The Journal of Immunology

Cutting Edge: Depletion of $CD4^+CD25^+$ Regulatory T Cells Is Necessary, But Not Sufficient, for Induction of Organ-Specific Autoimmune Disease

Rebecca S. McHugh and Ethan M. Shevach

J Immunol 2002; 168:5979-5983; ;
doi: 10.4049/jimmunol.168.12.5979
http://www.jimmunol.org/content/168/12/5979

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

CUTTING EDGE

Cutting Edge: Depletion of CD4$^+$CD25$^+$ Regulatory T Cells Is Necessary, But Not Sufficient, for Induction of Organ-Specific Autoimmune Disease

Rebecca S. McHugh and Ethan M. Shevach[1]

Thymectomy of BALB/c mice on day 3 of life results in the development of autoimmune gastritis (AIG) due to the absence of CD4$^+$CD25$^+$ regulatory T cells. However, depletion of CD4$^+$CD25$^+$ T cells by treatment with anti-CD25 rarely resulted in AIG. Depletion was efficient, as transfer of splenocytes from depleted mice induced AIG in *nu/nu* mice. One explanation for this result is that CD4$^+$CD25$^-$ T cells upon transfer to nude recipients undergo lymphopenia-induced proliferation, providing a signal for T cell activation. Cotransfer of CD25$^+$ T cells did not inhibit initial proliferation but did suppress AIG. Surprisingly, immunization with the AIG target Ag, H/K ATPase, in IFA failed to induce disease in normal animals but induced severe AIG in CD25-depleted mice. These results demonstrate that second signals (nonspecific proliferation, TCR activation, or inflammation) are needed for induction of autoimmunity in the absence of CD25$^+$ regulatory T cells. ***The Journal of Immunology*****, 2002, 168: 5979–5983.**

Experimental organ-specific autoimmune disease in susceptible strains of mice has been induced by neonatal thymectomy on day 3 of life (d3Tx)[2] and other protocols that result in removal or delay in the development of regulatory CD4$^+$ T cells, primarily CD4$^+$CD25$^+$ T cells (1, 2). Reconstitution of d3Tx mice with purified CD4$^+$CD25$^+$, but not CD25$^-$, T cells on day 10 of life completely prevents the induction of autoimmunity. Similarly, transfer of CD4$^+$CD25$^-$ T cells to immunoincompetent recipients results in a spectrum of autoimmune disease that closely resembles that seen after d3Tx; cotransfer of CD4$^+$CD25$^+$ T cells prevents the development of disease.

The mechanism by which CD4$^+$CD25$^+$ T cells prevent the development of autoimmune disease remains unknown (3). One difficulty in the interpretation of many of the earlier studies is that most of the protocols, including d3Tx (4), involved induction of a generalized state of partial lymphocyte depletion or transfer of cells to a lymphopenic host in addition to depletion of CD4$^+$CD25$^+$ T cells. Indeed, it has been proposed that the lymphopenic state itself may promote the activation of autoreactive effector cells and that reconstitution of a depleted animal with either CD4$^+$CD25$^+$ or CD4$^+$CD25$^-$ would prevent disease development (4, 5). A much more direct test of the role of CD4$^+$CD25$^+$ T cells in regulating the development of autoimmunity in vivo would be their selective removal from the intact animal. In the present report, we have attempted to define the requirements for induction of autoimmune disease following depletion of CD4$^+$CD25$^+$ T cells. We demonstrate that selective depletion of CD4$^+$CD25$^+$ only rarely results in the development of autoimmunity and that an additional signal is required to activate autoreactive effector CD4$^+$CD25$^-$ T cells. This signal may be supplied by the induction of proliferation following transfer to a lymphopenic environment or by activation of the effector T cells via strong stimulation of their TCR with specific Ag.

Materials and Methods

Mice, Abs, and reagents

BALB/c females (6–8 wk old), 14-day gestation BALB/c females, BALB/c *nu/nu* females (6–8 wk old), and C.B-17 SCID females (6–8 wk old) were purchased from the National Cancer Institute animal facility (Frederick, MD) and housed under specific pathogen-free conditions. H/K ATPase was obtained by purification of microsomes from rabbit stomach (6). PC61 (anti-CD25) hybridoma was purchased from American Type Culture Collection (Manassas, VA) and ascites was produced in C.B-17 SCID mice. For all injections, an ammonium sulfate cut of PC61 ascites was used. Abs purchased from BD PharMingen (San Diego, CA) were FITC-anti-CD25 (7D4) and PE-anti-CD25 (PC61). Tri-color (TC)-anti-CD4 was purchased from Caltag Laboratories (Burlingame, CA). Rat IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). CFSE was purchased from Molecular Probes (Eugene, OR).

In vivo depletion, immunization with H/K ATPase, and assessment of AIG

BALB/c mice were given 1 mg PC61 every 3 days i.p. beginning day 10 of life for 2 wk. Alternatively, mice were given three injections from days 17 to 23 or one injection on day 23 of life only. Depletion of CD25$^+$ cells was confirmed by flow cytometry of PBMC using anti-CD4-Tri-color and anti-CD25-FITC (clone 7D4). All flow cytometry was performed on a BD FACScan or FACSCalibur and analyzed using CellQuest software (BD Immunocytometry Systems, San Jose, CA). After depletion, animals were followed until 3 mo of age and were analyzed for the production of anti-parietal cell Abs and gastric pathology (6).

PC61 treated or nontreated BALB/c mice were immunized s.c. in the hind flank with 50 μg of H/K ATPase containing rabbit microsomes emulsified in CFA or IFA in a volume of 50 μl. These animals were then assayed 6–10 wk later for anti-parietal cell Abs and gastric pathology.

Cellular Immunology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Received for publication March 18, 2002. Accepted for publication April 22, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Ethan M. Shevach, Cellular Immunology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11N311, 9000 Rockville Pike, Bethesda, MD 20892. E-mail address: eshevach@niaid.nih.gov

[2] Abbreviations used in this paper: d3Tx, thymectomy on day 3 of life; AIG, autoimmune gastritis.

0022-1767/02/$02.00

Purification of cells and transfer to recipient animals

Splenocytes (10–20 $\times$ 10^6) from depleted and nondepleted animals were harvested and transferred i.v. into 6- to 8-wk-old BALB/c wild-type and *nu/nu* females. These animals were analyzed for anti-parietal cell Abs and gastric pathology at 5–6 wk posttransfer.

CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells were purified and in vitro suppression assays were performed as described previously (7). For some experiments cells were labeled with CFSE at a final concentration of 1 μM in PBS for 8 min at room temperature. Labeled CD4$^+$CD25$^-$ or CD4$^+$CD25$^+$ T cells (5 $\times$ 10^6) were injected i.v. alone, or labeled CD4$^+$CD25$^-$ T cells were injected in combination with unlabeled CD4$^+$CD25$^-$ or CD4$^+$CD25$^+$ T cells (5 $\times$ 10^6) into BALB/c *nu/nu* animals. Labeled CD4$^+$CD25$^-$ cells were also injected into BALB/c hosts as nonproliferating controls. Cell division was measured by loss of CFSE on live-gated CD4$^+$ cells at various time points posttransfer by flow cytometry using a BD FACSCalibur and analyzed using CellQuest software.

Results

In vivo depletion of CD4$^+$CD25$^+$ T cells does not result in the development of AIG

The anti-CD25 mAb, PC61, is capable of depleting CD25$^+$ cells in vivo (8). We initially injected 1 mg of PC61 every other day for 2 wk beginning on day 10 of life. One day after the last injection (day 24 of life), CD25$^+$ T cells were markedly depleted from peripheral blood (Fig. 1*A*, 10.5 to 0.8%). Identical results were observed when mice were injected once on day 23 of life or every other day between days 17 and 23 (data not shown). The depleted mice were followed until they were 3 mo of age. A low (10%) but consistent incidence of spontaneous autoimmune gastritis (AIG) was observed as measured by the presence of autoantibodies to gastric parietal cells or gastric pathology (Fig. 1*B*). In contrast, when BALB/c mice are subjected to d3Tx, the incidence of AIG is ~60%. To rule out the possibility that depletion was not complete and that the low number of surviving CD4$^+$CD25$^+$ cells were capable of controlling the induction of autoimmunity, we transferred splenocytes from 24-day-old PC61-depleted animals to BALB/c *nu/nu* recipients. All (100%) of the PC61-depleted splenocyte recipients developed severe destructive gastritis (Fig. 1*C*). As a positive control for suppression of the development of disease in recipients, we cotransferred CD4$^+$CD25$^+$ T cells from normal adult mice with these splenocytes into *nu/nu* recipients; none of the recipients of the cotransferred cells developed AIG (data not shown).

Control of autoimmunity in CD25-depleted animals does not result from repopulation of CD25$^+$ T cell pool

As we did not thymectomize the animals following depletion of the CD25$^+$ T cells, it remained possible that CD25$^+$ cells could be re-emerging from the thymus and repopulating the periphery, controlling the induction of autoimmunity. Indeed, analysis of the percentage of CD4$^+$CD25$^+$ population at different time points following depletion did reveal a slow, partial recovery of the percentage of CD25$^+$ T cells in splenocytes, as well as peripheral blood with 3–4% of the CD4 pool expressing CD25 at 6 wk postdepletion (Fig. 2*A*). To determine whether this low number of CD25$^+$ cells is capable of suppressing the induction of AIG, we transferred splenocytes from 24-day-old depleted animals to *nu/nu* recipients at various time points following depletion and monitored

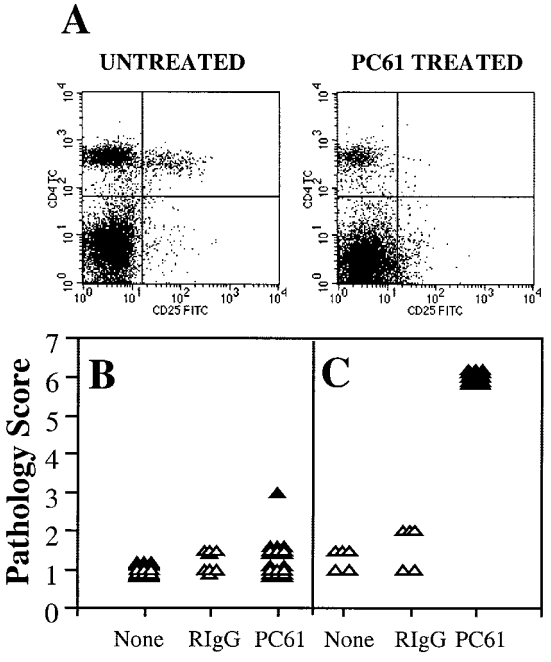

FIGURE 1. In vivo treatment with PC61 depletes CD25$^+$ cells but does not induce AIG. *A*, BALB/c mice (day 10) were injected every other day for 2 wk with PC61 ascites. One day following the final injection, PBMC were analyzed for the presence of CD4$^+$CD25$^+$ cells by flow cytometry. *B*, BALB/c mice treated with PC61 or control rat IgG, as in *A*, or left untreated were monitored for the production of anti-parietal cell Abs and gastric pathology at 3 mo of age. ▲, Animals positive for anti-parietal cell Abs. *C*, Splenocytes from BALB/c mice, treated as in *B*, were harvested 1 day after final Ab injection and transferred to BALB/c *nu/nu* mice. Recipient mice were analyzed 5–6 wk posttransfer for AIG.

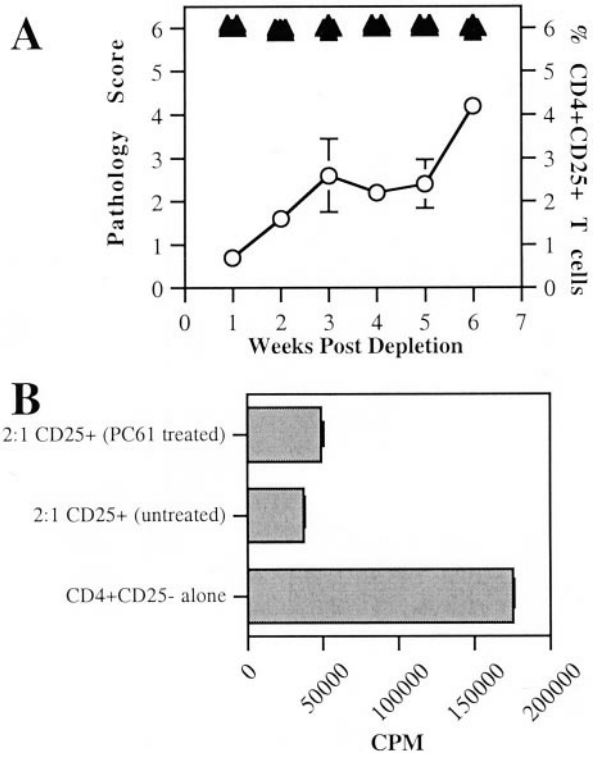

FIGURE 2. CD4$^+$CD25$^+$ cells do repopulate following depletion but do not control AIG upon transfer to BALB/c *nu/nu* mice. *A*, BALB/c mice (day 17) were treated with PC61 every other day for 1 wk. Splenocytes were harvested at various time points following depletion and transferred to BALB/c *nu/nu* animals. At the time of transfer, the percentage of CD4$^+$CD25$^+$ T cells was measured in the donor splenocytes. Animals were analyzed for AIG 5–6 wk posttransfer. *B*, CD4$^+$CD25$^+$ T cells were purified from animals 6 wk postdepletion or from untreated BALB/c mice. These cells were used at a 2:1 responder to suppressor cell ratio in an in vitro suppression assay.

them for the presence of AIG. Surprisingly, all *nu/nu* recipients had severe destructive AIG (Fig. 2*A*), even when the transferred $CD4^+$ population contained as many as 4% $CD25^+$ T cells. To further substantiate that the $CD25^+$ T cells, which repopulated the depleted animals, belonged to the naturally occurring $CD4^+CD25^+$ pool and had not acquired the expression of CD25 due to activation in vivo, we purified $CD25^+$ T cells from animals 6 wk after depletion and evaluated their capacity to suppress the proliferative response of $CD25^-$ T cells in vitro. The recovered $CD25^+$ T cells were as suppressive as $CD25^+$ T cells from normal mice (Fig. 2*B*).

$CD4^+CD25^+$ regulatory T cells do not control the initial proliferation of transferred $CD4^+CD25^-$ T cells into a lymphopenic environment

Many groups have demonstrated that $CD4^+$ T cells will rapidly divide nonspecifically in a lymphopenic environment to restore T cell homeostasis (9–12). As shown in Fig. 3*A*, CFSE-labeled

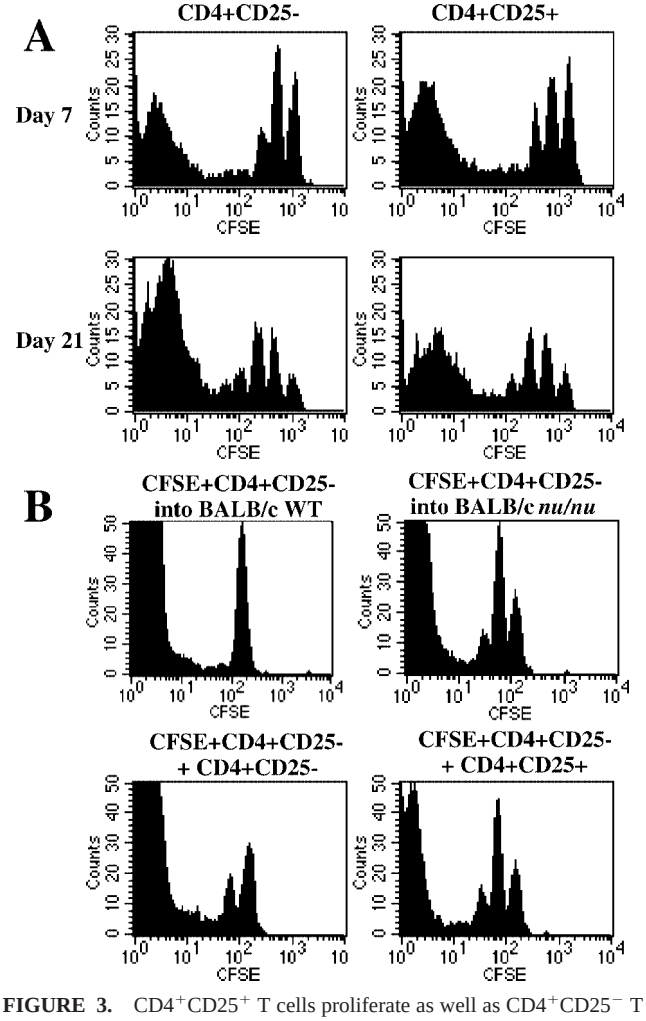

FIGURE 3. $CD4^+CD25^+$ T cells proliferate as well as $CD4^+CD25^-$ T cells in BALB/c *nu/nu* mice but do not affect the lymphopenia-induced proliferation of $CD4^+CD25^-$ T cells. *A*, CFSE-labeled $CD4^+CD25^-$ and $CD4^+CD25^-$ cells (5×10^6) were transferred to BALB/c *nu/nu* mice. Proliferation of the $CD4^+$ cells was measured by dilution of CFSE on $CD4^+$ T cells at day 7 or 21 posttransfer. *B*, CFSE-labeled $CD4^+CD25^-$ T cells (5×10^6) were transferred alone into BALB/c wild-type or *nu/nu* hosts, or in combination with equal numbers of unlabeled $CD4^+CD25^-$ or $CD4^+CD25^+$ T cells into BALB/c *nu/nu* hosts. CFSE dilution of $CD4^+$ cells was analyzed on day 14 posttransfer. Two to three mice from each group were analyzed per time point. These data are representative of two independent experiments.

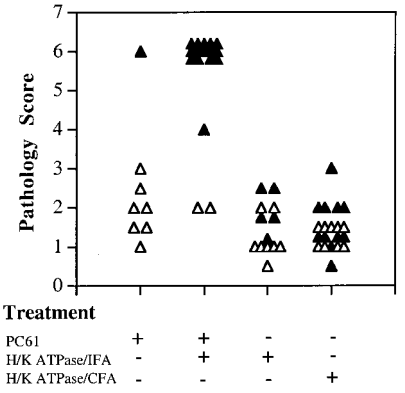

FIGURE 4. Immunization of CD25-depleted mice with H/K ATPase in IFA results in AIG. BALB/c mice were treated with PC61 starting on day 17 of life or left untreated. One day following the last injection, mice were immunized s.c. with H/K ATPase in IFA or CFA as indicated. Mice were monitored for anti-parietal cell Abs and gastric pathology 6–10 wk postimmunization. ▲, Mice with anti-parietal cell Ab titers.

$CD4^+CD25^-$ and $CD4^+CD25^+$ T cells proliferated comparably in the BALB/c *nu/nu* recipients; both populations showed at least two divisions by day 7 and continued to divide 21 days posttransfer. It has been proposed that one mechanism by which $CD4^+CD25^+$ T cells can control the induction of autoimmunity is to control the nonspecific proliferation that takes place in a lymphopenic environment. If this were the case then cotransfer of $CD25^-$ and $CD25^+$ T cells should resemble the proliferation seen upon transfer into a wild-type BALB/c recipient (Fig. 3*B*, *upper left*). To evaluate the ability of $CD4^+CD25^+$ T cells to control lymphopenia-induced proliferation, we transferred CFSE-labeled $CD4^+CD25^-$ T cells alone or with unlabeled $CD4^+CD25^-$ or $CD4^+CD25^+$ T cells to wild-type or *nu/nu* recipients. Analysis of CFSE-labeled $CD4^+CD25^-$ T cells in the presence of either unlabeled $CD4^+CD25^-$ (Fig. 3*B*, *lower left*) or $CD4^+CD25^+$ (Fig. 3*B*, *lower right*) T cells revealed a similar proliferation profile seen upon labeled $CD4^+CD25^-$ T cells transferred alone (Fig. 3*B*, *upper right*) into a BALB/c *nu/nu* host. Similar results were observed when the $CD4^+CD25^+$ T cells were injected 2 wk before injection of CFSE-labeled $CD4^+CD25^-$ T cells (data not shown). As a control, some recipient animals were monitored for development of AIG. All BALB/c *nu/nu* animals receiving $CD4^+CD25^-$ T cells alone developed AIG, whereas all recipients of cotransferred $CD4^+CD25^+$ T cells were free of gastric pathology (data not shown).

Immunization of animals depleted of $CD25^+$ cells with H/K ATPase in IFA results in severe, destructive AIG

Immunization of BALB/c animals with the AIG target Ag H/K ATPase in CFA has failed to induce sustained severe AIG in mice, although mice can develop anti-parietal cell Abs. Gastric pathology is evident while immunizations are ongoing but resolves once immunization is ceased (13). We investigated whether immunization of animals that were depleted of $CD25^+$ cells would result in severe AIG. Animals were either depleted of $CD25^+$ cells or left untreated and immunized the following day with H/K ATPase in IFA. Mice immunized with H/K ATPase in IFA were positive for anti-parietal cell Abs, but only animals that were depleted of $CD25^+$ cells and immunized with Ag and IFA had severe gastric pathology 5 wk postimmunization (Fig. 4). As a control, untreated mice immunized with H/K ATPase in CFA failed to show signs of gastric pathology. Induction of nonspecific inflammation by injection of IFA alone did not result in the induction of AIG (data not shown).

Discussion

The general approach used to analyze the role of regulatory T cell function in vivo is to compare the incidence of autoimmune disease in the presence of T cell populations that have been depleted of regulatory T cells with the incidence of disease in T cell populations that contain regulatory T cells. These studies are performed by transfer of the T cell populations to a T cell-deficient recipient, most frequently an immunoincompetent *nu/nu*, SCID, or recombination-activating $gene^{-/-}$ mouse. A major difficulty interpreting the prevention of disease by cotransfer of regulatory T cells is that T lymphocytes undergo a rapid, vigorous proliferative response when transferred to such a recipient. Therefore, it has been proposed that a major component of regulatory T cell function is to nonspecifically inhibit this lymphopenia-induced expansion.

In an attempt to more accurately define a role for $CD4^+CD25^+$ suppressor T cells in the control of organ-specific autoimmune disease in the absence of lymphopenia-induced proliferation, we selectively depleted them with Ab. Depleted animals only rarely developed AIG, the most common manifestation of a deficiency of regulatory T cell function in BALB/c mice. This result must be compared with that of Taguchi and Takahashi (8), who observed a significant increase in organ-specific autoimmune disease using a very similar protocol. This difference may be secondary to the different animal strains used, as our studies involved the induction of AIG in BALB/c mice, whereas Taguchi and Takahashi (8) used $(B6 \times A/J)F_1$ animals, which are more susceptible to diseases of the reproductive system following d3Tx. Curiously, CD25-depleted $(B6 \times A/J)F_1$ mice developed gastritis, normally not observed in that strain after d3Tx, while they failed to develop diseases of the reproductive system. Importantly, our data are consistent with studies (14, 15) in which depletion of $CD25^+$ cells to induce tumor immunity did not enhance susceptibility to autoimmune disease. One trivial explanation for our failure to observe autoimmune disease following depletion of $CD25^+$ T cells in euthymic animals is that the depletion was inadequate or that new thymic emigrants rapidly repopulated the $CD4^+CD25^+$ pool. This explanation is highly unlikely, because transfer of T cells from CD25-depleted animals to *nu/nu* recipients for up to 6 wk following depletion resulted in the induction of autoimmune disease in 100% of recipients, even when partial reconstitution of the $CD25^+$ population was observed.

The disparity between the ability to transfer disease to a lymphopenic animal, while the lymphocyte-sufficient, CD25-depleted donor does not develop autoimmune disease, forced us to reexamine the contribution of lymphopenia for the induction of organ-specific autoimmunity. Annacker et al. (16) have proposed that a primary function of $CD4^+CD25^+$ T cells is to regulate the homeostatic proliferation of peripheral T cells, most likely by the secretion of IL-10. However, the concept of homeostatic proliferation must be clearly defined. In the lymphopenic animal, transferred T cells are stimulated through TCR/MHC interactions to proliferate and fill the "empty" space to reach T cell homeostasis (17). Unseparated $CD4^+$, $CD4^+CD25^-$ ($CD45RB^{high}$), and $CD4^+CD25^+$ T cells are capable of proliferating in a lymphopenic host with proliferation beginning on days 3–4 posttransfer and reaching as many as eight divisions by days 21–28 (12, 16). We did not observe a suppressive effect of cotransfer of $CD4^+CD25^+$ T cells on this early phase of proliferation of $CD4^+CD25^-$ T cells, particularly when compared with cotransfer of an equivalent number of $CD4^+CD25^-$ T cells. It is likely that the modest reduction of the proliferation of $CD4^+CD25^-$ T cells observed by Annacker et al. (16) by cotransfer of $CD4^+CD25^+$ T cells was secondary to an overall increase in the number of injected T cells (12). Cotransfer of $CD4^+CD25^-$ T cells was not examined by Annacker et al. (16).

In contrast to the lack of an effect of $CD4^+CD25^+$ T cells on the early phase of the expansion of $CD4^+CD25^-$ T cells, regulatory T cells have been reported to exert a profound effect on the accumulation or steady state numbers of $CD4^+CD25^-$ T cells when the recipient animals are studied 2–6 mo after cell transfer (16, 18). This suppressive effect of cotransfer of $CD4^+CD25^+$ T cells on the late accumulation of $CD4^+CD25^-$ T cells must be interpreted with caution. Recipients of $CD4^+CD25^-$ T cells frequently develop autoimmune diseases (1, 19) and therefore have undergone Ag-specific stimulation and expansion in addition to lymphopenia-driven T cell proliferation. $CD4^+CD25^+$ T cells are known to inhibit the induction of these autoimmune diseases. Indeed, the requirement for IL-10 to control the late expansion of the $CD4^+CD25^-$ in BALB/c recombination-activating gene $2^{-/-}$ mice reported by Annacker et al. (16) is most consistent with the requirement for IL-10 production by $CD4^+CD25^+$ T cells to prevent the induction of inflammatory bowel disease that develops in these recipients (20, 21).

Collectively, our data are most consistent with a model in which $CD4^+CD25^+$ T cells play no role in the control of lymphopenia-induced proliferation, but that the proliferative response functions as a second signal that is required for the differentiation of $CD4^+CD25^-$ autoreactive effector cells. Our ability to readily induce AIG in CD25-depleted, but not normal, animals by stimulation with the target Ag in IFA also suggests that strong TCR stimulation can provide the necessary second signal. We have not yet tested what other environmental inflammatory insults might be able to precipitate induction of autoimmunity in the CD25-depleted animal. Last, these results have important implications with regard to the therapeutic manipulation of $CD4^+CD25^+$ T cells in vivo. Depletion of $CD4^+CD25^+$ T cells greatly enhances the ability of the host to respond to immunization with a weak tumor vaccine (14, 15, 22). Depletion of $CD4^+CD25^+$ T cells might also be useful in the enhancement of immune responses to weak vaccines to infectious agents or to promote sterilizing immunity in the setting of chronic indolent infections. Although young adult euthymic mice appear to repopulate the $CD4^+CD25^+$ T cell pool within 2 mo following depletion, extrapolation of this finding to humans should be undertaken with caution in the absence of data on the capacity of the adult human thymus to produce $CD4^+CD25^+$ T cells.

References

1. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunological self-tolerance maintained by activated T-cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155:1151.*
2. Suri-Payer, E., A. Z. Amar, A. M. Thornton, and E. M. Shevach. 1998. $CD4^+CD25^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J. Immunol. 160:1212.*
3. McHugh, R., E. Shevach, and A. Thornton. 2001. Control of organ-specific autoimmunity by immunoregulatory $CD4^+CD25^+$ T cells. *Microbes Infect. 3:919.*
4. Bonomo, A., P. J. Kehn, and E. M. Shevach. 1995. Post-thymectomy autoimmunity: abnormal T-cell homeostasis. *Immunol. Today 16:61.*
5. Stockinger, B., T. Barthlott, and G. Kassiotis. 2001. T cell regulation: a special job or everyone's responsibility. *Nat. Immunol. 2:757.*
6. SuriPayer, E., P. J. Kehn, A. W. Cheever, and E. M. Shevach. 1996. Pathogenesis of post-thymectomy autoimmune gastritis: identification of anti-H/K adenosine triphosphatase-reactive T cells. *J. Immunol. 157:1799.*
7. McHugh, R. S., M. J. Whitters, C. A. Piccirillo, D. A. Young, E. M. Shevach, M. Collins, and M. C. Byrne. 2002. $CD4^+CD25^+$ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity 16:311.*
8. Taguchi, O., and T. Takahashi. 1996. Administration of anti-interleukin-2 receptor α antibody in vivo induces localized autoimmune disease. *Eur. J. Immunol. 26:1608.*

9. Rocha, B., N. Dautigny, and P. Pereira. 1989. Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios "in vivo." *Eur. J. Immunol. 19:905.*
10. Freitas, A. A., and B. Rocha. 2000. Population biology of lymphocytes: the flight for survival. *Annu. Rev. Immunol. 18:83.*
11. Marrack, P., J. Bender, D. Hildeman, M. Jordan, T. Mitchell, M. Murakami, A. Sakamoto, B. C. Schaefer, B. Swanson, and J. Kappler. 2000. Homeostasis of $\alpha\beta$ TCR^+ T cells. *Nat. Immunol. 1:107.*
12. Dummer, W., B. Ernst, E. LeRoy, D. Lee, and C. D. Surh. 2001. Autologous regulation of naive T cell homeostasis within the T cell compartment. *J. Immunol. 166:2460.*
13. Scarff, K. J., J. M. Pettitt, I. R. VanDriel, P. A. Gleeson, and B. H. Toh. 1997. Immunization with gastric H^+/K^+-ATPase induces a reversible autoimmune gastritis. *Immunology 92:91.*
14. Onizuka, S., I. Tawara, J. Shimizu, S. Sakaguchi, T. Fujita, and E. Nakayama. 1999. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. *Cancer Res. 59:3128.*
15. Shimizu, J., S. Yamazaki, and S. Sakaguchi. 1999. Induction of tumor immunity by removing $CD25^+CD4^+$ T cells: a common basis between tumor immunity and autoimmunity. *J. Immunol. 163:5211.*
16. Annacker, O., R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano, and A. Bandeira. 2001. $CD25^+CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J. Immunol. 166:3008.*
17. Beutner, U., and H. R. MacDonald. 1998. TCR-MHC class II interaction is required for peripheral expansion of CD4 cells in a T cell-deficient host. *Int. Immunol. 10:305.*
18. Annacker, O., O. Burlen-Defranoux, R. Pimenta-Araujo, A. Cumano, and A. Bandeira. 2000. Regulatory CD4 T cells control the size of the peripheral activated/memory CD4 T cell compartment. *J. Immunol. 164:3573.*
19. Powrie, F., M. W. Leach, S. Mauze, S. Menon, L. B. Caddle, and R. L. Coffman. 1994. Inhibition of Th1 responses prevents inflammatory bowel-disease in SCID mice reconstituted with $CD45Rb^{hi}$ $CD4^+$ T cells. *Immunity 1:553.*
20. Asseman, C., S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie. 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med. 190:995.*
21. Suri-Payer, E., and H. Cantor. 2001. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by $CD4^+CD25^+$ T cells. *J. Autoimmun. 16:115.*
22. Steitz, J., J. Bruck, J. Lenz, J. Knop, and T. Tuting. 2001. Depletion of $CD25^+CD4^+$ T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon α-induced, $CD8^+$ T-cell-dependent immune defense of B16 melanoma. *Cancer Res. 61:8643.*