24. O. Wiborg, M. S. Pederson, A. Wind, L. E. Berglund, K. A. Marcker, J. Vuust, *EMBO J.* **4**, 755 (1985).
25. E. Ozkaynak, D. Finley, A. Varshavsky, *Nature (London)* **312**, 663 (1984).
26. M. Siegelman, M. W. Bond, W. M. Gallatin, T. St. John, H. T. Smith, V. A. Fried, I. L. Weissman, *Science* **231**, 823 (1986).
27. V. A. Fried, H. T. Smith, M. Morrison, in preparation.
28. R. A. Reichert, W. M. Gallatin, E. C. Butcher, I. L. Weissman, *Cell* **38**, 89 (1984).
29. R. I. Fox and I. L. Weissman, *J. Immunol.* **122**, 1697 (1979); I. A. Wilson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
30. S. Vijay-Kumar, C. E. Bugg, K. D. Wilkinson, W. J. Cook, *Cell* **82**, 3582 (1985).
31. M. O. Dailey, C. G. Fathman, E. C. Butcher, E. Pillemer, I. Weissman, *J. Immunol.* **128**, 2134 (1982); P. J. Fink, W. M. Gallatin, R. A. Reichert, E. C. Butcher, I. L. Weissman, *Nature (London)* **313**, 233 (1985).
32. T. St. John *et al.*, in preparation.
33. We thank C. Nottenburg for advice on experiments and critical review of the manuscript, and J. Carlson, B. Sher, and G. Griffiths for critical review of the manuscript. Supported by NIH grant AI19512 to I.W., and in part by NIH grants GM 31461 and the American Lebanese Syrian Associated Charities (V.A.F.); a grant from the American Cancer Society, California Division (W.M.G. and T.St.J.) and Tumor Biology Training Grant CA 09151 (T.St.J. and M.S.).

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Long-Term Cultures of HTLV-III–Infected T Cells: A Model of Cytopathology of T-Cell Depletion in AIDS

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Long-term cultures were established of HTLV-III–infected T4 cells from patients with the acquired immune deficiency syndrome (AIDS) and of T4 cells from normal donors after infection of the cells in vitro. By initially reducing the number of cells per milliliter of culture medium it was possible to grow the infected cells for 50 to 60 days. As with uninfected T cells, immunologic activation of the HTLV-III–infected cells with phytohemagglutinin led to patterns of gene expression typical of T-cell differentiation, such as production of interleukin-2 and expression of interleukin-2 receptors, but in the infected cells immunologic activation also led to expression of HTLV-III, which was followed by cell death. The results revealed a cytopathogenic mechanism that may account for T4 cell depletion in AIDS patients and suggest how repeated antigenic stimulation by infectious agents, such as malaria in Africa, or by allogeneic blood or semen, may be important determinants of the latency period in AIDS.

THE PATHOGENESIS OF THE ACquired immune deficiency syndrome (AIDS) involves a decrease in the number and function of mature T4 lymphocytes. When cultured in vitro, these cells are the main target of infection by the human T-lymphotropic retrovirus designated HTLV-III/LAV (*1–3*), and it is from these cells that HTLV-III/LAV is usually isolated (*4*). T4 lymphocytes from normal donors infected by HTLV-III in vitro, as well as HTLV-III–infected primary T4 cells from AIDS patients, have been difficult to maintain in culture for longer than 2 weeks (*4, 5*), and it has often been assumed that the virus has a direct cytolytic effect on these cells (*4, 5*). However, in this report we describe culture conditions that permit the long-term growth of HTLV-III–infected T cells derived from AIDS patients and of normal donor T cells infected with HTLV-III in vitro. In the long-term cultures, the expression of HTLV-III was always preceded by the initiation of interleukin-2 secretion, both of which occurred only when T cells were immunologically activated. Thus, the immunologic stimulation that was required for IL-2 secretion also induced viral expression, which led to cell death.

For these experiments we obtained heparinized peripheral blood lymphocytes (PBL) from six patients with AIDS and from three normal donors. The T cells were separated from mononuclear cells by Ficoll-Hypaque gradients. The sera of the six AIDS patients were positive for antibodies to HTLV-III, whereas sera from the normal donors were negative. The PBL (6×10^5 per milliliter) were activated by 0.1 percent (PHAp; Difco) in round-bottom tissue culture tubes (white caps RBTC; Falcon) containing 2 ml of medium composed of RPMI 1640, 20 percent fetal calf serum (FCS), sheep antiserum to α-interferon (neutralizing titer 6 IU at 10^{-5} dilution), and goat antiserum to human γ-interferon (neutralizing titer 1 IU at 10^{-5} dilution). After 2 to 3 days, IL-2 was added to the cultures. The medium was changed twice each week.

The primary PBL cultures from AIDS patients proliferated for about 12 to 15 days and consisted of adherent macrophages, T cells, and a few B cells. After this period they exhibited abundant cell lysis. However, when these cultures were activated with phytohemagglutinin (PHA), they first secreted IL-2 (days 1 and 2 after activation) and then transiently produced virus (days 6 to 12), as indicated by reverse transcriptase (RT) activity in the culture supernatants (*3–5*) and by the presence of HTLV-III p15 and p24 antigens on the surface of a few acetone-fixed cells detected by specific monoclonal antibodies (*6*). These observations suggested that agents that inhibit IL-2 production might enhance virus expression [for example, hydrocortisone (*6, 7*)] and favor cell death, whereas inhibitors of virus production might be associated with increased IL-2 secretion (for example, γ interferon) (*8*).

To obtain cultures of T cells from AIDS patients, we used the conditions described previously for the long-term growth of normal T-cell clones, with certain modifications (*9*). The modifications consisted of dilution of the cell number from 10^5 to 10^6 cells per milliliter (usual culture conditions) to 10^5 to 10^4 cells per milliliter and the addition of a feeder cell layer of 10^5 to 10^6 irradiated (4000 rads) PBL pooled from 10 to 20 normal human donors (Fig. 1). With these conditions, the HTLV-III–infected T cells could be maintained in the presence of exogenous IL-2 for 50 to 60 days. The initial reduction of the cell concentration was sufficient to select out those cells that could survive; the lack of further antigenic stimulation and, presumably, the reduced concentrations of toxic substances released by the mature cells, permitted cell survival at concentrations ranging from 5×10^5 to 1×10^6 per milliliter. However, after this period, cell degeneration occurred in a manner similar to that of normal T cells grown under the same conditions (*9*).

The cultures of infected T cells showed other similarities to cultures of normal T cells. When T-cell surface antigens were measured by the rosette technique in the presence of specific monoclonal antibodies (*10*), the proportion of infected cell cultures exhibiting T4, T8, and Tac antigen were in the same range as normal T cells. No cytogenetic changes occurred in the T-cell cultures from three AIDS patients when the cultures were stained by the Giemsa banding tech-

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nique. As reported for cultured T-cell clones (*11*) and shown in this study (Table 1, column 3), uninfected, normal T-cell cultures secreted large amounts of IL-2 only when they were activated by PHA in the presence of macrophages and B cells. We found the same results with cultured T cells from AIDS patients and with T cells from normal donors after infection with HTLV-III in vitro (Table 1).

The T cells from AIDS patients grown in the presence of exogenous IL-2 did not express HTLV-III even though they proliferated. However, when they were stimulated with PHA under the same conditions used to generate IL-2 production, RT activity was found transiently in the culture supernatants 5 to 8 days after activation (Table 1, column 4). As shown in Fig. 1, from 0.5 to 3.0 percent of the PHA-activated, RT-positive cultures of T cells from AIDS patients also expressed p15 and p24 HTLV-III antigens, identified by indirect immunofluorescence with specific monoclonal antibodies (*3, 4*). Cell death occurred 7 to 10 days after PHA activation and virus expression. These results show that long-term cultures of T cells from AIDS patients, like fresh PBL from AIDS patients, express HTLV-III after immunologic stimulation and that the sequence of the events after such stimulation in both systems is IL-2 secretion, virus expression, and cell death.

Similar results have been reported with PBL from normal donors infected with HTLV-III in vitro (*6*). In the present studies, T cells from normal donors that were infected with HTLV-III in vitro, after stimulation with PHA, followed the same pattern of secretion of IL-2 (day 1), production of HTLV-III, and cell death (Table 2). Again, the cells did not produce IL-2 or express virus without immunologic activation. Stimulation with PHA also induced variations in the T-cell differentiation antigens, such as loss of the T4 and Tac (IL-2 receptor), on day 6. This loss was more marked in the HTLV-III–infected cells (Table 3), although the differentiation of infected cells paralleled that of the normal uninfected cells. When T4 or T8 cells were removed by complement-dependent cytotoxicity in the presence of corresponding monoclonal antibodies, only PHA-activated subpopulations of T cells, including T4 cells, secreted IL-2 and expressed virus (Table 2).

Although immunologic activation is required for viral expression, it is not necessary for HTLV-III infection of T cells. As shown in Table 4, incubation of normal T cells with HTLV-III resulted in infection, but virus was expressed only after PHA activation.

We also studied the IL-2–secreting human leukemic T4 cell lines HUT-78 (*4*) and JM (*6*). When these cells are infected with HTLV-III, after PHA stimulation, many constitutively express virus and die 4 to 6 days after infection. In some of our experiments, the cells were infected with HTLV-III after treatment with PHA in the presence of macrophages and B cells. This resulted in increased IL-2 production and virus expression (Table 5).

In patients with AIDS or the AIDS-related complex (ARC), the number of T cells progressively decreases and the secretion of IL-2 is dramatically impaired (*8*). Impaired production of IL-2 in turn leads to decreased T-cell proliferation and a reduction in IL-2 receptors, as well as a reduction in γ-interferon and other effector molecules. The results described here reveal some of the events that may lead to the cytopathogenetic disorders of T cells in AIDS patients. (i)

Table 1. Production of IL-2 and RT in long-term T-cell cultures from PBL of AIDS patients and normal donors before and after activation with PHA. Activated and nonactivated T-cell cultures (20 ± 5 days old) derived from AIDS patients and normal donors were assayed for IL-2 production and RT activity. For activation, T cells (6×10^5) were washed twice and incubated in an IL-2–free medium containing PHAp (0.1%), and a feeder cell layer consisting of macrophages (5×10^4) and B cells (2×10^4) derived from normal PBL. Culture supernatants were collected after 24 hours for IL-2 assay and replaced by a culture medium composed of exogenous IL-2 and hydrocortisone (5 μg) which allowed growth of the cells for more than two additional weeks, during which RT activity was monitored. Nonactivated T-cell cultures were similarly treated, except that they were incubated for 24 hours in an IL-2–free medium without PHA or feeder cells.

Patients*	Origin of T cells	HTLV-III serology	PHA activation	IL-2 production† (IL-2 U/2×10^5 cells)	RT activity‡ (10^4 cpm/ml)	T4 cell (% of PBL)	Ratio of T_4 to T_8
			Experiment 1				
European (M)	AIDS (RK)‖	+	−	<260	<1	<5	0.2
			+	>1400	28		
European (M)	AIDS (JMB)	+	−	<260	<1	7	0.4
			+	>1400	6.6		
European (F)	Normal$_1$ (MF)	−	−	<260	<1	39	1.9
			+	>1400	<1		
			Experiment 2				
Zairian (F)	AIDS (BS)	+	−	<260	<1	9	0.7
			+	>1400	17		
European (M)	AIDS (AC)	+	−	<260	<1	5	0.4
			+	>1400	3.2		
European (F)	Normal$_2$ (BR)	−	−	<260	<1	45	2.4
			+	>1400	<1		
			Experiment 3				
Zairian (M)	AIDS (AD)	+	−	<260	<1	<5	0.2
			+	>1400	8.4		
European (M)	AIDS (MP)	+	−	<260	<1	6	0.6
			+	>1400	31		
European (F)	Normal$_1$ (MF)	−	−	<260	<1	39	1.9
			+	>1400	<1		

*The five male patients were hospitalized for opportunistic infections; the Zairian female had Kaposi's sarcoma. M, male; F, female. †For the IL-2 assay, 12- to 15-day-old normal T cells (2×10^4) were cultured in 200-μl round-bottom wells (Nunc RBTC) containing 150 μl of fresh RPMI 1640/FCS (RPMI 1640 medium with 20% FCS) and 50 μl of test supernatants. After 4 days of culture, [^{3}H]thymidine (1 μCi per well) was added and supernatants were collected after 12 hours. In control wells the test supernatant was replaced by (i) RPMI 1640/FCS; (ii) RPMI 1640/FCS with PHAp (0.1%); or (iii) RPMI 1640/FCS containing HTLV-III. To prepare this last medium, 10 ml of H9/HTLV-III culture fluid was centrifuged at 50,000*g* for 1 hour, the supernatant was removed, and the pellet was resuspended in 1 ml of RPMI 1640/FCS. Test supernatants were derived from cultures of T cells derived from AIDS patients and normal donors. IL-2 content was measured in triplicate in test supernatants previously described (*11*). Mean results from triplicate samples are given in Transgene IL-2 units and the count variability was less than 10%. Extrapolation from radioactivity to IL-2 units (Transgene) was made between 10,500 and 510,500 cpm corresponding to linear variation of IL-2 concentration from 280 to 1400 units as determined experimentally. Radioactive thymidine uptake for <280 mU of IL-2 ranged between 1,500 and 10,000 cpm, whereas for 71,400 mU of IL-2 the range was between 500,000 and 850,000 cpm. ‡For the RT assay, samples of supernatant from activated T-cell cultures were collected at different periods after PHA stimulation. The culture fluid (1 ml) was centrifuged for 10 minutes at 300*g*. Virus particles were precipitated from cell-free supernatants and the RT assay was performed as described (*3*). Nonstimulated samples were always below 1000 cpm/ml. The results are for samples from nonactivated and activated T cells at the period of maximum RT activity, which usually occurred 6 to 8 days after lectin stimulation. ‖Letters in parentheses are code initials for patient samples.

Table 2. Effect of PHA activation on IL-2 and virus production in long-term cultures of T cells from PBL of normal donors after infection with HTLV-III in vitro. Activated and nonactivated cultures of T cells (20 days old) derived from the same normal donor were assayed for IL-2 production and RT activity. After activation by PHA for 24 hours, the T cells from the donor were treated with polybrene (2 μg/ml), incubated in the presence of HTLV-III–containing culture supernatant fluid obtained from the H9/HTLV-III–B2 cell line for 1 hour at 37C°, and cultured in RPMI 1640 and FCS containing exogenous IL-2, antibody to α-interferon, and hydrocortisone (*6*). Non-T_4 and non-T_8 T-cell subpopulations were obtained after treatment of the total T-cell population by complement-dependent cytotoxicity in the presence of OKT4 and OKT8 monoclonal antibodies, respectively. Nonactivated cells were used as controls. IL-2 production and RT activity were measured as described in Table 1.

T-cell culture	PHA activation	IL-2 production (IL-2 U/2 × 10^4 cells)	RT activity (10^3 × cpm/ml)
		Mixed cell populations	
Uninfected control	−	<260	<1
	+	>1400	<1
Infected in vitro	−	<260	<1
	+	1400	46
		Non-T4 subpopulation	
Infected in vitro	−	<260	<1
	+	<260	<1
		Non-T8 subpopulation	
Infected in vitro	−	<260	<1
	+	>1400	26.5

Table 3. Antigenic variation in long-term cultured T cells from normal donors after infection with HTLV-III in vitro. The cultured (20 days) T cells were activated with PHA in the presence of macrophages and B cells and either uninfected or infected with HTLV-III as described in Table 2. Nonactivated normal T cells were also used as controls. T4 and Tac antigens were tested in triplicate for each culture on days 0, 2, and 6 after PHA stimulation. Variability in these samples was less than 10% and the results presented are the mean values.

T-cell culture	HTLV-III/LAV infection	Number of positive cells (%)					
		T4			Tac		
		Day 0	Day 2	Day 6	Day 0	Day 2	Day 6
Nonactivated	−	34	38	25	42*	31	38
PHA-activated	−	34	28	10	42	54	20
PHA-activated	+	34	30	3	42	51	7

*The high percentage of Tac^+ (IL-2 receptor–positive) cells on day 0 of the experiment is due to the previous long-term culture (20 days) of these cells.

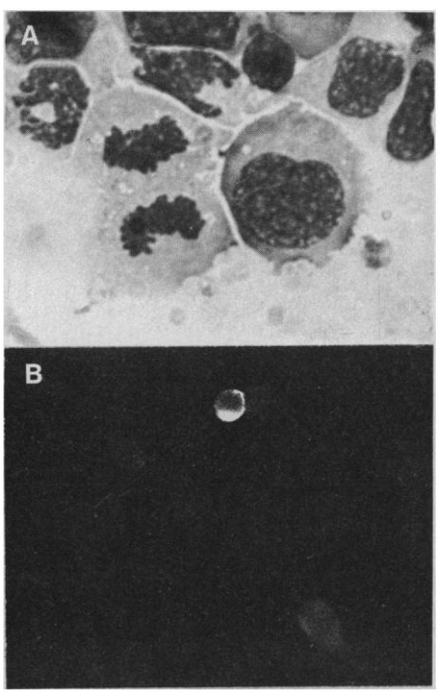

Fig. 1. Long-term cultures of T cells derived from PBL of AIDS patients were obtained as follows. The PBL were seeded at low density (0.1×10^4 to 1.0×10^4 cells per milliliter) in 2-ml round-bottom tissue culture tubes (Falcon) in the presence of a feeder cell layer of 0.5×10^6 to 1.0×10^6 irradiated (4000 rads) PBL pooled from 10 to 20 normal donors; the medium contained RPMI 1640; FCS (20%); antibody to human α- (1:200) and γ- (1:500) interferon; and PHAp (0.1%); IL-2 (10%) was added after 2 days. T-cell cultures grew in the presence of IL-2 at a concentration ranging from 0.5×10^6 to 1×10^6 cells per milliliter for 50 to 60 days. (A) Light microscopic examination of 30-day-old cultured T cells treated with Giemsa-Wright stain. (B) Detection of HTLV-III p24 antigen in PHA-activated 30-day-old cultured T cells. Cells were activated by PHA as described in Table 1. The assay was by indirect immunofluorescence as previously described (*4, 6*). Cells were collected from RT-positive cultures, spotted on a slide, dried, and fixed in acetone. Fixed cells were incubated with monoclonal antibody to HTLV-III p24 (p15 in other experiments), and after three washes they were treated with the fluorescein-conjugated antiserum. Controls consisted of (i) cells treated with murine monoclonal antibodies to immunoglobulin in place of the specific antibodies and (ii) cells treated only with the fluorescein conjugate. Fluorescent cells were observed only in samples treated with monoclonal antibody to p15 or p24, as shown here.

Immunologic activation of T cells induces IL-2 secretion and is required for viral expression. (ii) Infection of T4 cells with HTLV-III does not require immunologic activation. (iii) The expression of HTLV-III follows immunologic activation but is a late event in this process. (iv) Virus expression in turn promotes cell death (Fig. 2).

Although there are major differences between stimulation with PHA and antigenic stimulation, we propose that, in vivo, antigenic stimulation of an infected T cell results in virus expression which is followed by lysis of the differentiated cell and, consequently, virus release and the probability of other T4 cells becoming infected. Thus, multiple rounds of immunologic stimulation may initially generate more and more infected T cells but will ultimately result in decreased numbers. Therefore, the presence of other infections, such as malaria in Africa, or repeated exposure to other antigens, such as allogeneic leukocytes from blood or spermatozoan antigens from semen, may be important determinants of the latency period in AIDS.

The expression of the IL-2 gene and of HTLV-III after immunologic activation appear to be coordinated. Recent nucleotide sequence studies show a region of homology between sequences upstream from the IL-2 gene and a segment of the HTLV-III long terminal repeat (LTR) (*13*). Since immunologic activation of T4 cells leads to IL-2 production followed by HTLV-III expression, it is possible that these homologous regions are common sequences involved in recognition of immunologic activation signals. Factors within the cell, or from outside, may favor one effector pathway (either IL-2 secretion or virus production) and thus may account in part for the clinical heterogeneity observed in HTLV-III–infected individuals.

Experiments in vitro on PBL from AIDS patients showed that when a reagent that blocks viral replication (Suramin) and an immunologic regulator such as γ-interferon are used together with antibodies to α-interferon, the immune defect in the T cells may be repaired, at least as assessed by IL-2 production (*8*). The mechanism by which immunologic stimulation of infected cells results in activation of viral or cellular genes that in turn promote T-cell terminal differentiation may in part involve the process known as *trans*-acting transcriptional activation (*13–16*). This hypothesis is attractive in view of the recent demonstration that a product of HTLV-III promotes activity of the HTLV-III LTR (*14*). This phenomenon is reminiscent of the *trans*-acting transcriptional activation of the chloramphenicol acetyltransferase (CAT) gene of HTLV-I and

HTLV-II LTR complexes (*16*) by the x-*lor* proteins (now termed tat_I and tat_{II} genes, respectively) derived from the 3′ ends of the HTLV-I and the HTLV-II genomes. These proteins may promote both viral and cellular gene expression.

The major functional product of the HTLV-III gene (tat_{III}) responsible for *trans*-acting transcriptional activation results from a double-spliced messenger RNA (mRNA) similar to the mRNA of HTLV-I and HTLV-II (*15, 16*). The size of tat_{III}, however, is smaller and its position in the viral genome different from tat_I and tat_{II} (*16*). While tat_I and tat_{II} may activate genes involved in T-cell proliferation, tat_{III} may augment the expression of genes involved in terminal differentiation leading to cell death. When primary cultures of T cells from AIDS patients are maintained by standard procedures, most of the cells die within 8 to 15 days (*4*). However, our studies show that it is possible to select some of them for long-term growth by using low cell concentrations and a feeder layer of irradiated cells. Thus, their premature death in bulk culture may be due to the presence in the medium of products released by the numerous degenerating infected cells. Similar results were previously observed with T cells derived from lymph nodes and semen of AIDS patients (*17, 18*). Our results also suggest that multiple rounds of antigenic stimulation in vivo, as a result of infection with various microorganisms or exposure to allogeneic cells such as semen or blood, may promote HTLV-III expression, T4 cell death, further spread of the virus, and ultimately an immunodeficiency syndrome (*19*).

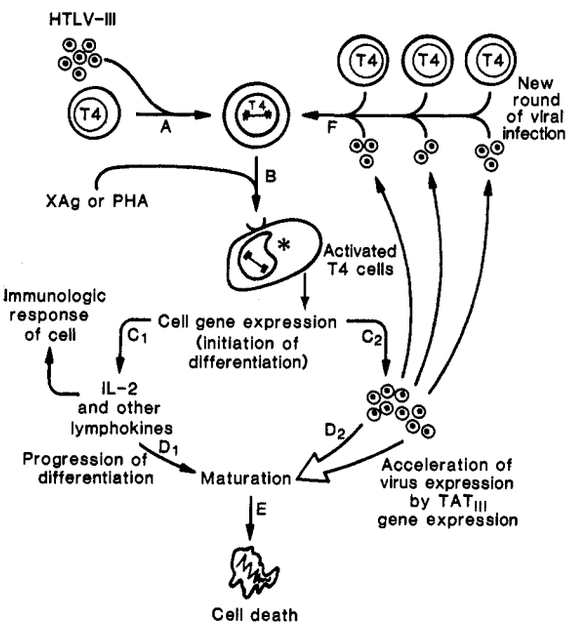

Fig. 2. Schematic representation of biological events leading to HTLV-III/LAV infection, viral expression, and death of T4 cells. At stage A, T4 lymphocytes, either activated or nonactivated, are preferential target cells for HTLV-III/LAV. At stage B, infected as well as noninfected T4 cells are activated either specifically by a corresponding antigen (XAg) or by exposure to blood or semen or, as in the experiments in vitro, by PHA. This activation initiates effector differentiation by expression of cellular genes (stage C_1) which leads to promotion of IL-2 secretion (physiological pathway, stage D_1) as well as accelerated HTLV-III/LAV expression (pathologic pathway, stages C_2 and D_2). In activated T4 cells maturation occurs progressively; in infected cells viral expression is accelerated through tat_{III} gene product which greatly enhances virus expression and in turn leads to stage E, terminal differentiation, and cell death.

Table 4. Viral expression in long-term cultures of T cells from normal donors after infection with HTLV-III in vitro. The T-cell cultures (25 days old) were treated for 0 to 3 days with polybrene (2 μg/ml), incubated in the presence of HTLV-III/LAV–containing culture supernatants for 1 hour at 37C°, and cultured again in the presence of IL-2, cortisone, and sheep antiserum to human α-interferon. After 1 to 2 hours, the infected cultures were washed twice and divided into two cultures. One was maintained in the same medium containing IL-2 (nonactivated) and the other (PHA-activated) was stimulated by PHA in the presence of macrophages and B cells, as described in Table 1. This experiment was repeated twice with similar results.

PHA stimulation	Time between viral infection and PHA stimulation	RT activity* (10^3 cpm/ml)
	Experiment A	
+	0	46
−	0	0
	Experiment B	
+	2 hours	27
−	2 hours	0
	Experiment C	
+	1 day	38
−	1 day	0
	Experiment D	
+	3 days	19
−	3 days	0

*Results show the peak activity of RT in culture supernatants as described in Table 1.

Table 5. IL-2 secretion and HTLV-III production in infected human T_4 leukemic cell lines before and after activation with PHA. HUT-78 and JM cell lines were treated with PHA for 24 hours in the presence of macrophage and B cells as described in Table 1. Nonactivated and activated cells were incubated with HTLV-III–containing culture fluid for 1 hour at 37°C, washed twice, and cultured in RPMI 1640/FCS.

Cell line*	PHA activation	IL-2 secretion† (IL-2 U/10^4 cells)	HTLV-III‡ production
HUT-78	+	1400	++++
	−	850	++
JM	+	1100	++++
	−	800	++

*Uninfected HUT-78 and JM cells do not express any RT activity in the culture supernatant before or after stimulation with PHA. †IL-2 secretion was measured in culture supernatants from cells activated with PHA for 24 hours and from nonactivated cells just before HTLV-III infection. IL-2 assays were performed as described in Table 1, and the results are given in IL-2 units (Transgene) (mean of triplicate samples). Variability in triplicate samples was less than 10%. ‡HTLV-III production was measured by RT activity as described in Table 1. RT activity was measured by [^{3}H]thymidine incorporation into trichloracetic acid–precipitable DNA and the radioactivity ranged from $10^4 \times 10^5$ to 5×10^5 cpm/ml. Relative scores are indicated by the number of plus signs.

REFERENCES AND NOTES

1. F. Wong-Staal and R. C. Gallo, *Nature (London)* **317**, 395 (1985); R. C. Gallo, Henry Kaplan Memorial Lecture, Princess Takatmatsu, Symposia on Retroviruses in Human Lymphoma/Leukemia, M. Miwa *et al.*, Eds. (Sci. Soc. Press, Tokyo, 1985).
2. S. Broder and R. C. Gallo, *N. Engl. J. Med.* **316**, 1297 (1984); F. Wong-Staal and R. C. Gallo, *Blood* **65**, 253 (1985).
3. R. C. Gallo *et al.*, *Science* **224**, 500 (1984).
4. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.*, p. 497.
5. F. Barré-Sinnousi *et al.*, *ibid.* **220**, 868 (1983).
6. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *Science* **224**, 506 (1984); S. Z. Salahuddin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5530 (1985).
7. G. H. Reem and N.-H. Yeh, *Science* **225**, 429 (1984).
8. D. Zagury *et al.*, *Lancet* **1985-II**, 449 (1985).
9. D. Zagury, D. A. Morgan, M. Fouchard, *J. Immunol. Methods* **43**, 67 (1981).
10. J. Bernard, T. Ternynck, D. Zagury, *J. Immunol. Lett.* **4**, 66 (1982).
11. D. Zagury, D. Morgan, G. Lenoir, M. Fouchard, M. Feldman, *Int J. Cancer* **31**, 427 (1983); D. Zagury, J. Bernard, D. Morgan, M. Fouchard, M. Feldman, *ibid.*, p. 705.
12. J. W. Mier and R. C. Gallo, *J. Immunol.* **128**, 1122 (1982); P. S. Sarin and R. C. Gallo, *CRC Crit. Rev. Immunol.* **4**, 279 (1984).
13. L. Ratner *et al.*, *Nature (London)* **323**, 277 (1985); M. A. Muesing *et al.*, *ibid.*, p. 45.
14. J. G. Sodroski *et al.*, *Science* **227**, 171 (1985).
15. S. K. Arya, C. Guo, S. F. Josephs, F. Wong-Staal, *ibid.* **229**, 69 (1985); J. Sodroski, R. Patarca, C. Rosen, F. Wong-Staal, W. Haseltine, *ibid.*, p. 74.
16. J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *ibid.* **225**, 381 (1984).
17. D. Zagury *et al.*, *Cancer Res. (Suppl.)* **45**, 4595 (1985).
18. D. Zagury *et al.*, *Science* **226**, 449 (1984).
19. R. R. Redfield *et al.*, *J. Am. Med. Assoc.* **253**, 1571 (1985).

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