or whether about half of them have completely lost their ability to stimulate adenylate cyclase. They do demonstrate that the receptors are the locus of the alteration in this system.

The methods described here open the way to exploring the fundamental biochemical mechanisms of these processes. Thus, it should be possible to attempt to mimic the functional alterations of the receptors by directly modifying their structure—for example, using appropriate kinases to phosphorylate the purified receptors in vitro. The use of these methods to assess the biological activating function of receptors can help to elucidate the molecular mechanisms that regulate receptor activity. These methods provide a major advance in attempts to understand receptor function and its regulation.

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Isolation of Lymphocytopathic Retroviruses from San Francisco Patients with AIDS

Abstract. *Infectious retroviruses have been detected in 22 of 45 randomly selected patients with acquired immune deficiency syndrome (AIDS) and in other individuals from San Francisco. The AIDS-associated retroviruses (ARV) studied in detail had a type D morphology, Mg^{2+}-dependent reverse transcriptase, and cytopathic effects on lymphocytes. The viruses can be propagated in an established adult human T cell line, HUT-78. They cross-react with antiserum to the lymphadenopathy-associated retrovirus isolated from AIDS patients in France. Antibodies to ARV were found in all 86 AIDS patients and in a high percentage of 88 other homosexual men in San Francisco. This observation indicates the widespread presence of these lymphocytopathic retroviruses and their close association with AIDS.*

Acquired immune deficiency syndrome (AIDS) has affected more than 4000 individuals in the world; in San Francisco, over 600 cases have been reported (*1*). In addition, there are many patients with unexplained chronic lymphadenopathy which may be caused by the agent responsible for AIDS (*1*). Last year, two different retroviruses were isolated from AIDS patients. One of these, human T cell leukemia virus (HTLV-I) (*2*), which is associated with T cell leukemias in man (*3*), has a type C morphology as determined by electron microscopy; this virus can immortalize T cells to produce continuous cell lines and is primarily cell-associated (*3*). The other, lymphadenopathy-associated retrovirus (LAV), was isolated from the lymph node of a patient with lymphadenopathy (*4*) and has subsequently been recovered from patients with AIDS (*5*). LAV has a type D morphology, causes cytopathic changes in T cells, and is infectious in culture fluids. A third retrovirus, HTLV-III, was recently reported in AIDS pa-

Table 1. Peripheral mononuclear cell (PMC) cultures were established from 10 to 30 ml of heparinized blood from individuals seen at the Kaposi's sarcoma clinic, University of California, San Francisco, or the AIDS clinic, San Francisco General Hospital. Patients were selected at random intervals from the sequence of individuals appearing at the clinics for evaluation. All patients and most of the clinically healthy individuals had lived in San Francisco for at least 2 years. The PMC were separated on Ficoll-Hypaque gradients (*9*). Washed cells were plated at approximately 2×10^6 per milliliter in RPMI 1640 containing 10 percent fetal bovine serum and antibiotics (penicillin, 100 unit/ml; streptomycin 100 μg/ml). To this medium was added interleukin-2 (Meloy) (0.5 μg/ml) and Polybrene (1 μg/ml). At initiation of the cultures, phytohemagglutinin (Wellcome), approximately 2.5 μg/ml, was added. Some cultures also received $10^{-5}M$ β-mercaptoethanol and sheep antisera to interferon-α (*4*) provided by the National Institutes of Health (lot 61220); K. Cantell, Helsinki; or F. Barré-Sinoussi, Paris. These antisera were used at a dilution that neutralized 700 to 1000 units of interferon-α per milliliter of culture. The culture supernatants were routinely assayed for Mg^{2+}-dependent reverse transcriptase activity (see legend to Fig. 1) every 3 to 6 days. The cells were studied for the presence of HTLV-I and LAV antigens by standard indirect immunofluorescence assays. For these studies, cells were put on glass slides, air dried, and fixed in cold acetone for 15 minutes. A monoclonal antibody to HTLV p19 provided by Robert-Guroff and Gallo, National Institutes of Health, and a monoclonal antibody to adult T cell leukemia virus (ATLV) p19/p28 provided by Y. Hinuma, Kyoto, Japan (*10*), were used. For detection of LAV, human serum (from patient B.R.U.) provided by Barré-Sinoussi, Paris, was used (*4*). Peripheral mononuclear cells producing ARV were also tested for reactivity with sera from AIDS patients from San Francisco. For the sera we examined, the results were the same as those obtained with the B.R.U. serum. The fluorescein-conjugated antibodies for these immunofluorescence assays were either goat antiserum to mouse immunoglobulin G or goat antiserum to human immunoglobulin G. Some cultures were examined for virus by electron microscopy (see legend to Fig. 2). Any cultures that gave positive results repeatedly by any of these tests were considered positive for virus.

Subjects	No. tested	Positive results	
		No.	Percent
Patients with diagnosis of			
AIDS with Kaposi's sarcoma	41	22	53.6
AIDS with opportunistic infection	4	0	0
Lymphadenopathy syndrome	10	5	50.0
Other individuals			
Male sexual partners of AIDS patients*	14	3	21.4
Clinically healthy homosexual men†	9	2	22.2
Clinically healthy heterosexual individuals†	23	1	4.0

*Clinically healthy individuals who had steady sexual contact with a patient for at least 6 months before the patient became ill. †Some of these individuals volunteered for the study.

tients and related syndromes (*6*). This virus has some cross-reactivity with HTLV-I and HTLV-II but, like LAV, has a type D morphology and causes cytopathic changes in lymphocytes.

In attempts to isolate the infectious agent responsible for AIDS in homosexual patients from San Francisco, we have identified lymphocytopathic retroviruses that are similar to LAV. In addition, serologic studies show a high prevalence of antibodies to these AIDS-associated retroviruses (ARV) in individuals from San Francisco.

Using interleukin-2 and phytohemagglutinin stimulation (*3*), we established more than 100 cultures of peripheral mononuclear cells (PMC), which after 1 week in culture, were primarily of the T cell lineage (*7*). When supernatants from cultures prepared from 41 homosexual AIDS patients were examined, 22 were positive for Mg^{2+}-dependent reverse transcriptase activity (Table 1). The viruses were found primarily in PMC of patients in the early stages of disease. The reverse transcriptase activity was detected in the PMC usually within the first 2 weeks of culture, with the peak of activity observed by 12 to 16 days (Fig. 1A). When bone marrow aspirates were cultured, three of nine cultures from AIDS patients showed evidence of retroviruses (data not shown). With some cell cultures, the use of antiserum to interferon helped to demonstrate the presence of virus (*4*). When reverse transcriptase activity diminished in the cultures after 2 to 3 weeks, the addition of fresh lymphocytes from normal donors sometimes reestablished this activity (see legend to Fig. 1A). Supernatant fluids from positive cultures (some stored at −70°C for more than 3 months) also induced reverse transcriptase activity in uninfected fresh lymphocyte cultures. Mg^{2+}-dependent reverse transcriptase activity was also observed repeatedly in PMC cultures from patients with lymphadenopathy syndrome, steady male sexual partners of AIDS patients, clinically healthy homosexual men, and one healthy young heterosexual man (Table 1).

The viruses detected in seven of the PMC cultures were grown in high titer and had similar characteristics. One virus (ARV-2) was recovered within 2 weeks directly from the PMC of a patient approximately 1 month before the onset of AIDS (Fig. 1A). AIDS-associated retroviruses were isolated from subsequent cultures taken 2 and 6 months later, after the onset of AIDS. Multiple time-spaced samplings from five of six other patients with AIDS have also yielded retroviruses (data not shown).

The reverse transcriptase activity of these viruses was associated with particles banding in a sucrose gradient at 1 :14 to 1:16 g/ml (Fig. 1B). With poly(rA) · oligo(dT) or poly(rC) · oligo (dG) as template primers, the viral enzyme had up to an eightfold cation preference for Mg^{2+} over Mn^{2+}. With Mg^{2+}, reverse transcriptase levels higher than 3.5×10^6 cpm per milliliter of culture supernatant could be reached.

The isolated viruses induced multinucleated cells in lymphocyte cultures and

Table 2. The presence of antibodies to HTLV-I in sera from patients and controls was assessed with slides containing acetone-fixed smears of the M-2 cell line (provided by Y. Hinuma) in which 100 percent of the cells demonstrate HTLV or ATLV antigens (*3*). The controls for this experiment included the M-1 line (from Y. Hinuma), which has less than 1 percent positive cells, and the HUT-78 cell line (provided by A. Gazdar, Bethesda) (*8*), which lacks expression of HTLV (*11*). The cells were fixed in acetone and then assayed by standard indirect immunofluorescence procedures; heated (56°C, 30 minutes) sera from AIDS patients and other patients and controls were used at an initial dilution of 1:10. Antibodies to LAV were detected with fixed cells obtained from Barré-Sinoussi. About 10 percent of these lymphocytes were infected with LAV. Antibodies to ARV were detected with an HUT-78 line in which 40 percent of the cells were infected with ARV-2. Most sera were tested as coded samples. The data are the number of individuals showing positive results as a fraction of the number of individuals tested, with the corresponding percentages given in parentheses.

Subjects	HTLV-I	LAV	ARV
Patients with diagnosis of			
AIDS with Kaposi's sarcoma	7/55 (13)	20/38 (53)	59/67* (88)
AIDS with opportunistic infection	1/5 (20)	2/2 (100)	19/19 (100)
Lymphadenopathy syndrome	1/13 (8)	1/4 (25)	22/27* (81)
Other individuals			
Male sexual partners of AIDS patients	1/19 (5)	7/18 (39)	13/14 (93)
Clinically healthy homosexual men	1/13 (8)	1/4 (25)	27/47* (57)
Clinically healthy heterosexual individuals	0/12 (0)	0/9 (0)	0/56* (0)

*When antibody negative–sera were tested at a 1:5 dilution against the ARV-infected cells, all eight AIDS patients (giving 67 of 67, or 100 percent positive), three of five lymphadenopathy syndrome patients (giving 25 of 27, or 92 percent positive), and 3 of 20 healthy homosexual controls (giving 30 of 47, or 64 percent positive) showed reactivity. None of the sera from healthy heterosexual individuals was positive at this dilution.

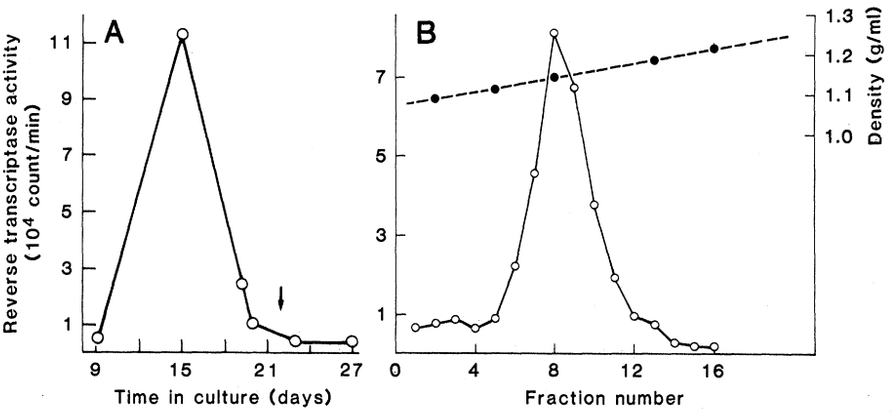

Fig. 1. (A) Kinetics of reverse transcriptase activity detected in peripheral mononuclear cell (PMC) cultures as illustrated by a representative culture. Culture medium (1 to 5 ml) from PMC was centrifuged (Beckman SW41 rotor; 40,000 rev/min, 4°C, 45 minutes). The pellets were assayed in 50 μl of a mixture containing 40 m*M* tris-HCl (*p*H 7.8), 60 m*M* KCl, 2.2 m*M* dithiothreitol, 10 m*M* $MgCl_2$, 0.1 percent Triton X-100, 30 μCi of [^{3}H]thymidine triphosphate (specific activity, 78 Ci/mmole), and poly(rA) · oligo(dT) (50 μg/ml) (P-L Biochemicals). Samples were incubated at 0°C for 15 minutes; the reaction was then run for 1 hour at 37°C and stopped with 4 ml of a mixture of 5 percent trichloroacetic acid, 0.005*M* sodium pyrophosphate, and 0.5*N* HCl. Precipitates were collected on filters (Whatman GF/A), washed, dried, and counted in a liquid scintillation counter (LKB). A high level of reverse transcriptase activity was seen in the culture on day 15. On day 22 (arrow), when the reverse transcriptase activity was low, the supernatant fluid was removed and inoculated onto fresh human PMC stimulated 3 days before with phytohemagglutinin. Supernatants from this culture, within 6 days, contained reverse transcriptase activity at levels of 650,000 cpm/ml and yielded the virus isolate ARV-2. (B) Density of retrovirus particles. Supernatant from a culture of ARV-infected PMC was concentrated by centrifugation (Beckman SW55 rotor; 45,000 rev/min, 4°C, 30 minutes). The pellet was suspended in 100 μl of a mixture containing 10 m*M* tris-HCl (*p*H 8.0), 100 m*M* NaCl, and 1 m*M* EDTA (*p*H 8.0) and layered on a 20 to 60 percent (by weight) sucrose gradient in the same mixture, and centrifuged (Beckman SW55 rotor; 35,000 rev/min, 4°C, 16 hours). Fractions (200 μl) were collected from the top and assayed for reverse transcriptase activity. Density of sucrose was determined by refractive index measurements.

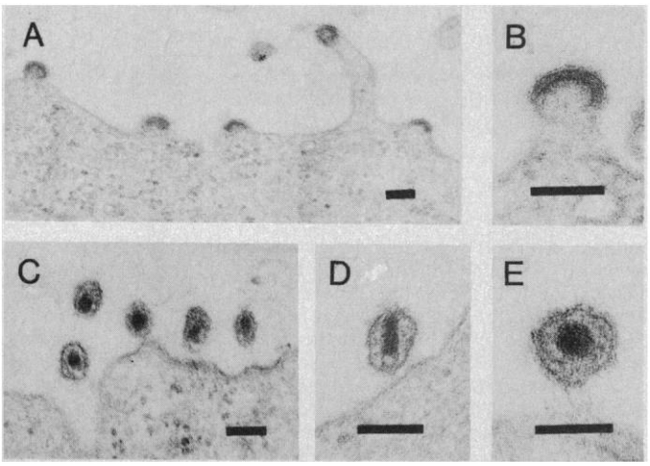

Fig. 2. Transmission electron microscopy of thin sections of peripheral mononuclear cells producing ARV. Cells were fixed in glutaraldehyde, washed, postfixed in osmium tetroxide, and embedded in Araldite. Thin sections were stained in uranyl acetate and lead citrate. (A and B) Budding forms typical of type C and D particles; (C to E) complete extracellular forms of type D morphology. Scale bar, 100 nm.

did not immortalize the cells. Particles with characteristic type D retrovirus morphology were detected by electron microscopy (Fig. 2). Budding forms showed early features of both type C and type D particles, but only mature type D particles were observed in the cultures.

When antibodies to HTLV-I and LAV were used to detect viral antigens in the cultures infected with these seven isolates (see legend to Table 2), only the antibody to LAV (serum from patient B.R.U.) reacted with cells; up to 20 percent of the cells in these cultures had LAV-related antigens. Sera from AIDS patients in San Francisco reacted with the infected cells in a similar manner (see below).

In attempts to establish ARV in continuous culture, we infected human T cell lines in the presence of antiserum to interferon and Polybrene. The MOLT-4 and CCRF-CEM lines could not be successfully infected, but in the HUT-78 line (*8*) virus was replicated in substantial titer. This cell line has been useful for the continuous propagation of ARV and the detection of antibodies against virus-infected cells.

A HUT-78 line infected for several weeks with ARV-2 was used to look for antibodies to ARV in sera from patients with AIDS or lymphadenopathy syndrome and in sera from other individuals. The sera were also examined for antibodies to HTLV-I and LAV (Table 2). The results indicated a high prevalence of antibodies to ARV in sera from patients with AIDS and lymphadenopathy syndrome and in sera from steady sexual partners of AIDS patients and healthy homosexual men. When the antibody-negative sera from patients with AIDS and lymphadenopathy syndrome were tested at a lower dilution, all sera from AIDS patients and all but two sera from lymphadenopathy patients reacted with the ARV-infected cells (footnote to Table 2). No antibodies were detected in the sera of healthy heterosexual individuals from San Francisco. All available sera of individuals from whom ARV was isolated had antibodies to the virus. Some patients' sera titered at 1:640. The high frequency of these antibodies in healthy homosexuals may reflect a bias resulting from the use of some volunteers for these studies. Nevertheless, these results indicate the close association of ARV with AIDS and lymphadenopathy syndrome, the widespread presence of ARV in the homosexual community, and the detection of greater numbers of individuals with antibodies to ARV than with infectious virus. The data support the contention that lymphadenopathy syndrome is related to AIDS. The prevalence of antibodies to LAV in the patients is in agreement with data from others (*4*). The lower frequency of antibodies to LAV than of antibodies to ARV could be related to the difficulty in reading the results of the immunofluorescence tests, with only 10 percent of the cells infected. All sera that reacted with LAV also contained antibodies to ARV. The low incidence of antibodies to HTLV-I in our patients was consistent with previous reports (*5*, *6*).

These studies indicate that patients with AIDS and lymphadenopathy syndrome as well as the steady sexual partners of these patients and healthy homosexual men in the San Francisco area have retroviruses in their PMC and bone marrow (Table 1). The retroviruses studied in detail have the characteristics of LAV. Serologic examination showed a high prevalence of antibodies to ARV and LAV in randomly selected patients and healthy homosexual men in the San Francisco area. We have also detected antibodies to ARV in sera from AIDS patients living elsewhere in the world. These results are the first independent confirmation of LAV-like viruses in patients outside Europe. Our data cannot reflect a contamination of our cultures with LAV since the original French isolate was never received in our laboratory. The relation of ARV to the recently described HTLV-III is still unknown. However, the similarity of the San Francisco isolates to LAV, the same reverse transcriptase cation preference, the same morphology under electron microscopy, and cytopathic effects similar to those of HTLV-III, suggest that all three virus types belong to the same retrovirus subgroup. Although no conclusion can yet be made concerning their etiologic role in AIDS, their biologic properties and prevalence in AIDS patients certainly suggest that these retroviruses could cause this disease.

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