness of IL-7 neutralization in vivo (Fig. 4, *B* and *C*). Taken together, these data strongly suggest that effector T cells do not depend on IL-7 any more than do naive T cells.

Our data suggest that IL-7/IL-7R signaling is not critical for effector CD4$^+$ T cells to survive the contraction of the response. We showed that the neutralization of IL-7 in vivo had little effect on the magnitude of the contraction of the response, despite a near complete ablation of immature B cells in the BM. It remains formally possible that IL-7R still plays a major role in the selection of effector T cells because it also binds thymic stromal lymphopoietin. However, the similarities between IL-7-deficient and IL-7R-deficient mice suggest that thymic stromal lymphopoietin plays a limited role in peripheral T cell survival (18, 19). Thus, while therapeutic manipulation of IL-7 levels in vivo may enhance memory T cell numbers, IL-7 levels themselves do not appear to control the process.

The transition from naive to effector T cell is accompanied by substantial changes in gene expression (20). We and others have shown that effector T cells have significantly decreased expression of Bcl-2 (15, 21, 22). Because Bcl-2 acts as a survival factor for naive T cells (23, 24), its decreased expression in effector T cells likely contributes to their shorter half-life (15). Although it is possible that a small percentage of Bcl-2-expressing T cells survive contraction, our data here strongly argue against this possibility. We previously showed in both superantigen and LCMV models that the Bcl-2 antagonist Bim is critical for the contraction of the CD4$^+$ and CD8$^+$ T cell response (8, 15). Thus, effector T cells that survive contraction likely need to counteract the effects of Bim in an IL-7- and Bcl-2-independent manner.

In summary, we have shown that while IL-7 administration can increase the number of T cells that survive contraction, this effect requires the induction of anti-apoptotic Bcl-2 family members. However, neither IL-7 nor Bcl-2 appears to be necessary to limit the normal contraction of the CD4$^+$ T cell response and allow memory cell survival.

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Disclosures

The authors have no financial conflict of interest.

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