5. M. Sarngadharan *et al.*, *ibid.*, p. 506.
6. M. Popovic *et al.*, *ibid.* **219**, 856 (1983).
7. H. M. Rho *et al.*, *Virology* **112**, 355 (1981).
8. J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *Science* **225**, 381 (1984); J. Sodroski *et al.*, *ibid.* **227**, 171 (1985).
9. M. Essex *et al.*, *ibid.* **221**, 1061 (1983); H. W. Jaffe *et al.*, *ibid.* **223**, 1309 (1984); M. Essex *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984), p. 355; M. Essex, M. F. McLane, T. H. Lee, in *Acquired Immune Defiency Syndrome*, M. S. Gottlieb and J. E. Groopman, Eds. (Liss, New York, 1984), p. 91; M. Popovic *et al.*, *Science* **226**, 459 (1984).
10. M. Essex *et al.*, *Science* **220**, 859 (1983).
11. J. Sodroski *et al.*, *ibid.* **225**, 421 (1984).
12. I. S. Chen *et al.*, *Nature (London)* **305**, 502 (1983).
13. T. H. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7579 (1984).
14. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
15. B. Hahn *et al.*, *Nature (London)* **312**, 166 (1984).
16. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
17. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985).
18. T. H. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3856 (1984).
19. M. A. Gonda *et al.*, *Science* **227**, 173 (1985).
20. J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, *ibid.* **224**, 607 (1984).
21. L. Kitchen *et al.*, *Nature (London)* **312**, 367 (1984); F. Barin *et al.*, *Science* **228**, 1094 (1985).
22. R. Stohrer and E. Hunter, *J. Virol.* **32**, 412 (1979).
23. T. Taniyama and H. T. Holden, *J. Exp. Med.* **150**, 1367 (1979); D. C. Flyer, S. J. Burakoff, D. V. Faller, *Nature (London)* **305**, 815 (1983).
24. J. E. Coligan *et al.*, *Methods Enzymol.* **91**, 413 (1983); J. E. Coligan and T. J. Kindt, *J. Immunol. Methods* **47**, 1 (1981).
25. Supported by NIH grants CA 37466, CA 13885, and 2T32-CA09031. We thank R. Gallo for reference reagents and P. Fischinger and G. Robey for stimulating discussions.

8 February 1985; accepted 12 April 1985

Virus Envelope Protein of HTLV-III Represents Major Target Antigen for Antibodies in AIDS Patients

Abstract. *In this study, two glycoproteins (gp160 and gp120) that are encoded by human T-cell lymphoma virus type III (HTLV-III) were the antigens most consistently recognized by antibodies found in patients with the acquired immune deficiency syndrome (AIDS) and with the AIDS-related complex (ARC) and in healthy homosexual males. The techniques used to detect the glycoproteins were radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE). Although most antibody-positive samples from ARC patients and from healthy homosexual males also reacted with the virus core protein p24, less than half of the AIDS patients revealed a positive band with p24 under the same conditions. The ability to detect antibodies against a profile of both the major* env *gene encoded antigens and the* gag *gene encoded antigens suggests that the RIP/SDS-PAGE may be a valuable confirmatory assay for establishing the presence or absence of antibodies to HTLV-III in human serum samples.*

One of the first suggestions that a retrovirus might have a role in the etiology of the acquired immune deficiency syndrome (AIDS) was the finding that a minority of patients with AIDS and AIDS-related complex (ARC) had antibodies that reacted with antigens found in cells infected with human T-cell leukemia virus type I (HTLV-I) (*1*). The low titers of these antibodies and the observation that only about 25 percent of the patients had antibodies that would precipitate the HTLV-I encoded gp61 or p24 proteins suggested that cross-reacting antibodies to a related agent could be responsible for the initial observation (*1*, *2*). Subsequently, retroviruses that were cytopathic for T-helper lymphocytes were isolated from numerous AIDS and ARC patients. Although these viruses were usually designated HTLV-III (*3*), some were designated lymphadenopathy-associated virus (LAV) (*4*, *5*) or AIDS-related virus (ARV) (*6*). HTLV-III, LAV, and ARV probably represent the same class of agent (*7*, *8*), but on the basis of their antigenic cross-reactivity and several short stretches of amino acid homology they appear to be only distantly related to HTLV-I (*2*, *7*, *9*). However, both HTLV-I and HTLV-III infect T-helper lymphocytes and have a Mg^{2+}-dependent reverse transcriptase (*3*).

The major envelope (*env*) gene products of HTLV-III have been identified as glycoproteins of 160 kD (gp160) and 120 kD (gp120 (*10*). These two glycoproteins have the same amino acid sequence at the amino terminus (*10*). We now report that gp120 and gp160 are the HTLV-III proteins detected most readily in radioimmunoprecipitation assays of serum samples from patients with AIDS or ARC. In AIDS patients, the *env* gene encoded proteins are detected about twice as readily as p24, which is the *gag* gene encoded protein of HTLV-III that represents the major antigen found in virus particles.

Serum samples were initially screened for antibodies to HTLV-III by indirect cell membrane immunofluorescence (MIF) using the H9/HTLV-III cell line as described (*11*) (Table 1). Whereas 48 of 50 (96 percent) of the AIDS patients and 43 of 50 (86 percent) of the ARC patients were positive, 34 of 73 (47 percent) of the healthy homosexual males but none of 27 healthy laboratory workers were positive.

All of the samples from the same 190 individuals were also tested by radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE) with [^{35}S]cysteine-labeled H9/HTLV-III and uninfected H9 cells (*11*). As shown in Table 1, samples that were positive by MIF were also positive by RIP/SDS-PAGE, and one sample from an AIDS patient and three samples from ARC patients that were negative by MIF were also positive by RIP/SDS-PAGE.

Figure 1 shows the RIP/SDS-PAGE reactions for 15 representative samples of human serum that were positive by MIF (lanes 2 to 16) and one sample that was negative (lane 1). Whereas all the HTLV-III membrane antigen antibody-positive samples regularly reacted with the *env* gene encoded gp120 and gp160 proteins, only 85 of 131 (62 percent) also detected the p24 protein of HTLV-III. Although

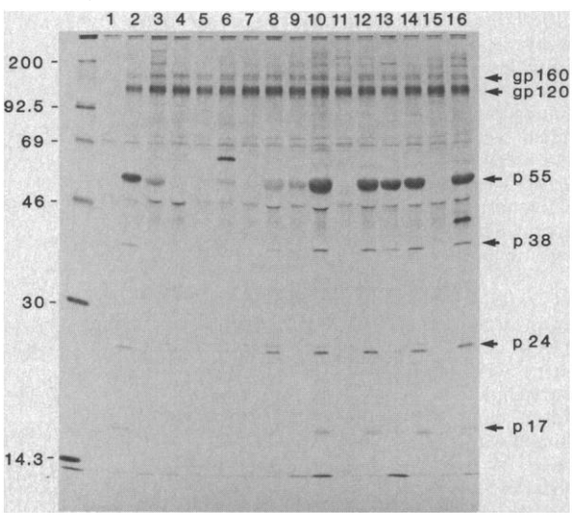

Fig. 1. Reactivity of serum samples for antibody to HTLV-III proteins as determined by RIP/PAGE (*10*, *11*). Cell lysates were prepared from [^{35}S]cysteine-labeled HTLV-III–infected H9 cells and immunoprecipitated with sera from the following individuals: a laboratory worker whose serum was negative for antibody to HTLV-III membrane antigens (lane 1); seven ARC patients (lanes 2, 6, 7, 10, 11, 12, and 16); three AIDS patients (lanes 3, 5, and 15); and five healthy homosexuals who were positive for antibody to HTLV-III membrane antigens (lanes 4, 8, 9, 13, 14).

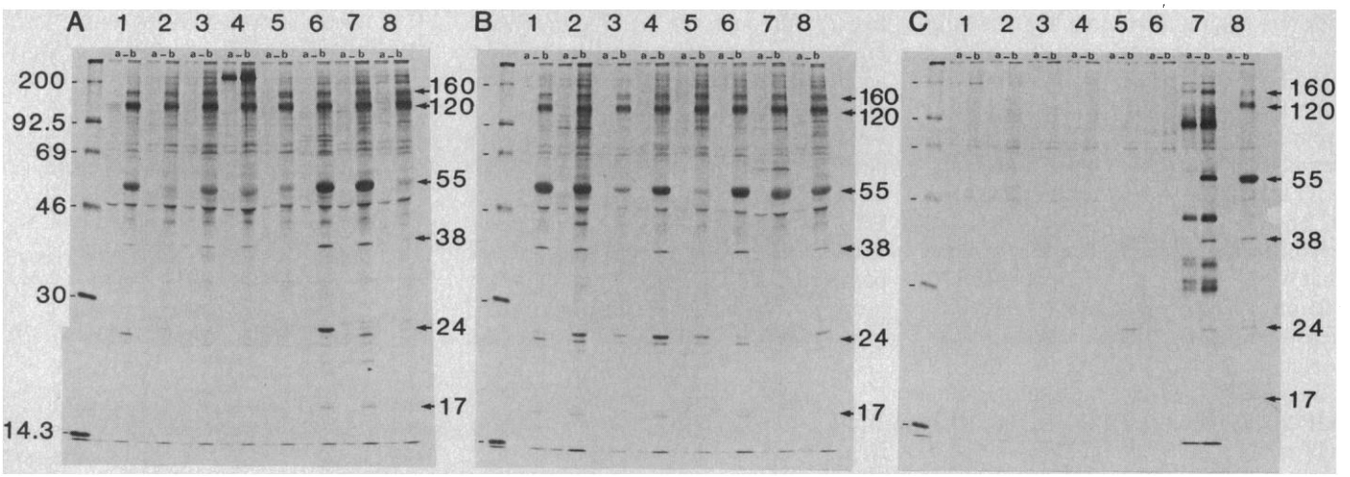

Fig. 2. Analysis of human serum samples for antibodies to HTLV-III proteins. Soluble cell lysates labeled with [^{35}S]cysteine from uninfected H9 cells (a) and infected H9 cells (b) were reacted with (A) sera from eight AIDS patients (lanes 1 to 8); (B) sera from four ARC patients (lanes 1 to 4), and four positive healthy homosexual males (lanes 5 to 8); and (C) sera from two healthy homosexual males (lanes 1 and 2) and two laboratory workers (lanes 3 and 4), all of whom were negative for antibody to HTLV-III membrane antigens; mouse monoclonal antibody to p24 of HTLV-III (lane 5), normal rabbit serum (lane 6), reference rabbit antiserum to disrupted HTLV-III (lane 7), and a positive control from an ARC patient (lane 8).

some sera from patients with AIDS and ARC patients and some samples from healthy homosexual males have readily detectable antibodies to p24 or to the *gag* gene precursor protein p55 (for example, Fig. 2, lanes 2, 10, 12 to 14, and 16), others lack readily detectable antibodies to these proteins (for example, Fig. 1, lanes 4, 5, 7, 11, and 15). Several other bands were precipitated in various proportions from the sera of AIDS and ARC patients, including proteins of about 41 kD (gp41), 38 kD (p38), 17 kD (p17), and a second band near p24 that migrates close to the 24-kD band precipitated by reference rabbit antisera to whole disrupted HTLV-III (Fig. 2C, lane 7b) or by a reference monoclonal antibody to HTLV-III p24 (Fig. 2C, lane 5b).

To establish the specificity of these sera for HTLV-III proteins, we tested all of them by RIP/SDS-PAGE on uninfected H9 cells. The serum samples from eight highly reactive AIDS patients failed to detect proteins of similar sizes in uninfected control cells (Fig. 2A). The results were similar for five ARC patients (Fig. 2B, lanes 1 to 4, and Fig. 2C, lane 8) and for four antibody-positive healthy homosexual males (Fig. 2B, lanes 5 to 8). No bands of the same sizes were detected on either the HTLV-III infected or the uninfected H9 cells when sera from antibody-negative healthy homosexual males (Fig. 2C, lanes 1 and 2) or healthy laboratory workers (Fig. 1, lane 1, and Fig. 2C, lanes 3 and 4) were checked.

Representative antibody-positive sera were also tested on glycoprotein preparations of H9/HTLV-III cells enriched through the use of a lentil lectin column. Samples from four antibody-positive AIDS patients precipitated proteins of about 120 kD, 160 kD, and 41 kD (Fig. 3, lanes 1 to 4; these are the same sera as in Fig. 2A, lanes 1 to 4). Similar results were obtained with two antibody-positive ARC patients (Fig. 3, lanes 5 and 6; these are the same sera as in Fig. 2B,

Table 1. Presence of antibodies to membrane antigens (HTLV-III-MA) and to HTLV-III gp120 and p24 proteins in patients with AIDS or ARC and in healthy homosexual controls and healthy laboratory workers. Positivity for HTLV-III-MA was determined by MIF (*11*) and the presence of antibodies to gp120 and p24 was determined by RIP/SDS-PAGE (*11*).

Category*	Number tested	Number and percent positive for			Ratio of number positive for p24 to number positive for gp120
		HTLV-III-MA	gp120	p24	
AIDS	50	48 (96)	49 (98)	22 (44)	0.45
ARC	50	43 (86)	46 (92)	37 (74)	0.80
Healthy homosexual males	73	34 (47)	36 (49)	26 (36)	0.72
Healthy laboratory workers	27	0	0	0	

*Serum samples were taken from subjects who came to a community health clinic in a high-risk area and to area hospitals in 1983–1984. The samples were coded to prevent experimental bias.

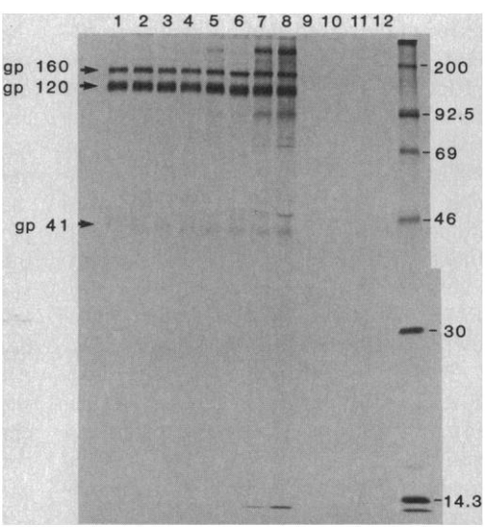

Fig. 3. Analysis of glycoproteins from HTLV-III–infected cells. Cell lysates from [^{35}S]cysteine-labeled H9 cells infected with HTLV-III were enriched for glycoproteins by lentil lectin affinity chromatography as described (*10*, *11*). The bound fraction was reacted with antibodies in the sera of four AIDS patients (lanes 1 to 4, same as in Fig. 2A, lanes 1 to 4); two ARC patients (lanes 5 and 6, same as in Fig. 2B, lanes 1 and 2); two healthy homosexual males who were positive for antibodies to HTLV-III membrane antigens (lanes 7 and 8, same as Fig. 2B, lanes 5 and 6); two healthy homosexual males who were negative for antibodies to HTLV-III membrane antigens (lanes 9 and 10, same as Fig. 2C, lanes 1 and 2); and two laboratory workers (lanes 11 and 12, same as Fig. 2C, lanes 3 and 4).

lanes 1 and 2) and with two antibody positive healthy homosexual males (Fig. 3, lanes 7 and 8; these are the same sera as in Fig. 2B, lanes 5 and 6). No proteins of related sizes were detected in sera from antibody-negative healthy homosexual males (Fig. 3, lanes 9 and 10) or with sera from laboratory workers (Fig. 3, lanes 11 and 12).

None of the human serum samples that we tested contained antibodies to p24 or other *gag*-related HTLV-III antigens without also containing readily detectable antibodies to gp120 and gp160. Conversely, when examined in the same RIP/SDS-PAGE preparation, less than half of the AIDS patients that had antibodies to gp120 also revealed detectable antibodies to p24 (Table 1). Although this ratio was higher for ARC patients and antibody-positive healthy homosexual males, only about three-fourths of the individuals in these categories that had readily detectable antibodies to gp120 and gp160 also had readily detectable antibodies to p24. Other, more sensitive assay systems could conceivably reveal a higher proportion of individuals with antibodies to p24. However, for assays based on equimolar amounts of undenatured antigen, the gp120/160 complex, which has been mapped to the *env* gene of HTLV-III, would presumably be the antigen of choice. This might be particularly important for patients involved in differential diagnoses for AIDS, since such individuals had lower amounts of antibody to p24.

We recently reported that asymptomatic hemophiliacs that tested positive by MIF also regularly had antibodies to gp120/160 (*11*). At that time we had not established that these proteins were encoded by HTLV-III. However, as we now find with AIDS patients, ARC patients, and healthy homosexual males, a significant fraction of the asymptomatic hemophiliacs had antibodies to gp120/gp160 in the absence of antibodies to p24 that could be detected with the same procedure. We also recently reported that four healthy individuals from "high risk" backgrounds carried infectious HTLV-III but had no antibodies detectable by the MIF, ELISA (enzyme-linked immunosorbent assay), or Western blotting procedures (*12*). Three of these individuals were also tested by RIP/SDS-PAGE and were also found to be negative by this procedure.

These results suggest that RIP/SDS-PAGE should be considered as a possible confirmatory test for establishing reactivity of selected serum samples that give uninterpretable results by procedures such as ELISA and Western blotting, especially potential "false positives." Although the ELISA and Western blotting procedures are clearly more adaptable for use in broad-scale screening, to our knowledge the RIP/SDS-PAGE described here is the only assay currently available that regularly reveals a reactivity with a spectrum of the major HTLV-encoded proteins of both the *gag* and *env* genes, virtually eliminating the possibility that false positives would be a problem with this procedure.

F. BARIN
M. F. MCLANE
J. S. ALLAN
T. H. LEE
Department of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts 02115
J. E. GROOPMAN
Departments of Pathology and *Medicine, New England Deaconess Hospital, Boston, Massachusetts 02115*
M. ESSEX
Department of Cancer Biology, Harvard University School of Public Health

References and Notes

1. M. Essex *et al. Science* **220**, 859 (1983).
2. T.-H. Lee *et al., Proc. Natl. Acad. Sci. U.S.A.* **81**, 7579 (1984); M. Essex, M. F. McLane, T. H. Lee, in *Acquired Immune Deficiency Syndrome*, M. S. Gottlieb and J. E. Groopman, Eds. (Liss, New York, 1984), pp. 91–100.
3. M. Popovic *et al., Science* **224**, 497 (1984); R. C. Gallo *et al., ibid.*, p. 500; J. Schüpbach *et al., ibid.*, p. 503; M. Sarngadharan *et al., ibid.*, p. 506; J. Groopman *et al., ibid.* **226**, 447 (1984); D. Zagury *et al., ibid.*, p. 449; D. D. Ho *et al., ibid.*, p. 453; G. M. Shaw *et al., Adv. Intern. Med.* **30**, 1 (1984).
4. F. Barré-Sinoussi *et al., Science* **220**, 868 (1983).
5. L. Montagnier *et al., ibid.* **225**, 63 (1984); P. M. Feorino *et al., ibid.*, p. 69.
6. J. A. Levy *et al., ibid.*, p. 840.
7. L. Ratner *et al., Nature (London)* **313**, 277 (1985).
8. S. Wain-Hobson *et al., Cell* **40**, 9 (1985); R. Sanchez-Pescador *et al., Science* **227**, 484 (1985).
9. S. K. Arya *et al., Science* **223**, 927 (1984).
10. J. S. Allan *et al., ibid.* **228**, 1091 (1985).
11. L. W. Kitchen *et al., Nature (London)* **312**, 367 (1984).
12. S. Z. Salahuddin *et al., Lancet* **1984-II**, 1418 (1984).
13. Supported by NIH grants CA 37466, CA 13885, and 2T32-CA09031. F.B. is a visiting scientist from Université Francois Rabelais in Tours, France and was supported by the Association pour le Development de la Recherche sur le Cancer, the Fondation pour la Recherche Medicale, and the Phillipe Fondation. We thank R. Gallo and M. Sarngadharan for the reference rabbit antisera and mouse monoclonal antibodies to HTLV-III and for the H9/HTLV-III cells.

13 February 1985; accepted 12 April 1985

In Vivo Function and Membrane Binding Properties Are Correlated for *Escherichia coli* LamB Signal Peptides

Abstract. *Wild-type and pseudorevertant signal peptides of the* lamB *gene product of* Escherichia coli *interact with lipid systems whereas a nonfunctional deletion mutant signal peptide does not. This conclusion is based on (i) interaction of synthetic signal peptides with a lipid monolayer-water surface, (ii) conformational changes induced by presence of lipid vesicles in an aqueous solution of signal peptide, and (iii) capacities of the peptides to promote vesicle aggregation. Analysis of the signal sequences and previous conformational studies suggest that these lipid interaction properties may be attributable to the tendency of the functional signal peptides to adopt α-helical conformations. Although the possibility of direct interaction between the signal peptide and membrane lipids during protein secretion is controversial, the results suggest that conformationally related amphiphilicity and consequent membrane affinity of signal sequences are important for function in vivo.*

Secreted proteins are generally synthesized as precursors with an amino-terminal extension termed a signal sequence (*1–4*). The signal sequence is required for protein export, but the mechanism of its action is unclear. Biochemical and genetic evidence suggests that the signal sequence plays a number of roles, interacting at various times in the secretion process with one or more species including the ribosome, soluble and membrane-bound proteins, lipid head groups, and hydrocarbon chains. Although signal sequences exhibit little sequence homology, they may be interchanged with retention of function, even among widely different proteins and organisms (*5–9*). Several investigators (*10–12*) have proposed a "conformational homology" as the basis for this interchangeability; that is, signal sequences all take on the same general conformation (or conformations) during the secretion process. Inspection of known signal sequences (*13*) supports this idea. Signal sequences are similar in length, generally having 15 to 30 amino acid residues. One to three charged residues, usually basic, are located near the NH_2-terminus, and are adjacent to a stretch of hydrophobic residues. This hydrophobic core region is usually about 9 to 18 residues long, and has been predicted (*14*) and observed in synthetic signal peptides (*15, 16*) to adopt an α-helical conformation, a structural feature often found in membrane-binding proteins. Biochemical and biophysical studies have, in fact, shown