Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3856–3860, June 1984
Medical Sciences

Human T-cell leukemia virus-associated membrane antigens: Identity of the major antigens recognized after virus infection

(human T-cell leukemia virus/gp61/membrane immunofluorescence/radiosequence analysis)

T. H. LEE*, J. E. COLIGAN†, T. HOMMA*, M. F. MCLANE*, N. TACHIBANA*‡, AND M. ESSEX*§

*Department of Cancer Biology, Harvard University School of Public Health, Boston, MA 02115; and †Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205

Communicated by Elkan R. Blout, February 13, 1984

ABSTRACT **Specific antibodies to cell membrane antigens found on human T-cell leukemia virus (HTLV)-infected cells have been detected in Japanese patients with adult T-cell leukemia/lymphoma and in asymptomatic carriers, using a live cell-membrane immunofluorescence assay. Reactivity of the positive antisera was analyzed using radioimmunoprecipitation and NaDodSO$_4$/PAGE with the HTLV-infected tumor cell line Hut 102 (clone B2). The major cell-associated antigens identified include two glycoproteins of ≈61 and 45 kDa, which appear to be the most immunogenic species in exposed people, a nonglycosylated species of 42 kDa, and four additional species that contain *gag* gene-encoded antigens with sizes ranging from 19 to 55 kDa. The two glycoproteins (gp61 and gp45) are encoded, at least in part, by the *env* gene of HTLV as evidenced by amino acid sequence analysis.**

Human T-cell leukemia virus (HTLV) is a type C retrovirus, initially isolated from a U. S. patient with a malignant variant form of mycosis fungoides (1). Many additional isolates have since been identified in different parts of the world (2–5), primarily from patients with clinical manifestations of adult T-cell leukemia/lymphoma (ATLL) (6). HTLV or a variant of the virus has also been considered as a candidate agent in the acquired immune deficiency syndrome (7–9).

Several lines of evidence have indicated that HTLV is distinct from the previously known retroviruses of lower species. These include nucleic acid hybridization studies (10) and serological studies with antisera directed to the major *gag* gene-encoded proteins p24 and p19 (11, 12). Using these procedures, HTLV was found to be distantly related to the bovine leukemia virus (13) and more closely related to a virus recently described in Old World monkeys (14).

Two areas of the world where both the rate of infection with HTLV and the rate of occurrence of ATLL have been found to be increased are the southwestern Japanese island of Kyushu and the Caribbean Basin. Based on serological analyses with a fixed-cell immunofluorescence assay (15) and a radioimmunoassay for antibodies to gag proteins (16), the prevalence rate for HTLV infection in the Kagoshima and Nagasaki districts of Kyushu was 10%–30%.

We examined sera from ATLL patients and from healthy adult blood donors from Miyazaki, Japan, which is on the island of Kyushu and adjacent to Kagoshima. After the serum samples were screened by membrane immunofluorescence for antibodies to HTLV-associated cell-membrane antigens (HTLV-MA), we used representative positive human sera to determine which HTLV-related proteins were most frequently precipitated from virus-infected cells. Two proteins regularly precipitated by HTLV-MA positive human sera were designated gp61 and gp45 and were found to be encoded, at least in part, by the *env* gene of HTLV.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cells. The HTLV-infected tumor cell line Hut 102 clone B2 (Hut 102), derived from a U. S. patient with ATLL, has been described (1, 17). MT-2 is a HTLV-infected T-cell line of Japanese origin (3). Uninfected T-cell lines used as controls included 8402 (18), MOLT-4 (19), and Jurkat (20). NC 37 is a B lymphoid line (21), and HL 60 is of myeloid origin (22). All were cultured according to conditions described (1, 3, 7, 17–22).

Membrane Immunofluorescence. All human serum samples were initially screened at a 1:4 dilution on both Hut 102 and MT-2 cells, using indirect membrane immunofluorescence. Samples that gave specific fluorescence on at least 40% of the HTLV-infected cells and on <20% of the negative control cells tested (8402 and NC 37) were considered positive. Those that were positive at a 1:4 dilution were also screened at a 1:16 dilution. Details of the procedure used were recently published (7).

Sera. Reference reagents, goat antiserum to p24, and monoclonal antibody to p19 were made available to us by R. C. Gallo (National Cancer Institute). The specificity of these reagents for the *gag* gene-encoded peptides has been described in detail (11, 12). Serum samples were also collected from healthy adult residents of Miyazaki, Japan, as well as from adults with T-cell lymphoma (ATLL) and other tumors. All the human serum samples were collected at the Miyazaki Medical School in Kyushu.

Radioimmunoprecipitation and NaDodSO$_4$/PAGE. Detailed procedures for these analyses have been described (7). Two major modifications used in the present study are (*i*) soluble cell lysate without any preabsorption directly reacted with serum bound to protein A-Sepharose CL-4B and (*ii*) for amino acid sequence analysis, antigens were eluted from immune complexes by heating at 60°C for 15 min.

Tunicamycin and Endo-β-*N*-acetylglucosaminidase H (Endo-H) Treatment. For tunicamycin treatment, Hut 102 cells were incubated in the presence of tunicamycin (20 μg/ml) for 2 hr. At the end of incubation, cells were washed and radiolabeled as described (7), except that the tunicamycin concentration was maintained at 20 μg/ml and the labeling period was 2–3 hr. For Endo-H treatment, the immune complex was first resuspended in 0.15 M Na citrate, pH 5.5/1 mM phenylmethylsulfonyl fluoride/trypsin inhibitor (0.02 mg/ml)/0.1% NaDodSO$_4$, and then boiled for 2 min. Eluted antigens were divided into two portions; one portion was incubated with 0.25 μg of Endo-H at 37°C for 3 hr and the other portion was incubated with buffer only as a control. Tunicamycin, trypsin inhibitor, and NaDodSO$_4$ were

Abbreviations: ATLL, adult T-cell leukemia/lymphoma; Endo-H, endo-β-*N*-acetylglucosaminidase H; HTLV, human T-cell leukemia virus; HTLV-MA, HTLV-associated cell-membrane antigen.

‡Present address: Miyazaki Medical School, Kiyotake, Miyazaki, 889-16 Japan.
§To whom reprint requests should be addressed.

purchased from Sigma. Endo-H was obtained from Health Inc. (Albany, NY).

Amino Acid Sequence Analysis. [^{35}S]Cysteine-labeled gp61 and gp45 were precipitated from 36 × 10^6 Hut 102 cells metabolically labeled with 5 mCi of [^{35}S]cysteine (specific activity, 1011.2 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) in 30 ml of cysteine-free RPMI 1640 medium containing 15% fetal bovine serum (GIBCO) for 10 hr. The two glycoproteins were excised from NaDodSO$_4$/polyacrylamide gels and then individually subjected to electrophoretic elution in the presence of 50 mM Tris acetate, pH 7.8/0.01% NaDodSO$_4$ for 12–16 hr. Samples were dialyzed once with 10 mM ammonium bicarbonate buffer containing 0.002% NaDodSO$_4$ for 12–16 hr. One more dialysis was done with 10 mM ammonium bicarbonate buffer without NaDodSO$_4$ for 12–16 hr. The sample was then lyophilized and amino acid sequences were analyzed. Extensive details of the radiochemical amino acid sequencing procedures have been described (23, 24). Briefly, automated Edman degradation of radiolabeled peptides was carried out using a Beckman 890C sequencer with cold-trap modification and 0.1 M Quadrol program 121078. The butyl chloride extracts from each sequence step were dried using N_2 evaporation in 7.0-ml scintillation vials. After addition of Biofluor (New England Nuclear), radioactivity was determined on a Beckman LS9000 liquid scintillation counter.

RESULTS

Membrane Immunofluorescence. Serum samples from ATLL patients, patients with tumors other than ATLL, and healthy blood donors living in the same endemic region were first analyzed by indirect living cell membrane immunofluorescence (7). The results of this survey are summarized in Table 1. All of the eight individuals with ATLL that were examined had specific antibodies to HTLV-MA on Hut 102 and MT-2 cells but did not react with the negative control cells. Conversely, only ≈10% of the samples from the healthy blood donors had such activity. However, for those that gave a positive reaction, no apparent differences in intensity were observed between the ATLL patients and the healthy donors or the individuals with other tumors.

Analysis of Sera Positive for HTLV-MA by Immunoprecipitation. Serum samples from ATLL patients and healthy blood donors living in the same endemic region who were positive for antibodies to HTLV-MA, with a titer of at least 1:16, were examined by immunoprecipitation and NaDodSO$_4$/PAGE. Serum samples from individuals who were negative for antibodies to HTLV-MA were similarly examined. The spectrum of reactivities observed with representative serum samples using [^{35}S]cysteine-labeled Hut 102 cell lysate as antigen is shown in Fig. 1.

Major HTLV-related antigens recognized by the human sera positive for HTLV-MA (lanes 5–8 and 11–14) can be grouped into two categories. Included in the first category are four major species of approximately 55 kDa (p55), 29 kDa (p29), 24 kDa (p24), and 19 kDa (p19). Among these,

Table 1. Prevalence of antibodies to HTLV-MA in ATLL patients, in patients with other tumors, and in healthy adult blood donors from Miyazaki, Kyushu, Japan

Health status	Number tested	Number and % positive at dilution 1:4	1:16
ATLL	8	8 (100)	7 (87.5)
Other tumors*	13	3 (23.1)	2 (15.4)
Healthy blood donor	264	27 (10.2)	17 (6.4)

*Included four non-T-cell lymphoid tumors, four myeloid leukemias (one positive), three hepatomas (one positive), and two carcinomas (one positive).

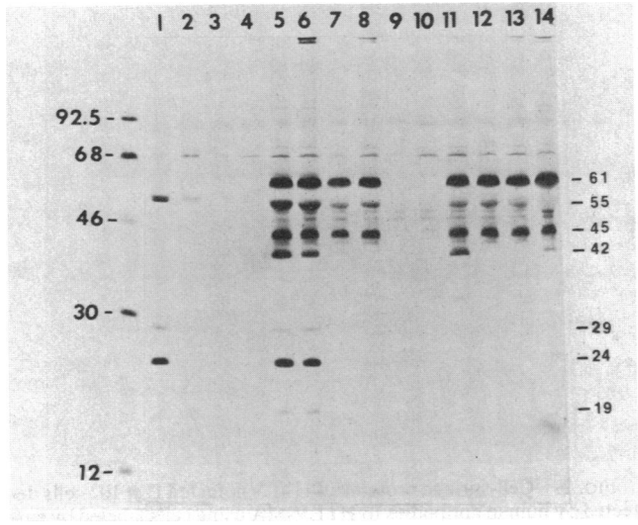

FIG. 1. Analysis of HTLV-related proteins detected by human antibodies to HTLV-MA using NaDodSO$_4$/PAGE. Soluble lysate derived from [^{35}S]cysteine-labeled Hut 102 cells reacted with 3 μl of reference goat antiserum to p24 of HTLV (lane 1), 10 μl of mouse monoclonal antibody to p19 of HTLV (lane 2), 5 μl of serum samples that were positive for HTLV-MA from four representative asymptomatic individuals living in the same endemic region as the ATLL patients (lanes 5–8), 5 μl of serum samples that were negative for HTLV-MA from two patients with non-ATLL type of leukemia lymphoma (lanes 9, 10), and 5 μl of serum samples that were negative for HTLV-MA from two representative asymptomatic individuals (lanes 3, 4). The approximate molecular sizes in kDa for the major HTLV-specific antigens are indicated next to lane 14. Marker proteins (7) are shown in the extreme left lane.

p55, p29, and p24 can all be precipitated by a heterologous goat antiserum to the purified major core antigen of HTLV, p24 (lane 1), while mouse monoclonal antibody to another core antigen, p19, precipitates p55, p29, and p19 (lane 2). Grouped in the second category are three other antigens with approximate sizes of 42 kDa, 45 kDa, and 61 kDa, respectively. The latter three species were not precipitated by antibody to either p24 or p19 of HTLV.

The two categories of antigens appear to be HTLV specific, because they cannot be detected in several lymphoid cell lines that do not contain HTLV sequences such as 8402, NC37, MOLT-4, Jurkat, and HL60 (data not shown). Results seen with four representative serum samples from two healthy blood donors and from two patients with non-ATLL leukemia/lymphoma, which are negative for antibody to HTLV-MA, are shown in Fig. 1 (lanes 3, 4, 9, and 10). Thirty randomly chosen serum samples that were negative for HTLV-MA were analyzed. Only in one case was any reactivity seen to the proteins listed above. In this case, weak reactivity of the 61-kDa antigen was observed.

Of the eight representative serum samples positive for HTLV-MA shown in Fig. 1, all precipitated the 61-kDa and 45-kDa species equally well. In contrast, the degree of reactivity of the same sera with p42, p29, p24, and p19 was quite variable. This result suggests that the 61-kDa and the 45-kDa species are the two most immunogenic proteins, as recognized by people exposed to HTLV.

HTLV-Specific Membrane Antigens. As shown in Fig. 2, four representative HTLV-MA positive sera from Japanese ATLL patients and five representative HTLV-MA positive sera derived from healthy individuals living in the same endemic region as the ATLL patients precipitated a 61-kDa antigen from surface-iodinated Hut 102 cells (lanes 3–7 and 11–14). The 61-kDa species was not precipitated by HTLV-MA negative sera. Results of five negative sera (three from healthy donors and two from patients with other neoplastic

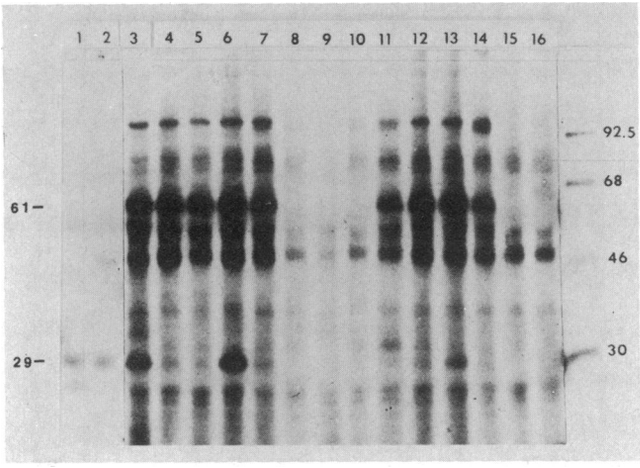

FIG. 2. Cell-surface proteins of HTLV-infected Hut 102 cells detected by human antibodies to HTLV-MA using cells labeled by surface iodination. Cell lysate iodinated by the lactoperoxidase method reacted with 5 μl of reference goat antiserum to p24 of HTLV (lane 1), 10 μl of monoclonal antibody to p19 of HTLV (lane 2), 10 μl of serum samples that were positive for HTLV from four representative ATLL patients (lanes 11–14), 10 μl of serum samples that were positive for HTLV-MA from five representative healthy individuals living in the same endemic region (lanes 3–7), 10 μl of serum samples that were negative for HTLV-MA from two representative non-ATLL leukemia/lymphoma patients (lanes 15, 16), and 10 μl of serum samples that were negative for HTLV-MA from three representative healthy individuals living in the same region (lanes 8–10). The approximate molecular sizes in kDa of the major HTLV-specific antigens are indicated in the extreme left lane. Marker proteins are shown in the extreme right lane.

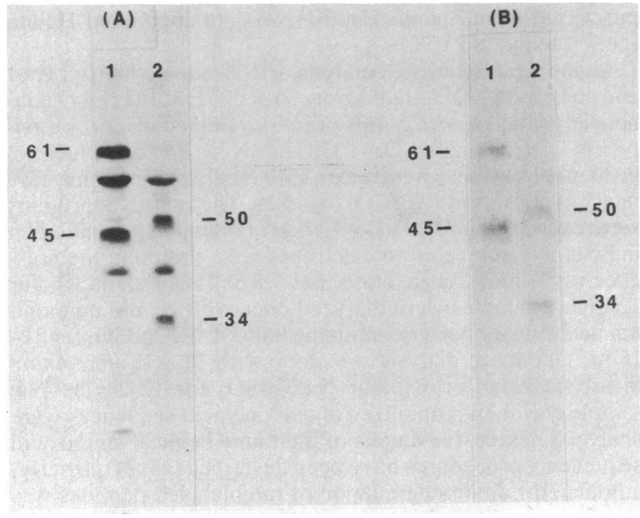

FIG. 3. Tunicamycin and Endo-H treatment of major HTLV-specific glycoproteins. (*A*) Tunicamycin treatment. Hut 102 cells were metabolically labeled with [^{35}S]cysteine in the presence of tunicamycin at 20 μg/ml (lane 2) or in the absence of tunicamycin (lane 1). Soluble lysates from each then reacted with 3 μl of a human serum sample known to have antibody to HTLV-MA. Immunoprecipitation and NaDodSO$_4$/PAGE were done as described (7). Molecular sizes in kDa of the glycosylated species are shown on the left side of lane 1. The approximate molecular sizes in kDa of the deglycosylated species are shown on the right side of lane 2. (*B*) Endo-H treatment. Radioimmunoprecipitation and NaDodSO$_4$/PAGE were done as described (7), except that Hut 102 cells were pulse-labeled for only 15 min. Lane 1 shows antigens that were treated only with the buffer solution. Lane 2 shows the species detected after Endo-H digestion. The approximate molecular sizes in kDa of these species are indicated.

diseases, all living in the same endemic area) are shown in lanes 8–10, 15, and 16.

Heterologous goat antiserum to purified p24 of HTLV and a mouse monoclonal antibody to p19 of HTLV do not precipitate the 61-kDa species. Instead, the p29 species was precipitated by these two reference reagents (lanes 1 and 2) and with some of the human serum samples that were positive for antibodies to HTLV-MA (lanes 3, 6, and 13). Thus, our data indicate that both the 61-kDa species and p29 species are expressed at the cell surface. Whether the 45-kDa species is also expressed on the cell surface is not clear. Nonetheless, serum samples that are known to precipitate gp45 from [^{35}S]cysteine-labeled cell lysate reproducibly give a stronger than background signal (such as those shown in lanes 4, 6, 7, 12, and 13).

HTLV-Specific Glycoproteins. The 61- and 45-kDa species are glycoproteins, as they are sensitive to treatment with tunicamycin or Endo-H. Tunicamycin is a glycosylation inhibitor that blocks the addition of *N*-acetylglucosamine to dolichol phosphate, thus preventing the formation of core oligosaccharide (25, 26). Serum samples positive for HTLV-MA precipitate a new species of ≈50 kDa and another new species of ≈34 kDa from Hut 102 cells metabolically labeled in the presence of tunicamycin (20 μg/ml). A representative result is shown in Fig. 3*A*.

Endo-H cleaves between two proximal *N*-acetylglucosamine residues of *N*-linked oligosaccharides with a high concentration of mannose and leaves one sugar residue behind (27, 28). Antigens dissociated from the immune complex were divided into two aliquots. One aliquot was treated with Endo-H and compared with the other aliquot, which was not. Results of Endo-H digestion are compatible with those from the experiment of tunicamycin treatment and are shown in Fig. 3*B*. These two lines of evidence not only indicate that the 61- and 45-kDa species are glycoproteins (thus, they are designated as gp61 and gp45, respectively), but also show that representative human sera positive for HTLV-MA are not directed only to the sugar moieties of these glycoproteins.

NH_2-Terminal Amino Acid Sequence Analysis. Because gp61 and gp45 are glycoproteins of the approximate size that might be predicted as *env* gene products for HTLV (29), and the *env* products for this virus have not yet been identified, we addressed the question of whether these proteins might be encoded by *env* by analysis of the amino acid residues present at the NH_2 terminus of the proteins. Based on the published nucleotide sequence of the *env* gene of HTLV (29), we predicted that within the first 40 NH_2-terminal amino acid residues of gp61, there should be four cysteine residues with respective spacing of 0, 13, and 6 residues (ordered from the NH_2 terminus to the COOH terminus) between two neighboring cysteines. This assumes that the cleavage site for the leader sequence or signal peptide lies between the 17th and the 21st NH_2-terminal residues and that cysteine residues are conserved between HTLV-I_{KK} (from which the nucleotide sequence was derived) and HTLV-I_{CR} (the strain of HTLV present in Hut 102 cells).

Automated sequencing procedures were followed to determine the NH_2-terminal sequence of the [^{35}S]cysteine-labeled gp61. Fig. 4 shows ^{35}S peaks at residues 6, 7, 21, and 28, indicating the presence of cysteine at these positions in the protein sequence. The repetitive yield for this sequence was 90%, indicating that all the residues are in the same sequence. These results indicate that gp61 is encoded, at least in part, by the *env* gene of HTLV and that the leader sequence of the *env* gene consists of 20 residues.

Similar analysis was carried out with [^{35}S]cysteine-labeled gp45. Cysteine residues were detected at positions 6, 7, and 21 when the first 22 NH_2-terminal residues were analyzed (data not shown). This result suggests that gp45 is also en-

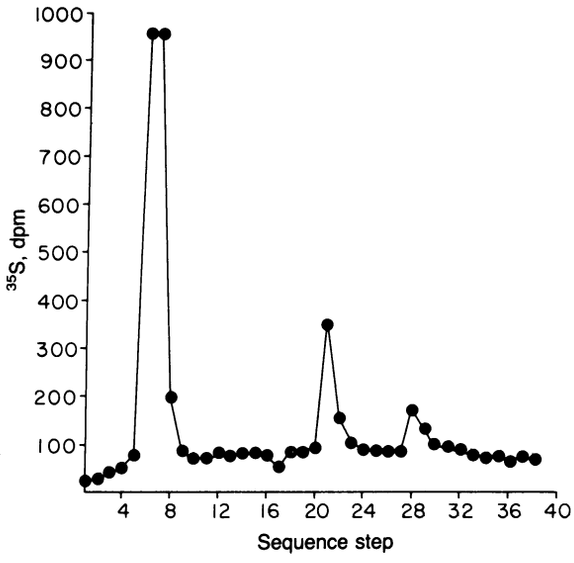

FIG. 4. NH_2-terminal amino acid sequence analysis of [^{35}S]cysteine-labeled gp61. Automated Edman degradation was performed and the radioactivity in the fraction obtained from each cycle is listed on the vertical axis in disintegrations per minute (dpm). Radioactive cysteine was detected at cycles 6, 7, 21, and 28.

coded, at least in part, by the same NH_2-terminal end of the *env* gene.

DISCUSSION

Using procedures that ranged from fixed-cell immunofluorescence to radioimmunoassays for antibodies to p24 and p19, various investigators have shown that people with ATLL usually have detectable antibodies to HTLV-related antigens (11, 15, 16, 30–34). Similarly, 5%–30% of the healthy individuals from southwestern Japan and the Caribbean Basin were also found to have such antibodies (15, 16, 30, 33, 34). We obtained similar results using indirect membrane immunofluorescence, demonstrating that representative proportions of people from the same populations also have antibodies that react with antigens at the surface of HTLV-infected cells (designated HTLV-MA).

Serum samples from people with antibodies to HTLV-MA were able to precipitate a *gag* gene-related protein designated p55, as well as two glycoproteins designated gp61 and gp45. Some of the positive human antisera also precipitated other species such as p42, p29, p24, and p19. The p55 is a *gag* precursor polyprotein that can be chased into mature form of virus core proteins (unpublished data). Another *gag* gene-encoded protein found with the human sera was p29, a species that contained determinants of both p19 and p24. In other unpublished experiments, we have found that p29 is a polymorphic phosphoprotein whose size varies in different HTLV-infected cells. This species can be precipitated with some, but not all, monoclonal antibodies to p19 and p24. The identity of p42 remains unknown. It was not precipitable with reference antisera to p24 or p19 and it did not appear to be glycosylated. The detection of the p55, p24, and p19 peptides with antisera specific for *gag* gene products confirms earlier reports (11, 12).

The major species that was detectable at the cell surface using the lactoperoxidase ^{125}I-labeling procedure was gp61. Among all the antigens detected by the human sera positive for HTLV-MA, gp61 and gp45 appear to be the most immunogenic species recognized by HTLV-exposed individuals, as not all people positive for HTLV-MA had detectable levels of antibodies to the other antigens. When taken in combination, these observations suggest that gp61 is the major target antigen recognized by human sera that are positive in the living cell-membrane immunofluorescence assay.

The question of whether gp61 and gp45 might be encoded by HTLV was also investigated. Based on analysis of the nucleic acid sequence, the estimated molecular size of the unprocessed *env* gene polyprotein precursor was 49–54 kDa, and the estimated size of the processed major *env* gene product (the NH_2-terminal peptide) is ≈32 kDa (29). Our observation that the sizes of the deglycosylated gp61 and gp45 are 50 kDa and 34 kDa, respectively, is compatible with the hypothesis that gp61 may represent the *env* gene polyprotein precursor, and gp45 may represent the major cleaved *env* gene product. Also compatible with this hypothesis was the observation that gp61 and gp45 were highly immunogenic in HTLV-exposed people, because animals exposed to natural infections with retroviruses usually mount the greatest immune response to the *env* gene glycoproteins.

NH_2-terminal amino acid sequence analysis of the proteins labeled with a single amino acid is the strategy we used to delineate the coding origin of gp61 and gp45. If our hypothesis that the gp61 and gp45 are encoded by the *env* gene is correct, there would be enough cysteine residues located in the NH_2 terminus of the proteins to elucidate their origin. We calculate that the probability of finding a protein containing 4 cysteine residues with spacing of 0, 13, and 6 in the first 39 NH_2-terminal residues by chance alone is $(19/20)^{35} \times (1/20)^4$—i.e., 1.04×10^{-6}—and the probability of finding a protein containing 3 cysteine residues with spacing of 0 and 13 by chance alone in the first 22 NH_2-terminal residues is $(19/20)^{19} \times (1/20)^3$—i.e., 4.72×10^{-5}. We thus can conclude with almost complete certainty from the amino acid sequence analysis of gp61 and gp45 that these two glycoproteins are encoded, at least in part, by the *env* gene of HTLV.

We conducted several pulse–chase experiments to address the question of whether gp61 and gp45 can be processed into smaller species. We were unsuccessful in our attempt to show that the gp61 could be processed into gp45 or any other new species. In the same experiments, the *gag* precursor polyprotein p55 was readily shown to undergo further processing to form p24 and p19 (unpublished data). We cannot rule out the possibility that gp61 may be processed into gp45 at an unusually rapid pace, but an alternative hypothesis compatible with the experimental findings is that gp61 may represent an *env* gene product that is defective for processing. This in turn could explain why HTLV appears to be made by infected cells only in very low titers and is difficult to transmit in nature.

Aside from their presence in Japanese ATLL patients and in healthy individuals from the HTLV-endemic region of Japan, antibodies to HTLV-MA and/or gp61 have been found in patients with the acquired immune-deficiency syndrome (7) and in asymptomatic hemophiliacs (35, 36). This suggests that a sensitive immunoassay directed to purified gp61 would be of considerable value for future seroepidemiological studies involving HTLV.

The authors are grateful to Drs. W. Haseltine, J. Sodroski, G. Franchini, F. Wong-Staal, and R. C. Gallo for sharing their unpublished data. We also thank Drs. T. Copeland and S. Oroszlan for discussion and criticism, Dr. C. Terhorst and P. Snow for valuable technical advice, Dr. R. C. Gallo for reference antisera to p24 and p19, and Drs. K. Tsuda and Y. Tsunetoshi for their interest and cooperation. T.H.L. was supported by Institutional Research Service Award 2-T32-CA09031 and the research was supported in part by Grant CA 18216 from the National Institutes of Health.

1. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D. & Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7415–7419.
2. Catovsky, D., Greaves, M. F., Rose, M., Galton, D. A. G., Goolden, A. W. G., McCluskey, D. R., White, J. M., Lam-

pert, I., Bourikas, G., Ireland, R., Brownell, A. I., Bridges, T., Blattner, W. A. & Gallo, R. C. (1982) *Lancet* **i**, 639–643.
3. Yoshida, M., Miyoshi, I. & Hinuma, Y. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2031–2035.
4. Haynes, B. F., Miller, S. E., Palker, T. J., Moore, T. O., Dunn, P. H., Bolognesi, D. P. & Metzgar, R. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2054–2058.
5. Popovic, M., Sarin, P. S., Robert-Guroff, M., Kalyanaraman, V. S., Mann, D., Minowada, J. & Gallo, R. C. (1983) *Science* **219**, 856–859.
6. Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. (1977) *Blood* **50**, 481–492.
7. Essex, M., McLane, M. F., Lee, T. H., Falk, L., Howe, C. W. S., Mullins, J. I., Cabradilla, C. & Francis, D. P. (1983) *Science* **220**, 859–862.
8. Gelmann, E. P., Popovic, M., Blayney, D., Masur, H., Sidhu, G., Stahl, R. E. & Gallo, R. C. (1983) *Science* **220**, 862–865.
9. Gallo, R. C., Sarin, P. S., Gelmann, E. P., Robert-Guroff, M., Richardson, E., Kalyanaraman, V. S., Mann, D., Sidhu, G. D., Stahl, R. E., Zolla-Pazner, S., Leibowitch, J. & Popovic, M. (1983) *Science* **220**, 865–867.
10. Reitz, M. S., Poiesz, B. J., Ruscetti, F. W. & Gallo, R. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1887–1891.
11. Kalyanaraman, V. S., Sarngadharan, M. G., Poiesz, B. J., Ruscetti, F. W. & Gallo, R. C. (1981) *J. Virol.* **38**, 906–915.
12. Robert-Guroff, M., Ruscetti, F. W., Posner, L. E., Poiesz, B. J. & Gallo, R. C. (1981) *J. Exp. Med.* **154**, 1957–1964.
13. Oroszlan, S., Sarngadharan, M. G., Copeland, T. D., Kalyanaraman, V. S., Gilden, R. V. & Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1291–1294.
14. Miyoshi, I., Yoshimoto, S., Fujishita, M., Taguchi, H., Kubonishi, I., Niiya, K. & Minezawa, M. (1982) *Lancet* **ii**, 658.
15. Hinuma, Y., Komoda, H., Chosa, T., Kondo, T., Kohakura, M., Takenaka, T., Kikuchi, M., Ichimaru, M., Yunoki, K., Sato, I., Matsuo, R., Takiuchi, Y., Uchino, H. & Hanaoka, M. (1982) *Int. J. Cancer* **29**, 631–635.
16. Robert-Guroff, M. & Gallo, R. C. (1983) *Blut* **47**, 1–12.
17. Gazdar, A. F., Carney, D. N., Bunn, P. A., Russell, E. K., Jaffe, E. S., Schechter, G. O. & Guccion, J. G. (1980) *Blood* **55**, 409–417.
18. Huang, C. C., Hou, Y., Woods, L. K., Moore, G. E. & Minowada, J. (1974) *J. Natl. Cancer Inst.* **53**, 655–660.
19. Minowada, J., Ohnuma, T. & Moore, G. E. (1972) *J. Natl. Cancer Inst.* **49**, 891–895.
20. Kaplan, J., Tilton, J. & Peterson, W. D., Jr. (1976) *Am. J. Hematol.* **1**, 219–225.
21. Minowada, J., Sagawa, K., Lok, M. S., Kubonishi, I., Nakazawa, S., Tatsumi, E., Ohnuma, T. & Goldblum, N. (1980) in *International Symposium on New Trends in Human Immunology and Cancer Immunotherapy*, eds. Serrou, B. & Rosenfeld, C. (Doin, Paris), pp. 198–199.
22. Collins, S. J., Gallo, R. C. & Gallagher, R. E. (1977) *Nature (London)* **270**, 347–349.
23. Coligan, J. E. & Kindt, T. J. (1981) *J. Immunol. Methods* **47**, 1–11.
24. Coligan, J. E., Gates, F. T., III, Kimball, E. S. & Maloy, W. L. (1983) *Methods Enzymol.* **91**, 413–434.
25. Takatsuki, A., Fukui, Y. & Tamura, A. (1975) *Agric. Biol. Chem.* **39**, 2089–2091.
26. Tkacz, J. S. & Lampen, J. O. (1975) *Biochem. Biophys. Res. Commun.* **65**, 248–257.
27. Tarentino, A. L. & Maley, F. (1974) *J. Biol. Chem.* **249**, 811–817.
28. Tarentino, A. L., Plummer, T. H. & Maley, F. (1974) *J. Biol. Chem.* **249**, 818–824.
29. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3618–3622.
30. Saxinger, C. & Gallo, R. C. (1983) *Lab. Invest.* **49**, 371–377.
31. Robert-Guroff, M., Fahey, K., Maeda, M., Nakao, Y., Ito, Y. & Gallo, R. C. (1982) *Virology* **122**, 297–305.
32. Yamamoto, N., Schneider, J., Koyanagi, Y., Hinuma, Y. & Hunsmann, G. (1983) *Int. J. Cancer* **32**, 281–287.
33. Schüpbach, J., Kalyanaraman, V. S., Sarngadharan, M. G., Blattner, W. A. & Gallo, R. C. (1983) *Cancer Res.* **43**, 886–891.
34. Gallo, R. C., Kalyanaraman, V. S., Sarngadharan, M. G., Sliski, A., Vonderheid, E. C., Maeda, M., Nakao, Y., Yamada, K., Ito, Y., Gutensohn, N., Murphy, S., Bunn, P. A., Jr., Catovsky, D., Greaves, M. F., Blayney, D. W., Blattner, W., Jarrett, W. F. H., zur Hausen, H., Seligmann, M., Brouet, J. C., Haynes, B. F., Jegasothy, B. V., Jaffee, E., Cossman, J., Broder, S., Fisher, R. I., Golde, D. W. & Robert-Guroff, M. (1983) *Cancer Res.* **43**, 3892–3899.
35. Essex, M., McLane, M. F., Lee, T. H., Tachibana, N., Mullins, J. I., Kreiss, J., Kasper, C. K., Poon, M.-C., Landay, A., Stein, S. F., Francis, D. P., Cabradilla, C., Lawrence, D. N. & Evatt, B. L. (1983) *Science* **221**, 1061–1064.
36. Evatt, B. L., Stein, S. F., Lawrence, D. N., McLane, M. F., McDougal, J. S., Lee, T. H., Spira, T. J., Cabradilla, C., Mullins, J. I., Francis, D. P. & Essex, M. (1983) *Lancet* **ii**, 698–701.