Requirement for IL-13 Independently of IL-4 in Experimental Asthma

Gabriele Grünig, Martha Warnock, Adil E. Wakil, Rajeev Venkayya, Frank Brombacher, Donna M. Rennick, Dean Sheppard, Markus Mohrs, Debra D. Donaldson, Richard M. Locksley, David B. Corry*

The pathogenesis of asthma reflects, in part, the activity of T cell cytokines. Murine models support participation of interleukin-4 (IL-4) and the IL-4 receptor in asthma. Selective neutralization of IL-13, a cytokine related to IL-4 that also binds to the α chain of the IL-4 receptor, ameliorated the asthma phenotype, including airway hyperresponsiveness, eosinophil recruitment, and mucus overproduction. Administration of either IL-13 or IL-4 conferred an asthma-like phenotype to nonimmunized T cell–deficient mice by an IL-4 receptor α chain–dependent pathway. This pathway may underlie the genetic associations of asthma with both the human 5q31 locus and the IL-4 receptor.

Allergic asthma is a complex disorder characterized by local and systemic allergic inflammation and reversible airway obstruction. Asthma symptoms, especially shortness of breath, are primarily related to airway obstruction, and death is almost invariably due to asphyxiation (*1*). Increased airway responsiveness to provocative stimuli, termed airway hyperresponsiveness (AHR), and mucus hypersecretion by goblet cells are two of the principal causes of airway obstruction observed in asthma patients (*2*). Data from animal models consistently reveal a critical role for T_H2 (T helper 2) cells and potentially important roles for the cytokines IL-4 and IL-5 (*3–7*).

T_H2 cells selectively develop and expand in the presence of IL-4 (*8*). To separate direct effects of IL-4 from developmental effects on T_H2 cells in an asthma model, we compared the ability to establish the asthma phenotype in BALB/c mice deficient in either IL-4 or the IL-4 receptor α chain (IL-4Rα) (*9*). After intranasal challenge with the antigen ovalbumin (OVA), BALB/c mice developed a stereotyped asthma phenotype characterized by eosinophil influx of the airways, goblet cell metaplasia with mucus overproduction, and an increase in AHR as revealed by enhanced sensitivity to acetylcholine challenge (*6, 7*). IL-4 and IL-4Rα–deficient mice showed incremental attenuation of each of these asthma indices (Fig. 1, C through E) (*10*). Thus, in agreement with prior studies (*5–7*), IL-4 contributes to the asthma phenotype, but these data suggest an independently greater contribution by IL-4Rα.

IL-13 is a cytokine closely related to IL-4 that binds to IL-4Rα and is also expressed by T_H2 cells from asthma patients (*11*). To assess whether IL-13 might contribute to the asthma

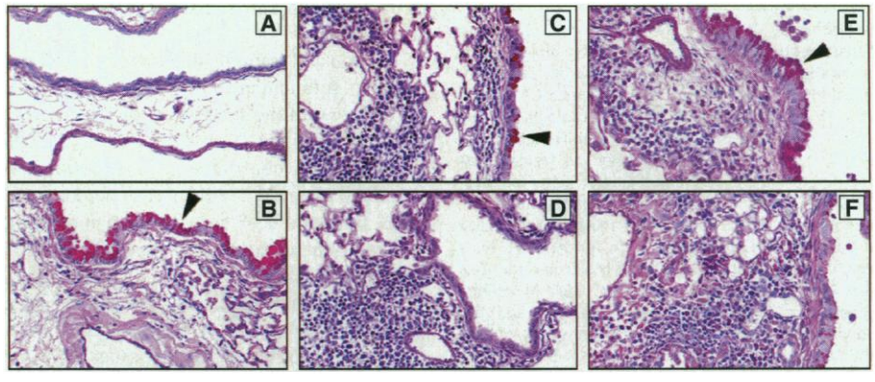

Fig. 1. PAS-stained histologic sections of murine lungs. Arrowheads point to goblet cells within the respiratory epithelium. (**A**) Wild-type mice were primed with OVA and challenged with PBS intranasally. (**B**) Wild-type mice were administered IL-13 intranasally. (**C**) IL-4–deficient and (**D**) IL-4Rα–deficient mice were primed with OVA and challenged with OVA intranasally. Wild-type mice were primed with OVA and challenged intranasally with (**E**) OVA and human Fc control protein or with (**F**) OVA and IL-13R-Fc. Note the marked reduction in goblet cells in (**D**) and (**F**).

assay (ELISA). Sample wells were coated with a 0.01% OVA solution in PBS, blocked with 10% fetal bovine serum (FBS) in PBS, and washed with 0.05% Tween-20 in PBS. Serum samples were diluted 1:10 and 1:100 with 10% FBS in PBS. After an overnight incubation, plates were washed with 0.05% Tween-20 in PBS, and biotin-conjugated anti-mouse IgE (Pharmingen, San Diego, CA) was added. After a wash, avidin peroxidase (0.0025 mg/ml) (Sigma) in 10% FBS/PBS was added, and plates were developed with 2.2′-azino-di(3-ethyl-benzthiazone sulfonate) (Kirkegaard and Perry, Gaithersburg, MD). Plates were read at 405 nm within 30 min. Reported optical density (OD) values are from serum samples that were diluted 1:10, because these values were proven to be below the saturation point of the assay by comparison of OD values of serum samples diluted 1:100 with 10% FBS/PBS.

19. P. D. Mehlhop *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1344 (1997).
20. T. Aikawa *et al.*, *Chest* **101**, 916 (1992).
21. L. Cohn *et al.*, *J. Exp. Med.* **186**, 1737 (1997).
22. DNA encoding a honeybee melittin leader [D. C. Tessier, D. Y. Thomas, H. E. Khouri, F. Laliberte, T. Vernet, *Gene* **2**, 177 (1991)] followed by a six-His tag was fused by an enterokinase cleavage site (His_6-EK) to the mature region of murine IL-13 at Gly^{21} and was constructed in the mammalian expression vector pHTop. His_6-EK murine IL-13 protein was produced from stably transfected CHO cells and purified through Ni—nitrilotriacetic acid chromatography to >97% purity as determined by SDS–polyacrylamide gel electrophoresis. The protein concentration was determined by absorption at 280 nm, and endotoxin contamination was <30 endotoxin units/mg as measured by the limulus amebocyte lysate assay (Cape Cod Associates, Woods Hole, MA). The median effective dose of His_6-EK murine IL-13 as determined by the Ba/F3.IL-13Rα1 proliferation assay was 1 ng/ml. Murine rIL-13 (5 μg in a total volume of 50 μl) was administered daily by intratracheal instillation to naïve mice that had been anesthetized with a mixture of ketamine and xylazine (45 and 8 mg/kg, respectively).
23. M. Goebeler *et al.*, *Immunology* **91**, 450 (1997).
24. A murine IgE-specific ELISA was used to quantitate total IgE concentrations in serum with complementary antibody pairs for mouse IgE (R35-72 and R35-92) (Pharmingen), according to the manufacturer's instructions. Duplicate samples (of a 1:10 dilution in 10% FBS in PBS) were examined from each animal. OD readings of the samples were converted to picograms per milliliter with the values that were obtained from standard curves generated with known concentrations of recombinant mouse IgE (5 to 2000 pg/ml).
25. C. L. Emson *et al.*, *J. Exp. Med.* **188**, 399 (1998).
26. L. R. Friedhoff and D. G. Marsh, *Int. Arch. Allergy Immunol.* **100**, 355 (1993).
27. To examine the effects of rIL-13 on the mucus cell content of the airway epithelium, lungs were excised and fixed in 10% formalin. They were then washed in 70% ethanol; dehydrated; embedded in glycol methacrylate; cut into 10-μM sections; mounted on slides; and stained with hematoxylin, eosin, and periodic acid–Schiff. Four sections were examined per animal, and four fields were scored per lung section. Sections were scored on a scale from 1 to 4, with 1 representing no mucus cell content.
28. J. Luyimbazi, X. Xu, M. Wills-Karp, unpublished data.
29. M. Humbert *et al.*, *J. Allergy Clin. Immunol.* **99**, 657 (1997); S. K. Huang, *J. Immunol.* **155**, 2688 (1995).
30. D. G. Marsh *et al.*, *Science* **264**, 1152 (1996).
31. L. J. Rosenwasser, *N. Engl. J. Med.* **337**, 1766 (1977).
32. G. K. Hershey *et al.*, *ibid.*, p. 1720.
33. J. F. Urban *et al.*, *Immunity* **8**, 255 (1998).
34. This work was supported by grants from NIH (HL58527) and the Center for Indoor Air Research to M.W.-K. We thank B. Annis and Z. Lu at Genetics Institute for murine rIL-13 and the Research Support Team at Genetics Institute for murine s13Rα2-Fc.

4 September 1998; accepted 6 November 1998

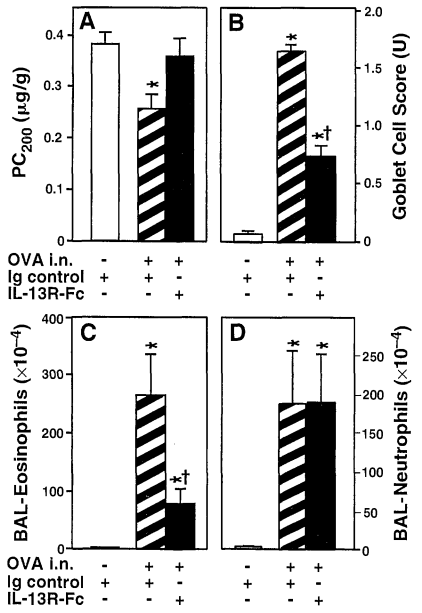

Fig. 2. Effect of neutralization of IL-13. Primed wild-type mice were administered intranasally human immunoglobulin (Ig control), Ig control and OVA, or IL-13R-Fc and OVA as indicated by (+). Data for (**A**) AHR, (**B**) goblet cell score, and numbers of (**C**) eosinophils and (**D**) neutrophils in the BAL fluid are plotted as means ±SEM. *$P < 0.05$ relative to PBS and Ig control–treated mice; †$P < 0.05$ relative to OVA and Ig control–treated mice. Data are representative of at least two comparable experiments with four to eight mice per group.

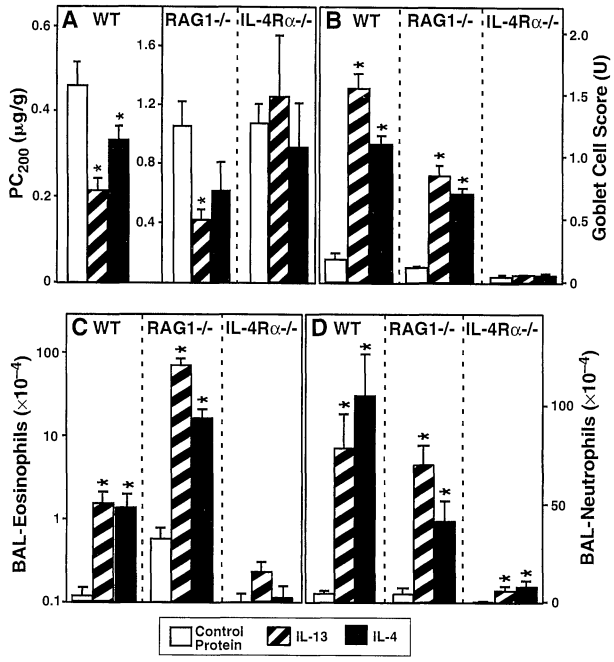

Fig. 3. Effect of recombinant IL-4 and IL-13. Wild-type (WT), RAG1-deficient (RAG1$^{-/-}$), and IL-4Rα–deficient (IL-4Rα$^{-/-}$) mice were administered IL-4, IL-13, or control protein intranasally. Data for (**A**) AHR, (**B**) goblet cell score, and numbers of (**C**) eosinophils and (**D**) neutrophils in the BAL fluid are plotted as means ±SEM. *$P < 0.05$ relative to mice receiving control protein. Data are representative of at least two comparable experiments with four to eight mice per group.

phenotype, we administered a soluble IL-13 recetor α_2-human Fc fusion protein (IL-13R-Fc) to BALB/c mice sensitized to OVA and compared them to mice that received control protein (*12*). IL-13R-Fc selectively binds to and neutralizes murine IL-13 but not IL-4 (*13*). This treatment significantly attenuated the asthma phenotype, although little effect was seen on neutrophil influx into bronchoalveloar lavage (BAL) (Figs. 1, E and F, and 2). Thus, IL-13, like IL-4 (*5*–*7*), can contribute to the acute effector phase of experimental asthma.

To assess the capacity of IL-13 and IL-4 to cause pathology independently of T and B cells, we administered each cytokine to nonimmunized BALB/c and RAG1 (recombinase activating gene 1)–deficient mice (*14*). Each cytokine alone induced the asthma phenotype (Figs. 1, A and B, and 3). In contrast, administration of either cytokine to IL-4Rα–deficient mice resulted in no significant changes in any asthma parameter, demonstrating that their effects were dependent on signals mediated by IL-4Rα. Further, adoptive transfer of OVA-specific T_H2 cells to IL-4Rα–deficient mice failed to elicit the asthma phenotype, whereas identical treatment of wild-type mice resulted in the full phenotype (*15, 16*). Thus, experimental asthma induced by antigen challenge, recombinant cytokine, or adoptive transfer of T_H2 cells, is mediated through a final pathway dependent on IL-4Rα.

Attenuated asthma phenotypes observed in IL-4–deficient mice may now be interpreted as representing the effects of residual IL-13 derived from IL-4–deficient T_H2 cells (*17*). Parallel observations in experimental intestinal helminth infections demonstrate roles for both IL-4 and IL-13 in mediating critical final effector pathways via IL-4Rα (*18*). It is possible that human asthma represents a spectrum of disease also linked by a shared receptor effector pathway. The common embryological origin of tissues from the gut and lung (*19*) would support the presence of stereotyped responses in these organs.

The relevance of our data to human asthma remains an important issue that cannot be entirely addressed, given the complexity of the disease and the inadequacies of any animal model. Linkage analysis has mapped susceptibility to asthma to a region on human chromosome 5q25-31, which includes the genes for both IL-4 and IL-13 (*20*), and to mutations in two domains of the α chain of the IL-4 receptor (*21*). A number of additional regions in the genome have been linked to asthma in human studies, suggesting a complex multifactorial phenotype (*22*). As we suggest, however, diverse forms of asthma might follow a final common effector pathway mediated through signals transduced by IL-4Rα, thus creating a unified target for potential intervention.

G. Grünig, R. Venkayya, D. Sheppard, D. B. Corry, Departments of Medicine and the Lung Biology Center at the San Francisco General Hospital, University of California San Francisco, San Francisco, CA 94143, USA. M. Warnock, Department of Pathology, University of California San Francisco, San Francisco, CA 94143, USA. A. E. Wakil, Department of Transplantation, California Pacific Medical Center, San Francisco, CA 94115, USA. F. Brombacher, Department of Immunology at the Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa. D. M. Rennick, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304, USA. M. Mohrs, Department of Microbiology/Immunology and the Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA 94143, USA. D. D. Donaldson, Genetics Institute, Cambridge, MA 02140, USA. R. M. Locksley, Departments of Medicine and Microbiology/Immunology, and the Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA 94143, USA.

*To whom correspondence should be addressed. E-mail: habari@itsa.ucsf.edu

References and Notes

1. N. A. Molfino, L. J. Nannini, A. N. Martelli, A. S. Slutsky, *N. Engl. J. Med.* **324**, 285 (1991).
2. R. A. Goldstein, W. E. Paul, D. D. Metcalfe, W. W. Busse, E. R. Reece, *Ann. Intern. Med.* **121**, 698 (1994).
3. S. H. Gavett, X. Chen, F. Finkelman, M. Wills-Karp, *Am. J. Respir. Cell Mol. Biol.* **10**, 587 (1994).
4. A. Watanabe *et al.*, *ibid.* **16**, 69 (1997); J. Garssen, F. P. Nijkamp, H. Van Der Vliet, H. Van Loveren, *Am. Rev. Respir. Dis.* **144**, 931 (1991); J. A. Rankin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7821 (1996); P. S. Foster, S. P. Hogan, A. J. Ramsay, K. I. Matthaei, I. G. Young, *J. Exp. Med.* **183**, 195 (1996); P. J. Mauser *et al.*, *Am. Rev. Respir. Dis.* **148**, 1623 (1993).
5. G. Brusselle, J. Kips, G. Joos, H. Bluethmann, R. Pauwels, *Am. J. Respir. Cell Mol. Biol.* **12**, 254 (1995).
6. D. B. Corry *et al.*, *J. Exp. Med.* **183**, 109 (1996).
7. D. B. Corry *et al.*, *Mol. Med.* **4**, 344 (1998).
8. A. K. Abbas, K. M. Murphy, A. Sher, *Nature* **383**, 787 (1996).
9. Wild-type, BALB/c IL-4$^{-/-}$ (*23*), and C57BL/6 RAG1$^{-/-}$ (*24*) mice were purchased from Jackson Laboratory. BALB/c IL-4Rα$^{-/-}$ were obtained at the Max-Planck-Institut für Immunbiologie. Mice were immunized and intranasally challenged with chicken egg OVA (*6, 7*). AHR was expressed as the provocative concentration of acetylcholine (in milligrams per kilogram) that increased baseline airway resistance 200% (PC_{200}) (*6, 7*). Periodic acid-Schiff (PAS)–stained lung sections were examined at 250× magnification. Twenty to 50 consecutive airways from saline- and OVA-

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