JOURNAL OF VIROLOGY, Nov. 1989, p. 4952–4957
0022-538X/89/114952-06$02.00/0

Vol. 63, No. 11

Antibody Reactivity to Different Regions of Human T-Cell Leukemia Virus Type 1 gp61 in Infected People

YI-MING A. CHEN,[1] TUN-HOU LEE,[1] KENNETH P. SAMUEL,[2] AKIHIKO OKAYAMA,[1,3] NOBUYOSHI TACHIBANA,[3] ISAO MIYOSHI,[4] TAKIS S. PAPAS,[5] AND MAX ESSEX[1]*

Department of Cancer Biology, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115[1]; Nucleic Acid and Protein Synthesis Laboratory, Program Resources, Inc.,[2] and Laboratory of Molecular Oncology,[5] National Cancer Institute–Frederick Cancer Research Facility, Frederick, Maryland 21701-1013; and Miyazaki Medical School, Miyazaki 889-16,[3] and Kochi Medical School, Nankoku,[4] Japan

Received 18 April 1989/Accepted 19 July 1989

The primary protein product of the human T-cell leukemia virus type 1 (HTLV-1) *env* gene, gp61, is cleaved to produce both the exterior (gp46) and the transmembrane (gp21) portions of the HTLV-1 envelope protein. To compare the reactivity with human antibodies of different regions of this gp61 protein, five plasmids (A, B, B1, C, and D) were constructed to express recombinant proteins (RPs) in *Escherichia coli*. RP-A, RP-B, RP-B1, and RP-C contain amino acid residues 26 to 165, 166 to 229, 166 to 201, and 229 to 308, respectively, of the exterior envelope protein gp46. Serum samples from HTLV-1-seropositive subjects were assayed for reactivity with these RPs by Western immunoblotting. The percentages of positive reactivity with each of the RPs were as follows: 18.9% (23 of 122) for RP-A, 89.6% (112 of 125) for RP-B, 70.2% (85 of 121) for RP-B1, and 92.9% (117 of 126) for RP-C. These results indicate that the C-terminal half of gp46 (RP-B plus RP-C) can detect 97.6% (123 of 126) of positive samples, while the N-terminal half of gp46 (RP-A) can only detect 18.9% of the HTLV-1-positive sera ($P < 0.005$). Furthermore, RP-A, -B, and -C, which together span the entire length of gp46 except the first five amino acids at the N terminus and the last four amino acids at the C- terminus, detected 99.2% (125 of 126) of the HTLV-1-positive subjects. In contrast, RP-D, which contains the HTLV-1 transmembrane envelope protein gp21 minus the first amino acid at the N terminus, had a lower rate of antibody reactivity at 73.7% (84 of 114) ($P < 0.005$). The difference in seropositive rates for RP-D between HTLV-1 carriers (55.6%) and adult T-cell leukemia patients (85.5%) is statistically significant ($P < 0.01$). This study therefore indicates that the C-terminal half of gp46, especially the amino acid sequence from 200 to 308, contains the most reactive epitopes of the HTLV-1 gp61 envelope glycoprotein.

The human T-cell leukemia virus type 1 (HTLV-1) has been shown to be etiologically associated with human adult T-cell leukemia and lymphoma (10, 20, 23, 30) and with a rare neurological disorder called tropical spastic paraparesis and HTLV-1-associated myelopathy (13, 21). Several lines of evidence suggest that the HTLV-1 *env* glycoprotein may be a critical viral antigen for targeting by the host immune system. It has been demonstrated that the HTLV-1 *env* protein is the first viral antigen recognized by antibodies in the sera of HTLV-1-infected subjects (8, 18). Also, the infectivity of pseudotypes of vesicular stomatitis virus bearing the HTLV-1 *env* protein can be neutralized by antibodies specific to this protein (4). It is therefore logical to assume that immune effector mechanisms directed to the HTLV-1 envelope protein represent an important mechanism for inhibiting infection and/or the spread of the virus (4, 28).

Previous studies on the HTLV-1 *env* protein have revealed interesting observations about its antibody reactivity (6, 7, 14, 25, 27). Experiments have shown dramatic variations in the reactivity of different regions of this protein by testing antibody reactivities to three synthetic peptides, each representing 11 to 15 amino acids of gp61 (6). Further experiments have studied a *Bam*HI-*Xho*I fragment of the HTLV-1 *env* gene cloned into plasmid pkS400 which, upon induction, expressed a recombinant protein containing the final four amino acids at the C terminus of gp46 and two-thirds of the length of gp21 (25). This recombination protein was able to detect 11 of 11 HTLV-1-positive test sera. A comparative study to determine which regions of gp61 are most reactive with human antibodies has not yet been described. This information may be useful for both blood screening and clinical diagnosis.

We therefore designed our study to provide a systematic exploration of the serologic reactivity of various portions of the gp61 protein. To that end, we constructed five plasmids and induced them to express recombinant proteins (RPs). Each RP represents a specific region of the HTLV-1 *env* protein, and together they span the entire length of gp61. Serum samples from asymptomatic Japanese HTLV-1 carriers and adult T-cell leukemia (ATL) patients were tested for antibody reactivity to these RPs by Western immunoblot assay. The results show that the most reactive region of gp61 is located in the C-terminal half of the HTLV-1 exterior glycoprotein gp46.

In this study, the HTLV-1 proviral DNA sequences reported by Seiki et al. (26) were used as the reference for the restriction enzyme sites for gene cloning and screening. As shown in Fig. 1, a 4.2-kilobase (kb) *Sph*I-*Sph*I fragment containing both the HTLV-1 *env* gene and the *x* region was excised from pMT2 (5) and subcloned into plasmid p806. Plasmid p806, a derivative of pUC18, contains an *Eco*RI-*Nco*I fragment from plasmid pXVR (9). To ensure that the inserted *env* gene was in the same reading frame as the preceding v-*ras*^{H} sequence, the DNA sequence in the junction region of plasmid psphI-env1 was analyzed by the dideoxynucleotide sequencing method (29).

To generate plasmid pS3 containing the distal two-thirds of the HTLV-1 *env* gene (nucleotides 5694 to 6665), psphI-

* Corresponding author.

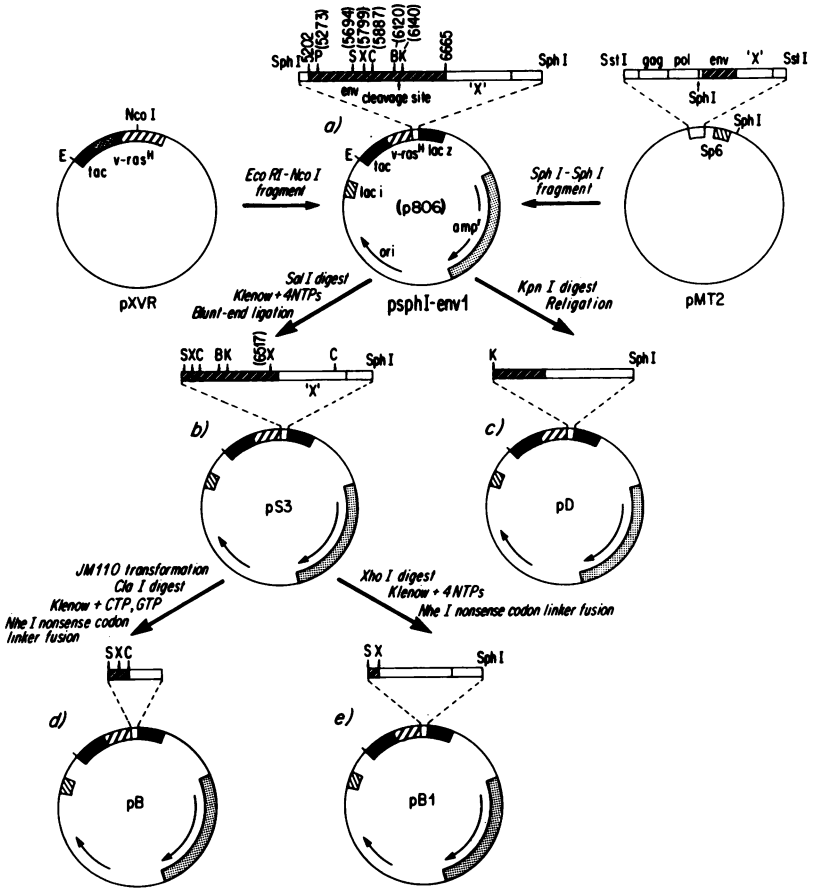

FIG. 1. Construction of plasmids containing specific regions of the HTLV-1 *env* gene. Abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; S, *Sal*I; X, *Xho*I; 'X', *x* region of the HTLV-1 provirus. Nucleotide numbers are shown in parentheses.

env1 DNA was digested with *Sal*I, which cuts at nucleotide 5694 of the *env* gene and at the *Sal*I sequence in the polylinker site of the plasmid. The sticky ends of the linearized plasmid DNA were repaired with Klenow fragment and then fused by blunt-end ligation (Fig. 1b). Plasmid pD (Fig. 1c), which contains the gene corresponding to the HTLV-1 transmembrane protein gp21 (nucleotides 6140 to 6665), was similarly generated by digesting psphI-env1 DNA with *Kpn*I, which cuts at nucleotide 6140 of the *env* gene and at the *Kpn*I sequence of the polylinker site. The plasmid DNA was then religated.

To construct plasmids expressing the middle regions of gp46, we took plasmid pS3 DNA, which had been isolated from DNA methylase-free cells (strain JM110), digested it with either *Cla*I or *Xho*I, repaired the ends with Klenow fragment, and then re-fused them by blunt-end ligation with an *Nhe*I nonsense codon linker, d(CTAGCTAGCTAG) (New England BioLabs, Beverly, Mass.) (Fig. 1d and e). These new plasmids, called pB and pB1, contained HTLV-1 *env* gene nucleotide sequences 5694 to 5887 and 5694 to 5799, respectively.

Another expression vector used in this study was pJL6 (17). It contains a bacteriophage λ p_L promoter and the N-terminal fragment of the λ *c*II gene, including the ribosome-binding site and an ATG start codon. As shown in Fig. 2a, a 422-base-pair (bp) *Pvu*II-*Sal*I fragment (nucleotides 5273 to 5694), obtained from pMT2 and encoding the N-terminal half of the HTLV-1 envelope exterior glycoprotein gp46, was fused in-frame by blunt-end ligation of the repaired *Hin*dIII cloning site of pJL6 (plasmid pA). Plasmid pC (Fig. 2b), which encodes the C-terminal region of gp46, was constructed by force-cloning a 234-bp *Cla*I-*Bam*HI fragment (nucleotides 5887 to 6120) from plasmid pMT2 into plasmid

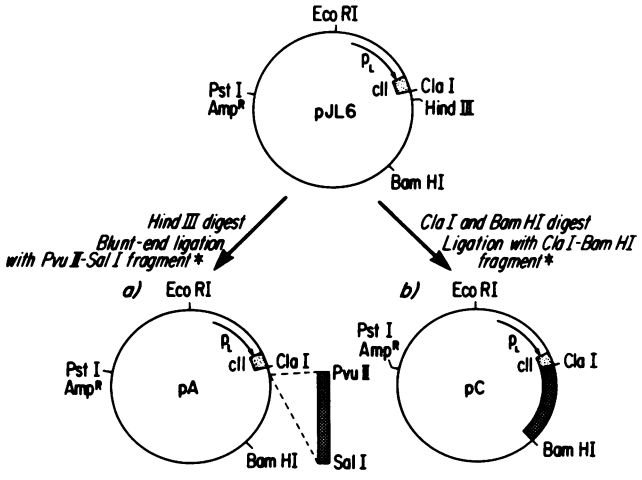

FIG. 2. Construction of plasmids containing specific regions of the HTLV-1 *env* gene. Fragments from the HTLV-1 provirus clone pMT2 are marked (*).

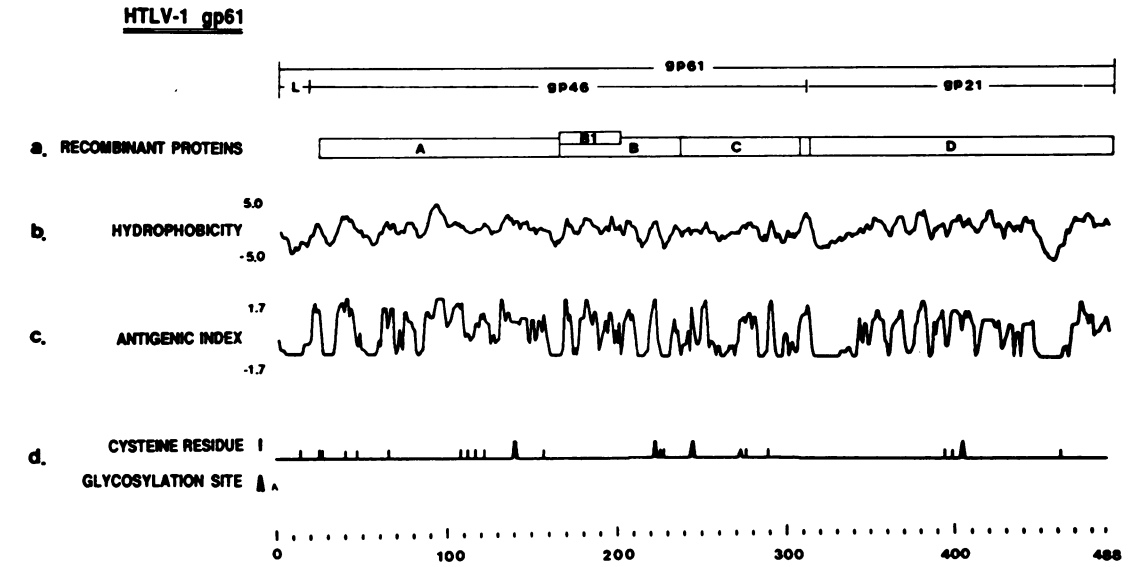

FIG. 3. Schematic diagram of the hydrophobicity plot, predicted antigenic index, and the positions of predicted cysteine residues and glycosylation sites of regions contained within specific RPs along the HTLV-1 envelope protein (gp61). The primary envelope protein gp61 is proteolytically cleaved at the N terminus (to remove a short leader [L] sequence) and within the gp61 precursor to generate the mature envelope glycoprotein comprising the gp46 (exterior) and gp21 (transmembrane) proteins. The scale refers to amino acid sequence number.

pJL6, which had been linearized by *Cla*I and *Bam*HI digestion.

RPs containing specific regions (Fig. 3) of HTLV-1 gp61 were expressed either by vector p806 (pB, pB1, and pD) or by vector JL6 (pA and pC) in two different bacterial culture systems. *Escherichia coli* X-90 cells (22) carrying plasmids derived from p806 were induced to express RPs by treatment with IPTG (isopropyl-β-D-thiogalactopyranoside). *E. coli* DC1148 was used to carry recombinant plasmids derived from pJL6. This strain is a lysogen that contains a mutant temperature-sensitive repressor encoded by the phage λ gene *c*I857 and can be induced to express RPs when exposed to a temperature shift from 32 to 42°C.

The characteristics of the RPs expressed by these plasmids are summarized in Table 1. In general, the RPs were initially identified by staining sodium dodecyl sulfate-polyacrylamide (SDS-PA) gels containing both controls and the induced clones with Coomassie blue. Reactivity of the RPs was then confirmed by Western blot with high-titer HTLV-1-positive sera for all of the RPs and goat anti-v-*ras*^{H} antiserum for the fusion proteins expressed by pB, pB1, and pD. As shown in Fig. 4, the observed molecular masses of the RPs corresponded to the predicted values.

In the case of RP-D there were two bands, one with a molecular mass of 32 kilodaltons (kDa) and the other of 30 kDa, which reacted by Western blot with most of the HTLV-1-positive sera (Fig. 4) and with a rabbit anti-gp21 antiserum (data not shown).

We initially tried and failed to induce plasmid psphI-env1 to express the full length or plasmid pS3 to express two-thirds of the length of gp61. Several possibilities may be considered to explain this phenomenon: message instability, codon usage preference, a toxic effect of the expressed proteins on the bacterial cells (1), and metabolic instability of the protein itself (2). Nevertheless, after the gene fragments were shortened for cloning, all the RPs were expressed at levels high enough to allow direct detection by Coomassie blue staining. The RPs, which ranged from 15 to 32 kDa, constituted approximately 98% of the HTLV-1 *env* precursor complex, as shown in Fig. 3. While both bacterial systems produced adequate quantities of the RP products, the pJL6 system produced them in greater quantities than did the p806 system. The reason for this difference is not clear, although the two systems are not identical; protein expression with the pJL6 vector is induced by temperature shift, while that of the p806 system is induced by chemical treatment with IPTG.

Serum samples from two groups of Japanese HTLV-1-positive subjects, healthy carriers, and ATL patients were used to study the prevalence of antibodies to each of the

TABLE 1. Characteristics of HTLV-1 envelope RPs

Plasmid	Expression vector	gp61 amino acid sequence	Region of HTLV-1 envelope gp61[a]	Observed mass[b] (kDa)	Approx amt of protein synthesized (%)[c]
pA	pJL6	26–165	N-gp46	18	10
pB	p806	166–229	M	25	2
pB1	p806	166–201	M	23	5
pC	pJL6	229–308	C-gp46	15	8
pD	p806	313–488	gp21	32, 30	5

[a] N, N terminus; M, middle region; and C, C terminus of gp46, the exterior domain of gp61; gp21, the transmembrane domain of gp61.
[b] In most instances, the observed mass deviates slightly from the predicted mass, which is an intrinsic property of the input gene sequence.
[c] Values are rough estimates of the relative quantities of recombinant proteins expressed against a background of total bacterial proteins as observed in Coomassie blue-stained SDS-PA gels.

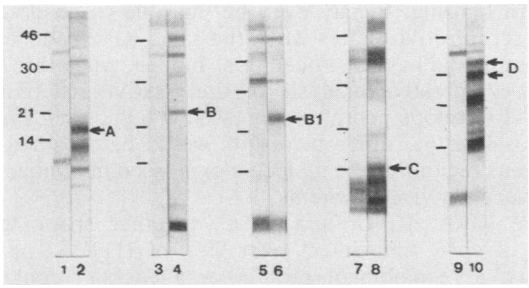

FIG. 4. Western blotting assay of the five recombinant proteins (arrows) detected by HTLV-1-positive sera (lanes 2, 4, 6, 8, and 10). Control sera are in lanes 1, 3, 5, 7, and 9. Sizes (in kilodaltons) are labeled at the left side of each of the reaction pairs. RP preparations from partial purification (19) were run on a 15% SDS–PA gel (16). After electrophoresis, the proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, N.H.) and assayed by the method described previously (3).

specific RPs. Initial screening for HTLV-1 seropositivity was standardized by using both the particle agglutination test and the immunofluorescence test (12). All samples were then confirmed as positive by both a Western blot assay (3) with MJ cell (24) lysates and a radioimmunoprecipitation assay with [^{35}S]cysteine-labeled MT-2 (20) antigens. As shown in Table 2, the results of the studies assaying for antibody reactivity to the RPs were as follows: 22.2% (10 of 45) of samples from the carriers and 16.9% (13 of 77) of samples from the ATL patients were reactive to RP-A; 80% (36 of 45) of samples from the carriers and 95% (76 of 80) of samples from the ATL patients were reactive to RP-B; 72.7% (32 of 44) of samples from the carriers and 68.8% (53 of 77) of samples from the ATL patients were reactive to RP-B1; and 91.1% (41 of 45) of samples from the carriers and 93.8% (76 of 81) of samples from the ATL patients were reactive to RP-C.

Together, the antibody reactivity rates for all the serum samples, as assayed by Western blot, were as follows: 18.9% (23 of 122) reacted with RP-A, 89.6% (112 of 125) reacted with RP-B, 70.2% (85 of 121) reacted with RP-B1, and 92.9% (117 of 126) reacted with RP-C. These results indicate that the C-terminal half of gp46 (RP-B plus RP-C) detected 97.6% (123 of 126) of the HTLV-1-positive sera, a percentage which is significantly higher ($P < 0.005$) than that detected by the N-terminal half of gp46 (RP-A). RP-A, RP-B, and RP-C, which together span the entire length of gp46 except the first five amino acids at the N terminus and the last four amino acids at the C terminus, detected 99.2% (125 of 126) of the HTLV-1-positive subjects. Interestingly, this same combination of RPs detected every ATL patient in this study. In contrast, RP-D, which contains the transmembrane envelope protein gp21 minus the first amino acid at the N terminus, had only a 73.7% (84 of 114) reactivity rate. The difference in the rates of antibody response between gp46 (RP-A plus RP-B plus RP-C) and gp21 (RP-D) is statistically significant ($P < 0.005$).

The results of the Western blot assay shown in Fig. 4 and the serological data summarized in Table 2 indicate that many antigenic determinants of the gp61 protein retain their antibody-binding specificities in the denatured forms of the RPs. Indeed, all of the RPs, with the exception of RP-A, were able to react with over 70% of the HTLV-1-positive sera.

RP-A, on the other hand, detected a much smaller percentage of the HTLV-1-positive sera, although it does contain many potentially reactive epitopes (see antigenic index of HTLV-1 envelope protein in Fig. 3c). It is possible that many epitopes residing on the N terminus of gp46 may be hindered by interactions between gp46 and other molecules such as gp21. Such a scenario was in fact considered in an analysis of the human immunodeficiency virus type 1 (HIV-1) external glycoprotein (15). For this study, Kowalski et al. (15) constructed a panel of HIV-1 envelope mutants and analyzed the phenotypic changes corresponding to each genetic mutation. Their results indicated that among the regions tested, those responsible for the association of HIV-1 gp120 with HIV-1 gp41 (analogous to HTLV-1 gp46 and gp21, respectively) are clustered at the N-terminal half of the gp120 molecule.

Conformational epitopes may also play an important role in determining the relative immunogenicity of the N-terminal half of gp46. Thus, when the proteins are not in their natural configurations, they may not be recognized by the same antibodies which react with the native protein. As shown in Fig. 3d, there are 10 cysteine residues in the N-terminal half of gp46, but only 4 in the C-terminal half. It is therefore possible that disulfide bonds between these cysteine residues might be especially significant to the tertiary structure of the gp46 protein at its N terminus. Since all the RPs used for the Western blot assays were solubilized in either a 3 or 8 M urea–Tris hydrochloride buffer solution, their conformational epitopes were denatured and could not be studied by the assay.

Nevertheless, when comparing the relative antibody reactivities of the various regions of HTLV-1 gp61 by using denatured RPs in Western blot assays (Table 2), we found that the C-terminal half of gp46 (RP-B plus RP-C) was the most immunogenic region of the protein. This region reacted with over 95% of the HTLV-1-positive sera, whereas the N-terminal half of gp46 (RP-A) had a much lower reaction rate. Perhaps more interesting still is the fact that RP-B1, which contains only 36 amino acids from the middle region of HTLV-1 gp46, reacted with 70% of the HTLV-1-positive sera. If one examines antibody reactivity as a function of relative polypeptide length, RP-B1 may be considered a highly reactive domain in the HTLV-1 envelope protein. As illustrated in Fig. 3c, RP-B1 did have a rather high antigenic index in its predicted peptide sequence. This index was calculated by summing several weighted measures of sec-

TABLE 2. Rates of antibody positivity for various groups of HTLV-1-positive subjects to RPs of the HTLV-1 envelope protein

Serum source	Reactivity[a] [no. positive/no. tested (% positive)] with:						
	RP-A	RP-B	RP-B1	RP-C	RP-D	RP-B + RP-C	RP-A + RP-B + RP-C
HTLV-1 carriers	10/45 (22.2)	36/45 (80.0)	32/44 (72.7)	41/45 (91.1)	25/45 (55.6)	43/45 (95.6)	44/45 (97.8)
ATL patients	13/77 (16.9)	76/80 (95.0)	53/77 (68.8)	76/81 (93.8)	59/69 (85.5)	80/81 (98.8)	81/81 (100)
Total	23/122 (18.9)	112/125 (89.6)	85/121 (70.2)	117/126 (92.9)	84/114 (73.7)	123/126 (97.6)	125/126 (99.2)

[a] Rates of reactivity to RP-D versus RP-B plus RP-C were statistically significant ($P < 0.005$).

TABLE 3. Patterns of antibody reactivity to different regions of the HTLV-1 envelope protein in HTLV-1 carriers and ATL patients

Pattern	Reactivity with:			No. (%) of samples with pattern		
	RP-A (N-gp46)	RP-B + RP-C (C-gp46)	RP-D (gp21)	ATL patients	HTLV-1 carriers	Total
1	−	+	−	8 (24.2)	13 (28.9)	21 (26.9)
2	−	+	+	20 (60.6)	21 (46.7)	41 (52.6)
3	+	+	−	0	2 (4.4)	2 (2.6)
4	+	+	+	4 (12.1)	7 (15.6)	11 (14.1)
5	+	−	−	1 (3.0)	1 (2.2)	2 (2.6)
6	−	−	−	0	1 (2.2)	1 (1.3)

ondary structure, including the hydrophobicity, the surface probability, the flexibility, the Chou-Fasman structure, and the Robson-Garnier structure (11). It was used in this paper for reference, but we do not imply that our analysis is in any way meant to test the validity of this model.

Seropositivity rates for RP-D (HTLV-1 gp21) were significantly lower than those for RP-B or RP-C (C terminus of gp46) in the HTLV-1 carrier and ATL patient groups and in the two groups combined ($P < 0.005$). In an earlier report, workers in one of our laboratories described a higher degree of reactivity for a recombinant polypeptide representing HTLV-1 gp21 (pKS400; see Introduction and reference 25). However, this study was based on the analysis of a relatively small panel of HTLV-1-positive serum samples, and the recombinant protein expressed by pKS400 contains several amino acids from the gp46 C-terminal region, whereas RP-D represents sequences from gp21 only.

Of further interest in the present study was the difference in rates of RP-D seroreactivity between HTLV-1 carriers (55.6%) and ATL patients (85%) ($P < 0.01$). Whether this difference bears any direct relationship to disease development cannot be determined from the current studies.

As controls, 50 serum samples from HTLV-1 antibody-negative Japanese people living in the same HTLV-1-endemic region were tested for their reactivities to the various RPs. None of the control sera reacted with any of the HTLV-1 *env* RPs.

Table 3 provides an analysis of antibody reactivity patterns found in sera from HTLV-1 carriers and ATL patients. The regions of HTLV-1 gp61, as expressed in *E. coli*, are defined as RP-A for the N-terminal half of gp46 (N-gp46), RP-B plus RP-C for the C-terminal half of gp46 (C-gp46), and RP-D for the gp21 transmembrane protein. In the most common serological pattern, pattern 2, there was a negative reaction to N-gp46 and a positive reaction to both C-gp46 and gp21. However, 24.2% to 28.9% of HTLV-1-positive serum samples showed antibodies to C-gp46 only (pattern 1). Eleven of 78 (14.1%) of the HTLV-1-positive serum samples had antibodies to all regions of gp61 represented by the RPs (pattern 4). Surprisingly, two cases (one an ATL patient, and the other a healthy carrier) had antibodies only to N-gp46, which according to the results of this study is the least reactive region of the entire HTLV-1 envelope protein.

The serum of one HTLV-1 carrier was also found to show no antibody reactivity to any of the RPs tested. However, both the Western blot assay and radioimmunoprecipitation showed that this patient's serum had antibodies to HTLV-1 p19 and p24 *gag* gene proteins (group-specific antigens). It is possible that this person was actually infected with an HTLV-1-related virus or that antibodies to gp46, gp61, and/or the RPs may be detected by techniques other than Western blotting. It may even be possible to observe different seropositivity rates when the same RPs are tested by different immunoassay procedures. Indeed, since this study was a comparative analysis of the relative reactivity of HTLV-1 envelope peptides expressed in a bacterial system, it is possible that different results would be obtained with analogous regions of the molecule expressed in nonbacterial (e.g., vaccinia virus) systems.

Since RP-A, RP-B, and RP-C, together spanning the length of gp46, can detect over 99% of HTLV-1-positive subjects, a recombinant protein or a cocktail containing various proteins representing this region may be a logical candidate for use in blood-screening and epidemiological studies. Such an antigen preparation would probably provide greater specificity than current diagnostic assays based on disrupted whole virus and/or infected-cell antigen preparations. Furthermore, since the rate of in vitro production for HTLV-1 is quite low, the use of bacterially expressed RPs as antigens may in fact provide a more economical and abundant source of well-defined envelope proteins for the assay systems currently in use.

This work was supported in part by Public Health Service grant 1 ROI CA 38450 from the National Institutes of Health, grant I-P50-HL-33774 from the Center for Blood Research, and Outstanding Investigator Award CA39805 from the National Cancer Institute.

We thank S. Finkelstein for manuscript preparation, G. W. Smythers for computer analysis, and M. F. McLane, X. F. Yu, Z. Matsuda, M. Matsuda, M. Chen, B. Du, W.-J. Syu, and G. C. DuBois for technical assistance and helpful discussions.

LITERATURE CITED

1. **Amann, E., M. Broker, and F. Wurm.** 1984. Expression of herpes simplex virus type 1 glycoprotein C antigens in *Escherichia coli*. Gene **32**:203–215.
2. **Bachmair, A., D. Finley, and A. Varshavsky.** 1986. In vitro half life of a protein is a function of its amino-terminal residue. Science **234**:179–186.
3. **Barin, F., S. M'Boup, F. Denis, P. Kanki, J. S. Allan, T. H. Lee, and M. Essex.** 1985. Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of West Africa. Lancet **ii**:1387–1389.
4. **Clapman, P., K. Nagy, and R. A. Weiss.** 1984. Pseudotypes of human T-cell leukemia virus type 1 and 2: neutralization by patients' sera. Proc. Natl. Acad. Sci. USA **81**:2886–2889.
5. **Clarke, M. F., E. P. Gelmann, and M. S. Reitz, Jr.** 1983. Homology of human T-cell leukaemia virus envelope gene with class I HLA gene. Nature (London) **305**:60–62.
6. **Copeland, T. D., W. Tsai, Y. D. Kim, and S. Oroszlan.** 1986. Envelope proteins of human T-cell leukemia virus type 1: characterization by antisera to synthetic peptides and identification of a natural epitope. J. Immunol. **137**:2945–2951.
7. **Eiden, M., M. Newman, A. G. Fisher, D. L. Mann, P. M. Howley, and M. S. Reitz.** 1985. Type 1 human T-cell leukemia virus small envelope protein expressed in mouse cells by using a bovine papillomavirus-derived shuttle vector. Mol. Cell. Biol. **5**:3320–3324.
8. **Essex, M., P. Kanki, J. Allan, F. Barin, M. F. McLane, T. H. Lee, L. Kitchen, T. Homma, G. Malone, I. Yokota, N. Tachibana, and J. Groopman.** 1985. HTLV and immunosuppression, p. 309–318. *In* M. Miwa, H. Sugano, T. Sugimura, and R. A. Weiss (ed.), Retroviruses in human lymphoma/leukemia. Japan Scientific Society Press, Tokyo.
9. **Feig, L. A., B. T. Pan, T. M. Roberts, and G. M. Cooper.** 1986. Isolation of *ras* GTP-binding mutants using an in situ colony-binding assay. Proc. Natl. Acad. Sci. USA **83**:4607–4611.
10. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. Kinoshita, S. Shirakawa, and I. Miyoshi.** 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc. Natl. Acad. Sci. USA **78**:6476–6480.

11. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78**:3824–3828.
12. **Ishizaki, J., A. Okayama, N. Tachibana, T. Yakota, E. Shishime, K. Tsuda, and N. Mueller.** 1988. Comparative diagnostic assay results for detecting antibody to HTLV-1 in Japanese blood donor samples: higher positive rates by particle agglutination assay. J. AIDS **1**:340–345.
13. **Jacobson, S., C. S. Raine, E. S. Mingiolio, and D. E. McFarlin.** 1988. Isolation of an HTLV-1-like retrovirus from patients with tropical spastic paraparesis. Nature (London) **331**:540–543.
14. **Kiyokawa, T., H. Yoshikura, S. Hattori, M. Seiki, and M. Yoshida.** 1984. Envelope proteins of human T-cell leukemia virus: expression in *Escherichia coli* and its application to studies of *env* gene functions. Proc. Natl. Acad. Sci. USA **81**:6202–6206.
15. **Kowalski, M., J. Potz, L. Basiripour, D. Tatyana, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lautenberger, J. A., A. Seth, C. Jorcyk, and T. S. Papas.** 1984. Useful modifications of *Escherichia coli* expression plasmid pJL6. Gene Anal. Technol. **42**:49–57.
18. **Lee, T. H., T. Homma, K. T. Schultz, M. F. McLane, N. Tachibana, C. W. S. Howe, and M. Essex.** 1984. Antigens expressed by human T-cell leukemia virus-transformed cells, p. 111–120. *In* R. C. Gallo, M. E. Essex, and L. Gross (ed.), Human T-cell leukemia/lymphoma virus. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Matsuda, Z., M. J. Chou, M. Matsuda, J. Huang, Y. Chen, R. Redfield, K. Mayer, M. Essex, and T. H. Lee.** 1988. Human immunodeficiency virus type 1 has an additional coding sequence in the central region of the genome. Proc. Natl. Acad. Sci. USA **85**:6968–6972.
20. **Miyoshi, I., I. Kubonishi, S. Yoshimoto, T. Akagi, Y. Ohtsuki, Y. Shiraoshi, K. Nagata, and Y. Hinuma.** 1981. Type C virus particles in a cord T-cell line derived by co-cultivating normal cord leukocytes and human leukaemic T-cells. Nature (London) **294**:770–771.
21. **Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Amitani, A. Igata, M. Matsumoto, and M. Tara.** 1986. HTLV-I associated myelopathy, a new clinical entity. Lancet **i**:1031–1032.
22. **Pallas, D. C., C. Sohley, M. Mahoney, E. Harlow, B. S. Schaffhausen, and T. M. Roberts.** 1986. Polyomavirus small t antigen: overproduction in bacteria, purification, and utilization for monoclonal and polyclonal antibody production. J. Virol. **60**:1075–1084.
23. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77**:7415–7419.
24. **Popovic, M., P. S. Sarin, M. Robert-Gurroff, V. S. Kalyanaraman, D. Mann, J. Minowada, and R. C. Gallo.** 1983. Isolation and transmission of human retrovirus (human T-cell leukemia virus). Science **219**:856–859.
25. **Samuel, K. P., J. A. Lautenberger, C. L. Jorcyk, S. Josephs, F. Wong-Staal, and T. S. Papas.** 1984. Diagnostic potential for human malignancies of bacterially produced HTLV-1 envelope protein. Science **226**:1094–1097.
26. **Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida.** 1983. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc. Natl. Acad. Sci. USA **80**:3618–3622.
27. **Shida, H., T. Tochikura, T. Sato, T. Konno, K. Hirayoshi, M. Seki, Y. Ito, M. Hatanaka, Y. Hinuma, M. Sugimoto, F. Takahashi-Nishimaki, T. Maruyama, K. Miki, K. Suzuki, M. Morita, H. Sashiyama, and M. Hayami.** 1987. Effect of the recombinant vaccinia viruses that express HTLV-1 envelope gene on HTLV-1 infection. EMBO J. **6**:3379–3384.
28. **Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for $CD4^+$ cell depletion. Cell **54**:561–575.
29. **Toneguzzo, F., S. Glynn, E. Levi, S. Mjolsness, and A. Hayday.** 1988. Use of a chemically modified T7 DNA polymerase for manual and automated sequencing of supercoiled DNA. Biotechniques **6**:460–469.
30. **Yoshida, M., M. Seiki, K. Yamaguchi, and K. Takatsuki.** 1984. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proc. Natl. Acad. Sci. USA **81**:2534–2537.