both are RNPs, both bind to anion-exchange columns (although they elute at different ionic strengths), and both sediment as a 15S particle in glycerol gradients (*6, 10*). Furthermore, RNase P and RNase MRP are both RNA processing enzymes that make a single endoribonucleolytic cleavage in their RNA substrates, a function that could be dependent on a common polypeptide. In addition, both H1 RNA and RNase MRP RNA have a site, approximately in the middle, at which cleavage occurs (*6, 10*). Whether a common polypeptide component of RNase P and RNase MRP is responsible for creating a labile phosphodiester bond and possibly cleaving that bond remains to be determined. It will be important to ascertain the function of the full-length versus the cleaved RNA species in each of these enzymes. Several other RNAs may be cleaved and remain functional (*15, 16*).

Both RNase P and RNase MRP are present in the nucleus and mitochondria of mammalian cells and, at least in the case of RNase P, are known to have the same function in both locations. Since RNase MRP is transported to the mitochondria (*10*), it is possible that RNase P is also transported to the mitochondria and that a common protein is responsible for mitochondrial targeting of these enzymes.

REFERENCES AND NOTES

1. E. M. Tan, *Adv. Immunol.* **33**, 167 (1982); J. A. Hardin, *Arthritis Rheum.* **29**, 457 (1986); J. Craft and J. A. Hardin, *J. Rheum.* **14**, 106 (1987).
2. J. A. Steitz in *Molecular Biology of RNA*, M. Inouye and B. S. Dudock, Eds. (Academic Press, New York, 1987), p. 69–95.
3. H. A. Gold, J. Craft, J. A. Hardin, M. Bartkiewicz, S. Altman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5483 (1988).
4. S. Altman, M. F. Baer, C. Guerrier-Takada, A. Vioque, *Trends Biochem. Sci.* **11**, 515 (1987).
5. C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace, S. Altman, *Cell* **35**, 849 (1983).
6. M. Bartkiewicz, H. A. Gold, S. Altman, *Genes Dev.* **3**, 488 (1989).
7. J. A. Hardin *et al.*, *J. Clin. Invest.* **70**, 141 (1982).
8. C. Hashimoto and J. A. Steitz, *J. Biol. Chem.* **258**, 1379 (1983); R. Reddy, E. M. Tan, D. Henning, K. Nohga, H. Busch, *ibid.*, p. 1383; R. Reddy *et al.*, *ibid.* **256**, 8452 (1981).
9. G. Reimer, I. Raska, U. Scheer, E. M. Tan, *Exp. Cell Res.* **176**, 117 (1988).
10. D. D. Chang and D. A. Clayton, *EMBO J.* **6**, 409 (1987); *Science* **235**, 1178 (1987); *Cell* **56**, 131 (1989).
11. J. N. Topper and D. A. Clayton, in preparation.
12. H. A. Gold, J. N. Topper, D. A. Clayton, J. Craft, unpublished observations.
13. J. Craft and H. A. Gold, unpublished observations.
14. A. Vioque, J. Arnez, S. Altman, *J. Mol. Biol.* **202**, 835 (1988).
15. C. Guerrier-Takada and S. Altman, *Cell* **45**, 177 (1986).
16. C. W. Greider and E. H. Blackburn, *Nature* **337**, 331 (1989).
17. J. Craft, T. Mimori, T. L. Olsen, J. A. Hardin, *J. Clin. Invest.* **81**, 1716 (1988).
18. T. E. England, A. G. Bruce, O. C. Uhlenbeck, *Methods Enzymol.* **65**, 65 (1980).
19. H. Donis-Keller, A. Maxam, W. Gilbert, *Nucleic Acids Res.* **4**, 2527 (1977).
20. E. Jay, R. Bambara, R. Padmanabhan, R. Wu, *ibid.* **1**, 331 (1974).
21. M. Tabler, M. Schnoelzer, H. L. Saenger, *Biosci. Rep.* **5**, 143 (1985).

22. We thank S. Altman for helpful discussions of this work and of protein binding sites on M1 RNA, J. Hardin for advice and critical review of this work, K. Tyc for his help in performing the wandering spot analysis and for his advice on RNA sequencing, and M. Bartkiewicz for her advice on the RNA sequencing and many helpful discussions. J.N.T. thanks R. Karwan for helpful discussions. J.N.T. is a Medical Scientist Training Program Trainee of the National Institute of General Medical Sciences. Supported by grants from the National Institutes of Health (AI 26853 to J.C. and GM 33088-19 to D.A.C.), from the United Scleroderma Foundation (to J.C.), from the Arthritis Foundation and its Connecticut Chapter (to J.C.), and from the American Cancer Society (NP-90 to D.A.C.). J.C. is a Pew Scholar in the Biomedical Sciences.

10 April 1989; accepted 10 July 1989

Synergism Between HIV gp120 and gp120-Specific Antibody in Blocking Human T Cell Activation

ROBERT S. MITTLER AND MICHAEL K. HOFFMANN*

The human immunodeficiency virus (HIV) binds to CD4-positive cells through interaction of its envelope glycoprotein (gp120) with the CD4 molecule. CD4 is a prominent immunoregulatory molecule, and chronic exposure to antibody against CD4 (anti-CD4) has been shown to cause immunodeficiency in mice. T cell–dependent in vitro immune responses can also be inhibited by anti-CD4. Experimental findings reported here indicate that CD4-bound gp120 attracts gp120-specific antibodies derived from the blood of HIV-seropositive individuals to form a trimolecular complex with itself and CD4. Thus targeted to CD4, the gp120-specific antibody functions as an antibody to CD4; it cross-links and modulates the CD4 molecules and suppresses the activation of T cells as measured by mobilization of intracellular calcium (Ca_i^{2+}). The synergism between gp120 and anti-gp120 in blocking T cell activation occurs at low concentrations of both components. Neither gp120 nor anti-gp120 inhibits T cell activation by itself in the concentrations tested.

THE CD4 RECEPTOR EXPRESSED ON lymphoid cells is an immunoregulatory molecule through which the immune system regulates its function (*1, 2*). Antibody reactive with CD4 suppresses T cell–dependent immune responses in vitro and in vivo. HIV, thought to be the causative agent of acquired immunodeficiency syndrome (AIDS), is a ligand of CD4; the virus uses CD4 as receptor to facilitate its entry into cells (*3–5*). The possibility has been considered that, through its interaction with CD4, HIV may influence the host's immune response. After ligation with the viral envelope glycoprotein gp120, CD4 can activate or suppress the function of T cells (*6, 7*). CD4 may undergo phosphorylation as a consequence of its ligation with gp120 (*8*).

HIV-induced pathology becomes manifest after the virus engages the immune system. We speculated that the immune system may participate in the pathogenesis of AIDS and play a contributory role in its own destruction. We report experimental evidence to support this speculation. We show that through its interaction with CD4, gp120 may target the CD4 receptor for antibody that the virus has induced in its host. HIV-specific antibodies can thus react indirectly with the CD4 receptor and, like a typical CD4-reactive antibody, may block an early step in the T cell receptor–mediated activation of gp120-laden CD4 lymphocytes.

The basic experiment to demonstrate a synergism between gp120 and immunoglobulin (Ig) isolated from an AIDS patient's serum in blocking T cell activation is shown in Fig. 1. Human peripheral blood T cells enriched for $CD4^+$ cells were treated with an antibody reactive with a monomorphic determinant of the T cell antigen receptor (Ti). This antibody, WT/31, activates T cells to proliferate when immobilized on a solid support (*9*) and can induce them to mobilize intracellular stores of Ca^{2+} when cross-linked with a second antibody, anti-mouse IgG (Fig. 1A). T cells failed to mobilize intracellular calcium when treated, before stimulation with WT/31, with gp120 and the serum IgG fraction from an HIV-seropositive individual (Fig. 1B). Neither treatment with gp120 alone nor treatment with IgG from an HIV patient alone had an inhibitory effect on intracellular Ca^{2+} mobi-

R. S. Mittler, Bristol-Myers Company, Wallingford, CT 06492.
M. K. Hoffmann, Sloan-Kettering Institute, New York, NY 10021.

*To whom correspondence should be addressed.

lization mediated by WT/31. Both reagents were tested in various doses ranging up to 2000 μg/ml for gp120 and up to 30 μg/ml for the immunoglobulin. In the absence of antibody WT/31, neither gp120 nor anti-gp120, alone or together, in high or low doses (gp120 at 1.5 and 1000 ng/ml; anti-gp120 at 100 and 10,000 ng/ml) caused noticeable change in the basal level of intracellular calcium (*10*). It would thus appear that ligation and cross-linkage of the CD4 receptor with gp120–anti-gp120 complexes might be the cause of the inhibition of the calcium response observed in the presence of gp120 and IgG from an AIDS patient. Via gp120, the gp120-specific patient IgG may take the role of a CD4-reactive antibody. Cross-linkage of the CD4 molecule with CD4-specific antibody has indeed been shown by others (*11*) to inhibit anti-Ti–mediated Ca_i^{2+} mobilization (Fig. 1C).

We speculated that by generating antibodies to gp120 the immune system may play a contributory role in the generation of HIV-dependent immunodeficiency. To test this hypothesis, we screened HIV-positive sera for gp120 reactivity, as measured by indirect immunofluorescence, and for the ability of the sera to block Ti-mediated Ca_i^{2+} mobilization in T cells. Immunoglobulin preparations from the sera of 14 AIDS patients contained gp120-reactive antibodies, and all 14 preparations synergized with gp120 to inhibit Ca_i^{2+} mobilization in normal T cells. Control sera were obtained from patients infected with other viruses: human T cell lymphotropic virus (HTLV-1), cytomegalovirus, rubella, and hepatitis virus. Control sera gave negative results in both tests (Table 1).

Table 1. Reactivity of gp120 in sera from virus-infected individuals. All sera were heat-inactivated and centrifuged for 1 hour at 100,000*g*. The Ca_i^{2+} mobilization assay was performed as described for Fig. 1, except that gp120 at 150 μg/ml was used to incubated T cells.

Virus in patient serum	Number of sera tested	Number of sera with gp120 reactivity	Suppression of Ca_i^{2+} mobilization (%) at various serum dilutions				
			Untreated		gp120-treated		
			1:100	1:1000	1:100	1:1000	1:3000
HIV	14	14	5–17	3–9	60–90	60–90	35–80
Cytomegalovirus	4	0	3–7	NT	2–12	5–8	NT
Rubella	5	0	3–11	NT	0–5	NT	NT
HTLV-I	4	0	6–13	5–11	6–11	5–14	2–7
Hepatitis B	2	0	3–7	NT	7–10	4–13	NT

We determined the sensitivity of T cells to suppression caused by gp120–anti-gp120 in terms of gp120 dose and antibody concentration. Significant inhibition of Ca_i^{2+} mobilization can be achieved with gp120 as low as 15 ng/ml in the presence of antibody to gp120 (Fig. 2). The end point dilution of antibody activity was also determined at low concentrations. Using an unfractionated patient serum, we found an end point dilution below 1:30,000.

A further indication that gp120 directed gp120-specific antibody to the CD4 molecule was discovered in experiments designed to study antigenic modulation. When peripheral T cells were treated with gp120, the detection of virus envelope protein by indirect immunofluorescence remained relatively unchanged for at least 24 hours. However, when gp120 addition was followed by administration of gp120-reactive antibody, the resulting antigen-antibody complexes modulated within 1 hour to form caps that cleared from the T cell surface within 2 hours. This microscopic observation was supported by flow cytometric analysis (Fig. 3). In one experiment, T cells were treated in short sequence with gp120 and anti-gp120 (Fig. 3A); in another experiment, T cells were treated with gp120 and incubated for 1 hour at 37°C before the antibody was added (Fig. 3B). The fluorescence profiles are similar in both experimental settings. However, if T cells were exposed to gp120 and anti-gp120 and left at 37°C for 1 hour, we found a considerable loss of fluorescence intensity consistent with the modulation of antigen-antibody complexes from the cell surface observed microscopically (Fig. 3C). The observation was confirmed by a concomitant loss of CD4 fluorescence intensity.

Our data support the idea that humoral immune responses to HIV infection may indirectly cause the suppression of T cell activation. These data differ from those of Kornfeld *et al.* (*6*), who showed that gp120

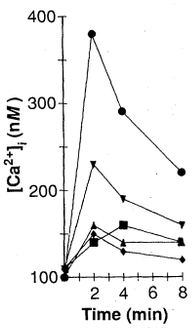

Fig. 2. Titration of gp120 in gp120–anti-gp120–dependent inhibition of Ca_i^{2+} mobilization. Indo-1–loaded T cells were treated with gp120 as described in Fig. 1, washed twice, and incubated with patient serum IgG (30 μg/ml). Thirty minutes later, the cells were stimulated with WT/31 and rabbit antiserum to mouse IgG and subjected to the calcium mobilization assay. The following additions were made together with anti-gp120 (50 μg of patient IgG per milliliter): WT31 plus second antibody (●); the same plus gp120 (15 ng/ml) (▼), plus gp120 (45 ng/ml) (■), plus gp120 (150 ng/ml) (▲), plus gp120 (450 ng/ml) (◆).

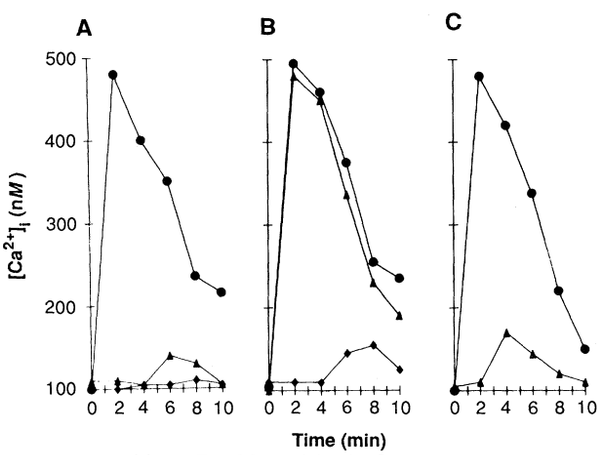

Fig. 1. Inhibiton of Ca_i^{2+} mobilization in peripheral blood T cells by gp120 and gp120-reactive antibodies. $CD4^+$ T cells prepared by negative selection (*12*) were loaded with 2 μ*M* Indo-1 (Molecular Probes, Eugene, Oregon) (*13*). Calcium mobilization was carried out in a two-step process. T cells (1×10^6) were incubated at room temperature for 5 min in 100 μl of RPMI containing 250 ng of WT/31 (Becton Dickinson, Mountainview, California). RPMI 1640 (900 μl) containing 2 μl of rabbit antiserum to mouse IgG was added to the cell suspension, and calcium mobilization was measured by flow cytometry with an EPICS model 753 flow cytometer (Coulter Electronics, Hialeah, Florida) (*12*). To determine the effects of gp120 and anti-gp120–reactive or control antibodies on Ca_i^{2+} mobilization, we modified the procedure: T cells maintained at 37°C were incubated with gp120 for 30 min at 37°C before Indo-1 loading. The cells were washed twice and incubated for 30 min at 4°C with or without patient serum or IgG fractions prepared by protein A chromatography of patient or control sera. T cells were then incubated with WT/31 and cross-linked as described above. Unfractionated sera were ultracentrifuged at 100,000*g* for 1 hour to remove immune complexes. (**A**) WT/31 + second antibody (●); WT/31 alone (▲); second antibody alone (◆). (**B**) Additions before WT/31 and second antibody were gp120 (2 mg/ml) + anti-gp120 (patient Ig, 50 μg/ml) (◆); gp120 alone (●); anti-gp120 alone (▲). (**C**) Additions before WT/31 and second antibody were biotinylated, anti-CD4(OKT4D, 5 μg/ml) + avidin (▲); WT/31 + second antibody alone (●).

in the supernatants of HIV-infected T cells can activate normal T cells to mobilize calcium in the absence of gp120-specific antibody. This discrepancy cannot be resolved at present. We have tested two different preparations of gp120 (one of which is a fusion protein) and obtained the same results with both. However, unlike Kornfeld *et al.* (*6*), we did not use supernatants of HIV-infected T cells as a source of gp120. In agreement with the findings presented here, Ledbetter *et al.* (*11*) showed that a monoclonal antibody to CD4 inhibits anti-CD3–mediated Ca_i^{2+} mobilization in resting T cells when CD4 is cross-linked before the administration of anti-CD3. We have confirmed this observation with WT/31. These studies suggest that ligation and cross-linking of CD4 downregulates T cell function before the T cell receptor is engaged.

Whether or to what extent gp120-specific antibodies contribute to establishing immunodeficiency in HIV-infected individuals cannot be determined from our results. It appears conceivable that gp120 disseminated by HIV-replicating cells forms bloodborne complexes with anti-gp120, thus exposing noninfected CD4-bearing T cells to continuous downregulatory signals that may eventually lead to immunodeficiency of the kind seen in mice after treatment with anti-CD4. The exquisite efficacy of gp120–anti-gp120 complexes in inhibiting T cell receptor–mediated calcium flux may also be viewed as favorable to a gp120-mediated autoimmune mechanism. It places this regulatory phenomenon in a dose range of contributory reagents (gp120 and anti-gp120) that can reasonably be expected to occur in HIV-infected individuals.

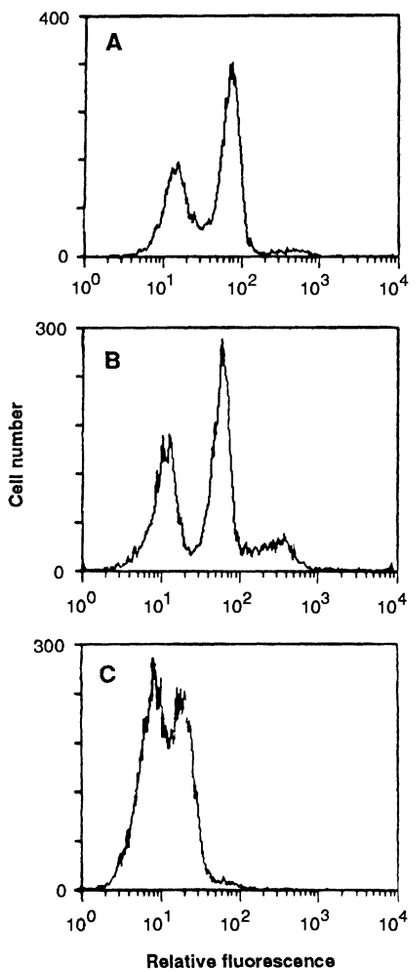

Fig. 3. Modulation of CD4 on the T cell surface by gp120–anti-gp120. (**A**) T cells treated with gp120 (150 μg/ml) were immediately exposed to IgG from AIDS patients (30 μg/ml) and fluorescein isothiocyanate–conjugated second antibody. (**B**) The gp120-treated T cells were incubated for 2 hours at 37°C before anti-gp120 and second antibody were added. (**C**) The gp120-treated T cells were incubated for 2 hours with anti-gp120 before the fluoresceinated second antibody was added.

REFERENCES AND NOTES

1. B. E. Bierer, B. P. Sleckman, S. E. Ratnofsky, S. D. Burakoff, *Annu. Rev. Immunol.* **7**, 579 (1989).
2. S. Stober, *Immunol. Today* **7**, 153 (1986).
3. D. Klatzman *et al.*, *Nature* **312**, 767 (1984).
4. J. S. McDougal, *Science* **231**, 382 (1986).
5. P. J. Maddon *et al.*, *Cell* **47**, 333 (1986).
6. H. Kornfeld *et al.*, *Nature* **355**, 445 (1988).
7. P. C. Diamond *et al.*, *J. Immunol.* **141**, 3715 (1988).
8. A. P. Fields, D. Bednarik, A. Hess, W. Stratford-May, *Nature* **333**, 278 (1988).
9. H. Spits *et al.*, *J. Immunol.* **135**, 1922 (1985).
10. R. S. Mittler, unpublished data.
11. J. A. Ledbetter *et al.*, *Eur. J. Immunol.* **18**, 525 (1988).
12. G. Gandernack, T. Leivestad, J. Ugelstad, E. Thorsby, *J. Immunol. Methods* **90**, 179 (1986).
13. P. S. Rabinowitch, C. H. June, A. Grossmann, J. A. Ledbetter, *J. Immunol.* **137**, 952 (1986).

14. We thank K. Small, K. Budzko, and D. Armstrong for providing us with patient sera, and H. F. Oettgen and S. Koenig for their comments on the manuscript. Recombinant gp120 preparations were kindly provided by Genetic Systems and by Genentech. We thank B. Forbes for manuscript preparation.

21 June 1989; accepted 7 August 1989

Mutant Potassium Channels with Altered Binding of Charybdotoxin, a Pore-Blocking Peptide Inhibitor

RODERICK MACKINNON* AND CHRISTOPHER MILLER

The inhibition by charybdotoxin of A-type potassium channels expressed in *Xenopus* oocytes was studied for several splicing variants of the *Drosophila Shaker* gene and for several site-directed mutants of this channel. Charybdotoxin blocking affinity is lowered by a factor of 3.5 upon replacing glutamate-422 with glutamine, and by a factor of about 12 upon substituting lysine in this position. Replacement of glutamate-422 by aspartate had no effect on toxin affinity. Thus, the glutamate residue at position 422 of this potassium channel is near or in the externally facing mouth of the potassium conduction pathway, and the positively charged toxin is electrostatically focused toward its blocking site by the negative potential set up by glutamate-422.

CHARYBDOTOXIN (CTX) IS A 37–amino acid peptide found in the venom of the scorpion *Leiurus quinquestriatus*. This peptide inhibits, at nanomolar concentrations, several types of K^+-specific ion channels, including Ca^{2+}-activated K^+ channels from many vertebrate tissues (*1*), *Aplysia* neurons (*2*), and red blood cells (*3*), and voltage-dependent K^+ channels from lymphocytes (*4*). The molecular mechanism by which CTX inhibits K^+ channels is understood. The peptide inhibitor binds to the channel with one-to-one stoichiometry, and physically occludes its outer "mouth," thus blocking K^+ permeation through the pore. This conclusion emerged from mechanistic studies on the high-conductance Ca^{2+}-activated K^+ channel from rat skeletal muscle, reconstituted into planar lipid bilayers (*5*). Potassium ions moving through the channel pore from the internal solution can destabilize CTX on its blocking site (*6*) and tetraethylammonium ion, which blocks the channel pore from the external solution, competitively prevents CTX from binding (*7*).

Charybdotoxin also inhibits the A-type K^+ channel coded by the *Shaker* gene of *Drosophila melanogaster* expressed in *Xenopus* oocytes (*8*). CTX inhibition of this K^+ channel is mechanistically similar to the peptide's effects on the well-studied Ca^{2+}-activated K^+ channel. Since cDNA clones of the *Drosophila* K^+ channel have been isolated (*9*), it is a good candidate for a detailed molecular investigation of the CTX-channel interaction. We therefore examined the CTX inhibition of four natural variants within the

Howard Hughes Medical Institute, and Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

*Present address: Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115.