PHA and PMA. Since the NF-κB protein is not only restricted to lymphoid cells, but is also detectable in PMA-stimulated HeLa cells (*19*), additional positive and negative elements located further 5′ in the HIV-1 LTR might regulate expression of the LTR in a cell- or tissue-specific manner. In particular, the sequences homologous to the IL-2 and IL-2 receptor gene promoters are of interest since a segment containing these imperfect repeats is important for mitogen activation of the IL-2 promoter that is largely restricted to T cells (*20*).

The increased HIV-1 LTR activity observed with combinations of the mitogens and *tat*-III suggests that these agents act through independent mechanisms (*4*) and underscores the large changes in LTR activity that can occur when both agents are present (for example, >9000-fold increase in CAT activity in the presence of PMA and *tat*-III). Thus, even relatively weak mitogenic stimuli, such as the effect of *tat*-1 on the full-length HIV-1 LTR, may lead to marked changes in viral gene expression by triggering production of the *tat*-III protein.

The capacity of the trans-activator gene product of HTLV-I to stimulate the HIV-1 LTR when present in the same cell is similar to the effects of many DNA viruses (herpes simplex, papovavirus, and varicella zoster) (*21*). This finding may be of potential clinical importance in view of the incidence (27%) of dual HIV-1 and HTLV-I infection in some populations of intravenous drug abuse patients (*22*).

Note added in proof: Similar results regarding the mitogen inducibility of the HIV-1 LTR have recently been described by Tong-Starksen *et al.* (*23*).

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5 May 1987; accepted 5 October 1987

Identification of Nuclear Receptors for VIP on a Human Colonic Adenocarcinoma Cell Line

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Vasoactive intestinal peptide (VIP) is a neuropeptide with broad tissue distribution. Although its precise function is unknown, it is thought to exert its effect, at least in part, by interacting with cell surface receptors. Nuclear receptors for VIP have now been identified by specific binding of ^{125}I-labeled VIP to nuclei of a human colonic adenocarcinoma cell line (HT29) and by cross-linking of ^{125}I-labeled VIP to its receptor on intact nuclei. In contrast, ^{125}I-labeled transferrin shows only background binding to nuclei but significant binding to intact cells. Purity of the isolated nuclei was further substantiated by electron microscopy. The apparent molecular sizes of the VIP–cross-linked nuclear and cell surface receptors are similar but not identical.

PEPTIDE HORMONES INDUCE THEIR responses by interacting with cell surface receptors of their target tissue (*1*). Nuclear receptors have been reported for several polypeptide hormones (*2–4*), including epidermal growth factor (EGF) and nerve growth factor (NGF) (*4*). The biologic role that these nuclear receptors play has not been clearly defined, but evidence exists for direct action in the nucleus (*2–5*). Thus far, the putative nuclear receptors for polypeptide hormones have not been characterized well biochemically and, in the case of the NGF nuclear receptor, a different molecular size species was immunoprecipitated compared to that for the cell surface receptor (*6*).

Vasoactive intestinal peptide (VIP) is a 28–amino acid residue peptide that belongs to the secretin-glucagon family (*7*). It has been considered to be a neurotransmitter with various activities, but its precise physiologic role has not been established (*7*). After binding to its cell surface receptor, VIP causes an increase in intracellular adenosine 3′,5-monophosphate via adenylate cyclase (*8*). In the human colon adenocarcinoma cell line HT29 there is evidence for rapid internalization of VIP after binding at 37°C (*9*). The receptor for VIP has been identified by detergent solubilization (*10*), by cross-linking ^{125}I-labeled VIP to its receptor on intact cells or membrane preparations (*11, 12*), and by photoaffinity labeling (*13*).

In this study, binding of ^{125}I-labeled VIP was tested on intact HT29 cells and purified nuclei. There was similar binding of ^{125}I-labeled VIP to both HT29 cells and nuclei (Fig. 1), which was competitively displaced in the presence of unlabeled VIP. Since there is a significant amount of cell surface transferrin (Tr) receptors on HT29 cells (*14*), we compared the presence of Tr recep-

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tors on nuclei, if any, to that on intact cells. The binding of ^{125}I-labeled Tr to its receptor on intact HT29 cells was saturable, as determined by competitive displacement (Fig. 1). In contrast, binding of ^{125}I-labeled Tr to nuclei was negligible. From Scatchard analysis (*15*), the approximate number of receptors on HT29 cells is 76,000 ± 12,000 per cell with a dissociation constant (K_D) of 1.1 ± 0.6 n*M* (n = 2) for Tr and 140,000 ± 103,000 per cell with a K_D of 0.95 ± 0.6 n*M* (n = 4) for VIP. The number of VIP receptors on nuclei was similar to the number of VIP cell surface receptors and in some experiments was somewhat higher. Muller *et al.* (*12*) reported 20,000 VIP receptors per cell on intact HT29 cells with a K_D of 0.5 n*M*. This discrepancy may be accounted for by the different conditions used in their incubation binding assay and the potential errors inherent in Scatchard analysis (*16*).

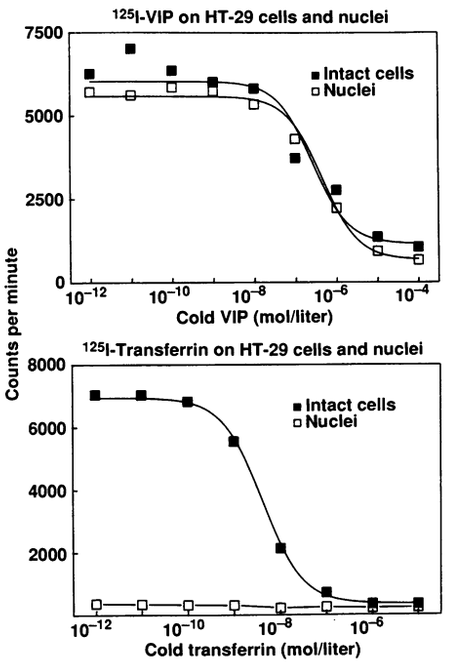

Fig. 1. Competitive inhibition of ^{125}I-labeled VIP and ^{125}I-labeled Tr binding on intact HT29 cells or HT29 purified nuclei. A fixed amount of ^{125}I-labeled ligand was added in the presence of increasing concentration of unlabeled ligand. The amount of ligand bound was measured in counts per minute. Each data point corresponds to an average from duplicate samples. Binding was performed on 5×10^5 cells or nuclei at 4°C for 3 hours with intermittent mixing. For binding studies or for preparation of nuclei, HT29 cells (*26*) were rinsed once with RPMI 1640 medium and then incubated in RPMI 1640 medium without serum for 45 minutes at 37°C. Monolayers were then detached by incubating with 1 m*M* EDTA in phosphate-buffered saline (PBS), *p*H 7.3 (37°C, 20 minutes). Cells were washed three times with binding buffer (0.2% bovine serum albumin in PBS); PBS alone was used in the cell washing for cross-linking experiments or for nuclear preparation. Nuclei were prepared as described (*27*) except that cells were disrupted by nitrogen cavitation for 5 minutes at 34 atm, and the lower portion of the sucrose gradient was 2.1*M* instead of 2.3*M*. ^{125}I-labeled Tr was prepared by the chloramine T method (*28*), and free ^{125}I was separated on a Sephadex G-25 column. For ^{125}I-labeled VIP (New England Nuclear) and ^{125}I-labeled Tr binding, 20,000 cpm and 48,000 cpm were added, respectively. The total volume of the binding reaction was 0.1 to 0.2 ml. At the end of a binding assay, samples were washed twice with 1 ml of binding buffer and then counted.

The contamination of nuclei with rough endoplasmic reticulum, cytoskeletal, and other nonnuclear elements has made it difficult to interpret and differentiate true nuclear binding from extranuclear binding (*17*). Absence of specific ^{125}I-labeled Tr binding to our nuclear preparation suggests that extranuclear contamination is unlikely. Electron microscopy of HT29 nuclei was used to test this critical point. The HT29 nuclei showed minimal contamination (Fig. 2A). In contrast, when nuclei from a human cutaneous T lymphoma cell line [HUT 78, (*18*)] were prepared in a manner identical to that used for HT29 nuclei, significant binding of ^{125}I-labeled Tr was noted (from 60 to 70% of that observed on intact HUT 78 cells). This suggested probable contamination of the HUT 78 nuclei, which was confirmed by electron microscopy (Fig. 2B). Thus, our data from ^{125}I-labeled Tr binding to nuclei and the electron microscopy analysis are complementary (*19*).

We also performed biochemical tests on the interaction of ^{125}I-labeled VIP with its receptor. The noncleavable cross-linking agent disuccinimidyl suberate (DSS) specifically cross-links ^{125}I-labeled VIP to its receptor on intact HT29 cells or nuclei (Fig. 3). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showed that the major cross-linked species has an apparent molecular size of approximately 48,000 daltons (48 kD). This band was absent when excess unlabeled VIP was added or when the cross-linking agent was absent (Fig. 3). Specificity of the cross-linked receptor was further verified since the related unlabeled peptides, glucagon and secretin, either partially or completely interfered with visualization of the ^{125}I-labeled VIP cross-linked receptor (^{125}I-labeled VIP-R), in agreement with their relative potency in blocking ^{125}I-labeled VIP binding to its putative receptor (*11*). However, the ligands peptide T (*20*), CCK octapeptide, and transferrin did not affect specific ^{125}I-

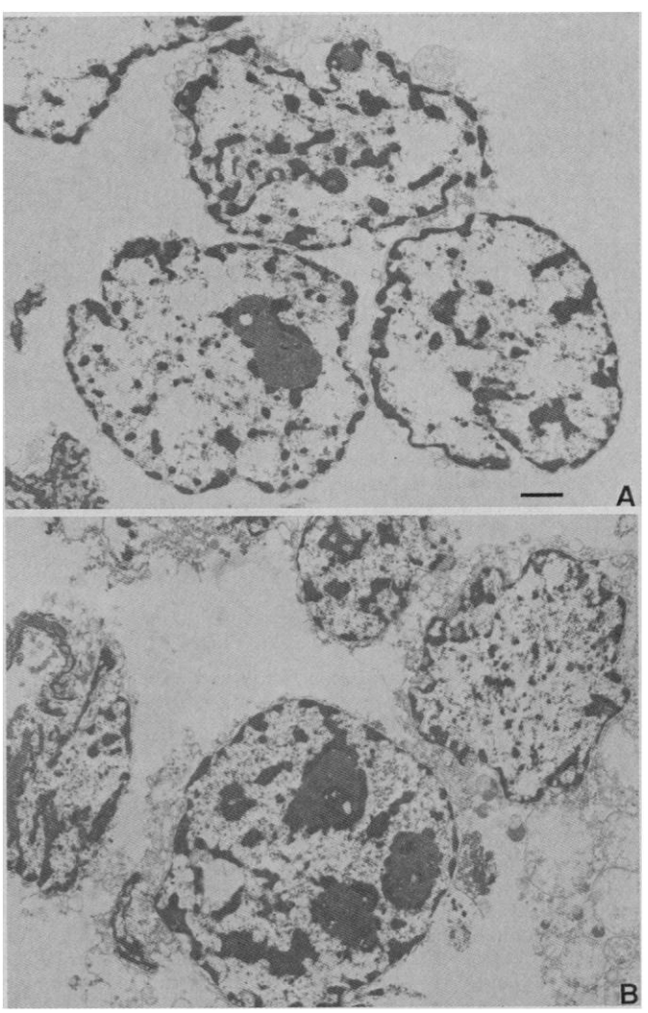

Fig. 2. Electron micrographs of (**A**) HT29 nuclei and (**B**) HUT 78 nuclei. Nuclei from both cell lines were prepared in an identical manner (legend to Fig. 1). Electron microscopy was performed as described by Finlay *et al.* (*29*). Bar corresponds to 1 μm. Data shown in Figs. 1 through 4 were from a single nuclear preparation. Similar data were obtained from two additional nuclear preparations.

labeled VIP cross-linking to its receptor either on nuclei or intact cells (Fig. 3). The pattern for cross-linked ^{125}I-labeled VIP-R complex in the presence of different ligands is similar on nuclei and cells. Two other faint bands with molecular sizes of approximately 70 kD and 200 kD were seen in some experiments. As reported (*11*), the 70-kD species may represent bovine serum albumin, which is present in commercial preparations of ^{125}I-labeled VIP. The 200-kD species may represent cross-linked aggregates or may be related to a 200- to 250-kD species recently identified by a photoactivated affinity probe for the VIP receptor. The 200- to 250-kD species was identified together with a 50-kD form (*13*).

The size of our cross-linked VIP receptor from HT29 cells is in agreement with previous reports of VIP receptors identified by cross-linking from HT29 cells, human lymphoid cell lines (Molt 4b and Nalm 6), rat frontal cortex (*11*), and rat liver plasma membranes (*13*). In these reports, the range from 47 to 50 kD compared with our estimated 48 kD. We also observed a 48-kD species (*14*) when ^{125}I-labeled VIP was cross-linked to intact HUT 78 lymphoid cells or to a mouse T cell line [AKR1, (*21*)].

Closer examination of the molecular size of our cross-linked species showed that the cell surface–cross-linked VIP receptor migrated slightly faster in SDS-polyacrylamide gels (under reducing conditions) than the nuclear cross-linked receptor. This was reproducible in three separate experiments and in the presence of multiple protease inhibitors (Fig. 4). Both the cell surface and nuclear VIP receptors from HT29 cells retained their electrophoretic pattern under nonreducing conditions. This is consistent with the observation that interchain disulfide bonds are absent in the human lymphoid cell surface VIP receptor (*11*). The reason for the observed difference in apparent molecular size and the physiologic significance, if any, of nuclear VIP receptors remains to be determined. Possible considerations include nuclear translocation of the cell surface VIP receptor, as hypothesized for the insulin (*5*) and the EGF receptor (*2*). The nuclear envelope also may serve as a major source of cell surface receptor synthesis, as reported for the vesicular stomatitis G protein (*22*). Both considerations would require structural modification to explain the difference in molecular size observed for the cell surface and nuclear receptors. Differences in phosphorylation, as has been shown for H1 histone (*23*) and protein kinase C (*24*), could account for the different migration rates in SDS-polyacrylamide gels. Alternatively, there may be differential sorting of nuclear and cell surface receptors, with potential nuclear signal sequence involvement (*25*).

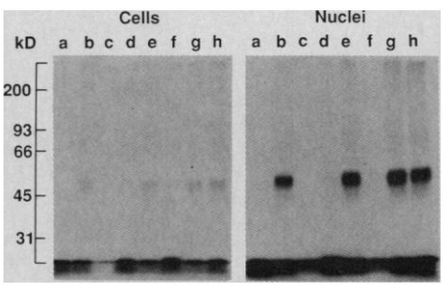

Fig. 3. Specificity of cross-linking of ^{125}I-labeled VIP to HT29 intact cells or nuclei. Autoradiograph from SDS-PAGE of cells or nuclei that had been incubated with: (lanes a and b) ^{125}I-labeled VIP, (lane c) ^{125}I-labeled VIP with an excess of unlabeled VIP, (lane d) glucagon, (lane e) peptide T, (lane f) secretin, (lane g) CCK octapeptide, or (lane h) transferrin. DSS was used for cross-linking in all samples except for the one in lane a. Molecular size of standard marker proteins are shown on the left. Cells or nuclei (1×10^6 per sample) were prepared as described in Fig. 1. Incubation with various peptides (10 μM) and ^{125}I-labeled VIP (0.6 nM) was performed for 20 minutes at 21°C followed by washing twice with PBS. Samples in lanes b through h were then incubated with 1 mM DSS for 15 minutes at 21°C. Cross-linking was quenched with 40 mM glycine. Cells or nuclei were solubilized for 40 minutes at 4°C with 50 μl of 1% Nonidet P40 in PBS containing: aprotinin, 400 KIU per milliliter; leupeptin, 10 μM; pepstatin, 10 μM; and PMSF, 1 mM. Solubilized material was analyzed on an 8% SDS-polyacrylamide gel with subsequent autoradiography. Exposure was for 9 days with an intensifying screen. Efficiency of cross-linking on intact cells compared to nuclei was somewhat variable and ranged from a minimum seen in this figure to that shown in Fig. 4.

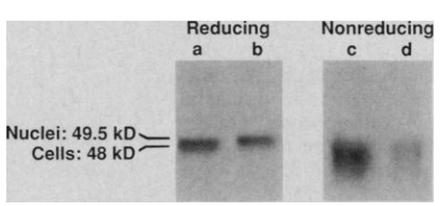

Fig. 4. Apparent molecular sizes for ^{125}I-labeled VIP cross-linked to its receptor on HT29 nuclei or intact cells. As shown in the autoradiogram, the estimated size of the nuclear cross-linked VIP receptor is 49.5 kD compared with 48 kD for the cell surface receptor. (Lanes a and c) Cross-linked cell surface receptor under reducing and nonreducing conditions, respectively. (Lanes b and d) Cross-linked nuclear receptor under reducing and nonreducing conditions, respectively. Cross-linking was as described in Fig. 3. Samples were analyzed by SDS-PAGE on an 8% gel after treatment with 2-mercaptoethanol (reducing) or with 100 mM iodoacetamide in the absence of 2-mercaptoethanol (nonreducing). Control tracks in the absence of the cross-linking agent or in the presence of excess unlabeled VIP are not shown but were identical to those shown in lanes a and c of Fig. 3. Similar variation in molecular sizes was seen when the incubation with ^{125}I-labeled VIP and cross-linking were done at 4°C.

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HTLV-V: A New Human Retrovirus Isolated in a Tac-Negative T Cell Lymphoma/Leukemia

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A new human retrovirus was isolated from a continuous cell line derived from a patient with CD4$^+$ Tac$^-$ cutaneous T cell lymphoma/leukemia. This virus is related to but distinct from human T cell leukemia/lymphoma virus types I and II (HTLV-I and HTLV-II) and human immunodeficiency virus (HIV-1). With the use of a fragment of provirus cloned from one patient with T cell leukemia, closely related sequences were found in DNA of the cell line and of tumor cells from seven other patients with the same disease; these sequences were only distantly related to HTLV-I. The phenotype of the cells and the clinical course of the disease were clearly distinguishable from leukemia associated with HTLV-I. All patients and the wife of one patient showed a weak serological cross-reactivity with both HTLV-I and HIV-1 antigens. None of the patients proved to be at any apparent risk for HIV-1 infection. The name proposed for this virus is HTLV-V, and the date indicate that it may be a primary etiological factor in the major group of cutaneous T cell lymphomas/leukemias, including the sporadic lymphomas known as mycoses fungoides.

PREVIOUS ANALYSES OF SERA FROM patients with mycosis fungoides (*1, 2*) led to the suggestion that a retrovirus related to human T cell leukemia virus types I and II (HTLV-I and HTLV-II) (*3*) might be associated with this form of T cell lymphoma. Mycosis fungoides begins with cutaneous lesions and progresses slowly. There is often no evidence of visceral infiltration for several years, and the disease has a much better prognosis than adult T cell leukemia/lymphoma (ATLL) (*4*).

In recent studies on HTLV-I involvement in T cell malignancies in Italy (*5*), the presence of DNA sequences homologous to, but not identical to, HTLV-I provirus was shown by hybridization in two patients. One patient (G.T.) had T cell leukemia, and the other (G.P.) had cutaneous T cell lymphoma (mycosis fungoides). Both patients appeared to be infected by a variant of HTLV-I (*6, 7*). Here we report on the isolation of a new retrovirus from a cell line derived from a male patient (G.B.) with a cutaneous T cell lymphoma in the leukemic phase. The peripheral blood mononuclear cells of this patient were >90% CD3$^+$, CD4$^+$, and Tac$^-$. Only a few cells expressed antigens recognized by Leu 2b (CD8) or Leu 16 (CD20) monoclonal antibodies (Table 1).

Samples of serum from patient G.B. showed a slight reaction by enzyme-linked immunosorbent assay (ELISA) with both HTLV-I and HIV-1 (*8, 9*) antigens, a very faint p24 by Western blot analysis, and negative immunocompetition with both antigens. The patient did not come from an HTLV-I endemic area and did not appear to be at risk for HIV-1 infection.

Lymphocytes from patient G.B. were maintained in RPMI 1640 medium without the addition of any growth factors. After 6 to 7 weeks in culture, a small number of cells began to proliferate spontaneously and form large clumps. Most of the cells were large and generally mono- or polynucleated. These cells were markedly different from most fresh peripheral blood mononuclear cells in that they lacked T cell markers such as CD3, CD4, and CD8 and expressed surface immunoglobulins and B cell antigens recognized by Leu 12 (CD19), Leu 16 (CD20), and CR2 (CD21) monoclonal antibodies. The cells in culture also expressed activation markers such as transferrin and interleukin-2 receptors (CD25 or Tac) and major histocompatibility class II antigens. The cell phenotype, which was examined every 2 weeks, remained relatively stable for several months (Table 1). Electron microscopic examination revealed typical type C virus budding from cell membranes with most particles present as immature and mature extracellular virions; no particles with eccentric or cylindrically shaped nucleoids were found (Fig. 1).

Supernatants from cultured cells showed reverse transcriptase (RT) activity that increased with passages until it stabilized on a level comparable to that present in culture fluids of the HTLV-I–producing cell line MT2 (*1*).

DNA extracted from fresh and cultured cells from patient G.B. was hybridized with probes derived from cloned HTLV-I, HTLV-II, and HIV-1 genomes. The HTLV-I probe (*10*) hybridized specifically, but only under low stringency conditions [3× standard saline citrate (SSC) at 60°C], with the same bands in the DNA from fresh and cultured cells. This indicates that the provirus is integrated in the same way in both cell populations (Fig. 2D). The

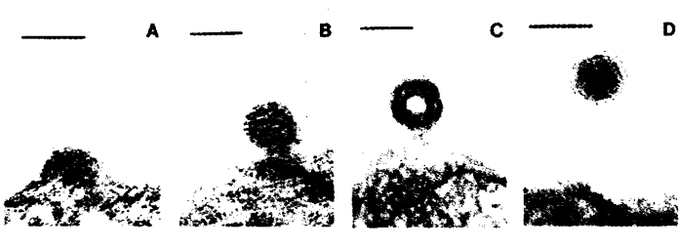

Fig. 1. Electron micrographs of virus particles from patient G.B. Cells were fixed in cacodylate-buffered 2.5% glutaraldehyde, postfixed in osmium tetroxide, and then embedded in Epon 81. Ultrathin sections were stained with uranyl acetate–lead citrate. Mature virus particles show a centrally located round dense core (scale bar, 100 nm). (**A**) Budding with crescent-shaped nucleoid. (**B**) Virus in formation. (**C**) Extracellular immature virus. (**D**) Extracellular mature virus sectioned perpendicular to the core axis.

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