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This information is current as of September 17, 2021.

*J Immunol* 2001; 167:1137-1140; ;  
doi: 10.4049/jimmunol.167.3.1137  
<http://www.jimmunol.org/content/167/3/1137>

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## Cutting Edge: Control of CD8<sup>+</sup> T Cell Activation by CD4<sup>+</sup>CD25<sup>+</sup> Immunoregulatory Cells

Ciriaco A. Piccirillo and Ethan M. Shevach<sup>1</sup>

**CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells inhibit organ-specific autoimmune diseases induced by CD4<sup>+</sup>CD25<sup>-</sup> T cells and are potent suppressors of CD4<sup>+</sup>CD25<sup>-</sup> T cell activation in vitro. We demonstrate that CD4<sup>+</sup>CD25<sup>+</sup> T cells also suppress both proliferation and IFN- $\gamma$  production by CD8<sup>+</sup> T cells induced either by polyclonal or Ag-specific stimuli. CD4<sup>+</sup>CD25<sup>+</sup> T cells inhibit the activation of CD8<sup>+</sup> responders by inhibiting both IL-2 production and up-regulation of IL-2R $\alpha$ -chain (CD25) expression. Suppression is mediated via a T-T interaction as activated CD4<sup>+</sup>CD25<sup>+</sup> T cells suppress the responses of TCR-transgenic CD8<sup>+</sup> T cells stimulated with soluble peptide-MHC class I tetramers in the complete absence of APC. These results broaden the immunoregulatory role played by CD4<sup>+</sup>CD25<sup>+</sup> T cells in the prevention of autoimmune diseases, but also raise the possibility that they may hinder the induction of effector CD8<sup>+</sup> T cells to tumor or foreign Ags. *The Journal of Immunology*, 2001, 167: 1137–1140.**

**S**tudies in a number of experimental models have demonstrated the existence of regulatory T cell populations that prevent the activation of autoreactive T cells (1–3). The most useful marker to date for identification of regulatory T cells is the CD25 (IL-2R $\alpha$ -chain) Ag that is present on 5–10% of CD4<sup>+</sup> T cells in normal animals (4–8). The functional properties of murine CD4<sup>+</sup>CD25<sup>+</sup> T cells have been extensively studied in vitro. They demonstrate profound anergy to stimulation via their TCR, and this anergic state cannot be reversed by costimulation with anti-CD28 (9, 10). More importantly, when CD4<sup>+</sup>CD25<sup>+</sup> T cells are cocultured with CD4<sup>+</sup>CD25<sup>-</sup> T cells, they induced profound suppression of T cell activation by down-regulating IL-2 production in the responding CD4<sup>+</sup>CD25<sup>-</sup> T cells (10). The suppressive activity of the CD4<sup>+</sup>CD25<sup>+</sup> T cells requires that they be activated via their TCR and is cell contact dependent but cytokine independent (10, 11).

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Received for publication April 30, 2001. Accepted for publication June 4, 2001.

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<sup>2</sup> Abbreviations used in this paper: NP, nucleoprotein; LCMV, lymphocytic choriomeningitis virus; Tg, transgenic; TdS, T-depleted spleen cells.

The mechanism by which CD4<sup>+</sup>CD25<sup>+</sup> T cells mediate their suppressive effects is poorly understood. The physiologic ligand recognized by their TCR is unknown, and considerable controversy exists as to their cellular target. Thornton and Shevach (12) have demonstrated that the suppressors do not modulate APC function, whereas other laboratories have raised the possibility that they act by suppressing APC function (13) or by competing for APC-derived costimulatory signals (9). The potential suppressive activity of CD4<sup>+</sup>CD25<sup>+</sup> T cells on non-CD4<sup>+</sup> responder cells has not been studied in detail. Here, we demonstrate that CD4<sup>+</sup>CD25<sup>+</sup> T cells suppress CD8<sup>+</sup> T cell proliferation and IFN- $\gamma$  production induced by polyclonal or Ag-specific stimuli. In addition, the effects of the CD4<sup>+</sup>CD25<sup>+</sup> T cells on CD8<sup>+</sup> cells are more complex than their effects on CD4<sup>+</sup>CD25<sup>-</sup> responders, because they suppress both IL-2 production and CD25 expression. Finally, we made use of peptide-MHC tetramers to stimulate CD8<sup>+</sup> responders in a two-cell suppressor assay system to formally demonstrate that CD4<sup>+</sup>CD25<sup>+</sup> cells mediate their suppressor function via a T-T cell interaction and in the absence of APC.

### Materials and Methods

#### Mice

Female C57BL/6 and BALB/c mice were obtained from the National Cancer Institute (Frederick, MD). OT-I (specific for OVA<sub>257–264</sub> peptide), F5 (specific for nucleoprotein (NP)<sub>366–374</sub> influenza peptide), and P14 (specific for lymphocytic choriomeningitis virus (LCMV) gp33–41 peptide) CD8<sup>+</sup> TCR-transgenic (Tg) mice were obtained from Taconic Farms (Germantown, NY). All mice used were 6–12 wk of age.

#### The mAbs

The following Abs were used for flow cytometry experiments: biotin-anti-CD25 (7D4 clone), FITC-streptavidin, PE-anti-CD4, PE-anti-CD8, FITC-anti-CD25, FITC-anti-CD69, and purified anti-CD3 $\epsilon$  (2C11), all of which were purchased from BD PharMingen (San Diego, CA).

#### Peptides

OT-I OVA<sub>257–264</sub>, F5 NP<sub>366–374</sub>, and LCMV gp33–41 peptides were provided by R. Germain and J. Yewdell (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). Peptides were synthesized and purified by the Laboratory of Molecular Structure Peptide Synthesis Laboratory (National Institute of Allergy and Infectious Diseases, National Institutes of Health).

#### Tetramers

MHC class I H-2K<sup>b</sup>-OVA<sub>257–264</sub> tetramer solutions were prepared at the National Institute of Allergy and Infectious Diseases tetramer facility.

#### Cell purification and culture

CD4<sup>+</sup>CD25<sup>+</sup> T cells were isolated on a FACStar cell sorter (BD Biosciences, San Jose, CA) as described previously (10). The purity of the final CD4<sup>+</sup>CD25<sup>+</sup> preparation was typically >95%. T-depleted spleen cells (10; TdS) were irradiated at 3000 rad and pulsed for 30 min at 37°C with

an appropriate peptide. Activated CD4<sup>+</sup>CD25<sup>+</sup> cells were prepared as previously described (12). Briefly, cell-sorted CD4<sup>+</sup>CD25<sup>+</sup> cells were cultured with irradiated APC (1:1 ratio), anti-CD3 (0.5 μg/ml), and human IL-2 (5 ng/ml, 100 U/ml) for 72 h and were then split and maintained in IL-2 medium for ~7–14 days. CD8<sup>+</sup> T cells were purified either by negative (depletion of B220-, CD4-, and I-A<sup>b</sup>-positive cells) or positive selection (using CD8α magnetic beads) on the AutoMACS magnetic separation system (Miltenyi Biotec, Auburn, CA). For experiments involving tetramer stimulation, OT-I CD8<sup>+</sup> T cells were FACS purified using Abs against Thy1.2 and CD8α molecules, with final purities of >99%.

#### Proliferation assays

Proliferation assays were performed by culturing CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) in flat-bottom microtiter plates (0.2 ml) with peptide-pulsed APC ( $1-2 \times 10^5$ ) and resting or activated CD4<sup>+</sup>CD25<sup>+</sup> or CD4<sup>+</sup>CD25<sup>-</sup> T cells for 72 h at 37°C in complete medium (10). Human rIL-2 was purchased from Peprotech (Rocky Hill, NJ). Cell cultures were pulsed with [<sup>3</sup>H]TdR for the last 8 h. All data represent the average cpm of triplicate determinations. All proliferation experiments were repeated at least three times.

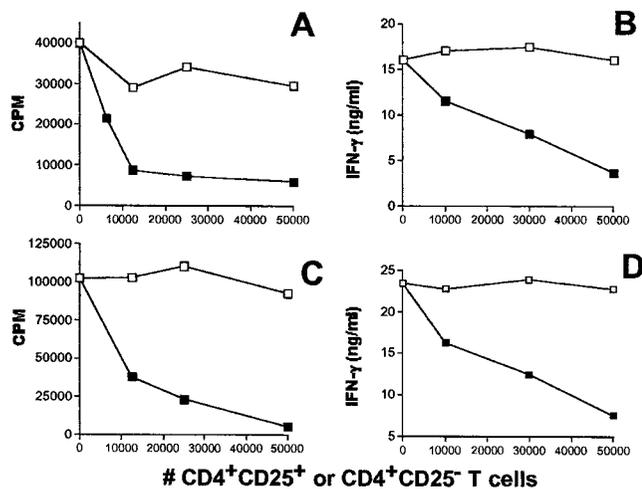
For cytokine production, supernatants were taken at 72 h, and the production of IFN-γ was measured using an ELISA kit (R&D Systems, Minneapolis, MN).

#### Flow cytometry

Cells were collected and stained with PE-CD8α and FITC-CD69 or FITC-CD25 and analyzed with a FACScan flow cytometer (BD Biosciences).

### Results and Discussion

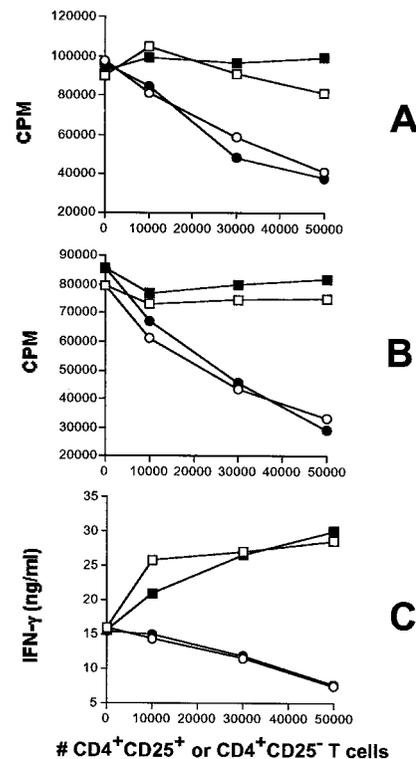
When freshly explanted CD4<sup>+</sup>CD25<sup>+</sup> T cells from normal mice were cocultured with F5 TCR-Tg CD8<sup>+</sup> T cells, significant cell-dose-dependent suppression of proliferation was observed with soluble anti-CD3 as the stimulus in the presence of APC (Fig. 1A). When the TCR-Tg cells were stimulated with specific peptide, no inhibition of proliferation was observed (data not shown). When activated CD4<sup>+</sup>CD25<sup>+</sup> T cells from normal mice were used, marked suppression of proliferation of F5 CD8<sup>+</sup> TCR-Tg T cells was observed with peptide stimulation (Fig. 1C). This finding confirms previous studies (12) using CD4<sup>+</sup> T cells as responders in which the CD4<sup>+</sup>CD25<sup>+</sup> T cells required activation via their TCR to manifest suppressor function, but following activation, suppressor effector function was Ag-nonspecific and did not require re-stimulation of the suppressors via their TCR.



**FIGURE 1.** CD4<sup>+</sup>CD25<sup>+</sup> T cells suppress CD8<sup>+</sup> T cell proliferation and effector function. Tg F5 CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) were stimulated with anti-CD3 and TdS ( $2 \times 10^5$ ; A and B) or NP<sub>366-374</sub> peptide-pulsed TdS ( $2 \times 10^5$ ; C and D) and in the presence of either freshly isolated (A and B) or activated (C and D) CD4<sup>+</sup>CD25<sup>+</sup> (■) or CD4<sup>+</sup>CD25<sup>-</sup> (□) T cells. Proliferation (A and C) and IFN-γ production (B and D) were measured. Results from a representative experiment are shown.

Because CD8<sup>+</sup> T cells will produce significant amounts of IFN-γ in the absence of previous priming, we next studied the effects of CD4<sup>+</sup>CD25<sup>+</sup> T cells on the capacity of CD8<sup>+</sup> T cells to produce IFN-γ. Freshly explanted CD4<sup>+</sup>CD25<sup>+</sup> T cells readily suppressed IFN-γ production by CD8<sup>+</sup> T cells stimulated with anti-CD3 (Fig. 1B), and activated CD4<sup>+</sup>CD25<sup>+</sup> T cells suppressed IFN-γ production when the CD8<sup>+</sup> T cells were stimulated with specific peptide (Fig. 1D). We have consistently shown >50% suppression of both proliferation and IFN-γ secretion at a CD25<sup>+</sup>:CD8 ratio of 1:2, and >75% suppression at a 1:1 cell ratio. CD4<sup>+</sup>CD25<sup>+</sup> T cells (Fig. 1, A and C) cultured alone do not proliferate and do not secrete IFN-γ (data not shown). CD4<sup>+</sup>CD25<sup>-</sup> T cells (Fig. 1C) cultured in the absence of CD8<sup>+</sup> T cells do not produce IFN-γ (data not shown).

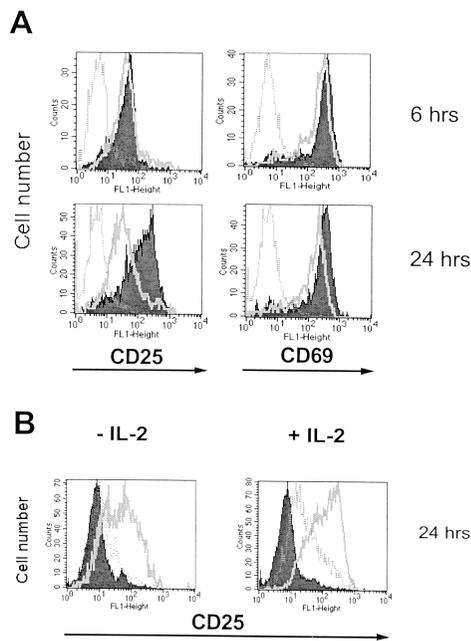
IL-2 will reverse suppression when CD4<sup>+</sup>CD25<sup>+</sup> suppressors are cocultured with CD4<sup>+</sup>CD25<sup>-</sup> responders. It has been proposed that IL-2 can directly act on the suppressors and reverse their anergic phenotype and consequently disable their suppressive capability (14). Alternatively, the addition of exogenous IL-2 may simply be circumventing the block in IL-2 production induced in the responders by the CD4<sup>+</sup>CD25<sup>+</sup> suppressors. When fresh CD4<sup>+</sup>CD25<sup>+</sup> T cells (Fig. 2A) or activated CD4<sup>+</sup>CD25<sup>+</sup> T cells (Fig. 2B) were cocultured with OT-I CD8<sup>+</sup> T cells, significant suppression of proliferation (~60%) and IFN-γ production (Fig. 2C) was observed with soluble anti-CD3 or peptide stimulation.



**FIGURE 2.** IL-2 does not reverse CD4<sup>+</sup>CD25<sup>+</sup>-mediated suppression. A, Tg OT-I CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) were stimulated with anti-CD3 (0.5 μg/ml) and TdS ( $2 \times 10^5$ ) and either freshly isolated CD4<sup>+</sup>CD25<sup>+</sup> (circles) or CD4<sup>+</sup>CD25<sup>-</sup> (squares) T cells in the presence (filled symbols) or absence (open symbols) of exogenous IL-2 (100 U/ml). B, Tg OT-I CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) were stimulated with OVA<sub>257-264</sub> peptide-pulsed TdS ( $2 \times 10^5$ ) and either activated CD4<sup>+</sup>CD25<sup>+</sup> (circles) or CD4<sup>+</sup>CD25<sup>-</sup> (squares) T cells in the presence (filled symbols) or absence (open symbols) of exogenous IL-2 (100 U/ml). Proliferation was assayed as in Fig. 1C. Supernatants from B were collected at 72 h, and IFN-γ ELISA was performed.

Suppression was not reversed by the addition of exogenous IL-2 at all suppressor:responder ratios or by enhancement of endogenous IL-2 production by the addition of anti-CD28 (data not shown). Increasing the amount of IL-2 added to the cocultures to 100 U/ml also had no effect. It should be noted that the proliferative response of CD4<sup>+</sup>CD25<sup>+</sup> T cells to IL-2 is modest when compared with activated CD4<sup>+</sup>CD25<sup>-</sup> T cells. Thus, the contribution of the activated CD4<sup>+</sup>CD25<sup>+</sup> T cells to the proliferative responses in the cocultures performed in the presence of IL-2 is also minimal but may contribute some residual proliferation to the culture. The hypothesis (9) that IL-2 abrogates suppressor function is not supported by our studies on CD8<sup>+</sup> responders because suppression is clearly maintained in the presence of IL-2.

We next examined the expression of CD25 on F5 CD8<sup>+</sup> T cells stimulated either in the presence or absence of CD4<sup>+</sup>CD25<sup>+</sup> T cells. Following 6 h of stimulation with peptide-pulsed APC, up-regulation of CD25 expression on the F5 CD8<sup>+</sup> T cell responders was similar in presence or absence of CD4<sup>+</sup>CD25<sup>+</sup> T cells (Fig. 3A). However, no further up-regulation of CD25 on the CD8<sup>+</sup> responders was seen in the CD4<sup>+</sup>CD25<sup>+</sup>/CD8<sup>+</sup> cocultures (Fig. 3A). The expression of CD69 was identical on CD8<sup>+</sup> responders stimulated in the presence or absence of CD4<sup>+</sup>CD25<sup>+</sup> cells at both time points tested (Fig. 3A). In multiple (*n* = 5) experiments, addition of activated CD4<sup>+</sup>CD25<sup>+</sup> T cells resulted in 73 ± 5% suppression of the induction of CD25 when the responding CD8<sup>+</sup> T cells were assayed for CD25 expression following 24 h of stimu-

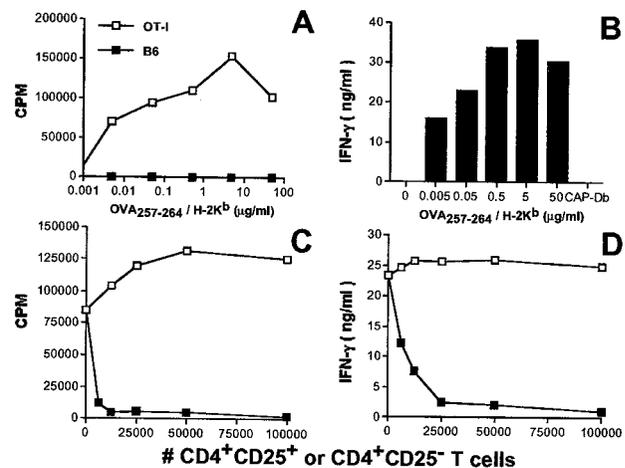


**FIGURE 3.** CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells suppress IL-2R $\alpha$  expression on responding CD8<sup>+</sup> T cells, and IL-2 fails to rescue its expression. *A*, Tg F5 CD8<sup>+</sup> T cells ( $5 \times 10^5$ ) were stimulated with NP<sub>366–374</sub> peptide-pulsed TdS ( $2 \times 10^6$ ) either alone (filled line) or with activated CD4<sup>+</sup>CD25<sup>+</sup> T cells ( $5 \times 10^5$ ; solid line). FACS analysis for CD25 and CD69 was performed at 6 and 24 h and compared with unstimulated CD8<sup>+</sup> T cells (dotted line). *B*, Tg P14 CD8<sup>+</sup> T cells ( $5 \times 10^5$ ) were stimulated with LCMV gp33–41 peptide-pulsed TdS ( $2 \times 10^6$ ) either alone (solid line) or with activated CD4<sup>+</sup>CD25<sup>+</sup> T cells ( $5 \times 10^5$ ; dotted line) in the presence of exogenous IL-2 (100 U/ml). FACS analysis for CD25 was performed at 24 h and compared with unstimulated CD8<sup>+</sup> T cells (filled line). All histograms were gated on CD8<sup>+</sup> T cells. CD8<sup>+</sup> T cells stimulated in the presence of activated CD4<sup>+</sup>CD25<sup>-</sup> cells displayed CD25 and CD69 expression levels indistinguishable to those observed when CD8<sup>+</sup> T cells were cultured alone (data not shown).

lation. The addition of exogenous IL-2 (100 U/ml) also failed to restore the level of CD25 expression on suppressed responders to levels seen on responders cultured alone (Fig. 3B) or responders cultured with activated CD4<sup>+</sup>CD25<sup>-</sup> T cells (data not shown).

Soluble tetramer stimulation of OT-I CD8<sup>+</sup> T cells resulted in robust T cell proliferation (Fig. 4A) and IFN- $\gamma$  production (Fig. 4B), confirming previous studies (15–17) showing that CD8<sup>+</sup> T cells can be efficiently activated in the absence of APC and APC-derived costimulatory signals. CD8<sup>+</sup> T cells from normal C57BL/6 mice did not proliferate at any tetramer concentration (Fig. 4A), nor did an irrelevant soluble tetramer (H-2D<sup>b</sup>/CEA antigenic peptide) preparation induce proliferation (data not shown) or IFN- $\gamma$  production (Fig. 4B). To directly assess whether CD4<sup>+</sup>CD25<sup>+</sup> T cells suppress CD8<sup>+</sup> T cell responders by modulating APC function or by direct T-T contact, we stimulated highly purified OT-I CD8<sup>+</sup> T cells (>99% pure) with soluble MHC I H-2K<sup>b</sup>-OVA<sub>257–264</sub> tetramers, in the presence or absence of titrated numbers of CD4<sup>+</sup>CD25<sup>+</sup> T cells. Marked suppression of both proliferation and IFN- $\gamma$  production was seen in the presence of CD4<sup>+</sup>CD25<sup>+</sup> T cells, but not CD4<sup>+</sup>CD25<sup>-</sup> cells, even at low CD4<sup>+</sup>CD25<sup>+</sup>:CD8<sup>+</sup> ratios (Fig. 4, C and D). Taken together, the results from this two-cell system conclusively demonstrate that CD4<sup>+</sup>CD25<sup>+</sup>-mediated suppression occurs via a T-T cell interaction, and the APC is not directly required for the delivery of the suppressive signal to responding CD8<sup>+</sup> T cells.

CD8<sup>+</sup> T cells may contribute to the immunopathogenesis of many autoimmune diseases (18–21). Therefore, it is desirable that regulatory T cells be able to control autoreactive CD8<sup>+</sup> T cells as well as CD4<sup>+</sup> T cells. Most of the effects of regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells on CD8<sup>+</sup> responders were similar to those seen with CD4<sup>+</sup> responders. However, several important differences should be noted. First, in addition to T cell proliferation, the capacity of fresh CD8<sup>+</sup> T cells to manifest effector function such as the production of IFN- $\gamma$  was also suppressed. Second, whereas CD4<sup>+</sup>CD25<sup>+</sup> T cells inhibit the activation of CD4<sup>+</sup> responders by primarily blocking IL-2 production, CD4<sup>+</sup>CD25<sup>+</sup> T cells regulate



**FIGURE 4.** Suppression mediated by CD4<sup>+</sup>CD25<sup>+</sup> T cells is APC-independent. *A*, Tg (OT-I) and non-Tg (wild-type B6) CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) were stimulated with varying concentrations of soluble MHC I H-2K<sup>b</sup>-OVA<sub>257–264</sub> tetramers. Proliferation was measured as in Fig. 1. *B*, Culture supernatants were harvested at 48 h, and IFN- $\gamma$  ELISA was performed. *C*, Tg OT-I CD8<sup>+</sup> T cells ( $5 \times 10^4$ ) were stimulated with soluble MHC I H-2K<sup>b</sup>-OVA<sub>257–264</sub> tetramers (0.5  $\mu$ g/ml) and titrated numbers of activated CD4<sup>+</sup>CD25<sup>+</sup> (■) or CD4<sup>+</sup>CD25<sup>-</sup> (□) T cells, and proliferation was assayed. *D*, Supernatants were collected at 72 h, and IFN- $\gamma$  ELISA was performed.

CD8<sup>+</sup> T cell responses both by blocking IL-2 production as well as by lowering responsiveness to exogenous IL-2 and thereby potentially disrupting CD4 help for CD8<sup>+</sup> T cells. Finally, CD4<sup>+</sup>CD25<sup>+</sup> T cells can inhibit T cell activation by directly acting on responder CD8<sup>+</sup> T cells in the absence of APC. However, this result does not exclude the possibility that CD4<sup>+</sup>CD25<sup>+</sup> T cells might also exert inhibitory/deactivating effects on APC or use the APC surface as a platform on which the suppressor cells physically interact with CD4<sup>+</sup> or CD8<sup>+</sup> effectors in vivo.

Although immunoregulatory CD4<sup>+</sup>CD25<sup>+</sup> cells function beneficially in vivo to protect the host against the development of autoimmunity, they may simultaneously prevent the host from mounting an immune response to autoantigens such as tumor Ags. Because IL-2 responsiveness by CD8<sup>+</sup> T cells is a critical factor for cytokine production (IFN- $\gamma$ ) and cytolytic activity (22, 23), our demonstration that CD4<sup>+</sup>CD25<sup>+</sup> T cells down-regulate both IL-2 production and CD25 expression on CD8<sup>+</sup> T cells may represent a significant impediment to the use of tumor or viral vaccines. Indeed, deletion of CD4<sup>+</sup>CD25<sup>+</sup> T cells before the use of such vaccines may be needed for optimal immunotherapy.

## References

1. Shevach, E. M. 2000. Suppressor T cells: rebirth, function and homeostasis. *Curr. Biol.* 10:R572.
2. Fowell, D., and D. Mason. 1993. Evidence that the T cell repertoire of normal rats contains cells with the potential to cause diabetes: characterization of the CD4<sup>+</sup> T cell subset that inhibits this autoimmune potential. *J. Exp. Med.* 177:627.
3. Sakaguchi, S. 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101:455.
4. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor  $\alpha$ -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155:1151.
5. Powrie, F., M. W. Leach, S. Mauze, L. B. Caddle, and R. L. Coffman. 1993. Phenotypically distinct subsets of CD4<sup>+</sup> T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int. Immunol.* 5:1461.
6. Sakaguchi, S., M. Toda, M. Asano, M. Itoh, S. S. Morse, and N. Sakaguchi. 1996. T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. *J. Autoimmun.* 9:211.
7. Asano, M., M. Toda, N. Sakaguchi, and S. Sakaguchi. 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med.* 184:387.
8. Salomon, B., D. J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, and J. A. Bluestone. 2000. B7/CD28 costimulation is essential for the homeostasis of the CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12:431.
9. Takahashi, T., Y. Kuniyasu, M. Toda, N. Sakaguchi, M. Itoh, M. Iwata, J. Shimizu, and S. Sakaguchi. 1998. Immunologic self-tolerance maintained by CD25<sup>+</sup>CD4<sup>+</sup> naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10:1969.
10. Thornton, A. M., and E. M. Shevach. 1998. CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J. Exp. Med.* 188:287.
11. Takahashi, T., T. Tagami, S. Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, T. W. Mak, and S. Sakaguchi. 2000. Immunologic self-tolerance maintained by CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* 192:303.
12. Thornton, A. M., and E. M. Shevach. 2000. Suppressor effector function of CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory T cells is antigen nonspecific. *J. Immunol.* 164:183.
13. Cederbom, L., H. Hall, and F. Ivars. 2000. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *Eur. J. Immunol.* 30:1538.
14. Itoh, M., T. Takahashi, N. Sakaguchi, Y. Kuniyasu, J. Shimizu, F. Otsuka, and S. Sakaguchi. 1999. Thymus and autoimmunity: production of CD25<sup>+</sup>CD4<sup>+</sup> naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol.* 162:5317.
15. Wang, B., R. Maile, R. Greenwood, E. J. Collins, and J. A. Frelinger. 2000. Naive CD8<sup>+</sup> T cells do not require costimulation for proliferation and differentiation into cytotoxic effector cells. *J. Immunol.* 164:1216.
16. Lu, P., Y. L. Wang, and P. S. Linsley. 1997. Regulation of self-tolerance by CD80/CD86 interactions. *Curr. Opin. Immunol.* 9:858.
17. Sepulveda, H., A. Cerwenka, T. Morgan, and R. W. Dutton. 1999. CD28, IL-2-independent costimulatory pathways for CD8 T lymphocyte activation. *J. Immunol.* 163:1133.
18. Vizler, C., N. Bercovici, A. Cornet, C. Cambouris, and R. S. Liblau. 1999. Role of autoreactive CD8<sup>+</sup> T cells in organ-specific autoimmune diseases: insight from transgenic mouse models. *Immunol. Rev.* 169:81.
19. Herrera, P. L., D. M. Harlan, and P. Vassalli. 2000. A mouse CD8 T cell-mediated acute autoimmune diabetes independent of the perforin and Fas cytotoxic pathways: possible role of membrane TNF. *Proc. Natl. Acad. Sci. USA* 97:279.
20. Wong, F. S., I. Visintin, L. Wen, R. A. Flavell, and C. A. Janeway. 1996. CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. *J. Exp. Med.* 183:67.
21. Wong, F. S., and C. A. Janeway. 1997. The role of CD4 and CD8 T cells in type I diabetes in the NOD mouse. *Res. Immunol.* 148:327.
22. Sad, S., and L. Krishnan. 1999. Cytokine deprivation of naive CD8<sup>+</sup> T cells promotes minimal cell cycling but maximal cytokine synthesis and autonomous proliferation subsequently: a mechanism of self-regulation. *J. Immunol.* 163:2443.
23. Mosmann, T. R., S. Sad, L. Krishnan, T. G. Wegmann, L. J. Guilbert, and M. Belosevic. 1995. Differentiation of subsets of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. *Ciba Found. Symp.* 195:42.