# IL-7/Anti-IL-7 mAb Complexes Restore T Cell Development and Induce Homeostatic T Cell Expansion without Lymphopenia[1]

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**IL-7, a member of the common γ-chain family of cytokines, is essential for B and T lymphocyte development and homeostasis of mature T cell subsets. Thus, naive and memory T cells are both dependent on IL-7 for survival and homeostatic proliferation under lymphopenic conditions. In line with prior findings with IL-2, we show in this study that the biological activity of IL-7 in vivo is greatly increased by association with anti-IL-7 mAb. Under in vivo conditions, IL-7/mAb complexes displayed 50- to 100-fold higher activity than free IL-7 and induced massive expansion of pre-B cells. IL-7/mAb complexes also increased thymopoiesis in normal mice and restored thymopoeisis in IL-7-deficient mice. For mature T cells, IL-7/mAb complexes induced marked homeostatic proliferation of both naive and memory CD4$^+$ and CD8$^+$ cell subsets even under normal T cell-replete conditions. Finally, IL-7/mAb complexes were able to enhance the magnitude of the primary response of Ag-specific naive CD8$^+$ cells. The strong stimulatory activity of IL-7/mAb complexes could be useful for treatment of immunodeficiency and cancer.** ***The Journal of Immunology,*** **2008, 180: 7265–7275.**

Cytokines are small molecular mass soluble mediators produced by various types of cells of the immune system and regulate development, survival, and function of a multitude of cell types, including B and T lymphocytes. For T cells, the most essential cytokines are three members of the common γ (γc)[4] chain receptor family, namely, IL-2, IL-7, and IL-15 (1, 2). IL-2 is a pleiotropic cytokine produced mainly by activated T cells to promote autologous cell proliferation and acquisition of effector function. IL-2 is also essential for development and survival of regulatory T cells (Tregs), which are essential to control excessive immune responses and overt autoimmunity (3). Thus, defective IL-2 signaling leads to spontaneous lymphoproliferative and autoimmune diseases in mice and humans because of impaired development and function of Tregs (4). Unlike IL-2, IL-7, and IL-15 are produced mainly by non-T cells, such as epithelial and reticular cells and APC, and are largely involved in supporting development and homeostasis of T cells (1, 5, 6). Thus, IL-7 is essential for T cell development in the thymus and for survival of mature T cells in the secondary lymphoid tissues (1, 7). Similarly, IL-15 is crucial for development and survival of memory T, as well as NKT and NK cells (6, 8).

Despite their essential function, the therapeutic potential of these three cytokines has yet to be fully realized. IL-2 was approved by the FDA for the treatment of metastatic cancer in 1992 and 1998, but has to be administered at very high doses to be beneficial and generally causes severe side effects (9, 10). A key problem is that the administered IL-2 often does not display high biological activity, presumably because of its short lifespan. To overcome this problem, various attempts have been made to increase the in vivo half life of IL-2, e.g., through amino acid modifications and conjugation of IL-2 to polyethylene glycol, albumin, or IgG (11–13). An alternative approach, first described in the early 1990's, to achieve this goal is to bind IL-2 to an anti-IL-2 mAb before its administration; the method was reported to be applicable also to other cytokines, including IL-3, IL-4, IL-6, and IL-7 (14–16). Recently, we have serendipitously realized the power of this approach to immensely augment the in vivo biological activity of IL-2. Thus, complexes of IL-2/anti-IL-2 mAb were found to display vastly superior in vivo activity than free IL-2 and induced strong expansion of T cells and NK cells. In addition, depending on the fine specificity of the particular anti-IL-2 mAb used, the IL-2/mAb complexes either induced preferential expansion of CD122$^{high}$ memory CD8$^+$ T cells and NK cells or elicited selective expansion of the CD25$^+$ CD4$^+$ regulatory T cells (Tregs) (14). In an analogous fashion, the activity of IL-15 was also found to be greatly increased upon binding to its natural presenting receptor, IL-15Rα-chain, and such complexes were found to induce strong expansion of CD122$^{high}$ memory CD8$^+$ T cells and NK cells (17, 18).

IL-2/mAb and IL-15/IL-15Rα complexes predominantly activate memory CD8$^+$ T cells, NK cells, and Tregs, but not naive CD4$^+$ and CD8$^+$ T cells unless the latter are stimulated in the absence of competing CD122$^{high}$ cells (19, 20). In the present study, we sought to determine whether naive T cells could be driven to undergo proliferation in response to IL-7/anti-IL-7 mAb complexes, because IL-7R is highly expressed on naive and memory CD4$^+$ and CD8$^+$ subsets of T cells. Indeed, administration of

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[4] Abbreviations used in this paper: $\gamma_c$, common γ chain; Treg, regulatory T cell; LN, lymph node; rh, recombinant human; rm, recombinant murine; OVA-HSP, OVA conjugated to heat shock protein; LCMV, lymphocytic choriomeningitis virus; BM, bone marrow; DP, double positive; MP, memory-phenotype.

IL-7/mAb complexes into normal non-lymphopenic mice induced strong expansion of both naive and memory $CD8^+$ T cells; similar observations applied to $CD4^+$ T cells, though these cells required higher doses of IL-7/mAb complexes. In addition to their strong effect on naive and memory T cells, IL-7/mAb complexes also efficiently restored thymopoiesis in IL-7-deficient mice. These findings indicate that IL-7/mAb complexes may have therapeutic utility for restoring naive T cell numbers in patients undergoing T cell depleting regimens and for expanding preexisting T cell populations to enhance immunity against cancer or infectious agents.

## Materials and Methods

### *Mice*

C57BL/6 (B6), B6.PL (Thy-1.1), and B6.SJL (Ly5.1) mice were purchased from The Jackson Laboratory. IL-7-deficient ($IL\text{-}7^{-/-}$) (7), TAP-deficient ($TAP^{-/-}$) (21), Thy-1.1 OT-I (22), P14 (23), and Smarta (24) mice were maintained in our animal facility. All mice were housed under specific pathogen-free conditions at The Scripps Research Institute and used at 3–6 mo of age. Thymectomy and sham operation was performed on young B6 mice as previously described (25). Experiments involving the use of animals were approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute.

### *Abs and cytokines used in vivo*

Recombinant human IL-7 (rhIL-7) was obtained from NCI Biological Resources Branch and recombinant murine IL-2 (rmIL-2) and rmIL-7 were purchased from R&D Systems and eBioscience. The hybridomas for the anti-IL-7 mAb M25 (mouse IgG2b) (26), anti-IL-7R$\alpha$ mAb A7R34 (rat IgG) (27), anti-mIL-2 mAb S4B6 (28) were provided by Dr. Philippa Marrack (Denver, CO) with permissions from originators. Mouse mAbs specific to mIL-7 (IgG2b, MAB407, clone 204626) and hIL-7 (IgG1, MAB207, clone 7417) were purchased from R&D Systems. Fab fragments of M25 were generated by digesting 1 mg/ml M25 with 0.1 mg/ml pepsin (Worthington Biochemical) for 15–30 min in 0.1 M NaOAc buffer (pH 4.0) as previously described (29).

### *Flow cytometry and cell sorting*

Cell suspensions of spleen or pooled (inguinal, axillary, cervical, and mesenteric) lymph nodes (LN) were prepared according to standard protocols and stained for FACS analysis as previously described (14, 17, 30) with the following mAbs (from eBioscience or BD Biosciences unless otherwise stated): PerCP-Cy5.5-conjugated anti-CD3 (145-2C11); Alexa Fluor 405-conjugated anti-CD4 (RM4–5; Caltag Laboratories); PerCP-Cy5.5- or APC-Cy7-conjugated anti-CD8$\alpha$ (53-6.7); PE-conjugated anti-CD8$\beta$ (H35-17.2); allophycocyanin-conjugated anti-CD25 (PC61.5); allophycocyanin-conjugated anti-CD44 (IM7); PE-conjugated Ig$\kappa$ (187.1); allophycocyanin-conjugated anti-CD45.1 (A20); biotinylated anti-CD62 ligand (MEL-14) plus streptavidin-conjugated APC; biotinylated anti-CD69 (H1.2F3) plus streptavidin-conjugated APC; allophycocyanin-conjugated anti-CD90.1 (HIS51); FITC-conjugated anti-CD122 (TM-$\beta$1); and PE-conjugated anti-CD127 (SB/14). Stained cells were analyzed using a BD LSR II digital flow cytometer.

### *Adoptive transfer of T cell subsets*

Whole LN cells or purified lymphocytes from Thy1.1- or CD45.1-congenic B6 mice were labeled with CFSE and adoptively transferred i.v. to B6 or mutant mice in a B6 background (30). For unirradiated hosts, donor cells were injected at a dose of $10–20 \times 10^6$ cells/mouse and at a dose of $1–2 \times 10^6$ cells/mouse to irradiated B6 hosts. The hosts then received i.p. injections of PBS, rhIL-7, anti-IL-7 mAb M25, or IL-7 plus M25 (at 2:1 to 1:1 molar ratio) every other day for the indicated duration and donor T cells analyzed by flow cytometry. T cell subsets were purified from pooled LN cells by one of three methods: either killing non-T cells using mAbs plus complement, followed by panning on mAb coated plates (30), by MACS columns (14), or by sorting on a flow cytometer after staining for the desired populations of cells. For sorted naive and memory-phenotype (MP) cells, purities of these two cell subsets were >99% for $CD4^+CD44^{low}$ or $CD8^+CD44^{low}$ cells, and >98% for $CD4^+CD44^{high}$ or $CD8^+CD44^{high}$ cells.

### *Generation of Ag-specific effector and memory $CD4^+$ and $CD8^+$ T cells*

Thy-$1.1^+$ OT-I $CD8^+$ cells ($2 \times 10^5$) were transferred into normal B6 mice and then the hosts were immunized subcutaneously with a total of 3 or 10 $\mu$g of fusion protein comprised of OVA conjugated to heat shock protein (OVA-HSP) in PBS and analyzed 6 days later (31). Smarta TCR transgenic $CD4^+$ T cells specific to the lymphocytic choriomeningitis virus (LCMV) gp61–80 epitope, or $CD8^+$ T cells from P14 mice responding to the LCMV $gp_{33–41}$ epitope were used to generate Ag-specific memory T cells. Spleen cells from $Thy1.1^+$ Smarta or P14 mice were adoptively transferred to normal B6 mice at $5 \times 10^4$ cells/mouse and infected with $2 \times 10^5$ PFU of the LCMV strain Armstrong and left for >40 days to allow for generation of memory cells (32).

## Results

### *Effects of IL-7/mAb complex administration on lymphoid tissues*

Normal B6 mice were injected i.p. three times at 2-day intervals with complexes of 1.5 $\mu$g rhIL-7 plus 15 $\mu$g of anti-hIL-7 mAb M25 (mouse IgG2b). Analysis of the lymphoid tissues 7 days after the start of the injections revealed prominent enlargement of the spleen and LN, whereas only minimal changes occurred in control mice injected with only PBS, rhIL-7, or M25 alone. The spleen and LN of mice injected with rhIL-7/M25 complexes were enlarged 5- to 10-fold in cellularity (Fig. 1). As previously reported (16), splenomegaly was mainly due to expansion of pre-B cells (Fig. 1*A*). Thus, whereas $B220^+$ $IgM^-$ ($Ig\kappa^-$) immature B cells were rare in the spleen of control mice, they represented >60% of cells in the spleen of mice injected with rhIL-7/M25 complexes (Fig. 1*B*, *left*), representing a 200- to 400-fold expansion of total numbers of these cells. In the bone marrow (BM), immature B cells comprised ~40% of all cells in control mice but 97% of the all cells in the BM of mice injected with rhIL-7/M25 complexes, thereby causing the BM to appear white rather than red (Fig. 1*B* and data not shown). Additionally, a moderate 2- to 3-fold expansion of mature B ($B220^+IgM^+$) and T cells was apparent in the spleen (Fig. 1*A*). The preferential expansion of immature over mature B cells correlated with the relative expression levels of IL-7R, which are considerably higher on immature B cells than on mature B cells (data not shown). The proliferative potential of immature B cells to IL-7 also appeared to be much greater than for mature lymphocytes as mature T cells only underwent ~2- to 3-fold expansion in the spleen in response to rhIL-7/M25 complexes, even though mature T cells expressed slightly higher levels of IL-7R than immature B cells (data not shown).

In the LN of B6 mice injected with rhIL-7/M25 complexes, there was prominent expansion of T cells and moderate expansion of mature B cells (Fig. 1, *A* and *B*); as expected, there were very few immature B cells in LN. For T cells, CD8 cells underwent a greater (4-fold) expansion than CD4 cells (2-fold), and cell expansion was associated with an increased accumulation of $CD44^{high}$ cells, especially for the CD8 subset, without any change in the proportion of $CD25^+$, $CD69^+$, or $CD62L^+$ T cells, including $CD25^+$ $CD4^+$ cells (Fig. 1*B* and data not shown).

The thymus of B6 mice injected with rhIL-7/M25 complexes, had 15–20% higher cellularity than PBS-injected mice, mostly from a rise in numbers of CD4/CD8 double positive (DP) cells (Fig. 2*A*). To better assess the effect of IL-7 on thymopoiesis, groups of $IL\text{-}7^{-/-}$ mice, which have a very small thymus (7, 33), were injected with rhIL-7/M25 complexes, rhIL-7 alone, or PBS. Two injections of 1.5 $\mu$g rhIL-7 plus 15 $\mu$g M25, 3 days apart, caused the thymus of $IL\text{-}7^{-/-}$ mice to greatly enlarge and show a 50- to 100-fold increase in cellularity by 7 days; by contrast, injection of 1.5 $\mu$g rhIL-7 alone induced only a relatively minor

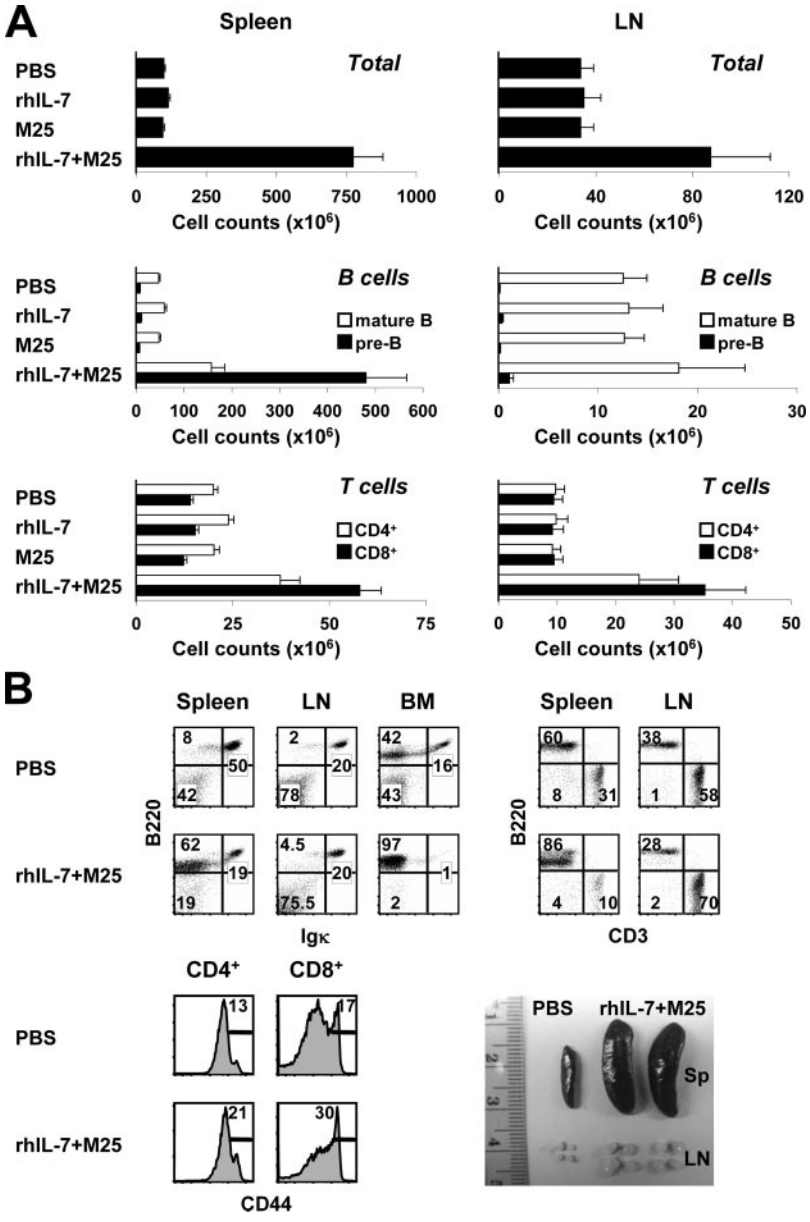


**FIGURE 1.** IL-7/mAb complexes induce enlargement of spleen and LN. *A*, Young normal B6 mice were i.p. injected either with PBS, 1.5 $\mu$g rhIL-7, 15 $\mu$g anti-IL-7 mAb M25, or a mixture of 1.5 $\mu$g rhIL-7 plus 15 $\mu$g M25 three times at 2 day intervals. Spleen (*left column*) and LN cells (*right column*) were analyzed 7 days after the start of the injections by flow cytometry after staining for B220 vs Ig$\kappa$ or CD3, CD4 vs CD8 as described in *Materials and Methods*. Shown are total number of spleen cells (*top*), immature and mature B cells (*middle*), and T cell subsets (*bottom*). *B*, Spleen, LN, and BM cells from representative mice injected with PBS or rhIL-7/M25 complexes in *A* are shown for expression of B220 vs Ig$\kappa$ and B220 vs CD3 (*top*). CD44 expression on $CD4^+$ and $CD8^+$ cells from LN of the same mice are shown (*bottom left*), together with a photograph of the enlarged spleens and LN from the injected mice (*bottom right*). The results are representative of at least three separate experiments involving two to three mice per group.

2- to 3-fold increase in cell numbers (Fig. 2*A*). Analysis of the $CD4^-CD8^-$ (DN) population of thymocytes revealed that injection of rhIL-7/M25 complexes induced the selective emergence of $CD25^+CD44^-$DN3 and $CD25^-CD44^-$DN4 cells, which were severely deficient in IL-$7^{-/-}$ mice injected with PBS or rhIL-7 alone (Fig. 2*A*). The restoration of thymopoiesis induced by rhIL-7/M25 complexes was transient as the thymus of the injected IL-$7^{-/-}$ mice reverted back to a hypocellular state by 3 wk after injecting the complexes (Fig. 2*B*, *left*). It should be mentioned that in contrast to the marked effect on the thymus, injection of rhIL-7/M25 complexes caused only a 2-fold increase in the cellularity of the spleen of IL-$7^{-/-}$ mice (data not shown).

To estimate the relative biological activity of rhIL-7/M25 complexes, IL-$7^{-/-}$ mice were injected twice over 7 days with a moderate dose (1 + 5 $\mu$g) of rhIL-7/M25 complexes vs titrated doses (1, 10, and 100 $\mu$g) of rhIL-7 alone. The striking finding was that the enlargement of the thymus induced by 1 $\mu$g rhIL-7 bound to M25 was equivalent to the increase in the thymus size elicited by 100 $\mu$g of free rhIL-7 (Fig. 2*B*, *right*).

### *IL-7/mAb complexes induce homeostatic proliferation of naive T cells*

The rise in peripheral T cell numbers induced by injection of rhIL-7/M25 complexes in normal B6 mice could be due to increased thymic output and/or expansion of preexisting peripheral T cells. The former idea is a possibility because IL-$7^{-/-}$ mice injected with rhIL-7/M25 complexes possessed detectable, albeit low, numbers of naive phenotype T cells in the spleen, which are usually extremely rare in these mice (data not shown). However, measuring an increase in thymic output in normal B6 mice given rhIL-7/M25 complexes is problematic because of the difficulty of distinguishing recent thymic emigrants from preexisting T cells. Therefore, we examined the capacity of IL-7/M25 complexes to expand the mature T cell pool in the absence of thymic output, i.e., by using thymectomized B6 mice. Notably, injection of rhIL-7/M25 complexes (1.5 + 7.5 $\mu$g, three times over 7 days) into thymectomized B6 mice induced a relatively similar extent of peripheral T cell expansion as that observed in normal B6 mice (data

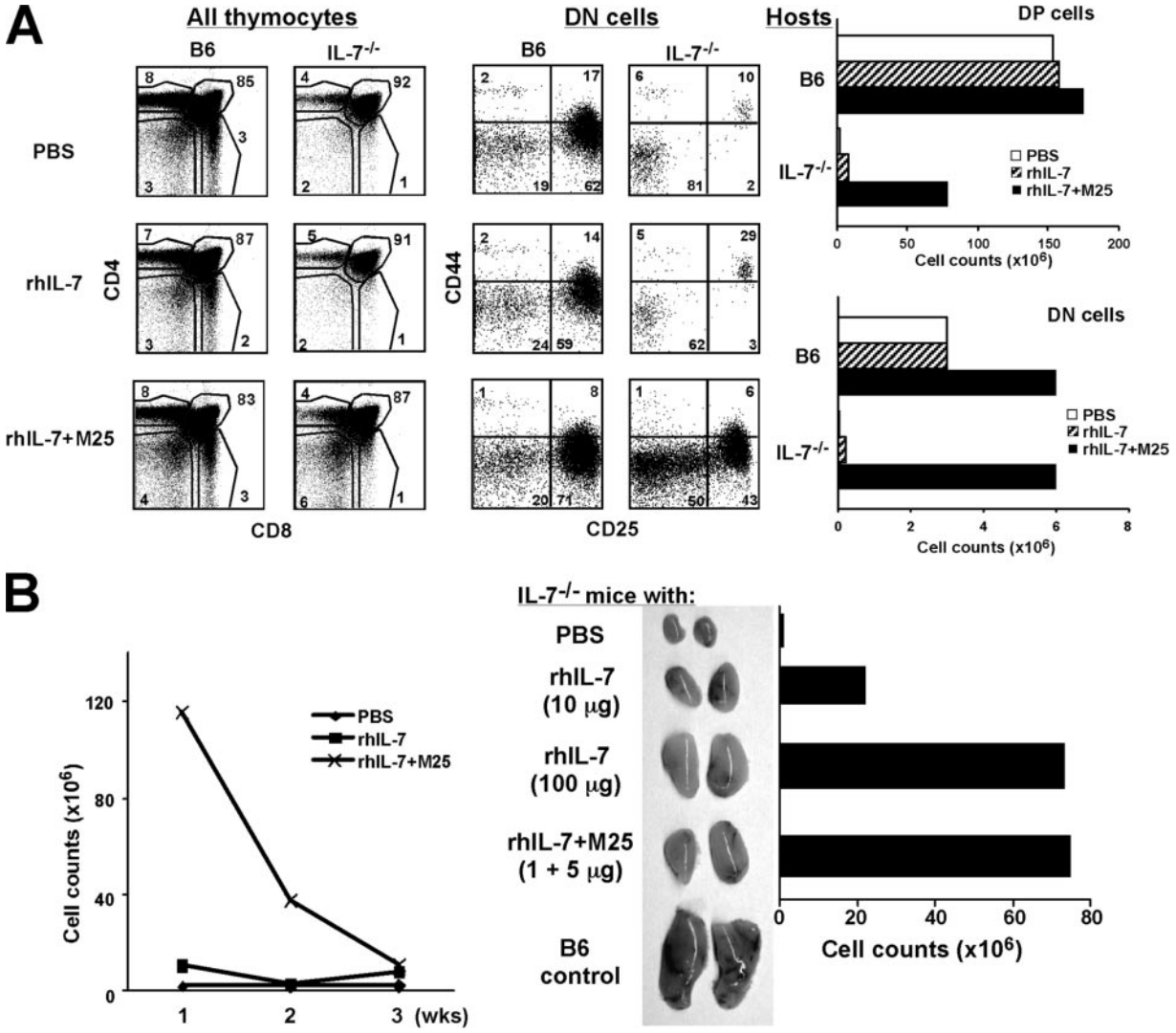


**FIGURE 2.** IL-7/mAb complexes enhance and restore thymopoiesis. *A*, Normal young B6 or B6.IL-7$^{-/-}$ mice were injected with rhIL-7 plus M25 (1.5 + 15 $\mu$g) on days 0 and 3 and analyzed on day 7 by staining thymocytes for CD8, CD8, CD44, and CD25. Control mice received either PBS or rhIL-7 alone. Shown are profiles of CD4 vs CD8 on all thymocytes (*left*), CD44 vs CD25 on CD4$^-$CD8$^-$ double negative (DN) cells (*middle*) and total numbers of CD4$^+$8$^+$ DP and CD4$^-$CD8$^-$ cells (*right*). *B*, Groups of B6 mice were injected as described in *A* and the total number of thymocytes analyzed 1, 2, and 3 wk after start of the injections (*left*). Groups of B6.IL-7$^{-/-}$ mice were injected twice, 3 days apart, with PBS, or the indicated amounts of free rhIL-7 or IL-7 plus M25 and the total number of thymocytes analyzed (*right*). The results are representative of at least two separate experiments with two to four mice per group.

not shown), suggesting that the complexes acted largely on peripheral T cells rather than by enhancing thymic export.

To better assess the ability of IL-7/M25 complexes to induce expansion of mature T cells, CFSE-labeled CD45-congenic LN cells were adoptively transferred into unirradiated normal B6 mice. These hosts were injected with rhIL-7/M25 complexes (1.5 + 7.5 $\mu$g, three times over 7 das) and the fate of donor cells analyzed; control hosts received only PBS, rhIL-7, or M25. Notably, although donor mature B and T cells did not proliferate in control hosts, injections of rhIL-7/M25 complexes induced up to four to five rounds of proliferation of most donor CD8$^+$ cells, one round of proliferation of about one half of the donor CD4$^+$ cells, and minimal proliferation of donor B220$^+$ mature B cells (Fig. 3*A*). Although the majority of the donor T cells underwent slow cell division, a population of fast dividing (CFSE$^-$) cells also emerged in some hosts. For T cells undergoing slow pace of proliferation, it is likely that the rhIL-7/M25 complexes caused "homeostatic" proliferation of donor T cells, despite being in normal T cell-replete hosts. The following three findings are consistent with this notion. First, the dividing CD8$^+$ cells up-regulated CD44, but did not down-regulate CD62L or up-regulate the acute activation markers CD25 and CD69 (data not shown); this phenotype is characteristic of cells undergoing IL-7-driven homeostatic proliferation under lymphopenic conditions (34–36). Second, self-MHC/peptide ligands appeared to be driving rhIL-7/M25-induced proliferation of naive CD8$^+$ cells as injection of the complexes elicited proliferation of two lines of naive TCR transgenic CD8$^+$ cells tested (OT-1 in Fig. 3*B* and P14 in Fig. 6), and proliferation of naive OT-I cells was largely abrogated in the absence of MHC class I molecules, i.e., in TAP-1$^{-/-}$ hosts (Fig. 3*B*). Third, injection of rhIL-7/M25 complexes caused naive T cells to undergo homeostatic proliferation in IL-7$^{-/-}$ hosts, which induce only minimal homeostatic proliferation of donor naive T cells injected alone (Fig. 3*C*). In this study, control injection of rhIL-7 at the same dose without M25 failed to increase donor T cell proliferation (Fig. 3*C*). It should be mentioned that rhIL-7/M25 induced

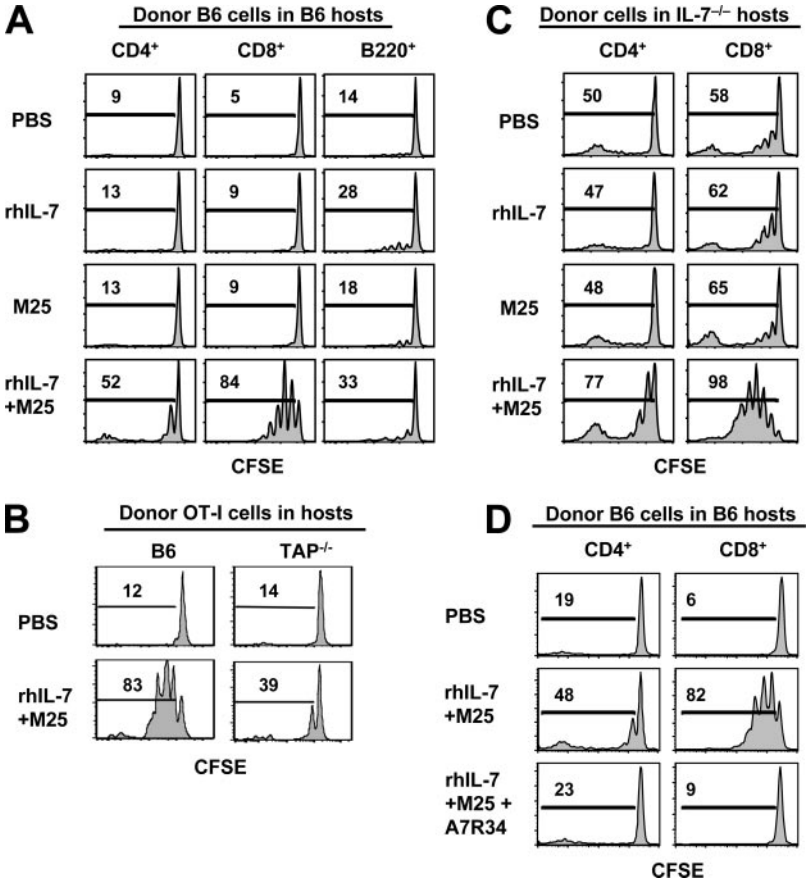


**FIGURE 3.** IL-7/mAb complexes induce homeostatic proliferation of mature T cells in response to a combination TCR and IL-7R signals. *A*, CFSE-labeled CD45.1-congenic B6 LN cells were transferred into normal CD45.2$^+$ B6 mice and the hosts were then injected three times, every 2 days, with rhIL-7 plus M25 (1.5 + 7.5 $\mu$g); control mice received PBS, rhIL-7, or M25 alone. CFSE profiles of donor CD4$^+$, CD8$^+$, and B220$^+$ cells in host LN were analyzed 8 days later by staining for CD45.1, CD4, CD8, or B220 by flow cytometry as described in *Materials and Methods*. *B*, Purified Thy-1.1$^+$ naive OT-I TCR transgenic cells were CSFE-labeled and transferred into normal B6 and TAP$^{-/-}$ hosts. The hosts were injected with rhIL-7 plus M25 complexes or PBS as described in *A* and analyzed 7 days later. The CFSE profiles of donor T cells analyzed by flow cytometry after staining host spleen cells for Thy-1.1, CD4, and CD8 are shown. *C*, CFSE-labeled Thy-1-congenic B6.PL LN cells were injected into B6.IL-7$^{-/-}$ mice, followed by injections of the hosts with the indicated reagents as in *A* and analyzed on day 7. The CFSE profiles of donor T cells analyzed by flow cytometry after staining host spleen cells for Thy-1.1, CD4, and CD8 are shown. *D*, CFSE-labeled B6.PL LN cells were transferred into normal B6 mice and then the hosts were injected with PBS, rhIL-7 plus M25 (1.5 + 7.5 $\mu$g) complexes or rhIL-7 plus M25 (1.5 + 7.5 $\mu$g) complexes together with anti-IL-7R$\alpha$ mAb A7R34 (50 $\mu$g) every other day for 7 days. The CFSE profiles of donor T cells in host LN analyzed by flow cytometry after staining for Thy-1.1, CD4 and CD8 are shown. The results are representative of two to three experiments with two to three mice per group.

proliferation of donor T cells by directly engaging IL-7R, as proliferation was completely abrogated when anti-IL-7R$\alpha$ mAb A7R34 was coinjected with the complexes (Fig. 3*D*).

Correlating with the data on thymus regeneration, IL-7/M25 complexes were ~100-fold more effective than IL-7 alone at inducing homeostatic proliferation of mature T cells in IL-7$^{-/-}$ hosts. In this study, the percentage of donor CD8$^+$ cells that divided more than three times after injection of 1 $\mu$g IL-7 plus M25 was higher than with administration of 10 $\mu$g or 100 $\mu$g of IL-7 alone (data not shown).

As for homeostatic proliferation to endogenous IL-7 in lymphopenic hosts (30, 37), injection of rhIL-7/M25 complexes caused much stronger proliferation of naive CD8$^+$ cells than naive CD4$^+$ cells, both in normal and lymphopenic hosts. Because rhIL-7 appears to bind to mouse IL-7R with a slightly lower affinity than rmIL-7 (38) we examined the effects of rmIL-7/M25 on naive CD4$^+$ cells. Notably, rmIL-7/M25 complexes displayed 2- to 3-fold greater biological activity than rhIL-7/M25 complexes and induced efficient proliferation of donor CD4$^+$ and CD8$^+$ donor cells in normal B6 hosts (Fig. 4*A*). Proliferation of both subsets was also seen with rhIL-7/M25 complexes when these complexes were injected at higher doses (Fig. 4*B*). Unfortunately, the prohibitively high cost of rmIL-7 has limited further studies with this reagent.

The above experiments were performed using whole LN cells from young mice as donor cells. Because 80–90% of the T cells in

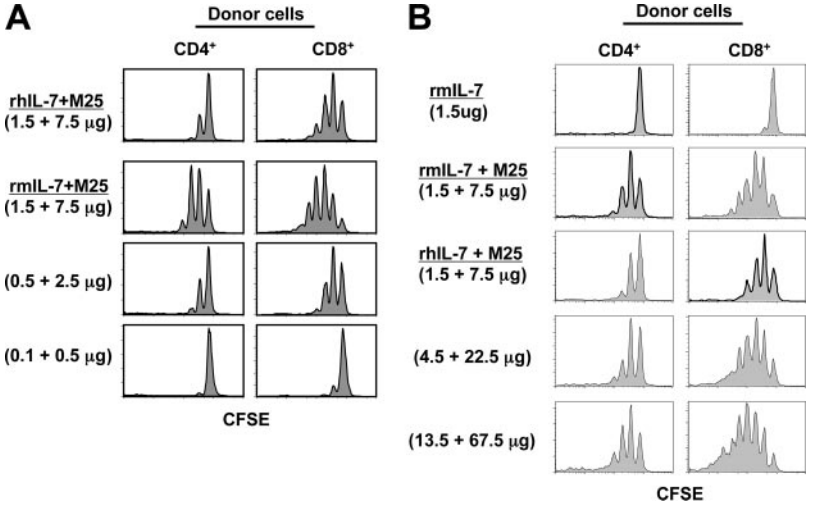


**FIGURE 4.** Mouse IL-7/M25 complexes display slightly higher biological activity than human IL-7/mAb complexes. *A*, Titration of mouse rIL-7/M25 complexes. CFSE-labeled B6.PL LN cells were injected into normal B6 mice; the hosts injected with indicated doses of rhIL-7/M25 or rmIL-7/M25 complexes three times and analyzed on day 7. The CFSE profiles of donor T cells in host LN are shown. *B*, Titration of human IL-7/M25 complexes. CFSE-labeled B6.PL LN cells were transferred into normal B6 mice and the hosts injected three times with indicated rmIL-7/M25 or rhIL-7/M25 complexes. The CFSE profiles on donor T cells in host LN were analyzed 7 days after start of the injections.

young LN are naive cells, most of the responding cells were presumably naive T cells. To seek direct evidence on this issue, the experiments were repeated using sorted $CD44^{low}$ naive-phenotype donor T cells. These studies showed that both naive $CD4^+$ and $CD8^+$ T cells were able to undergo efficient homeostatic proliferation in response to rhIL-7/M25 complexes (Fig. 6*A*).

*Requirement for activity of IL-7/mAb complexes*

The above results demonstrated that binding IL-7 to anti-IL-7 mAb M25 before injection greatly increases the in vivo biological activity of the cytokine. Moreover, the ability of M25 to boost the activity of exogenous IL-7 applies to both human and mouse IL-7. Interestingly, despite the ability of M25 to augment activity of exogenous mouse IL-7, injection of M25 alone failed to have any effect on $IL\text{-}7R^+$ cells (Figs. 1 and 3), indicating that M25 cannot boost the activity of preexisting endogenous IL-7. Therefore, M25 is different from the anti-IL-2 mAbs which when injected alone were able to enhance the biological activity of endogenous IL-2 (14). Why this is the case is unknown but could reflect the fact that M25 is a mouse mAb raised against rhIL-7 (26), and its cross-reactivity for mIL-7 maybe too low to enhance the activity of endogenous mIL-7. In addition, or maybe alternatively, the basal level of endogenous IL-7 under normal conditions could be too low to be impacted by M25 injection. To evaluate the validity of these ideas, the effect of injecting M25 into hosts known to possess elevated levels of endogenous IL-7 was analyzed, namely, in lymphopenic mice. However, even repeated injection of M25 (7.5 $\mu$g three times over 7 days) alone failed to augment T cell proliferation in irradiated B6 hosts (Fig. 5*A*), implying no discernible effect on endogenous IL-7. Because M25 displays higher affinity toward hIL-7 than mIL-7, we then tested whether injecting M25 is effective in mice with elevated levels of hIL-7, i.e., in mice injected with hIL-7 just before administration of M25. However, even injecting rhIL-7 only 1 min before administration of M25 failed to enhance the activity of rhIL-7 (Fig. 5*B*). These findings suggest that M25 is able to boost the activity of IL-7 only when prebound to the cytokine and is not able to enhance the activity of the endogenous cytokine.

Precisely why cytokine/mAb complexes display so much greater activity than free cytokines under in vivo conditions is not known, but previous work with an anti-IL-2 mAb has revealed that the Fc portion of the mAb must be present, implying involvement of Fc receptors (14). To determine whether the same requirement applies to anti-IL-7 mAb M25, M25 was digested with pepsin to remove the Fc portion of the mAb. Only Fab, instead of $F(ab)_2'$ fragments, were generated from pepsin digestion of M25, presumably because M25 is a mouse IgG2b, an isotype known to yield only Fab fragments upon pepsin digestion (39). Despite the caveat that a Fab fragments is a monomer with 2-fold lower avidity than a dimeric $F(ab)_2'$ fragment, the potent biological activity of rhIL-7/M25 complexes generated with intact M25 mAb was largely abrogated when the complexes were generated with Fab fragments of M25 (Fig. 5*C*).

One of the main mechanisms previously proposed to be involved in enhancing the activity of IL-7 by M25 binding is the prolongation of the cytokine lifespan under in vivo conditions (16). To assess this idea, normal B6 mice were injected with rhIL-7/M25 complexes (1.5 + 15 $\mu$g, one time only) at various time points before injecting CFSE-labeled Thy-1-congenic LN cells to determine the duration the complexes will display biological activity under in vivo conditions. Analysis of donor T cells 7 days after donor cell injection revealed that the mitogenic effect of the complexes was still observed even as late as 24 h post injection (Fig. 5*D*). One caveat of this finding is that the binding of the

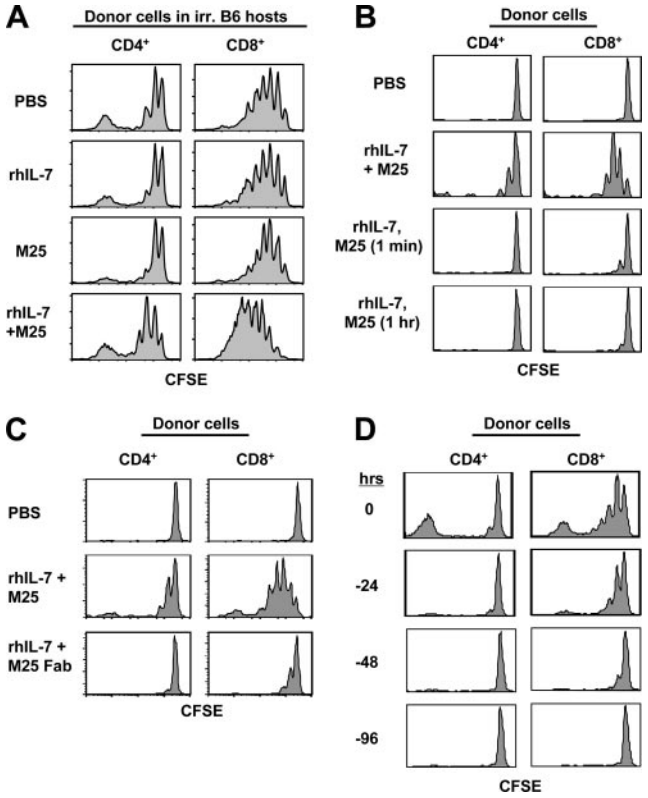


**FIGURE 5.** The requirements of IL-7/M25 complexes to induce homeostatic proliferation of T cells. *A*, Injection of M25 alone is unable to boost the activity of elevated IL-7 levels present under lymphopenic conditions. CFSE-labeled B6.PL LN cells were injected into irradiated (600 cGy) B6 mice, which then received three injections of either PBS, M25 (7.5 $\mu$g), or rhIL-7 (1.5 $\mu$g), or rhIL-7 plus M25 (1.5 + 7.5 $\mu$g) and analyzed on day 7. The CFSE profiles of donor T cells in host LN are shown. *B*, IL-7 and M25 need to be injected concurrently to induce T cell proliferation. CFSE-labeled B6.PL LN cells were transferred into normal B6 mice and the hosts injected once with rhIL-7 plus M25 (2 + 10 $\mu$g) complexes or rhIL-7 first followed 1 min or 1 h later with M25. The CFSE profiles on donor T cells in host LN were analyzed 7 days after start of the injections. *C*, IL-7/mAb complexes generated with Fab fragmentsof M25 do not display enhanced IL-7 activity. CFSE-labeled B6.PL LN cells were injected into normal B6 mice, which then received injections of either rhIL-7 plus M25 (1.5 + 10 $\mu$g) or IL-7 plus M25 Fab fragments (1.5 + 10 $\mu$g) every other day. The CFSE profiles of donor T cells in host LN were analyzed 7 days later. *D*, IL-7/mAb complexes induce T cell proliferation for ~24 h. CFSE-labeled B6.PL LN cells were injected into normal B6 mice that were injected once with rhIL-7 plus M25 (2 + 10 $\mu$g) complexes right afterward or at 24, 48, or 96 h before injection of the donor T cells. The CFSE profiles of donor T cells in host LN were analyzed 7 days after injection of the T cells. All results are representative of two to four experiments with two to four mice per group.

IL-7/mAb complexes to host $IL\text{-}7R^+$ cells may have abbreviated the half-life of the complexes. Arguing against this possibility, the complexes displayed a similar duration of biological activity when the experiments were repeated in $IL\text{-}7^{-/-}$ hosts, which are severely deficient in $IL\text{-}7R^+$ cells (data not shown). These findings suggest that extension of the in vivo cytokine activity is a major mechanism by how M25 boosts the activity of IL-7.

*IL-7/mAb complexes induce homeostatic proliferation of memory T cells*

We next analyzed the effect of injecting IL-7/M25 complexes on memory T cells. For memory $CD8^+$ cells, CFSE-labeled sorted $CD44^{high}$ MP Thy-1-congenic $CD8^+$ cells were transferred into

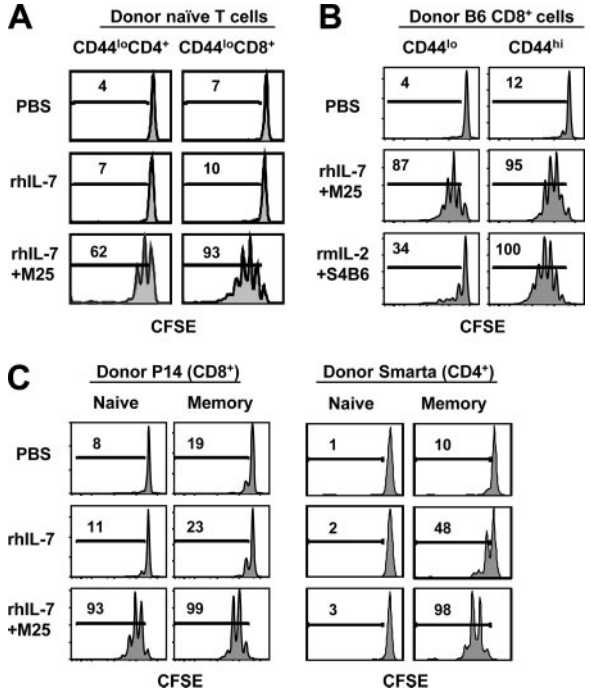


**FIGURE 6.** IL-7/mAb complexes induce homeostatic proliferation of naive and memory T cells. *A*, B6.PL LN cells were sorted by flow cytometry for $CD44^{low}CD4^+$ and $CD44^{low}CD8^+$ cells, labeled with CFSE and transferred into normal B6 mice. The hosts were injected three times, ever 2 days, with rhIL-7 plus M25 (3 + 15 $\mu$g); control mice received PBS or rhIL-7 (3 $\mu$g). CFSE profiles of donor T cells in host LN were analyzed by flow cytometry on 7 d after staining for Thy-1.1, CD4, and CD8. *B*, Sorted $CD44^{low}$ and $CD44^{high}$ B6.PL LN $CD8^+$ cells were labeled with CFSE and transferred into normal B6 mice. The hosts were injected three times with rhIL-7 plus M25 (1.5 + 10 $\mu$g); control mice received PBS or rmIL-2 plus S4B6 (1.5 + 10 $\mu$g). CFSE profiles of donor T cells in host LN were analyzed by flow cytometry on day 7 after staining for Thy-1.1 and CD8. *C*, CFSE-labeled naive and memory Thy-$1.1^+$ $CD8^+$ P14 or $CD4^+$ Smarta TCR transgenic T cells were transferred into normal B6 mice, the hosts injected with rhIL-7 plus M25, and analyzed as in *B*. The hosts transferred with Smarta cells were injected with a slightly higher dose of rhIL-7/M25 complexes than usual (3 + 15 $\mu$g of rhIL-7 + M25). Shown are CFSE profiles of naive and memory donor P14 (*left*) and Smarta (*right*) cells in host spleen. Memory P14 and Smarta cells were generated by transferring small numbers of naive P14 cells into B6 mice, immunizing the hosts with LCMV, and waiting for ≥6 wk. All results are representative of at least two separate experiments with two to four mice per group.

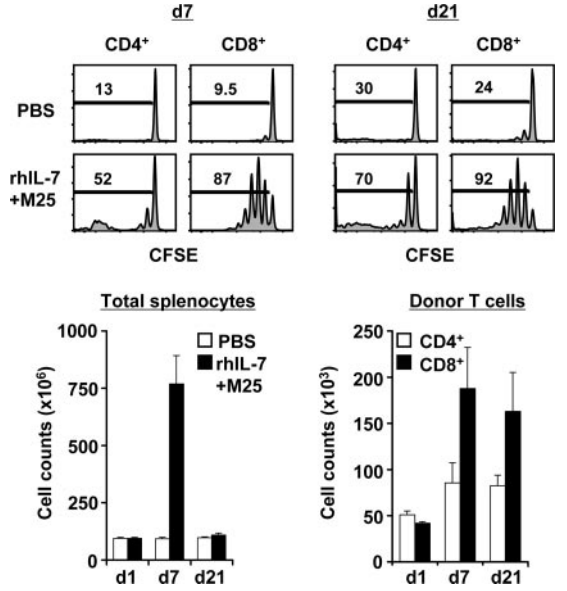


**FIGURE 7.** Fate of lymphocytes expanded by IL-7/mAb complexes. CFSE-labeled B6.PL LN cells were transferred into B6 hosts, injected three times with PBS or rhIL-7 plus M25 (1.5 + 10 $\mu$g), every 2 days, and CFSE profiles of donor T cells analyzed 7 and 21 days after start of the injections. Some mice were analyzed 1 day after donor cell injection as controls. Shown are CFSE profiles of donor cells in host spleen on days 7 and 21 (*top*) and the recoveries of total number of lymphocytes (*bottom left*) and donor T cells (*bottom right*) in host spleen on days 1, 7, and 21. The results are representative of two separate experiments.

normal B6 mice, which then received rhIL-7/M25 complexes (1.5 + 7.5 $\mu$g, three times over 7 days). As controls, the response of MP $CD8^+$ cells following administration of IL-2/mAb complexes and the responses of $CD44^{low}$ naive cells to injection of the two types of complexes were analyzed. Significantly, rhIL-7/M25 complexes induced prominent proliferation of both naive and MP donor $CD8^+$ cells in normal B6 hosts (Fig. 6*B*). In contrast, control rmIL-2/S4B6 complexes elicited much stronger proliferation of MP $CD8^+$ cells than of naive $CD8^+$ cells (Fig. 6*B* and Ref. 14). In addition to MP $CD8^+$ cells, rhIL-7/M25 also promoted strong homeostatic proliferation of LCMV-specific P14 TCR transgenic memory $CD8^+$ cells in normal hosts (Fig. 6*C*). Indeed, both naive and memory P14 cells proliferated in response to rhIL-7/M25 complexes in normal T-sufficient hosts at a rate that was similar to that observed for these cells in lymphopenic hosts (Fig. 6*C* and data not shown).

To study the effect of IL-7/mAb complexes on memory $CD4^+$ cells, we used a line of LCMV-specific TCR transgenic $CD4^+$ cells on a B6 background, designated Smarta (24), to generate memory $CD4^+$ cells. These cells lack the population of fast-dividing effector-like cells found in normal MP $CD4^+$ cells (40). When CFSE-labeled Thy-$1.1^+$ Smarta memory $CD4^+$ cells were transferred into normal B6 hosts, injecting slightly higher doses of rhIL-7/M25 complexes (3 + 15 $\mu$g, three times over 7 days) caused the donor memory Smarta cells to undergo efficient homeostatic proliferation (Fig. 6*C*). By contrast, naive Smarta cells failed to respond to rhIL-7/M25 complexes (Fig. 6*C*), consistent with the fact that these cells cannot undergo homeostatic proliferation in lymphopenic hosts (40).

### *Lifespan of IL-7/mAb-expanded lymphocytes*

To determine the fate of the lymphocytes expanded by IL-7/mAb complexes, CFSE-labeled Thy-1-congenic LN cells were transferred into normal B6 mice, which were then injected with rhIL-7/M25 (1.5 + 10 $\mu$g, three times over 7 days) and analyzed 2–3 wk later; some mice were analyzed at days 1 and 7 as controls. As expected, the donor T cells, especially $CD8^+$ cells, underwent considerable proliferation upon injection of the cytokine/mAb complexes during the first 7 days; at this stage there was prominent splenomegaly (Fig. 7), mainly due to the marked expansion pre-B cells (Fig. 1). However, during the next 2 wk, the spleen returned to normal size and the donor cells showed little change in their CFSE profiles, suggesting that the T cells had ceased to proliferate (Fig. 7). Moreover, although the expanded pre-B cells largely disappeared during this time, there was no decline in the numbers of the expanded T cells between days 7 and 21, and the expanded cells retained the phenotype of memory cells ($CD44^{high}$) (data not shown). The maintenance of expanded $CD44^{high}$ T cells also applied to host T cells, indicating that the naive-to-memory conversion induced by the injected complexes was followed by survival of these cells for ≥2 wk.

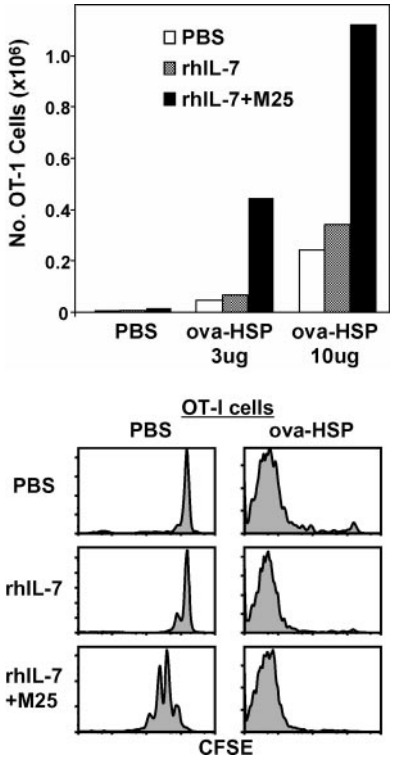


**FIGURE 8.** IL-7/M25 complexes augment primary $CD8^+$ cell response to foreign Ags. CFSE-labeled Thy-$1^+$ OT-I TCR transgenic cells were purified and adoptively transferred to normal B6 hosts and immunized with either 3 or 10 $\mu$g OVA-HSP fusion protein; control mice receive only PBS. The hosts were also injected twice, on days 0 and 4, with rhIL-7 plus M25 (1.5 + 10 $\mu$g) or with only rhIL-7 or PBS. The recoveries (*top*) and CFSE profiles (*bottom*, from mice injected with 10 $\mu$g OVA-HSP) of donor cells in host spleen were analyzed by flow cytometry on day 6. Virtually identical CFSE profiles of donor OT-I cells were observed from mice injected with 3 $\mu$g OVA-HSP (data not shown). The results are representative of at least two separate experiments.

### *IL-7/mAb complexes boost T cell responses to foreign Ags*

To determine whether IL-7/M25 complexes can enhance T cell responses to foreign Ags, OVA-specific Thy-$1.1^+$ $CD8^+$ OT-I cells were transferred into normal B6 mice, which were then immunized with a low and high dose of OVA protein covalently linked to a heat shock protein with or without rhIL-7/M25 complexes (1.5 + 10 $\mu$g, injected on days 1 and 4). As controls, some mice were injected with OVA-HSP plus free rhIL-7, whereas other mice received only the cytokines without OVA-HSP. Analysis of the hosts on day 6 showed that OVA-HSP alone at both doses induced rapid proliferation of OT-I cells, but expansion was much more pronounced with the high dose (~25-fold) than at the low dose (~5-fold) (Fig. 8). When supplemented with rhIL-7/M25 complexes the expansion of OT-I cells was even greater, and the augmentation was highest for OT-I cells responding to the low dose of OVA-HSP. Thus, although the response to the high dose of OVA-HSP was enhanced ~4-fold, the response to the low dose of OVA-HSP was ~10-fold greater than that observed with OVA-HSP alone (Fig. 8). In conclusion, IL-7/mAb complexes can augment T cell responses to foreign Ags, especially with suboptimal doses of Ag.

## Discussion

IL-7 was initially discovered on the basis of its non-redundant role in the growth and differentiation for B and T cell progenitors in the mouse (7, 41–43), and shown subsequently to be important for T cell, but not B cell, development in humans (44). More recently, IL-7 has also been found to be essential in supporting homeostasis of nearly all subsets of T cells. Thus, together with TCR signals from contact with self-MHC/peptide ligands, IL-7 is essential for sustaining the survival of naive $\alpha\beta$ T cells, and also for inducing naive T cells to undergo "spontaneous" homeostatic proliferation under lymphopenic conditions (6, 45). Moreover, IL-7, together with IL-15, was found to be crucial for survival and homeostatic proliferation of memory $\alpha\beta$ T cells as well as $\gamma\delta$ T cells and NKT cells (46–48). The only exception among T cells for IL-7-dependency for survival thus far appears to be $CD4^+CD25^+$ Tregs, which express low levels of IL-7R (49, 50).

Reflecting its prominent role for T and B cell development, in vivo administration of IL-7 has been known for some time to augment lymphopoiesis and induce expansion of mature lymphocytes. In normal mice, IL-7 injection has been reported by a number of labs to induce strong expansion of pre-B cells and mature T cells with only minor effects on thymocytes and mature B cells (51–55). In mice exposed to lympho-depleting irradiation or cytolytic drugs, IL-7 injection accelerated restoration of T cell numbers in the periphery and enhanced thymopoiesis (54, 56, 57). Moreover, in primates, IL-7 therapy induced expansion of mature T cells in humans and monkeys, but had only a minimal effect on B cells, consistent with the notion that IL-7 is not essential for B cell development in primates (58–60). To be effective in vivo, however, relatively high amounts of IL-7 appear to be required for a prolonged duration. For instance, administration of IL-7 in quantities sufficient to raise blood levels 10- to 100-fold for 3 wk caused only a 3- to 7-fold increase in T cell numbers in humans (59). Hence, much of the administered IL-7 may have limited biological activity, perhaps because of short lifespan or from the failure to reach appropriate niches in the lymphoid tissues.

In this report, we describe an effective way to markedly boost the biological activity of IL-7 for T cells, namely, by binding the cytokine to an anti-IL-7 mAb M25 before administration. The ability of specific anti-cytokine mAbs to dramatically boost the in vivo activity of the bound cytokines was initially recognized in the early 1990s. This applied to several cytokines, including IL-2, IL-3, IL-4, IL-6, and IL-7 (15, 16, 61–63). Nonetheless, the potential utility of cytokine/mAb complexes remained largely unexplored. We and others (14, 64) have recently rekindled interest in this technology by demonstrating that the superagonistic activity of IL-2/mAb complexes is the mechanism behind the enigmatic ability of anti-IL-2 mAbs to enhance expansion of memory $CD8^+$ cells; here, anti-IL-2 mAb boosts the biological activity of endogenous IL-2. Unlike past studies on IL-2/mAb complexes, which used indirect methods to measure of T cell expansion, such as anti-tumor activity, these recent studies directly described the strong mitogenic potential of the IL-2/mAb complexes on T cells. Past studies defined the strong activity of IL-7/mAb on pre-B cells (16), but its effect on T cells remained undefined until now.

In the current work, we confirmed the previous finding by Finkelman et al. (16) that administration of IL-7/M25 complexes induces conspicuous expansion of pre-B cells. Pre-B cells appeared to be the most responsive cell type to these complexes, undergoing 200–400-fold expansion within 1 wk and causing the bone marrow to appear white and the spleen to expand dramatically. Progenitors of T cells also responded readily to IL-7/M25 complexes, but to a lesser degree than pre-B cells as numbers of $CD4^-CD8^-$ DN cells in the thymus increased only 2-fold in normal mice. Nonetheless, dramatic restoration of thymopoiesis was observed within 1 wk when IL-7/M25 complexes were injected into IL-$7^-$ mice; thus, the complexes induced prompt generation

of the late subset of $CD25^+$ DN cells, which are largely absent in $IL\text{-}7^-$ mice, and the rapid emergence of DP thymocytes.

At the level of mature lymphocytes, IL-7/M25 complexes had a strong mitogenic effect for T cells, but not B cells, closely reflecting the expression levels of IL-7R, which is high on T, but not B cells. The effect of the complexes on mature T cells was clearly evident from the prominent enlargement of the LN in normal B6 mice within 1 wk and the increase in the T:B cell ratio. Proliferation induced by IL-7/M25 complexes applied to both naive and memory cells for $CD4^+$ and $CD8^+$ subsets, although $CD4^+$ cells were clearly less responsive than $CD8^+$ cells. The characteristics of T cell expansion induced by IL-7/M25 complexes in normal mice closely resembled spontaneous homeostatic proliferation that occurs in response to severe lymphopenia. Thus, homeostatic proliferation of naive T cells in normal mice was accompanied by up-regulation of CD44, but not CD69, indicative of responses to low affinity self-MHC/peptide ligands (34–36). It should be mentioned that in an apparent discrepancy, one of the past studies reported that T cells in normal mice stimulated to undergo expansion with repeated injection of IL-7 did not acquire any activation or memory markers, including up-regulation of CD44 (52). However, these investigators analyzed the phenotype of the host T cells after only a 2-day period of IL-7 injections. Such a short duration is unlikely to be sufficient for T cells to have undergone multiple rounds of cell divisions, a prerequisite for up-regulation of CD44 (36). Another indication that IL-7/M25 complexes induced homeostatic proliferation was that blocking signaling through either IL-7R or TCR prevented proliferation of naive T cells. In addition, proliferation applied to a large repertoire of naive T cells, including TCR transgenic lines, indicating that TCR signaling is most likely derived from contact with self-MHC/peptide ligands. IL-7/M25 complexes also strongly augmented the responses of naive T cells to foreign Ags, presumably due to the costimulatory activity of IL-7. In essence, IL-7/M25 complexes have the ability to enhance TCR signaling, whether it is covert from contact with low affinity self-ligands or overt from contact with high affinity foreign Ag.

In two different assays, we found the IL-7/M25 complexes displayed 50- to 100-fold higher in vivo activity than free IL-7. Why the cytokine/mAb complexes are so much more potent than free cytokines is currently unknown. One essential component appears to be the intact Fc portion of the mAb, as M25 Fab fragments had only minimal capacity to boost the activity of bound IL-7, although some reduction in the activity is likely to be due to a 2-fold reduction in the avidity. Similarly, $F(ab)_2'$ fragments of an anti-IL-2 mAb S4B6 were also incapable of augmenting the activity of IL-2 (14). The exact role of the Fc portion remains unclear, but it appears not to mediate its effect through the Fc$\gamma$Rs (20, 65). However, it is possible that the neonatal Fc receptors, which are involved in prolonging the half-life of IgG (65, 66), could play an important role. Past reports have ascribed an increased in cytokine half-life as the main mechanism behind the intense activity of cytokine/mAb complexes (15, 16, 61–63). This idea is supported by the finding that IL-7/M25 complexes survived functionally for ~24 h, much longer than the ~20-min half-life typically assigned to free cytokines (14, 64). However, we believe that additional mechanisms are likely to be involved for the following two reasons. First, not all anti-cytokine mAbs can mediate superagonistic activity. Thus, in contrast to neutralizing M25 (mouse IgG2b) Finkelman et al. (16) has previously found that a non-neutralizing anti-IL-7 mAb M23 (mouse IgG1) failed to augment the activity of rhIL-7. Similarly, we also recently found that two additional anti-IL-7 mAbs, a neutralizing MAB207 (mouse IgG1; R&D Systems) and a non-neutralizing MAB407 (rat IgG2b; R&D Systems), were also ineffective in boosting the activity of bound IL-7 (data not shown). Second, at least for IL-2, fusion proteins are ineffective. Thus, we have recently found that rIL-2-mAb fusion proteins, which are also known to have extended half-life under in vivo conditions (67, 68), did not display the strong activity of IL-2/mAb complexes in terms stimulating MP $CD8^+$ cells and $CD25^+CD4^+$ Tregs (data not shown). The nature of the additional mechanism(s) involved in the high activity of cytokine/mAb complexes is currently under investigation.

One intriguing finding related to the above issue is that M25, as with S4B6 (the prototypic augmenting anti-IL-2 mAb), is noted for its ability to neutralize the activity of it target cytokine (26). However, because injection of large amounts of M25 is needed to block IL-7 activity, the question arises as why M25 enhances the activity of IL-7 at lower doses. One interesting hypothesis recently put forth by Phelan et al. (65) is that the cytokine may eventually need to dissociate from the mAb for cytokine/mAb complexes to display its strong activity, and this is unlikely to occur if mAb is present in large excess. A more trivial, but not mutually exclusive idea is that the affinity of the blocking mAb to the cytokine is lower than the affinity of the cytokine to its receptor, thus requiring a large excess of mAb to block the activity of the cytokine. At lower doses, as mentioned above, the neutralizing mAbs gain the unexpected capacity to enhance the cytokine activity, presumably by serving as a depot of cytokines and also by mediating some additional effect that is yet to be defined.

Irrespective of the mechanisms involved, the ability of IL-7/mAb complexes to induce prominent expansion of immature and mature lymphocytes is likely to be useful for a number of therapeutic applications. In humans, beneficial effects are likely to be limited to T cells, because B cell generation is fairly undisturbed in individuals with defective IL-7R signaling (44, 69) and administration of free IL-7 has minimal effect on B cells (59). Hence, the complexes could be useful for restoring the T cell pool for patients undergoing lympho-depleting treatments for cancer or organ transplantation. Treatment of immunodeficient individuals could also be considered, but only if they do not have a defect in signaling through the IL-7R. In this regard, humans with mutations in IL-7, possibly the ideal candidate for IL-7/mAb complexes, have yet to be reported. Another suitable group of individuals who may benefit are people of advanced age, who are often susceptible to new pathogens due to depleted numbers of naive T cells. Restoring the naive T cell counts through induction of thymopoeisis and expansion of preexisting naive T cells may lead to enhanced capacity to mount an effective immune response against both new and reoccurring pathogens. For practical purposes in terms of clinical applications, we have found both i.v. and i.p. routes of administration of both IL-7/mAb and IL-2/mAb complexes to be equally effective.

Finally, the capacity of IL-7/mAb complexes to augment T cell responses to foreign Ags could be beneficial in two respects. First, these complexes may potentiate the efficacy of vaccines, especially weak vaccines with low immunogenic non-replicating recombinant proteins. Second, IL-7/mAb complexes might serve to boost T cell immunity to tumors, particularly weakly immunogenic tumors, and also to promote responses to infectious agents. Stimulation of Tregs is unlikely because these cells are relatively independent of IL-7 (49, 50).

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## Disclosures

A patent listing O.B., J.S., and C.D.S. as inventors describing the main technology described in the manuscript, namely, the potent activity of cytokine/mAb complexes, is currently pending.

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