Translational Control of Interleukin 2 Messenger RNA as a Molecular Mechanism of T Cell Anergy

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Summary

T cell stimulation by triggering through the T cell receptor (TCR) in the absence of costimulatory signals or by calcium ionophore induces unresponsiveness in T cells to further stimulation, a phenomenon known as anergy. In freshly isolated T cells, calcium ionophore induces expression of interleukin (IL)-2 messenger (mRNA), but this mRNA is not translated and not loaded with ribosomes. In addition, while plate-bound anti-CD3 stimulation of resting T cells leads to IL-2 mRNA expression and IL-2 secretion, in cells pretreated with calcium ionophore before anti-CD3 stimulation, the IL-2 mRNA remains polysome unloaded and no IL-2 is produced. These observations show that IL-2 expression is controlled at the translational level, by differential ribosome loading. Furthermore, our data suggest that translational control of IL-2 mRNA may be a molecular mechanism by which anergy is attained.

Antigenic stimulation of resting T lymphocytes induces expression of more than 100 different genes (1), secretion of lymphokines, and proliferation in response to IL-2 both in an autocrine and paracrine fashion (2). Antigenic stimulation requires triggering of the TCR as well as additional costimulatory signals (3–5), and can be mimicked by a combination of calcium ionophore and phorbol esters (6). Triggering of the TCR in the absence of costimulatory signals leads to a state of unresponsiveness to further stimulation known as anergy (7). Anergy can also be induced by calcium ionophore alone, in freshly isolated T cells and some T cell clones (8, 9). The biochemical base for anergy remain controversial (10–14). However, it is known that anergy can be reversed with either exogenous IL-2 (15, 16) or a combination of PMA and ionophore (8, 9, 17).

To study the mechanism of anergy induction, we have stimulated human peripheral blood T cells with calcium ionophore. This signal, although sufficient to induce the expression of IL-2 mRNA (18–20), did not lead to secretion of IL-2. Indeed, here we describe that upon ionophore stimulation, IL-2 mRNA is not translated and not loaded with ribosomes. Upon subsequent stimulation of these cells with plate-bound anti-CD3, the IL-2 mRNA remains unloaded and no IL-2 is produced. Furthermore, the translational blockade, like anergy, can be reversed by a combination of phorbol esters and calcium ionophore. Taken together, our data suggest that translational control of IL-2 may represent a mechanism by which anergy is reached.

Materials and Methods

Cell Purification and Culture Conditions. Fresh T cells were isolated from buffy coats by Ficoll-Hypaque followed by Percoll gradient centrifugation (Pharmacia Biotech Inc., Piscataway, NJ). T cells were resuspended in DMEM supplemented with 10 mM Hepes, 2 mM glutamine, 5×10^{-5} M β-mercaptoethanol, and either 5% heat-inactivated autologous or FCS (Readysysteme, Zurzach, Switzerland). Cells were cultured for 12 h (unless otherwise indicated) at 37°C in a humidified incubator containing 5% CO_2 in the presence or absence of 10 μg/ml plate-bound anti-CD3 mAb (clone 66.1), calcium ionophore (1 μg/ml, A23187), or calcium ionophore in combination with 1 ng/ml PMA. IL-2 biological activity was measured in a bioassay using CTLL-2 cells (21), and IL-2 units were determined by comparison to a standard.

Preparation and Analysis of RNA. Total RNA was prepared using the guanidinium thiocyanate/acid phenol method and analyzed by Northern blotting as described (22, 23). Northern blots were subsequently hybridized with ^{32}P-labeled full-length IL-2 and HLA (24) probes.

Biosynthetic Labeling and Immunoprecipitations. Purified T cells were stimulated for 11 h with calcium ionophore, either alone or in combination with PMA. After several washes in methionine-free media, cells were pulsed for 15 min with [^{35}S]methionine in methionine-free media. Cell lysis and immunoprecipitations were done as described (25). Sequential immunoprecipitations of each lysate were performed in the following order with rabbit preimmune serum, rabbit anti–IL-2 antisera (catalogue no. 1300008; Boehringer Mannheim GmbH, Mannheim, Germany), and mouse anti-HLA class I mAb (W6/32). After immunoprecipitation and washes, samples were separated on a 10% SDS-PAGE under reducing conditions, and the gel was exposed to an x-ray film.

Polysome Gradients. Total cytoplasmic extracts were fractionated in 15–40% sucrose gradients (26–28). After fractionation and deproteinization, RNA samples were denatured with 4.6 M formaldehyde at 65°C, blotted onto nylon membranes using a slot blotter (Schleicher & Schuell, Inc., Keene, NH), and immobilized by UV fixation. Filters were hybridized with a human IL-2 cDNA probe. As controls, the 0.3-kb EcoRI-EcoRI fragment of mouse β_2-microglobulin (29), the 1.1-kb PstI-PstI fragment of mouse β-actin (30), and an oligonucleotide 5′-ACGGGAGGT-

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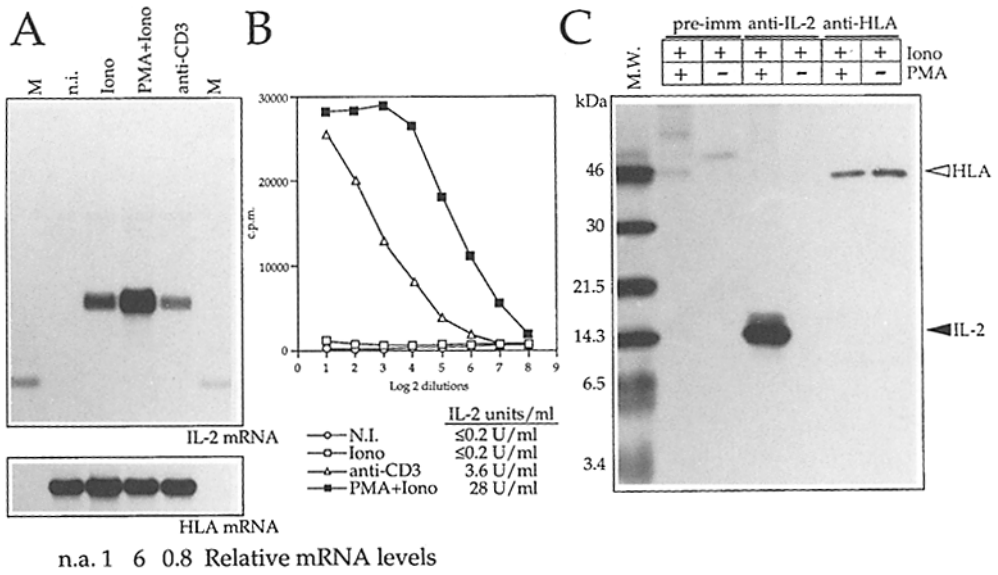


Figure 1. IL-2 expression after calcium ionophore stimulation of human peripheral blood T cells. (*A*) IL-2 mRNA levels from T cells noninduced (*n.i.*) or induced for 12 h with (*a*) plate-bound anti-CD3 mAb (*anti-CD3*); (*b*) calcium ionophore (*Iono*); or (*c*) calcium ionophore in combination with PMA (*PMA+Iono*). The specific signals for IL-2 and HLA class I mRNAs were quantified using a phosphoimager (Molecular Dynamics, Sunnyvale, CA). The relative IL-2 mRNA levels were obtained after dividing the signal obtained for IL-2 mRNA by the signal obtained for HLA class I mRNA and giving the value of 1.0 to ionophore-stimulated cells. (*B*) Secreted IL-2 into the supernatants of the cells induced as described in *A* and measured as biological activity in a bioassay using CTLL-2 cells. (*C*) Immunoprecipitation of IL-2 and HLA class I molecules after biosynthetic labeling of T cells stimulated with calcium ionophore, alone or in combination with PMA. As control, rabbit preimmune sera were used. The arrowheads indicate the migration of IL-2 and HLA class I proteins.

TTCTGTCCTCCC-3′ complementary to nucleotides 4205–4225 specific for human 28 S RNA were used. Hybridization and washing conditions were as described (23).

Results and Discussion

Calcium Ionophore Induces the Expression of IL-2 mRNA but not IL-2 Synthesis. It is well known that while resting T cells do not express detectable levels of IL-2 mRNA, stimulation with a combination of phorbol esters and calcium ionophore, or plate-bound anti-CD3 mAbs, induce the expression of this mRNA (Fig. 1 *A*), and secretion of IL-2 into the supernatant (Fig. 1 *B*). Furthermore, calcium ionophore by itself is able to induce expression of IL-2 mRNA (Fig. 1 *A*) (18–20). However, this signal alone does not lead to IL-2 secretion into the supernatant (≤0.2 U/ml; Fig. 1 *B*). The differences in IL-2 biological activity between supernatants from cells stimulated with calcium ionophore, alone or in combination with PMA (130–140-fold; Fig. 1 *B*), could not be explained by differences in IL-2 mRNA levels (6–8-fold; Fig 1 *A*), nor by differences in stimulation kinetics (data not shown). In addition, T cells stimulated with plate-coated anti-CD3 mAb secrete detectable IL-2 (3.6 U/ml; Fig. 1 *B*), although they express IL-2 mRNA levels comparable (0.8-fold) to ionophore-stimulated cells (Fig. 1 *A*). Taken together, these data clearly indicate that the lack of detection of secreted IL-2 is not caused by the differences in mRNA levels, and they suggest that IL-2 mRNA can be translationally regulated in ionophore-stimulated cells.

To determine if the lack of secreted IL-2 into the supernatant of calcium ionophore–stimulated cells was caused by a lack of IL-2 mRNA translation, newly synthesized IL-2 was immunoprecipitated from lysates of cells stimulated with calcium ionophore, alone or in combination with PMA, after metabolic labeling of the cells for 15 min with [^{35}S]methionine. As shown in Fig. 1 *C*, newly synthesized IL-2 was readily immunoprecipitated from lysates of cells stimulated with PMA and calcium ionophore. However, we failed to detect newly synthesized IL-2 in cells stimulated with calcium ionophore alone. Subsequent immunoprecipitation of the lysates with anti-HLA class I antibodies showed that the lack of translation of IL-2 is specific, since we could immunoprecipitate comparable amounts of newly synthesized class I molecules under both stimulation conditions (Fig. 1 *C*). Taken together, these data indicate that the lack of IL-2 biological activity in calcium ionophore–stimulated cells results from a specific lack of translation of IL-2 mRNA.

The Lack of IL-2 Translation Is Linked to a Differential Ribosome Loading of IL-2 mRNA. To dissect the defect of IL-2 mRNA translation in calcium ionophore–stimulated cells, we analyzed sucrose gradients from lysates of cells stimulated with calcium ionophore alone or in combination with

Figure 3. Ionophore stimulation of T cells induces unresponsiveness to subsequent stimulation with plate-bound anti-CD3 mAb. IL-2 biological activity from T cells stimulated with (*A*) plate-bound anti-CD3 mAb for 6 h after an overnight culture in either media (*n.i.=>@CD3*) or calcium ionophore (*I=>@CD3*). (*B*) Plate-bound anti-CD3 mAb, either alone (*@CD3*) or concommitantly with calcium ionophore (*Iono+@CD3*), for 24 h. (*C*) A combination of PMA and ionophore either for 24 h (*PMA+Iono*) or for 6 h after an overnight treatment with ionophore (*Iono=>PMA+Iono*). IL-2 was quantified in a CTLL-2 assay.

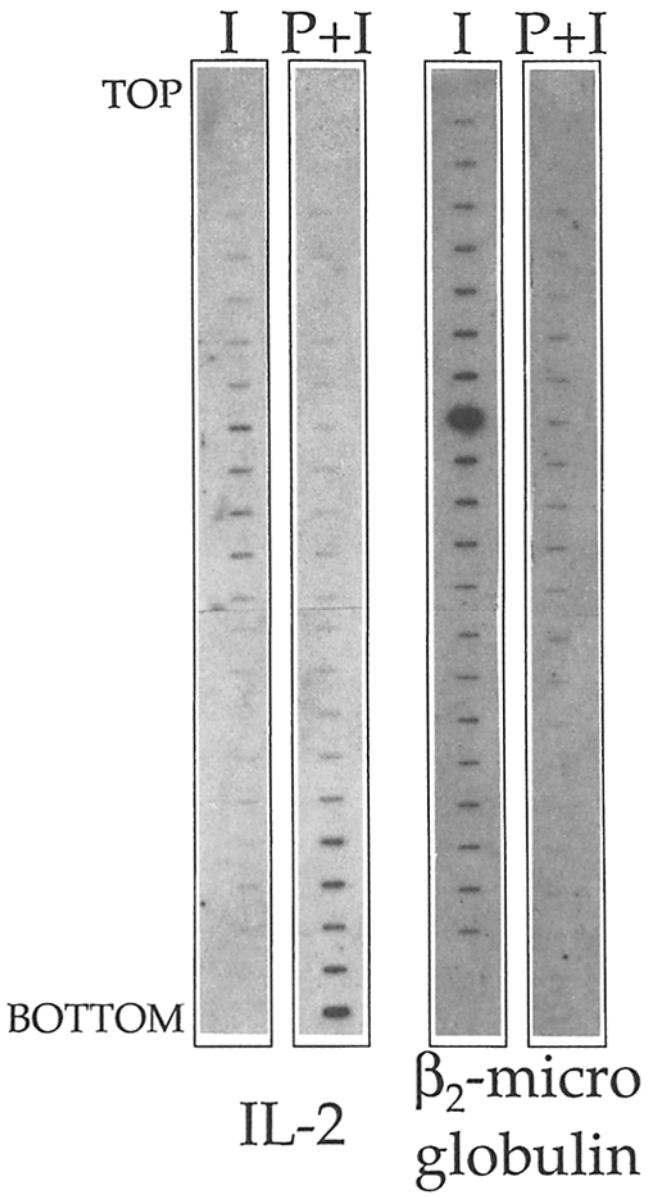
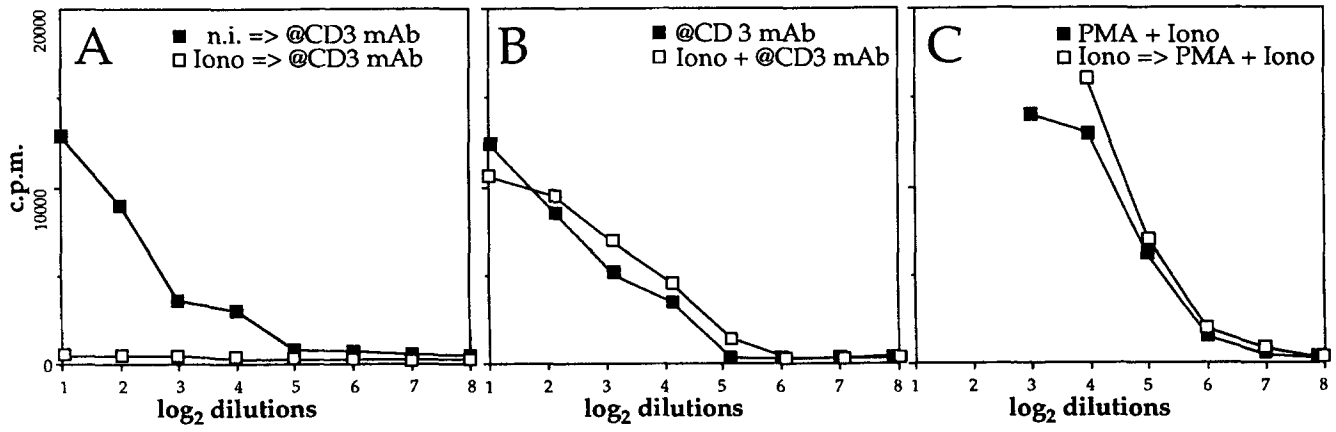


Figure 2. Polysome fractionation of cytoplasmic IL-2 mRNA. Distribution of IL-2 and β_2-microglobulin mRNA in polysome gradients from T cells stimulated with calcium ionophore, alone (*I*) or in combination with PMA (*PI*), for 12 h. Different cell numbers were used for each gradient to give similar signal strength for IL-2.

phorbol esters. These gradients allow us to investigate the distribution of a given mRNA species and to compare it to other samples, independent of the steady-state levels of such mRNA. Heavily translated mRNAs migrate to the bottom fractions of the gradients, while mRNAs having few or no ribosomes migrate to the top fractions of the gradients (26, 28). As expected, in cells stimulated with a combination of PMA and calcium ionophore, IL-2 mRNA was present in the bottom fractions of the gradient, corresponding to polysome loaded mRNA (Fig. 2). In gradients from calcium ionophore–stimulated cells, however, IL-2 mRNA was clearly shifted to the top fractions, corresponding to mRNAs containing very few or no ribosomes (Fig. 2). These data suggest that the defect in IL-2 mRNA translation in calcium ionophore–stimulated cells is linked to a differential ribosome loading of this mRNA. The following lines of evidence indicate that the effect is not caused by a general inhibition of translation: (*a*) HLA class I molecules were translated at similar rates in cells stimulated with ionophore, alone or in combination with PMA, as shown by immunoprecipitation (Fig. 1 *C*); (*b*) under both stimulation conditions, there was a fraction of β_2-microglobulin mRNA bound to polysomes (Fig. 2; similar results were obtained for HLA class I mRNA, data not shown); and (*c*) the distribution of β-actin mRNA was similar in cells pretreated or not with ionophore (see Fig. 4).

T Cell Anergy and Translational Repression Are Both Induced and Reversed by the Same Stimuli. T cell anergy is characterized by a lack of response to further stimulation (31). To determine whether pretreatment of freshly isolated T cells with ionophore prevented the response to a subsequent stimulation, T cells were stimulated with plate-bound anti-CD3 mAb under conditions leading to IL-2 secretion (32). T cells pretreated with calcium ionophore, unlike resting T cells, did not secrete detectable amounts of IL-2 after subsequent stimulation with plate-bound anti-CD3 mAb (Fig. 3 *A*). However, a combination of anti-CD3 mAb and calcium ionophore lead to secretion of IL-2 in amounts comparable to anti-CD3 mAb alone (Fig. 3 *B*). Taken together, these data indicate that pretreatment with ionophore induces an anergic phenotype, defined as the inability to produce IL-2 upon TCR complex stimulation (33–36). Furthermore, pretreatment with calcium ionophore not only



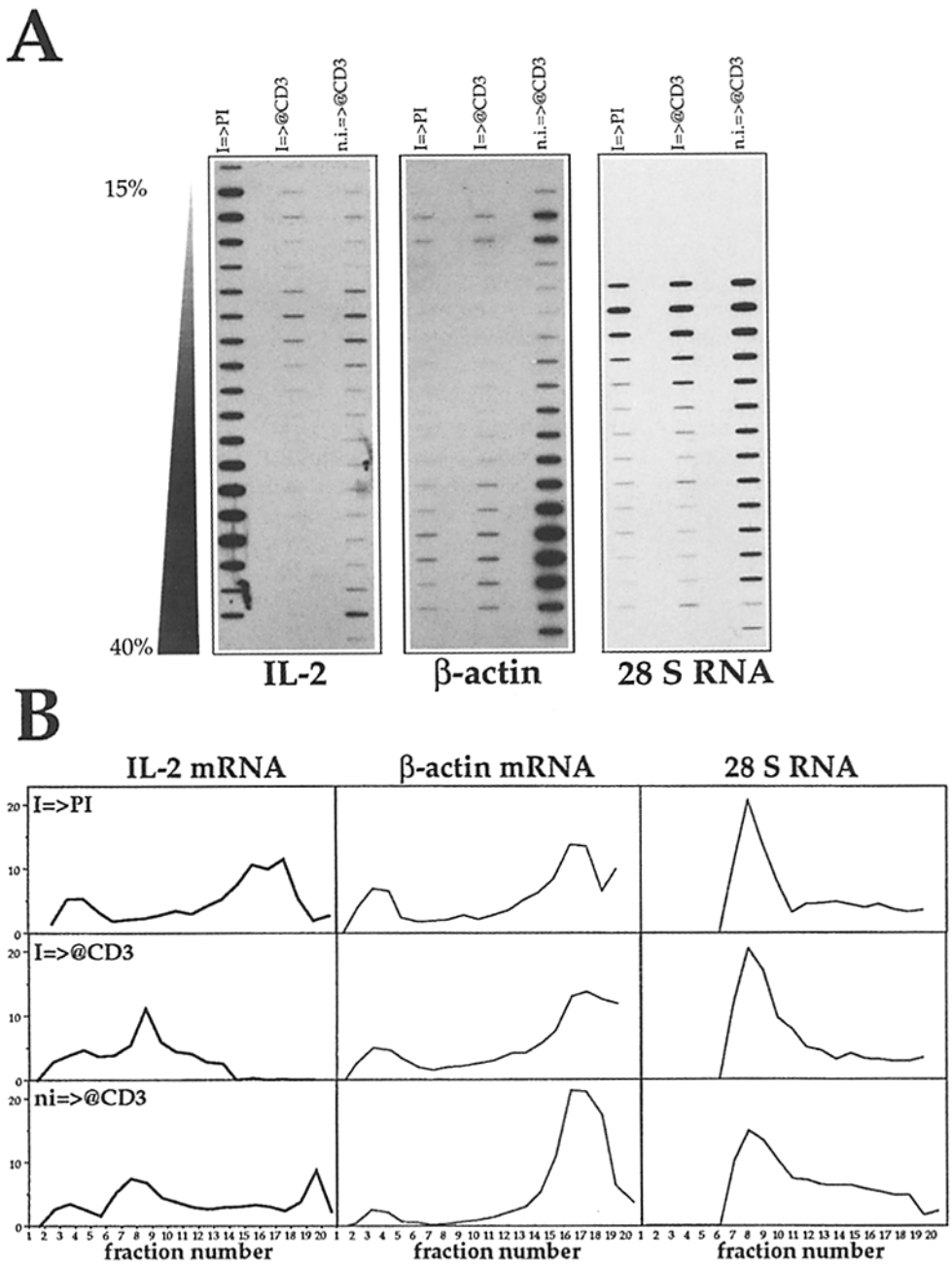


Figure 4. T cell unresponsiveness after ionophore stimulation is caused by translational regulation of IL-2 mRNA. Polysome gradients from cells stimulated overnight with ionophore followed by either PMA and ionophore (*I=>PI*) or anti-CD3 mAb (*I=>@CD3*). As a control, cells cultured in media overnight were stimulated with anti-CD3 mAb (*n.i. => @CD3*). (*A*) Filters from these gradients after sequential hybridization with IL-2, β-actin, and 28 S RNA-specific probes. (*B*) Distribution of IL-2, β-actin, and 28 S RNAs on the polysome gradients, after quantitation of the filters using a phosphoimager.

inhibited the response to anti-CD3 mAbs, but also the proliferative response in allogeneic stimulations (J.A. Garcia-Sanz, unpublished results).

To determine if the lack of IL-2 secretion in anti-CD3 mAb–stimulated T cells after pretreatment with calcium ionophore was caused by the translational inhibition of IL-2 mRNA, we analyzed the distribution of cytoplasmic mRNAs isolated from these cells in polysome gradients. Polysome gradients from cells stimulated with anti-CD3 mAb after overnight culture in media showed two peaks of IL-2 mRNA, one on the top fractions of the gradient, representing IL-2 mRNA with few or no ribosomes, and another at the bottom of the gradient, representing ribosome-loaded IL-2 mRNA (Fig. 4). In cells stimulated with anti-CD3 mAb after pretreatment with ionophore, however, we detected only one peak of IL-2 mRNA at the top of the gradient. The peak corresponding to ribosome-bound IL-2 mRNA was not present. As a control, we analyzed the distribution of β-actin mRNA under the same conditions. Despite the differences in β-actin mRNA steady-state levels detected in Fig. 4 *A*, which reflect differences in transcription rate upon stimulation (37, 38), its distribution pattern on the polysome gradients was similar (Fig. 4 *B*). As an additional control, we analyzed the distribution of 28 S

RNA in these gradients. The first fraction containing 28 S RNA corresponds to the fraction containing the 60 S ribosomal subunits, and the following two fractions correspond to mRNAs with one ribosome attached (39). As shown in Fig. 4, the fractions containing the peak of IL-2 mRNA after pretreatment with ionophore corresponded to the first three fractions containing 28 S RNA. This indicated that IL-2 mRNA was able to form a translation preinitiation complex, but only one ribosome was able to bind IL-2 mRNA.

To rule out the possibility that the lack of translation in anti-CD3–stimulated cells after pretreatment with calcium ionophore was caused by the toxicity of the ionophore, T cells were incubated overnight with ionophore and then stimulated for an additional 6 h with a combination of phorbol esters and ionophore. Under these conditions, anergy should be reversed and indeed, secreted IL-2 was detectable (Fig. 3 *C*). Furthermore, polysome gradients of these cells showed that beside a minor peak of free IL-2 mRNA (top of the gradient), most of IL-2 mRNA was present at the bottom of the gradient, corresponding to polysome bound mRNA. Again, the distribution of β-actin and 28 S RNAs was unchanged (Fig. 4). Thus, the translational repression of IL-2 was relieved by the same conditions that reversed T cell anergy.

Conclusions. The data presented here demonstrate that conditions which induce T cell anergy in freshly isolated human peripheral T cells are also able to induce a translational repression of IL-2 mRNA. This translational inhibition of IL-2 mRNA was maintained upon subsequent stimulation with plate-bound anti-CD3 mAb, but could be reversed by a combination of phorbol esters and ionophore, conditions that also reverse the anergic phenotype. The inhibition of IL-2 mRNA translation is demonstrated both by the lack of IL-2 synthesis upon biosynthetic labeling of the cells and by a shift in the distribution of IL-2 mRNA on polysome gradients towards the fractions containing a ribosomal subunit or a single ribosome. This shift of IL-2 mRNA upon ionophore treatment is consistent with a mechanism allowing the formation of the translation preinitiation complex and the loading of one ribosome, followed by a block of IL-2 mRNA translation. This block of IL-2 mRNA translation would be responsible for the anergic state of the cells. The reversal of both the anergic state and the specific block of IL-2 mRNA translation by phorbol esters in combination with ionophore is most likely caused by the higher levels of IL-2 mRNA expressed.

The phenomenon of T cell anergy has been described as the induction of a lack of response to further stimulation. At the molecular level, changes of phosphorylation patterns and lack of ZAP70 recruitment have been shown to play a role in the induction of anergy (10, 11). Furthermore, in some models, a lack of IL-2 transcription and a role for different DNA-binding proteins, including AP-1, NRE-α, PRE, as well as NFA-T, have been described (12–14). These data, together with functional data, suggest that anergy can be induced at different nonexclusive levels. Our data clearly indicates that translational regulation of IL-2 mRNA is a control point for T cell activation, which may be one of the mechanisms by which anergy is reached.

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References

1. Crabtree, G.R. 1989. Contingent genetic regulatory events in T lymphocyte activation. *Science (Wash. DC).* 243:355–361.
2. Weiss, A., J. Imboden, K. Hardy, B. Manger, C. Terhorst, and J. Stobo. 1986. The role of the t3/antigen receptor complex in T-cell activation. *Annu. Rev. Immunol.* 4:593–619.
3. Schwartz, R.H. 1992. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell.* 71:1065–1068.
4. Harding, F.A., J.G. McArthur, J.A. Gross, D.H. Raulet, and J.P. Allison. 1992. CD28-mediated signaling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature (Lond.).* 356:607–609.
5. Jenkins, M.K., P.S. Taylor, S.D. Norton, and K.V. Urdhal. 1991. CD28 delivers a costimulatory signal involved in antigen specific IL-2 production by human T cells. *J. Immunol.* 147:2461–2466.
6. Truneh, A., F. Albert, P. Golstein, and A.M. Schmitt-Verhulst. 1985. Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Na-*

ture (Lond.). 313:318–320.
7. Jenkins, M.K., D.M. Pardoll, J. Mizuguchi, T.M. Chused, and R.H. Schwartz. 1987. Molecular events in the induction of a nonresponsive state in interleukin-2 producing helper T-lymphocyte clones. *Proc. Natl. Acad. Sci. USA.* 84:5409–5413.
8. Jenkins, M.K., D.M. Pardoll, J. Mizuguchi, H. Quill, and R.H. Schwartz. 1987. T-cell unresponsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. *Immunol. Rev.* 95:113–135.
9. Essery, G., M. Feldmann, and J.R. Lamb. 1988. Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in cloned human T lymphocytes. *Immunology.* 64:413–417.
10. Sloan Lancaster, J., A.S. Shaw, J.B. Rothbard, and P.M. Allen. 1994. Partial T cell signaling: altered phospho-zeta and lack of zap70 recruitment in APL-induced T cell anergy [see comments]. *Cell.* 79:913–922.
11. Cho, E.A., M.P. Riley, A.L. Sillman, and H. Quill. 1993. Altered protein tyrosine phosphorylation in anergic Th1 cells. *J. Immunol.* 151:20–28.
12. Becker, J.C., T. Brabletz, T. Kirchner, C.T. Conrad, E.B. Brocker, and R.A. Reisfeld. 1995. Negative transcriptional regulation in anergic T cells. *Proc. Natl. Acad. Sci. USA.* 92: 2375–2378.
13. Wotton, D., J.A. Higgins, R.E. O'Hehir, J.R. Lamb, and R.A. Lake. 1995. Differential induction of the NF-AT complex during restimulation and the induction of T-cell anergy. *Hum. Immunol.* 42:95–102.
14. Kang, S.M., B. Beverly, A.C. Tran, K. Brorson, R.H. Schwartz, and M.J. Lenardo. 1992. Transactivation by AP-1 is a molecular target of T cell clonal anergy. *Science (Wash. DC).* 257: 1134–1138.
15. Beverly, B., S.M. Kang, M.J. Lenardo, and R.H. Schwartz. 1992. Reversal of in vitro T cell clonal anergy by IL-2 stimulation. *Int. Immunol.* 4:661–671.
16. DeSilva, D.R., K.B. Urdahl, and M.K. Jenkins. 1991. Clonal anergy is induced in vitro by T cell receptor occupancy in the absence of proliferation. *J. Immunol.* 147:3261–3277.
17. Schwartz, R.H. 1990. A cell culture model for T lymphocyte clonal anergy. *Science (Wash. DC).* 248:1349–1356.
18. Geller, R.L., G. Gromo, L. Inverardi, E. Ferrero, and F.H. Bach. 1987. Stepwise activation of T cells. Role of the calcium ionophore A23187. *J. Immunol.* 139:3930–3934.
19. Szamel, M., M. Kracht, B. Krebs, U. Hubner, and K. Resch. 1990. Activation signals in human lymphocytes: interleukin 2 synthesis and expression of high affinity interleukin 2 receptors require differential signalling for the activation of protein kinase C. *Cell. Immunol.* 126:117–128.
20. Lu, P., J.A. Garcia-Sanz, M.G. Lichtenheld, and E.R. Podack. 1992. Perforin expression in human peripheral blood mononuclear cells. Definition of an IL-2-independent pathway of perforin induction in $CD8^+$ T cells. *J. Immunol.* 148: 3354–3360.
21. Gillis, S., M.M. Ferm, W. Ou, and K.A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* 120:2027–2032.
22. Müllner, E.W., and J.A. Garcia-Sanz. 1996. Preparation of RNA. *In* Manual of Immunological Methods. I. Lefkovits, editor. Academic Press, London. In press.
23. Müllner, E.W., and J.A. Garcia-Sanz. 1996. Analysis of RNA expression by Northern blotting. *In* Immunology Methods Manual. I. Lefkovits, editor. Academic Press, London. In press.
24. Ploegh, H.L., H.T. Orr, and J.L. Strominger. 1980. Molecular cloning of a human histocompatibility antigen cDNA fragment. *Proc. Natl. Acad. Sci. USA.* 77:6081–6085.
25. Cosson, P., and J.S. Bonifacino. 1992. Role of transmembrane domain interactions in the assembly of class II molecules. *Science (Wash. DC).* 258:659–662.
26. Müllner, E.W., B. Neupert, and L.C. Kuhn. 1989. A specific mRNA binding factor regulates the iron-dependent stability of cytoplasmic transferrin receptor mRNA. *Cell.* 58:373–382.
27. Garcia-Sanz, J.A., and E.R. Podack. 1993. Regulation of perforin gene expression in a T cell hybrid with inducible cytolytic activity. *Eur. J. Immunol.* 23:1877–1883.
28. Müllner, E.W., and J.A. Garcia-Sanz. 1996. Polysome gradients. *In* Manual of Immunological Methods. I. Lefkovits, editor. Academic Press, London. In press.
29. Daniel, F., D. Morello, O. Le Bail, P. Chambon, Y. Cayre, and P. Kourlisky. 1983. Structure and expression of the mouse β2-microglobulin gene isolated from somatic and non-expressing teratocarcinoma cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1061–1065.
30. Alonso, S., A. Minty, Y. Bourlet, and M. Buckingham. 1986. Comparison of three actin-coding sequences in the mouse: evolutionary relationships between the actin genes of warm-blooded vertebrates. *J. Mol. Evol.* 23:11–22.
31. Quill, H., and R.H. Schwartz. 1987. Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. *J. Immunol.* 138:3704–3712.
32. Tax, W.J., H.W. Willems, P.P.M. Reekers, P.J.A. Capel, and R.A.P. Koene. 1983. Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. *Nature (Lond.).* 304:445–447.
33. Mueller, D.L., M.K. Jenkins, and R.H. Schwartz. 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol.* 7:445–480.
34. Schwartz, R.H., D.L. Mueller, M.K. Jenkins, and H. Quill. 1989. T-cell clonal anergy. *Cold Spring Harb. Symp. Quant. Biol.* 54:605–610.
35. Li, W., C.D. Whaley, A. Mondino, and D. Mueller. 1996. Blocked signal transduction to the ERK and JNK protein kinases in anergic $CD4^+$ T cells. *Science (Wash. DC).* 271: 1272–1276.
36. Fields, P.E., T.F. Gajevski, and F.W. Fitch. 1996. Blocked Ras activation in anergic $CD4^+$ T cells. *Science (Wash. DC).* 271:1276–1278.
37. McCairns, E., D. Fahey, G.E. Muscat, M. Murray, and P.B. Rowe. 1984. Changes in levels of actin and tubulin mRNAs upon the lectin activation of lymphocytes. *Mol. Cell. Biol.* 4: 1754–1760.
38. Muscat, G.E., A. Caputo, E. McCairns, and P.B. Rowe. 1985. Growth-related changes in specific mRNAs upon lectin activation of human lymphocytes. *DNA.* 4:377–384.
39. Savant, B.S., and D.W. Cleveland. 1992. Evidence for instability of mRNAs containing AUUUA motifs mediated through translation-dependent assembly of a >20S degradation complex. *Genes Dev.* 6:1927–1939.