the IL-2 sequence was present, but preceded by a six-histidine domain, expression was very low. Only the construction with the IL-2 fragment N-terminus sequence, an the six histidine domain at the carboxiterminus showed high expression levels of TMPA (20% of total bacteria protein), and the protein was recognized by the specific serum in W.B.

We, and others, have discussed that the presence of a human IL-2 encoding sequence, 5' to the protein of interest, provides an excellent stabilizer for protein expression in *E. coli*, either by conferring high stability to mRNA, or by reducing the probability of proteolysis (2, 4-5). This same effect has been found with the five other different viral proteins, and immunoglobulin fragments we have cloned into pPF-15, and its modified versions lacking the IL-2 fragment (unpublished results). Now we also show that the effect of the IL-2 sequence at the N-terminus can be partially affected by a preceding six-histidine domain.

REFERENCES

1. YOUNG, H. *et al* (1992). *International Journal of SDT and AIDS*, 3:391-413
2. EUROPEAN PATENT APPLICATION N.90202108.8
3. DOMÍNGUEZ, M. C. *et al.* (1994) Advances in Modern Biotechnology 2:93. Biotecnología Habana'94. Short Reports of the Congress, La Habana, Cuba, Nov.28-Dic.3,1994
4. DEUTSCHER, M. P. *et al* (1988). *TIBS* 13:103-115
5. LAVALLIE, E. R. *et al.* Biotechnology 11: 187-192

HIGH EXPRESSION LEVEL IN E. coli OF HTLV-I CORE PROTEINS

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INTRODUCTION

The Human T cell Leukemia Lymphoma virus type 1 (HTLV-I) was the first human retrovirus to be discovered (1). It is associated to adult T cell leukemia/lymphoma (ATLL) and a progressive neuromyelopathy named Tropical Spastic Paraparesis (TSP) (2). The antibodies of infected individuals are predominantly against the core proteins (p19 and p24) and the envelope protein gp46 (3). These proteins have been cloned in *E. coli*, but the expression levels obtained have been very low, less than 1% (4), in the specific case of p19-p24 and under the lactose promoter the expression level was 0.3% (5). In this work we cloned and expressed the core protein p19 and p24 fussed to IL-2 fragment, under tryptophan promoter, and the expression level obtained was 10% of total bacterial protein.

MATERIALS AND METHODS

The DNA fragment of 1042 bp corresponding to p19 and p24 proteins was amplified by PCR using specific primers. The fragment was cloned in the expression vector pR2M6 and transformed in the *E. coli* strain GC366. All the procedures for PCR, cloning and the analysis of the clones have been previously described (6). For the sequence analysis the Pharmacia sequencing Kit was used, following the manufacturer instructions. In one case, the induction of the tryptophan promoter was done in minimal media supplemented with glucose and casaminoacids at 37°C, by adding 0.012 mg/mL of ß-Indoleacrilyc acid after 2 h of the inoculation and growing the culture for 4 h at 37°C. Also, recombinant cells were inoculated in Luria Broth supplemented with 0.1 mg/mL of tryptophan and the culture was growth for 8 h at 37°C. The expression analysis was made by SDS-PAGE and Western blot (W.B) (7). In the latter, a sera from an infected patient diluted 1/10 in PBS-5% skim-milk and free of anti-E. coli antibodies, was used.

RESULTS AND DISCUSSION

The Plasmid named pGAG1, was obtained from the cloning of the PCR product corresponding to the p19-p24 gene in the pR2M6 expression vector. The clone was analyzed using the restriction enzymes XbaI and BamHI and by partial sequence of the ends of the gene, corroborating in frame insertion and absence of any mutation and stop codons. The induction of the clone in the E. coli strain GC366 by the two methods evaluated gave the following results. With minimal media and ß-Indoleacrilyc acid as inducer the expression was less than 1%, as previously reported for other cases. With rich media and the depletion of the repressor as induction method we obtained an increase in the expression

level up to 10%. This result is better than those previously reported in *E. coli* and even in yeast, which was 3% (5). The HTLV-I positive sera used in the WB recognized only the protein at the expected molecular weight (4500 Da) in the lanes corresponding to the induced clones. These results allows the production of enough quantity of core antigens from HTLV-I, which is very important for the development of diagnostic kits for HTLV-I.

REFERENCES

1. B.. J. POIESZ *et al.(1980) PNAS* **77:7415.**
2. G. de Thé and R. Bomford. (1993) AIDS Re. Hum. Ret. **65:** 1870.
3. B. L. RENU *et al.* (1991) Journal of Virology **65:** 1870.
4. S. ITARMURA *et al. (1985) Gene* **38:57.**
5. Y. FUJISAWA *et al.*(1989) European Patent Application. G. 352 060 A2.
6. J. SAMBROOK *et al.*(1989) *Molecular Cloning: A. Laboratory Manual.* Eds.Cold Spring Harbor Laboratories Press.
7. H. TOWBIN *et al.* (1989) *PNAS* **76:** 4350.

THE HEPATITIS C VIRUS INFECTION IN CUBA: ANTIBODY PATTERN AND GENOTYPES

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INTRODUCTION

Hepatitis C virus (HCV) is the responsible of more than 90% post-transfusion hepatitis and a high proportion of chronic cases. HCV infection is a serious health problem in Cuba (Padrón *et al.*, 1994) and the knowledge of its characteristics is an important cornerstone in order to structure control strategies. The aim of the present paper is to show the serologic pattern of this infection in Cuban hepatitis patients and to report the genotypes identified in our carriers.

MATERIALS AND METHODS

The antibody pattern study was performed using samples positive to anti-HCV from Cuban chronic and acute patients. The positivity to different HCV proteins was established using two line-dot systems (RIBA 2.0, Chiron Corp, USA and LiaTek HCV, Organon Teknika, Netherlands). Genotyping was performed by the cDNA-nested PCR procedure using specific primers for each described genotype and samples of individuals at higher risk of infection.

RESULTS AND DISCUSSION

The antibodies to core antigens appear earlier and are the most frequently found among Cuban HCV carriers (75% of acute and 100% of choronic cases) after RIBA 2.0, followed by antibodies to protein from NS3 region C33c (table 1). LiaTeck HCV showed a better performance of its core antigens (100% and 84.2% in chronic and acute cases, respectively), specially in the detection of acute cases during seroconversion (table 2). It yielded less indeterminate results in both acute and chronica individuals. The NS4 synthetic antigen in LiaTek showed a better performance (83.9%) than the recombinant proteins C-100-3 and 5-1-1 of RIBA 2.0 (58.5 and 62.3%, respectively).

The genotype II (Okamoto *et al.* 1992) was found in 26 out of 28 (92.6%) individuals tested (Table 3). This correlates with the high propensity toward chronic and more severe forms of the disease found in Cuban patients (Arús *et al*, 1994). The high rate of multiple genotypes in studied samples is due to the higher risk to HCV infection of the selected carriers, which increases the probability of multiple infection.

Table 1.
Antibody Pattern (RIBA 2.0) among Cuban patients (%)

	RIBA ANTIGENS					
PATIENTS	5-1-1	C-100-3	C33c	C22	INDET.	TOTAL
CHRONIC	29 (70.70	26 (63.4)	37 (90.2)	41 (100)	8 (19.5)	41
ACUTE	4 (33.3)	5 (41.7)	8 (66.7)	9 (75.0)	7 (58.3)	12
TOTAL	33 (62.3)	31 (58.5)	45 (84.9)	50 (94.3)	15 (28.3)	53

Table 2.
Antibody Pattern (LiaTek HCV) among Cuban patients (%)

	LiaTek ANTIGENS							
PATIENTS	NS4	NS5	C-1	C-2	C-3	C-4	INDET.	TOTAL
CHRONIC	57 (83.8)	36 (52.9)	52 (76.5)	56 (82.4)	44 (64.7)	49 (72.1)	6 (8.8)	68
ACUTE	16 (84.2)	13 (68.4)	15 (78.9)	15 (78.9)	11 (57.9)	13 (68.4)	5 (26.3)	19
TOTAL	73 (83.9)	49 (56.3)	67 (77.0)	71 (81.6)	55 (63.2)	62 (71.3)	11 (12.6)	87