Expression of the *x-lor* Gene of Human T-Cell Leukemia Virus I in *Escherichia coli*

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The 3'-terminal regions of the human T-cell leukemia virus I (HTLV-I) and HTLV-II genomes encode a novel gene product. We showed that expression of this region fused to the β-galactosidase gene in bacteria produces a protein recognized by adult T-cell leukemia-lymphoma patient sera. Rabbit antibodies raised against this protein specifically precipitated the 42-kilodalton *x-lor* gene protein from HTLV-I-infected cells.

Human T-cell leukemia viruses (HTLV) make up a family of retroviral isolates associated with human T-cell pathology (2). They include HTLV-I, a virus associated with an aggressive form of adult T-cell leukemia-lymphoma (ATLL) (7, 16); HTLV-II, derived from a patient with a benign form of hairy T-cell leukemia (1, 5); and HTLV-III, isolated from patients with acquired immune deficiency syndrome (8). Nucleotide sequence analysis of the HTLV-I provirus revealed that in addition to the *gag*, *pol*, and *env* genes typical of animal leukemia viruses, the genome includes a sequence of about 1600 nucleotides located between the 3' end of the envelope gene and the long terminal repeat called pX (10). A sequence of similar length is also found at the 3' end of the HTLV-II genome (4, 11). The 3' end of the HTLV-I and -II genomes can be divided into a 5' region that is not conserved and a 3' region approximately 1,000 nucleotides long that is well conserved between the two viruses. For both HTLV-I and -II, the 3' region, now designated the *x-lor* gene (long open reading frame [ORF]), encodes a polypeptide of molecular size 38 to 42 kilodaltons (kDa) (6, 12). For both viruses, the *x-lor* gene must be expressed as a fusion product, because there are splice acceptor sites at the 5' end of the long ORF but no in-frame methionine codons (14).

It has been proposed that the *x-lor* gene product is potentially important in mediating the *trans*-acting transcriptional effects of infection and the early steps in transformation by this virus (13). To facilitate purification of this protein for biochemical studies, we raised monospecific antibodies against the *x-lor* protein by the expression of a major portion of the *x-lor* gene in bacteria. We report here the fusion of the *x-lor* region into a bacterial ORF vector containing the β-galactosidase (β-gal) gene (15).

The structure of the plasmid used in these studies is shown in Fig. 1. The *x-lor* region is inserted between the 5' end of the *ompF* gene, and *Escherichia coli* gene encoding an abundant outer membrane protein, and the *lacZ* gene of *E. coli*. Both promoter and translation initiation signals are provided by the *ompF* gene, whereas the *lacZ* gene provides an indicator of gene expression. More than 95% of the 3' long ORF of the *x-lor* gene in HTLV-I (nucleotides 7289 to 8339 [10]) is present in this plasmid. A plasmid that contained an in-frame fusion product between the *ompF* and β-gal genes without an insert was also constructed. Both plasmids produced blue colonies in the appropriate *lacZ+*

indicator strain of *E. coli* indicating that the plasmids encoded proteins that contain functional β-gal.

The plasmids were transferred to *E. coli* TK1046, a strain that permits higher gene expression of the *ompF* promoter. Cells transformed by the *omp–lor–β*-gal plasmid expressed a large, approximately 150-kDa protein that was detected by Coomassie blue staining (Fig. 2). No protein of this size was present in either the TK1046 cells without the plasmid or cells transformed by the *omp–β*-gal plasmid alone. To determine whether the 150-kDa protein was expressed from the *omp–lor–β*-gal plasmid, we also labeled cells with either [^{35}S]methionine or [^{35}S]cysteine and analyzed the cells by immunoprecipitation with either anti-β-gal serum or ATLL patient serum that had previously been shown to contain antibodies directed against the *x-lor* gene product (6). Anti-β-gal serum precipitated a 116-kDa protein from the control *omp–β*-gal hybrid clone (the *ompF–β*-gal fusion protein) as

GAT CCG TCG ACG GAT CCC   AAC CAT GCT TAT........ATA TCC CCC   GGG GAT CCC

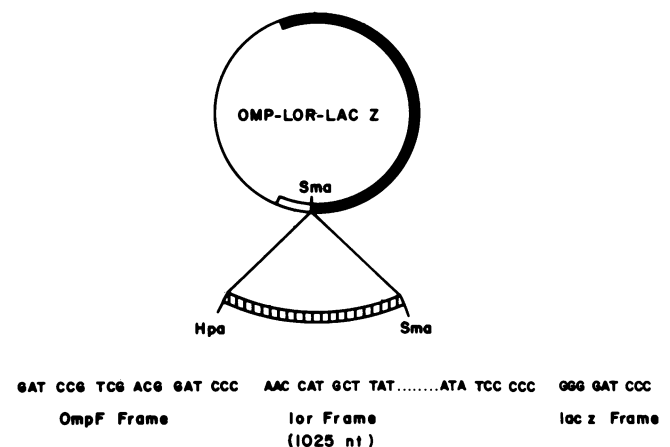OmpF Frame   lor Frame   lac z Frame
            (1025 nt)

FIG. 1. Construction of *omp–lor–β*-gal. The 3' terminal region of HTLV-I between the *env* gene and the 3' long terminal repeat of HTLV (strain CH) was digested with *Hpa*I and *Sma*I. This was blunt-end ligated into the *Sma*I site of pORF1 (13). Blue *lacZ+* strain MH3000 or TK1046 colonies were selected on Lbroth-ampicillin plates containing 5-bromo-4-chloro-3-indoxyl β-D-gal. The correct orientation of the plasmid was verified by restriction enzyme digests. At bottom is the frame of *ompF*, *x-lor*, and *lacZ*. The open bar indicates the *ompF* promoter and translation initiation signals, the dark bar indicates the *lacZ* gene, and the striped bar indicates the *x-lor* insert.
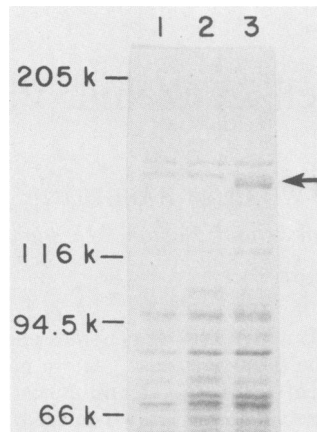
* Corresponding author.

FIG. 2. Protein expression in *lacZ*+ TK1046 colonies. Cells were grown in L broth medium at room temperature until the optical density at 550 nm was approximately 0.3 to 0.4. They were then spun down, suspended in loading buffer (125 mM Tris hydrochloride [pH 6.8], 2% sodium dodecyl sulfate, 9% glycerol, 0.7 M 2-mercaptoethanol, 0.0025% bromphenol blue), and boiled for 5 min before being loaded on a 5% sodium dodecyl sulfate-polyacrylamide gel. Proteins were analyzed by Coomassie blue staining. Lanes: 1, Coomassie blue staining of an untransformed TK1046 colony; 2, an *omp*–β-gal clone; and 3, an *omp*–*lor*–β-gal clone. k, kDa.

expected and the 150-kDa protein from an *omp*–*x*–*lor*–β-gal clone (Fig. 3, lanes 1 and 4). The 150-kDa protein was also precipitated by ATLL patient serum (lane 5) but not by normal human serum (lane 6). The ATLL patient serum, however, did not immunoprecipitate the 116-kDa protein from the *omp*–β-gal control cells. These results show that the 150-kDa protein contains antigenic sites recognized by the ATLL serum that were provided by the *lor* gene insert.

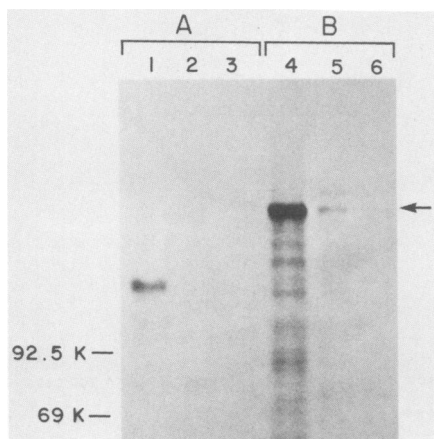As a further test to determine whether the fusion bacterial

FIG. 3. Immunoprecipitation analysis of *lacZ*+ TK1046 colonies. Cells were labeled in M63 glucose medium at room temperature with 100 μCi of [35S]methionine or [35S]cysteine per ml for 15 min. Labeled proteins were processed for immunoprecipitation as previously described (13). Panel A shows an analysis of a hybrid *omp*–β-gal fusion clone, and panel B shows the *omp*–*lor*–β-gal product. Lanes: 1 and 4, immunoprecipitates with rabbit anti-β-gal serum (Cappel Laboratories); 2 and 5, immunoprecipitates with ATLL patient serum; 3 and 6, immunoprecipitates with normal human serum. k, kDa.
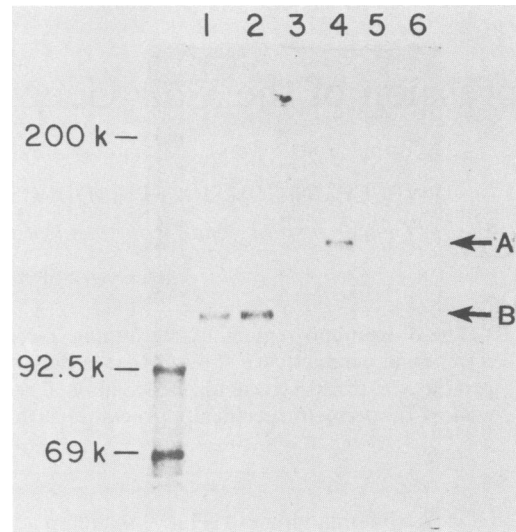

FIG. 4. Immunocompetition studies. TK1046 colonies transformed by an *omp*–β-gal plasmid (lanes 1 to 3) or an *omp*–*lor*–β-gal plasmid (lanes 4 to 6) were labeled in M63 glucose medium at room temperature with 100 μCi of [35S]cysteine per ml for 15 min. Labeled proteins were competed with cold lysates from C81-66-45 (lanes 1, 4, and 5) before immunoprecipitation with anti-β-gal (lanes 1, 2, and 4) or ATLL patient serum (lanes 3 and 5). Lane 6 shows immunoprecipitation of TK1046 colonies transformed by an *omp*–*lor*–β-gal plasmid with normal human serum. Arrows A and B indicate the 150kDa *omp*–*lor*–β-gal product and the 116-kDa β-gal protein, respectively. Labeled molecular size markers (Amersham Corp.) are shown in the leftmost lane. k, kDa.

protein contained antigenic determinants similar to that of the native *x*-*lor* product expressed in HTLV-I-infected cells, immunocompetition studies were done with lysates from a cell line (C81-66-45) (16) that had previously been shown to produce only the single viral 42-kDa *x*-*lor* product (6). The 150-kDa *omp*–*lor*–β-gal fusion protein was competed by cold unlabeled C81-66-45 lysates when ATLL patient serum was used for immunoprecipitation (Fig. 4, lane 5). C81-66-45 lysates, however, did not compete for precipitation of the 150-kDa protein when anti-β-gal serum was used (lane 4). These experiments also show that the bacterial *x*-*lor* fusion protein shares antigenic determinants with the authentic 42-kDa *x*-*lor* protein.

The production of a highly expressed bacterial protein that shares antigenic determinants with the *x*-*lor* product should make possible the production of monospecific antibodies against it. For that purpose, the bacterial 150-kDa *x*-*lor* fusion protein was purified either from gel slices or by affinity chromatography with a *p*-aminophenyl-β-D-thiogalactopyranoside substrate affinity matrix that binds the β-gal portion of the hybrid protein (3) and was injected into rabbits. Rabbit antiserum against the 150-kDa protein precipitated the 42-kDa *x*-*lor* protein from an HTLV-I producer T-cell line, HUT102 (2) (Fig. 5). Neither the *gag* nor *env* viral gene products present in the infected cell were recognized by the rabbit antiserum. A similar 42-kDa protein from C81-66-45, and HTLV-I-transformed but nonproducer cell line (9), was also precipitated by the rabbit antiserum. The results show that the rabbit antibodies raised to the 150-kDa protein are specific for the *x*-*lor* gene product.

The results presented here confirm previous reports that the *x*-*lor* region of HTLV-I encodes a functional protein as antiserum raised to the hybrid protein synthesized from the
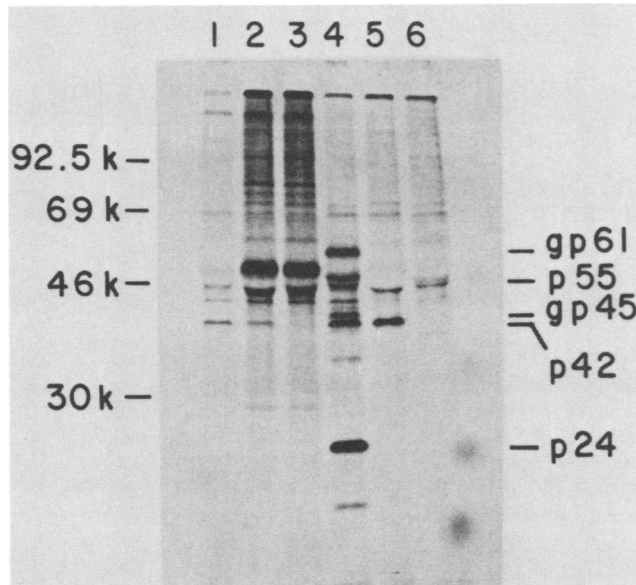
FIG. 5. Immunoprecipitation of HTLV-I-infected cells. Lysates from [^{35}S]cysteine-labeled HUT102 (lanes 4 to 6) and C81-66-45 cells (lanes 1 to 3) were reacted with ATLL patient serum (lanes 1 and 4), rabbit antiserum against the 150-kDa omp–lor–β-gal protein (lanes 2 and 5), and rabbit preimmune serum (lanes 3 and 6). k, kDa; p, protein; gp, glycoprotein.

x-lor region precipitates the 42-kDa HTLV-I-specific protein. This protein is antigenic in some ATLL patients, as the hybrid protein is recognized by antibodies in serum from patients.

The availability of monospecific antibodies should now allow the purification of the 42-kDa x-lor product. The availability of large amounts of easily purifiable bacterial protein that contains a large fraction of the HTLV x-lor gene product should also permit an assessment of its biochemical and physical parameters.

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