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Cutting Edge: Cell-Extrinsic Immune Regulation by CTLA-4 Expressed on Conventional T Cells

Chun Jing Wang, Rupert Kenefeck, Lukasz Wardzinski, Kesley Attridge, Claire Manzotti, Emily M. Schmidt, Omar S. Qureshi, David M. Sansom, and Lucy S. K. Walker

The CTLA-4 pathway is a key regulator of T cell activation and a critical failsafe against autoimmunity. Although early models postulated that CTLA-4 transduced a negative signal, in vivo evidence suggests that CTLA-4 functions in a cell-extrinsic manner. That multiple cell-intrinsic mechanisms have been attributed to CTLA-4, yet its function in vivo appears to be cell-extrinsic, has been an ongoing paradox in the field. Although CTLA-4 expressed on conventional T cells (Tconv) can mediate inhibitory function, it is unclear why this fails to manifest as an intrinsic effect. In this study, we show that Tconv-expressed CTLA-4 can function in a cell-extrinsic manner in vivo. CTLA-4$^{+/+}$ T cells, from DO11/rag$^{-/-}$ mice that lack regulatory T cells, were able to regulate the response of CTLA-4$^{-/-}$ T cells in cotransfer experiments. This observation provides a potential resolution to the above paradox and suggests CTLA-4 function on both Tconv and regulatory T cells can be achieved through cell-extrinsic mechanisms. *The Journal of Immunology*, 2012, 189: 1118–1122.

The immune system relies on rapid expansion of rare Ag-specific lymphocytes to effectively control replicating pathogens. Consequently, upon receiving the correct signals, T cells undergo efficient clonal expansion and differentiate to acquire effector function. However, for immune homeostasis to be maintained, it is essential that the decision to embark on a program of rapid cell division is tightly regulated. A key arbiter in this decision is the T cell protein CTLA-4, the deficiency of which leads to systemic immune dysregulation with lymphoproliferation, tissue infiltration, and death at 3–4 wk (1, 2). Although the critical importance of CTLA-4 has been clear for many years, the precise mechanisms underlying CTLA-4 function have been controversial.

The notion that CTLA-4 and CD28 played opposing roles in regulating T cell activation, with CTLA-4 inhibiting and CD28 promoting responses, arose from studies in which CTLA-4 was targeted with Abs in vitro (3–6). This concept received support from the observation that disease in CTLA-4$^{-/-}$ mice was abrogated when the CD28 pathway was interrupted by blockade (7) or deficiency (8) of their shared ligands (CD80 and CD86). However, the widely held belief that CTLA-4 worked via a "negative signal" was called into question when bone marrow chimeras, containing both wild-type and CTLA-4$^{-/-}$ cells, were shown to have normal immune systems with no T cell hyperactivation (9). Analysis of such chimeras following infection showed that CTLA-4$^{-/-}$ T cells proliferated and differentiated indistinguishably from CTLA-4$^{+/+}$ cells in the same mice (10–12). This was surprising because if CTLA-4 acts on the cell that expresses it, one would expect only CTLA-4$^{+/+}$ cells to exhibit the consequences of such regulation. Instead, these data pointed to an extrinsic function for the CTLA-4 pathway, such that a CTLA-4$^+$ cell could regulate the response of a CTLA-4$^-$ one.

The emergence of regulatory T cells (Treg), which specialize in dominant tolerance, reinforced the importance of cell-extrinsic immune regulation. Reports that Treg constitutively express CTLA-4 (13–15), together with the phenotypic similarities between CTLA-4–deficient and Treg-deficient mice, sparked interest in whether this protein might contribute to Treg function. Indeed, it is now established that CTLA-4 can serve as major mechanism of Treg suppression (16–22). Although this fits well with an extrinsic function for CTLA-4, it leaves unresolved how CTLA-4 functions on conventional T cells (Tconv).

Numerous studies have shown that Tconv-expressed CTLA-4 can inhibit immune responses (21–25). Such observations have generally been interpreted as evidence of a cell-intrinsic function for CTLA-4. To reconcile these observations with the behavior of CTLA-4$^{-/-}$ T cells in mixed bone marrow chimeras, we recently proposed that conventional T cells might use CTLA-4 in a cell-extrinsic, rather than cell-intrinsic, manner (26). However, this hypothesis has not been formally tested. In this study, we use TCR transgenic T cells to dem-

The online version of this article contains supplemental material.

onstrate that CTLA-4 expressed on one cohort of Tconv can regulate a second cohort of Tconv. These results modify our perspective on CTLA-4 function, suggesting that both Treg and Tconv may use CTLA-4 cell-extrinsically in vivo.

Materials and Methods

Mice

DO11.10 mice were purchased from The Jackson Laboratory and $RAG2^{-/-}$ mice from Taconic Farms. Rat insulin promoter-mice expressing OVA (RIP-mOVA) mice (from line 296-1B) were a gift from W. Heath (Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia). CTLA-$4^{-/-}$ mice were a gift from A. Sharpe (Harvard University, Cambridge, MA). Mice were housed at the University of Birmingham Biomedical Services Unit and used according to home office and institutional guidelines.

Flow cytometry

Abs were purchased from eBioscience or BD Pharmingen.

Analysis of T cell proliferation

$DO11^+$ T cells were injected i.v. into RIP-mOVA mice. Where indicated, donor mice were $CD28^{-/-}$, $rag^{-/-}$, or $CTLA-4^{-/-}$ and recipient mice were $CD28^{+/+}$ or $CD28^{-/-}$. Anti–CTLA-4 Ab (4F10) or control Ab was injected as indicated. For pancreas-draining lymph node (PanLN) analysis, cells from two or more donors were pooled.
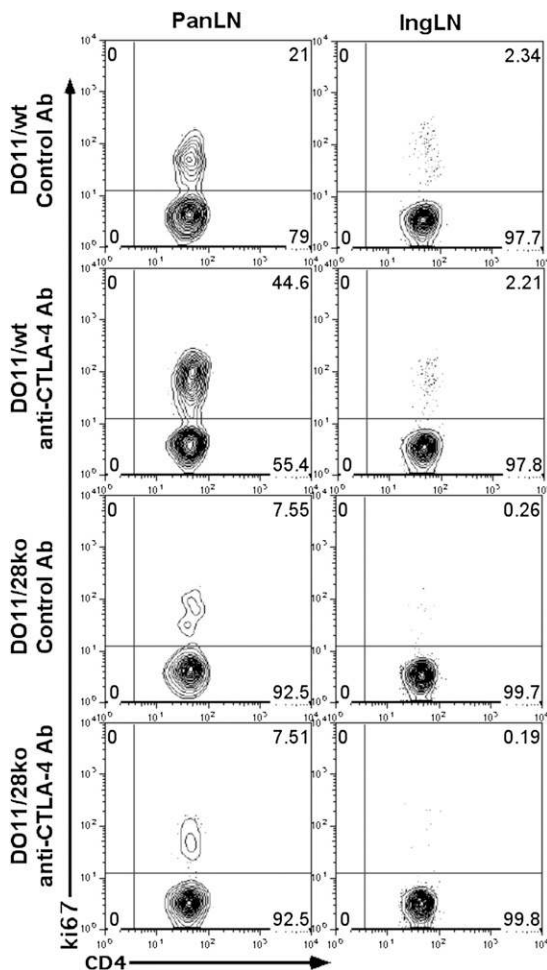

FIGURE 1. Modulation of the T cell response to pancreas-derived Ag by CD28 and CTLA-4. A total of 1.5×10^6 $DO11^+$ T cells (wild-type or $CD28^{-/-}$) were injected i.v. into RIP-mOVA recipients. Where indicated mice were injected with 500 μg anti–CTLA-4 Ab, or control Ab, i.p. on days 1 and 3. On day 4, PanLN and IngLN cells were analyzed by flow cytometry. Ki67 staining on gated $CD4^+DO11^+$ T cells is shown. Data are representative of four independent experiments.

Control of CTLA-4$^{-/-}$ disease

$Rag^{-/-}$ mice were injected i.v. with $CTLA-4^{-/-}$ lymph node (LN) cells alone or in combination with wild-type $CD4^+CD25^-$ or $CD4^+CD25^+$ cells. Percentage weight loss was determined 3 wk later.

Results and Discussion

Response of CD4 T cells to pancreas-derived Ag is modulated by CD28 and CTLA-4

We have previously shown that T cell responses to tissue-expressed self-Ag are more readily controlled by CTLA-4 than responses to Ag administered in immunogenic form (27). In this study, using a second TCR transgenic system, we examined the response of DO11 T cells to pancreas-expressed OVA. DO11 T cells were adoptively transferred into mice expressing OVA under the control of the rat insulin promoter (RIP-mOVA), and their proliferative response was examined 4d later. DO11 T cells in the PanLN had proliferated, as assessed by Ki67 staining, whereas those in the inguinal LN (IngLN) remained largely undivided (Fig. 1, *top panels*). PanLN proliferation depended on expression of OVA in the pancreas because DO11 T cells transferred into RIP-mOVA–negative littermates did not proliferate (data not shown). Injection of blocking anti–CTLA-4 Ab increased T cell proliferation in the PanLN, consistent with a role for CTLA-4 in regulating this stimulation (Fig. 1) (27).

The observation that disease in $CTLA-4^{-/-}$ mice is prevented by deficiency or blockade of CD80/CD86 indicates
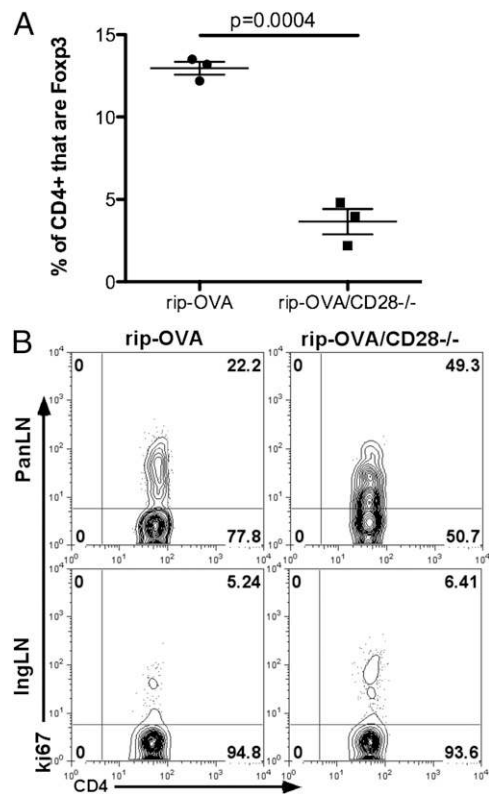
FIGURE 2. Increased response to pancreas-derived Ag in mice with reduced Treg. (**A**) Foxp3 expression in the PanLN of RIP-mOVA mice that were $CD28^{+/+}$ or $CD28^{-/-}$. The percentage of $Foxp3^+$ cells within the CD4 gate is shown. (**B**) A total of 1.5×10^6 $DO11^+$ T cells were injected i.v. into RIP-mOVA or RIP-mOVA/$CD28^{-/-}$ recipients. Ki67 expression on gated $CD4^+DO11^+$ T cells isolated from PanLN or IngLN at day 6 is shown. Data are representative of three independent experiments.

that CTLA-4 functions to control the CD28 pathway. It follows that responses that are subject to CTLA-4-regulation must be dependent on CD28 costimulation. To directly test whether T cell proliferation to pancreas-derived OVA required CD28 we compared the response of wildtype and CD28$^{-/-}$ DO11 T cells. The proliferative response in the PanLN was reduced when the T cells lacked CD28 and was not altered by CTLA-4 blockade (Fig. 1). Collectively these data indicate that pancreas-expressed Ag is presented in the draining LN in a manner that is influenced by CD28 and CTLA-4.

Reducing endogenous Treg increases the T cell response to pancreas-derived Ag

It has recently become clear that CTLA-4 can be used by Treg to elicit suppressive function (16–22, 28), and we have pre-

viously shown that the proliferative response to pancreas-derived OVA can be diminished by Ag-specific Treg (29). The ability of CTLA-4 blockade to augment T cell proliferation in the PanLN could therefore reflect its capacity to impair the suppressive function of endogenous Treg. To investigate the role of endogenous Treg, we took advantage of the fact that CD28-deficient animals have a known Treg deficit yet remain healthy. We therefore bred RIP-mOVA mice to a CD28$^{-/-}$ background. Analysis of RIP-mOVA/ CD28$^{-/-}$ animals revealed significantly reduced proportions of Treg in peripheral lymphoid organs, including the PanLN (Fig. 2A). Moreover, DO11 cells adoptively transferred into RIP-mOVA mice that were CD28$^{-/-}$ showed increased proliferation in the PanLN, consistent with a role for Treg in regulating this response (Fig. 2B). Collectively, these experiments suggested that the T cell response to pancreas-derived OVA was under Treg- and CTLA-4–mediated control; however, this did not preclude an additional role for CTLA-4 expressed on Tconv in regulating the response.

Cell-extrinsic regulation of the response to pancreatic Ag by Tconv-expressed CTLA-4

We recently described a novel mechanism of action for CTLA-4 whereby it physically removes its ligands from adjacent cells (30). Interestingly, this was a fundamental feature of the CTLA-4 protein and occurred regardless of whether it was expressed on Treg or Tconv. This suggested the possibility that Tconv could also use CTLA-4 to achieve cell-extrinsic regulation. To test this idea, DO11/rag$^{-/-}$ cells from CTLA-4$^{+/+}$ and CTLA-4$^{-/-}$ animals were transferred into mice expressing OVA in the pancreas either alone or as a mixed population distinguishable by the congenic marker Thy1. These cells are negative for Foxp3 (18) and therefore comprise pure populations of conventional T cells. To minimize the contribution of host Treg, RIP-mOVA/CD28$^{-/-}$ recipients were used. The proliferative response of DO11 T cells in the PanLN and IngLN was assessed 6 d later (Fig. 3).

When transferred into separate recipients, DO11/CTLA-4$^{-/-}$/ rag$^{-/-}$ cells proliferated more than DO11/rag$^{-/-}$ cells, confirming the ability of CTLA-4 to regulate this response. In contrast, CTLA-4$^{-/-}$ T cells cotransferred with CTLA-4$^{+/+}$ cells (that are devoid of Treg) no longer showed an increased proliferative response to pancreatic Ag. In fact, the response of
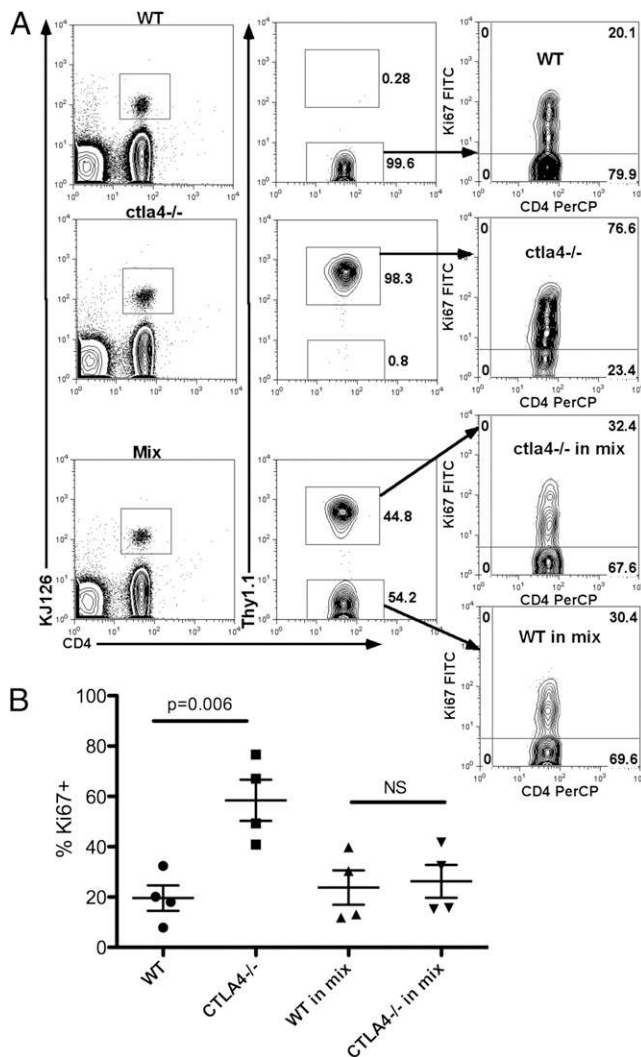
FIGURE 3. Cell-extrinsic regulation of the response to pancreatic Ag by Tconv-expressed CTLA-4. A total of 10^6 DO11$^+$ T cells from DO11/rag$^{-/-}$ (Thy1.1$^-$) or DO11/CTLA4$^{-/-}$/rag$^{-/-}$ (Thy1.1$^+$) donors were injected i.v. into RIP-mOVA/CD28$^{-/-}$ recipients. Cells were injected into separate recipients or coinjected as a 1:1 mix. Six days later, PanLN and IngLN cells were analyzed. CTLA4$^{+/+}$ and CTLA4$^{-/-}$ donor cells (within the CD4$^+$DO11$^+$ gate) were distinguished by Thy1.1 expression. (**A**) Ki67 staining for Thy1.1$^+$ (CTLA4$^{-/-}$) or Thy1.1$^-$ (CTLA4$^{+/+}$) populations within the CD4$^+$DO11$^+$ gate in PanLN is shown. (**B**) The percentage of Ki67$^+$ cells in multiple experiments. The experiment was repeated twice with CTLA4$^{-/-}$ cells being Thy1.1$^+$ and twice with CTLA4$^{+/+}$ cells being Thy1.1$^+$ (with no differences observed). WT, Wild-type.
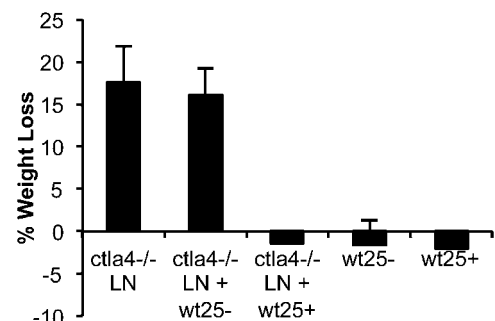
FIGURE 4. Tconv-expressed CTLA-4 is not sufficient to prevent disease induced by CTLA-4$^{-/-}$ cells. A total of 10–20 × 10^6 LN cells from 17-d-old CTLA-4$^{-/-}$ mice were injected i.v. into rag$^{-/-}$ mice either alone or with 10^6 wild-type (wt) CD4$^+$CD25$^-$ or CD4$^+$CD25$^+$ cells. Percentage weight loss is shown 3 wk later. Mice injected with LN cells from wt mice did not show weight loss. Data are compiled from two independent experiments with four recipients per condition.

the CTLA-4$^{+/+}$ and CTLA-4$^{-/-}$ cells in the same animal was indistinguishable. Similar results were obtained when proliferation was tracked using a cell division dye (Supplemental Fig. 1A). Although DO11/rag$^{-/-}$ cells can upregulate Foxp3 after encounter with tissue Ag in certain settings (31), this was not observed in our experiments (Supplemental Fig. 1B). These data therefore provide direct evidence that CTLA-4 expressed on conventional T cell populations can mediate cell-extrinsic regulation of Tconv lacking CTLA-4.

CTLA-4$^{+/+}$ Tconv are not sufficient to prevent CTLA-4$^{-/-}$ lymphoproliferative disease

The fact that the proliferative response of wild-type and CTLA-4$^{-/-}$ T cells was indistinguishable in cotransfer experiments is reminiscent of data obtained from mixed bone marrow chimeras (9). This raised the question of whether CTLA-4 expressed on Tconv alone can protect from disease induced by CTLA-4 deficiency. To test this, we took advantage of the fact that CTLA-4$^{-/-}$ lymphocytes can transfer disease to rag$^{-/-}$ recipient mice (18). LN cells from CTLA-4$^{-/-}$ donors were injected into rag$^{-/-}$ mice either alone or in the presence of CTLA-4–sufficient CD4$^+$CD25$^-$ or CD4$^+$CD25$^+$ cells. As expected, CTLA-4$^{-/-}$ lymphocytes induced wasting disease, and this was prevented by coinjection of CTLA-4–sufficient CD4$^+$CD25$^+$ cells. However, recipients of CTLA-4–sufficient CD4$^+$CD25$^-$ cells showed similar weight loss to recipients of CTLA-4$^{-/-}$ cells alone (Fig. 4). In a second model, involving transfer of CTLA-4$^{-/-}$ bone marrow, CTLA-4–sufficient CD4$^+$CD25$^-$ cells provided partial protection, whereas CD4$^+$CD25$^+$ cells were fully protective (Supplemental Fig. 2). Collectively, these data suggest that although CTLA-4 can elicit extrinsic immune regulation when expressed on Tconv, Treg-expressed CTLA-4 more efficiently regulates systemic autoimmunity.

Multiple cell-intrinsic mechanisms have been ascribed to CTLA-4 (reviewed in Refs. 32 and 33), and it has been tacitly assumed that one or more of these accounts for the actions of CTLA-4 on Tconv. However, in this paper, we provide evidence that CTLA-4 expressed on Tconv can function in a cell-extrinsic manner. This implies that Tconv can themselves contribute to immune regulation, perhaps self-regulating clonal expansion. Our experiments focus on the response of T cells to tissue-expressed Ag that is naturally presented in the absence of adjuvant or immunization. Responses to self-Ags may be particularly susceptible to CTLA-4–dependent regulation, likely due to the low levels of costimulatory ligands on Ag-bearing APC in the absence of inflammation [as originally postulated by Thompson and Allison (34)].

The inability of Tconv-expressed CTLA-4 to control disease induced by CTLA-4$^{-/-}$ cells is consistent with Treg being the dominant population for eliciting CTLA-4–dependent regulation and Tconv playing a secondary role. This may reflect the distinct kinetics of CTLA-4 expression on Treg and Tconv, with constitutive expression only in the former. Indeed, transgenic expression of CTLA-4 in Tconv under the control of the IL-2 promoter could protect CTLA-4$^{-/-}$ mice from death for over 12 mo (22). Because IL-2 is secreted within a few hours of T cell stimulation (35) whereas CTLA-4 induction takes ~2 d (3), the early appearance of CTLA-4 on Tconv in this IL-2 transgenic system could conceivably account for the greater protection. In contrast, naturally controlled expression of

CTLA-4 in Tconv (in mice lacking CTLA-4 specifically in Treg) was only able to extend the lifespan by a few weeks (16). The relative role of CTLA-4 in Treg and Tconv may also be influenced by the nature of the T cell response; one could envisage that upregulation of CTLA-4 on high-affinity Ag-specific T cells could elicit feedback regulation of clone size, whereas Treg-expressed CTLA-4 may be more important for preventing autoimmunity.

In summary, this study demonstrates that similar to Treg, Tconv can use CTLA-4 extrinsically to regulate T cell responses. Similar data have recently been obtained in a separate experimental system [see companion article (36)]. These findings obviate a requirement to postulate differing functions for CTLA-4 in Treg and Tconv and instead suggest a similar mechanism of action in both populations. This moves us toward a scenario in which intrinsic effects may not need to be invoked to explain the function of CTLA-4 in either the Tconv or the Treg compartment.

Acknowledgments

Disclosures

References

1. Tivol, E. A., F. Borriello, A. N. Schweitzer, W. P. Lynch, J. A. Bluestone, and A. H. Sharpe. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3: 541–547.
2. Waterhouse, P., J. M. Penninger, E. Timms, A. Wakeham, A. Shahinian, K. P. Lee, C. B. Thompson, H. Griesser, and T. W. Mak. 1995. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270: 985–988.
3. Walunas, T. L., D. J. Lenschow, C. Y. Bakker, P. S. Linsley, G. J. Freeman, J. M. Green, C. B. Thompson, and J. A. Bluestone. 1994. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1: 405–413.
4. Krummel, M. F., and J. P. Allison. 1995. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J. Exp. Med.* 182: 459–465.
5. Walunas, T. L., C. Y. Bakker, and J. A. Bluestone. 1996. CTLA-4 ligation blocks CD28-dependent T cell activation. *J. Exp. Med.* 183: 2541–2550.
6. Krummel, M. F., and J. P. Allison. 1996. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. *J. Exp. Med.* 183: 2533–2540.
7. Tivol, E. A., S. D. Boyd, S. McKeon, F. Borriello, P. Nickerson, T. B. Strom, and A. H. Sharpe. 1997. CTLA4Ig prevents lymphoproliferation and fatal multiorgan tissue destruction in CTLA-4–deficient mice. *J. Immunol.* 158: 5091–5094.
8. Mandelbrot, D. A., A. J. McAdam, and A. H. Sharpe. 1999. B7-1 or B7-2 is required to produce the lymphoproliferative phenotype in mice lacking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). *J. Exp. Med.* 189: 435–440.
9. Bachmann, M. F., G. Köhler, B. Ecabert, T. W. Mak, and M. Kopf. 1999. Cutting edge: lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. *J. Immunol.* 163: 1128–1131.
10. Bachmann, M. F., P. Waterhouse, D. E. Speiser, K. McKall-Faienza, T. W. Mak, and P. S. Ohashi. 1998. Normal responsiveness of CTLA-4–deficient anti-viral cytotoxic T cells. *J. Immunol.* 160: 95–100.
11. Bachmann, M. F., A. Gallimore, E. Jones, B. Ecabert, H. Acha-Orbea, and M. Kopf. 2001. Normal pathogen-specific immune responses mounted by CTLA-4–deficient T cells: a paradigm reconsidered. *Eur. J. Immunol.* 31: 450–458.
12. Homann, D., W. Dummer, T. Wolfe, E. Rodrigo, A. N. Theofilopoulos, M. B. Oldstone, and M. G. von Herrath. 2006. Lack of intrinsic CTLA-4 expression has minimal effect on regulation of antiviral T-cell immunity. *J. Virol.* 80: 270–280.
13. Metzler, B., C. Burkhart, and D. C. Wraith. 1999. Phenotypic analysis of CTLA-4 and CD28 expression during transient peptide-induced T cell activation in vivo. *Int. Immunol.* 11: 667–675.
14. Read, S., V. Malmström, and F. Powrie. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25$^+$CD4$^+$ regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192: 295–302.
15. 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$^+$CD4$^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* 192: 303–310.
16. Wing, K., Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, and S. Sakaguchi. 2008. CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science* 322: 271–275.

17. Read, S., R. Greenwald, A. Izcue, N. Robinson, D. Mandelbrot, L. Francisco, A. H. Sharpe, and F. Powrie. 2006. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. *J. Immunol.* 177: 4376–4383.

18. Schmidt, E. M., C. J. Wang, G. A. Ryan, L. E. Clough, O. S. Qureshi, M. Goodall, A. K. Abbas, A. H. Sharpe, D. M. Sansom, and L. S. Walker. 2009. Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. *J. Immunol.* 182: 274–282.

19. Sojka, D. K., A. Hughson, and D. J. Fowell. 2009. CTLA-4 is required by CD4+CD25+ Treg to control CD4+ T-cell lymphopenia-induced proliferation. *Eur. J. Immunol.* 39: 1544–1551.

20. Kolar, P., K. Knieke, J. K. Hegel, D. Quandt, G. R. Burmester, H. Hoff, and M. C. Brunner-Weinzierl. 2009. CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice. *Arthritis Rheum.* 60: 123–132.

21. Ise, W., M. Kohyama, K. M. Nutsch, H. M. Lee, A. Suri, E. R. Unanue, T. L. Murphy, and K. M. Murphy. 2010. CTLA-4 suppresses the pathogenicity of self antigen-specific T cells by cell-intrinsic and cell-extrinsic mechanisms. *Nat. Immunol.* 11: 129–135.

22. Jain, N., H. Nguyen, C. Chambers, and J. Kang. 2010. Dual function of CTLA-4 in regulatory T cells and conventional T cells to prevent multiorgan autoimmunity. *Proc. Natl. Acad. Sci. USA* 107: 1524–1528.

23. Greenwald, R. J., V. A. Boussiotis, R. B. Lorsbach, A. K. Abbas, and A. H. Sharpe. 2001. CTLA-4 regulates induction of anergy in vivo. *Immunity* 14: 145–155.

24. Eggena, M. P., L. S. Walker, V. Nagabhushanam, L. Barron, A. Chodos, and A. K. Abbas. 2004. Cooperative roles of CTLA-4 and regulatory T cells in tolerance to an islet cell antigen. *J. Exp. Med.* 199: 1725–1730.

25. Peggs, K. S., S. A. Quezada, C. A. Chambers, A. J. Korman, and J. P. Allison. 2009. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. *J. Exp. Med.* 206: 1717–1725.

26. Walker, L. S., and D. M. Sansom. 2011. The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. *Nat. Rev. Immunol.* 11: 852–863.

27. Walker, L. S., L. J. Ausubel, A. Chodos, N. Bekarian, and A. K. Abbas. 2002. CTLA-4 differentially regulates T cell responses to endogenous tissue protein versus exogenous immunogen. *J. Immunol.* 169: 6202–6209.

28. Friedline, R. H., D. S. Brown, H. Nguyen, H. Kornfeld, J. Lee, Y. Zhang, M. Appleby, S. D. Der, J. Kang, and C. A. Chambers. 2009. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J. Exp. Med.* 206: 421–434.

29. Clough, L. E., C. J. Wang, E. M. Schmidt, G. Booth, T. Z. Hou, G. A. Ryan, and L. S. Walker. 2008. Release from regulatory T cell-mediated suppression during the onset of tissue-specific autoimmunity is associated with elevated IL-21. *J. Immunol.* 180: 5393–5401.

30. Qureshi, O. S., Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E. M. Schmidt, J. Baker, L. E. Jeffery, S. Kaur, Z. Briggs, et al. 2011. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332: 600–603.

31. Thompson, L. J., A. C. Valladao, and S. F. Ziegler. 2011. Cutting edge: de novo induction of functional Foxp3+ regulatory CD4 T cells in response to tissue-restricted self antigen. *J. Immunol.* 186: 4551–4555.

32. Bour-Jordan, H., J. H. Esensten, M. Martinez-Llordella, C. Penaranda, M. Stumpf, and J. A. Bluestone. 2011. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol. Rev.* 241: 180–205.

33. Wing, K., T. Yamaguchi, and S. Sakaguchi. 2011. Cell-autonomous and -non-autonomous roles of CTLA-4 in immune regulation. *Trends Immunol.* 32: 428–433.

34. Thompson, C. B., and J. P. Allison. 1997. The emerging role of CTLA-4 as an immune attenuator. *Immunity* 7: 445–450.

35. Sojka, D. K., D. Bruniquel, R. H. Schwartz, and N. J. Singh. 2004. IL-2 secretion by CD4+ T cells in vivo is rapid, transient, and influenced by TCR-specific competition. *J. Immunol.* 172: 6136–6143.

36. Corse, E., and J. P. Allison. 2012. Cutting edge: CTLA-4 on effector T cells inhibits in trans. *J. Immunol.* 189: 1123–1127.