JOURNAL OF VIROLOGY, Apr. 1991, p. 1870–1876
0022-538X/91/041870-07$02.00/0

Vol. 65, No. 4

Characterization of Immunodominant Epitopes of *gag* and *pol* Gene-Encoded Proteins of Human T-Cell Lymphotropic Virus Type I

RENU B. LAL,[1]* DONNA L. RUDOLPH,[1] KEVIN P. GRIFFIS,[1] KATSUHIKO KITAMURA,[1] MITSUO HONDA,[2] JOHN E. COLIGAN,[3] AND THOMAS M. FOLKS[1]

Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia 30333[1]; AIDS Research Center, National Institute of Health, Kamiosaki, Tokyo 141, Japan[2]; and Biological Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20894[3]

Received 2 November 1990/Accepted 4 January 1991

A series of synthetic peptides derived from the corresponding regions of the *gag*, *pol*, and *env* proteins of human T-cell lymphotropic virus types I (HTLV-I) and II (HTLV-II) were used in an enzyme immunoassay to map the immunodominant epitopes of HTLV. Serum specimens from 79 of 87 (91%) HTLV-I-infected patients reacted with the synthetic peptide Gag-1a (amino acids [a.a.] 102 to 117) derived from the C terminus of the $p19^{gag}$ protein of HTLV-I. Minimal cross-reactivity (11%) was observed with serum specimens from HTLV-II-infected patients. Peptide Pol-3, encoded by the *pol* region of HTLV-I (a.a. 487 to 502), reacted with serum specimens from both HTLV-I- and HTLV-II-infected patients (94 and 86%, respectively). The antibody levels to Pol-3 were significantly higher ($P < 0.01$) in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis than in either adult T-cell leukemia patients or HTLV-I-positive asymptomatic carriers. None of the other peptides studied demonstrated significant binding to serum specimens obtained from HTLV-I- or HTLV-II-infected individuals. While Gag-1a did not react with serum specimens from normal controls, Pol-3 demonstrated some reaction with specimens from seronegative individuals (11.4%). The antibodies to Gag-1a and Pol-3 in serum specimens from HTLV-I-infected patients could be specifically inhibited by the corresponding synthetic peptides and by a crude HTLV-I antigen preparation, indicating that these peptides mimic native epitopes present in HTLV-I proteins that are recognized by serum antibodies from HTLV-I- and -II-infected individuals.

The human T-cell lymphotropic virus (HTLV) viral genome consists of four major regions, *gag*, *pol*, *env*, and *tax*. The gene products from each region are recognized as p19, p24, and p15; reverse transcriptase; gp46 and gp21; and p40, respectively (9). Persons infected with HTLV usually develop antibodies to *gag* and *env* proteins (26). Unlike the situation with human immunodeficiency virus (HIV) infection (29), natural antibodies to the *pol* gene product (25) have not been documented.

HTLV type I (HTLV-I) has been etiologically associated with adult T-cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (6). HTLV-I is endemic in southwestern Japan, the Caribbean, and some regions of Africa (2) and among certain risk groups, including intravenous drug users (IVDU) and prostitutes in the United States (2, 15). A low seroprevalence rate (0.025%) has also been documented for the normal blood donor population in the United States (1, 30). Another human retrovirus, HTLV-II, has been isolated from a patient with hairy cell leukemia (14). While little is known about the disease association and modes of transmission of HTLV-II, recent evidence suggests that certain IVDU populations may have elevated rates of infection with this virus (18).

Advances in diagnostic testing and vaccine development will require a clearer understanding of the humoral and cellular immune responses to infection with HTLV. One approach to identifying and mapping antigenic determinants on HTLV is to synthesize peptides representing potential antigenic regions of HTLV in order to analyze the binding of these peptides to antibodies from HTLV-infected patients. In addition, antipeptide antibodies can also be tested for other activities, such as virus neutralization and inhibition of syncytium formation. The general rules for selecting sequences for synthesis are that the peptides should be 10 to 20 amino acids in length and have a surface location as predicted by hydrophilicity and secondary-structure analyses (3, 11). Since we were also interested in analyzing type-specific antibodies, we chose several peptides that showed considerable differences in the primary sequences between HTLV-I and HTLV-II (27, 28). Using these criteria, we synthesized 18 peptides representing various proteins encoded by the HTLV *gag*, *pol*, and *env* genes. In the present report, we describe the characterization of the immunodominant epitopes represented by these peptides and provide the first evidence that the polymerase region of HTLV-I elicits a humoral immune response in persons infected with this virus.

MATERIALS AND METHODS

Peptide selection and synthesis. Using published amino acid sequences (27, 28), we aligned the *gag*, *pol*, and *env* regions of HTLV-I and HTLV-II sequences. The GenBank accession numbers are J02029 for HTLV-I and M10060 for HTLV-II. Several peptides were selected for synthesis by identifying regions in which HTLV-I and HTLV-II showed amino acid sequence differences. Their relative positions in the HTLV genome are shown in Fig. 1. Secondary-structure characteristics of the proteins were determined with the peptide structure and plot structure programs in the Sequence Analysis Software Package, version 5.0 (Genetics Computer Group, University of Wisconsin, Madison), and

* Corresponding author.

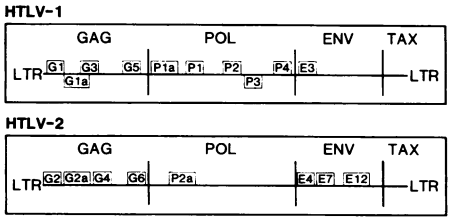

FIG. 1. Relative positions of synthetic peptides in the HTLV-I (upper panel) and HTLV-II (lower panel) genome. The position of each peptide is shown by the small boxes. LTR, Long terminal repeat.

hydrophilicity characteristics were calculated by the method of Hopp and Woods (11).

Synthetic peptides were made on the MillGen 9050 Pepsynthesizer with 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry, using the manufacturer's reagents and recommended chemistry cycles. Peptides were cleaved from the resin, precipitated, and extracted several times with anhydrous ether. Final purification was done by preparative high-performance liquid chromatography (HPLC) on a Waters C18 Delta-Pak (19 mm by 30 cm, 15-μm particle, 300-μm pore size), using 0.1% trifluoroacetic acid (TFA) in water as the starting solvent, followed by a 0 to 50% acetonitrile gradient in 0.1% TFA. Amino acid composition, amino acid sequence analysis, and analytical reverse-phase HPLC were performed to confirm peptide sequence and purity.

Human serum specimens. A total of 215 serum specimens from various study groups were chosen for this study (Table 1), including 122 specimens from subjects who were seropositive for HTLV-I or -II. All seropositive specimens from the U.S. study population have previously been characterized (17). All seropositive specimens were confirmed to be from HTLV-I- or HTLV-II-positive individuals by polymerase chain reaction (PCR) assays, performed with DNA derived from peripheral blood lymphocytes from these individuals (5). None of these specimens were from individuals infected with both HTLV-I and HTLV-II. All Japanese sera were confirmed to be HTLV-I positive by their reactivity in the HTLV-I-specific synthetic peptide immunoassay (unpublished data). For comparison, serum specimens from 15 patients with confirmed HIV infection manifesting as asymptomatic (n = 8) or AIDS (n = 7) were tested. Serum specimens representing various clinical diseases (n = 43), including 16 with viral infection (cytomegalovirus, 3; Epstein-Barr virus, 3; herpes simplex virus, 3; hepatitis B virus, 4; and rubella virus, 3) and 27 with parasitic infection (*Plasmodium falciparum*, 3; *Toxoplasma gondii*, 3; *Trypanosoma cruzi*, 5; *Schistosoma mansoni*, 5; *Strongyloides stercoralis*, 6; and *Wuchereria bancrofti*, 5) were included to test for nonspecific interference. Serum specimens from 35 normal blood donors served as negative controls.

TABLE 1. Standard serologic test results

Study group	No. tested	Origin	Serology test	
			HTLV-I/II	HIV
HTLV-I infected				
HAM/TSP	5	Japan	+	−
	13	U.S.	+	−
ATL	29	Japan	+	−
Asymptomatic	32	Japan	+	−
	8	U.S.	+	−
HTLV-II infected (asymptomatic)	35	U.S.	+	−
HIV-1 infected	15	U.S.	−	+
Other infection[a]	43	U.S.	−	−
Normal	35	U.S.	−	−

[a] As described in Materials and Methods.

Reference HTLV and HIV antibody tests. Serum specimens from all patients were initially tested for HTLV-I antibodies with a commercial HTLV-I enzyme-linked immunosorbent assay (ELISA; Du Pont, Wilmington, Del.), according to the manufacturer's recommendations. Specimens that were repeatedly reactive were further tested by immunoblotting and the radioimmunoprecipitation assay as described previously (8). A serum specimen was determined to be positive if antibody reactivity was detected to at least two different HTLV structural gene products ($p24^{gag}$ and $gp46^{env}$ and/or $gp68^{env}$). Antibodies to HIV proteins were determined by both ELISA and immunoblot analysis (Du Pont), and only those specimens that had antibodies to both *gag* and *env* proteins were included.

Quantitative assessment of antibodies to synthetic peptides. An enzyme immunoassay (EIA) was developed to detect antibodies to synthetic peptides in a way similar to that described previously (17). Briefly, polyvinyl plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 50 μl of synthetic peptides (100 μg/ml) in 0.01 M carbonate buffer, pH 9.6, and incubated overnight at 4°C. The plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-T) six times, and each well was incubated with 200 μl of 3% bovine serum albumin (BSA) in PBS-T for 1 h at 37°C to block excess reactive sites. After the wells were washed, a 1:20 dilution of each test serum was added to duplicate wells, and the plates were incubated for 90 min at room temperature, followed by addition of p-nitrophenyl phosphate (Sigma) substrate. The plates were read with an ELISA reader (SLT Lab Instruments, Ronkonkome, Austria) at 405 nm. Each serum specimen was also assayed in plates coated with BSA alone or an unrelated synthetic peptide to control for nonspecific antibody binding. Seropositivity was defined as any value greater than the mean of the normal controls + 2 standard deviations (SDs). For quantitative comparison, the amount of immunoglobulin G (IgG) antipeptide antibodies in each serum was calculated in arbitrary units, determined by comparison with a control serum pool defined as having 100 U/ml.

Inhibition of antibody binding to the peptide was carried out by adding increasing concentrations of peptide or purified HTLV-I or HTLV-II antigen (1 to 10 μg/ml) in the ELISA. The serum was mixed with the inhibition antigen immediately before it was added to the peptide-coated plate, followed by assay as described above. The results were calculated as the percent inhibition of antibody binding in three independent experiments.

Statistical analysis. Student's t test was used for statistical evaluation as noted.

RESULTS

Seroreactivity of synthetic peptides. A noncompetitive EIA was developed with synthetic peptides as a solid phase to detect antibodies in subjects infected with HTLV-I or -II. A total of 87 serum specimens from HTLV-I-infected persons, 35 from HTLV-II-infected persons, and 35 from normal donors were tested against the panel of peptides (Table 2). One synthetic peptide, Gag-1a (HTLV-I; amino acids [a.a.]

TABLE 2. Seroreactivity of serum specimens from HTLV-I- and HTLV-II-infected individuals with synthetic peptides

Peptide code	Amino acids	Sequence	Seroreactivity[a] (% of samples)	
			HTLV-I (n = 87)	HTLV-II (n = 35)
gag-encoded protein				
HTLV-I				
Gag-1	88–101	IQTQAQIPSRPAPP	5	0
Gag-1a	102–117	PPSSPTHDPPDSDPQI	90	11
Gag-3	126–141	APQVLPVMHPHGAPPN	2	1
Gag-5	416–429	ADIPHPKNFIGGEV	2	ND[b]
HTLV-II				
Gag-2	88–104	VKNQVSPSAPAAPVPTP	5	5
Gag-2a	108–123	TTTPPPPPPPSPEAHV	7	10
Gag-4	132–147	TTQCFPILHPPGAPSA	3	2
Gag-6	418–433	STSGTTEEKNSLRGEI	2	2
pol-encoded protein				
HTLV-I				
Pol-1a	11–27	TGASRPWARTPPKAPRN	10	2
Pol-1	296–315	PRDQIYLNPSQVQSLVQLRQ	5	ND
Pol-2	400–415	ISTQTFNQFIQTSDHP	5	ND
Pol-3	487–502	KQILSQRSFPLPPPHK	96	86
Pol-4	640–658	GGNPQHQMPRGHIRRGLLP	2	ND
HTLV-II				
Pol-2a	46–62	SHSKHHRPRTPSPTSPS	ND	5
env-encoded protein				
HTLV-I				
Env-3	3–24	KFLATLILFFQFCPLIFGDYSP	0	0
HTLV-II				
Env-4	3–19	NVFFLLLFSLTHFPLAQ	0	2
Env-7	45–60	TWNLDLNSLTTDQRLH	ND	5
Env-12	313–327	PSQPSLWTHCYQPRL	ND	7

[a] Seropositivity was defined as any value greater than the mean of the normal controls ± 2 standard deviations.
[b] ND, Not determined.

102 to 117), derived from the p19^{gag} protein of HTLV-I, demonstrated a high degree of reactivity (79 of 87, 91%) with serum specimens from HTLV-I-infected persons and some cross-reaction (4 of 35, 11%) with specimens from HTLV-II-infected persons (Table 2). None of the other peptides derived from the *gag* proteins of HTLV-I and HTLV-II demonstrated significant reactivity.

Of the six peptides derived from the *pol*-encoded proteins, only Pol-3 (HTLV-I; a.a. 487 to 502) reacted with serum specimens obtained from persons infected with HTLV-I (82 of 87, 96%) and HTLV-II (30 of 35, 86%) (Table 2). In addition, 4 of the 35 serum specimens (11.4%) from normal donors demonstrated low levels of reactivity to Pol-3. None of the 15 serum specimens from subjects with HIV infection or 43 of those from subjects with other infections reacted with any of these peptides (data not shown).

To further investigate the specificity of Gag-1a and Pol-3, a competitive inhibition experiment with serum specimens from five HTLV-I-infected patients was performed by incubating the serum specimens with Gag-1a peptide, Pol-3 peptide, and HTLV-I and HTLV-II antigen. The antibody reactivity against Gag-1a and Pol-3 could be specifically inhibited by preincubating the serum with the respective peptides and HTLV-I proteins in a dose-dependent manner, whereas incubation with HTLV-II proteins or unrelated peptides had a minimal effect (Fig. 2).

The envelope region of both HTLV-I and HTLV-II is known to be immunodominant in natural infection (9). Studies in our laboratories have recently identified two peptides from the outer envelope protein, gp46, of HTLV-I that contain HTLV-I-specific epitopes (17). In the present study, four additional peptides (Env-3, Env-4, Env-7, and Env-12) were chosen because the homology alignment between the envelope proteins of HTLV-I and HTLV-II showed them to correspond to regions of significant differences. All of the peptides demonstrated minimal reactivity (0 to 7%) with

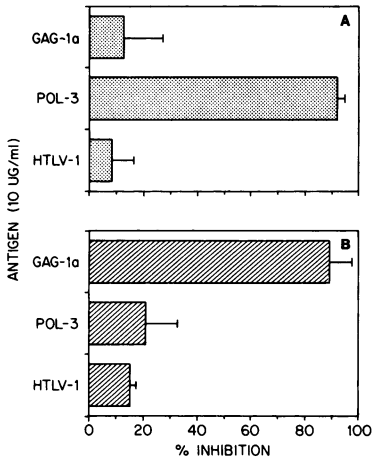

FIG. 2. Competitive inhibition of anti-Gag-1a (A) and anti-Pol-3 (B) antibodies in serum specimens from patients infected with HTLV-I by Gag-1a peptide, Pol-3 peptide, and HTLV-I antigen. The results are expressed as the mean percent inhibition for three separate experiments.

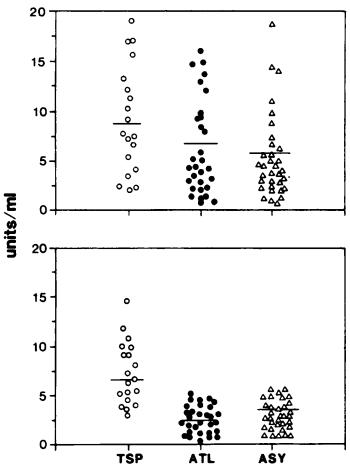

FIG. 3. IgG antibodies to Gag-1a (upper panel) and Pol-3 (lower panel) peptide in patients with HTLV-I infection with different clinical manifestations: HAM/TSP (○), ATL (●), and asymptomatic (ASY) (△). The horizontal bar represents the geometric mean of the IgG antibodies.

serum specimens from HTLV-I- and HTLV-II-infected persons (Table 2).

Levels of antibodies to Gag-1a and Pol-3. To evaluate the relative distribution of antibodies, serum levels of IgG antibodies to Gag-1a and Pol-3 were compared in HTLV-I-infected individuals with clinical symptoms (HAM/TSP and ATL) and asymptomatic individuals. IgG anti-Gag-1a antibody (Fig. 3) did not differ significantly among the clinical groups (81 ± 24 U/ml for HAM/TSP, 57 ± 32 U/ml for ATL, and 50 ± 32 U/ml for asymptomatic individuals; $P > 0.05$ for all comparisons). In contrast, the seroreactivity of Pol-3 was significantly higher ($P < 0.01$) in patients with HAM/TSP (55 ± 23 U/ml) than in either ATL (22 ± 8 U/ml) or asymptomatic (29 ± 9 U/ml) individuals.

Antigenic index characteristics of Gag-1a and Pol-3. The secondary-structure characteristics of *gag* and *pol* proteins from both HTLV-I and HTLV-II were analyzed by using the computer algorithms developed by Chou and Fasman (3). Figure 4 shows a secondary-structure prediction for the *gag* region of HTLV-I and HTLV-II. Superimposed on the structural backbone are domains of high antigenic indices. The antigenic index is an algorithm designed by Jameson and Wolf (13) to predict surface domains for combined values of flexibility, hydrophilicity, and surface probability. One of the four regions with high antigenic indices (a.a. 101 to 121) lies within the C terminus of the $p19^{gag}$ protein, which overlaps the Gag-1a domain. Three other regions with predicted high antigenic indices are located near the C terminus of the *gag* protein of HTLV-II (a.a. 334 to 340, 365 to 377, and 384 to 394). The three antigen index domains within HTLV-II are also located at the C terminus of Gag (a.a. 334 to 340, 365 to 377, and 384 to 394). The absence of an antigenic structural motif at the C terminus of p19 within the HTLV-II sequence that corresponds to the Gag-1a (a.a. 102 to 117) determinant agrees with the failure to detect antibodies reactive with the Gag-2a peptide in serum specimens from persons infected with HTLV-II.

A similar analysis of the HTLV-I *pol* proteins demonstrated Pol-3 to be located in an area of high antigenic index (data not shown). However, although other *pol* peptides, such as Pol-1a and Pol-4, were derived from the regions with a high antigen index, less than 10% of the serum specimens from infected persons reacted with these peptides.

DISCUSSION

Using a series of synthetic peptides from conserved amino acid regions predicted to be antigenic epitopes, we located structural motifs within HTLV-I that are recognized by antibodies in serum specimens from persons infected with HTLV-I or HTLV-II. Of the various peptides derived from the *gag*, *pol*, and *env* regions of both HTLV-I and HTLV-II, only two reacted with serum specimens from HTLV-infected individuals. Gag-1a, derived from the C terminus of the $p19^{gag}$ protein (HTLV-I; a.a. 102 to 117), was an immunodominant epitope and reacted with 90% of the HTLV-I-infected serum specimens; a small percentage of HTLV-II-infected specimens also reacted with this peptide (11%), reflecting some degree of antigenic homology within HTLV-I and HTLV-II. Serum antibody reactivity against Gag-1a could be specifically inhibited with HTLV-I protein, indicating that these peptides mimic conformationally native epitopes present on the *gag* protein of HTLV-I.

The immunodominance of the C terminus region of the $p19^{gag}$ protein has been documented previously (9, 12, 23). More recently, Kuroda et al. (16) reported an immunodominant epitope contained within amino acids 100 to 130 of the C terminus of p19 that reacts with 100% of serum specimens from HTLV-I-infected persons. The reactivity of this peptide with HTLV-II-infected serum specimens was not tested in their study. Furthermore, a number of potential B-cell epitopes from the *gag* region have also been identified by the epitope-mapping technique (12). However, when the most promising sequences were incorporated in larger (17 to 21 a.a.) synthetic peptides and tested against greater numbers of serum specimens from HTLV-I-infected patients, the only epitope that showed strong reactivity was found at the C terminus of p19, suggesting that most of the epitopes of retrovirus *gag*-encoded proteins may be discontinuous, i.e., may be dependent on the conformational structure of the protein. These studies and the data presented here confirm that the C terminus region of p19 is highly immunogenic and further demonstrate that the C terminus of HTLV-I p19 contains a strain-specific epitope that reacts predominantly with serum specimens from HTLV-I-infected patients.

Previous analysis of the primary and secondary structures of proteins has shown that regions exposed on the cell surface (highly hydrophilic) with predicted β-turn secondary structure represent immunodominant epitopes (11). Specifically, this has been demonstrated for hepatitis B virus surface antigen (19), Rauscher murine leukemia virus (10), adenovirus spike protein (7), and herpes simplex virus envelope glycoprotein D (25). Similar analysis of the *gag* region of HTLV demonstrated that one of the hydrophilic regions with predicted β-turns is found in the region at the C terminus of the p19 from which Gag-1a is derived. The absence of such an antigenic epitope within the HTLV-II sequence may relate to our failure to detect antibodies to peptides derived from this region in the sera of HTLV-II-infected persons.

The C terminus region of the $p19^{gag}$ protein of HTLV-I is rich in proline (21, 27) and showed 30 to 50% similarity to regions of other proline-rich viral proteins such as those from Epstein-Barr virus, equine adenovirus type 1, encephalomyocarditis virus type B, and herpes simplex virus type 1 (Fig. 5). Since serum obtained from individuals infected with Epstein-Barr virus and herpes simplex virus did not react

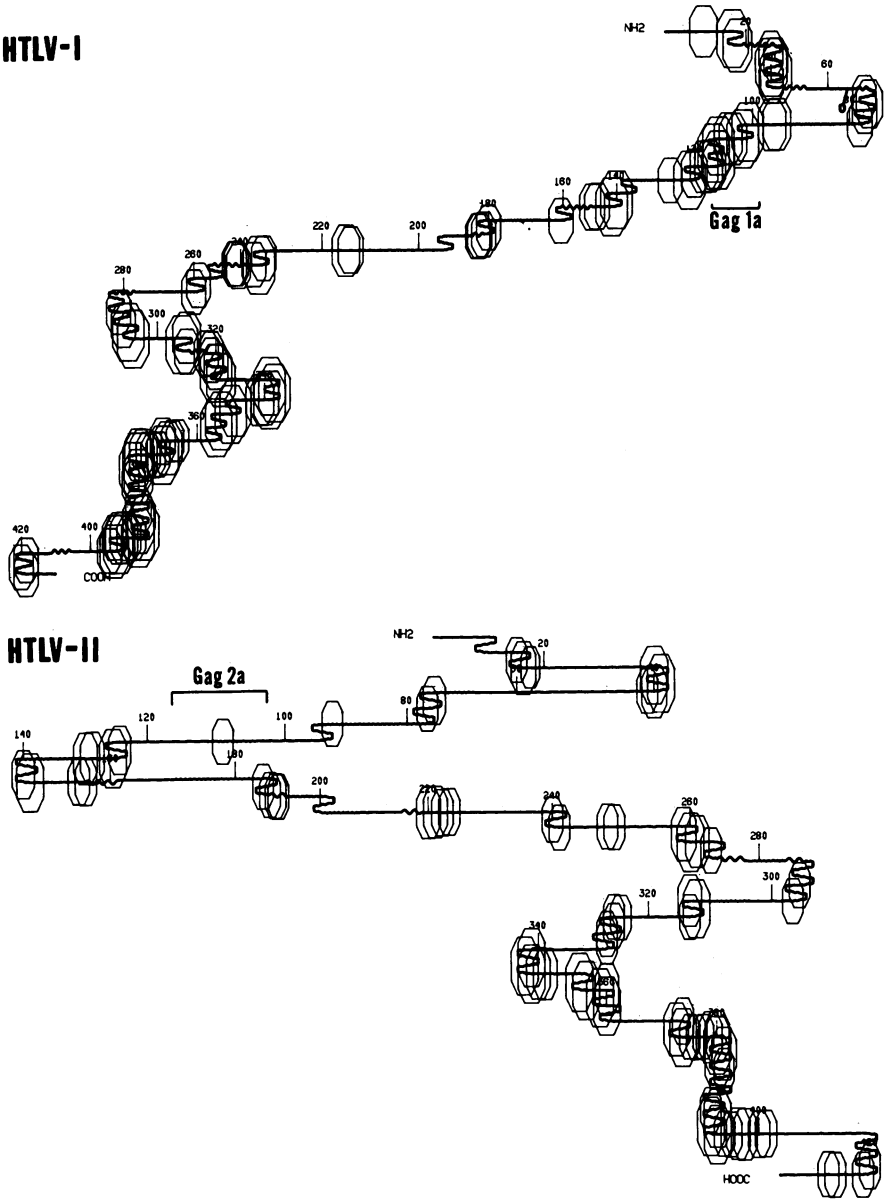

FIG. 4. Computer prediction of the secondary structure of *gag*-encoded protein of HTLV-I (top) and HTLV-II (bottom) superimposed with the antigenic index value. The radius of a circle over a residue is proportional to the mean antigenic index calculated for the residue plus the next five residues. The parameters for hydrophilicity, flexibility, and surface probability were averaged over five amino acid residues, with limits of 0.7 for hydrophilicity, 1.04 for flexibility, and 5.0 for surface probability to calculate the antigenic index.

with the Gag-1a peptide, the conserved regions presumably reflect the structural constraints on these proteins and the possible occurrence of a common precursor virus. The variations in the spacing of the conserved regions could be attributed to deletions or insertions that have occurred in the course of divergent evolution and may have structural as well as functional importance.

In a natural infection with a retrovirus, the host generally makes antibodies to the products of the *gag* and/or *env* gene products (2, 9). Antibody responses to the products of the *pol* gene during infection with HTLV-I or HTLV-II have not been described and could be attributed to the relative quantity of the *pol* gene product within the virus lysate. Indeed, recent studies with HTLV-II RNA indicate that two translation frameshift events are required for the synthesis of *pol* and that the relative amounts of *pol* and *gag* products are only about 1:100 (20). The results presented here demonstrate that most persons infected with HTLV-I or HTLV-II have readily detectable levels of antibodies to the peptide derived from the central region of the *pol* gene. While the significance of the immune response in humans to this protein remains to be established, these results clearly indicate that HTLV *pol* products induce antibody responses in a manner similar to antibody responsiveness to *pol* gene products during HIV infection (29). Therefore, it might now be advantageous to use the *pol* peptide to screen sera from patients with disease possibly caused by a retrovirus.

Of greater significance is the finding that patients with

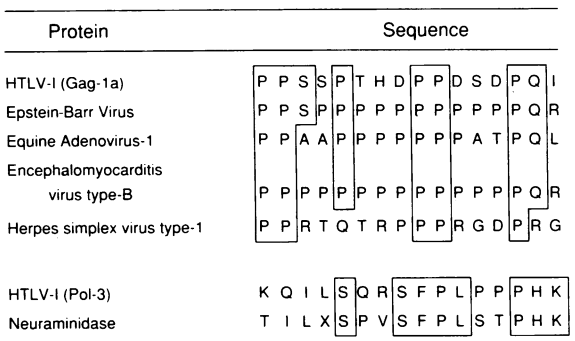

FIG. 5. Comparison of amino acid sequence from Gag-1a and Pol-3 with the protein data base by the Align program. Identical residues are boxed.

HAM/TSP had significantly higher levels of antibodies to Pol-3 than the asymptomatic carriers. This increased antibody production could result from more efficient replication of HTLV-I or preferential growth of infected cells in patients with HAM/TSP. Indeed, patients with HAM/TSP have been shown to induce significantly high levels of HTLV-I-specific antibodies (22). Furthermore, while the *pol* gene of HTLV has remarkable sequence homologies with that of other type C retroviruses (9), the Pol-3 amino acid sequence did not show homology to *pol* protein from other retroviruses. Instead, considerable homology (Fig. 5) was observed with neuraminidase, a surface glycoprotein of influenza virus A and B virions (4). Thus, it is conceivable that the antibody responsiveness to Pol-3 in normal donors could have been due to prior exposure to influenza virus.

In conclusion, we have identified two immunodominant epitopes present on the *gag*- and *pol*-encoded proteins of HTLV-I. Whether these epitopes are also involved in induction of protective immune responses remains to be determined. Finally, it seems likely that custom-made synthetic peptides will play an increasingly important role in the diagnosis and characterization of the immunodominant epitopes of retroviruses and a wide variety of other infectious pathogens.

ACKNOWLEDGMENTS

We thank J. Kaplan, R. Khabbaz, and M. Osame for providing us with serum specimens, M. Garfield for synthesis of peptides, John O'Conner for excellent editorial assistance, and LaWanda Hodo for manuscript preparation.

REFERENCES

1. **Anderson, D. W., J. S. Epstein, T. H. Lee, M. D. Lairmore, C. Saxinger, V. S. Kalyaraman, D. Slamon, W. Parks, B. J. Poiesz, L. T. Pierik, H. Lee, R. Montagana, P. A. Roche, and W. A. Blattner.** 1989. Serologic confirmation of human T-lymphotropic virus type I infection in healthy blood and plasma donors. Blood **74**:2585–2591.
2. **Blattner, W. A.** 1989. Retroviruses, p. 545–92. *In* A. A. Evans (ed.), Viral infections of humans: epidemiology and control, 3rd ed. Plenum Publishing Corp., New York.
3. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47**:45–48.
4. **Colman, P. M., J. N. Varghese, and W. U. Laver.** 1983. Structure of the catalytic and antigenic sites in influenza virus neuraminidase. Nature (London) **303**:41–44.
5. **De, B., and A. Srinivasan.** 1989. Detection of human immunodeficiency virus (HIV) and human T-lymphotropic virus type I or II dual infections by polymerase chain reaction. Oncogene **4**:1533–1535.
6. **Ehrlich, G. D., and B. J. Poiesz.** 1988. Clinical and molecular parameters of HTLV-I infections. Clin. Lab. Med. **8**:65–84.
7. **Gingeras, T. R., D. Sciaky, R. E. Gelinas, J. Bing-Dong, C. E. Yen, M. M. Kelly, P. A. Bullock, B. L. Parson, K. E. O'Neil, and R. J. Roberts.** 1982. Nucleotide sequences from the adenovirus-2 genome. J. Biol. Chem. **257**:13475–13491.
8. **Hartley, T. M., R. F. Khabbaz, R. O. Cannon, J. E. Kaplan, and M. D. Lairmore.** 1990. Characterization of antibody reactivity to human T-cell lymphotropic virus types I/II using immunoblot and radio immunoprecipitation assays. J. Clin. Microbiol. **28**: 646–650.
9. **Haseltine, W. A., J. G. Sodroski, and R. Patarca.** 1985. Structure and function of the genome of HTLV. Curr. Top. Microbiol. Immunol. **115**:177–209.
10. **Henderson, L. E., T. D. Copeland, R. Sowder, G. Smythers, and S. Oroszlan.** 1981. Primary structure of the low molecular weight nucleic acid-binding proteins of murine leukemia viruses. J. Biol. Chem. **256**:8400–8406.
11. **Hopp, T. T., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78**:3824–3828.
12. **Horal, P., S. Jeansson, L. Rymo, B. Svennerholm, and A. Vahlne.** 1990. Epitope profiles of HTLV-1 *env* and *gag* gene encoded proteins, p. 461–467. *In* W. A. Blattener (ed.), Human retrovirology: HTLV. Plenum Publishing Corp., New York.
13. **Jameson, B. A., and H. Wolf.** 1988. The antigenic index: a novel algorithm for predicting antigenic determinants. Comput. Appl. Biosci. **4**:181–186.
14. **Kalyaraman, V. S., M. G. Sarngadharan, R. M. Guroff, I. Miyoshi, D. Blayney, D. Golde, and R. C. Gallo.** 1982. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science **218**:571–573.
15. **Khabazz, R. F., W. W. Darrow, T. M. Hartley, J. Witte, J. B. Cohen, J. French, P. S. Gill, J. Potterat, R. K. Sikes, R. Reich, J. E. Kaplan, and M. D. Lairmore.** 1990. Seroprevalence and risk factors for HTLV-I/II infection among prostitutes in the United States. J. Am. Med. Assoc. **263**:60–64.
16. **Kuroda, N., Y. Washitani, H. Shiraki, H. Kiyokawa, M. Ohno, H. Sato, and Y. Maeda.** 1990. Detection of antibodies to human T-lymphotropic virus type I by using synthetic peptides. Int. J. Cancer **45**:865–868.
17. **Lal, R. B., D. L. Rudolph, M. D. Lairmore, R. F. Khabbaz, M. Garfield, J. E. Coligan, and T. M. Folks.** 1991. Serologic discrimination of HTLV-I and HTLV-II infection by using a synthetic peptide based enzyme immunoassay. J. Infect. Dis. **163**:41–48.
18. **Lee, H., P. Swanson, V. S. Shorty, J. A. Zack, J. D. Rosenblalt, and I. Chen.** 1989. High rate of HTLV-II infection in seropositive IV drug abusers in New Orleans. Science **244**:471–475.
19. **Lerner, R. A., N. Green, H. Alexander, F. T. Liu, J. G. Sutclitte, and T. M. Shinnick.** 1981. Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. Proc. Natl. Acad. Sci. USA **78**:3403–3407.
20. **Mador, N., A. Panet, and A. Honigan.** 1989. Translation of *gag*, *pro*, and *pol* gene products of human T-cell leukemia virus type II. J. Virol. **63**:2400–2404.
21. **Malik, K. T. A., J. Even, and A. Karpas.** 1988. Molecular cloning and complete nucleotide sequence of an adult T cell leukemia virus/human T cell leukemia virus type I (ATLV/HTLV-I) isolate of Caribbean origin: relationship to other members of the ATLV/HTLV-I subgroup. J. Gen. Virol. **69**: 1695–1710.
22. **Osame, M., M. Matsumoto, K. Usuku, S. Izumos, N. Ijichin, H. Amitani, M. Tara, and A. Igata.** 1987. Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type-1 and adult T-cell leukemia-like cells. Ann. Neurol. **21**:117–122.
23. **Palker, T. J., R. M. Scearce, T. D. Copeland, S. Oroszlan, and**

B. F. Haynes. 1986. C terminal region of human T-cell lymphotropic virus type I (HTLV-I) p19 core protein is immunogenic in humans and contains an HTLV-I specific epitope. J. Immunol. **136**:2393–2397.

24. **Pellett, P. E., K. G. Kousoulas, L. Pereira, and B. Roizman.** 1985. Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. J. Virol. **53**:243–253.
25. **Rho, H. M., B. Poiesz, P. W. Ruscetti, and R. C. Gallo.** 1981. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. Virology **112**:355–360.
26. **Schneider, J., N. Yamamoto, Y. Hinuma, and A. Hunsmann.** 1984. Sera from adult T-cell leukemia patients react with envelope and core polypeptides of adult T-cell leukemia virus. Virology **132**:1–11.
27. **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.
28. **Shimotohno, K., Y. Takahashi, N. Shimizu, T. Gojobori, D. W. Golde, I. S. Y. Chen, M. Miwa, and T. Sugimura.** 1985. Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: an open reading frame for the protease gene. Proc. Natl. Acad. Sci. USA **82**:3101–3105.
29. **Steimer, K. S., K. W. Higgins, M. A. Powers, J. C. Stephans, A. Gyenes, C. G. Nascimento, P. A. Lucine, P. J. Barr, R. A. Hallewell, and R. S. Pescador.** 1986. Recombinant polypeptide from the endonuclease region of the acquired immune deficiency syndrome retrovirus polymerase (*pol*) gene detects serum antibodies in most infected individuals. J. Virol. **58**:9–16.
30. **Williams, A. E., C. T. Fang, D. J. Slamon, B. J. Poiesz, S. G. Sandler, W. F. Darr II, G. Shulman, E. I. McGowan, D. K. Douglas, R. J. Bowman, F. Peetoom, S. H. Kleinman, B. Lenes, and R. Y. Dodd.** 1988. Seroprevalance and epidemiological correlation of HTLV-I infection in U.S. blood donors. Science **240**:643–646.