late denticity provided by the additional side arm (*17*). Further studies with this interesting compound are currently in progress.

The results obtained here with ^{212}Bi are not entirely consistent with those showing a prolongation in survival and cure of an experimental ovarian cancer with ^{211}At nonspecific radiocolloid (*18*). The longer 7.2-hour half-life of this α-particle emitter as well as its physical form may be responsible for the observed differences.

The experiments described in this report demonstrate that ^{212}Bi-labeled α-particle–emitting immunoconjugates exhibit high levels of antigen-selective cytotoxicity in vitro, requiring very few ^{212}Bi atoms per target cell to reduce [^{3}H]thymidine incorporation to background levels. These RICs are capable of antigen-specific cure of mice inoculated with malignant ascites.

Our curative therapy of an intraperitoneal murine lymphoma using a radionuclide with a short physical half-life coupled to an antibody of the IgM isotype that remains confined to the peritoneum for relatively long periods of time represents an attempt to design a class of RICs that may eventually prove valuable in the treatment of intraperitoneal malignancies such as ovarian carcinoma and metastatic carcinomatosis from gastrointestinal cancer. The high LET and 5- to 7-cell-diameter path length of the α-particle ejected from the ^{212}Bi nucleus make this radionuclide ideal for the treatment of tumors in which most clonogenic cells are accessible to antibody binding, and especially for the treatment of micrometastases. The short half-life of the isotope limits doses to nontarget structures in those cases where rapid antibody binding to target tissues can be achieved. Further progress in this field will be made by the judicious design of RICs that match the physical and chemical characteristics of the radionuclide and the antibody with the immunologic and clinical characteristics of the proposed tumor target.

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HIV-1 Production from Infected Peripheral Blood T Cells After HTLV-I Induced Mitogenic Stimulation

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The human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type I (HTLV-I) are two distinct human retroviruses that infect T cells. Recent epidemiologic studies have identified a cohort of individuals that are coinfected with both viruses. It is reported here that human peripheral blood leukocytes infected with HIV-1 in vitro can be induced to produce large quantities of HIV-1 after mitogenic stimulation by noninfectious HTLV-I virions. It is also shown that HTLV-I virions may exert this effect prior to, immediately following, or well after the cells are infected with HIV-1. These results provide further impetus for epidemiologic studies of dually infected individuals to determine whether HTLV-I may act as a cofactor for acquired immunodeficiency syndrome (AIDS).

THE HUMAN IMMUNODEFICIENCY virus type 1 (HIV-1) infects cells bearing the CD4 molecule, which include certain T cells and macrophages (*1*). The human T-cell leukemia viruses types I (HTLV-I) and II (HTLV-II) cause various forms of T-cell leukemia and lymphoproliferative disorders (*2*) and can immortalize peripheral blood T cells in vitro (*3*). Since some patients are infected with HIV-1 as well as HTLV-I or HTLV-II (*4–6*), we investigated whether HTLV would augment HIV production in vitro.

HTLV-I containing supernatants and particles prepared by high-speed centrifugation are mitogenic for T cells without directly infecting these cells (*7, 8*). Gradient-purified virions of HTLV-I and HTLV-II are similarly mitogenic (*9*). Human sera containing antibodies to HTLV-I as well as a monoclonal antibody to the HTLV-I envelope glycoprotein gp46 neutralize the virus-induced proliferation of T cells (*8*), suggesting that gp46 mediates this effect. Both $CD4^+$ and $CD8^+$ T cells respond to HTLV-I stimulation (*8, 9*). Because HTLV-I is mitogenic for the same cells ($CD4^+$) that may be infected by HIV-1, we tested whether HTLV-I induced proliferation of HIV-1–infected peripheral blood leukocytes (PBL) results in the increased production of HIV-1.

As shown in Table 1, HIV-1–infected PBL increased production of HIV-1 when exposed to HTLV-I (*10, 11*). A similar increase in HIV-1 production occurred when PBL were stimulated with phytohemagglutinin (PHA) (*12*). The increased production of HIV-1 was not due to HTLV-I–induced T cell transformation, because HTLV-I cell-free virions are generally not infectious (*13*). Furthermore, no immortalization of PBL incubated with purified HTLV-I virions occurred under these conditions.

The cumulative amount of HIV-1 produced by HTLV-I–stimulated PBL depended on the concentration of HTLV-I used to stimulate the cells (Fig. 1). The highest concentration of HTLV-I virions tested

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stimulated maximum thymidine incorporation, but did not induce maximum HIV-1 production. This suggests that mitogenic activity and virus production may be linked but separable phenomena. The dose of HTLV-I that stimulated maximum p24 antigen production was approximately equivalent to five times the concentration of the original virus-containing supernatant. Thus, gradient-purified HTLV-I virions can stimulate both DNA synthesis and HIV-1 production by infected PBL in a dose-dependent fashion.

Enhanced production of HIV-1 by HTLV-I–stimulated PBL was first seen at 5 days after stimulation (Fig. 2A). The daily production of HIV-1 attained a maximum at day 8 after infection and stimulation. HIV-1–infected PBL stimulated by the constant presence of PHA similarly produced detectable levels of virus on day 5; however, production did not attain the same magnitude and declined more rapidly, probably because of PHA-induced cytotoxicity.

Most individuals exposed to HIV-1 do not develop AIDS until a few years after infection (*14*). We therefore studied the effect of HTLV-I virions on latent HIV-1 harbored in quiescent PBL. Unstimulated PBL infected with HIV-1 harbor HIV-1 in a nonproductive state for at least 7 days. Virus can be induced from these infected cells by treatment with mitogenic agents such as lectin or specific antigens. The infected nonproductive state of the virus is probably similar to the latent state known as the "stationary cell intermediate" observed for most retroviruses (*15*). As shown in Fig. 2B, production of HIV-1 was induced by stimulation with HTLV-I virions added immediately after HIV-1 infection (also see Figs. 1 and 2A). In addition, HIV-1 production could be induced by HTLV-I stimulation applied up to 4 days after infection. These data show that HTLV-I virions can stimulate the replication of inactive HIV-1 harbored by quiescent PBL.

Stimulation of PBL by HTLV-I virions prior to infection with HIV-1 also resulted in increased production of HIV-1 (Table 2). PBL treated similarly with PHA also show an increased production of HIV-1 (*12*). These data show that stimulation of PBL with HTLV-I prior to infection with HIV-1 results in increased production of the AIDS virus.

The increased production of HIV-1 particles from infected PBL stimulated with HTLV-I appears to be a consequence of mitogenic proliferation of T cells and to be independent of any direct interactions between the two viruses. Although both HTLV-I and HIV-1 encode proteins [such as those produced by the *tax* (also known as *x*, *x-lor*, and *tat*-I/II) and *tat* genes, respectively] which can act in trans to stimulate viral gene expression, there is no evidence that these proteins will act on the intact heterologous viral long terminal repeats (LTRs). However, experiments addressing transactivation were not performed directly in primary human T cells. HTLV-II particles, like HTLV-I particles, also stimulate HIV-1–infected PBL to produce increased amounts of HIV-1. The HTLV-II *tax* gene product has a greater range of activity than the HTLV-I *tax*, being capable of functioning on both the HTLV-I and -II LTRs (*16, 17*). We therefore tested the transactivating capabilities of the HTLV-II transactivating protein and the HIV-1 transactivating protein on the heterologous LTRs, using a method that we developed to efficiently introduce biologically active DNA directly into primary human T cells (*11*). Expression constructs containing the genes encoding the HTLV-II or HIV-1 transactivating pro-

Table 1. Production of HIV-1 by infected PBL after stimulation with HTLV-I. Normal human PBL obtained by Ficoll-Hypaque purification were infected with one of three different HIV-1 isolates (*10*) by incubation for 2 hours in the presence of Polybrene (10 μg/ml). Infections were standardized by HIV-1 core antigen (10 ng per 5×10^6 cells). After infection, cells were cultured at 1×10^6 per milliliter for 7 days in RPMI 1640 containing 20% fetal bovine serum (FBS), either in the presence or absence of HTLV-I particles (1.6 μg of protein per milliliter) purified by sucrose density gradients (*23*). Culture supernatants were assayed for virus production by ELISA specific for the HIV-1 core antigen, p24 (Abbott) (*24*).

Isolate	Source of isolate	Stimulation	p24 produced (pg/ml)
HIV-1_{LA2}	PBL	None	<100
		HTLV-I	1280
HIV-1_{JR-FL}	Brain (frontal lobe)	None	<100
		HTLV-I	8604
HIV-1_{JR-CSF}	Cerebrospinal fluid	None	<100
		HTLV-I	9798

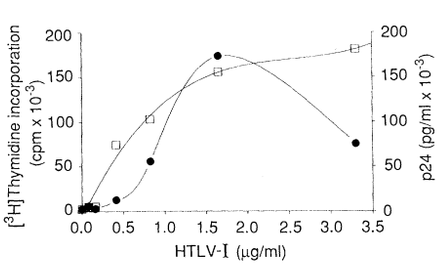

Fig. 1. Dose response of HTLV-I induced DNA synthesis and cumulative HIV-1 production by HIV-1 infected PBL. Human PBL (3×10^7) were infected with HIV-1_{JR-CSF} (60 ng of p24), rinsed, and seeded into 96-well plates at 10^5 cells per well in RPMI 1640 supplemented with 20% FBS. Cells were stimulated with various concentrations of HTLV-I virions and cultured for 5 days. DNA synthesis (□) was measured by incubating cells for 6 hours with [^{3}H]thymidine (10 μCi/ml), harvesting onto glass fiber filters, and determining incorporated radioactivity with a liquid scintillation counter. Triton X-100 (0.5%) was added to all cultures prior to harvesting to inactivate virus. HIV-1 production (●) was measured by ELISA specific for the HIV-1 p24 core antigen (Abbott). All values shown arose from pooled duplicate 0.1-ml cultures from a representative experiment (*24*).

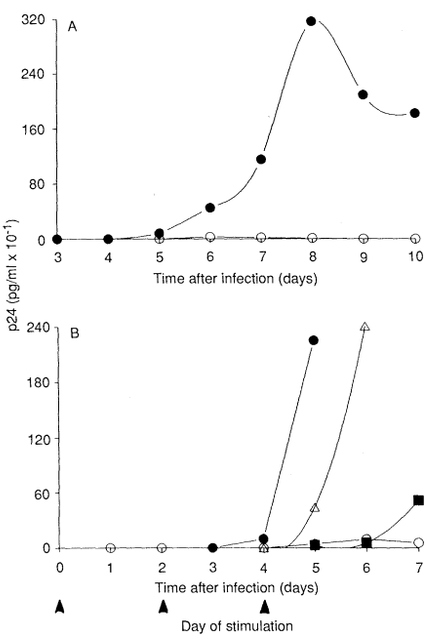

Fig. 2. (**A**) Daily production of HIV-1 by infected PBL stimulated with purified HTLV-I from a representative experiment. PBL were infected with HIV-1_{JR-CSF} and placed into culture (1×10^6 cells per milliliter) in the presence (●) or absence (○) of gradient-purified HTLV-I particles (0.8 μg/ml). Medium (RPMI 1640 supplemented with 20% FBS) was changed daily after day 3, and the cells were assayed for virus production by ELISA on the days indicated. The HTLV-I concentration was held constant in cultures receiving stimulation. (**B**) Activation of HIV-1 production from quiescent PBL from a representative experiment. Cells were infected with HIV-1_{JR-CSF} (see Fig. 1) and plated into microwells (10^5 cells per well) in RPMI 1640 supplemented with 20% FBS. Cells were left unstimulated or were stimulated with HTLV-I virions (1.6 μg/ml) on days 0, 2, or 4 after infection (see arrows). Culture supernatants were assayed for p24 core antigen by specific ELISA on the days indicated. Symbols: ○, no stimulation; ●, day 0 stimulation; △, day 2 stimulation; ■, day 4 stimulation.

teins were co-transfected into PHA-stimulated PBL, along with either the HTLV-I or HIV-1 LTRs linked to the indicator chloramphenicol acetyltransferase (CAT) gene, and LTR transcription was assayed in cell lysates. Although HTLV-II *tax* induced a 16-fold increase in CAT activity from the HTLV-I LTR (Fig. 3), it did not increase CAT activity from the HIV-1 LTR above background levels. Similarly, the *tat* gene product increased expression from the HIV-1 LTR, but had no effect on the HTLV-I LTR. These data demonstrate directly that transactivation of the intact HIV-1 LTR by the HTLV *tax* gene protein cannot account for the large increase in HIV-1 production from HIV-1-infected PBL. These results are in agreement with the recent observations of Siekevitz *et al.* (*18*), who showed that the HTLV-I *tax* gene protein can only transactivate the HIV-1 LTR after the deletion of some 5′ LTR sequences. Therefore, the interaction of HIV-1 with HTLV-I and -II differs from its interaction with members of the herpesvirus family, where the HIV-1 LTR can be directly activated by herpes virus-encoded transacting factors (*19*).

Table 2. Production of HIV-1 by PBL stimulated with HTLV-I prior to infection. Normal PBL were placed into culture (1×10^6 cells/ml) in RPMI 1640 supplemented with 20% FBS for 48 hours in the presence or absence of purified HTLV-I virions (1.6 μg/ml). Cells were then washed extensively, infected with HIV-$1_{JR\text{-}CSF}$ (10 ng of p24 per 5×10^6 cells), and placed back into culture (with the original adherent cells also washed). Culture supernatants were assayed for p24 core antigen by ELISA on days 4 and 5 after infection.

Day	Stimulation	p24 produced (pg/ml)
4	None	<100
	HTLV-I	62,200
5	None	260
	HTLV-I	174,300

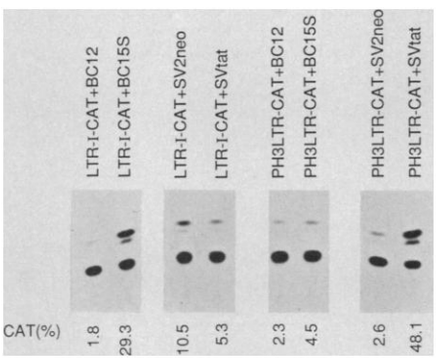

Fig. 3. Transactivation by HTLV Tax and HIV-1 Tat proteins. Recombinant constructs containing the chloramphenicol acetyltransferase (CAT) gene under the control of the LTR derived from HTLV-I or HIV-1 were introduced by electroporation (*11*) into PHA-stimulated PBL, along with equal amounts of constructs that express either the HTLV-II or HIV-1 transactivating proteins, or control vectors. After 24 hours, cells were lysed and assayed for CAT activity as described (*25*). The percent conversion of chloramphenicol to its acetylated forms is indicated for each sample. Constructs used were LTR-I-CAT (*26*), which contains the entire HTLV-I LTR inserted upstream from a promoterless CAT gene; the vector BC12 is BC12/CMV/IL2 (*27*); BC15S, which expresses the HTLV-II *tax* gene product (*17*), driven by the cytomegalovirus immediate early promoter of BC12/CMV/IL2; the vector pSV2neo (*28*); pSVtat (*29*), which expresses the *tat* gene product of HIV-1, driven by the SV40 early promoter; and pH3LTR-CAT, which contains the HIV-1 LTR (nucleotide positions 8915–531 derived from the unintegrated circular genome of strain ARV) (*30*) inserted upstream of the CAT gene.

Our results show that the increased production of HIV-1 is probably due to mitogenic stimulation of T cells by HTLV-I. The induction of cellular transcription factors after T cell activation, reported to be required for efficient HIV-1 LTR function and found in elevated amounts in activated T cells (*20*), is likely to be the mechanism. Other investigators have shown that the HIV-1 LTR contains a region responsive to T cell activation that is separable from the region responsive to viral *tat* gene transactivation (*18, 21*).

The number of patients simultaneously infected with HIV-1 and either HTLV-I or HTLV-II may continue to rise (*4, 5*). Within a dually infected individual, coinfection of a single cell with both viruses would not be expected, since only a small proportion of lymphocytes are actually infected by either virus (*22*). However, it is possible that released HTLV virions contacting an HIV-1–infected T cell would stimulate division of that cell, resulting in increased HIV-1 production. Therefore, in vivo, any HTLV-I–infected cell actively producing virus could theoretically result in activation of other T cells that harbor HIV-1. Patients coinfected with both viruses might thus produce more HIV-1 than those not infected with HTLV-I. One report (*5*) suggested that individuals coinfected with HIV-1 and HTLV-I had lower indices of immune function than those not dually infected. Since HIV-1 is likely to spread into areas endemic for HTLV as the number of AIDS cases increases (*5*), further studies need to be conducted on the effects of coinfection with the HTLVs.

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Early Signal Transduction by the Antigen Receptor Without Commitment to T Cell Activation

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The T lymphocyte antigen–receptor complex mediates antigen-specific cell activation, at least in part, through the production of inositolphospholipid-derived second messengers. Little is known about how second messenger events, typically measured within minutes of ligand binding, eventually lead to distal biologic responses such as expression of lymphokine genes. Several monoclonal antibodies directed against the receptor complex were tested for their ability to elicit transmembrane signaling in the parental Jurkat line and in a somatic mutant (J.CaM1) with a deficient receptor function. One antibody elicited substantial early Ca^{2+} mobilization responses in both cells but was unable to promote expression of the interleukin-2 gene in J.CaM1. In J.CaM1 there was a diminished production of phosphatidylinositol second messengers, and the elevation in intracellular free Ca^{2+} was transient. Thus, short-term Ca^{2+} mobilization does not always indicate complete signal transmission and lead to a full cellular response.

THE T LYMPHOCYTE ANTIGEN RECEPTOR recognizes foreign antigen and transduces this recognition into intracellular biochemical events that result in biologic responses. The receptor complex is a multimeric structure consisting of the antigen-binding disulfide-linked heterodimer (Ti) noncovalently associated with three to seven invariant proteins (CD3) (*1, 2*). This complex is functionally coupled to the phosphatidylinositol (PI) second messenger pathway (*3*) responsible for mobilization of intracellular free Ca^{2+} and activation of protein kinase C (PKC) (*4, 5*). These second messenger events contribute to later cellular responses, such as lymphokine gene expression (*2*).

Recently we described a system for isolating and characterizing T cell somatic mutants with deficient signal transduction by the antigen-receptor complex (*6*). The first such mutant, J.CaM1 (derived from the leukemic cell line Jurkat), was selected primarily for its inability to increase the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in response to monoclonal antibodies (mAbs) directed against the antigen receptor complex, which function as agonists for the wild-type Jurkat cells. In J.CaM1, lectins and mAbs against the idiotype of the receptor (Ti) or certain mAbs against the invariant CD3 molecules associated with the receptor (Fig. 1B) are ineffective in eliciting the rapid increase in $[Ca^{2+}]_i$ that is characteristic of Jurkat (Fig. 1A) and other cell lines (*7–11*). However, some mAbs that alone are nonagonists for J.CaM1 (for example, OKT3 and C305) when used in combination are potent activators of Ca^{2+} mobilization in J.CaM1 (*6*); in addition, at least one mAb directed against CD3 (mAb 235) (*12*) appears to act alone as an effective agonist for both Jurkat (Fig. 1C) and J.CaM1 (Fig. 1D). Thus, the CD3-Ti complex expressed on J.CaM1 appears to be inefficiently coupled to the PI pathway, but certain potent mAbs to CD3 can initiate PI metabolism in this cell.

In Jurkat, expression of the interleukin-2 (IL-2) gene can be induced by phorbol myristate acetate (PMA) combined with a Ca^{2+}-mobilizing agonist, such as a lectin, an ionophore, or a mAb against CD3-Ti (*7, 13*). Since the short-term Ca^{2+} responses to mAb 235 are comparable in the parental and mutant cell lines, we were surprised to find a discrepancy in the ability of mAb 235 to synergize with PMA to produce IL-2 in J.CaM1 and Jurkat. The parental Jurkat cells secreted substantial amounts of IL-2 during a culture overnight in the presence of PMA and mAbs directed against Ti (C305) or against CD3 (OKT3 or mAb 235), as expected (Fig. 2). However, neither the agonist combination of OKT3 and C305 nor mAb 235 alone synergized with PMA to elicit substantial production of IL-2 by J.CaM1 (Fig. 2) even though they elicited large immediate Ca^{2+} responses in this cell (Fig. 1) (*6*). Both cell lines produced substantial IL-2 in response to the ionophore ionomycin in the presence of PMA (Fig. 2), indicating that the failure to respond was not due to a defect in the distal gene expression apparatus.

Since production of IL-2 is regulated primarily pretranslationally (*2, 14*), we investigated the possibility that the failure of mAb 235 to stimulate production of IL-2 by J.CaM1 was also observable at the level of mRNA for IL-2. Analysis of IL-2 mRNA generation by these cells confirmed that mAb 235 was unable to synergize with PMA to promote transcription of the IL-2

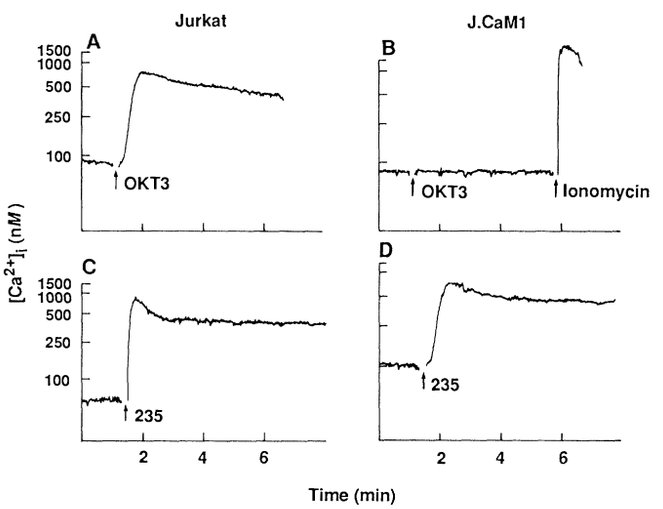

Fig. 1. Mobilization of Ca^{2+} by Jurkat and a signaling mutant derived from Jurkat. Cells were loaded with the fluorescent Ca^{2+}-sensitive indicator Indo-1 (*18*) at 3 μM, and fluorescence profiles were obtained with a Spex Fluorolog II spectrofluorometer as described (*6, 7*). Jurkat cells showed large increases in $[Ca^{2+}]_i$ in response to (**A**) OKT3 or (**C**) mAb 235 (ascites, 1:1000 dilution). J.CaM1 cells failed to respond to (**B**) OKT3 but showed a substantial response to (**D**) mAb 235, which was comparable to that of Jurkat cells. Ionomycin was added where indicated (1 μM) to demonstrate intracellular Indo-1 and releasability of Ca^{2+} stores.

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