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CD25⁺CD4⁺ Regulatory T Cells and Memory T Cells Prevent Lymphopenia-Induced Proliferation of Naive T Cells in Transient States of Lymphopenia

Christine Bourgeois and Brigitta Stockinger

Lymphopenia has been associated with autoimmune pathology and it has been suggested that lymphopenia-induced proliferation of naive T cells may be responsible for the development of immune pathology. In this study we demonstrate that lymphopenia-induced proliferation is restricted to conditions of extreme lymphopenia, because neither naive nor memory T cells transferred into T cell-depleted hosts proliferate unless the depletion exceeds 90% of the peripheral repertoire. Memory CD4 T cells as well as regulatory CD4 T cells proved to be relatively resistant to depletion regimes, and both subsets restrict the expansion and phenotypic conversion of naive T cells by an IL-7R-dependent mechanism. It therefore seems unlikely that lymphopenia-induced proliferation of peripheral T cells causes deleterious side effects that result in immune pathology in states of partial and transient lymphopenia. The Journal of Immunology, 2006, 177: 4558–4566.

In contrast to Ag-driven responses, the term lymphopenia-induced proliferation (LIP)³ refers to the expansion and activation of peripheral T cells in response to lymphopenia in the absence of their cognate Ags (1). This process, which has been extensively studied in experimental models in which naive T cells are transferred into hosts lacking any lymphocytes, is driven by recognition of self-peptide/MHC complexes and cytokine signaling (2). The extent of T cell expansion and the degree of phenotypic conversion are dependent on the number of cells that are transferred (3, 4). In response to lymphopenic episodes, T cell homeostasis is restored by two main pathways: thymic export and peripheral expansion. Although thymic export is the predominant source of T cell regeneration, peripheral expansion may participate in T cell restoration, especially when the thymic function is altered (5, 6). Peripheral expansion includes different mechanisms such as Ag-driven responses, basal homeostatic proliferation (or “self-renewal”) of memory T cells, and lymphopenia-induced proliferation of peripheral naive T cells. Although initially considered as a pathway of T cell restoration in the periphery and consequently termed “homeostatic proliferation” (2, 7), it is now clear that T cell expansion in response to acute lymphopenia cannot restore the naive T cell pool (8, 9) because it induces activation and conversion of naive cells to an activated/memory phenotype (7, 10). Some of these memory-like cells exhibit enhanced functional activity similar to those expected for genuine memory cells (11–13). It has therefore been hypothesized that lymphopenia-induced activation of peripheral cells may participate in the development of immune or autoimmune pathology (14–22).

Experimental models using transfer of peripheral T cells into genetically lymphopenic hosts to study lymphopenia-induced proliferation have two major drawbacks. First, although lymphopenia-induced proliferation may occur in response to endogenous self-peptide/MHC complexes, it is clear that polyclonal T cells transferred into T cell-deficient hosts mainly react to the high load of enteric bacteria in such hosts rather than to self-peptides (23, 24). It appears that, because of the lack of T cells, genetically immunodeficient mice (such as nude, SCID, or Rag-deficient mice) cannot regulate the presentation of Ags from normal commensal bacteria. As a consequence, it is not possible to distinguish between lymphopenia-induced proliferation and environmental Ag-driven responses in experiments involving adoptive transfer of polyclonal T cells. Similarly, irradiated wild-type hosts may present higher amounts of commensal bacterial Ags due to irradiation-induced gut damage as well as the release of multiple cytokines that may influence lymphopenia-induced proliferation of adoptively transferred naive T cells (25). Second, in contrast to experimental models with genetically lymphopenic or irradiated hosts, the physiological occurrence of lymphopenia throughout an individual’s lifetime due to viral infection (26–28), therapy-related toxicity (29–31), or age-related changes (neonatal conditions (32–34) and aging) rarely results in the depletion of all peripheral T cells. However, the incidence of lymphopenia-induced proliferation and homeostasis of peripheral T cell pools under conditions of partial and transient lymphopenia have not been studied in detail.

To mimic partial lymphopenic incidents, we treated normal mice with T cell-depleting Abs. This experimental setup allowed us to titrate the degree of lymphopenia to study the behavior of residual peripheral T cells as well as adoptively transferred T cells and to investigate the composition of the peripheral T cell pool following restoration of lymphopenia by thymic output. Our data show that there is no lymphopenia-induced proliferation of transferred T cells unless the degree of depletion exceeds 90% of the peripheral T cell pool. Even in severely depleted hosts, very few divisions of transferred cells were detectable, and there was no conversion to a memory-like phenotype. Any effects of residual depleting Abs on endogenous or transferred T cells was ruled out. Memory cells as well as CD25⁺CD4⁺ regulatory T cells were able to restrain the proliferation and phenotypic conversion of naive T cells via an

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IL-7R-dependent mechanism. This finding suggests that differential competition for this cytokine by T cell subsets usually prevents excessive proliferation and phenotypic conversion of naive T cells during states of transient lymphopenia. It is therefore unlikely that conditions of partial lymphopenia provoke excessive T cell responses to self-peptide/MHC molecules that could lead to autoimmune pathology.

**Materials and Methods**

**Mice**

A1 TCR transgenic (Tg) Rag1−/− (H-2b) female mice (harboring T cells specific for the male H-Y Ag) (35) and corresponding A1 mice expressing the Thy1.1 allotype, DO11.10 TCR Tg Rag2−/− (H-2b) specific for the OVA peptide (36), 6.5 TCR Tg Rag2−/− (H-2b) specific for an influenza hemagglutinin peptide (37), and AND TCR Tg Rag2−/− mice (H-2b) (38) as well as congenic CD45.1 AND mice between 8 and 12 wk of age were used. CBA, BALB/c, C57BL/10, and IL-7 were kept under specific pathogen-free conditions, and all animal experiments were conducted in accordance with institutional guidelines and Home Office (UK) regulations.

**Depleting Abs**

Anti-CD4 (YTS 191.1) and anti-CD8 (YTS 169.4.2) depleting Abs were rat IgG2b mAbs (39) prepared from cell line supernatants and purified by affinity chromatography on columns. 19E12.1 is a mouse mAb against Thy1.1 (40), and culture supernatants of this hybridoma were purified by Affigel protein A-Sepharose affinity chromatography (Bio-Rad). YTH-89.1, a rat IgG2b Ab specific for human glycophorin (41), was used as control Ab. All depleting Ab were injected i.v.

To achieve varying degrees of depletion of peripheral T cells, we injected 6- to 8-wk-old Thy1.1 CBA mice with a mix of either anti-CD4 and anti-CD8, anti-CD4, anti-CD8, and anti-Thy1.1 depleting Abs in different quantities. The dose dependency of depletion is shown in Fig. 1a, indicating that a single dose of 100 µg of anti-CD4 Ab per mouse is needed to deplete ~80% of the CD4 T cells, whereas as little as 50 µg of anti-CD8 Ab is sufficient for a similar degree of depletion of CD8 T cells. One hundred micrograms of anti-Thy1.1 Ab was sufficient to deplete ~80% of both the CD4 and the CD8 T cell pools. A combination of 100 µg of anti-CD4, 50 µg of anti-CD8, and 25 µg of anti-Thy1.1 Abs was used to achieve severe depletion of >90% of peripheral T cells, whereas the lower depletion regime used 50 µg of anti-CD4 and 12.5 µg of anti-CD8 Abs. Limited doses of anti-Thy1.1 Abs were used because this Ab has a slight effect on thymic cellularity, whereas anti-CD4 and anti-CD8 Abs dosed as high as 200 µg per mouse and 100 µg per mouse, respectively, had no effect on thymus cellularity or subpopulations (Fig. 1b). ELISA measurements of residual depleting Abs were done using a goat anti-rat IgG Ab, followed by the addition of test sera and development with alkaline phosphatase-conjugated mouse anti-rat IgG.

**Cell suspension, flow cytometry, and cellularity determinations**

Lymph node (axillary, inguinal, and mesenteric) and spleen cell suspensions were prepared in IMDM (Sigma-Aldrich). Cells were stained using the usual procedures with the following: allophycocyanin- and PE-CD4 (GK1.5); allophycocyanin- and FITC-TCR (H57-597); PE-CD44; biotinylated and PE-Thy1.2; PE-Thy1.1, allophycocyanin-IL-7Ra, and allophycocyanin-IgG2a isotype control. Streptavidin-PerCP was used to detect biotinylated Abs. All Abs were purchased from BD Pharmingen. Flow cytometry was performed using a FACScalibur cytometer (BD Biosciences), and data files were analyzed using FlowJo software (Tree Star).

Cell sorting was performed on a MoFlo cell sorter (DakoCytometion) using previously purified suspensions with autoMACS beads: CD25-expressing cells were selected by CD25 PE staining and PE beads before sorting; naive and memory CD4 T suspensions were derived from the CD25− fraction and positively selected for CD4. Cell sorting was based on CD4, CD44, and CD62 ligand staining for the naive and memory CD4 T cell populations and on CD4, CD25, and CD44 staining for the CD25−CD4+ regulatory T cell population. Cell numbers were determined using a Scharf Instruments Casy Counter. The total number of T cells of each phenotype in each organ was calculated from the frequency determined by FACS analysis and the total number of cells recovered from each organ. The total number of T cells recovered from the peripheral pools was considered equal to that from spleen, pooled lymph nodes, and peritoneal cavity.

**Generation of memory AI T cells**

Lymph node cells from A1 Rag1−/− female mice were transferred together with syngeneic bone marrow-derived dendritic cells pulsed with 1 µM H-Y peptide into syngeneic adoptive hosts by i.v. injection as previously described (42). Dendritic cells were generated from bone marrow cultures with GM-CSF (43). Memory T cells were harvested starting 6 wk after transfer.

**Polyinosinic-polycytidylic acid (poly(I:C)) treatment**

Three hundred micrograms of poly(I:C) sodium salt (Sigma-Aldrich) diluted in PBS were injected i.p. per mouse.

**CFSE labeling**

Cell division was assessed by CFSE labeling ( Molecular Probes) using standard methods. Cells were resuspended in PBS in a concentration of 107/ml and incubated with CFSE at final concentration of 2.5 µM for 10 min at 37°C, followed by two washes in IMDM medium containing 10% FCS. Labeled cells were i.v. injected into syngeneic recipients.

**Foxp3 intracellular staining**

Forfhead box P3 (Foxp3) staining was performed using the PE Foxp3 staining set (FJK-16s Ab) (eBioscience), as described in the protocol.

**Statistical analysis**

All p values were obtained using THE Mann-Whitney two-tailed t test.

**Results**

**Characteristics of T cell depletion induced by treatment with T cell depleting antibodies**

To assess the consequences of different degrees of lymphopenia on homeostasis in peripheral T cell compartments, we treated mice with different combinations of Abs to achieve partial depletion of ~50% of peripheral T cells or severe depletion of 90% of T cells (described in Materials and Methods and Fig. 1a). T cell differentiation in the thymus was not affected (Fig. 1b). Furthermore, a potential effect of depleting Abs on dendritic cells, in particular the CD8− subset of dendritic cells, was not apparent because the transfer of TCR Tg T cells into Rag-deficient hosts that had been pre-treated with anti-CD8 Abs showed equivalent division potential (Fig. 1c).

The peripheral lymphopenia induced by T cell-depleting Abs was transient in both cases, because peripheral T cell numbers recovered between 20 and 60 days after depletion, depending on the initial intensity of depletion (Fig. 2a). As shown in Fig. 2b, Ab treatment preferentially deleted naive T cells, such that only 6% of the original number of naive T cells remained 3 days after the severe depletion regime, whereas 28% of memory cells and 36% of regulatory T cells survived. The preferential loss of naive T cells resulted in a shift in activated/memory phenotype T cells expressing high levels of CD44 (Fig. 1d). This shift was already detectable 18 h after depletion (data not shown), confirming the differential sensitivity of T cell subsets to depletion rather than the selective proliferation of peripheral T cells with an activated phenotype. Similar observations of the preferential depletion of naive T cells compared with that of activated T cells under a number of conditioning regimes resulting in loss of T cells have been reported previously (44–46). CD25−CD4+ regulatory T cells, like memory T cells, were more resistant to Ab-mediated depletion and are therefore present in higher proportions in the peripheral T cell pool during a lymphopenic episode (as shown in Fig. 2b). The relative resistance of this T cell subset to various conditioning regimes (29, 47), as well as depletion by HIV infection (48, 49), has been reported previously.
Lymphopenia-induced proliferation of naive CD4 T cells is minimal in partial lymphopenia

Extrapolation from experimental models of lymphopenia in fully T cell-deficient mice would suggest that the transfer of CFSE-labeled T cells into hosts rendered partially lymphopenic by Ab-mediated T cell depletion would result in some degree of division. To study the proliferation of adoptively transferred naive peripheral T cells, we injected them into Thy1.1 congenic CBA mice that had been pretreated with either isotype control Ab (T cell-replete host) or with different doses of depleting Abs to achieve low or severe depletion of peripheral T cells. Rag-deficient hosts were used as positive controls for complete lymphopenia. FACS-sorted naive polyclonal Thy1.2 CD4 and CD8 T cells or Tg Thy1.2 CD4 T cells were CFSE labeled and transferred into the different hosts categorized as follows: "nondepleted" (100% residual T cells); "low depletion" (45% residual T cells); "high depletion" (10% residual T cells) and "Rag-deficient hosts" (0% T cells). The intensity of depletion was determined by sacrificing mice in each group and counting the peripheral T cells recovered from lymph nodes and spleen on the day of CFSE T cell injection. The proliferative response of naive CD4 or CD8 T cells from wild-type mice was assessed 12 days after transfer. Data shown in Fig. 3, a and b, indicate that the depletion of 50% of T cells induced only minimal proliferation of polyclonal naive T cells. In highly depleted hosts (90%) few divisions were detectable, but the majority of the naive T cells that were injected had divided less than twice irrespective of whether the naive cells were CD4 (Fig. 3 a) or CD8 (Fig. 3 b), which generally have been shown to exhibit a higher proliferative capacity (50). Furthermore, none of the dividing T cells changed their naive phenotype (Fig. 3 e). In contrast and as previously described, polyclonal naive T cells transferred into Rag-deficient hosts had undergone extensive division by day 12 after transfer, resulting in a large pool of cells that had completely lost the CFSE label. However, the transfer of polyclonal CD4 T cells into Rag-deficient hosts does not allow distinction between the relative roles of environmental Ag compared

FIGURE 1. Characteristics of depletion induced by depleting Ab. a, Dose-dependent effects of depleting Abs on peripheral CD4 and CD8 T cell numbers 3 days after treatment with anti-CD4 (left panel), anti-CD8 (middle panel), and Ab mix (right panel) treatment. Results are expressed as the percentage of numbers recovered in control nondepleted mice. Gray bars represent the CD4 subset, and open bars represent the CD8 subset. b, Percentage of residual double positive (DP) thymocytes after different doses of depleting Abs. Results are expressed as the percentage of numbers recovered compared with those of control nondepleted mice. c, CD8-depleting Ab (top panel) or control Ab (bottom panel) was injected into Rag-deficient (Rag neg) hosts followed 2 days later by injection of 0.5 $\times$ 10$^6$ CFSE-labeled 6.5 Tg CD4 T cells. CFSE profiles were analyzed 7 days after transfer. d, Correlation between the intensity of depletion and the frequency of CD44$^{high}$ T cells assessed 24 h after deple

FIGURE 2. T cell restoration following Ab treatment. a, Total T cells recovered from lymph nodes and spleens following treatment with low doses of anti-CD4 and anti-CD8 (0.025 and 0.0125 mg per mouse, respectively) (open circles) or a mix of high doses of anti-CD4, anti-CD8, and anti-Thy.1.1 Abs (0.1, 0.05, and 0.05, respectively) (black circles). T cell numbers were assessed on days 5, 8, 15, and 21 after T cell depletion. Mean values and SD for three mice per time point are shown. Stippled lines represent the mean value + SD of T cell numbers in control Ab-treated mice. The experiment has been repeated three times with similar results. b, Naive, memory, and regulatory T cell numbers recovered from partially (left panels) or severely (right panels) depleted hosts. Mean values and SD for three mice per time point are shown. The experiment was repeated three times with similar results.
with self-peptide/MHC complexes in proliferation. We therefore performed similar experiments with monoclonal A1 or AND TCR Tg CD4 T cells (Fig. 3, c and d), which do not encounter their cognate Ags in the adoptive host and therefore proliferate only in response to self-peptide/MHC complexes. As reported previously, A1 TCR Tg CD4 T cells divide very slowly, whereas AND TCR Tg CD4 T cells divide extensively in response to lymphopenia. Because of different proliferative capacities, CFSE profiles were determined on day 15 for hosts that had received CFSE-labeled A1 T cells and on day 7 for hosts of AND T cells. Similarly as polyclonal CD4 T cells and independently of their intrinsic proliferative capacity, no more than one division of both Tg T cell populations was observed when the host contained 50% residual peripheral T cells. More T cell proliferation was detectable in highly T cell-depleted hosts, but it was still considerably lower than the proliferation observed in Rag-deficient hosts and was not associated with conversion toward a memory-like phenotype as assessed by CD44 expression (data not shown).

We also tested the proliferative capacity of polyclonal CD4 and CD8 memory phenotype T cells in depleted hosts, because it was possible that memory T cells would have higher expansion capacity and might therefore be more sensitive to the available space created by T cell depletion. However, like their naive counterparts, memory phenotype T cells also failed to proliferate in partially depleted hosts (Fig. 3, f and g).

A potential problem for these experiments could be the persistence of depleting Abs in the adoptive hosts, which might subsequently affect the proliferative potential of transferred T cells. To test how long depleting Abs remain detectable in the circulation, we tested sera from mice injected with depleting Abs or control Abs. As shown in Fig. 4a, sera from mice that had received low-dose depleting Abs contained detectable amounts of Ab only 6 h after injection. With the high-depletion dose, some Ab was still detectable 3 days after injection, but not 6 days later. In contrast, control Abs that did not bind to any target cells in these mice were readily detectable at all time points tested. We further assessed the levels of surface bound Ab on endogenous T cells. Depleting Abs were detectable beginning 6 h after injection, but their detection gradually declined over the next 4 days. Recipients of the low-depleting dose no longer showed any surface bound Ab on day 3 after injection, whereas in recipients of the high-depleting dose, cell-bound Ab became undetectable by day 4 after injection. (Fig. 4b). To assess the proliferative capacity of residual T cells that were not depleted, naive T cells isolated from mice 3 days after injection of high-dose depleting Abs were CFSE labeled and transferred into Rag-deficient hosts. They proliferated to the same extent as naive T cells from control mice injected with control Ab when transferred into Rag-deficient hosts (Fig. 4c). This finding clearly indicates that there is no adverse effect of any residual Ab on the residual endogenous T cell population. To directly assess whether there is any free Ab in the circulation that would bind to the surface of adoptively transferred T cells, we adoptively transferred naive A1 T cells into hosts injected with a high-depleting dose of Ab 3 days previously and measured surface bound Ab levels 24 h later. No trace of depleting Abs was detectable on the transferred T cells (Fig. 4d). We therefore feel confident that the absence of lymphopenia-induced proliferation in partially lymphopenic hosts is not effected by the T cell-depleting Abs.

Partial and transient lymphopenia induced as a consequence of viral or bacterial infections is likely to be accompanied by substantial inflammation. To address the possibility that inflammatory signals are needed for lymphopenia-induced proliferation of naive T cells, we treated T cell-depleted hosts with poly(I:C) at the time of adoptive transfer of CFSE-labeled naive T cells. Transfer of polyclonal naive CD4 T cells into Ab-depleted hosts in the presence of an inflammatory signal elicited by poly(I:C) did not result in increased expansion of the transferred T cells (Fig. 5). Thus, our data show that the incidence of lymphopenia-induced proliferation of naive and memory CD4 and CD8 T cells is marginal under conditions of transient partial or even substantial depletion and does not increase in the presence of inflammatory signals. This finding suggests that lymphopenia-induced proliferation
of naive T cells is curbed by the presence of a small number of residual peripheral T cells that resisted depletion by Ab.

**Memory and regulatory T cell subsets inhibit LIP of naive T cells**

We have previously demonstrated that memory CD4 T cells are able to reduce the extent of lymphopenia-induced proliferation of naive CD4 T cells in T cell-deficient hosts. This was observed whether naive and memory CD4 T cells were cotransferred into T cell-deficient hosts or when naive T cells were transferred into hosts containing only a memory pool. Furthermore, the inhibition did not depend on naive and memory T cells sharing the same antigenic specificity (51). However, CD25+CD4+ regulatory T cells similar to memory T cells are also present in higher proportions in the peripheral T cell pool during a lymphopenic episode.

To test whether CD25+CD4+ T cells, like CD4 memory T cells, inhibit lymphopenia-induced proliferation of naive T cells, we transferred CFSE-labeled D011.10 TCR Tg CD4 T cells into Rag-deficient hosts on their own, with sorted polyclonal CD25+CD4+ T cells, or with sorted polyclonal memory CD4 T cells. The CFSE profiles obtained 10 days after transfer showed that cotransfer of CD25+CD4+ T cells or memory CD4 T cells stalled the division of naive D0.11.10 T cells, with ~50% remaining undivided compared with ~7% when transferred on their own (Fig. 6a). Based on the recovery of the naive T cell input, CD25+CD4+ T cells and memory T cells had similar inhibitory potential (Fig. 6b).

**Competition for IL-7 underlies the inhibition of LIP**

Because lymphopenia-induced proliferation of naive T cells has been shown to depend on the availability of IL-7 (52–55), we determined the respective inhibitory effect of memory or CD25+CD4+ T cells from IL-7-R-deficient or -competent hosts on the lymphopenia-induced proliferation of naive CD4 T cells. Although IL-7-R-deficient mice show severe deficiency of T cell selection in the thymus and have few if any naive T cells in the periphery, they do contain memory phenotype T cells as well as T cells with a regulatory phenotype (56).

To test the inhibitory capacity of both memory and regulatory T cells separately, we first transferred CFSE-labeled AND TCR Tg CD4 T cells expressing the CD45.1 allotype into Rag-deficient hosts either alone or with sorted IL-7-R-deficient or competent memory CD45.2 CD4 T cells. Fig. 7a shows that 8 days after transfer, ~30% of the transferred AND T cells had undergone more than seven divisions when transferred alone or cotransferred with IL-7-R-deficient memory T cells. In contrast, the presence of IL-7-R-competent memory cells reduced their proliferation such that only 9% of transferred AND T cells divided >7 times. The recovery of AND T cells transferred with wild-type memory cells was substantially reduced in agreement with their reduced proliferative capacity, whereas similar numbers of AND T cells were cotransferred with sorted IL-7-R-deficient memory T cells. To rule out the possibility that memory CD4 T cells were being depleted by the anti-CD4 Ab used to enrich for them, we performed a control experiment in which CFSE-labeled, sorted naive CD4 T cells (1 × 10^6) recovered from the depleted or control hosts were transferred into hosts containing only a memory pool. Furthermore, the inhibition of LIP of naive CD4 T cells was substantially reduced in agreement with their reduced proliferative capacity, whereas similar numbers of AND T cells were cotransferred with sorted IL-7-R-deficient memory T cells.
recovered following transfer on their own compared with cotransfer of IL-7R-deficient memory cells (Fig. 7b, left panel). The failure of IL-7R-negative memory cells to inhibit was not due to their own lower proliferative capacity, because 8 days after transfer similar numbers of IL-7R-deficient and -competent competitors were recovered from the adoptive hosts (Fig. 7b, right panel). It is also unlikely that the disrupted lymphoid architecture in IL-7R-deficient mice and the resulting potential defect in T cell selection and development are responsible for the failure of IL-7R-deficient memory T cells to inhibit lymphopenia-induced expansion, because T cells from IL-7 cytokine-deficient hosts, which have similar defects in T cell selection and development but express IL-7R, were as inhibitory as wild-type memory T cells (data not shown).

We next tested the inhibitory capacity of CD25+CD4+ T cells isolated from IL-7R-deficient hosts. CFSE-labeled CD45.1-expressing AND T cells were transferred into Rag-deficient hosts alone or with sorted IL-7R-deficient or -competent CD25+CD4+ T cells. The CFSE profile of AND Tg cells was analyzed 8 days after transfer. The cotransfer of CD25+CD4+ regulatory cells from wild-type mice reduced the fraction of AND T cells that had undergone more than seven divisions from 35 to 7%, whereas AND T cells cotransferred with CD25+CD4+ T cells from IL-7R-deficient donors divided as extensively as those transferred on their own (Fig. 7c). Recovery (Fig. 7d) of the naive AND T cell input was lower in the presence of wild-type CD25+CD4+ T cells compared with CD25+CD4+ T cells from IL-7R-deficient hosts (p = 0.03), whereas the recovery of the competitor cells was not significantly different whether they were derived from IL-7R-deficient or wild-type hosts (p = 0.19).

To address the issue of an IL-7R requirement in a more physiological context that does not involve cotransfer into T cell-deficient hosts, we injected AND T cells directly into either IL-7R-deficient hosts or Ab-depleted wild-type hosts. Rag-deficient mice served as an additional control. IL-7R-deficient hosts contained similar numbers of regulatory T cells and memory T cells (1.12 × 10^7 and 5.45 × 10^6, respectively) as Ab-depleted wild-type hosts (1 × 10^6 and 4.2 × 10^6). Nevertheless, AND T cells only divided...
in IL-7R-deficient hosts, similarly as Rag-deficient hosts, whereas they underwent only one division in Ab-depleted wild-type hosts (Fig. 7e). This finding supports the conclusion that neither memory nor regulatory T cells are able to inhibit lymphopenia-induced expansion if they cannot compete for IL-7.

To address whether the CD25⁺CD4 T cells recovered were genuine regulatory T cells rather than effector-like T cells, we performed intracellular staining for Foxp3. IL-7R-deficient CD25⁺CD4⁺ T cells expressed similar levels of Foxp3 as wild-type CD25⁺CD4⁺ T cells (Fig. 8a). Although the percentage of CD25⁺CD4⁺ regulatory T cells was higher in IL-7R-deficient mice because those mice lack naive T cells (56), absolute numbers were lower in IL-7R-deficient mice compared with wild-type mice (Fig. 8b). The data shown in Fig. 6 indicate that CD25⁺CD4⁺ regulatory T cells as well as memory T cells inhibit lymphopenia-induced expansion of other T cells by an IL-7R-dependent mechanism. This finding was unexpected in the case of CD25⁺CD4⁺ regulatory T cells, because these cells are reported to express relatively low levels of IL-7R. We therefore investigated the levels of IL-7R expression on naive, memory, and CD25⁺CD4⁺ regulatory T cells during the state of partial lymphopenia in mice that had undergone T cell-depletion regimes. CBA mice were depleted of ~50 or 90% of their peripheral T cells, and IL-7R levels were determined on the residual T cell populations 7 days later. As shown in Fig. 7c, both the naive and the memory CD4 T cells that remained following Ab-mediated depletion down-regulated IL-7R expression as compared with their counterparts in mice treated with control Abs. CD25⁺CD4⁺ regulatory T cells, in contrast, maintained a low but constant expression of IL-7R that did not change upon partial or severe depletion. Thus, IL-7R dependency of inhibition by regulatory T cells is conceivable, as they express similar levels of this receptor as both naive and memory T cells in a state of partial lymphopenia.

**Discussion**

Although the principles and mechanisms underlying the effects of lymphopenia on the behavior of peripheral T cells are mainly investigated in experimental models of extreme lymphopenia, episodes of transient lymphopenia will rarely reach such intensity of lymphocyte depletion. Bacterial and viral infections do not cause the loss of >50% of peripheral T cells (26–28, 57). Although aging is associated with a certain degree of lymphopenia due to the loss of T cell production by the thymus, the effect is not that drastic (51). The lymphopenia observed in NOD mice genetically disposed to develop diabetes is also not comparable with that seen in T cell-deficient hosts (18). During a short time frame in the neonatal phase, T cells seeding the periphery will encounter a severely lymphopenic environment, but the effect of lymphopenia-induced proliferation during this short time period remains unclear and appears minor overall (32, 34). Severe induction of lymphopenia may thus be restricted to clinical interventions such as radiation treatment or treatment with cytotoxic drugs that may, in addition to depleting mature peripheral T cells, also affect T cell replenishment by the thymus.

In this study we modeled partial lymphopenic incidents by treatment with T cell-depleting Abs. Surprisingly, even in a situation where 90% of peripheral host T cells had been eliminated, there was little evidence that the “space” created induced either a higher expansion of remaining T cells as assessed by analysis of BrdU incorporation (data not shown) or an expansion of adoptively transferred CFSE-labeled T cells, whether these were naive or memory phenotype. The lack of expansion of transferred T cells was not due to any potential effect of residual depleting Abs, as we ascertained that there was no free Ab present at the time of T cell transfer 3 days after Ab injection. All available Abs seemed to have been sequestered by peripheral T cells, such that there were not even sufficient Abs left in the circulation to affect differentiating T cells in the thymus as judged by the fact that thymus cellularity and composition of subpopulations remained unchanged after the injection of depleting Abs.

The lymphopenia induced by T cell-depleting Abs was transient, because numbers of peripheral T cells slowly increased over a period of 4–6 wk due predominantly to thymic export in agreement with previous reports (5). There was no indication of increased proliferation of residual memory T cells in the host as a response to partial lymphopenia, suggesting that the normal slow self-renewal of the memory pool is not accelerated in conditions of partial lymphopenia. Because T cell homeostasis and lymphopenia-induced proliferation are restrained by competition for survival/proliferation factors (58), it seemed reasonable to assume that residual T cell subsets were responsible for the abrogation of lymphopenia-induced proliferation in T cell-depleted mice. Indeed, both memory and CD25⁺CD4⁺ regulatory T cells that are preserved in higher numbers than naive T cells during lymphopenic

![FIGURE 8](http://www.jimmunol.org/)
episodes curbed lymphopenia-induced proliferation of naive T cells, and this effect depended on their expression of IL-7 receptors.

Thus, although the survival of naive, memory, and CD25⁺ CD4⁺ regulatory T cells under steady-state conditions depends on distinct factors, it appears that under conditions of extreme lymphopenia IL-7 becomes the dominant cytokine for which different T cell subsets compete. This finding was unexpected for CD25⁺ CD4⁺ regulatory T cells, because they express lower levels of IL-7R than both naive and memory T cells in replete hosts (39), which is in agreement with their reliance on other factors such as IL-2 for their survival (60, 61). However, naive and memory T cells down-regulate IL-7R under conditions of lymphopenia, whereas IL-7R levels on regulatory T cells remain constant irrespective of the presence or absence of other T cells. The correlation between IL-7R expression levels on naive and memory T cells and the intensity of depletion further emphasizes the central role of IL-7 (54) and CD25⁺ CD4⁺ T cells (62, 63) in the control of peripheral T cell homeostasis. We demonstrated here that in addition to their suppressive effect on Ag-driven responses, CD25⁺ CD4⁺ regulatory T cells also inhibit lymphopenia-induced activation of peripheral T cells, but by using a different suppressive mechanism based on IL-7 competition.

Although extrapolation from experimental models of T cell transfer into T cell-deficient hosts has prompted suggestions that lymphopenia-induced proliferation might underlie loss of tolerance and development of autoimmunity, it seems that the incidence of lymphopenia-induced proliferation of naive T cells is drastically restricted in a more physiological context of lymphopenia. Furthermore, our experimental model reflects transient incidents of lymphopenia that can be corrected by continuous thymic export. Under these conditions, excessive responses to self-peptide/MHC molecules or environmental Ags do not seem to occur, thus making it unlikely that transient and partial lymphopenic incidents are predisposing for autoimmune pathology.

Deleterious effects of lymphopenia as a consequence of exacerbated expansion of effector T cells responding to pathogens or aberrantly presented self-Ags (64–66) rather than the tonic stimulation of self-peptide/MHC complexes have been reported. However, in these instances also such responses were induced in T cell-deficient hosts, and there is not yet any information as to whether similar events would occur in partial and transient lymphopenia.

More extreme immune depletion protocols are used in a clinical setting with the aim of resetting the T cell repertoire to induce remission from autoimmune disease (67). It is possible that depletion regimens that spare regulatory T cells or memory T cells may be particularly successful, because these cells could potentially curb excessive pathological responses of reduced numbers of effector T cells. A remarkably successful strategy that inhibits clinical manifestations of diabetes is the treatment using anti-CD3 Abs, which, similar to the data described here, induce only partial and transient lymphopenia that appears to spare CD25⁺ CD4⁺ regulatory T cells (68, 69).

Alternatively, there may be treatment regimens that affect different CD4 T cell subsets such as regulatory T cells or memory T cells, and these might have opposing effects that allow the expansion of residual endogenous cells. Whether such regimens also result in deleterious responses to aberrantly presented self-Ags or pathogens remains to be determined.

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Disclosures

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