JOURNAL OF VIROLOGY, Aug. 1989, p. 3250–3256
0022-538X/89/083250-07$02.00/0

Vol. 63, No. 8

Identification, Using Synthetic Peptides, of the Minimum Amino Acid Sequence from the Retroviral Transmembrane Protein p15E Required for Inhibition of Lymphoproliferation and Its Similarity to gp21 of Human T-Lymphotropic Virus Types I and II

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Received 21 November 1988/Accepted 17 April 1989

Synthetic peptides containing portions of a highly conserved region of retroviral transmembrane proteins of human and animal retroviruses were tested for their ability to inhibit lymphoproliferation to determine the minimum amino acid sequence required. The previously reported immunosuppression mediated by the peptide CKS-17 was confirmed and further localized to a sequence of eight residues essentially identical to the sequence present in the transmembrane protein gp21 of human T-lymphotropic virus types I and II (HTLV-I and -II). To substantiate the physiological relevance of the inhibition of lymphoproliferation observed with the synthetic peptides and to relate this activity to the intact protein, we purified the Rauscher murine leukemia virus transmembrane protein p15E by immunoaffinity chromatography and report that this purified component presented in the form of protein micelles inhibited the interleukin-2-dependent proliferation of the murine T-cell line CTLL-2 in a dose-dependent manner, with a half-maximal inhibitory dose (ID_{50}) of ~16 nM. In comparison, the ID_{50} concentration of a recombinant form of p15E required to inhibit lymphoproliferation was ~2.2 μM. The results reported here support the hypothesis that the transmembrane protein gp21 of HTLV-I and -II participates in the mechanism of immunosuppression previously reported for the transmembrane proteins of feline leukemia virus and other animal retroviruses. Thus, the transmembrane protein of HTLV-I, the etiological agent of adult T-cell leukemia-lymphoma, may be partially responsible for the immunocompromised clinical course of this disease that results in fatal opportunistic infections in a majority of cases.

Retroviral infection is often accompanied by clinical immunosuppression in both humans and animals, leading to enhanced susceptibility to infections by opportunistic organisms (10, 20, 25, 31, 47). The in vivo immune functions suppressed by type C retroviruses include both the cellular and humoral arms of the immune response (2, 5, 42). This suppression was initially attributed to the cytotoxic effect of virus infection on lymphocytes. However, in the mid-1970s, several laboratories demonstrated that inactivated retroviruses abrogated resistance to subsequent challenge infection (29, 45). Additional studies of Cianciolo and Snyderman and colleagues (3, 11, 12, 18, 27, 28, 33) with synthetic peptides and a recombinant expression product of p15E have demonstrated that the active sequence is contained within the viral transmembrane protein p15E. Computer-assisted analysis of the amino acid sequences of various animal retroviral transmembrane proteins as well as that of human T-lymphotropic virus types I and II (HTLV-I and -II) showed that a very high degree of similarity exists within a 26-amino-acid region (4). A synthetic peptide, CKS-17, corresponding to the most highly conserved 17 amino acids was inhibitory in several assays of immune function: (i) mitogen- and alloantigen-stimulated proliferation of human lymphocytes (3); (ii) proliferation of the cloned murine CTLL-2 cell line (3); (iii) the respiratory burst of human monocytes (11); (iv) human natural killer cell-mediated cytotoxicity (12); (v) immunoglobulin secretion (27); (vi) interleukin-1 (IL-1) activity (18); and gamma interferon production (28). In addition, these investigators demonstrated that a recombinant expression product comprising the major hydrophilic domain of p15E, which contains the CKS-17 sequence, can inhibit lymphoproliferation (33).

The present study was undertaken to delineate the minimum amino acid sequence of p15E that is capable of inhibiting lymphoproliferation and to relate the inhibitory potency of the synthetic peptides to that observed with the native and recombinant forms of p15E.

MATERIALS AND METHODS

Synthetic peptides. Peptides were synthesized by automated Merrifield solid-phase techniques with an Applied Biosystems 430A peptide synthesizer as described previously (6). In each case, the peptides included the amino acid sequence Lys-Cys-Tyr-Gly-Gly at the N terminus: Lys and Cys for use in conjugation, Tyr for radioiodination, and the Gly-Gly dipeptide as a spacer. The peptides were cleaved and deprotected by the low-high hydrogen fluoride cleavage method (Multiple Peptide Systems, Solano Beach, Calif.). Following purification by reversed-phase high-performance liquid chromatography, the peptides were estimated to be >95% pure and the sequences were verified by sequencing with an Applied Biosystems 470A gas-phase protein microsequenator. Purified peptides were conjugated to bovine serum albumin (BSA) by a modification of the method described by Dolittle (8): 2.5 mM peptide and 0.075 mM BSA were mixed in 0.16 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-hydrochloride (pH 4.5) and rotated at 25°C for 4 h. The reaction was quenched by the addition of 0.1 volume of 2 M acetic acid–2 M ethanolamine (pH 4.5) followed by rotation overnight at 4°C. BSA in the absence of

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peptide was treated in the same manner and used as a control. The peptides were radioiodinated at the Tyr residue (15, 26) and included in conjugation reactions as a radiotracer to permit quantitation of the final peptide concentration of peptide-BSA conjugates. The conjugates were then exhaustively dialyzed against phosphate-buffered saline (PBS) before use in cellular assays.

Purification of native p15E. Rauscher murine leukemia virus (R-MuLV) was obtained as a twice-sucrose-banded preparation from the Frederick Cancer Research Center (39). Purification of p15E was accomplished by a two-step procedure. First, virus particles were solubilized essentially as described previously (41) with 50 mM Tris–100 mM NaCl–1 mM EDTA (pH 7.4) (TEN) with 2 mM phenylmethylsulfonyl fluoride, except that 1% Triton X-114 (TX-114) was substituted for TX-100. The mixture was submitted to three rounds of freeze-thawing and then exhaustively dialyzed against TEN with 0.1% TX-114. The lysate was centrifuged at 100,000 × *g* for 1 h at 4°C to remove insoluble material. The soluble fraction was then warmed to induce phase separation, and the p15E was extracted into the detergent phase exactly as described for integral membrane proteins (1). The second step of the purification procedure consisted of immunoaffinity chromatography. Purified monoclonal antibody 19F8 (immunoglobulin G2a) (22) was coupled to protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) and cross-linked by using the bifunctional reagent dimethyl pimelimidate (Pierce Chemical Co., Rockford, Ill.) as described previously (34). The TX-114 phase of the viral extract was diluted with 0.1 M borate (pH 8.0) to lower the detergent concentration to 1% and applied to the immunoaffinity column, which had been preequilibrated in the same buffer. After the column was washed with 0.1 M borate–0.5 M NaCl–0.1% TX-100 (pH 8.0), the p15E was eluted with 0.5 M propionic acid–0.1% TX-100 (pH 2.5) and the eluent was collected into tubes containing 2 M Tris–0.1% TX-100, pH 7.6. The eluent was then extensively dialyzed against 50 mM Tris–100 mM NaCl (TN) with 0.1% TX-100 (pH 7.4) prior to preparation of protein micelles (PM).

The purity of p15E was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (19) and silver staining (46). Samples were prepared by heating to 100°C for 2 min in sample buffer containing 1% SDS and 5% 2-mercaptoethanol and resolved by SDS-PAGE in a 12 to 22% linear gradient slab gel (1.5 by 180 mm).

PM. PM were prepared by ultracentrifugation of detergent-solubilized protein into a detergent-free sucrose gradient by the method of Simons et al. (37). Control PM (cntl PM) were generated by extracting 3×10^8 HaSV-NIH 3T3 cells (32) exactly as for R-MuLV in the presence of the following protease inhibitors: 10 μM leupeptin, 10 μM pepstatin A, and 0.5 trypsin inhibitor units of aprotinin per ml. Approximately 0.25 ml of the purified p15E and HaSV-NIH 3T3 TX-114 extract, each adjusted to a protein concentration (BCA Protein Assay; Pierce) of 1 mg/ml in TN with 1% TX-100, was layered onto density gradients consisting of a zone of 0.3 ml of TN–1% TX-100–5% (wt/wt) sucrose above a 12.5-ml gradient of 10 to 50% (wt/wt) sucrose in TN without detergent. After centrifugation in a Beckman SW41 rotor at 40,000 rpm for 20 h at 20°C, fractions containing p15E PM were identified by the dot-immunobinding assay described below. Fractions of sucrose gradients containing cntl PM were identified spectrophotometrically at 280 nm and pooled. Both p15E PM and cntl PM were exhaustively dialyzed against PBS before use in cellular assays.

An estimate of the molecular size of the p15E PM was obtained by sedimentation analysis and gel filtration chromatography. Sedimentation of TX-100-solubilized purified p15E into detergent-free sucrose gradients resulted in the formation of ordered micellar aggregates of the protein that were no longer dependent on detergent for solubility. The sedimentation coefficient of the p15E PM was calculated to be 12.0S by comparison with thyroglobulin (19.3S), apoferritin (17.6S), and alcohol dehydrogenase (7.6S) in velocity sedimentation analyses (24). An estimate of the molecular size of the p15E PM was obtained from the sedimentation coefficient alone, using the equation $s_1/s_2 = (M_{r1}/M_{r2})^{2/3}$ (24), where M_r is molecular weight. Assuming 669,000 as the M_r of thyroglobulin, the p15E PM had a M_r of 760,000. The M_r of the p15E PM was confirmed by gel filtration chromatography (44) with a column (1.5 by 100 cm) of Sephacryl S-300 (Pharmacia) calibrated with the same standards as were used for sedimentation. The M_r obtained, 780,000, is in good agreement with the value obtained by sedimentation analysis. The cntl PM exhibited a molecular size range of 250,000 to 1,000,000. The residual TX-100 content was determined with [phenyl-^{3}H(N)]TX-100 (specific activity, 1.3 mCi/mg; Dupont, NEN Research Products, Boston, Mass.) as described previously (13).

Dot-immunobinding assay. Dot-blot assays were done at room temperature, and all incubations and washes were performed on a rotary shaker. Nitrocellulose membranes (pore size, 0.22 μm; Schleicher & Schuell, Inc., Keene, N.H.) were washed in PBS for 15 min prior to spotting 2 to 50 μl (depending on the protein concentration) of fractions from sedimentation and gel filtration analyses. The membranes were then incubated in BLOTTO (16) for 1 h to reduce nonspecific binding and washed in PBS for 30 min with five changes of buffer, the third containing 0.05% Tween 20. All subsequent incubation steps were followed by the same washing procedure. Membranes were incubated with purified monoclonal antibody 19F8 (5 μg/ml in PBS with 10 mg of BSA per ml) for 1 h, washed, and incubated with biotinylated sheep anti-mouse immunoglobulin (Amersham Corp., Arlington Heights, Ill.) followed by avidin-linked horseradish peroxidase (Amersham), and bound antibody was detected with diaminobenzidine according to the instructions provided with the biotin-streptavidin system.

rp15E. A recombinant expression product comprising the hydrophilic domain of p15E (rp15E) was generated by subcloning the corresponding DNA fragment from the infectious proviral molecular clone pAKV623 (23) kindly provided by Neal Copeland of NCI-Frederick. The proviral clone obtained in pBR322 was digested with *Bgl*I, and the relevant 1.5-kilobase fragment was isolated by agarose gel electrophoresis. This fragment was added to *Kpn*I-digested pGEM-3Z (Promega Biotec, Madison, Wis.), and the mixture was blunt ended with T4 DNA polymerase in the presence of deoxynucleotide triphosphates followed by ligation with T4 DNA ligase. This construct was then digested with *Sty*I-*Sac*I, blunt ended, and reclosed with T4 DNA ligase as above. The resultant 0.3-kilobase insert in pGEM-3Z was excised with *Bam*HI-*Eco*RI, and the fragment was ligated into the corresponding sites of the expression vector pGEX-2T (38) (Amrad Corp., Sydney, Australia). This vector expresses a tripartite fusion protein consisting of glutathione-*S*-transferase (GST) followed by a thrombin cleavage site and then the inserted sequence. *Escherichia coli* DH5α was used for intermediate cloning steps, and strain HB101 was used for expression of the final construct.

For the production of fusion protein, an overnight culture was diluted 1:10 in Luria broth containing ampicillin (100

TABLE 1. Sequences of retroviral transmembrane protein-derived synthetic peptides

Source	Sequence	No. of peptides[a]
Viral sequences		
MuLV[b]	E V V L Q N R R G L D L L F L K E G G L	
HTLV-I, -II[c]	A Q N R R G L D L L F W E Q G G L	
Synthetic peptides		
MOLV.1 (K C Y G G)	L Q N R R G L D L L F L K E G G L	23
MOLV.2 (K C Y G G)	E V V L Q N R R G L D L L	17
MOLV.3 (K C Y G G)	L L F L K E G G L	29
MOLV.21 (K C Y G G)	E V V L Q N R R G L D	10
MOLV.22 (K C Y G G)	E V V L Q N R R G	16
MOLV.23 (K C Y G G)	L Q N R R G L D L L	17
MOLV.24 (K C Y G G)	L Q N R	12
MOLV.25 (K C Y G G)	Q N R R	10

[a] Number of peptides per molecule of BSA.
[b] Represents residues 436 to 455 of gp80^{env} of AKR MuLV (14).
[c] Represents residues 376 to 392 and 372 to 388 of gp67^{env} of HTLV-I and -II, respectively (39).

μg/ml), incubated for 1 h at 37°C, induced with isopropyl-β-D-thiogalactopyranoside (0.5 mM), and then incubated for an additional 4 h at 37°C. The cells were lysed, and the fusion protein was purified by glutathione affinity chromatography and cleaved with thrombin (Sigma Chemical Co., St. Louis, Mo.) exactly as described previously (38). The cleavage products were then subjected to immunoaffinity chromatography, and the rp15E was purified exactly as described above for native p15E. The flowthrough fraction was rechromatographed with glutathione-agarose to obtain purified GST. The purification was analyzed by SDS-PAGE exactly as for native p15E except that a 10 to 20% linear gradient slab gel (1.5 by 100 mm) was used and stained with Coomassie blue. The purified rp15E and GST were then exhaustively dialyzed against PBS prior to use in lymphoproliferation assays.

Cell culture. The T-cell lines CTLL-2 and EL-4.IL-2 were obtained from the American Type Culture Collection (Rockville, Md.). CTLL-2 cells were grown in RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 50 μM 2-mercaptoethanol, 50 μg of penicillin per ml, 50 μg of streptomycin sulfate per ml, and 100 μg of neomycin sulfate per ml (RPMI-complete) and supplemented with conditioned medium (final concentration, 50%) from rat splenocytes cultured for 48 h in RPMI-complete and stimulated with 2 μg of concanavalin A per ml. EL-4.IL-2 cells were grown in Dulbecco minimum essential medium supplemented with 2 mM glutamine and 10% fetal calf serum and were induced with 10 ng of phorbol myristate acetate (Sigma) per ml for 24 h at a density of 2×10^6 cells per ml to prepare conditioned medium for CTLL-2 proliferation assays. The 19F8 hybridoma (22) was kindly provided by Mark Lostrom (Genetic Systems, Seattle, Wash.) and was maintained in Dulbecco minimum essential medium supplemented with 10% fetal calf serum and 2 mM glutamine.

Proliferation assays. The inhibitory activity of the synthetic peptides and the p15E preparations was determined by measuring their effect on lymphoproliferation in vitro. CTLL-2 cells (10^4 in 200 μl per well) were cultured in 96-well microdilution plates in the presence of free peptide, peptide-BSA conjugates, and p15E preparations at the concentrations specified for 24 h at 37°C in humidified 5% CO_2. Culture medium was that used for propagation, with 1% conditioned medium from induced EL-4.IL-2 cells added as a source of IL-2. Proliferation was quantitated by pulsing cells with 1.0 μCi of [^{3}H]thymidine (specific activity, 50 Ci/mmol; Amersham) per well during the final 4 h of culture, harvesting cultures onto glass fiber filters, and determining incorporated radioactivity by scintillation spectrophotometry.

RESULTS

Inhibition of CTLL-2 proliferation by synthetic peptides. The minimum amino acid sequence required for inhibitory activity was delineated by measuring the effect on lymphoproliferation of synthetic peptides (Table 1) derived from a region of the retroviral transmembrane protein amino acid sequence that is highly conserved among a number of animal and human retroviruses (4) and is identical among more than 10 sequences available for MuLV. To extend the previous observations reported for the CKS-17 peptide, we synthesized these peptides (Table 1) to delineate the minimum sequence required for inhibition of lymphoproliferation.

We found that peptides MOLV.1, corresponding to the immunosuppressive CKS-17 sequence, and MOLV.2, comprising approximately the left half of this sequence, were almost equally potent in their dose-dependent inhibition of CTLL-2 proliferation (half-maximal inhibitory dose [ID_{50}] of ~30 μM) (Fig. 1A). In contrast, MOLV.3, comprising approximately the right half of the CKS-17 sequence, had no effect on cellular proliferation, nor did the BSA control. Deletion of the two C-terminal (MOLV.21) or three N-terminal (MOLV.23) residues from the MOLV.2 sequence did not significantly alter the ID_{50} (Fig. 1B). However, deletion of four residues from the C terminus of MOLV.2 (MOLV.22) resulted in a decrease in potency, and deletion of five (MOLV.25) or six (MOLV.24) residues abolished activity entirely (Fig. 1B). In agreement with previous observations (3), free peptides had no activity and required conjugation to carrier protein (data not shown).

The peptide conjugates were tested for their effect on the proliferation of NIH 3T3 fibroblast cells to determine whether the antiproliferative activity detected with CTLL-2 cells was specific for cells of the immune system. The peptides did not inhibit the proliferation of NIH 3T3 cells, suggesting that the inhibitory activity is dependent not only on the peptide sequence but also on the target cell type.

p15E PM. To substantiate the physiological relevance of the immunosuppressive activity observed with the synthetic peptides, we purified p15E and determined its effect on lymphoproliferation. As assessed by SDS-PAGE and silver

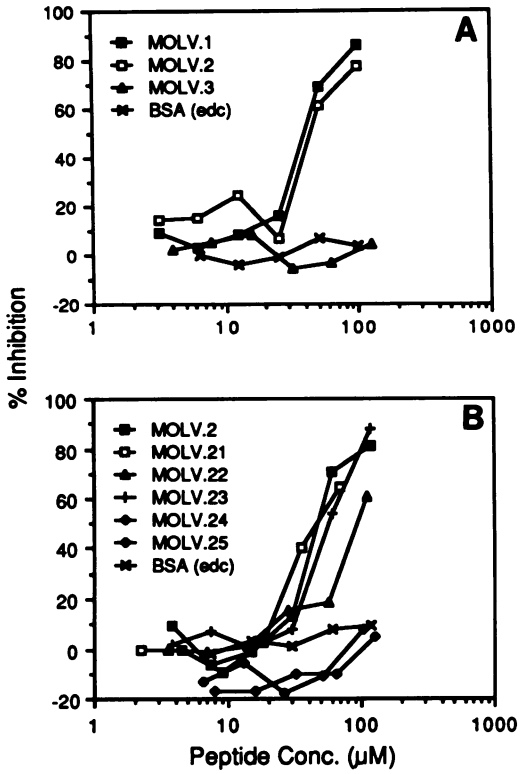

FIG. 1. Inhibition of proliferation of CTLL-2 by synthetic peptides. Percent inhibition was calculated by the following formula: % inhibition = [1 − (exp cpm − bkgd cpm)/(total cpm− bkgd cpm)] × 100 where bkgd cpm (counts per minute) is [^{3}H]thymidine uptake in the absence of IL-2 and synthetic peptides (2,471 ± 546), total cpm is uptake in the presence of IL-2 without peptides (83,193 ± 1,847), and exp cpm is uptake in the presence of IL-2 and peptides in the concentrations specified. These data and those in the following figures represent the mean of quadruplicate samples (the standard error of the mean of which averaged less than 5% of the mean in all experiments) and are representative of three separate experiments.

staining, the use of detergent-phase separation techniques and immunoaffinity chromatography resulted in the purification of p15E to apparent homogeneity (Fig. 2). Characterization of detergent-free p15E PM by velocity sedimentation and gel filtration chromatography showed that the M_r was about 780,000. This size value indicates that the p15E PM are composed of ~40 monomers. Although the transmembrane protein is designated p15E, its calculated molecular size based on sequence data is 19,850 daltons (36).

rp15E. A recombinant expression product of p15E (rp15E) consisting of *env* amino acids 499 to 606 from AKR MuLV was also constructed. After affinity chromatography, this 11-kilodalton hydrophilic domain of p15E was shown to be purified to apparent homogeneity as assessed by SDS-PAGE (Fig. 3).

Inhibition of CTLL-2 proliferation by p15E PM and rp15E. The p15E PM inhibited the IL-2-driven proliferation of the murine CTLL-2 cell line in a dose-dependent manner (Fig. 4A). The level of inhibition obtained at the highest concentration tested (60 μg/ml) was 95%, and the ID_{50} was ~10 μg/ml (Fig. 4A). The cntl PM had no significant effect on CTLL-2 proliferation.

The rp15E also inhibited CTLL-2 proliferati ,n in a specific and dose-dependent manner (Fig. 4B). The level of inhibition observed at the highest concentration tested (80 μg/ml) was

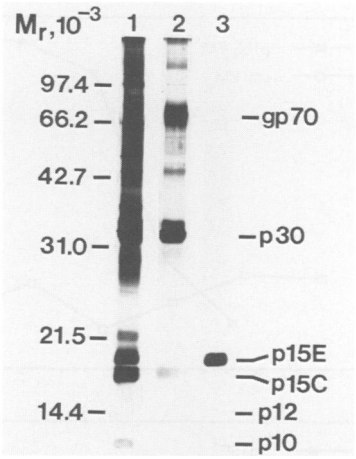

FIG. 2. SDS-PAGE of native p15E purification. Samples were treated as described in Materials and Methods. The samples analyzed were whole R-MuLV (lane 1), the aqueous fraction of the TX-114-extracted R-MuLV containing the major hydrophilic viral proteins (lane 2), and the eluent from immunoaffinity chromatography of the detergent phase of the TX-114 extraction (lane 3).

80% and the ID_{50} was ~30 μg/ml (Fig. 4B). The purified GST had no significant effect on CTLL-2 proliferation.

It was imperative to determine the residual detergent content of the p15E PM to ensure that any suppressive activity observed with the PM preparation could not be ascribed to the presence of cytotoxic concentrations of TX-100. When [^{3}H]TX-100 was included as a radiotracer in the p15E preparation prior to PM formation, it was determined that the residual TX-100 content of p15E PM was 1.1% on a weight/weight basis. The concentration of TX-100 associated with the p15E PM at its ID_{50} (Fig. 4A) was ~100-fold less than that found to inhibit proliferation (data not shown). Thus, TX-100 can be ruled out as contributing to the suppressive activity observed with the p15E PM.

DISCUSSION

We showed in this study that the sequence of eight residues shared by the peptides MOLV.21 and MOLV.23 is

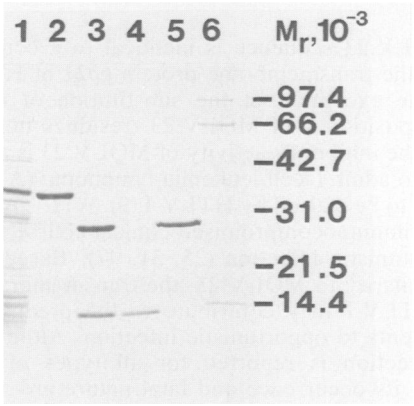

FIG. 3. SDS-PAGE of rp15E purification. Samples were treated as described in Materials and Methods. The samples analyzed were bacterial lysate (lane 1), purified GST-rp15E fusion protein (lane 2), thrombin cleavage products of GST-rp15E fusion protein (lane 3), purified rp15E (lane 4), and purified GST (lane 5).

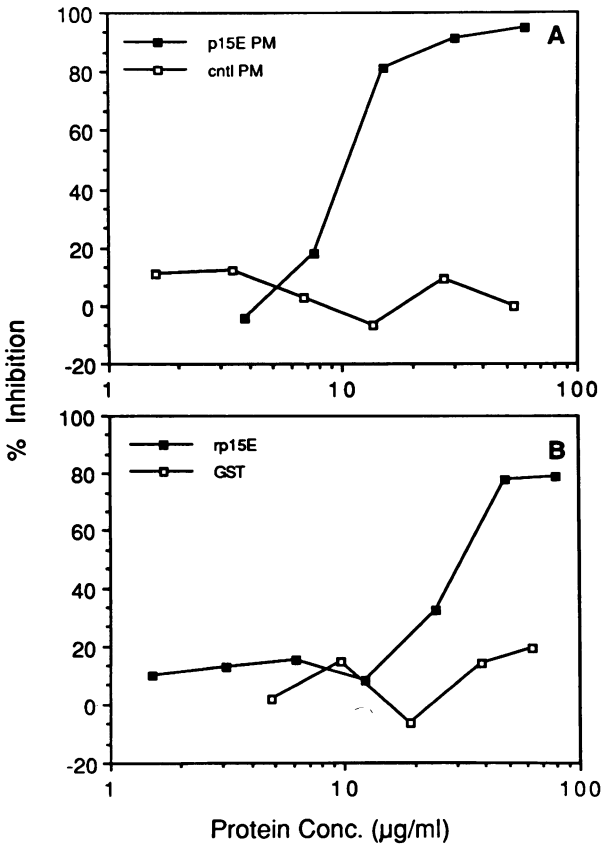

FIG. 4. Inhibition of proliferation of CTLL-2 by p15E PM (A) and rp15E (B). Data were obtained and calculated as described in the legend to Fig. 1. The p15E PM and rp15E, as well as their respective controls, cntl PM and GST, were obtained as described in Materials and Methods. The values for total cpm (counts per minute) and bkgd cpm in this experiment were 336,387 ± 12,988 and 1,347 ± 381, respectively.

sufficient to inhibit lymphoproliferation. This sequence represents approximately the left half of the CKS-17 peptide previously shown to mediate immunosuppression. In addition, the peptide MOLV.3, which comprises the right half of CKS-17, was shown to have no effect on lymphoproliferation.

The MOLV.23 sequence is identical to a conserved sequence of the transmembrane protein gp21 of HTLV-I and -II with the exception of the substitution of alanine for leucine at position 1 of MOLV.23 (residues underlined in Table 1). The inhibitory activity of MOLV.23 is of potential relevance to adult T-cell leukemia-lymphoma (ATLL), now recognized to be caused by HTLV-I (9). ATLL is characterized by its immunocompromised clinical course resulting in fatal opportunistic infection (25, 31, 47). Based on its sequence similarity to MOLV.23, the transmembrane protein gp21 of HTLV-I may contribute to the predisposition of ATLL patients to opportunistic infection. Although opportunistic infection is reported for all types of malignant lymphoma, its occurrence and fatal nature are as much as five times more prevalent in ATLL than for other forms of malignant lymphoma, e.g., T-cell malignant lymphoma (2, 17, 35). The impairment of humoral immunity in ATLL patients (2) is highly reminiscent of that observed in cats infected with feline leukemia virus (42). Cellular immune function is also affected by HTLV-I as measured by its suppressive effect on in vitro lymphoproliferation (43).

It has been well documented that immunosuppression can be achieved in vivo and in vitro with inactivated virus (7, 29, 45). Furthermore, as little as 0.2 ng of p15E administered systemically to mice has been shown to inhibit the accumulation of macrophages to inflammatory sites (5). Synthetic peptides can not be expected to optimally mimic native virus. This point is demonstrated by the dramatic increase in potency that we observed when synthetic peptide, p15E, and native p15E were compared (20 μM, 2 μM, and 16 nM, respectively, were required for half-maximal inhibition). The concentration of synthetic peptide required for inhibition of proliferation in vitro appears to be higher than the concentration of retroviral transmembrane protein expected during a normal infection in vivo. However, analogous differences between in vitro and in vivo concentrations of peptide required for immunologic stimuli have also been observed in other systems. In mapping of T-cell epitopes, a much greater concentration of synthetic peptide is required for in vitro stimulation than the concentration responsible for priming the immune response in vivo (21).

This report presents direct evidence that a purified form of native retroviral transmembrane protein p15E inhibits lymphoproliferative function in vitro. The purification of native p15E allowed us to generate PM that may more closely resemble the natural multivalent presentation of p15E on the viral envelope and the surface of infected cells and may therefore allow us to approximate more closely the immunosuppressive activity of this protein in vivo. Nevertheless, the purified forms of p15E may yet underestimate the true potency. The presence of additional viral components such as gp70 may facilitate the interaction between the p15E and its cellular target. The observed difference in inhibitory potency between the monomer (rp15E) and multimeric (p15E PM) preparations (discussed above) may be due to conformational differences between the two preparations. Nevertheless, because rp15E is soluble in monomeric form in the absence of detergent and is readily available, it may prove useful as a reagent for the characterization and identification of a putative cell surface receptor responsible for transduction of the inhibitory signal of these retroviral ligands.

We delineated the minimum amino acid sequence of the retroviral transmembrane protein p15E that is required for inhibition of lymphoproliferation and found this minimum sequence to be essentially identical to that present in the transmembrane protein gp21 of HTLV-I and -II, thus suggesting that this protein component of HTLV-I and -II is involved in the immunosuppression observed in vivo. This hypothesis is consistent with the clinical observation that HTLV-I infection and its manifestation as ATLL predispose these patients to suffer severe and usually fatal opportunistic infections owing to their immunosuppressed status. The prevalence of immune dysfunction in both ATLL patients and patients with acquired immunodeficiency syndrome has led us to test the hypothesis that the transmembrane protein gp41 of HIV may inhibit lymphoproliferation in a manner similar to that of p15E and gp21 (30).

ACKNOWLEDGMENTS

We thank Mark Lostrom of Genetic Systems and Neal Copeland of NCI-Frederick for gifts of reagents and cells, Deborah McClellan for editorial assistance, and J. Thomas August for critical reading of the manuscript.

This work was supported by Public Health Service grant CA-33470 and research training grant CA-09243 (to C.L.R. ad C.R.M.) from the National Institutes of Health.

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