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Inhibition of Lymphoproliferation by a Synthetic Peptide with Sequence Identity to gp41 of Human Immunodeficiency Virus Type 1

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Peptides were synthesized that contained sequences from two regions (*env* amino acids [aa] 581 to 597 and 655 to 671) of the transmembrane protein gp41 and one region of the external envelope glycoprotein gp120 (aa 457 to 464) of human immunodeficiency virus type 1. Selection of these sequences was based on their homology to the highly conserved and immunosuppressive sequence contained within the transmembrane proteins p15E and gp21 of animal and human retroviruses, respectively. Peptide aa581–597 was found to specifically inhibit human and murine lymphoproliferation, whereas peptides aa655–671 and aa457–464 had no activity. These results suggest a mechanism by which human immunodeficiency virus type 1 gp41 exerts a direct immunosuppressive effect in vivo, analogous to that postulated for p15E and gp21, which could contribute to the immune dysfunction observed in patients suffering from acquired immunodeficiency syndrome. It is of particular interest that the sequence aa 584 to 609, shown to contain B- and T-helper-cell epitopes, overlaps with the sequence aa 581 to 597 that is shown here to inhibit lymphoproliferation. The potential implications of this overlap of immunologic activities are discussed.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS), which is characterized by profound suppression of immune function leading to enhanced susceptibility to fatal opportunistic infection (reviewed in references 9, 12, and 13).

Initially, the immunosuppression observed in HIV-1-infected patients was attributed to the direct cytopathic effect of HIV infection of $CD4^+$ T cells; however, subsequent observations have suggested that HIV has immunomodulatory activity in vivo independent of its cytopathic effect (reviewed in reference 14). The inhibition of immune function observed in vivo is supported by experimental data indicating that noninfectious HIV-1 preparations inhibit mitogen- and antigen-driven proliferation of T lymphocytes in vitro as well as the differentiation responses of B cells (17). These findings suggest that some component of HIV-1 possesses immunomodulatory activity analogous to that of the transmembrane proteins p15E and gp21 (18). To test this hypothesis, we synthesized peptides corresponding to regions of HIV *env* with sequence similarity to p15E and tested them in immune function assays for inhibitory activity.

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): Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu, designated aa581–597; Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys, designated aa655–671; Leu-Leu-Leu-Thr-Arg-Asp-Gly-Gly, designated aa457–464; Leu-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Leu-Lys-Glu-Gly-Gly-Leu, designated MOLV.1 (corresponding to the sequence of CKS-17) (3). The HIV *env* amino acid (aa) position numbers and sequences used in this report correspond to the HIV-1 lymphadenopathy-associated virus type 1 isolate (22). In each case, the peptides included the aa 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, deprotected, purified, and conjugated to bovine serum albumin (BSA) exactly as described in the accompanying report (18).

Cell culture. The T-cell lines CTLL-2 and EL-4.IL-2 were obtained from the American Type Culture Collection (Rockville, Md.) and maintained as described in the accompanying report (18). The human helper T-cell (Th) clone PPD-1 (the gift of Elaine DeFreitas, Wistar Institute, Philadelphia, Pa.) was derived from the peripheral blood of a healthy volunteer and passaged weekly 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) supplemented with 20 U of recombinant human interleukin-2 (IL-2) per ml (the gift of Peter F. Sorter, Hoffmann-La Roche Inc., Nutley, N.J.) and 2×10^6 phytohemagglutinin-stimulated human peripheral blood mononuclear cells (PBMC) per ml (irradiated with 10,000 rads) as a source of accessory cells by methods previously described (7).

Proliferation assays. The synthetic peptides were tested for inhibitory activity by determining their effect on lymphoproliferation in vitro. The proliferation of CTLL-2 cells was measured as described in the accompanying report (18). Anti-CD3 stimulation of human lymphocytes was performed by incubating PBMC from normal human donors (2×10^5 per well) for 72 h in RPMI-complete in the presence of a 1/5,000 dilution (vol/vol) of ascites fluid obtained from mice injected with the hybridoma cell line OKT3. Proliferation was measured as for CTLL-2 cells by [^{3}H]thymidine pulse

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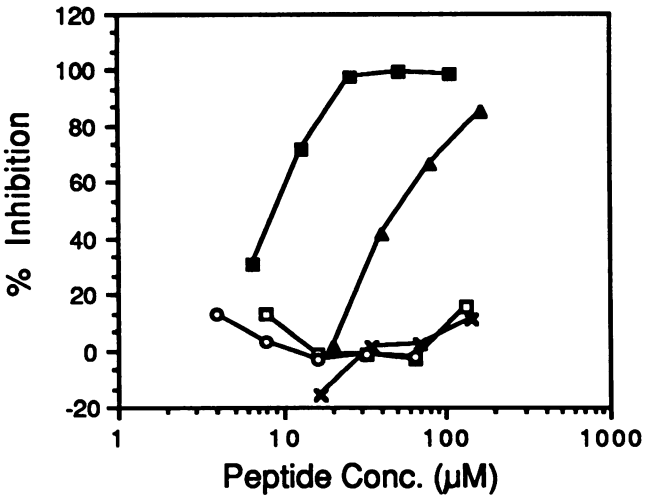

FIG. 1. Inhibition of proliferation of CTLL-2 by synthetic peptides. Percent inhibition was calculated by the following formula: % inhibition = [1 − (exp cpm − bkgd cpm)/(total cpm − bkgd cpm)] × 100, where bkgd cpm (counts per minute) is [^{3}H]thymidine uptake in the absence of IL-2 and/or other mitogens and synthetic peptides (2,402 ± 387), total cpm is uptake in the presence of IL-2 without peptides (246,914 ± 35,664), 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. Symbols: ▲, MOLV.1; ■, aa581–597; □, aa655–671; ×, BSA (EDC); ○, aa457–464.

during the final 6 h of culture. PPD-1 cells (1×10^4) and 10,000 rad-irradiated human PBMC (2×10^5) were cultured in 200 μl per well in the medium used for propagation for 72 h, and proliferation was measured as for CTLL-2 cells by [^{3}H]thymidine pulse during the final 18 h of culture.

RESULTS

Selection of sequences from HIV-1 envelope proteins. Computer-assisted sequence analysis has been utilized by other investigators to identify a highly conserved region of the transmembrane proteins of several animal and human retroviruses (4). The consensus sequence is represented by the synthetic peptide CKS-17, and this peptide has been shown previously to inhibit immune function (3). We performed computer-assisted alignment (8) of the CKS-17 sequence to that of HIV-1 transmembrane protein gp41 and external envelope protein gp120 and identified three regions with sequence similarity: aa 581–597, with 47% similarity over 17 residues; aa655–671, with 61% similarity over 13 residues; and aa457–464, with 88% similarity over 8 residues. All these sequences are highly conserved among different isolates of HIV-1. The sequences aa581–597 and aa655–671 are situated in the major hydrophilic domain of gp41, and aa457–464 is present in a hydrophilic portion of gp120. Thus, these sequences are predicted to be accessible at the surface of the intact envelope proteins. Peptides containing HIV *env* aa581–597, aa655–671, aa457–464 and the CKS-17 sequence (designated MOLV.1) were synthesized and tested in immune function assays for inhibitory activity.

Inhibition of CTLL-2 proliferation by synthetic peptides. We found that IL-2-dependent proliferation of CTLL-2 cells was specifically inhibited in a dose-dependent manner by the peptide aa581–597 and the positive control peptide MOLV.1 (Fig. 1). In contrast, neither the aa655–671 peptide, the aa457–464 peptide, nor the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)-treated BSA inhibited lymphoproliferation.

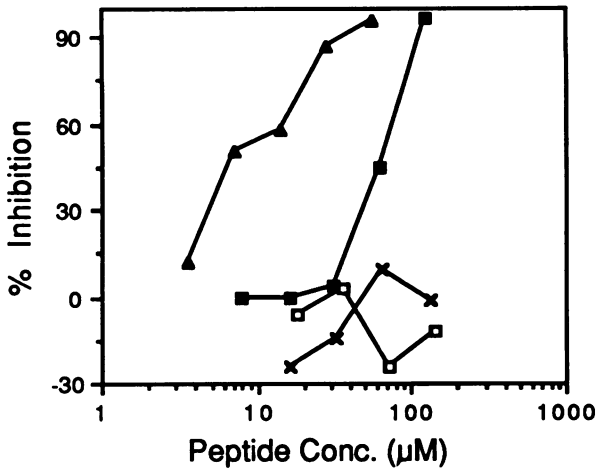

FIG. 2. Inhibition of proliferation of OKT3-stimulated human PBMC by synthetic peptides. Data were obtained and calculated exactly as described in the legend to Fig. 1. Values for total cpm and bkgd cpm were 233,642 ± 32,456 and 6,523 ± 986, respectively. Symbols are as in Fig. 1.

Inhibition of proliferation of human PBMC by synthetic peptides. To extend the results obtained with murine CTLL-2 cells, we tested the HIV-1-derived synthetic peptides and appropriate controls for their effect on the proliferation of normal human PBMC stimulated with the mitogenic monoclonal antibody OKT3, which binds the CD3 antigen of the T3-Ti T-cell receptor complex and presumably stimulates these cells to proliferate in a manner analogous to specific antigen. In agreement with the activities observed with the CTLL-2 cells, the peptide aa581–597 was found to reduce the anti-CD3-stimulated lymphoproliferation of human PBMC to background levels (Fig. 2). The MOLV.1 peptide inhibited proliferation to a similar degree, whereas the aa655–671 peptide and EDC-treated BSA had no inhibitory activity.

Inhibition of proliferation of human Th clone by synthetic peptides. To further characterize the inhibition of the mixed cell population present in the PBMC assay, we also tested the synthetic peptides for their effect on the IL-2-dependent proliferation of a human Th clone, PPD-1. Proliferation of the PPD-1 clone was reduced to control levels by peptides aa581–597 and MOLV.1, whereas the aa655–671 and aa457–464 peptides and EDC-treated BSA were not inhibitory (Fig. 3).

The peptide-BSA conjugates and EDC-treated BSA had no direct cytotoxic effect on any of the lymphocyte populations tested as determined by trypan blue exclusion. Furthermore, the free peptides MOLV.1 and aa581–597 did not inhibit lymphoproliferation and thus required conjugation to carrier protein for activity, in agreement with previous results (3, 18).

DISCUSSION

In this study, we identified a sequence, aa581–597, within HIV-1 gp41 that directly inhibits lymphoproliferation as measured in vitro. The sequence required for this activity

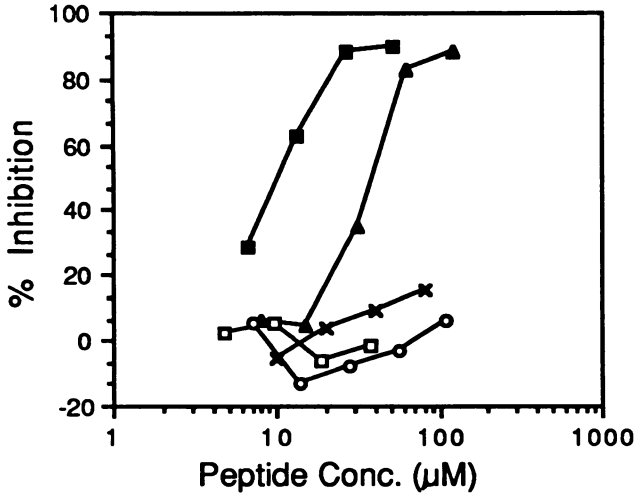

FIG. 3. Inhibition of proliferation of the human Th clone PPD-1 by synthetic peptides. Data were obtained and calculated exactly as described in the legend to Fig. 1. Values for total cpm and bkgd cpm were 20,144 ± 422 and 2,480 ± 132, respectively. Symbols are as in Fig. 1.

appears to be highly specific since the other two peptides, aa655–671 and aa457–464, were devoid of activity despite exhibiting a comparable degree of homology to CKS-17.

The lack of activity observed with peptides aa655–671 and aa457–464 also demonstrates that the addition of the aa Lys-Cys-Tyr-Gly-Gly at the N termini of the peptides does not contribute to the inhibitory activity, in agreement with our previous results (18). Furthermore, the peptides did not inhibit the proliferation of NIH 3T3 fibroblasts and thus may be specific for cells of the immune system.

Prostaglandins have been shown to inhibit many functions of the immune system (10). We therefore asked whether the inhibitory activity mediated by the synthetic peptides involved prostaglandins. Inhibition of lymphoproliferation by peptides aa581–597 and MOLV.1 was unaffected by the presence of the cyclooxygenase inhibitor indomethacin (data not shown), suggesting that the mechanism of inhibition mediated by these peptides is independent of the synthesis of prostaglandins.

We have shown previously (18) that the inhibitory potency of the synthetic peptide MOLV.1 is much less (~1,000-fold) than that of the native protein p15E. Moreover, in vitro assays of immune function appear to require higher concentrations of protein than is necessary for in vivo stimulation as exemplified by antigenic stimulation of T-cell proliferation (15, 19). Thus, we predict that the concentration of retroviral transmembrane protein that would be required to inhibit lymphoproliferation in vivo may be much less than the concentration of synthetic peptide required for activity in vitro.

Other investigators (20) have shown that both a 25- to 50-kilodalton ultrafiltrate fraction of AIDS sera and a recombinant gp41 preparation inhibit monocyte polarization in response to the chemotactant formyl-Met-Leu-Phe and that these inhibitory activities are adsorbed by a monoclonal antibody specific for gp41. Furthermore, synthetic peptides with sequence identity to regions of gp41 (aa733–750 and aa844–858) have been found to inhibit mitogen- and alloantigen-induced lymphoproliferation (2). However, these peptides show no homology to the immunosuppressive region of p15E represented by MOLV.1 or to the corresponding region of gp41 represented by peptide aa581–597.

The transmembrane protein p15E has been suggested to participate in the retrovirus-mediated immunosuppression observed in animals (5, 16, 24). The transmembrane protein gp21 of human T-lymphotropic virus types I and II may also be immunosuppressive in vivo since we have demonstrated in a separate study (18) that a synthetic peptide containing a sequence essentially identical to a region of the transmembrane protein gp21 of human T-lymphotropic virus types I and II inhibits lymphoproliferation in vitro. The results of the present study extend these findings and suggest that gp41 also inhibits lymphoproliferation and contributes to the in vivo immune dysfunction predisposing AIDS patients to fatal opportunistic infection.

The inhibitory region that we identified, aa581–597, is of particular interest because it has been shown to be associated with additional in vivo immunologic activities. Serodiagnostic assays have demonstrated that HIV-1 gp41 is the antigen most consistently recognized by antibodies in sera from patients with AIDS and AIDS-related complex (1), and epitope mapping has localized the gp41 immunogenic sites to the aa584–609 region (11, 23). Schrier et al. (19) have recently shown in the mouse that the immunoglobulin G antibody response to the aa598–609 sequence is T-cell dependent. They have also demonstrated that 7 of 29 HIV-1-seropositive but 0 of 13 HIV-1-seronegative donors exhibited Th-cell responses to the gp41 aa584–609 peptide (19). Thus, an overlap exists between the immunogenic B- and Th-cell epitopes of HIV-1 gp41 and the inhibitory sequence aa581–597 that we identified. The potential significance of these immune responses is unknown. However, studies of Thiel et al. (21) with the AKR murine leukemia model have suggested that anti-p15E antibodies play a role in preventing the immunosuppressive effects of p15E. This hypothesis has recently been extended to HIV, for which it was reported that in HIV-1-seropositive individuals antibody reactivity with the HIV-1 gp41 aa581–597 peptide was strongly associated with the absence of HIV-related disease (11). Thus, an antibody response against the aa581–597 region might be capable of abrogating its immunosuppressive effects.

The results of the present study are of potential relevance to vaccine development because of the overlap of inhibitory sequences with B- and Th-cell epitopes. The data obtained from this and other studies indicate that the balance between the suppressive and immunogenic activities localized to this region may be of prime importance for immunoprophylactic and therapeutic intervention in AIDS.

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ADDENDUM IN PROOF

Subsequent to the preparation of this manuscript, Cianciolo et al. (G. J. Cianciolo, H. Bogerd, and R. Snyderman, Immunol. Lett. **19**:7–14, 1988) reported inhibition of lymphoproliferation by a synthetic peptide essentially identical to that of aa581–597 described here.

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