challenged mice were categorized according to the abundance of PAS$^+$ goblet cells and assigned numerical scores (0: <5% goblet cells; 1: 5 to 25%; 2: 25 to 50%; 3: 50 to 75%; 4: >75%). The sum of the airway scores from each lung was divided by the number of airways examined for the histologic goblet cell score (expressed as arbitrary units; U). BAL samples were analyzed as described (7). Statistical significance was calculated using Student's *t* test (PC_{200}) or Wilcoxon test (goblet cell score, BAL cytology).

10. PC_{200} of saline-treated controls was 0.55 ± 0.06 mg/kg. PC_{200} of OVA-challenged wild-type, IL-4–deficient, and IL-4Rα–deficient mice were 0.24 ± 0.025, 0.36 ± 0.026, and 0.45 ± 0.058 mg/kg, respectively.
11. A. Minty *et al.*, *Nature* **362**, 248 (1993); J. Punnonen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3730 (1993); T. Naseer *et al.*, *Am. J. Respir. Crit. Care Med.* **155**, 845 (1997); S. Till *et al.*, *Immunology* **91**, 53 (1997).
12. After subcutaneous priming with OVA on days –14 and –7, BALB/c wild-type mice were administered intranasally 55 μl of IL-13R-Fc (*13*) or immunoglobulin control protein (Ig control) in phosphate-buffered saline (PBS). We administered 110 μg of IL-13R-Fc on days 0, 1, 2, 3, 3.5, 4, and 4.5, and 110 μg of Ig control was given on days 0, 1, 2, and 4. Both proteins were administered with 750 μg of OVA on days 1, 2, and 4. Data were collected on day 5 (9).
13. D. D. Donaldson *et al.*, *J. Immunol.* **161**, 2317 (1998).
14. Wild-type, IL-4Rα$^{-/-}$, and RAG1$^{-/-}$ mice were administered 3 to 5 μg of recombinant IL-4 or IL-13, or control protein (bovine serum albumin or irrelevant rat antibody) intranasally on days 1, 3, and 5, and data were collected 12 to 15 hours later (9). For clarity, only data from BALB/c wild-type mice are shown.
15. CD4$^+$ T cell lines were prepared using splenocytes from D011.10 T cell receptor (TCR) transgenic mice that express an OVA-specific TCR transgene (*25*). Equal numbers of T cells and antigen presenting cells (mitomycin c–treated and T cell–depleted BALB/c splenocytes) were incubated with 1 μM OVA peptide, IL-4 (300 IU/ml), and antibody to IFN-γ (R46A2; 100 μg/ml) for 5 days. Wild-type or IL-4Rα$^{-/-}$ mice were reconstituted with 1.2×10^7 washed cells intravenously. The mice were challenged intranasally with OVA for 6 consecutive days. Data were collected on day 7 (9).
16. G. Grünig and D. B. Corry, unpublished data.
17. S. P. Hogan *et al.*, *J. Immunol.* **161**, 1501 (1998); L. Cohn, R. J. Homer, A. Marinov, J. Rankin, K. Bottomly, *J. Exp. Med.* **186**, 1727 (1997).
18. A. J. Bancroft, A. N. McKenzie, R. K. Grencis, *J. Immunol.* **160**, 3453 (1998); J. F. Urban Jr. *et al.*, *Immunity* **8**, 255 (1998); J. F. Urban Jr., I. M. Katona, W. E. Paul, F. D. Finkelman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5513 (1991); M. Barner, M. Mohrs, F. Brombacher, M. Kopf, *Curr. Biol.* **8**, 669 (1998).
19. A. A. Ten Have-Opbroek, *Exp. Lung Res.* **17**, 111 (1991).
20. D. S. Postma *et al.*, *N. Engl. J. Med.* **333**, 894 (1995).
21. H. Mitsuyasu *et al.*, *Nature Genet.* **19**, 119 (1998); G. K. Hershey, M. F. Friedrich, L. A. Esswein, M. L. Thomas, T. A. Chatila, *N. Engl. J. Med.* **337**, 1720 (1997).
22. S. E. Daniels *et al.*, *Nature* **383**, 247 (1996); A. Sandford, T. Weir, P. Pare, *Am. J. Respir. Crit. Care Med.* **153**, 1749 (1996).
23. N. Noben-Trauth, G. Kohler, K. Burki, B. Ledermann, *Transgenic Res.* **5**, 487 (1996).
24. P. Mombaerts *et al.*, *Cell* **68**, 869 (1992).
25. K. M. Murphy, A. B. Heimberger, D. Y. Loh, *Science* **250**, 1720 (1990).

26. We thank D. Erle, M. Wills-Karp, R. Coffman, and F. Finkelman for helpful discussions, the Research Support Team of Genetics Institute for IL-13Rα$_2$-Fc protein, and R. Coffman for IL-4. Supported by NIH grants T32 HL07185 (G.G.), 03344 (D.B.C.), 47412, 53949, 33259 (D.S.), 09883 (R.V.), and P01-HL56385; the Crohn's and Colitis foundation and the Hefni Scholars Fund (A.E.W.); Howard Hughes Medical Institute (M.M. and R.M.L); and Schering-Plough Corporation (D.M.R.).

3 September 1998; accepted 4 November 1998

Molecular Basis of T Cell Inactivation by CTLA-4

Kyung-Mi Lee,* Ellen Chuang,* Matthew Griffin, Roli Khattri, David K. Hong, Weiguo Zhang, David Straus, Lawrence E. Samelson, Craig B. Thompson, Jeffrey A. Bluestone†

CTLA-4, a negative regulator of T cell function, was found to associate with the T cell receptor (TCR) complex ζ chain in primary T cells. The association of TCRζ with CTLA-4, reconstituted in 293 transfectants, was enhanced by p56^{lck}-induced tyrosine phosphorylation. Coexpression of the CTLA-4–associated tyrosine phosphatase, SHP-2, resulted in dephosphorylation of TCRζ bound to CTLA-4 and abolished the p56^{lck}-inducible TCRζ–CTLA-4 interaction. Thus, CTLA-4 inhibits TCR signal transduction by binding to TCRζ and inhibiting tyrosine phosphorylation after T cell activation. These findings have broad implications for the negative regulation of T cell function and T cell tolerance.

CTLA-4 is a T cell activation molecule essential for normal homeostasis of T cell reactivity. Engagement and cross-linking of CTLA-4 blocks production of interleukin-2, cell cycle progression, and cell differentiation, whereas in vivo blockade of CTLA-4–B7 interaction enhances autoreactive and tumor-specific T cell activity (*1*). Although it has been proposed that CTLA-4 affects signals downstream of initial T cell signaling events, several lines of evidence suggest that the negative signaling may occur at the T cell "activation cap" (*2*). Therefore, we investigated whether engagement of CTLA-4 directly affects proximal events of TCR-induced signaling pathways.

Primary T cells were activated for 2 days with monoclonal antibodies (mAbs) to CD3 and CD28 for optimal CTLA-4 expression and then rested to maximize anti-CD3-mediated signaling events. CTLA-4 cross-linking during restimulation with anti-CD3 mAbs resulted in decreased tyrosine phosphorylation of multiple intracellular proteins migrating between 18 and 40 kD (Fig. 1). Immunoblot analyses demonstrated that the affected proteins migrating at 18 to 23 kD represented the TCRζ chains, whereas the protein migrating at 36 kD was LAT (linker for activation of T cells) (*3*), an adaptor molecule critical for TCR signaling. In addition, tyrosine phosphorylation of mitogen-activated protein kinases stimulated by the anti-CD3 mAbs was reduced after CTLA-4 cross-linking (*4*). These data suggest that CTLA-4 can inhibit early TCR signaling events within the TCR complex.

To define the molecular mechanism by which CTLA-4 affected TCR signaling events, we analyzed anti-CTLA-4 immunoprecipitates, prepared from metabolically labeled and activated T cells, by two-dimensional SDS–polyacrylamide gel electrophoresis (PAGE) to identify interacting proteins (Fig. 2A). The bands migrating at 32 kD (nonreduced) and16 kD (reduced) were demonstrated to be TCRζ based on immunoblot analyses with antibodies to TCRζ (Fig. 2B) (*5*), which suggests that CTLA-4 was associated with TCRζ in these cells. The specificity of CTLA-4–TCRζ binding was confirmed as the 16-kD band was absent in anti-CTLA-4 immunoprecipitates prepared from CTLA-4-deficient T cells (Fig. 2C) and could be specifically blocked by the addition of CTLA-4 immunoglobulin during immunoprecipitation (*4, 6*).

The interaction of CTLA-4 with TCRζ in activated T cells is likely to be complex and may require additional T cell–specific proteins. Therefore, we examined the CTLA-4–TCRζ association in a non–T cell transfection system. Human embryonic kidney epithelial (293) cells were transiently transfected with a plasmid containing murine TCRζ and one encoding murine CTLA-4. The 16-kD TCRζ chain was coprecipitated with CTLA-4 (Fig. 3A). The TCRζ association was specific as mAb to CTLA-4 did not precipitate the 16-kD protein from cells transfected with vector alone or with a truncated form of CTLA-4 lacking the cytoplasmic tail. The importance of the CTLA-4 tail in the TCRζ interaction was confirmed by coexpressing a construct

K.-M. Lee, M. Griffin, R. Khattri, D. K. Hong, J. A. Bluestone, Ben May Institute for Cancer Research, and Committee on Immunology, University of Chicago, Chicago, IL 60637, USA. E. Chuang, Committee on Immunology, and Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, IL 60637, USA. W. Zhang and L. E. Samelson, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA. D. Straus, Committee on Immunology, and Department of Pathology and Medicine, University of Chicago, Chicago, IL 60637, USA. C. B. Thompson, Howard Hughes Medical Institute, Ben May Institute for Cancer Research, Committee on Immunology, and Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, IL 60637, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: jbluest@immunology.uchicago.edu

Downloaded from http://science.sciencemag.org/ on November 19, 2019

encoding a concatamer of three cytoplasmic tails of CTLA-4 fused to a glutathione *S*-transferase [GST-$(CTLA\text{-}4)_3$] with TCRζ (Fig. 3B). To ensure that the interaction of TCRζ with CTLA-4 occurred at the cell membrane, we examined CTLA-4–TCRζ association in 293 cells transfected with a cell surface–expressed chimeric Tac–ζ construct (extracellular and transmembrane domains of Tac fused to the cytoplasmic domain of TCRζ) (*7*). A significant amount of Tac–ζ, migrating between 43 and 60 kD, was coprecipitated with CTLA-4 in these transfectants (Fig. 3C). Thus, the association of CTLA-4 with TCRζ can occur at the membrane and does not depend on other T cell–specific proteins.

Previous studies have shown that $p56^{lck}$ can regulate phosphorylation of both CTLA-4 and TCRζ (*8*). Therefore, we examined the effect of $p56^{lck}$ on CTLA-4–TCRζ association. Cotransfection of $p56^{lck}$ resulted in increased CTLA-4–TCRζ association, especially with the higher, phosphorylated form (p18) of TCRζ (Fig. 3A). $p56^{lck}$ also enhanced TCRζ binding to the GST-$(CTLA\text{-}4)_3$ fusion protein (Fig. 3B). A kinase-defective mutant $p56^{lck}$ (K273A, KA) was not able to enhance CTLA-4–TCRζ binding and failed to recruit p18 TCRζ to CTLA-4 (Fig. 3D). These results suggest that the tyrosine kinase activity of $p56^{lck}$ was required for enhanced association of CTLA-4 with TCRζ. Two tyrosines (Y201 and Y218) located in the cytoplasmic domain of CTLA-4 are substrates for src family tyrosine kinases (*8*). Thus, the role of these tyrosines in mediating TCRζ association was examined in cells expressing a tyrosine-deficient [Tyr^{201} to Phe/Tyr^{218} to Phe (Y201F/Y218F)] CTLA-4 double mutant. The mutant CTLA-4 bound to TCRζ to a similar extent as wild-type CTLA-4 (Fig. 3A), which suggests that CTLA-4 tyrosine phosphorylation was not required for TCRζ binding. Therefore, the enhancement of TCRζ–CTLA-4 association by $p56^{lck}$ likely depends on tyrosine phosphorylation of TCRζ, although $p56^{lck}$-mediated phosphorylation of other unidentified molecules may be involved.

CTLA-4 can also bind to a tyrosine phosphatase, SHP-2 (*9*). Thus, we explored the possibility that CTLA-4 formed complexes with SHP-2 and TCRζ in activated T cells, which accounts for the lack of p18 TCRζ in CTLA-4 immunoprecipitates (Fig. 4A). SHP-2 coprecipitated with antibodies to both TCRζ and CTLA-4. Reciprocal immunoprecipitation of SHP-2 and subsequent immunoblotting with mAbs to TCRζ or CTLA-4 as well as transfection studies (Fig. 4B) confirmed the existence of a multimolecular complex of CTLA-4–SHP-2–TCRζ. Anti-CTLA-4 immunoprecipitates prepared from cells transfected with CTLA-4 and TCRζ contained small but detectable amounts of endogenous SHP-2 present in 293 cells that were significantly enhanced by cotransfecting $p56^{lck}$ (Fig. 4B). Overexpression of SHP-2 increased the amount of SHP-2 coprecipitated with CTLA-4. However, SHP-2 overexpression eliminated the binding of p18 TCRζ to CTLA-4. The failure to observe p18 TCRζ binding to CTLA-4 in SHP-2–overexpressing cells was not due to alterations in protein expression, as similar amounts of CTLA-4 (*4*) and TCRζ were detected. Likewise, SHP-2 overexpression did not alter the overall amounts of the p18 form of TCRζ within the cell, as equal amounts of p18 TCRζ were present in SHP-2–transfected and control cells. Similar results were obtained with the cells expressing the Tac–ζ chimera (*4*).

Similar to wild-type CTLA-4, the tyrosine mutant CTLA-4 could interact with SHP-2

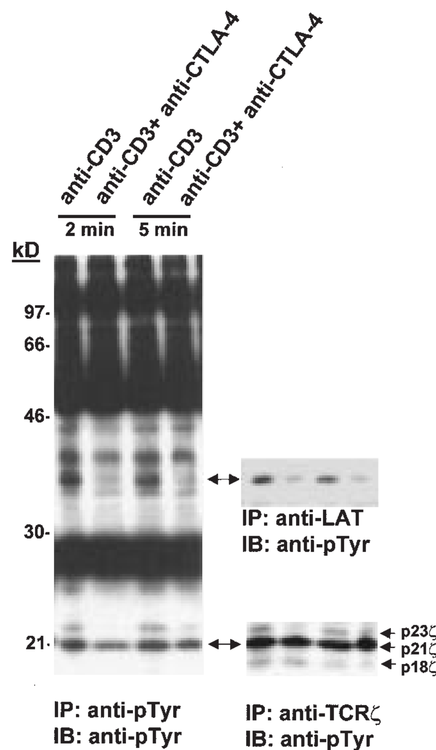


Fig. 1. Anti-CD3–induced LAT and TCRζ tyrosine phosphorylation is inhibited by anti-CTLA-4 engagement. Activated T cells (5×10^6) (*19*) were admixed with 293 cells (2.5×10^6) transiently expressing a membrane-bound single-chain mAb to CD3 in the presence or absence of a membrane-bound single-chain mAb to CTLA-4 (*20*). The cells were incubated at 37°C for the time indicated and then subjected to lysis in buffer (LB). Immunoprecipitates (IPs) were prepared with mAb FB2 to phosphotyrosine (pTyr), rabbit antiserum to LAT (3), or mAb H146-968 to TCRζ (*21, 22*). IPs separated on a reducing SDS–12% polyacrylamide gel were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA) and subsequently immunoblotted (IB) with mAb 4G10 to pTyr (Upstate Biotechnology Inc., Lake Placid, NY). Bound proteins were detected by chemiluminescence (Pierce, Rockford, IL). CTLA-4 cross-linking resulted in a consistent but incomplete reduction of tyrosine phosphorylated p23TCRζ and LAT. Results are representative of three independent experiments.

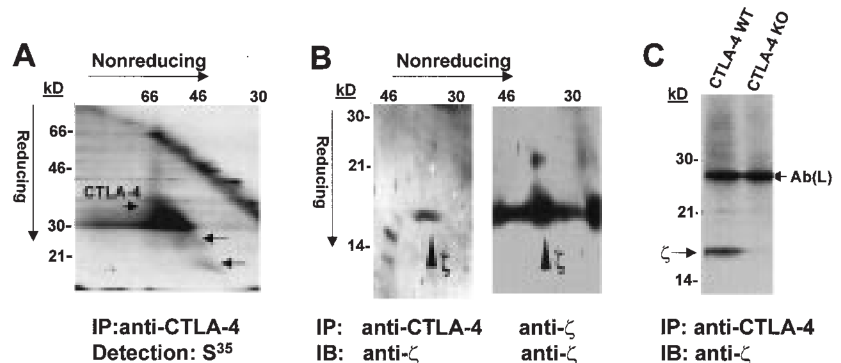


Fig. 2. (**A**) CTLA-4 coprecipitation of metabolically labeled proteins. Activated T cells (20×10^6 cells) (*23*) were labeled with ^{35}S-methionine and ^{35}S-cysteine (Amersham, Arlington Heights, IL). CTLA-4 was immunoprecipitated from cell lysates with mAb UC10-4F10 to CTLA-4 (*15*). Immune complexes were subjected to two-dimensional SDS-PAGE analysis with 10% acrylamide gels in both the first (nonreduced) and second (reduced) dimensions (*5*). Arrows indicate proteins coprecipitated with UC10-4F10. (**B** and **C**) Identification of p16 TCRζ by immunoblotting. Activated T cells (100×10^6 cells per condition) (*23*) were lysed and subjected to immunoprecipitation with mAb UC10-4F10 to CTLA-4 or mAb H146-968 to TCRζ. (B) IPs run on two-dimensional SDS–polyacrylamide gel (10% nonreduced, 12% reduced) were subjected to immunoblotting with a rabbit antiserum to TCRζ (Ab 387) (*24*). The bound proteins were visualized by chemiluminescence. Upper bands in the anti-ζ IP (right) represented higher phosphorylated forms of TCRζ and TCRη. (C) Anti-CTLA-4 IPs prepared from CTLA-4 wild-type (WT) or knockout (KO) mice were separated by one-dimensional reducing SDS-PAGE and immunoblotted with rabbit antiserum to ζ (Ab 387). Ab(L) represents antibody light chain. Independent experiments with similar results were performed five times.

Downloaded from http://science.sciencemag.org/ on November 19, 2019

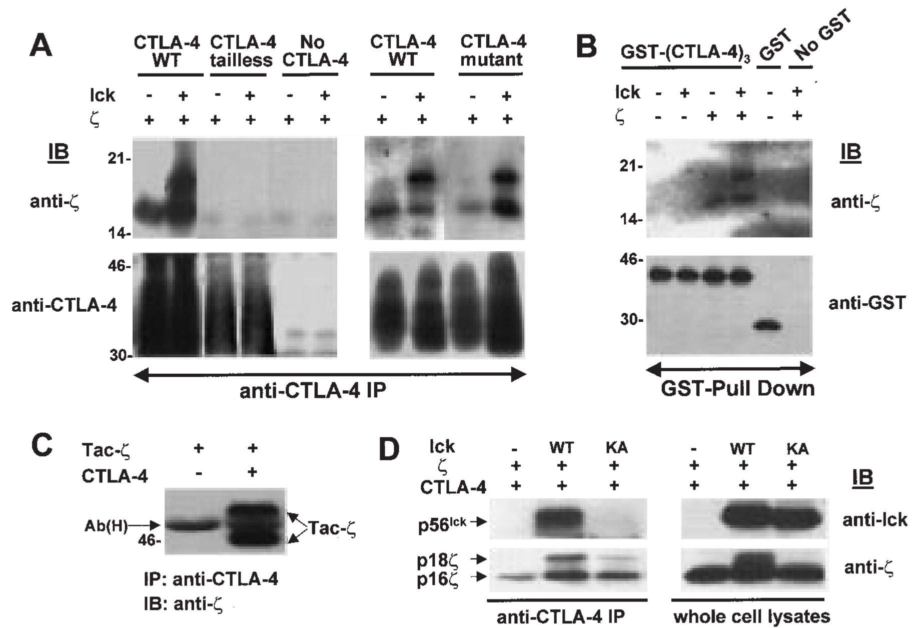
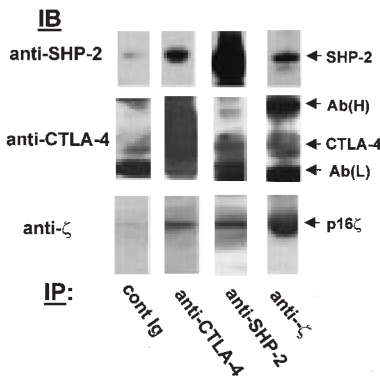


Fig. 3. Analysis of CTLA-4-TCRζ association in 293 cell transfectants. (**A**) Coimmunoprecipitation of CTLA-4 with TCRζ. 293 cells were transiently transfected with cDNA constructs encoding murine CTLA-4 WT, cytoplasmic domain-deficient (tailless) (*12*) CTLA-4, or CTLA-4 tyrosine double mutant (Y201F/Y218F) (*25*) in the absence or presence of WT $p56^{lck}$ and murine TCRζ (*25*). Cells were incubated for 40 hours and lysates were prepared. CTLA-4 was precipitated from an equal amount of protein [700 μg per IP (left); 300 μg per IP (right)]. IPs were analyzed by reducing SDS-PAGE and immunoblotted with the antibodies listed. The expression vectors used were pCDNA3 (CTLA-4 and TCRζ, 2.5 μg each) and pEF ($p56^{lck}$, 1.25 μg). (**B**) GST–$(CTLA\text{-}4)_3$ fusion protein binds TCRζ. 293 cells were transiently transfected with cDNA encoding GST–$(CTLA\text{-}4)_3$ (*12*) in the presence or absence of $p56^{lck}$ and TCRζ. GST proteins were precipitated from the lysates (200 μg per sample) with glutathione Sepharose beads (12.5 μl, Pharmacia) and analyzed by electrophoresis and immunoblotting with either rabbit antiserum to TCRζ (Ab 387), or GST mAb (Santa Cruz, Santa Cruz, CA). GST and GST–$(CTLA\text{-}4)_3$ proteins migrated at 25 and 40 kD, respectively. (**C**) CTLA-4 coprecipitates Tac–ζ in 293 transfectants. 293 cells were transiently transfected with cDNAs for Tac–ζ chimera (*7*) in the absence or presence of WT CTLA-4. CTLA-4 IPs were subjected to SDS-PAGE and immunoblotted with Ab 387. Chimeric Tac–ζ, migrated as a doublet when detected with Ab 387. Ab(H) represents antibody heavy chain. (**D**) Kinase-defective lck (KA) failed to enhance TCRζ association. 293 cells were transiently transfected with cDNAs for WT CTLA-4, murine TCRζ, and either WT or kinase-defective $p56^{lck}$ (*26*) and analyzed as described above.

(Fig. 4B) and abolished p18 TCRζ binding to CTLA-4. These results are in contrast to the reported dependence of the in vitro interaction between CTLA-4 and SHP-2 on phosphotyrosines and SH2 domains (*9*). The difference may be due to the in vivo rather than in vitro analysis and suggests that there may be a phosphotyrosine-dependent as well as -independent association of CTLA-4 and SHP-2. As CTLA-4 does not possess SH2 domains, it is possible that TCRζ binding to CTLA-4 is indirect and may depend on SHP-2 or the phosphotyrosine-dependent non-SH2 domain binding in CTLA-4.

A model can be envisioned to explain the process by which CTLA-4 regulates T cell responses. T cell activation, initiated by TCR ligation and CD28 costimulation, results in the recruitment of $p56^{lck}$ to the TCR cap and phosphorylation of multiple substrates including TCRζ, ZAP-70, and LAT. CTLA-4, newly expressed (*1*) or preexisting in resting cells (*10*), is exported to the cell surface and binds to B7 molecules present in antigen presenting cells (*11*). CTLA-4 membrane localization may be facilitated by colocalization to the site of TCR engagement through direct interaction with TCRζ and $p56^{lck}$-induced tyrosine phosphorylation of CTLA-4 (*12*). The interaction of TCRζ and CTLA-4 brings the phosphatase, SHP-2, into the complex where it promotes TCRζ dephosphorylation either directly by acting on TCRζ or indirectly by regulating $p56^{lck}$ kinase activity. In this regard, several studies have demonstrated a relationship between src family kinase activity and SHP-2 function (*13*). Currently, the molecular nature of TCRζ–CTLA-4 binding is not known. Given the fact that $p56^{lck}$ enhanced TCRζ binding to the Y201F/Y218F CTLA-4 mutant, CTLA-4–TCRζ interaction most likely depends on tyrosine phosphorylation of TCRζ or other molecules that might be critical for their association. Further studies will help to elucidate the molecular basis of CTLA-4–TCRζ binding. Finally, previous studies have shown that the ordered phosphorylation of TCRζ establishes the threshold for T cell activation (*14*). The ability of CTLA-4 to bind to and dephosphorylate the p23 form of phosphorylated TCRζ may decrease the extent and duration of TCRζ phosphorylation and thereby antagonize TCR signal transduction. Further studies are needed to elucidate the functional importance of CTLA-4 in tolerance and antagonist peptide activity. However, the cur-



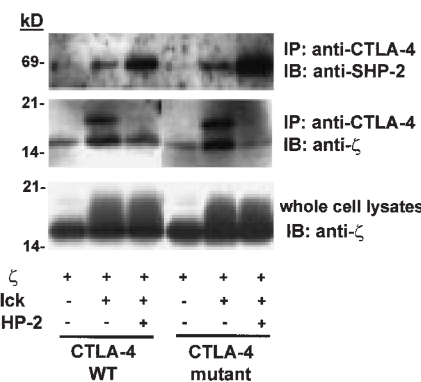


Fig. 4. SHP-2 associates with CTLA-4–TCRζ complexes and regulates binding of TCRζ to CTLA-4. (**A**) Equal numbers (100×10^6) of activated T cells (*23*) were lysed and subjected to IP with mAbs to CTLA-4 (UC10-4F10), rabbit antiserum against SHP-2 (*27*), and mAb to TCRζ (H146). IPs were analyzed by SDS-PAGE and immunoblotted with a rabbit antiserum to SHP-2 (*27*), goat polyclonal antibodies to CTLA-4 (Q20, Santa Cruz, Santa Cruz, CA), or rabbit antiserum to TCRζ (Ab 387). (**B**) 293 cells were transiently transfected with either WT CTLA-4 or mutated CTLA-4 (Y201F/Y218F) and TCRζ in the presence or absence of $p56^{lck}$ or SHP-2. CTLA-4 IPs prepared from lysates (400 μg per sample) or whole cell lysates (35 μg per lane) were electrophoresed and immunoblotted with rabbit antibody to SHP-2 (*27*) or antiserum to TCRζ (Ab 387). Results are representative of three separate experiments.

Downloaded from http://science.sciencemag.org/ on November 19, 2019

rent studies provide a conceptual framework for developing approaches to regulate T cell function through CTLA-4.

References and Notes

1. J. A. Bluestone, *J. Immunol.* **158**, 1989 (1997); A. I. Sperling and J. A. Bluestone, *Immunol. Rev.* **153**, 155 (1996).
2. C. R. Calvo, D. Amsen, A. M. Kruisbeek, *J. Exp. Med.* **186**, 1645 (1997), P. S. Linsley *et al.*, *Immunity* **4**, 535 (1996); M. Griffin and J. A. Bluestone, unpublished observations.
3. W. Zhang *et al.*, *Cell* **92**, 83 (1998).
4. K.-M. Lee *et al.*, unpublished observation.
5. A. M. Weissman, L. E. Samelson, R. D. Klausner, *Nature* **324**, 480 (1986); M. Baniyash *et al.*, *J. Biol. Chem.* **263**,18225 (1988).
6. P. W. Wallace *et al., Transplantation* **58**, 602 (1994).
7. F. Letourneur and R. Klausner, *Science* **255**, 79 (1992).
8. J. D. Bradshaw *et al., Biochemistry* **36**, 15975 (1997); E. Chuang *et al.*, *J. Immunol.*, in press.
9. L. E. Marengere *et al*., *Science* **272**, 1170 (1996).
10. P. J. Blair *et al., J. Immunol.* **160**, 12 (1998).
11. A. Kupfer and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8216 (1988); C. B. Thompson and J. P. Allison, *Immunity* **7**, 445 (1997).
12. E. Chuang *et al.*, *J. Immunol.* **159**, 144 (1997); Y. Zhang and J. P. Allison, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9273 (1997).
13. G. Feng *et al.*, *Science* **259**, 1607 (1993); W. Vogel *et al.*, *ibid.*, p. 1611; T. Tauchi *et al.*, *J. Biol. Chem.* **269**, 15381 (1994).
14. E. N. Kersh, A. S. Shaw, P. M. Allen *Science* **281**, 572 (1998).
15. T. L. Walunas *et al., Immunity* **1**, 405 (1994).
16. O. Leo *et al., Proc. Natl. Acad. Sci. U.S.A.* **84**, 1374 (1987).
17. R. Abe *et al., J. Immunol.* **154**, 985 (1995).
18. B. A. Houlden, R. Q. Cron, J. E. Coligan, J. A. Bluestone, *ibid.* **141**, 3753 (1988).
19. T cells from BALB/c lymph nodes were purified (*15*) and cultured for 60 hours in a dish coated with mAb to CD3 (anti-CD3) (145-2C11; 2 μg/ml) (*16*) and mAb to CD28 (anti-CD28) (PV-1; 2 μg/ml) (*17*). Cells were then washed three times to remove any residual antibodies present on the cell surface and rested at 37°C for additional 4 hours.
20. M. Griffin *et al.*, unpublished data.
21. Immunoprecipitations were performed with lysates prepared in LB [1% Nonidet P-40, 50 mM tris-HCl (pH 7.4), 150 mM NaCl, 20 mM EDTA (pH 8.0), 1 mM sodium vanadate, leupeptin (10 μg/ml), 10 μM aprotinin, 1 mM phenylsulfonylsulfoxide]. Lysates were precleared twice with protein A beads and once with protein A beads coated with a mAb from a control hamster (UC3-10A6) (*18*) before precipitating antibodies were added. Immunoprecipitation was performed overnight at 4°C. Immune complexes were washed five times with LB and subjected to SDS-PAGE.
22. M. M. Rozdzial, R. T. Kubo, S. L. Turner, T. H. Finkel *J. Immunol.* **153**, 1563 (1994).
23. BALB/c whole lymph node and spleen T cells were activated with mAb 145-2C11 (1 μg/ml) plus mAb PV-1 (1 μg/ml) for 60 hours. Cells were washed and rested as described (*19*). Viable cells were enriched by passage over Ficoll/Hypaque. Over 90% of viable cells were CD3$^+$ by fluorescence-activated cell sorter analysis.
24. D. G. Orloff *et al., J. Biol. Chem.* **264**, 14812 (1989).
25. The CTLA-4 tyrosine mutant (Y201F/Y218F) was generated by site-directed mutagenesis (Chameleon site-directed mutagenesis kit, Stratagene) and the presence of mutations was verified by DNA sequencing. A cDNA sequence incorporating the coding region of murine TCRζ was generated by reverse transcription and polymerase chain reaction from RNA extracted from activated murine T cells and cloned into the mammalian expression vector pCDNA3.1(+).
26. A. C. Carrera *et al., Proc. Natl. Acad. Sci. U.S.A.* **90**, 442 (1993).
27. T. Yin, R. Shen, G. S. Feng, Y. C. Yang, *J. Biol. Chem.* **272**, 1032 (1997).

28. Supported by the National Institutes of Health (PHS PO1 AI35294-6). Additional support was provided by a Howard Hughes Medical Institute Medical Student Research Training Fellowship (D.K.H.) and a Mayo Foundation Scholarship (M.G.). We thank G. Feng for the reagents; A. Sharpe for CTLA-4 knockout mice; N. Patel and C. Long for technical assistance; S. Thomas, M. Fonstein, and W. Buikema for services provided at the Cancer Research Center (CRC) DNA Sequencing Facility; and M. Clark for critical reading of the manuscript.

10 August 1998; accepted 10 November 1998

A Receptor/Cytoskeletal Movement Triggered by Costimulation During T Cell Activation

Christoph Wülfing and Mark M. Davis*

During T cell activation, the engagement of costimulatory molecules is often crucial to the development of an effective immune response, but the mechanism by which this is achieved is not known. Here, it is shown that beads attached to the surface of a T cell translocate toward the interface shortly after the start of T cell activation. This movement appears to depend on myosin motor proteins and requires the engagement of the major costimulatory receptor pairs, B7-CD28 and ICAM-1–LFA-1. This suggests that the engagement of costimulatory receptors triggers an active accumulation of molecules at the interface of the T cell and the antigen-presenting cell, which then increases the overall amplitude and duration of T cell signaling.

The central event in T cell activation is the interaction of the T cell receptor (TCR) with the antigenic peptide presented by the major histocompatibility complex (MHC) of the antigen-presenting cell (APC). However, because the number of agonist peptide–MHC complexes can be very low, in the range of 10 to 100 per APC (*1*), and because the TCR is continuously modulated from the T cell surface (*2*), sustained T cell activation is likely to require signal amplification (*3, 4*). An important component of this amplification is thought to be provided by costimulatory molecules on the T cell, although the mechanism by which they accomplish it is unclear (*5*). The most important of the costimulatory receptors on T cells and their ligand on APCs are CD28-B7 (*6*) and LFA-1–ICAM-1 (*7*). Many different receptor couples, including TCR–peptide-MHC and LFA-1–ICAM-1, accumulate at the T cell–APC interface (*8, 9*). This accumulation has been assumed to be a passive, diffusion-limited cocapping mechanism (*10*). Here, we describe an active, cytoskeletal mechanism that appears to drive receptor accumulation at the T cell–APC interface. This mechanism requires the APC to express B7 and ICAM-1 and is independent of TCR signaling. We suggest that this mechanism is a central part of costimulation, as it would effectively amplify any TCR-mediated signals.

Howard Hughes Medical Institute and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA.

*To whom correspondence should be addressed.

To study whether receptor accumulation at the T cell–APC interface could be actively driven by the T cell cytoskeleton, we monitored the general movement of the cortical actin cytoskeleton and linked receptors (*11*) using the classical technique of attaching large beads to the surfaces of antigen-specific T cells. We coated 4.5-μm beads with an antibody to the T cell surface antigen ICAM-1 (*12*). Cross-linking ICAM-1 by beads in this way is not expected to influence T cell function (*13*). As has been observed with fibroblasts (*11, 14*), we find that in migrating 5C.C7 transgenic T cells (*9, 15*) the beads translocate from the anterior to the posterior end of the cell (*16*). We then mixed bead-loaded 5C.C7 T cells with B cell lymphoma cells that express the appropriate MHC molecule (I-E^k) and have been pulsed with the moth cytochrome c peptide 88-103. After contact with the APC, the T cells rapidly become activated (*9, 17*) and the beads move from the posterior end of the T cell to the newly formed interface with the B cells (Fig. 1, movie 1) beginning 4 ± 1 min after the first rise in intracellular calcium (n = 13). This suggests that the T cell cortical actin cytoskeleton reorients toward the T cell–APC interface soon after the start of T cell activation. The ensuing cytoskeletal flow would allow receptors that are linked to the actin cytoskeleton to be transported to the newly formed T cell–APC interface.

To rule out ICAM-1–specific effects, we also attached beads to T cells in two other ways. First, we surface-biotinylated the 5C.C7 T cells with an amine-reactive form of biotin and used

Downloaded from http://science.sciencemag.org/ on November 19, 2019

Science

Molecular Basis of T Cell Inactivation by CTLA-4
Kyung-Mi Lee, Ellen Chuang, Matthew Griffin, Roli Khattri, David K. Hong, Weiguo Zhang, David Straus, Lawrence E. Samelson, Craig B. Thompson and Jeffrey A. Bluestone

Science **282** (5397), 2263-2266.
DOI: 10.1126/science.282.5397.2263

ARTICLE TOOLS http://science.sciencemag.org/content/282/5397/2263

REFERENCES This article cites 14 articles, 9 of which you can access for free
http://science.sciencemag.org/content/282/5397/2263#BIBL

PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 1998 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works.

Downloaded from http://science.sciencemag.org/ on November 19, 2019