Exp. Zool. **249**, 271 (1989).
5. V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951).
6. Vigorous and jerky movements of both legs were seen in a number of embryos on day 7 after paramedian section of the floor plate at stage 12 to 13. Such movements are due to the spontaneous firing of the motor neurons in the spinal cord. Histological sections of two of these embryos confirmed the absence of the floor plate and ventral horn motor neurons on the operated side.
7. M. Wagner, C. Thaller, T. Jessell, G. Eichele, *Nature* **345**, 819 (1990).
8. C. Tickle, B. Alberts, L. Wolpert, J. Lee, *ibid.* **296**, 564 (1982); D. Summerbell, *J. Embryol. Exp. Morphol.* **78**, 269 (1983); G. Eichele, C. Tickle, B. M. Alberts, *J. Cell Biol.* **101**, 1913 (1985).
9. M. Maden, D. E. Ong, D. Summerbell, F. Chytil, *Development (Suppl.)* **109** (1989); M. Maden, D. E. Ong, D. Summerbell, F. Chytil, E. A. Hirst, *Dev. Biol.* **135**, 124 (1989); M. J. Vaessen *et al.*, *Differentiation* **40**, 99 (1989); A. V. Perez-Castro, L. E. Toth-Rogler, L. N. Wei, M. C. Nguyen-Huu, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8813 (1989); M. Maden, D. E. Ong, F. Chytil, *Development* **109**, 75 (1990); M. Momoi *et al.*, *Dev. Brain Res.* **54**, 161 (1990).
10. Eggs of Japanese quail embryos were incubated at 37.5°C and in a humidity of about 90%. On the second day of incubation, a window was made in the egg shell and India ink was injected beneath the embryo for visualization. Hensen's node was cut with a fine stainless steel needle at stage 10 and removed with a micropipette. Median and paramedian incisions of the floor plate were also made with a fine needle at stage 12 to 13. All microsurgery was performed under aseptic conditions. After surgery the egg shell window was covered with Parafilm and embryos were reincubated and killed on embryonic day 5.
11. The results presented here were based on observations of a majority of these embryos. Some embryos turned out to be unsuitable for the study because of incomplete operations, retarded growth, and gross malformations.
12. For histology, embryos were fixed in Bouin's solution, embedded in paraffin, serially sectioned at 15 μm thickness, and stained with hematoxylin and eosin. For Golgi staining, embryos were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and processed for routine rapid Golgi impregnation.
13. We thank D. Bockman and M. Mulroy for a critical reading of the manuscript, and C. Motes for typing the manuscript. Supported by NIH grants HD 17800 and HD 18280.

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Regulation of Interleukin-2 Gene Enhancer Activity by the T Cell Accessory Molecule CD28

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The mechanism by which cell surface molecules regulate T cell production of lymphokines is poorly understood. Production of interleukin-2 (IL-2) can be regulated by signal transduction pathways distinct from those induced by the T cell antigen receptor. Stimulation of CD28, a molecule expressed on most human T cells, induced the formation of a protein complex that bound to a site on the *IL-2* gene distinct from previously described binding sites and increased *IL-2* enhancer activity fivefold. The CD28-responsive complex bound to the *IL-2* gene between −164 and −154 base pairs from the transcription start site. The sequence of this element is similar to regions conserved in the 5′ flanking regions of several other lymphokine genes.

T LYMPHOCYTES HAVE AN IMPORTANT role in most immunological responses. To exert their effector functions, relatively quiescent T cells are activated during a complex interaction with antigen-presenting cells. The specificity of a T cell response to antigen is mediated by the T cell antigen receptor (TCR) complex, which contains a ligand binding subunit, the immunoglobulin-like heterodimer Ti, noncovalently associated with at least five invariant proteins (*1*). Interaction of the TCR with antigen in association with the major histocompatibility complex (MHC) or antibodies to the TCR results in the initiation of signal transduction events and cellular activation. Perturbation of the TCR activates a tyrosine kinase and phospholipase C, which results in tyrosine phosphorylation and phosphatidylinositol 4,5-biphosphate hydrolysis, respectively (*2*). These events are thought to lead to a variety of cellular responses, one of which is lymphokine production.

However, stimulation of the TCR alone is insufficient to activate most T cells. In addition to the TCR, a number of other T cell surface molecules appear to contribute to T cell activation and proliferation (*3*). One of these, CD28, is a 44-kD glycoprotein that is expressed as a homodimer on the majority of human T cells (*4*). In T cells activated via their TCR or by pharmacological agents that mimic TCR-induced phospholipase C activation, the addition of antibody to CD28 (anti-CD28) causes a marked increase in the mRNA and secretion of several T cell lymphokines, including interleukin-2 (IL-2), tumor necrosis factor–α, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and lymphotoxin (*5*). The nature of the CD28-generated transmembrane signal in response anti-CD28 treatment is unclear, but is probably distinct from those of the T cell antigen receptor (*6*).

The induction of *IL-2* gene transcription during T cell activation is mediated primarily by a region extending 326 bp upstream of the transcription start site (*7*). This region contains binding sites for several nuclear proteins (*8, 9*) (Fig. 1). To investigate whether CD28 stimulation affects *IL-2* gene transcription, we tested constructs that contain this region of the *IL-2* gene linked to the reporter genes luciferase (Luc) or chloramphenicol acetyltransferase (CAT) in transient expression studies. In contrast to an earlier report that showed an increased stability of *IL-2* message but did not detect an effect on *IL-2* transcription (*10*), our studies indicate that CD28 stimulation caused a significant increase in *IL-2* enhancer activity. We have identified a previously uncharacterized element within the *IL-2* enhancer that is a target sequence for a CD28-regulated nuclear binding complex.

The plasmid pIL-2–Luc, a recombinant reporter plasmid that contains sequences from −326 to +46 of the human *IL-2* gene directing transcription of the firefly luciferase gene (*8*), was transfected into the Jurkat T cell line. The transfected cells were then treated for 8 hours with combinations of ionomycin and phorbol 12-myristate 13-acetate (PMA), agents that mimic the effect of TCR-induced phospholipase C activation by increasing the cytoplasmic Ca^{2+} concentration and activating protein kinase C, respectively. The transfected cells were stimulated in the presence or absence of monoclonal antibodies (MAbs) to either CD28 or class 1 histocompatibility antigens

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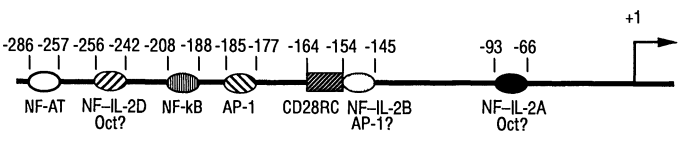

Fig. 1. The *IL-2* enhancer with known protein binding sites. The numbers at the top of the figure represent the position in base pairs relative to the initiation of transcription site.

Table 1. Anti-CD28 induces increased *IL-2* enhancer activity in Jurkat cells treated with PMA and ionomycin. The induced activity of the indicated plasmid constructs were assayed in transiently transfected Jurkat cells (*8*). After 40 hours, the cells were treated with the indicated combinations of PMA (P; 50 ng/ml), ionomycin (I; 1 μM), MAb W6/32 (anti-HLA: 1:10,000 dilution of ascites), or MAb 9.3 (anti-CD28; 1:10,000 dilution of ascites). Results are expressed as the mean fold induction of treated cells over that of untreated cells. The fold increase ± SE by anti-CD28 was calculated by comparing Jurkat cells transfected with the indicated plasmid constructs treated with PMA and ionomycin to those treated with PMA, ionomycin, and 1:10,000 dilution of MAb 9.3. The number (*n*) of independent experiments is indicated for each plasmid construct. ND, not determined.

Constructs	P + I	P + I + anti-HLA	P + I + anti-CD28	Fold increase
IL-2(−326 to +46)–Luc (*n* = 3)	37	39	180	4.9 ± 0.8
IL-2(−326 to −51)–CAT (*n* = 3)	10	11	51	4.9 ± 0.8
NFAT(4×)–CAT (*n* = 4)	74	ND	73	0.99 ± 0.1
NFIL2A(4×)–CAT (*n* = 4)	25	ND	25	1.0 ± 0.1
IL-2(−326 to −165)$_2$–CAT (*n* = 3)	9.0	9.1	17	1.8 ± 0.1
IL-2(−164 to −51)$_2$–CAT (*n* = 3)	13	14	96	7.5 ± 0.4

(HLA). Treatment of the transfected Jurkat with PMA or ionomycin alone had no detectable effect on enhancer activity, but the combination of PMA and ionomycin increased luciferase activity (*11*) (Table 1). Anti-CD28 treatment alone or anti-CD28 and PMA had little or no detectable effect on basal luciferase activity, but the addition of anti-CD28 in the presence of PMA and ionomycin induced five times more luciferase activity than that induced by PMA and ionomycin alone (Table 1). This induced activity was a specific response to anti-CD28 because MAb to HLA had little effect. We observed a similar degree of synergy by anti-CD28 treatment in Jurkat treated with MAbs to TCR and PMA (*12*). These results were consistent with the concentration of secreted IL-2 protein detected in the supernatants of the treated cell cultures (*12*). The time course of the CD28-induced increase in luciferase activity paralleled that induced by PMA and ionomycin (Fig. 2).

To address whether anti-CD28 might influence the post-transcriptional regulation of the transcribed *IL-2* sequences contained in the pIL-2–Luc plasmid, we tested the effect of CD28 stimulation on the IL-2–CAT plasmid (*8*). This construct contains from −326 to −51 of the *IL-2* gene linked to the γ-fibrinogen promoter (−54 to +36) and the CAT gene and therefore contains no transcribed *IL-2* sequences (*13*). In the presence of PMA and ionomycin, anti-CD28 treatment again increased reporter gene activity by approximately fivefold (Table 1). Thus, the −326 to −51 region of the *IL-2* gene contains a CD28-response element or elements.

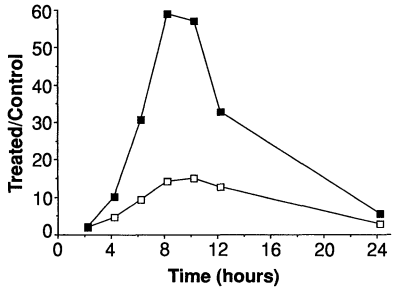

Fig. 2. Time course of the anti-CD28–induced luciferase activity. Jurkat cells were transfected with the pIL-2–Luc plasmid, which contains from −326 to +47 of the *IL-2* gene (*8*). After 40 hours the cells were treated with either PMA (50 ng/ml) and ionomycin (1 μM) (□) or PMA, ionomycin, and anti-CD28 (1:1000 dilution of ascites) (■). At the indicated times, portions of the cells were harvested and the luciferase activity was determined (*25*). The results shown are representative of three independent experiments.

To localize the CD28-response element, we tested constructs containing subfragments of the *IL-2* enhancer region (Table 1). Anti-CD28 treatment had no detectable effect on the activity of multimers of NF-AT or NF–IL-2A elements linked to the γ-fibrinogen promoter and CAT (*8*). A construct that contained two copies of the −326 to −165 region of the *IL-2* enhancer had a small but reproducible increase in response to anti-CD28 treatment in the presence of PMA and ionomycin. In contrast, a construct with two copies of the −165 to −51 fragment, a region that contains binding sequences for NF–IL-2A and NF–IL-2B responded with a 7.5-fold increase in activity after anti-CD28 treatment (Table 1).

To further localize the CD28 response elements, we tested a number of deletion mutants of the *IL-2* enhancer, focusing on the −164 to −51 region. This analysis suggested that the CD28-response element was either at or very close to the AP-1–like NF–IL-2B element (*8, 14, 15*). But results from deletions of this binding site were difficult to interpret, as consistent with another report (*8*), disruption of this region caused an 80% to 90% decrease in the PMA- and ionomycin-induced activity (*15*).

We performed gel mobility shift analysis to determine whether anti-CD28 treatment affected the protein-DNA complexes formed within this region (*16*). Nuclear extracts from untreated Jurkat cells contained little binding activity to a double-stranded oligonucleotide that corresponded to −164 to −140 of the human *IL-2* gene, which contains the NF–IL-2B binding site (*8, 13*) (Fig. 3A). Treatment with PMA and ionomycin induced the binding of a single complex (NF–IL-2B, lanes 4 and 5). Nuclear extracts from Jurkat treated with PMA, ionomycin, and anti-CD28 formed two distinct complexes, one with identical mobility to that induced in the PMA and ionomycin-treated cells and another complex of more rapid mobility (CD28RC) that was specific to the anti-CD28 treated T cells (lanes 6 and 7). Neither PMA alone, anti-CD28 alone, nor PMA and anti-CD28 treatment induced detectable binding (*15*). The CD28RC binding activity was not detected when PMA- and ionomycin-treated cells were costimulated with anti-TCR antibodies (*15*), suggesting the specificity of the anti-CD28 induction of CD28RC.

Competition with 10- or a 100-fold molar excess of unlabeled double-stranded IL-2 (−164 to −140) oligonucleotide specifically inhibited the binding of both complexes to the labeled (−164 to −140) probe; competition with a 100-fold molar excess of the NF-AT binding site (*17*) had no detectable

Table 2. Conserved sequences in the 5′ flanking region of cytokine genes. The sequences have been compared to the human *IL-2* sequence. Nonconserved bases are shown in lowercase letters. The numbering is relative to the transcription start site. The h refers to the human gene, m for the murine gene.

Gene	Region	Sequence
hIL-2	−162 to −152	AAGAAATTCCA
mIL-2	−164 to −154	AAGAAATTCCA
hGM-CSF	−96 to −86	AgGAgATTCCA
mGM-CSF	−108 to −98	AgGAgATTCCA
hIL-3	−119 to −109	tgGAggTTCCA
mIL-3	−115 to −105	tgGAggTTCCA
hG-CSF	−188 to −178	cAGAgATTCCA
mG-CSF	−192 to −182	cAGAgATTCCc
hγ-IFN	−163 to −153	AgGAAAcTCtA
mγ-IFN	−170 to −160	AgGAAAcTCtA

effect (Fig. 3B). A double-stranded oligonucleotide that contained a consensus AP-1 binding site (*17*) competed with the IL-2-(−164 to −140) oligonucleotide for binding to the NF–IL-2B complex, but not with the CD28-induced complex. This result indicates that NF–IL-2B and CD28RC have distinct DNA binding specificities.

To more precisely define the recognition sequences of the two complexes, we performed competition analysis with oligonucleotides that contained contiguous four base substitutions dispersed throughout the −164 to −140 region (Fig. 3C). Oligonucleotides M1 and M2, which contain mutations in −162 to −159 and −159 to −156, did not compete with the wild-type sequence for binding to the CD28-induced complex, indicating that this sequence is critical for the recognition and binding of the CD28RC. In contrast, competition for the PMA- and ionomycin-induced NF–IL-2B complex was marginally affected by mutations between −162 and −155 (M1 and M2), and oligonucleotides containing mutations between −153 and −146 competed more poorly, with the weakest competitor being the M4 oligonucleotide, which contains mutations within the AP-1–like motif (−149 to −146). Thus, the CD28-responsive complex binds 5′ to the AP-1–like NF–IL-2B element within −164 to −154 (AAAGAAATTCC). This sequence is similar to 5′ flanking regions of several lymphokine genes including GM-CSF (*18*), interleukin-3 (IL-3) (*19*), granulocyte colony stimulating factor (G-CSF (*20*), and IFN-γ (*21*) (Table 2). There is also an inverted copy of this sequence just 5′ of the *IL-2* NF-AT binding site (−295 to −284), which may explain why the IL-2(−326 to $-165)_2$-CAT construct was weakly responsive to anti-CD28 treatment (Table 1).

To determine whether the CD28RC region was required for the anti-CD28 induced activity, we used site-directed mutagenesis (*22*) to introduce the M2 mutation that did not bind CD28RC (Fig. 3C) into the pIL-2–Luc construct. This mutation resulted in the loss of more than 90% of the CD28-induced luciferase activity (Fig. 4). This demonstrates that this region of the *IL-2* enhancer is required for responsiveness to CD28 stimulation.

These studies indicate that, in addition to the effect of CD28 stimulation on IL-2 mRNA stability (*10*), signal transduction through CD28 can greatly increase IL-2 enhancer activity. The effect of CD28 on *IL-2* gene transcription may not have been previously detected because of the low sensitivity of run-on transcription assays for weakly expressed genes. The region of the *IL-2* enhancer that responds to CD28 stimulation is absolutely conserved between the human and murine *IL-2* genes and similar sequences are present in the 5′ flanking regions of several other lymphokine genes. This suggests an important function for this signal transduction pathway and DNA binding complex. Studies on murine $CD4^+$ T cell clones indicate that antigen-specific T cell activation requires the activity of a signal transduction pathway in addition to those generated via the TCR complex (*23*). Incubation of T cell clones with either peptide antigen plus purified MHC in planar membranes or antigen presented by chemically fixed antigen-presenting cells fails to induce T cell activation or proliferation and instead causes a prolonged nonresponsive state. Analysis of these T cells indicates that the failure of these cells to respond and proliferate is primarily the result of insufficient lymphokine secretion, particularly that of IL-2 (*24*). The large increases in both IL-2 and other T cell lymphokine expression induced by CD28 stimulation suggests that this cell surface molecule and the CD28RC complex that it induces may be part of this costimulatory pathway.

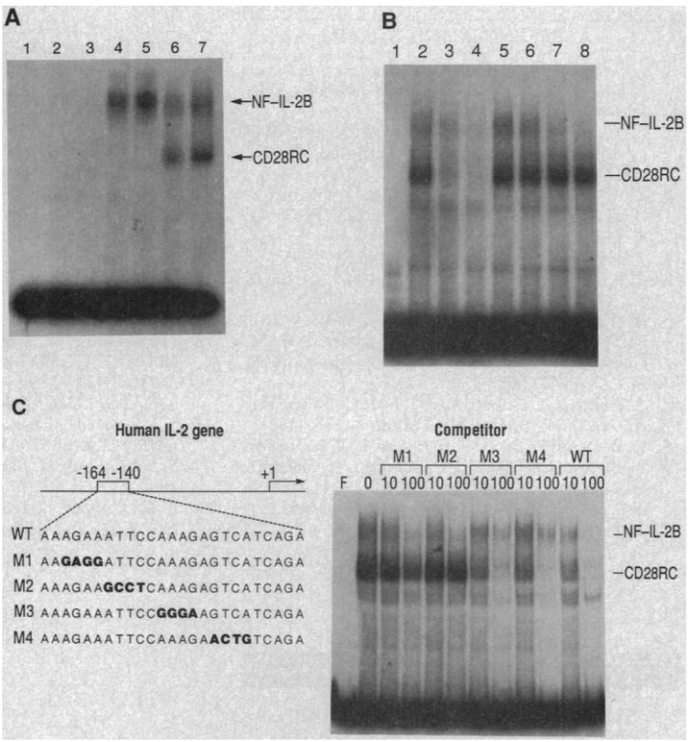

Fig. 3. Stimulation of CD28 induces the formation of a novel DNA-protein complex. Nuclear extracts were prepared from Jurkat T cells essentially as described by Dignam *et al.* (*26*). DNA-protein binding assays were done by incubating nuclear extracts for 20 min at 25°C in 15 μl of reaction buffer containing 15 mM Hepes (pH 7.8), 70 mM NaCl, 0.5 mM dithiothreitol, 2% glycerol, 3 μg of poly(dI-dC), 100 ng of pUC9 DNA, and 1 ng of ^{32}P-labeled IL-2 probe (−164 to −140). Complexes were separated on 4.5% polyacrylamide gels with 22.5 mM tris-borate (pH 8.0) and 1 mM EDTA buffer. (**A**) Lane 1, probe alone; lanes 2 and 3, 2 or 4 μg, respectively, of extract from untreated Jurkat; lanes 4 and 5, 2 or 4 μg, respectively, of extract from Jurkat cells treated with 1 μM ionomycin and PMA (50 ng/ml) for 2 hours; lanes 6 and 7, 2 or 4 μg, respectively, of extract from Jurkat cells treated with 1 μM ionomycin PMA (50 ng/ml), and 1:2000 dilution of MAb 9.3 ascites for 2 hours. Free probe and protein-DNA complexes were detected by autoradiography. (**B**) Nuclear extract from Jurkat T cells treated for 2 hours with PMA, ionomycin, and MAb 9.3 were incubated with ^{32}P-labeled IL-2 probe (−164 to −140) in the presence of 10- or 100-fold molar excess of (lanes 3 and 4) unlabeled IL-2 (−164 to −140), (lanes 5 and 6) NF-AT, or (lanes 7 and 8) AP-1 competitor oligonucleotides (*17*). (**C**) Mutational analysis of the CD28RC and NF–IL-2B binding sites. Nuclear extract from Jurkat T cells treated for 2 hours with PMA, ionomycin, and MAb 9.3 were incubated with ^{32}P-labeled IL-2 probe (−164 to −140) in the presence of 10- or 100-fold molar excess of the indicated unlabeled oligonucleotide.

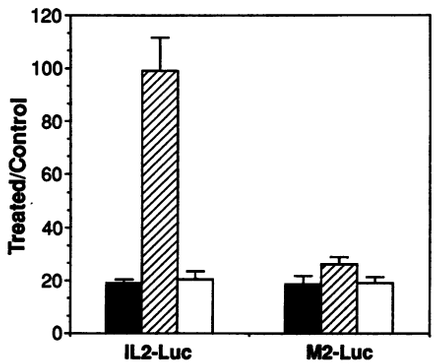

Fig. 4. Mutation of the CD28RC region of the *IL-2* enhancer renders the enhancer unresponsive to anti-CD28 treatment. Site-directed mutagenesis was used to introduce the M2 (Fig. 3C) mutation into the pIL2-Luc plasmid. The resulting construct, pM2-Luc was transfected into Jurkat cells as described for Table 1. After 40 hours the cells were treated with either PMA (50 ng/ml) and ionomycin (1 μM) (black bars), PMA, ionomycin, and MAb 9.3 (anti-CD28, 1:10,000 dilution of ascites) (striped bars), or PMA, ionomycin, and MAb W6/32 (anti-HLA, 1:10,000 dilution of ascites) (white bars). After 8 hours of treatment, the cells were harvested and the luciferase activity was measured. The results reflect cumulative data from three experiments. Error bars indicate standard error of the mean.

REFERENCES AND NOTES

1. J. P. Allison and L. L. Lanier, *Annu. Rev. Immunol.* **5**, 503 (1987); A. Weiss and J. B. Imboden, *Adv. Immunol.* **41**, 1 (1987).
2. L. E. Samelson and M. D. Patel, *Cell* **46**, 1083 (1986); J. B. Imboden and J. D. Stobo, *J. Exp. Med.* **161**, 446 (1985).
3. T. A. Springer, M. L. Dustin, T. K. Kishimoto, S. D. Marlin, *Annu. Rev. Immunol.* **5**, 223 (1987); D. R. Littman, *ibid.*, p. 561.
4. T. Hara *et al.*, *J. Exp. Med.* **161**, 1513 (1985).
5. C. B. Thompson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1333 (1989).
6. A. Weiss, B. Manger, J. Imboden, *J. Immunol.* **137**, 819 (1986); C. H. June, J. A. Ledbetter, M. M. Gillespie, T. Lindsten, C. B. Thompson, *Mol. Cell. Biol.* **7**, 4472 (1987).
7. T. Fujita *et al.*, *Cell* **46**, 401 (1986); U. Siebenlist *et al.*, *Mol. Cell. Biol.* **6**, 3042 (1986).
8. D. B. Durand *et al.*, *Mol. Cell. Biol.* **8**, 1715 (1988).
9. B. Hoyos, D. W. Ballard, E. Böhnlein, M. Siekevitz, W. C. Greene, *Science* **244**, 457 (1989); K. Muegge *et al.*, *ibid.* **246**, 249 (1989); G. R. Crabtree, *ibid.* **243**, 355 (1989).
10. T. Lindsten, C. H. June, J. A. Ledbetter, G. Stella, C. B. Thompson, *ibid.* **244**, 339 (1989).
11. D. B. Durand, M. R. Bush, J. G. Morgan, A. Weiss, G. R. Crabtree, *J. Exp. Med.* **165**, 395 (1987).
12. J. D. Fraser and B. A. Irving, unpublished observations.
13. The γ-fibrinogen–CAT construct that lacked the −326 to −51 IL-2 gene insert did not respond with inducible CAT activity to any of the stimuli tested. The basal activity of this construct transfected into Jurkat cells was approximately 0.2% conversion.
14. E. Serfling *et al.*, *EMBO J.* **8**, 465 (1989).
15. J. D. Fraser, unpublished observations.
16. M. Fried and D. M. Crothers, *Nucleic Acids Res.* **9**, 6505 (1981).
17. The sequences of the oligonucleotides are as follows: NF-AT, 5′-GGAGGAAAAACTGTTTCATACAGA-AGGCGT-3′(−285 to −254 of the IL-2 gene); AP-1, 5′-GATCTAGTGATGAGTCAGCCGGATC-3′.
18. S. Miyatake, T. Otsuka, T. Yokota, F. Lee, K. Arai, *EMBO J.* **4**, 2561 (1985); E. Stanley, D. Metcalf, P. Sobieszczuk, N. M. Gough, A. R. Dunn, *ibid.*, p. 2569.
19. H. D. Campell, S. Ymer, M. C. Fung, I. G. Young, *Eur. J. Biochem.* **150**, 297 (1985); Y. C. Yang and S. C. Clark, *Lymphokines* **15**, 375 (1987).
20. S. Nagata *et al.*, *EMBO J.* **5**, 575 (1986); M. Tsuchiya, Y. Kaziro, S. Nagata, *Eur. J. Biochem.* **165**, 7 (1987).
21. P. W. Gray and D. W. Goeddel, *Nature* **298**, 859 (1982). Murine interferon-γ is available through GenBank, accession no. M28381.
22. S. N. Ho, H. D. Hunt, R. M. Horton, J. K. Pullen, L. R. Pease, *Gene* **77**, 51 (1989).
23. D. L. Mueller, M. K. Jenkins, R. H. Schwartz, *Annu. Rev. Immunol.* **7**, 445 (1989).
24. H. Quill and R. H. Schwartz, *J. Immunol.* **138**, 3704 (1987); M. K. Jenkins, D. M. Pardoll, J. Mizuguchi, T. M. Chused, R. H. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5409 (1987).
25. J. R. de Wet, K. V. Wood, M. DeLuca, D. R. Helinski, S. Subramani, *Mol. Cell. Biol.* **7**, 725 (1987).
26. J. D. Dignam, R. M. Lebovitz, R. G. Roeder, *Nucleic Acids Res.* **11**, 1475 (1983).
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Technical Comments

Microwave Sounding Units and Global Warming

In their research article "Precise monitoring of global temperature trends from satellites," R. W. Spencer and J. R. Christy assert (*1*) that satellite microwave sounding units (MSUs) exhibit superb stability and may be used to monitor global warming in a way that is not possible by other means.

During the first 10 years of MSU measurements, global warming models predicted surface air temperature increases of approximately 0.2 K (*2*). The average temperature of the troposphere was expected to increase by about the same amount. The analysis by Spencer and Christy shows that globally averaged MSU brightness temperatures exhibit fluctuations of approximately 0.4 K over months and years. If one equates brightness temperature with average tropospheric air temperature, as the authors do, it becomes difficult to discern such a small "signal" embedded in so much "noise." That is why the authors can state that "[t]here is no obvious long-term trend . . ." in their data. They do not state that the expected global warming signal is not present in the MSU data, just that it cannot be detected. The main value of their analysis is to point out that short-term fluctuations are so large that it may be difficult for any investigator to evaluate the magnitude of long-term trends without more than one decade of data.

The data in Spencer and Christy's figure 5A (*1*) exhibits a slope of +0.06 K per decade (*3*) and a standard error of 0.07 K per decade (our estimate). [Spencer finds smaller slopes for an upgraded MSU data set (*3*)]. Because of the sinusoidal components apparent in the MSU time series, the slope uncertainty could actually be much greater than 0.07 K per decade. Statistically speaking, the 10-year trend is almost as consistent with the "expected warming" hypothesis as it is with the "no warming" hypothesis.

Five MSU instruments contributed to the data set used by Spencer and Christy. Another recent publication by these authors (*4*) describes in detail how the respective data sets were combined. Instrument intercalibration is a crucial issue for detecting trends, and it is important to know the sensitivity of trend solutions to alternative intercalibration approaches.

The reader who is looking for evidence of the expected 0.2 K global warming signal in the MSU data should be clearly informed that global warming models predict secondary effects, in addition to tropospheric warming, that can influence long-term brightness temperature trends. These include increasing water vapor, changing cloud cover and liquid content, and changing soil moisture (which, in turn, changes land emissivity). Most of these small effects tend to decrease the magnitude of any increase in the brightness temperature of MSU channel 2 resulting from global warming. Spencer and Christy's reference 8, stating that "[o]ther, smaller signals are also present in the measurements. . . . and have been determined to be small for MSU channel 2 (0.01°C or less)," should have been expanded to reveal their modeling assumptions. Such information may be critical to estimates of second-order contributions (*4*).

In response to the research article by Spencer and Christy, we have modified existing computer programs for calculating microwave observables and have studied several potential second-order effects, two of which are *not* noted in Spencer and Christy's reference 8. Specifically, we have evaluated the impact of MSU channel 2 frequency drifts and the effect of stratospheric cooling which others have suggested will occur with tropospheric warming (*5*). All effects that we have evaluated are small (0.01 to 0.05 K), but these and other potentially important effects merit further study.

Our tentative conclusion is that remote sensing using satellite microwave radiometers can in fact provide a means for monitoring troposphere-averaged air temperature, but for this to be successful more than one decade of data will be needed to overcome the apparent inherent variability of global average air temperature. The provocative data set reported by Spencer and Christy should be subjected to careful and rigorous review before it is interpreted as evidence of the presence or absence of global warming.

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