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Recombinant hydrophilic region of murine retroviral protein p15E inhibits stimulated T-lymphocyte proliferation

(immunosuppression/neoplasia/acquired immunodeficiency syndrome/expression vector)

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ABSTRACT **Retroviral envelope protein p15E and antigenically related proteins have been implicated as potential mediators of immune dysfunction associated with retroviral infections and with neoplasia. Due to its extreme hydrophobicity, purified p15E has not been available in a nondenatured form or in sufficient quantities for detailed studies on the mechanisms of its immunosuppressive effects. Therefore, a plasmid was constructed to direct the synthesis in *Escherichia coli* of the major hydrophilic region of murine p15E. The purified recombinant p15E derivative, soluble under physiological conditions, inhibited by up to 60% (EC_{50} = 7.5 nM) the anti-CD3-driven proliferation of human T lymphocytes but had no effect on the proliferation of the transformed T-cell line Jurkat. The recombinant protein also inhibited, by up to an average of 92% (EC_{50} = 2.1 μM), the proliferation of the murine T-cell line CTLL-2. These data (*i*) provide direct evidence that a retroviral envelope protein can itself inhibit lymphoproliferative function and (*ii*) map the inhibitory activity to a specific region of p15E. The availability of soluble, recombinant p15E should facilitate studies of the pathogenesis of the immunosuppression accompanying retroviral infections and neoplastic diseases.**

Retroviral infections and neoplasms are both associated with immunosuppression in humans and in animals (1, 2). The precise mechanisms of such suppression in either case are unclear, but evidence suggests that they may share a common feature. Retroviral envelope proteins or related proteins expressed by apparently uninfected tumor cells appear to be capable of directly inhibiting leukocyte functions. Inactivated murine, feline, and human retrovirus particles inhibit lymphocyte activities *in vitro* (3–6), and purified retroviral protein of the same molecular weight as the transmembrane envelope protein p15E of murine and feline leukemia viruses has suppressive activity *in vitro* and *in vivo* (7–11). p15E and related molecules may also function as immunosuppressive agents in neoplastic diseases, since extracts prepared from carcinogen-induced primary murine tumors and human malignant effusions contain inhibitors that can be specifically absorbed by anti-p15E monoclonal antibodies (12). Moreover, p15E-related antigens have been detected in human squamous cell carcinomas of the head and neck (13). Thus p15E and antigenically related proteins may contribute to the pathogenesis of immune dysfunction associated with retroviral infection and neoplasia (14).

Efforts to directly study the biological activity of retroviral p15E have been hampered by its limited availability in retrovirus preparations and by its insolubility in physiological solutions (15). The insolubility may be due to two distinct hydrophobic regions in the molecule. p15E consists of four distinct sequential regions: an N-terminal hydrophobic sequence, a larger central hydrophilic sequence, a second hydrophobic domain, and a final short C-terminal hydrophilic region. This general structure is typical for the transmembrane envelope proteins of retroviruses (16–19). Three of the four regions are thought to play assignable roles (16, 19, 20). The N-terminal hydrophobic region is probably extracellular and may serve as a domain for interaction with the exterior envelope glycoprotein and with the membrane of target cells during virus infection. The second hydrophobic domain is assumed to be the membrane-spanning region. The C-terminal hydrophilic region is probably cytoplasmic and may interact with internal viral core proteins during virus assembly. The function of the central large hydrophilic region of p15E is unknown. That it may be important for the biological activity of p15E is suggested by a high degree of conservation among retroviruses of a sequence internal to this region (18, 21). In addition, the hydrophilic region is probably located on the surface of the virion or the cell expressing p15E and thus would be accessible for interaction with the surrounding immunological environment (22).

To generate a water-soluble derivative of p15E, we have constructed a plasmid to express its major hydrophilic region in the absence of the flanking hydrophobic domains. We hypothesized that this region is responsible for some or all of the immunosuppressive activity ascribed to p15E.

MATERIALS AND METHODS

Construction of Expression Plasmids. Expression plasmids were constructed from subclones of pMo-env, a derivative of cloned proviral Moloney murine leukemia virus (23), by standard techniques (24). Plasmids were digested with the restriction enzymes indicated in Fig. 1. Fragments were modified by using the Klenow fragment of DNA polymerase to fill-in 5′ protruding ends when it was necessary to generate blunt ends, and by the ligation of synthetic 10- or 12-mer *Bam*HI linkers (New England Biolabs) chosen to properly adjust the translational reading frame in each final construct. End-modified fragments were isolated from gels after electrophoresis and ligated into the *Bam*HI site of expression vector pJG200 (25) to generate pME-e1, pME-e2, pME-e3, and pME-e4. These plasmids encode tripartite fusion proteins, consisting of an N-terminal p15E sequence followed by a segment of chicken collagen and enzymatically active *Escherichia coli* β-galactosidase. The ATG initiation codon (which overlaps the *Bam*HI insertion site) and a ribosomal binding site are contributed by the pJG200 vector. Transcription is under control of the λ phage P_R promoter, regulated by the thermolabile cI857 repressor protein, also encoded by the plasmid.

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Abbreviations: IL-2, interleukin 2; mAb, monoclonal antibody.

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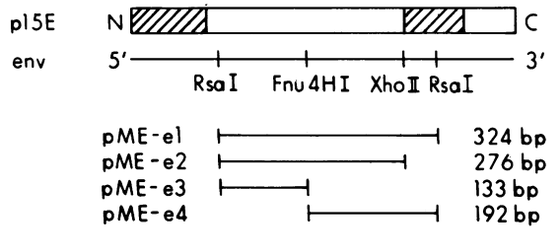

FIG. 1. Structural model for p15E and construction of expression plasmids. Hatched regions are hydrophobic, unhatched regions are predominantly hydrophilic. This schematic representation is based on published computer-assisted analyses (18). The indicated restriction sites in the *env* gene of Moloney murine leukemia virus (17) were utilized for construction of the expression plasmids. The portion of the gene incorporated into each expression plasmid is indicated. bp, Base pairs.

Plasmid pME-e2 was converted from a pBR322 derivative to pME-e2a, a pUC derivative, by the following manipulations. An *Ava* I site in pME-e2 just 3′ to the end of the β-galactosidase gene *lacZ* was converted to an *Eco*RI site by means of 10-mer *Eco*RI linkers (New England Biolabs). The *Pvu* II sites at positions 306 and 624 of pUC13 (26) were also converted to *Eco*RI sites with deletion of the intervening sequence to give plasmid pUC-E. pME-e2a was then constructed by ligation of three fragments: the large *Xba* I–*Eco*RI fragment of pUC-E, the *Xba* I–*Nde* I fragment of pME-e2 (which contains all of the tripartite gene through an *Nde* I site just 5′ to the end of *lacZ*), and the *Nde* I–*Eco*RI fragment (containing the final 3′ portion of *lacZ*) for which the *Eco*RI site was created by the linker modification described above. The conversion from pME-e2 to pME-e2a left the promoter and fusion-protein coding region unchanged but essentially converted the plasmid backbone from a pBR322 derivative to a pUC derivative.

Bacterial strains HB101, JM105, and JM109 (26) were used for plasmid construction. The Lac$^-$ strains MC1000 and MC1009 (27) were used as hosts for the final expression constructs.

Optimization of Expression Levels. The conversion of pME-e2 to pME-e2a resulted in a doubling of the level of expression. A second doubling of expression levels was obtained by growing the bacteria in a very rich "superbroth" (35 g of tryptone, 20 g of yeast extract, and 5 mg of NaCl per liter) (28) instead of standard Luria broth. Additionally, because of the relative stability of the protein in *E. coli*, it was possible to extend the induction period and thereby obtain even higher yields of protein. A 4-hr induction of MC1009 with pME-e2a in superbroth gave expression levels of ≈75 mg per liter of culture, based on β-galactosidase determinations (29).

Antibodies. Anti-p15E monoclonal antibodies (mAbs) 4F5, 19F8, 19VIIIE8, and 9E8 have been described (11, 30). Anti-p15E mAb 372 was kindly provided by M. Cloyd (Duke University, Durham, NC) (31), and anti-β-galactosidase mAb 8.4 was kindly provided by R. Greenspan (Princeton, NJ). Rabbit anti-p15E antiserum has been described (11). Rabbit anti-β-galactosidase antiserum was obtained by immunization of New Zealand White rabbits with *E. coli* β-galactosidase (Bethesda Research Laboratories).

Protein Blotting and Immunodetection. Protein samples were denatured in 2% $NaDodSO_4$, electrophoresed in 8% acrylamide gels, and transferred to nitrocellulose (70 V for 2 hr in 25 mM Tris/192 mM glycine/20% methanol). Remaining protein-binding sites on the nitrocellulose were blocked by overnight incubation in 5% bovine serum albumin. Strips of the nitrocellulose were incubated (3 hr to overnight) in blotting buffer (150 mM NaCl/50 mM Tris·HCl/5 mM EDTA/0.1% bovine serum albumin/0.25% gelatin/0.05% Tween 20/0.005% thimerosal, pH 7.4) with antibodies at various dilutions. After washing, strips were incubated with ^{125}I-iodinated *Staphylococcus* protein A (0.5×10^6 cpm/ml; specific activity 3–6 μCi/μg; 1 Ci = 37 GBq) and reactivity was detected by autoradiography.

Protein Purification. MC1000 or MC1009 cells containing pME-e2 or pME-e2a were grown at 32°C to late logarithmic phase and were induced for expression by warming to 40–42°C for 4 hr. Cells were washed, treated with lysozyme (1 mg/ml, 4°C, 30 min), and frozen. While the lysate was thawing, reagents were added to give final concentrations of 500 mM NaCl, 100 mM Tris (pH 7.8), 12 mM $MgCl_2$, 2 mM EDTA, 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 2% *n*-octyl glucoside in a volume of 30 ml per liter of initial culture. The lysate was sonicated and centrifuged at $100,000 \times g$ for 30 min, and the supernatant was applied to an affinity column with specificity for β-galactosidase (32). After washing, bound material was eluted with 100 mM sodium tetraborate, pH 10.0/10 mM $MgCl_2$/1 mM 2-mercaptoethanol/1.2% *n*-octyl glucoside. The eluate was neutralized and applied to a column of anti-p15E mAbs 4F5 and 19F8 coupled to Sepharose. This column was washed, and then bound material was eluted with 500 mM NaCl/100 mM Tris, pH 11.0/1.2% *n*-octyl glucoside. After neutralization with sodium phosphate buffer, protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μM pepstatin, 20 μM leupeptin), $CaCl_2$ (1 mM), and collagenase (from *Achromobacter iophegus*, Boehringer Mannheim, used at an enzyme/substrate ratio of 1:50) were added and digestion was allowed to proceed at room temperature for 1 hr. After centrifugation, the digest was passed through a β-galactosidase affinity column and the effluent was adsorbed to a fresh anti-p15E column. This column was washed overnight with detergent-free buffer. Material retained on the column was eluted with buffer containing detergent, and the eluate was concentrated and then dialyzed for 3 days against several changes of Hanks' balanced salts solution. The β-galactosidase portion of the cleaved fusion protein was also purified by first passing the digest through an anti-p15E column to adsorb any uncleaved fusion protein and then by use of the β-galactosidase affinity column. Like the p15E derivative, it was concentrated and dialyzed against Hanks' solution for use as a control in biological systems. The nonionic, dialyzable detergent *n*-octyl glucoside was included in the early steps of purification in order to improve the yield of fusion protein. The detergent was removed from the final purified products by extensive washing of the cleaved purified derivatives while adsorbed to the affinity resins, followed by elution and exhaustive dialysis. The recombinant p15E derivative and the β-galactosidase contained <0.1 ng of endotoxin per ml (*Limulus* lysate assay, performed by T. Hamilton, Duke University, Durham, NC). Protein was determined with the Bio-Rad protein assay kit. β-Galactosidase determinations were performed as described (29).

Anti-CD3-Stimulated Lymphoproliferation. Human mononuclear cells were isolated from healthy volunteers by density-gradient centrifugation of blood using Lymphocyte Separation Medium (LSM, Litton Bionetics). The cells were resuspended to 2×10^6 lymphocytes per ml in RPMI 1640 medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential amino acid solution (1%; GIBCO), and either 2% fetal bovine serum or 1% Nutridoma (Boehringer Mannheim). One-tenth milliliter of cell suspension was added to each well of a 96-well tissue culture plate with 50 μl of test material and 50 μl of anti-CD3 (OKT3) antibody (Ortho Pharmaceutical; 1:2500 dilution) in medium and the plate was incubated for ≈68 hr at 37°C. Fifty microliters of medium containing 1.0 μCi of [*methyl*-^{3}H]thymidine (New England Nuclear; 6–7 Ci/nmol) was added for an additional 4 hr at 37°C, the cells were harvested by filtration onto glass-fiber

filters, and incorporated radioactivity was determined by liquid scintillation spectrophotometry. All samples were run in quadruplicate. OKT3-stimulated human mononuclear cells in the presence of medium alone usually incorporated 45,000–60,000 cpm per well, and the standard error of each quadruplicate sample averaged <5%.

Proliferation of CTLL-2 Cells. CTLL-2 cells (5×10^3 per well in 96-well culture plates) were incubated for 20 hr at 37°C in supplemented RPMI 1640 culture medium with 2% fetal bovine serum and 1% partially purified human interleukin 2 (IL-2) (Electro-Nucleonics, Silver Spring, MD). Cells were cultured in the presence or absence of various amounts of p15E derivative or β-galactosidase derived from pME-e2. [^{3}H]Thymidine (1 μCi) was added to each well and incubation was continued for an additional 4 hr. Cultures were harvested onto glass-fiber filters and incorporated [^{3}H]thymidine was measured by liquid scintillation counting. Each sample was tested in quadruplicate and standard errors were <5% of the mean. The average incorporation by untreated cells was 50,000–75,000 cpm per well.

RESULTS

Expression of p15E in *E. coli*. The *env* gene of cloned proviral Moloney murine leukemia virus was used to construct prokaryotic expression plasmids (Fig. 1). Four constructs were made, which included different portions of p15E coding sequence. These varied significantly in the stability of the protein product in *E. coli* and in the level of expression. Since the recombinant proteins are expressed as tripartite fusion proteins, it is possible to compare the stability of the different products by detection of intact or degraded components with anti-p15E and anti-β-galactosidase antibodies. Fig. 2 shows the major anti-p15E- and anti-β-galactosidase-reactive bands detectable on immunoblots of $NaDodSO_4$/PAGE-separated proteins in lysates of *E. coli*, each harboring one of the four expression plasmids. The 117-kDa marker in Fig. 2 indicates the position of anti-β-galactosidase-reactive protein expressed from pJG200 without a p15E insert. Reactivity with anti-β-galactosidase but not with anti-p15E at this position in lysates of cells carrying pME constructs indicates degradation of the p15E portion of the fusion protein. For all constructs, β-galactosidase freed from N-terminal p15E sequence was the major detectable degradation product (with polyclonal as well as monoclonal anti-β-galactosidase). Reactivity at a higher molecular mass with both anti-p15E and anti-β-galactosidase indicates the presence of intact fusion protein. The blots indicate that the ranking of protein stability is pME-e2 > pME-e1 > pME-e3, pME-e4.

The level of protein expression can be estimated by the intensity of anti-β-galactosidase bands on immunoblots and by measurements of β-galactosidase activity (Fig. 2, Table 1). β-Galactosidase activity levels reflect contributions from both intact fusion protein and free enzyme generated by degradation of the p15E portion. Since degradation products may be undetectable by our antibodies or without enzymatic activity, neither method is truly quantitative. With these caveats, it appears that pME-e3 > pME-e2 > pME-e1 > pME-e4 in levels of expression. The constructs that retain hydrophobic regions (pME-e1 and pME-e4) were expressed at lower levels than the constructs not encoding hydrophobic portions of p15E (pME-e2 and pME-e3). Because of the stability and appreciable level of expression of their products, pME-e2 and its higher-expression-level derivative pME-e2a were chosen for use in biological studies.

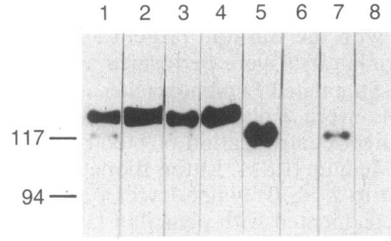

FIG. 2. Expression of intact fusion proteins and β-galactosidase degradation products. Proteins encoded by pME-e1 (lanes 1 and 2), pME-e2 (lanes 3 and 4), pME-e3 (lanes 5 and 6), and pME-e4 (lanes 7 and 8) were detected with anti-β-galactosidase mAb (lanes 1, 3, 5, and 7) or rabbit anti-p15E antibodies (lanes 2, 4, 6, and 8) after protein blotting. Cultures were induced at 42°C for 90 min before lysis. The 117-kDa marker indicates the position of the β-galactosidase product of pJG200 without any p15E insert (not shown) and is therefore also the position of degradation products in which the p15E portion has been cleaved from the fusion protein. Only the upper portion of the blot is shown.

Reactivity of Recombinant p15E with mAbs. pME-e2 fusion protein was tested for reactivity with anti-p15E mAbs. The 4F5, 19F8, 19VIIIE8, 9E8, and 372 antibodies were all reactive with blotted recombinant protein (Fig. 3). This maps the epitopes of these antibodies to the major hydrophilic region of p15E encoded by pME-e2.

Purification of Recombinant p15E. pME-e2 and pME-e2a direct the synthesis of a tripartite fusion protein, consisting of p15E sequence and enzymatically active β-galactosidase connected by a short segment of collagen (25). This permitted purification of the fusion protein by sequential use of affinity resins specific for β-galactosidase and for p15E (Fig. 4). The p15E portion of the purified fusion protein was liberated from β-galactosidase by digestion with collagenase and was further purified by anti-p15E affinity chromatography. The resulting purified p15E product was pure (≈99%) by scanning densitometry of a Coomassie blue-stained $NaDodSO_4$/polyacrylamide gel. The final p15E derivative proved to be soluble in physiological buffer (Hanks' balanced salts solution). In the absence of detergent, this material remained in the supernatant after centrifugation at 100,000 × *g* for 1 hr (data not shown).

Inhibition of Stimulated T-Lymphocyte Proliferation by Recombinant p15E. Soluble recombinant p15E was introduced into cultures of human mononuclear cells stimulated to proliferate by addition of anti-CD3 antibody OKT3. Dose-dependent inhibition of proliferation (Fig. 5) was observed, reaching a maximum of 29%, 50%, and 60% in three different experiments using leukocytes from three different healthy donors. The concentration required for half-maximal inhibition (EC_{50}) was 45, 144, and 35 ng per ml in these experiments, for an average EC_{50} of 7.5 nM. The β-galactosidase component of the cleaved fusion protein, purified under nearly identical conditions as the p15E derivative, was used as a control and gave no significant inhibition at equivalent protein concentrations (Fig. 5).

In experiments with the continuous human T-cell leukemia line Jurkat, neither the p15E derivative nor the β-galactosidase control inhibited proliferation at concentrations up to 2.88 μg/ml during 24 hr of culture in two experiments or during 72 hr of culture in a third experiment (data not shown). This indicates that the dose that inhibits the activation of normal human peripheral T cells does not have an antiproliferative effect on this transformed T-cell line.

Table 1. Relative levels of expression of β-galactosidase activity

Plasmid	β-Galactosidase activity, units/ml
pME-e1	1480
pME-e2	2410
pME-e3	2990
pME-e4	600

MC1000 cells harboring the indicated plasmids were grown in Luria broth to an OD_{550} of 0.260–0.300 and induced for expression. β-Galactosidase activity per ml of culture was determined after 90 min (29). Data are representative of three experiments.

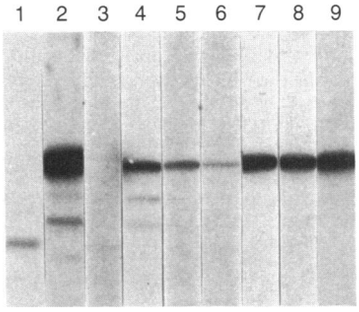

FIG. 3. Reactivity of the pME-e2 fusion protein with anti-p15E antibodies as demonstrated by immunoblotting. Lanes: 1, preimmune rabbit serum; 2, rabbit anti-p15E; 3, control myeloma antibody P3X63; 4–8, anti-p15E mAbs 4F5, 19F8, 19VIIIE8, 9E8, and 372, respectively; 9, anti-β-galactosidase mAb 8.4. The major band of reactivity in each lane corresponds to a band, appearing in Coomassie blue-stained gels of MC1000/pME-e2 but not control MC1000 lysates (data not shown), that has a migration consistent with the molecular weight predicted for the protein encoded by pME-e2. The extraneous lower molecular weight bands seen in lanes 1, 2, and 4 probably represent reactivity of these antisera with antigens of *E. coli*, since these bands were also reactive in lysates of control cultures. The portion of the blot corresponds to a protein size range of 80–180 kDa.

The recombinant proteins were also tested for their ability to inhibit the proliferation of the IL-2-dependent murine T-cell line CTLL-2. Inhibition was observed, reaching 89% at a dose of ≈45 μg/ml with an EC_{50} of ≈1.8 μM (Fig. 6). In three different experiments the maximal inhibition averaged 92% with an EC_{50} of 2.1 μM. β-Galactosidase at the highest protein concentrations tested did not inhibit proliferation.

DISCUSSION

The p15E component of retroviruses has been postulated to be a mediator of immune dysfunction (14). Previous studies using retroviral protein preparations, however, have relied on indirect methods such as physiochemical properties or antibody absorption as evidence that the active material was p15E (7–9, 12). The results presented here show directly that a soluble purified recombinant p15E derivative inhibits lymphoproliferative function *in vitro*. Moreover, the activity is localized to the central hydrophilic region of p15E, a potential domain for which there had been no previously demonstrated function. Interestingly, a synthetic peptide corresponding to a portion of this region that is highly conserved amongst retroviruses of numerous species (18, 21) was recently shown

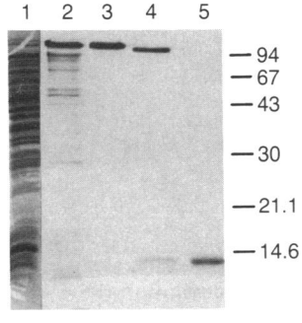

FIG. 4. Purification of recombinant p15E derivative. Coomassie blue-stained 15% polyacrylamide gel shows samples from various stages of purification. Lanes: 1, crude bacterial lysate; 2, material eluted from β-galactosidase affinity column; 3, purified fusion protein eluted from anti-p15E affinity column; 4, fusion protein digested with collagenase; 5, p15E derivative after final purification step on anti-p15E column. Markers at right show size (kDa) of standard proteins run in parallel.

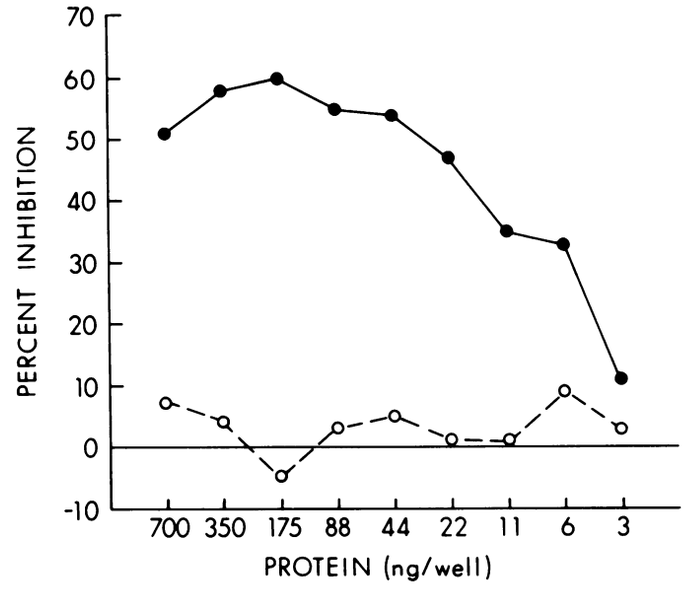

FIG. 5. Inhibition of anti-CD3 stimulated T-cell proliferation by recombinant p15E. Human mononuclear cells to which recombinant p15E (●) or recombinant β-galactosidase (○) was added were stimulated with OKT3 antibody. Percent inhibition of [^{3}H]thymidine incorporation is plotted vs. the amount of recombinant protein added per well. Data represents one of three experiments.

to inhibit certain lymphocyte (33, 34) and monocyte (35) functions. Unlike the recombinant p15E, however, the synthetic peptide was active only when coupled to a carrier protein and required substantially (>100-fold) higher doses, particularly for inhibition of anti-CD3-stimulated lymphocyte proliferation (ref. 33 and unpublished data). These observations suggest that the conserved sequence within the hydrophilic region of p15E is an important determinant of suppressive activity but requires a structural context provided by a larger carrier protein in order to be biologically active. The complete hydrophilic domain of p15E itself may provide the best context.

The varying stabilities of the different recombinant p15E constructs support the argument that the extracellular hydrophilic region constitutes a structural domain as well as a functional (immunosuppressive) region. Of the four different constructs, the one that most nearly isolated the entire

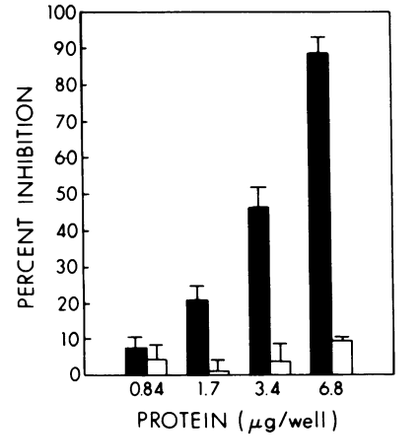

FIG. 6. Inhibition of IL-2-dependent proliferation of murine CTLL-2 T cells by recombinant p15E. p15E (solid bars) or β-galactosidase (open bars) was added to overnight cultures of CTLL-2 cells. The percent inhibition of [^{3}H]thymidine incorporation by each dose of recombinant protein presented here is representative of three experiments.

hydrophilic region yielded the most stable product. This might indicate that the hydrophilic region encoded by pME-e2 actually defines one or more structural domains, since the stability of foreign proteins expressed in *E. coli* appears to be related in part to the ability of the protein to fold properly into a native configuration (36).

The dose of the recombinant p15E required to inhibit the IL-2-dependent murine CTLL-2 cells was comparable to that reported for a low molecular weight protein (presumably p15E) isolated from feline leukemia virus (7). However, the recombinant p15E was ≈300 times more active in inhibiting anti-CD3-stimulated lymphoproliferation than it was in inhibiting the proliferation of CTLL-2 cells. There are numerous alternative possibilities to explain the differences in dose requirements. One is that the activation of resting T cells, which requires multiple sequential events, may be more sensitive to the inhibitory effects of p15E than is the ongoing proliferation of a T-cell line already expressing IL-2 receptors and dependent solely upon IL-2 for continued proliferation. Different mechanisms of inhibition may also be involved. For example, in the case of anti-CD3, the mechanism of p15E inhibition could include effects upon monocytes, which participate in the activation process and are known to be affected directly by p15E (10, 12) or the synthetic peptide corresponding to the conserved region of p15E (35). The greater efficacy of inhibition of anti-CD3-stimulated proliferation by p15E suggests that the corresponding mechanism may be of greater biological relevance than the mechanism of CTLL-2 inhibition.

The finding that the anti-CD3-driven proliferation could only be inhibited by 29–60%, even at high doses, raises the possibility that only certain populations of T cells are susceptible to p15E-mediated inhibition. This possibility will require investigation.

The relevance of these findings to potential *in vivo* mechanisms of p15E-mediated immune dysfunction remains to be explored. It will be important to determine the doses of p15E required to affect immunosuppression *in vivo* and to determine whether such levels are achieved during natural retroviral infection or neoplasia. The availability of recombinant p15E should make these determinations feasible, the latter through the development of competitive ELISA or other immunoassays.

The inhibition of stimulated T-lymphocyte proliferation by purified recombinant retroviral envelope protein at nanomolar concentrations may prove to be relevant to our understanding of immune dysfunction associated with certain human diseases. Human T-lymphotropic virus type I (HTLV-I) infection may be more frequently associated with immunosuppression than with T-cell lymphoma (37), and inactivated HTLV-I particles inhibit certain leukocyte functions *in vitro* (6). Human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), certainly causes immunosuppression through the destruction of T cells, but HIV envelope proteins may play an accessory role, particularly in the early phases of the disease (38). The finding that malignant effusions from humans with cancer contain a suppressive activity that can be absorbed by anti-p15E mAbs has led to speculation that p15E-related molecules, aberrantly expressed from endogenous loci during the course of tumorigenesis, could also play a role in the immunosuppression associated with cancer (14). In this regard, retroviral sequences that contain p15E-like coding regions have been identified in the normal human genome (39). We expect that the availability of soluble, recombinant p15E derivatives will facilitate studies of the cellular and molecular mechanisms of immunosuppression associated with both retroviral and neoplastic diseases.

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