International Immunology, Vol. 13, No. 6, pp. 763–768

T lymphocytes need IL-7 but not IL-4 or IL-6 to survive *in vivo*

Laëtitia Vivien, Christophe Benoist[1] and **Diane Mathis[1]**

Institut de Genetique et de Biologie Moleculaire et Cellulaire (CNRS/INSERM/ULP), 1 rue Laurent Fries, 67404 Illkirch, C. U. de Strasbourg, France

[1]Present address: Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA

Keywords: cytokine, homeostasis, IL, signaling, survival, T cell

Abstract

The role of IL-4, -6 and -7 in the survival of T lymphocytes was studied *in vivo*. The decay of polyclonal populations of CD4$^+$ and CD8$^+$ T cells was monitored in thymectomized anti-cytokine receptor mAb-treated and/or cytokine-deficient mice. The lack of IL-4 or -6 did not have any detectable effect on T cell survival, but IL-7 played an important role in the survival of the naive T cell compartment, especially of naive CD4$^+$ T cells.

Introduction

Like most cells in multicellular organisms, T lymphocytes residing in the peripheral lymphoid organs undergo homeostatic regulation (1). However, this control can sometimes be subverted—genetically, by infection, or due to artificial perturbations such as drugs or irradiation. It is important to elucidate the factors regulating T cell homeostasis because this could suggest novel strategies to promote population recovery in lymphopenic individuals or to maintain a vigorous immune system after involution of the thymus in older individuals.

Another equilibrium that the immune system needs to maintain is that between naive and memory lymphocytes. The continuous arrival in the periphery of T cells newly produced in the thymus should not compromise the memory pool, preselected to make a fast, effective response to already encountered antigens; and successive infections should not diminish the diversity of the naive pool, ready to mount a primary response against new challenges. At least for the CD8$^+$ T lymphocyte compartment, the homeostatic regulation of naive cells is independent of that of memory cells (2,3); it is not known whether the same is true of the CD4$^+$ lymphocyte compartment.

Some factors are already known to be important for the peripheral survival of T lymphocytes. Chief amongst these are MHC molecules. Naive CD8$^+$ T cells disappeared very quickly in an environment deprived of MHC class I molecules or in one expressing a class I allele different from that responsible for positive selection in the thymus (4,5). The data are more controversial for memory CD8$^+$ T cells. In some studies, memory CD8$^+$ cells required class I molecules in the periphery, though not necessarily the positively selecting allele (4,6). In others, MHC molecules were not required for the survival of CD8$^+$ memory cells (7). Likewise, naive CD4$^+$ T cells needed to encounter MHC class II molecules in the periphery in order to survive, even if they appeared to disappear significantly less quickly than CD8$^+$ T cells deprived of MHC class I molecules (8–10); survival required the positively selecting class II allele (11,12) and expression of class II molecules on dendritic cells sufficed (13). Again, there is controversy over the MHC dependency of memory cells. Although it is generally agreed that the positively selecting class II allele is not required, it has been reported that memory CD4$^+$ T cells need just any (14) or no (15) class II molecules.

Other factors thought to be involved in T cell survival are cytokines. There seems to be a consensus that IL-15 is important for maintenance of the memory CD8$^+$ T cell compartment (16–20). Most of the results obtained on naive T lymphocytes derive from *in vitro* studies. IL-4 was able to rescue naive T cells from the rapid apoptosis they normally undergo in culture (21–23); IL-6 could rescue murine (24) but not human (21) T cells; IL-7, which plays a crucial role in early thymocyte differentiation (25,26), was also an important T cell survival factor in culture (21–23,27–29). One *in vivo* study showed that at least one of the cytokines whose receptor uses the common γ chain was necessary for the survival of naive but not memory CD4$^+$ T cells (30). The only other two *in vivo* studies have reported contradictory findings. In the

Correspondence to: C. Benoist and D. Mathis, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA

Transmitting editor: M. Reth *Received* 13 December 2000, *accepted* 20 February 2001

first case, depletion of IL-7 or -4 alone did not affect the survival of naive cells, but removal of both led to their rapid disappearance (12). In the second, depletion of IL-7 alone had a significant influence (31). The root of this contradiction could be the different cell populations examined [$CD4^+$ T cells in a class II-restricted TCR transgenic line (12) versus $CD8^+$ cells in a class I-restricted TCR transgenic (31)] and/or the different assays employed [measuring T cell survival after mAb-mediated cytokine blockade in thymectomized animals (12) versus after transfer of cytokine-deficient cells into normal hosts (31)].

Here, we have addressed the role of IL-4, -6 and -7 *in vivo*. We have studied polyclonal populations of both $CD4^+$ and $CD8^+$ T cells, and have monitored population decay after thymectomizing anti-cytokine mAb-treated and/or cytokine-deficient mice. Our results highlight the role of IL-7 in homeostasis of the naive T lymphocyte compartment, in particular of the $CD4^+$ population.

Methods

Mice

C57/Bl6 (B6) and B10.BR mice were bred and kept under specific-pathogen-free conditions or were purchased from the Jackson Laboratories (Bar Harbor, ME); IL-$4^{0/0}$ mice on the NOD genetic background were bred and kept under conventional conditions (32); IL-$4^{0/0}$ (33) and IL-$6^{0/0}$ (34) mice on the B6 background were obtained from Jackson Laboratories. All mice on the NOD background were checked for diabetes by monitoring glucose in the urine every week; diabetic mice were excluded from the analysis.

Thymectomy

Thymectomies were performed as described (9). In brief, 4- to 7-week-old mice were anesthetized and immobilized face-up. The skin was opened with a short longitudinal incision from the apex of the mandibular to the middle of the sternum and was pushed sideways to free the rib-cage. The submandibular salivary glands were displaced towards the head of the animal. One blade of a blunt pair of scissors was inserted into the rib-cage from the suprasternal notch such that the tip of the scissor reached the second rib. A sharp, upward movement was made as the manubrium was snipped open. A small opening was made in the pleura. Both thymic lobes were extracted with curved narrow forceps.

Antibody treatment and lymph node sampling

The anti-IL-7 receptor (IL-7R) A7R34 hybridoma was a gift from Dr S. I. Nishikawa. Thymectomized mice were injected 3 times per week i.p. with 1 mg of purified A7R34 mAb (35) or purified isotype-matched control antibody. Treatment was started between 3 and 5 days after thymectomy.

To follow the T cell population in individual animals, we removed lymph nodes surgically, as described (9). Four data points were obtained for each mouse: the first from the left inguinal lymph node at the time of thymectomy, then the right inguinal node, and then the left and right axillary nodes.

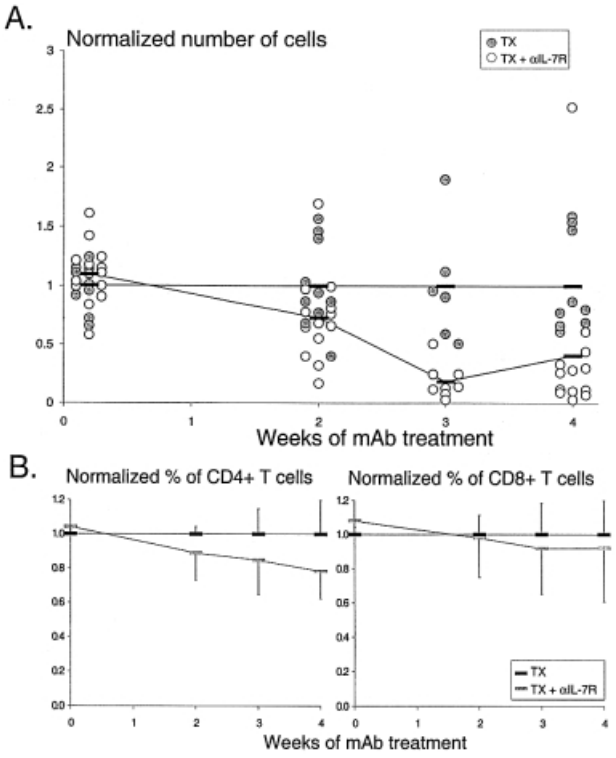

Fig. 1. Decay of lymphocytes in the absence of an IL-7 signal. (A) B6 or B10.BR mice were thymectomized and treated during 4 weeks with A7R34 (open circles) or control mAb (shaded circles). The number of living cells in excised lymph nodes was determined and was normalized according to the average of control points (see Methods). (B) Percentages of $CD4^+$ and $CD8^+$ T cells in the removed lymph nodes of mice injected with A7R34 (shaded symbols) or control mAb (closed symbols) were determined by flow cytometry and normalized on the average of the control. These data represent a compilation of results from four experiments, each having three or four control mice, and between three and five treated mice.

Flow cytometry and population analyses

Single-cell suspensions from lymph nodes were stained with mAb of the following specificities: anti-CD4–phycoerythrin (PE) (Caltag, Le Perray en Yvelines, France), anti-CD8–TriColor (Caltag), anti-CD44–biotin (PharMingen, San Diego, CA), anti-CD25–biotin (PharMingen), and anti-CD69–biotin (PharMingen) revealed by Red 613–streptavidin (Gibco/BRL, Gaithersburg, MD); anti-CD62L–FITC (Mel14), anti-$V_\alpha 2$ (B20.1), anti-$V_\beta 4$ (KT4.10), anti-$V_\beta 6$ (44.22.1) and anti-$V_\beta 8$ (KJ16) revealed by anti-rat Ig–FITC (Caltag). All mAb were used at saturating quantities. mAb dilutions or cell washes were done in FacsWash (PBS supplemented with 30 mM HEPES, 5% horse serum and 0.1% Na azide). Flow cytometry was performed on a Coulter (Hialeah, FL) Epics XL-MCL and data stored as list mode for analysis. Analysis of list-mode files was performed on Expo II MFA software, generating dot-plots or histograms. Live cells were gated on forward versus side scatter profiles.

The number of cells per lymph node and the percentages of $CD4^+$ and $CD8^+$ T cells were normalized according to the average of control points for each experiment. Briefly, for

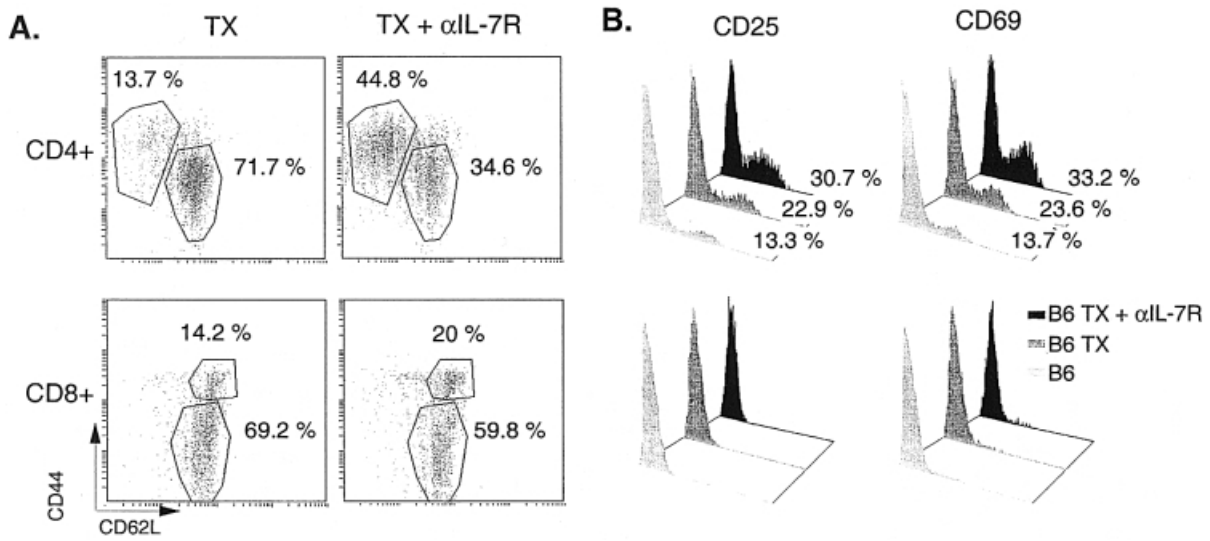

Fig. 2. T cell activation markers after 4 weeks of anti-IL-7R mAb treatment. Cell suspensions from lymph nodes of mice thymectomized and treated during 4 weeks with A7R34 or control mAb were stained for CD4, CD8, CD62L and CD44 (A), or for CD4, CD8, CD25 or CD69 (B).

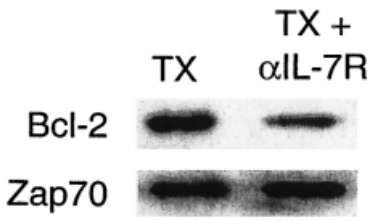

Fig. 3. Decrease in Bcl-2 expression in the absence of an IL-7 signal. Lysates of purified $CD4^+$ cells from thymectomized mice treated with A7R34 or control mAb were analyzed by Western blotting with an anti-Bcl-2 antibody. The membrane was stripped and reprobed with an anti-Zap70 antibody to permit quantitation. This result is representative of two experiments.

each time-point of each experiment, the average of the control values was calculated and the value for each mouse at this time-point was divided by this average. This was done to account for the variability between inguinal and axillary lymph nodes, and for the fluctuations between experiments.

Isolation of $CD4^+$ T cells and Western blot analysis

$CD4^+$ cells were purified using a MoFlo cytometer. Cell suspensions from lymph nodes were stained with anti-CD4 mAb (Caltag) in DMEM without phenol red (Gibco, Grand Island, NY), complemented with 2% FCS, 50 mM β-mercaptoethanol and 1 mM sodium pyruvate. $CD4^+$ cells were sorted using a MoFlo cytometer connected to an ice-cold waterbath and cells were collected in the medium maintained on ice. Live cells were gated on forward versus side scatter profiles.

Purified $CD4^+$ cells were washed several times with ice-cold PBS and were lysed at ~10^7 cells/ml in Laemmli loading buffer (62.5 mM Tris–HCl, pH 6.8, 0.72 M β-mercaptoethanol, 2% SDS, 7% glycerol and 0.1% bromophenol blue). Lysates were resolved on 12% SDS–PAGE and then transferred to a PVDF membrane (Millipore, Bedford, MA). After transfer, the membrane was blocked in Superblock solution (Pierce, Rockford, IL) and blotted with one of the following mAb: anti-Bcl2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Bcl-X (PharMingen), anti-Bax (PharMingen), anti-Bad (Becton Dickinson, Mountain View, CA), anti-phosphotyrosine (Upstate Biotechnology, Lake Placid, NY) or anti-Zap-70 (1E7.2) revealed by anti-mouse Ig or anti-rabbit Ig peroxidase-coupled antibody and the chemoluminescent reagent ECL Supersignal (Pierce).

Results

Importance of IL-7

Because of the crucial role of IL-7 in the differentiation of thymocytes, it is problematic to use IL-7-deficient mice to study the role of this cytokine in the periphery. Instead, we thymectomized wild-type B6 or B10.BR animals, thereby generating T cell compartments that had undergone normal differentiation but no longer underwent replenishment and injected them with the mAb A7R34, directed against the IL-7R and effective at blocking IL-7–IL-7R interactions. We monitored the decay of lymphocyte populations by counting cells in lymph nodes harvested after various times of treatment. The values reported in Fig. 1(A) were normalized on the average of the control values for each time-point in order to account for variable cell numbers in inguinal versus axillary lymph nodes and for experimental fluctuations. As we reported previously (9,10), cell numbers in the peripheral lymph nodes of untreated mice decreased only slightly over this time period (not shown), reflecting the long half-life of naive peripheral lymphocytes. Cell numbers in animals treated with anti-IL-7R mAb declined much more rapidly. The observed half-life of between 2 and 3 weeks was reminiscent of that found for T cells in an MHC-deficient periphery (9,10). The proportions of specific lymphocyte populations at different time-points

indicated that the decrease in T lymphocytes did not account for the overall decrease in cell numbers, suggesting that B lymphocyte survival was also affected by anti-IL-7R treatment. The decrease was greater for $CD4^+$ than for $CD8^+$ T cells (Fig 1B). This might be due to the higher sensitivity of $CD4^+$ naive cells to the lack of IL-7 (discussed below).

Phenotype of the surviving cells

To analyze the phenotype of the surviving lymphocytes, we stained the cells after 4 weeks of treatment. As illustrated in Fig. 2(A and B), the $CD8^+$ T cell population from unmanipulated or control mAb-treated mice contained mainly naive cells, expressing CD62L but not CD44, CD25 or CD69. The phenotype of $CD8^+$ cells surviving the anti-IL-7R mAb treatment was essentially the same. The $CD4^+$ T cell population showed an increase in the proportion of activated/memory cells after control mAb injection, which was repeatably augmented after administration of anti-IL-7R mAb. This finding correlates with the fact that naive $CD4^+$ T cells show a slightly higher expression of IL-7R than activated/memory $CD4^+$ or $CD8^+$ cells (data not shown).

MHC class II-deficient mice have a small $CD4^+$ T cell population with a special phenotype (36). These cells look activated and use a restricted set of V_α and V_β segments in their TCR. We wondered whether the $CD4^+$ T cells that preferentially survived anti-IL-7R treatment represented this population. Thus, we stained them with mAb recognizing several individual V_α or V_β segments. No bias in the V_α and V_β usage by these cells was detected (data not shown).

Pathway of IL-7R signaling

In order to compare results on mice deprived of IL-7–IL-7R interactions with those previously obtained on MHC class II-deficient animals (9,10), we focused on signaling changes in the $CD4^+$ T cell compartment.

The phenotypic defect in the thymus of mice incapable of IL-7–IL-7R interactions can be rescued by artificially expressing Bcl-2 (37,38), and the effect of removing IL-7R on *in vitro* T cell survival was linked to a decrease in Bcl-2 (38) or in Bcl-2 and Bcl-X_L (23) expression. To determine whether Bcl-2 is also implicated in the IL-7R-mediated survival signal *in vivo*, we sorted $CD4^+$ T cells from peripheral lymphoid organs of mice treated for 2 weeks with anti-IL-7R or control mAb and performed Western blotting on lysates of these cells with antibodies directed against Bcl-2, Bcl-X, Bax or Bad. The amount of Bcl-2 was reduced ~2-fold in the $CD4^+$ T cell compartment of mice treated with anti-IL-7R (Fig. 3). We did not see any differences in levels of expression of Bcl-X, Bax or Bad (data not shown).

We were also interested in the phosphorylation state of signaling molecules in these cells because we have previously demonstrated alterations in $CD4^+$ T cells dying due to a lack of stimulation by MHC class II molecules—a reduction in the partial CD3ζ phosphorylation that occurs in normal $CD4^+$ T cells and reduced ZAP-70 association (10). However, Western blots performed with an anti-phosphotyrosine antibody did not show any significant differences in the proteins from cells derived from control or anti-IL-7R mAb-treated mice, even for CD3ζ (data not shown).

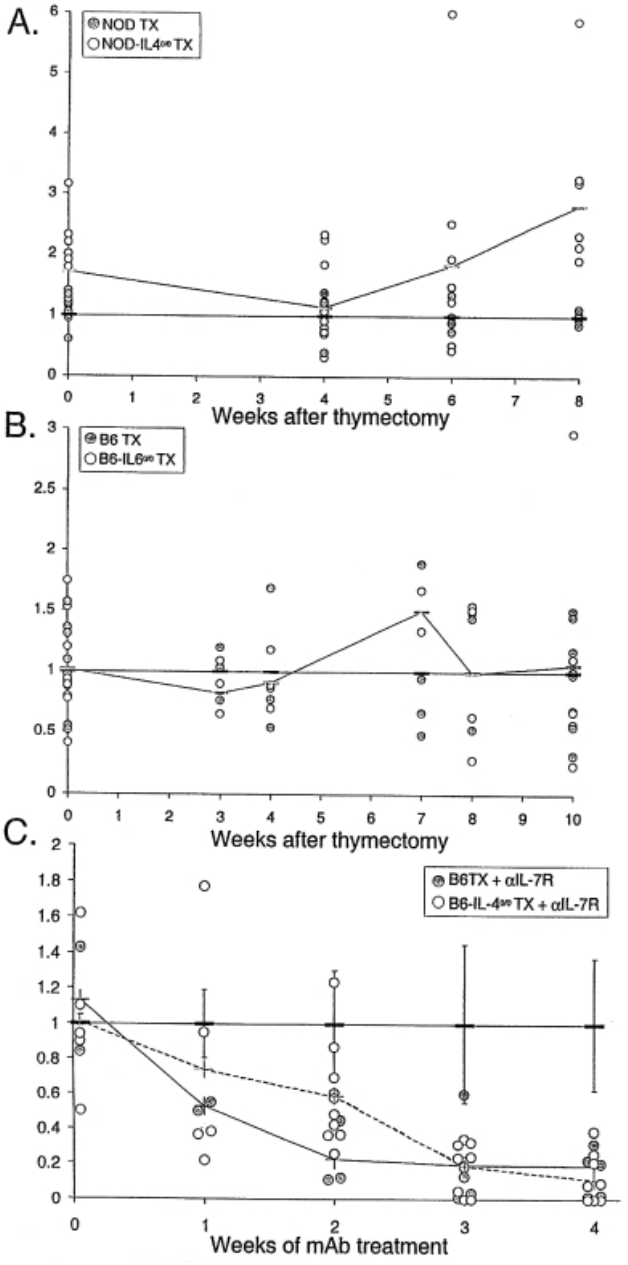

Fig. 4. Numbers of cells in the lymph nodes after thymectomizing cytokine-deficient mice. (A) NOD-IL4$^{0/0}$ mice (open circles) and NOD wild-type mice (shaded circles) were thymectomized, and cell numbers from excised lymph nodes determined and normalized. Six control and 11 mutant mice were analyzed. (B) B6-IL6$^{0/0}$ mice (open circles) and B6 wild-type mice (shaded circles) were thymectomized, and cell numbers from removed lymph nodes determined and normalized. Eleven control and 10 mutant mice were analyzed. (C) B6-IL4$^{0/0}$ mice (open circles) and B6 wild-type mice (shaded circles) were thymectomized and treated during 4 weeks with A7R34. Numbers of cells were normalized according to the averages for B6-IL4$^{0/0}$ or B6 mice treated with PBS (horizontal line). Four treated and three control B6 mice, and 10 treated and 10 control B6-IL4$^{0/0}$ mice were analyzed.

Role of IL-4 and IL-6

Finally, we also wanted to address the role of IL-4 and IL-6 in peripheral T cell survival. Since the differentiation of T cells was reported to be normal in both IL-$4^{0/0}$ (33) and IL-$6^{0/0}$ mice (34), we could just thymectomize these animals to obtain populations of T cells deprived of IL-4 or -6 in the absence of replenishment. Lymph node cells were removed as above, and lymphocyte populations counted and analyzed cytofluorimetrically. Figure 4(A and B) shows the evolution of lymph node cell numbers normalized on the control average at different times after thymectomy. The cell numbers did not significantly decrease in IL-$4^{0/0}$ mice compared with those in control animals, actually tending to be somewhat greater. No decrease in lymph node cell numbers relative to control values was seen in IL-$6^{0/0}$ mice either. The proportions of the different T cell subpopulations also remained normal in these mice (data not shown).

Since some investigators could see an effect of double depletion of IL-4 and IL-7 on the survival of naive lymphocytes, but not single depletion of either one of these cytokines, we wanted to see whether treating B6-IL-$4^{0/0}$ mice with anti-IL-7R mAb would have a stronger effect than treating standard B6 mice. As indicated in Fig. 4(C), the decrease in cell numbers observed with anti-IL-7R blockade was not any greater in the absence of IL-4.

Discussion

The crucial role of IL-7 in thymocyte differentiation complicates an assessment of its role in peripheral T lymphocyte survival. Several reports have suggested that preventing IL-7–IL-7R interactions may perturb T cell homeostasis in the peripheral lymphoid organs (35,39,40), but the maturation of T cells was disturbed in these studies and this might have been the root of any differences in survival that were observed. In the system we have studied, T cells differentiate in a normal thymic environment and thymectomy prevents the subsequent arrival of new T cells in the periphery. We observed a clear decrease in the number of T cells in the lymph nodes when IL-7–IL-7R interactions were prevented. B cell numbers seemed affected too, but we did not determine the extent to which this was due to a survival defect versus a defect in renewal from the bone marrow, given that IL-7 is known to play an important role in the differentiation of B cells (25,26). We have shown that the naive $CD4^+$ T cell population is the compartment most perturbed by abrogating IL-7–IL-7R interactions. This may reflect the fact that this population expresses a slightly higher level of IL-7R on the cell surface (data not shown).

The signal induced by IL-7–IL-7R engagement has been linked to Bcl-2 expression in several contexts. Bcl-2 overexpression in the thymus of IL-7- or IL-7R-deficient mice can restore T cell differentiation (37,38,41). In addition, adding IL-7 to T cell cultures increases Bcl-2 expression (23,38), and in some cases Bcl-X_L (23). Here we have shown that preventing IL-7–IL-7R interactions decreases Bcl-2 expression in $CD4^+$ lymph node cells, but not expression of Bcl-X_L, Bax or Bad. This result does not conflict with the recent report that IL-7 influences Bax expression (42), because in that case it modified Bax localization, which would not have been picked up in our assay.

IL-4 and IL-6 were claimed to be important T cell survival factors *in vitro* (21–24). However, we have found that T lymphocytes deprived of these cytokines survive normally in the periphery of mice. The differentiation of T cells in IL-$4^{0/0}$ (33) and IL-$6^{0/0}$ (34) mice was shown to be normal, but one might argue that the physiology of these cells is still disturbed in a way that somehow trains them to survive without these factors. This scenario cannot be formally ruled out. Another possible explanation for the differences observed in the *in vivo* and *in vitro* experiments is that the concentration of cytokines added to the culture *in vitro* is higher than *in vivo* or that *in vitro* the cytokines can replace some cell–cell interactions that occur *in vivo* in the cytokine-deficient mice.

Finally, it could be that other cytokines compensate for the lack of IL-4 or -6 in the cytokine-deficient mice. Boursalian and Bottomly have suggested that IL-4 and -7 can compensate for each other *in vivo*, and that only the depletion of both of them has an effect on T cell survival (12). The fact that these investigators did not see an effect when they blocked IL-7–IL-7R interactions only is not really in conflict with our own results because they only treated their mice for one week. We did treat thymectomized IL-4-deficient animals to see whether depletion of the two cytokines would have a stronger effect than just depriving them of IL-7–IL-7R interactions. Lack of the two cytokines did not appear to have a more drastic effect. The divergent results with the two systems could be related to the fact that the Boursalian and Bottomly study used TCR transgenic mice. The T cell population we monitored was polyclonal and one can imagine that cells displaying different specificities, especially a very restricted versus a diverse set, might have different needs. This could be the case, for example, if the MHC molecule-mediated and cytokine-mediated signals are somehow linked. All cells might require a certain level of survival signal, which could be provided either via MHC molecules or IL-7. Different TCR–MHC affinities would, then, need to be supplanted more or less by the cytokine-mediated signal. It may be possible to resolve this issue by monitoring T cell decline in the periphery of mice in the absence of both types of signal. The fact that phosphorylation of the CD3ζ chain was not affected by the anti-IL-7R treatment suggests that at least the initial signaling steps are different for these two factors, but the cascades might still interact downstream.

Acknowledgments

We would like to thank Dr L. Radvanyi for discussions, C. Ebel and P. Gerber for purifying mAb, H. Robinson for cell sorting, F. Fischer and P. Michel for taking care of the mice, and K. Elder for administrative assistance. The A7R34 hybridoma was provided by Drs S. I. Nishikawa and K. Rajewsky. This work was supported by institute funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique and the Centre Hospitalier Universitaire Régional. L. V. received fellowships from the Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche and from the Association pour la Recherche Contre le Cancer.

Abbreviations

B6	C57/Bl6
IL-7R	IL-7 receptor
PE	phycoerythrin

References

1 Freitas, A. A. and Rocha, B. 2000. Population biology of lymphocytes: the flight for survival. *Annu. Rev. Immunol.* 18:83.

2 Tanchot, C. and Rocha, B. 1995. The peripheral T cell repertoire: independent homeostatic regulation of virgin and activated $CD8^+$ T cell pools. *Eur. J. Immunol.* 25:2127.
3 Bruno, L., von Boehmer, H. and Kirberg, J. 1996. Cell division in the compartment of naive and memory T lymphocytes. *Eur. J. Immunol.* 26:3179.
4 Tanchot, C., Lemonnier, F. A., Pérarnau, B., Freitas, A. A. and Rocha, B. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 276:2057.
5 Nesic, D. and Vukmanovic, S. 1998. MHC class I is required for peripheral accumulation of $CD8^+$ thymic emigrants. *J. Immunol.* 160:3705.
6 Markiewicz, M. A., Girao, C., Opferman, J. T., Sun, J., Hu, Q., Agulnik, A. A., Bishop, C. E., Thompson, C. B. and Ashton-Rickardt, P. G. 1998. Long-term T cell memory requires the surface expression of self-peptide/major histocompatibility complex molecules. *Proc. Natl Acad. Sci. USA* 95:3065.
7 Murali-Krishna, K., Lau, L. L., Sambhara, S., Lemonnier, F., Altman, J. and Ahmed, R. 1999. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science* 286:1377.
8 Takeda, S., Rodewald, H. R., Arakawa, H., Bluethmann, H. and Shimizu, T. 1996. MHC class II molecules are not required for survival of newly generated $CD4^+$ T cells, but affect their long-term life span. *Immunity* 5:217.
9 Rooke, R., Waltzinger, C., Benoist, C. and Mathis, D. 1997. Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. *Immunity* 7:123.
10 Witherden, D., van Oers, N., Waltzinger, C., Weiss, A., Benoist, C. and Mathis, D. 2000. Tetracycline-controllable selection of $CD4^+$ T cells: half-life and survival signals in the absence of major histocompatibility complex class II molecules. *J. Exp. Med.* 191:355.
11 Kirberg, J., Berns, A. and von Boehmer, H. 1997. Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex-encoded molecules. *J. Exp. Med.* 186:1269.
12 Boursalian, T. E. and Bottomly, K. 1999. Stability of naive and memory phenotypes on resting CD4 T cells *in vivo*. *J. Immunol.* 162:9.
13 Brocker, T. 1997. Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expressing dendritic cells. *J. Exp. Med.* 186:1223.
14 Garcia, S., DiSanto, J. and Stockinger, B. 1999. Following the development of a CD4 T cell response *in vivo*: from activation to memory formation. *Immunity* 11:163.
15 Swain, S. L., Hu, H. and Huston, G. 1999. Class II-independent generation of CD4 memory T cell from effectors. *Science* 286:1381.
16 Lodolce, J. P., Boone, D. L., Chai, S., Swain, R. E., Dassopoulos, T., Trettin, S. and Ma, A. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 9:669.
17 Zhang, X., Sun, S., Hwang, L., Tought, D. F. and Sprent, J. 1998. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells *in vivo* by IL-15. *Immunity* 8:591.
18 Gasser, S., Corthesy, P., Beerman, F., MacDonald, H. R. and Nabholz, M. 2000. Constitutive expression of a chimeric receptor that delivers IL-2/IL-15 signals allow antigen-independent proliferation of $CD8^{-}CD44^{high}$ but not other T cells. *J. Immunol.* 164:5659.
19 Ku, C. C., Murakami, M., Sakamoto, A., Kappler, J. and Marrack, P. 2000. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. *Science* 288:675.
20 Kennedy, M. K., Glaccum, M., Brown, S. N., Butz, E. A., Viney, J. L., Embers, M., Matsuki, N., Charrier, K., Sedger, L., Willis, C. R., Brasel, K., Morrissey, P. J., Stocking, K., Schuh, J. C., Joyce, S. and Peschon, J. J. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191:771.
21 Boise, L. H., Minn, A. J., June, C. H., Lindsten, T. and Thompson, C. B. 1995. Growth factors can enhance lymphocyte survival without committing the cell to undergo cell division. *Proc. Natl Acad. Sci. USA* 92:5491.
22 Vella, A., Teague, T. K., Ihle, J., Kappler, J. and Marrack, P. 1997. Interleukin 4 (IL-4)or IL-prevents the death of resting T cell: stat6 is probably not required for the effect of IL-4. *J. Exp. Med.* 186:325.
23 Soares, M. V. D., Borthwick, N. J., Maini, M. K., Janossy, G., Salmon, M. and Akbar, A. N. 1998. IL-7-dependent extrathymic expansion of $CD45RA^+$ T cells enables preservation of naive repertoire. *J. Immunol.* 161:5909.
24 Teague, T. K., Marrack, P., Kappler, J. W. and Vella, A. T. 1997. IL-6 rescues resting mouse T cells from apoptosis. *J. Immunol.* 158:5791.
25 Peschon, J. J., Morrissey, P. J., Grabstein, K. H., Ramsdell, F. J., Maraskovsky, E., Gliniak, B. C., Park, L. S., Ziegler, S. F., Williams, D. E., Ware, C. B., Meyer, J. D. and Davison, B. L. 1994. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J. Exp. Med.* 180:1955.
26 von Freeden-Jeffry, U., Vieira, P., Lucian, L. A., McNeil, T., Burdach, S. E. G. and Murray, R. 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* 181:1519.
27 Lynch, D. H. and Miller, R. E. 1994. Interleukin 7 promotes long-term *in vitro* growth of antitumor cytotoxic T lymphocytes with immunotherapeutic efficacy *in vivo*. *J. Exp. Med.* 179:31.
28 Hassan, J. and Reen, D. J. 1998. IL-7promotes the survival and maturation but not differentiation of human post-thymic $CD4^+$ T cells. *Eur. J. Immunol.* 10:3057.
29 Webb, L. M., Foxwell, B. M. and Feldmann, M. 1999. Putative role for interleukin-7 in the maintenance of the recirculating naive CD + T-cell pool. *Immunology* 98:400.
30 Lantz, O., Grandjean, I., Matzinger, P. and Di Santo, J. P. 2000. γ chain required for naive $CD4^+$ T cell survival but not for antigen proliferation. *Nat. Immunol.* 1:54.
31 Schluns, K. S., Kieper, W. C., Jameson, S. C. and Lefrancois, L. 2000. Interleukin-7 mediates the homeostasis of naive and memory CD8 T *in vivo*. *Nat. Immunol.* 1:426.
32 Wang, B., Gonzalez, A., Katz, J., Höglund, P., Benoist, C. and Mathis, D. 1998. Interleukin-4 deficiency does not worsen disease in NOD mice. *Diabetes*, in press.
33 Kuhn, R., Rajewsky, K. and Muller, W. 1991. Generation and analysis of interleukin-4 deficient mice. *Science* 254:707.
34 Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H. and Kohler, G. 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* 368:339.
35 Sudo, T., Nishikawa, S., Ohno, N., Akiyama, M., Tamakoshi, M., Yoshida, H. and Nishikawa, S. 1993. Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc. Natl Acad. Sci. USA* 90:9125.
36 Cardell, S., Tangri, S., Chan, S., Kronenberg, M., Benoist, C. and Mathis, D. 1995. CD1-restricted $CD4^+$ T cells in major histocompatibility complex class II-deficient mice. *J. Exp. Med.* 182:993.
37 Maraskovsky, E., O'Reilly, L. A., Teepe, M., Corcoran, L. M., Peschon, J. J. and Strasser, A. 1997. Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant *rag*-$1^{-/-}$ mice. *Cell* 89:1011.
38 von Freeden-Jeffry, U., Solvason, N., Howard, M. and Murray, R. 1997. The earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression. *Immunity* 7:147.
39 Grabstein, K. H., Waldschmidt, T. J., Finkelman, F. D., Hess, B. W., Alpert, A. R., Boiani, N. E., Namen, A. E. and Morrissey, P. J. 1993. Inhibition of murine B and T lymphopoiesis *in vivo* by an anti-interleukin 7 monoclonal antibody. *J. Exp. Med.* 178:257.
40 Maraskovsky, E., Teepe, M., Morrissey, P. J., Braddy, S., Miller, R. E., Lynch, D. H. and Peschon, J. J. 1996. Impaired survival and proliferation in IL-7 receptor-deficient peripheral T cells. *J. Immunol.* 157:5315.
41 Akashi, K., Kondo, M., Freeden-Jeffry, U., Murray, R. and Weissman, I. L. 1997. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell* 89:1033.
42 Khaled, A. R., Kim, K., Hofmeister, R., Muegge, K. and Durum, S. K. 1999. Withdrawal of IL-7 induces Bax translocation from cytosol to mitochondria through a rise in intracellular pH. *Proc. Natl Acad. Sci. USA* 96:14476.