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Expression and Characterization of Human T-Cell Leukemia Virus Type-I Env and Gag Proteins

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Abstract Human T-cell leukemia virus Type-I (HTLV-I) is etiologically associated with rare adult T-cell leukemia, a malignant T-cell disorder. cDNAs encoding p24 (*gag*), gp21 (*env*), and pXII of HTLV-I were amplified by polymerase chain reaction (PCR) using the genomic DNA extracted from HUT102 cell line as a template. The amplified cDNAs were cloned into the *Escherichia coli* expression vectors and over-expression of the recombinant proteins were achieved by adding IPTG into the culture media in order to induce the promoter. The molecular weights of the recombinant p24, gp21, and pXII, determined by SDS-PAGE, were found to be approximately 28 kDa, 23 kDa, and 15 kDa, respectively. Reactivity of the recombinant proteins with human sera was tested by the immunoblot assay. The gp21 and p24 reacted against the sera obtained from HTLV-I-infected individuals but not against the sera obtained from normal persons. These results suggest that the recombinant proteins expressed in *E. coli* were recognized by antibodies in sera from HTLV-I infected patients. These recombinant proteins would be applicable for detecting the presence of antibodies against HTLV-I in human blood samples.

Key words: HTLV-1, expression vector, immunoblot, sera

Since the human T-cell leukemia virus Type-I (HTLV-I) was isolated from a patient with cutaneous T-cell lymphoma [17, 21], it has been considered as a cause of a hematopoietic malignancy and adult T-cell leukemia/lymphoma (ATL) [5, 23], as well as a progressive myelopathy called tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) [4]. It was also reported that HTLV-I causes transformation in tissue culture cell and stimulates target cell proliferation by activating growth promoting cellular lymphokines and cytokines [3].

Although the route of transmission for HTLV-I is similar to those of human immunodeficiency virus (HIV), transmission of HTLV-I is almost exclusively a cell-associated blood transfusion. This observation was supported by studies performed with blood transfusion. Recipients of HTLV-I-infected samples containing cellular blood products were seroconverted, while recipients of plasma from HTLV-I-infected individuals were not [9].

The HTLV-I genome consists of single-stranded RNA approximately 9000 nucleotides long, and encodes structural proteins (*gag* and *env*), enzymes (reverse transcriptase, integrase, and protease), and regulatory proteins (tax and rex). The 5' and 3' ends of the HTLV-I proviral DNA contain long terminal repeats (LTR). LTRs are necessary for viral replication and contain various regulatory sequences for transcription. Besides, they may be required for the integration of proviral DNA into the host genome [1].

The *env* and *gag* proteins contain multiple immunogenic epitopes to which HTLV-I-infected individuals develop an antibody response [13, 6]. Antibodies that react with HTLV-I proteins have been found in the sera of HTLV-I infected patients. These antibodies recognize both the *gag* core antigens [7] and the envelope proteins of the virus [19]. Anti-HTLV-I antibodies have been detected in the sera of the most patients with ATL or HAM/TSP and asymptomatic carriers [5]. In addition to the patients with verified ATL or HAM/TSP, asymptomatic carriers of HTLV-I infection has been identified in sero-epidemiologic studies.

HTLV-I infection is usually diagnosed by examining serum antibodies against the viral antigens [10] and the diagnosis is designed to detect viral gene products from peripheral blood mononuclear cells (PBMNCs) of patients [11].

Typically, antibodies produced in response to natural infection by HTLV-I are directed against the viral *gag*

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(p24, p19) and *env* (gp21, gp46) gene products. In this study, for the development of HTLV-I diagnostic kit by recombinant DNA technology, cDNAs coding part of the *gag* and *env* region of HTLV-I were cloned into the *E. coli* expression vector and over expression of the recombinant proteins were achieved by inducing the promoter with IPTG. The expressed proteins were identified by immunoblot (IB) analysis with human sera. We suggest that the recombinant p24 and gp21 expressed in this study would be useful for detecting the presence of antibodies against HTLV-I in human blood sample.

MATERIALS AND METHODS

Cell Line

HUT102 (ATCC-162: cutaneous T-cell lymphoma), a cell line of human T-cell infected with human T-cell leukemia virus type I (HTLV-I), was cultured at 37°C in RPMI 1640 (GIBCO BRL Gaithersburg, U.S.A.) supplemented with 10% fetal bovine serum (GIBCO BRL) under 5% CO_2.

Extraction of HTLV-I Proviral DNA

HUT102 cells (10^5-10^6 cells/ml) were harvested by centrifugation at 10,000-13,000×g for 5 min at 4°C. The cell pellets, resuspended in 100 µl of K buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.6), 2.5 mM $MgCl_2$, 0.45% Tween20) containing 2.5 µl of proteinase K (100 mg/ml), were incubated at 55°C for 1 h and then boiled for 10 min. Extracted DNA was used for PCR.

Polymerase Chain Reaction (PCR)

The PCR mixture contained, in a total volume of 100 µl, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM each of dATP, dCTP, dGTP, and dTTP, 20 pmole each of antisense and sense primer, 2.5 U of AmpliTaqGold™ DNA polymerase (Perkin-Elmer, Branchburg, U.S.A.), and 2 µl of template DNA. After denaturation at 95°C for 12 min, samples were subjected to 30 cycles of reaction consisted of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and polymerization at 72°C for 1 min, followed by a final extension at 72°C for 5 min. Five microliters of the first amplification products were used as a template DNA for the nested-PCR. The primers were designed to have *Bam*HI and *Pst*I sites for sense primer and an *Hin*dIII site for antisense primer. All primers involved in this study are listed in Table 1 with a detailed description. Five microliters of the completed PCR reaction was mixed with 1 µl of loading gel buffer and analyzed by a 0.8% agarose gel electrophoresis.

Construction of Recombinant Plasmids

The amplified cDNAs digested with either *Bam*HI and *Hin*dIII or *Pst*I and *Hin*dIII were separated on a 0.8% agarose gel electrophoresis. Each of the amplified DNA fragments with expected size was purified by GENECLEANII® Kit

Table 1. Oligonucleotide primers used for PCR.

Primers	Polarity	Sequence (5' to 3')	Size (nt)	Position
Sp19	sense	ACACAGTTGGGGCTCGTCCG	21	751-771
Ap19	antisense	GGTTGGAGGGGCACCATGTG	21	1225-1205
Sp24	sense	TCATCCTCCACCCACGACCC	21	1111-1205
Ap24	antisense	AGTGGCCTGCTTTCCCGCACC	21	1897-1877
Sgp26	sense	CATGCCCAAGACCCGTCGGA	20	4123-5142
Agp46	antisense	AGACAAGCCAGACCGCCACCG	21	6143-6123
Sgp21	sense	TGGACCCACTGCTTTGACCCC	21	5996-6016
Agp21	antisense	CTGGAGGCGATGTGGTTGCAATA	23	6683-6661
SpXII	sense	TCCTCCTCCTCCTTGTCCTTTA	22	7159-7180
ApXII	antisense	GAAGGGGAGTATTTGCGCAT	21	7642-7622
eSp19	sense	CCCTTTCTGCAGGGATCCATGGGCCAAATGTTTCC	36	784-819
eAp19	antisense	ATGTGGAAGCTTCTATTAAAGGACTTGGGGGGCTGT	36	1209-1174
eSp24	sense	ACAGCCCTGCAGGATCCCCAGTCATGCACCCACAT	36	1174-1209
eAp24	antisense	TTTTTTAAGCTTCTATCATAACACTTTGGTTTTGTC	36	1851-1816
eSgp46	sense	AAAAAGCTGCACCCATCCCCAGTCATGCACCCACAT	36	5162-5197
eAgp46	antisense	GACCGCAACGTTTCATTATCGGCGGGAGCGGGATCC	36	6132-6097
eSgp21	sense	GGATCCCTGCAGGGATCCGCAGTACCGGTGGCGGTC	36	6097-6132
eAgp21	antisense	AATTGTAAGATTCTATTACAGGGATGACTCAGG	33	6661-6629
eSpXII	sense	ACGGATCTGCAGGGATCCATGCTTATTATCAGCCCA	36	7269-7304
eApXII	antisense	TCCTTAAAGCTTTCATTAGAGGTTCTCTGGGTG	33	7566-7534

(Bio101, Vista, CA, U.S.A.). The pRSET expression vector (Invitrogen, Carlsbad, U.S.A.) designed for high level gene expression in *E. coli* using T7 promoter was digested with either *Bam*HI and *Hin*dIII or *Pst*I and *Hin*dIII. The purified cDNAs coding for p24, gp21, gp46, and pXII were ligated into the pRSET expression vector by T4 DNA ligase (TaKaRa OTSU, Shiga, Japan). After transformation of the ligation mixture into *E. coli* Top10F', the recombinant colonies were screened by ampicillin resistance and identified by digestion with restriction endonucleases. The recombinant plasmids carrying p24, gp21, gp46, and pXII were named pRSET-p24, pREST-gp21, pREST-gp46, and pRSET-pXII, respectively. Nucleotide sequences were determined by the dideoxy chain termination method with primer binding on the T7 promoter region. Sequenase^{R} Version 2.0 DNA sequencing kit was used according to the manufacturer's instructions. The DNASIS^{TM} and PROSIS^{TM} (Pharmacia Uppsala, Sweden) programs were used to analyze nucleotide sequences and deduced amino acid sequences, respectively.

Expression of Recombinant Proteins

Each of the recombinant plasmids was transformed into *E. coli* BL21(DE3)pLysS [F^{-} *ompT hsdS*$_{B}$ ($r_B^- m_B^-$) *gal dcm* (DE3)pLysS(Cm^{R})]. Induction of the *lacUV5* promoter by isopropyl-β-D-thiogalactopyranoside (IPTG) results in the expression of the T7 RNA polymerase, which, in turn, transcribes the foreign DNA inserted in the pRSET vector. The *lacUV5* promoter of the transformed *E. coli* BL21(DE3)pLysS was induced by addition of 0.4 mM IPTG. The cells were incubated at 37°C and harvested at various time intervals. Cell pellets collected from 1 ml cultures at each time interval were resuspended with 150 μl solution I (50 mM glucose, 25 mM Tris-HCl, pH 7.5, 10 mM EDTA) and 100 μl 2× sample buffer was added. Each sample disrupted by freezing and thawing was denatured by boiling for 10 min and centrifuged at 12,000×g for 5 min. Proteins in supernatant were separated by 15% SDS-PAGE [12] and visualized by staining with Coomassie blue (0.1% Coomassie Brilliant blue R250, 45% methanol, 10% acetic acid).

Purification of the Recombinant Proteins

For purification of polyhistidine fusion protein from cell lysates, affinity chromatography using Ni^{2+}-NTA resin (GIBCO BRL) was performed. Overnight culture of *E. coli* BL21(DE3)pLysS carrying recombinant pRSET vector was inoculated into fresh Luria-Bertani broth in the presence of ampicillin (50 μg/ml) and chloramphenicol (34 μg/ml). When the cell density (O.D. at 600 nm) was reached to 0.4–0.5, IPTG was added to a final concentration of 0.4 mM. After 4 h incubation at 37°C, the cells were harvested by centrifugation at 5000 rpm for 15 min at 4°C. The cell pellet was resuspended in 20 ml of guanidine HCL lysis buffer (pH 7.8) per liter of original culture volume. The cell suspension was lyzed with French-pressure flow-through twice and centrifuged at 10,000×g for 20 min at 4°C. The collected supernatant was loaded onto the Ni^{2+}-NTA column. After washing the column with washing buffer (8 M urea, 20 mM $NaPO_4$, 500 mM NaCl, pH 6.0), the retained protein was eluted with elution buffer (8 M urea, 20 mM $NaPO_4$, 500 mM NaCl, pH 4.0). The purified recombinant proteins were used for immunoblot analysis.

Immunoblot Analysis

Purified recombinant proteins were separated under reducing condition on a 12% SDS-PAGE. The resolved proteins were eletrotransferred onto a nitrocellulose membrane (Schleicher & Schuell) at 15 V for 2–3 h in transfer buffer (125 mM Tris-HCl pH 7.8, 192 mM glycine, 20% methanol, 0.1% SDS). The membrane was blocked by 6% skim milk in Tris-buffered saline/Tween20 (TTBS, 100 mM Tris-HCl, 0.95% NaCl, 0.5% Tween20, pH 7.5) for 2 h or overnight. For immunologic detection, the nitrocellulose membrane was incubated over right at 4°C with antisera diluted into 1:250 in blocking buffer (6% skim milk in TTBS) and then washed three times with TTBS. The bound antibodies were detected by incubation with biotin-conjugated anti-human IgG diluted 1:2000 in blocking buffer. Immunodetection was accomplished by binding the biotinylated anti-human IgG with avidin-horseradish peroxidase conjugate diluted 1:2000 in blocking buffer. The second conjugate was allowed to incubate for 1 h followed by three 5-min rinses with wash buffer, and color development was achieved by incubation with 4-chloro-1-naphthol dissolved in cold ethanol and 0.1% H_2O_2.

RESULTS AND DISCUSSION

Amplification of HTLV-I cDNA by Nested PCR

cDNAs coding for p24 (*gag*), gp46 (*env*), gp21 (*env*), and a regulatory protein (pXII) within pX of HTLV-I were amplified by the polymerase chain reaction. The whole cell DNA extracted from the HUT102 HTLV-I proviral cell line (ATCC-162) was used as a template. The oligonucleotide primers shown in Table 1 were used to amplify cDNA.

Four cDNAs were amplified by nested PCR. The amplified cDNAs were separated on a 0.8% agarose gel and the sizes of the amplified cDNAs for p24, gp46, gp21, and pXII, were estimated to be 680 bp (Fig. 1, lane 2), 980 bp (Fig. 1, lane 3), 530 bp (Fig. 1, lane 4), and 290 bp (Fig. 1, lane 5), respectively.

Cloning of the PCR Products

The amplified cDNAs were separated on agarose gel and eluted from the gel. The purified cDNAs were digested

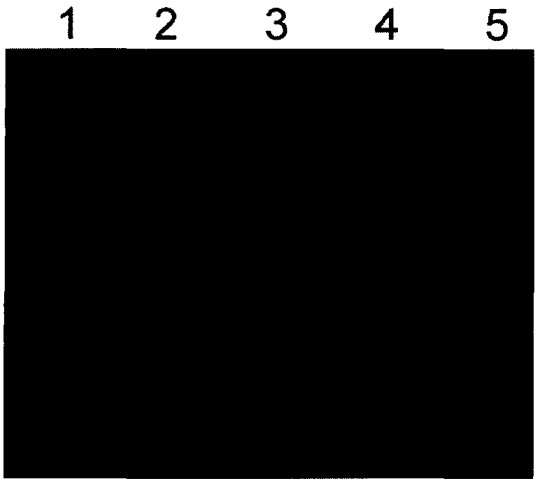

Fig. 1. Agarose gel electrophoresis of p24 (680 bp), gp46 (980 bp), gp21 (530 bp), and pXII (290 bp) PCR products.
Lane 1, size marker (1 kb ladder); lane 2, p24; lane 3, gp46; lane 4, gp21; lane 5, pXII.

with either *Bam*HI and *Hin*dIII or *Pst*I and *Hin*dIII and cloned into the pRSET expression vector as described in the Materials and Methods. pRSET vector consists of a T7 promoter and a prokaryotic ribosome binding site for high-level expression in *E. coli,* and a six-histidine affinity tag for easy purification.

The insertion of the p24, gp21, gp46, and pXII cDNAs in each of the recombinant plasmids was confirmed by digestion with either *Bam*HI and *Hin*dIII or *Pst*I and *Hin*dIII. The resulting DNA fragments were separated on a 0.8% agarose gel. Insert DNA fragments with expected size of p24 (680 bp), gp46 (980 bp), gp21 (530 bp), and pXII (290 bp) were observed (data not shown).

To confirm that the insert DNAs were derived from HTLV-I proviral DNA, the recombinant plasmids were subjected to nucleotide sequence determination. Comparative analysis of nucleotide sequences with that of HTLV-I isolated from an adult T-cell leukemia (ATL) patient [18] showed nucleotide sequence homology of 90%. These results indicated that the amplified cDNAs were derived from the HTLV-I proviral DNA.

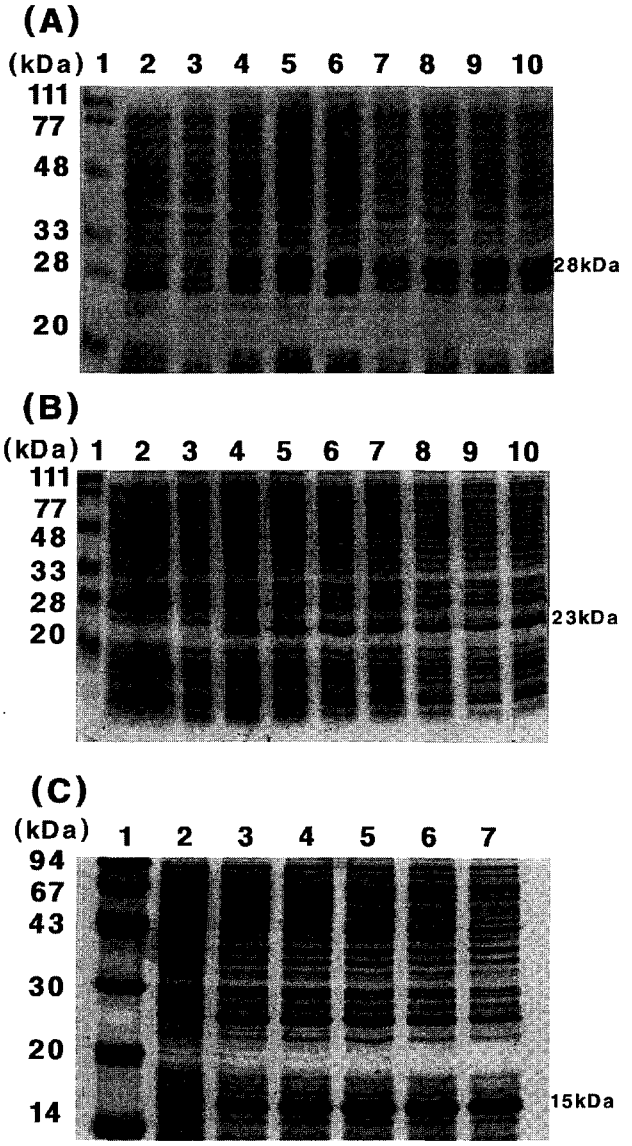

Fig. 2. SDS-PAGE of HTLV-I p24 (A), gp21 (B), and pXII (C) expressed in *E. coli.*
Lane 1, low molecular weight markers; lane 2, cell lysates of BL21(DE3)pLysS; lanes 3, 4, 5, 6, 7, 8, 9, and 10, cell extracts harvested at 0 h, 1 h, 2 h, 3 h, 5 h, 6 h, and overnight, respectively, after the promoter was induced by addition of IPTG.

Over Expression of the Recombinant Proteins

E. coli BL21(DE3)pLysS transformed with the recombinant plasmids were grown at 37°C and expression of the recombinant proteins were induced by adding 0.4 mM IPTG when the O.D. at 600 nm (cell density) was reached to 0.5. The cells were harvested at various time intervals and the expression levels of the fusion proteins were analyzed by a 10% SDS-PAGE.

The recombinant proteins, expressed for 1 h after addition of IPTG into the culture media, were separated on SDS-PAGE. The recombinant proteins expressed from the pRSET vector were expected to have an additional 4 kDa-oligopeptide that is derived from the coding sequences located at the multiple cloning site of the vector. About 28 kDa recombinant protein was expressed from pRSET-p24 (Fig. 2(A), lanes 3-10), 23 kDa recombinant protein was expressed from pRSET-gp21 [Fig. 2(B), lanes 3-10], and 15 kDa recombinant protein was expressed from pRSET-pXII [Fig. 2(C), lanes 4-7]. gp46 was not expressed in *E. coli* transformed with pRSET-gp46. Several methods attempted to express gp46, including various IPTG concentrations (1-0.1 mM), induction time, incubation temperature, and host strains, were not successful. Although

we do not know why gp46 was not expressed in *E. coli* at the present time, we are trying to express gp46 because it is an important antigen for diagnosis of HTLV-I-infected individuals. Lipka *et al.* [13] reported that recombinant HTLV-I *env* products containing epitopes of gp46 were sensitive and type-specific for the detection of HTLV-I antibody.

To investigate antigenic properties of the recombinant proteins (p24, gp21, and pXII), over expression of the proteins was achieved by the addition of IPTG and incubation for 1-5 h. Total cell lysates were subjected to a 15% SDS-PAGE and immunoblot analysis with anti-HTLV-I positive sera was performed. The result showed that both p24 and gp21 expressed in *E. coli* was strongly immunoreactive, but the recombinant pXII protein was not (data not shown). The recombinant proteins (p24 and gp21) reactive against anti-HTLV-I human sera were purified by affinity chromatography for further characterization.

Purification of Recombinant Proteins

In order to purify recombinant proteins, a metal-chelate affinity chromatography using Ni^{2+}-NTA resin was performed. The oligohistidine located at the N-terminal tail of the proteins will bind to the Ni^{2+}-NTA resin. Both soluble and insoluble proteins were purified by this column since the protein in native and denatured forms will be bound to the resin. High-level expression of foreign proteins in bacteria and other cells frequently results in poor solubility of the expressed protein. Before insouble proteins in inclusion bodies can be purified, they must be extracted and solubilized. The solubilization of inclusion body proteins requires denaturation with reagents such as concentrated solutions of urea, guanidine HCl, or SDS.

The p24 and gp21 were eluted from the resin and analyzed by separating on a 15% SDS-PAGE. Lanes 5, 6, 7, (p24) and, 8, 9, 10 (gp21) of Fig. 3 show the purified recombinant proteins. The concentrations of purified recombinant proteins determined by the Bicinchoninic Acid (BCA) protein assay were estimated to be 1.69 mg/ml and 1.12 mg/ml, respectively.

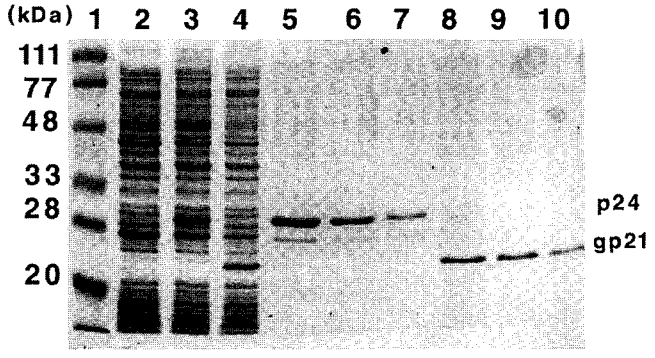

Fig. 3. SDS-PAGE of recombinant p24 and gp21 expressed in *E. coli*.
The recombinant proteins were purified by affinity chromatography. Lane 1, low molecular weight markers; lane 2, cell lysates BL21(DE3)pLysS; lane 3, whole cell lysates of BL21(DE3)pLysS carrying plasmid for gp21 expression; lanes 5, 6, and 7, purified recombinant p24 proteins; lanes 8, 9, and 10, purified recombinant gp21 protein.

Immunoblot Analysis

To investigate the antigenicity of the purified recombinant proteins, immunoblot analysis was performed with anti-HTLV-I positive sera and normal sera. Each of the ten HTLV-I-positive sera was used for testing antigenicity of purified recombinant p24 and gp21 by immunoblot analysis. As shown in Figs. 4(A) and 4(B), the purified proteins reacted with the sera from the HTLV-I-infected individual [Fig. 4(A), lanes 5-10] while the proteins did not react with the sera from the normal person [Fig. 4(B), lanes 5-10].

When the purified recombinant p24 protein was used as antigen for immunoblot analysis, five out of ten HTLV-I positive sera reacted strongly, while the rest of the 5 HTLV-I positive sera reacted weakly. When the purified recombinant gp21 protein was used as an antigen for

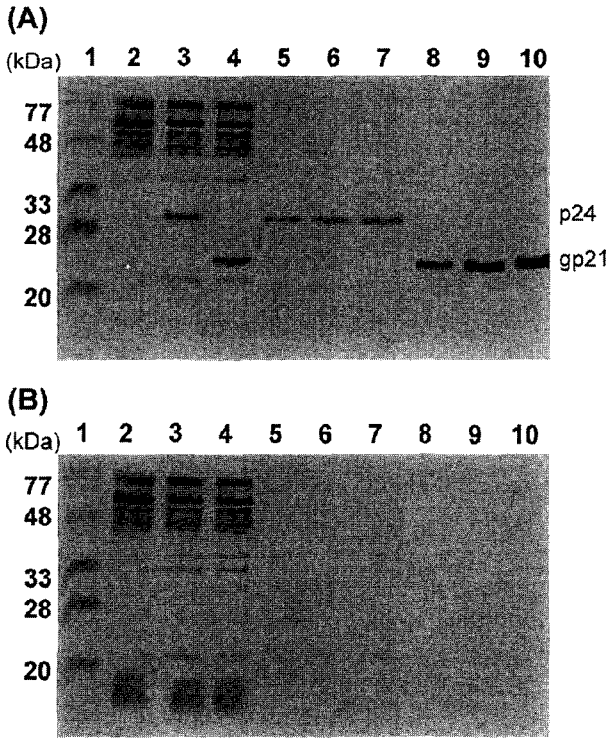

Fig. 4. Immunoblot analysis of recombinant HTLV-I proteins expressed in *E. coli*.
The recombinant proteins were separated on a 15% SDS-PAGE and subjected to immunoblot analysis by using HTLV-I positive sera (A) and HTLV-I negative human sera (B). Lane 1, low molecular weight marker; lane 2, cell lysates BL21(DE3)pLysS; lane 3, whole cell lysates of BL21(DE3)pLysS carrying plasmid for p24 expression; lane 4, whole cell lysates of BL21(DE3)pLysS carrying plasmid for gp21 expression; lanes 5, 6, and 7, purified recombinant p24 proteins; lanes 8, 9, and 10, purified recombinant gp21 protein.

immunoblot analysis, all HTLV-I positive sera were strongly reacted (data not shown). These results indicated that the recombinant proteins expressed in *E. coli* exhibited good antigenicity. Recently, cloning and preliminary analysis of several recombinant HTLV antigens were reported [2, 15]. Serologic confirmation of HTLV-I infection depends upon the presence of antibodies to both *gag* and *env* gene products [5, 20].

Recombinant proteins containing portions of the HTLV-I gp21 transmembrane envelop protein were demonstrated to be a very strong marker for HTLV infection [13]. Recombinant gp21 has been used to develop assays that not only yield a higher sensitivity of *env* product detection but also provide an excellent marker for early seroconversion [16]. In view of the extremely high sensitivity of gp21 and its ability to detect early infection [13], it could be included with other recombinant external envelop proteins for serologic confirmation of HTLV infection.

Furthermore, a recombinant gp21 protein has a false-positive rate of 1% to 4% with sera from uninfected individuals [14]. Indeed, our data indicated that specimens from 100% of HTLV-I-positive individuals reacted with recombinant gp21 protein. These results are in general agreement with those of previous reports in which recombinant gp21 immunoblot assay and enzyme immunoassay (EIA) were capable of correctly identifying nearly 100% of individuals infected with HTLV-I or HTLV-II [13, 14].

The purified recombinant proteins should be useful for detecting the presence of antibodies against HTLV-I in human blood sample and applied to development of an HTLV-I diagnostic system [8, 22]. But determination of the complete utility of these recombinant proteins in confirmation assays for HTLV-I infections requires further clinical studies.

In summary, two structural gene products of HTLV-I expressed in *E. coli* have been demonstrated to be bound to the antibodies in HTLV-I-infected individuals and may be useful in the screening and confirmation of HTLV-I infections.

Acknowledgments

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REFERENCES

1. Bhat, N. K., Y. Adachi, K. P. Samuel, and D. Derse. 1993. HTLV-I gene expression by defective proviruses in an infected T-cell line. *Virology* **196:** 15-24.
2. Chen, Y. A., T. H. Lee, S. Z. Wiktor, G. M. Shaw, E. L. Murphy, W. A. Blattner, and M. Essex. 1990. The specific antigens for serological discrimination of HTLV-I and HTLV-II infection. *Lancet* **336:** 1153-1160.
3. Franchini, G. 1995. Molecular mechanisms of human T-cell leukemia lymphotropic virus type I infection. *Blood* **86:** 3619-3639.
4. Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, and A. Calender. 1985. Antibodies to human T-lymphotrophic virus type-I in patients with tropical spastic parapatesis. *Lancet* **8452:** 407-410.
5. 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.
6. Horal, P., W. W. Hall, B. Svennerholm, J. Lycke, S. Jeansson, L. Rymo, M. H. Kaplan, and A. Vahlne. 1991. Identification of type specific linear epitopes in the glycoproteins gp46 and gp21 of human T-cell leukemia viruses type I and type II using synthetic peptides. *Proc. Natl. Acad. Sci. USA* **88:** 5754-5758.
7. Kalyanaraman, V. S., M. G. Sarngadharan, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1981. Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus. *Nature* **294:** 271-273.
8. Kleinman, S. H., J. E. Kaplan, R. F. Khabbaz, M. A. Calabro, R. Thomson, and M. Busch. 1994. Evaluation of a p21e-spiked western blot (immunoblot) in confirming human T-cell lymphotropic virus-type I or II infection in volunteer blood donors. *J. Clin. Microbiol.* **32:** 603-607.
9. Kleinman, S., P. Swanson, J. P. Allain, and H. Lee. 1993. Transfusion transmission of human T-lymphotropic virus type I and II serologic and polymerase chain reaction results in recipients identified through look-back investigation. *Transfusion* **33:** 14-18.
10. 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.
11. Kwok, S., G. Ehrlich, B. Poieze, R. Kalish, and J. J. Shinsky. 1988. Enzymatic amplification of HTLV-I viral sequence from peripheral blood mononuclear cell and infected tissue. *Blood* **72:** 1117-1123.
12. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685.
13. Lika, J. J., K. Bui, G. R. Reyes, R. Moechi, S. Z. Wiktor, W. A. Blattner, E. L. Murphy, G. M. Shaw, C. V. Hanson, J. J. Snisky, and S. K. Foung. 1990a. Determination of a unique and immunodominant epitope of human T-cell lymphotropic virus type I. *J. Infect. Dis.* **162:** 353-357.
14. Lika, J. J., P. Santiago, L. Chan, G. R. Reyes, K. P. Samuel, W. A. Blattner, G. M. Shaw, C. V. Hanson, J. J. Sninsky, and S. K. Foung. 1990b. Modified western blot assay for confirmation and differentiation of human T-cell lymphotropic virus type I and II *J. Infect. Dis.* **164:** 400-403.

15. Lillehoj, E. P., S. S. Alexander, C. J. Dubrule, S. Wiktor, R. Andams, C. C. Tai, A. Manns, and W. A. Blattner. 1990. Development and evaluation of a human T-cell leukemia virus type I serological assay incorporating a recombinant envelope polypeptide *J. Clin. Microbiol.* **28:** 2653-2658.
16. Manns, A., E. L. Murphy, R. Wilks, G. Haynes, J. P. Figueroa, B. Hanchard, M. Barnett, J. Drummond, D. Waters, M. Cerney, J. R. Seals, S. S. Alexander, H. Lee, and W. A. Blattner. 1991. Detection of early human T-cell lymphotropic virus type I antibody pattern during seroconversion among transfusion recipients. *Blood* **77:** 896-905.
17. Poisz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. D. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and culture lymphocytes of patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **77:** 7415-7419.
18. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. J. Horn, K. B. Mullis, and H. A. Erlich. 1983. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:** 487-491.
19. Samuel, K. P., J. A. Lautenberger, C. L. Jorcyk, S. Josephs, F. Wong-Staal, and T. S. Papis. 1984. Diagnostic potential for human malignancies of bacterial produced HTLV-I envelope protein. *Science* **226:** 1094-1097.
20. Schneider, J., N. Yamamoto, Y. Hinuma, and G. Hunsmann. 1984. Sera from adult T-cell leukemia patients reactive with envelope and core polypeptides of adult T-cell leukemia virus. *Virology* **132:** 1-11.
21. Uchiyama, Y., J. Yoda, K. Sagawa, K. Takatsuki, and H. Uchino. 1977. Adult T-cell leukemia: Clinical and hematologic features of 16 cases. *Blood* **50:** 481-492.
22. Varma, M., D. L. Rudolph, M. Knuchel, W. M. Switzer, K. G. Hanlock, M. Velligan, L. Chan, S. K. H. Foung, and R. B. Lal. 1995. Enhanced specificity of truncated transmembrane protein for serologic confirmation of human T-cell lymphotropic virus type 1 (HTLV-I) and HTLV-2 infections by western blot (immunoblot) assay containing recombinant envelope glycoproteins. *J. Clin. Microbiol.* **33:** 3239-3244.
23. Yoshida, M., I. Miyoshi, and Y. Hinuma. 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implications in the disease. *Proc. Natl. Acad. Sci. USA* **79:** 2031-2035.