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Isolation of a Type D Retrovirus from B-Cell Lymphomas of a Patient with AIDS

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An atypical syncytial variant of a high-grade Burkitt's-type B-cell lymphoma from a patient with AIDS who was seropositive for human immunodeficiency virus type 1 was studied. A productive type D retrovirus infection was identified in early-passage cell lines derived from two lymphomas from this patient. Nucleotide and amino acid sequence analysis as well as immunologic reactivity indicated that the isolated virus was highly related to Mason-Pfizer monkey virus (MPMV). MPMV is an immunosuppressive type D retrovirus that causes an AIDS-like syndrome in rhesus macaques. Amplification of DNA from the patient's diagnostic bone marrow biopsy specimen by polymerase chain reaction generated the appropriate MPMV-specific fragments and indicated that the patient was infected with the MPMV-like retrovirus. In addition, the patient's serum contained antibodies which recognized type D viral *env* proteins (gp70 and gp20) and *gag* proteins (p27 and p14). Although there have been reports of human cell lines infected with type D retroviruses and of type D-reactive human sera, this is the first evidence of a type D retrovirus infection in a human confirmed by virus isolation, serum reactivity, and viral DNA identification in tumor tissue.

Patients with AIDS often show active infections by viruses other than human immunodeficiency virus (HIV), such as cytomegalovirus, herpes simplex, and herpes zoster (28, 40). Retroviruses other than HIV may also be viral cofactors, as antibodies for human T-cell lymphotropic virus type I (HTLV-I) and/or HTLV-II are occasionally found in patients with AIDS, particularly in intravenous drug abusers (19). In this article, we describe a retrovirus isolated from a patient with AIDS and lymphoma which is virtually identical to a prototype simian type D retrovirus. Simian type D retroviruses are highly prevalent in rhesus macaques in U.S. primate colonies and can cause an AIDS-like syndrome in the infected animals (16, 27). There have been several related type D serotypes isolated from rhesus monkeys in the last 10 years. The first type D isolate, however, was obtained from a breast carcinoma of a female rhesus monkey in 1970 (4). This virus isolate, Mason-Pfizer monkey virus (MPMV), when inoculated into juvenile rhesus macaques, causes a wasting disease accompanied by lymphoid depletion, thymic atrophy, and opportunistic infections (2, 14). This syndrome, simian AIDS, is caused by several different types of type D virus isolates as well as by the lentiviral simian immunodeficiency viruses (1, 5, 6, 16, 26, 27, 33). Since its initial isolation, MPMV has rarely been reisolated from macaques (2). Related type D viruses, such as simian retrovirus-1 (SRV-1), SRV-2, and D/New England, are currently associated with macaques having simian AIDS (16).

Type D retroviruses, apparently of simian origin, have never been convincingly associated with humans. Extensive serological surveys in the past failed to show evidence of human type D retrovirus infection (3, 17). However, a number of isolated reports have appeared over the last 15 years describing serological reactivity of human sera to type D virus antigens (22, 34, 35, 44, 45). In addition, a number of established human cell lines producing type D retroviruses have been described (11, 16, 24, 25, 37, 47). Several of these cell line-derived type D isolates have been physically mapped with restriction endonucleases, and at least two of them have been molecularly cloned and sequenced. One cloned isolate was closely related to SRV-1 (30), and the other was very similar to the squirrel monkey retrovirus (36). Two of the physically mapped isolates appeared to be identical to MPMV (46). It was assumed that these viruses and many of the other isolates from established lines were a result of prior laboratory contamination (30, 47).

In this article, we describe a type D retrovirus virtually identical to MPMV that was produced by two early-passage B-cell lymphoma lines established from a patient with AIDS-related lymphomas. We provide evidence that the isolated virus was not a laboratory contaminant but was derived from the original patient. Histological examination of the patient's lymphoma tissue showed that his neoplastic B cells clearly formed syncytia, unprecedented in human B-cell lymphomas but quite characteristic of type D virus infections of the Raji Burkitt's lymphoma B-cell line in vitro (5). Polymerase chain reaction (PCR) analysis of the patient's diagnostic bone marrow biopsy specimen with MPMV-specific primers indicated that MPMV DNA sequences were present in the biopsy material. Finally, the patient's serum demonstrated a strong reactivity to MPMV antigens by both immunoblot and immunoprecipitation analyses. These data are consistent with a type D retrovirus infection of the reported patient. However, the role of the virus in the disease state of this individual remains unclear.

MATERIALS AND METHODS

Patient. The patient from whom the type D retrovirus was isolated was a 32-year-old male who complained of abdominal pain, fever, and night sweats for several months prior to admission. He was admitted to M. D. Anderson Cancer Center with generalized lymphadenopathy and a diagnosis of AIDS and B-cell lymphoma of the Burkitt's type. A serum sample obtained at admission showed HIV type 1 (HIV-1) seropositivity, and a bone marrow biopsy revealed extensive

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involvement with high-grade malignant lymphoma. A combination chemotherapy regimen was begun and achieved a partial remission. In spite of this treatment, however, the patient developed B-cell lymphomas at multiple sites and died approximately 9 months after admission. Permission to perform an autopsy was denied, and no tissues could be obtained for further analysis.

Primary cells and lymphoma cell lines. Primary cells were obtained from normal donors by Ficoll-Hypaque isolation of peripheral blood lymphocytes and separated into T-cell, monocyte/macrophage, and B-cell fractions by rosetting with sheep erythrocytes, anti-CD14-coated magnetic beads, and negative selection for B cells, respectively. Immunophenotyping with a Becton-Dickinson fluorescence-activated cell sorting (FACS) analyzer and human lymphoid lineage-associated monoclonal antibodies (Becton Dickinson) were used to verify the homogeneity of the fractionated cell types.

Lymphoma biopsy specimens were obtained under sterile conditions from the bone marrow and kidney either by excisional biopsy or by fine-needle aspiration and cultured in vitro by the procedure of Ford et al. (15). Peripheral blood was obtained by antecubital venipuncture with azide-free heparinized tubes. Established cell lines were obtained from the bone marrow (RC_{BM}), kidney (RC_{KD}), and peripheral blood samples (RC_L). These cells were grown in RPMI 1640 medium with 10% heat-inactivated fetal calf serum (Hyclone) and supplemented with 10% partially purified commercial human B-cell growth factor (Cellular Products). These lines were demonstrated to be B cell in origin by immunophenotypic and genotypic analysis with multiple lineage-associated monoclonal antibodies and with Southern blot analysis of immunoglobulin heavy-chain (IgH) (J_H) gene rearrangements, respectively. Each lymphoma line was also determined to have a unique clonal origin by IgH rearrangement patterns and karyotypic analyses (15a).

Antisera. Goat antisera to total viral proteins of MPMV and p27 of MPMV were obtained from Microbiological Associates. Rabbit antiserum to MPMV gp70 was generously provided by Eric Hunter (University of Alabama, Birmingham). Preston Marx (University of California, Davis) generously donated a monoclonal antibody to SRV-1 gp20 and monkey anti-SRV-1 serum. Mouse hyperimmune sera to the RC_{BM} and RC_{KD} viral isolates were generated by multiple injections with heat-inactivated, detergent-disrupted, purified virus preparations.

Electron microscopy. Ultrastructural analysis was performed on glutaraldehyde-fixed, pelleted lymphoma cells that were embedded in Epon, thin sectioned, and stained with uranyl acetate and lead citrate. The sections were examined on a Jeol 1200 EX electron microscope.

Virus isolation. Purified virus was obtained from the kidney- and bone marrow-derived lymphoma lines in the following manner. Low-speed centrifugation (to remove cell debris) of the virus-containing medium was followed by centrifugation of the supernatant through a 50% glycerol cushion for 4 h at 17°C at 28,000 rpm in a Beckman SW28 rotor. The pelleted virus was then resuspended in TNE (10 mM Tris-HCl [pH 8.0], 100 mM NaCl, 1 mM EDTA) and layered onto a 50 to 75% glycerol gradient prior to centrifugation in an SW41 rotor at 36,000 rpm for 10 h. Gradient fractions were collected and analyzed for peak virus activity by the reverse transcriptase assay (see below).

Reverse transcriptase assay. Reverse transcriptase assays were performed by solubilizing 5 μl of gradient-purified virus (concentrated 20- to 30-fold) in 15 μl of solubilization buffer (0.5% Triton X-100, 800 mM NaCl, 50 mM Tris-HCl [pH 8.2], 1% Trasylol protease inhibitor [Mowbray Pharmaceuticals], 5% glycerol) at room temperature for 15 min, followed by the addition of 25 μl of template-dNTP buffer [0.01 OD_{260} units of either poly(rC)-oligo(dG) or poly(rA)-oligo(dT) (Pharmacia), 10 mM $MgCl_2$, 2 μCi of [^{3}H]dTTP or [^{3}H]dGTP, 50 mM Tris-HCl (pH 8.2)] and subsequent incubation of this mixture for 1 h at 37°C. The reaction mix was absorbed onto a 10-mm^2 piece of Whatman DE-81 DEAE-cellulose paper at room temperature for 25 min. The DEAE paper was washed four times (5 min per wash) in 0.25 M $Na_2H_2PO_4$, once in distilled water, and once in 95% ethanol, air dried, and then immersed in 1.5 ml of Scintiverse II cocktail (Fisher Scientific) before radioactivity was counted on a Beckman LS3801 scintillation counter. The counts obtained in a reverse transcriptase reaction are divided by those obtained for the average of the negative controls (reaction mixes without virus) to obtain a ratio. Ratios of 3.0 or above were considered virus positive. Concentrated Rous sarcoma virus (RSV) and HIV-1 were used as positive controls to test the reaction components.

Amino acid sequencing. Glycerol gradient-purified virus from the bone marrow lymphoma line (RC_{BM}) was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The separated viral proteins were electrophoretically transferred onto Immobilon-P nylon membranes. After visualization with Coomassie blue, the 14- and 27-kDa bands were excised from the membrane, and the 20 N-terminal amino acids were sequenced, using an automated amino acid sequencer, by Richard Cook (Baylor College of Medicine amino acid sequencing core facility).

PCR. To identify the retrovirus produced by the lymphoma cell lines, reverse transcriptase-PCR (RT-PCR) experiments were performed as described previously by Donehower et al. (9) with degenerate primers derived from the conserved regions of *pol* present in all retroviruses. The patient's diagnostic bone marrow biopsy specimen was prepared for PCR by extracting the DNA from Formalin-fixed, paraffin-embedded tissue by the procedure of Wright and Manos (48). The isolated lymphoma DNA and other control DNAs were subjected to 40 cycles of PCR amplification with MPMV *env*-specific primers (5′ primer, 5′-CTCA ATGCTTCCCAACCCAGTTTAGCC-3′; 3′ primer, 5′-GG AGGCTGTAGAAACGTTATAATAG-3′). A second set of PCRs were performed with p27^{gag}-specific primers (5′ primer; 5′-GCCGCCACCATGGTCCCAGTGACTGAAACCGTT GATGGGC-3′; 3′ primer, 5′-TTACATGGCCAGGCCTTGC TG-3′ [Onasco Biotechnologies]) and 30 cycles of amplification. The reactions were performed in a thermal cycler (Perkin Elmer Cetus) with Amplitaq polymerase (Perkin Elmer Cetus) under standard conditions. The parameters of each cycle were 94°C for 15 s, 55°C for 30 s, and 72°C for 1 min. Aliquots (20 μl) of the PCR mixes were electrophoresed on a 2% agarose gel and transferred to a Zetaprobe nylon membrane (Bio-Rad) in 0.4 M NaOH by the protocol of Reed and Mann (39). The membranes were hybridized to a ^{32}P-radiolabeled MPMV *env* DNA fragment or a labeled p27^{gag} fragment generated by the technique of Feinberg and Vogelstein (10). Hybridizations were carried out at 68°C as described previously (39). After hybridization, the filters were washed several times with 0.1× SSC–1% SDS at 50°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

Immunoblot analysis. Immunoblotting was performed essentially as described by Harlow and Lane (20). Glycerol gradient-purified virus derived from the bone marrow-derived lymphoma cell line was disrupted in 4% SDS–4% 2-mercaptoethanol, heated to 100°C in sample buffer, and

electrophoresed on a 12% polyacrylamide–SDS gel. Proteins were electrophoretically transferred to a nitrocellulose membrane. The membrane was rinsed with phosphate-buffered saline, blocked with 5% nonfat dry milk (agitation for 2 h at room temperature) or Megga-Block IIb (Onasco Biotechnologies), and cut into strips. Individual strips were incubated with various anti-MPMV monoclonal antibodies and polyclonal sera or with human sera from the studied patient, other patients with AIDS, or healthy individuals. Bound antibodies were detected by an alkaline phosphatase-labeled secondary antibody with BCIP-NBT (5-bromo-4-chloro-3-indolylphosphate toluidinium–nitroblue tetrazolium) substrates (Promega Biotec Protoblot System). For the immunoblots shown in Fig. 5, the secondary antibody used was a nonconjugated goat anti-human immunoglobulin antibody. In addition, the immunoblots were incubated with an alkaline phosphatase-conjugated swine anti-goat immunoglobulin tertiary antibody.

Radioimmunoprecipitation. An in vitro transcription-translation system was used to generate ^{35}S-labeled MPMV major core protein as a substrate for immunoprecipitation assays with selected sera. A plasmid expression vector, pSP73 (Promega Biotec), was engineered so that it expressed the MPMV major core protein, p27, from its T7 promoter. An MPMV plasmid clone, generously supplied by Eric Hunter, was subjected to PCR with primers derived from the 5′ and 3′ ends of the p27 coding region. The 5′ primer had an additional Met codon preceded by a standard eucaryotic translation initiation motif (29) (5′ primer, 5′-GCCGCCACCATGGTC CCAGTGACTGAAACCGTTGATGGGC-3′; 3′ primer, 5′-TTACATGGCCAGGCCTTGCTG-3′ [Onasco Biotechnologies]). The resulting PCR fragment was inserted into pSP73 at the *Sma*I site. In vitro transcription of the recombinant plasmid was done with T7 RNA polymerase (Promega Biotec), and in vitro translation was done with a wheat germ extract (Promega Biotec) and [^{35}S]methionine to yield a ^{35}S-labeled p27. The p27 was further purified by electrophoresis on a 12% polyacrylamide–SDS gel, followed by excision of the p27 protein from the gel and electroelution of the protein. The isolated protein was resuspended in 50 mM Tris-HCl (pH 8.0)–100 mM NaCl–1% Nonidet P-40–1% Trasylol protease inhibitor, and aliquots were incubated with the appropriate antiserum. After overnight incubation at 4°C, the antigen-antibody complexes were precipitated with a 1:1 mixture of Omnisorb and Pansorbin (Calbiochem) for 1 h at 4°C, rinsed four times with the resuspension buffer, heated to 90°C in Laemmli buffer (20), and electrophoresed on a 12% polyacrylamide–SDS gel. The gel was fixed, treated with Amplify scintillation fluor (Amersham), dried, and autoradiographed.

RESULTS

Detection of a retrovirus in an AIDS-related B-cell lymphoma. Recently, an HIV-1-seropositive patient with AIDS was admitted to the University of Texas M. D. Anderson Cancer Center after presenting with high-grade B-cell lymphoma of the Burkitt's (small noncleaved cell) type. The lymphoma tissues displayed marked syncytium formation at diagnosis (Fig. 1A, B, and C). Multinucleated giant cells were interspersed among the usual small noncleaved cells characteristic of Burkitt's lymphoma. The presence of syncytia in these tumors in vivo was a distinctly unusual and previously unreported pathologic phenomenon. Our initial hypothesis was that an HIV-1 with altered biological properties had infected the lymphoma cells and caused the observed syncytium formation. In order to isolate a potentially novel HIV variant, two cell lines derived from separate B-cell tumors (bone marrow and kidney) and a lymphoblastoid B-cell line from peripheral blood lymphocytes of this patient were established in culture and were designated RC_{BM}, RC_{KD}, and RC_{L}, respectively. Both Southern blot analysis for immunoglobulin (J_H) and T-cell receptor genes and FACS analysis for B-cell and T-cell markers demonstrated these cells to be of B-cell lineage. In addition, cytogenetic analysis of each tumor cell line and analysis of immunoglobulin gene rearrangement suggested that the two lines were of different clonal origin, as they exhibited different chromosome numbers and J_H immunoglobulin rearrangement patterns.

Electron microscopic examination of the cell lines RC_{BM} and RC_{KD} revealed intracellular retroviral particles. Further microscopic examination of the virus-containing medium indicated that large numbers of retroviruses (greater than 10^8 particles per ml of medium) were released by these two lines. The particles in the infected cells were approximately 110 to 120 nm in diameter, and many appeared to be present in intracellular vesicles as well as in extracellular spaces (Fig. 1D). The presence of intracellular viral particles and the barrel-shaped core morphology sometimes observed in the mature extracellular virions are characteristic of type D retroviruses rather than lentiviruses or type C retroviruses (11).

Reverse transcriptase assays were performed on gradient fractions of virus purified from the RC_{BM} and RC_{KD} cell lines. Peak enzymatic activity was observed at a gradient density of 1.17 g/ml, indicating that typical retroviral particles were present (43). The viral reverse transcriptase from purified virions displayed a cation preference for magnesium over manganese, typical of type D retroviruses (11). The reverse transcriptase also had significantly greater activity in the presence of a poly(rC)-oligo(dG) template-primer combination than with the standard poly(rA)-oligo(dT) template-primer (Table 1). These reverse transcriptase activities are in marked contrast to those of HIV-1 and RSV, both of which demonstrate a poly(rA)-oligo(dT) template-primer preference. However, this substrate preference is in agreement with a previous report (21) which showed that MPMV reverse transcriptase had higher activity in the presence of poly(rC)-oligo(dG). None of the retroviruses showed significant activity with a poly(dA)-oligo(dT) template, indicating that contamination by cellular DNA polymerases was not a problem (data not shown).

The ability of the new viral isolates to infect B cells, T cells, and monocyte/macrophages was compared with that of HIV-1. Successful infection was measured by reverse transcriptase assays at 5 days postinfection (Table 2). The virus isolates from cell lines RC_{BM} and RC_{KD} infected both primary human peripheral blood B cells and T cells efficiently, whereas HIV-1 infected only T cells. Neither the isolates nor HIV-1 appeared to productively infect the monocyte/macrophage cells by this assay. During the course of infection of primary B cells with the viral isolates, we noted the appearance of syncytia within about 7 to 10 days postinfection (Fig. 1E). Syncytia were also evident when the Raji B-cell lymphoma line was infected with the same viral isolates (Fig. 1F). Syncytium formation in Raji cells has previously been reported to be characteristic of simian type D retroviruses (5).

Confirmation that the lymphoma cell lines were not infected with HIV-1 (or other known human retroviruses) was

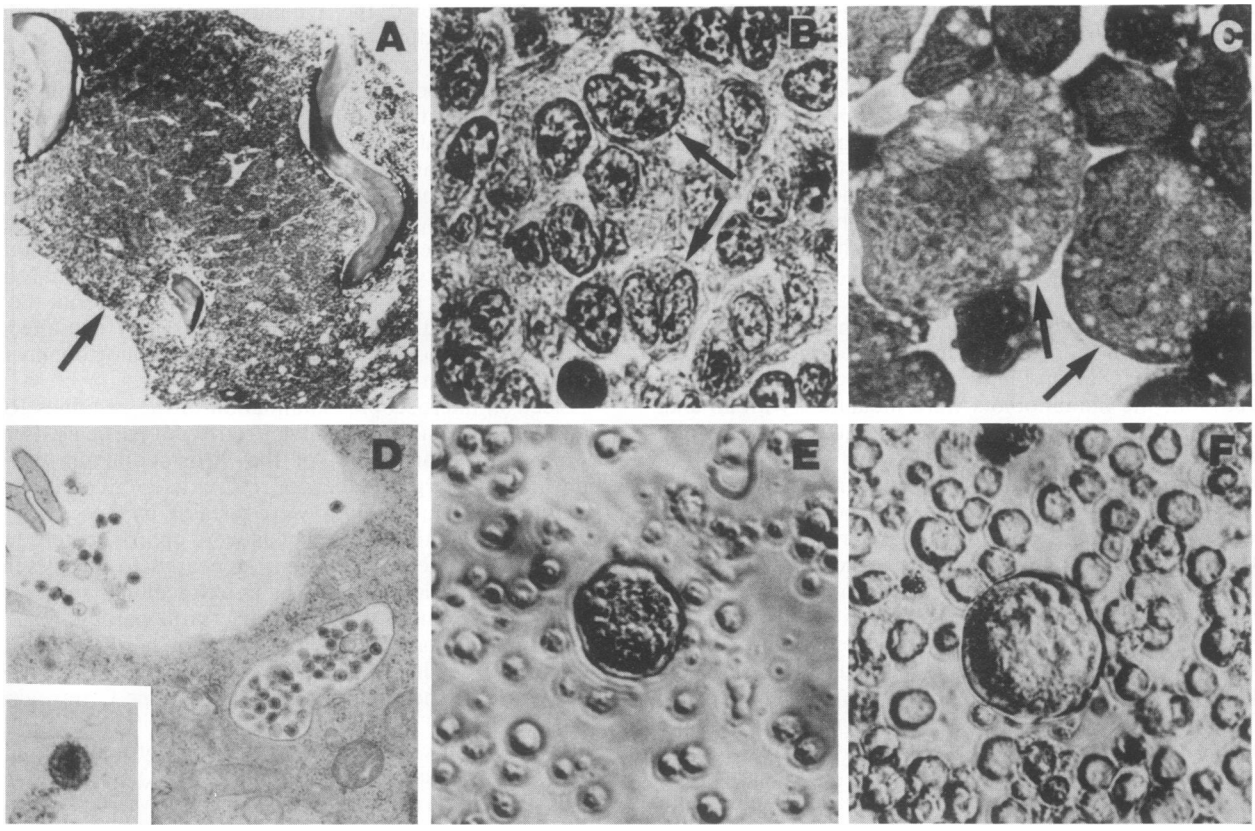


FIG. 1. Microscopic examination of diagnostic biopsy material and virus-infected cells. (A) Low-power photomicrograph of diagnostic bone marrow biopsy specimen from patient with AIDS-related lymphomas, showing replacement of normal marrow (between bony spicules) with monomorphous lymphoma cells (arrow). (B) High-power photomicrograph (×1,000) presenting detail of lymphoma cells shown in panel A. Arrow points to multinucleate syncytial variant Burkitt's lymphoma cells. (C) High-power photomicrograph (×1,000) of original aspirate of a kidney lymphoma (RC_{KD}) from our patient. Syncytia are indicated by arrows. (D) Electron micrograph (original magnification, ×20,000) of cell line RC_{BM}, established from a bone marrow lymphoma of our patient. Typical type D retrovirus particles are present in intracellular vesicles and outside the plasma membrane. Inset photo shows a fourfold enlargement of one of the retroviruses, with a barrellike core indicative of type D retroviruses. (E) Photomicrograph (×400) of a syncytium formed in normal peripheral blood B cells 10 days after infection with 0.2 μM filtered culture medium from RC_{KD} cells. (F) Photomicrograph (×400) of a syncytium formed in Raji cells 10 days after infection with filtered culture medium from RC_{KD} cells.

provided by a series of molecular analyses. Genomic DNA from the two virus-positive cell lines (RC_{BM} and RC_{KD}) failed to show evidence of HIV-1 or HTLV proviruses following Southern blot hybridization with radiolabeled HIV-1, HTLV-I, and HTLV-II probes (data not shown). PCR amplification with two different sets of HIV-1-specific primers generated no HIV-1-specific DNA fragments whereas HIV-1-infected H9 cells did exhibit the appropriately sized fragments. An anti-HIV-1 p24 monoclonal antibody failed to react with either viral isolate (from RC_{BM} or RC_{KD}) by immunoblot analysis, and an HIV-1 p24 antigen capture assay (Abbott Diagnostic) failed to detect HIV-1

TABLE 1. Reverse transcriptase template-primer preferences of selected known retroviruses and retroviruses produced by two AIDS-related lymphoma cell lines

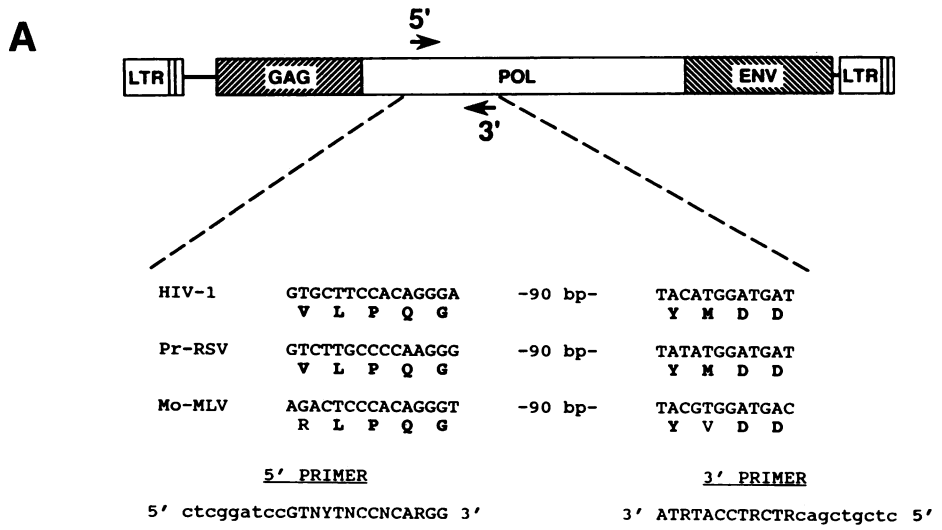
Virus	Reverse transcriptase assay (cpm in sample:cpm in negative control)[a]	
	Poly(rA)-oligo(dT)	Poly(rC)-oligo(dG)
RSV (highly concentrated)	2,635:1	270:1
HIV-1	279:1	18:1
RC_{KD} isolate	13:1	37:1
RC_{BM} isolate	5:1	14:1

[a] Results of reverse transcriptase assays are expressed as counts per minute incorporated in the experimental sample:average counts per minute of the negative-control samples (without virus). Negative-control sample values ranged from 150 to 180 cpm for the poly(rA)-oligo(dT) substrate and 300 to 400 cpm for the poly(rC)-oligo(dG) substrate. Ratios are rounded off to the nearest whole number.

TABLE 2. Infectivity of retroviruses obtained from two AIDS-related lymphoma cell lines as assayed by reverse transcriptase activity[a]

Virus	Reverse transcriptase activity (cpm in sample:cpm in negative control)		
	B cells	T cells	Macrophages
$HIV\text{-}1_{IIIB}$	2:1	196:1	1:1
RC_{BM} isolate	25:1	23:1	1:1
RC_{KD} isolate	29:1	23:1	1:1
None	1:1	1:1	1:1

[a] See Table 1, footnote *a*.



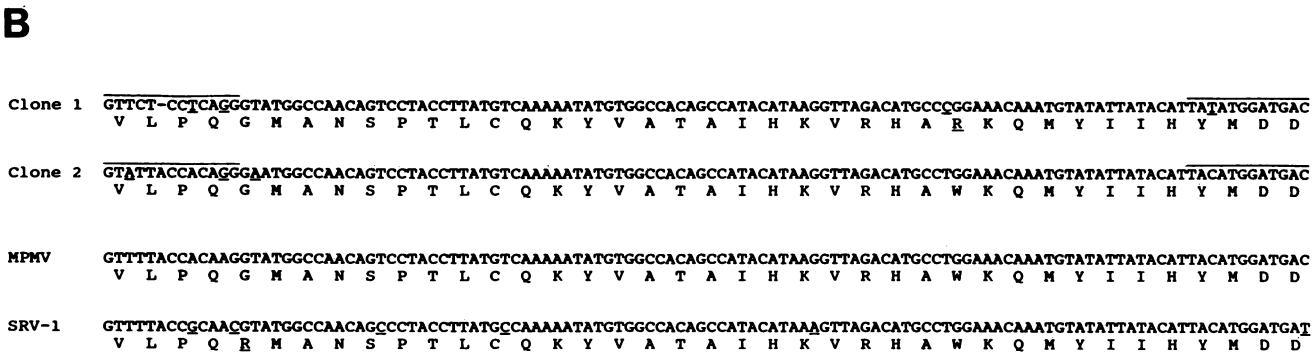


FIG. 2. Identification of the RC_{BM} retrovirus by PCR with degenerate primers from highly conserved regions of *pol*. (A) The conserved sequences of *pol* present in three widely divergent retroviruses are compared, and their approximate locations in the retrovirus genome are illustrated at the top. LTR, long terminal repeat. The 5' and 3' degenerate primers derived from these conserved sequences are indicated below the retroviral sequences. Standard single-letter abbreviations are used to designate amino acids below the nucleotide sequence. All nucleotide sequences, except for the 3' primer, are oriented in the 5' to 3' (sense) direction. Primer nucleotides in lowercase letters represent noncomplementary 5' extensions that contain recognition sequences for *Bam*HI (5') and *Sal*I (3') restriction endonucleases. N, Y, and R represent any nucleotide, pyrimidines, and purines, respectively. Pr-RSV, Prague C strain of RSV; Mo-MLV, Moloney murine leukemia virus. (B) Comparison of the two sequenced amplified fragment clones derived from the RC_{BM} virus isolate with the corresponding MPMV and SRV-1 sequences. The two sequenced clones are shown at the top, and both DNA sequences and derived amino acid sequences are indicated. The primer sequences are overlined. Nucleotide and amino acid differences with MPMV are underlined for the sequenced clones and for SRV-1.

core proteins in the isolated viral preparations (data not shown).

Identification of the AIDS-related lymphoma retrovirus. Since the viral isolates showed no similarity to known human retroviruses, we used a rapid molecular approach to identify them. A previously described PCR-based procedure (32, 41) was adapted that utilizes degenerate oligonucleotide primers derived from two regions in the *pol* gene (about 100 nucleotides apart) conserved in amino acid sequence among all retroviruses (Fig. 2A). The degenerate short primers anneal at lower temperatures to the appropriate sequences in any retroviral isolate. Initial experiments demonstrated that a specific 135-bp fragment could be amplified from four different retroviral DNAs (9). To test the new retroviral isolate, we synthesized cDNA from viral RNA purified from glycerol gradient-purified viral preparations obtained from cell line RC_{BM}. The cDNA was then amplified by PCR with the two degenerate *pol* primers. Following 40 cycles of amplification, a characteristic 135-bp fragment was obtained. The fragment was molecularly cloned, and two of the resulting clones were sequenced by the dideoxy chain termination procedure. Comparison of the sequences with sequences in the GenBank nucleotide sequence data bank revealed that the viral sequences showed a high degree of similarity to MPMV, an immunosuppressive type D retrovirus that causes an AIDS-like syndrome in rhesus monkeys (16, 27). In Fig. 2B, the alignment of the two sequenced clones with the corresponding region of MPMV and SRV-1 indicates that the clones are 96.6 and 97.4% similar to MPMV and 91.5 and 92.3% similar to SRV-1.

The identity of the viral isolate as MPMV-related was confirmed by several methods. Southern blot hybridization with MPMV DNA probes and PCR amplification with MPMV-specific primers showed that the genomic DNA of

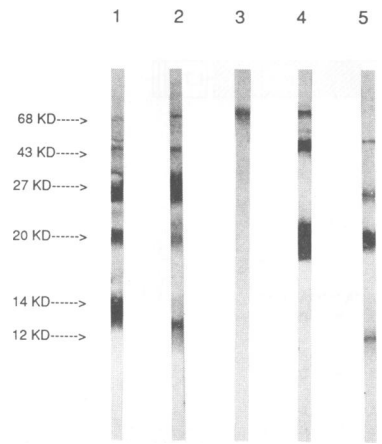


FIG. 3. Reactivity of MPMV- and SRV-1-specific antisera with the RC_{BM} viral isolate. Immunoblot strips containing viral proteins from the RC_{BM} isolate were prepared and probed with antisera. Antisera used were a 1:500 dilution of mouse hyperimmune serum to the purified RC_{BM} viral isolate (lane 1); a 1:100 dilution of goat anti-MPMV p27 hyperimmune serum (lane 2); a 1:100 dilution of rabbit anti-MPMV gp70 (*env*) hyperimmune serum (lane 3); a 1:100 dilution of a mouse monoclonal antibody to SRV-1 gp20 (lane 4); and a 1:100 dilution of pooled rhesus macaque sera from SRV-1-infected monkeys (lane 5). Sizes are shown in kilodaltons.

one of the virus producer lines contained at least three MPMV-like proviruses (data not shown). Murine antibodies were generated to the viral isolates from cell lines RC_{BM} and RC_{KD} and used to detect the viral proteins of disrupted virions (from both isolates) by immunoblot analysis (as typified in Fig. 3, lane 1). Major RC_{BM} and RC_{KD} viral protein bands at 14, 20, 27, and 70 kDa were observed, and these sizes correlate well with the known sizes of major MPMV *gag* (p14 and p27) and *env* (gp20 and gp70) proteins (8). The RC_{BM} and RC_{KD} viral proteins also reacted strongly with specific antisera to MPMV *gag* (p27) and *env* (gp70) proteins (Fig. 3, lanes 2 and 3) and with antisera to proteins of the closely related simian type D retrovirus SRV-1 (Fig. 3, lanes 4 and 5).

The 14- and 27-kDa *gag* viral proteins of the RC_{BM} isolate were purified and subjected to N-terminal amino acid sequencing. The amino-terminal 20 amino acids of the p14 of the viral isolate were identical to those for p14 of MPMV (42) and SRV-1 (38) (Table 3). Sequencing of the 20 amino-terminal residues of p27 showed a match of 19 of 20 amino acids with the MPMV and SRV-1 p27 major core proteins (Table 3).

Detection of MPMV-specific DNA sequences in bone marrow. The identification of AIDS-related lymphoma-derived viral isolates highly similar to a prototype simian type D retrovirus raised the possibility that these viruses might be laboratory contaminants. Previous investigators have observed MPMV or MPMV-related viruses in established human cell lines (18, 24, 25, 30, 36, 37, 46, 47). Although we identified the virus in early passages of the cultured lymphoma cells and our laboratories have never worked with monkeys, simian retroviruses, or known MPMV-infected human cell lines, the possibility of laboratory contamination could only be ruled out by direct demonstration of the virus in the patient from whom the lymphomas were obtained. Unfortunately, by the time the viral isolates had been identified, the patient had died of chemotherapy-resistant lymphoma, and we were limited for our analyses to a small sample of the patient's serum and a Formalin-fixed diagnostic bone marrow biopsy specimen embedded in a paraffin block. The biopsy specimen was prepared for PCR analysis by a published procedure (48). DNA extracted from this biopsy specimen was subjected to 40 cycles of PCR amplification with MPMV-specific *env* primers that could distinguish between MPMV and the closely related SRV-1. The PCR products were subjected to agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized to a labeled MPMV *env* DNA probe under stringent conditions (Fig. 4A). The patient material and positive controls generated a hybridizing band of approximately 300 bp, the expected size for an MPMV-specific fragment. PCR amplification of DNA from peripheral blood lymphocytes of six normal persons with the MPMV primers failed to generate any MPMV-specific fragments (data not shown). Repeated PCR experiments with the biopsy material (under conditions designed to avoid DNA contamination) generated the 300-bp MPMV-specific band, indicating that the lymphoma that infiltrated the patient's bone marrow was infected with this MPMV-like retrovirus. Southern blot hybridization (under stringent conditions) with the MPMV *env* probe and subsequent sequence analysis of the PCR-amplified 300-bp fragment derived from the bone marrow biopsy specimen confirmed the identity of the fragment as MPMV-like. Similar PCR analysis of the biopsy material performed with $p27^{gag}$-specific primers and 30 cycles of amplification showed that only DNA from the virus producer line RC_{BM} and the patient biopsy material generated the expected 700-bp signal (Fig. 4B).

Reactivity of serum with MPMV antigens. To provide further evidence that the patient was infected with an MPMV-like virus, a serum sample was tested for reactivity to proteins of the MPMV-like viral isolate by immunoblot analysis. The patient's serum sample strongly recognized the two *env* proteins gp70 and gp20 and the *gag* proteins p27 and p14 (and possibly p12). Serum samples from 10 patients with AIDS were negative for type D virus antibodies by this immunoblot assay (Fig. 5).

TABLE 3. Comparison of amino acid sequences of two RC_{BM} isolate proteins to $p14^{gag}$ and $p27^{gag}$ proteins of MPMV and SRV-1[a]

Protein	N-terminal amino acid sequence
$p27^{gag}$	
MPMV	NH_2-Pro-Val-Thr-Glu-Thr-Val-Asp-Gly-Gln-Gly-Gln-Ala-Trp-Arg-His-His-Asn-Gly-Phe
RC_{BM} isolate	NH_2-Pro-Val-Thr-Glu-Thr-Val-Asp-Gly-Gln-Gly-Gln-Ala-Ala-Arg-His-His-Asn-Gly-Phe
$p14^{gag}$	
MPMV	NH_2-Ala-Ala-Ala-Phe-Ser-Gly-Gln-Thr-Val-Lys-Asp-Phe-Leu-Asn-Asn-Lys-Asn-Lys-Glu
RC_{BM} isolate	NH_2-Ala-Ala-Ala-Phe-Ser-Gly-Gln-Thr-Val-Lys-Asp-Phe-Leu-Asn-Asn-Lys-Asn-Lys-Glu

[a] Single divergent amino acid in RC_{BM} viral isolate p27 is indicated by underlining.

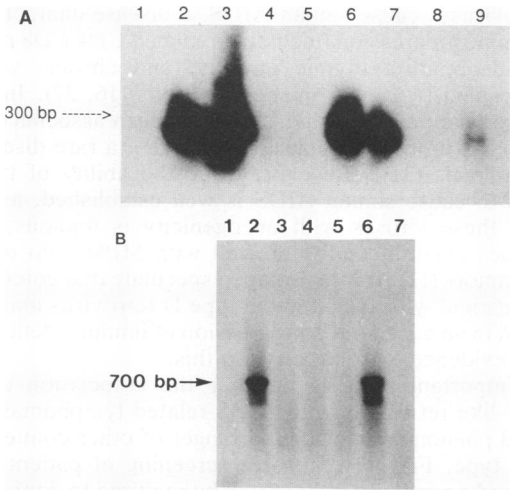


FIG. 4. Southern blot hybridization of PCR-amplified fragments from the diagnostic bone marrow biopsy specimen. (A) PCR analysis with *env* primers. The DNA samples that were amplified and tested were the amplification mixture without any template (lane 1); 5 pg of MPMV plasmid (lane 2); 1 μg of cell line RC_{BM} chromosomal DNA (lane 3); 1 μg of chromosomal DNA from H9 cells infected with HIV-1 (lane 4); 1 μg of chromosomal DNA from primary human foreskin cells (lane 5); 20 μl of chromosomal DNA extracted from the Formalin-fixed, paraffin-embedded sample of the patient's diagnostic bone marrow biopsy (lane 6); 10 μl of the same DNA as in lane 6 (lane 7); 20 μl of chromosomal DNA extracted from Formalin-fixed, paraffin-embedded human MT-4 cells (lane 8); 20 μl of chromosomal DNA extracted from a mixture of 99% MT-4 cells and 1% RC_{BM} cells that were Formalin fixed and paraffin embedded (lane 9). (B) PCR analysis with $p27^{gag}$ primers. The amplified samples were derived from the master mix minus template (lane 1), patient biopsy material (lane 2), H9 cells (lane 3), H9 cells infected with HIV ($HTLV\text{-}III_B$) (lane 4), human foreskin (lane 5), RC_{BM} virus producer cell line (template DNA concentrated fourfold) (lane 6), and MT-4 cells (lane 7).

In addition to our own immunoblot analyses, we sent the patient serum and other normal human serum samples to Nicholas Lerche at the California Regional Primate Research Center for independent verification of type D retrovirus antibodies. The serum from our patient showed antibodies to type D viral antigens gp70, p27, and gp20 in immunoblot assays, confirming our data.

To further establish that our patient had specific MPMV-reactive antibodies, we performed immunoprecipitation analyses with a labeled purified MPMV *gag* antigen, p27. The MPMV p27 protein was derived from a plasmid clone of MPMV generously provided by Eric Hunter. The p27-encoding region of MPMV was precisely obtained by PCR amplification with primers derived from the 5′ and 3′ ends of p27. A methionine codon and translational initiation signal were added to the 5′ primer, and a terminator codon was added to the 3′ primer. The PCR-amplified p27 fragment was inserted into the vector pSP73, which was designed for T7 in vitro transcription analyses. The resulting recombinant plasmid was in vitro transcribed with T7 RNA polymerase, and then the transcripts were in vitro translated to generate a ^{35}S-labeled p27 protein. This protein was gel purified and immunoprecipitated with the patient's serum, positive control anti-MPMV serum, and seven normal human sera. The results, shown in Fig. 6, demonstrate that only the patient's serum and anti-MPMV serum strongly reacted with purified p27, while normal sera failed to react at all with this antigen.

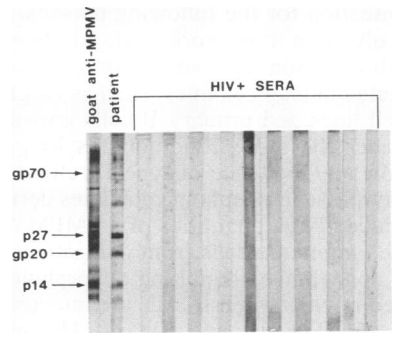


FIG. 5. Reactivity of patient serum with the MPMV-like retroviral proteins derived from the RC_{BM} lymphoma cell line. Immunoblot strips containing viral proteins from the RC_{BM} isolate were prepared and probed with antisera. The sera tested were a 1:500 dilution of goat anti-MPMV (whole virus) serum (lane 1); a 1:100 dilution of the patient's serum (lane 2); and a 1:100 dilution of 10 sera from patients with AIDS (lanes 3 through 12). All Western immunoblot strips except the positive control in lane 1 were incubated with a primary human serum, then with secondary goat anti-human immunoglobulins (IgG, IgM, and IgA), and finally with tertiary alkaline phosphatase-conjugated swine anti-goat IgG antibody. The lane 1 Western strip was incubated with the primary goat anti-MPMV antiserum and a secondary alkaline phosphatase-conjugated swine anti-goat IgG antibody.

DISCUSSION

The discovery of a simian type D retrovirus in two lymphomas from a patient with AIDS was unexpected. Since the discovery of MPMV in a rhesus macaque mammary adenocarcinoma in 1970 (4), attempts have been made to link this virus or closely related simian type D viruses to humans. The results have been inconclusive. A number of established human cell lines were found to harbor MPMV or other type D viruses (11, 18, 24, 25, 30, 36, 37, 46, 47), but these infections were usually ascribed to laboratory contaminations (30, 47). Other investigators have described MPMV-reactive antisera from normal humans (22, 35, 45) and those with AIDS-related or non-AIDS-related lymphadenopathy (34). However, there has been no conclusive evidence for active type D retrovirus infections in humans. We believe that the individual described in this report had a type D

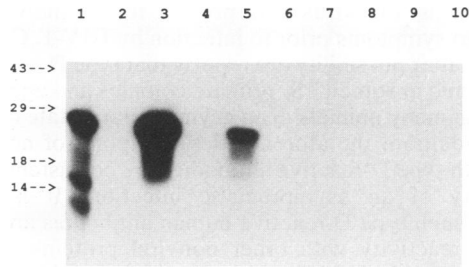


FIG. 6. Immunoprecipitation of purified ^{35}S-labeled MPMV p27 by normal sera and our patient's serum. A 3-μl amount of each serum was used. Lane 1 contains 1 μl of in vitro translation mix containing ^{35}S-labeled MPMV p27. Sera assayed for immunoprecipitation were normal human serum 1 (lane 2); goat anti-MPMV p27 (lane 3); normal human serum 2 (lane 4); our patient's serum (lane 5); and normal human sera 3 through 7 (lanes 6 through 10, respectively). Sizes are shown in kilodaltons.

retrovirus infection for the following reasons. (i) The laboratories involved in this work had not been previously working with any monkeys, simian retroviruses, or known MPMV-infected human cell lines, such as HeLa cells. Other lymphoid cell lines and primary B cells grown at the same time and under the same conditions as RC_{KD} and RC_{BM} failed to show any evidence of type D retrovirus infection. (ii) Two early-passage lymphoma cell lines derived from our patient produced large quantities of an MPMV-like retrovirus. (iii) The lymphoma cells from our patient were clearly syncytial in appearance, exhibiting a morphology similar to that observed in vitro when B cells are infected with type D retroviruses (5) (Fig. 1E and F). (iv) The patient's bone marrow biopsy specimen was repeatedly positive for an MPMV-like provirus by PCR analysis. (v) The patient's serum had a strong reactivity to the major structural proteins of the MPMV-like isolate by immunoblot analysis and also reacted strongly with purified MPMV p27 in an immunoprecipitation assay.

While we regard the above evidence for a human type D viral infection to be relatively strong, there are caveats which must be raised with respect to the data. For example, the PCR data which we gathered must be considered in light of the fact that PCR is notorious for generating false-positive results. While we took every precaution to minimize the possibility of contamination in our PCR experiments, false-positive results cannot be absolutely ruled out. In addition, the failure to observe contaminating type D virus in our other lymphocyte cultures does not preclude its contamination of the patient's cells in vitro. However, assuming that this patient was infected with the type D virus, we are presented with a dilemma. What could be the possible explanation for such an unusual retroviral infection? The unprecedented nature of this observation and the fact that this virus has been frequently associated with laboratory contaminations indicates that our results will be received with some skepticism, so that confirmation of type D virus infection in other individuals will be required to eliminate the uncertainty engendered by a single human infection event. Verification of type D virus infection in other patients is being vigorously pursued by our laboratories.

As far as can be determined, our patient had no contact with monkeys. However, since this individual was severely immunocompromised by infection with HIV-1, it is possible that he acquired this type D retrovirus under conditions in which an individual with a normal immune system would resist such an infection. Alternative possibilities are that (i) the virus was transmitted to the patient simultaneously with HIV-1 or (ii) the virus was present in the individual but caused no symptoms prior to infection by HIV-1. Consistent with the latter possibility are reports that type D retroviruses are endemic in some U.S. primate colonies and appear to be present in many animals in an asymptomatic state (7, 12, 13, 17). In addition, the aforementioned reports of normal humans with type D-reactive antibodies are consistent with the possibility of an asymptomatic infection. It is unclear whether such type D-reactive human antibodies are a result of cross-reactivity with other nonviral proteins, technical artifacts, or real viral infections. It should also be stated that findings of this type are disputed by major serological screens reported in 1977, which failed to find evidence of type D retrovirus-reactive antibodies in human populations (3, 17).

It is not known whether the MPMV-like retrovirus isolate played a role in the pathogenesis of the patient's depressed immune state. In rhesus macaques, MPMV and related type D retroviruses cause simian AIDS, a disease characterized by immunosuppression (including reduced CD4/CD8 ratios), lymphadenopathy, thymic atrophy, and chronic wasting accompanied by opportunistic infections (16, 27). In addition, type D primate retroviruses have been associated with lymphomas in infected macaques (23, 31), a rare disease in these animals (31). However, while the ability of type D viruses to cause simian AIDS is well established, a direct link of these viruses with oncogenicity is tenuous, since macaques experimentally infected with MPMV do not develop tumors (11). It is tempting to speculate that coinfection of our patient with HIV-1 and a type D retrovirus may have resulted in an accelerated progression of immune deficiency, but no evidence exists to confirm this.

An important issue is whether the association of this MPMV-like retrovirus with AIDS-related lymphomas is an isolated phenomenon or the harbinger of other coinfections of this type. Further extensive screening of patients with AIDS and normal individuals will be required to confirm the validity of this observation and to ascertain whether type D retroviruses have actually entered the human population in significant numbers.

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REFERENCES

1. **Benveniste, R. E., L. O. Arthur, C. C. Tsai, R. Sowder, T. D. Copeland, L. E. Henderson, and S. Oroszlan.** 1986. Isolation of a lentivirus from a macaque with lymphoma: comparison with HTLV-III/LAV and other lentiviruses. J. Virol. **60**:483–490.
2. **Bryant, M. L., M. B. Gardner, P. A. Marx, D. H. Maul, N. W. Lerche, K. G. Osborn, L. J. Lowenstine, A. Bodgen, L. O. Arthur, and E. Hunter.** 1986. Immunodeficiency in rhesus monkeys associated with the original Mason-Pfizer monkey virus. J. Natl. Cancer Inst. **77**:957–965.
3. **Charman, H. P., R. Rahman, M. H. White, N. Kim, and R. V. Gilden.** 1977. Radioimmunoassay for the major structural protein of Mason-Pfizer monkey virus: attempts to detect the presence of antigen or antibody in humans. Int. J. Cancer. **19**:498–504.
4. **Chopra, H. C., and M. M. Mason.** 1970. A new virus in a spontaneous mammary tumor of a rhesus monkey. Cancer Res. **30**:2081–2086.
5. **Daniel, M. D., N. W. King, N. L. Letvin, R. D. Hunt, P. K. Sehgal, and R. C. Desrosiers.** 1984. A new type D retrovirus isolated from macaques with an immunodeficiency syndrome. Science **223**:602–605.
6. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers.** 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science **228**:1201–1204.
7. **Daniel, M. D., N. L. Letvin, P. K. Sehgal, D. K. Schmidt, D. P. Silva, K. R. Solomon, F. S. Hodi, D. J. Ringler, R. D. Hunt, N. W. King, and R. D. Desrosiers.** 1988. Prevalence of antibod-

ies to 3 retroviruses in a captive colony of macaque monkeys. Int. J. Cancer **41**:601–608.
8. **Dickson, C., R. Eisenman, H. Fan, E. Hunter, and N. Teich.** 1982. Protein biosynthesis and assembly, p. 513–648. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Donehower, L. A., R. C. Bohannon, R. J. Ford, and R. A. Gibbs.** 1990. The use of primers from highly conserved *pol* regions to identify uncharacterized retroviruses by the polymerase chain reaction. J. Virol. Methods **28**:33–46.
10. **Feinberg, A., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
11. **Fine, D., and G. Schochetman.** 1978. Type D retroviruses: a review. Cancer Res. **38**:3123–3139.
12. **Fine, D. L., and L. O. Arthur.** 1981. Expression of natural antibodies against endogenous and horizontally transmitted macaque retroviruses in captive primates. Virology **112**:49–61.
13. **Fine, D. L., S. G. Devare, L. O. Arthur, H. P. Charman, and J. R. Stephenson.** 1978. Type D retroviruses: occurrence of natural antibodies to Mason-Pfizer monkey virus in rhesus monkeys. Virology **86**:567–571.
14. **Fine, D. L., J. C. Landon, R. J. Pienta, M. T. Kubicek, M. J. Valerio, W. F. Loeb, and H. C. Chopra.** 1975. Responses of infant rhesus monkeys to inoculation with Mason-Pfizer monkey virus materials. J. Natl. Cancer Inst. **54**:651–658.
15. **Ford, R. J., A. Goodacre, I. Ramirez, S. R. Mehta, and F. Cabanillas.** 1990. Establishment and characterization of human B-cell lymphoma cell lines using B-cell growth factor. Blood **75**:1311–1318.
15a. **Ford et al.** Submitted for publication.
16. **Gardner, M. B., P. Luciw, N. Lerche, and P. Marx.** 1988. Nonhuman primate retrovirus isolates and AIDS. Adv. Vet. Sci. Comp. Med. **32**:171–226.
17. **Gardner, M. B., S. Rasheed, S. Shimizu, R. W. Rongey, B. E. Henderson, R. W. McAllister, V. Klement, H. P. Charman, R. V. Gilden, R. L. Heberling, and R. J. Huebner.** 1977. Search for RNA tumor viruses in humans. Cold Spring Harbor Conf. Cell Proliferation **4**:1235–1251.
18. **Gelderblom, H., H. Bauer, H. Ogura, R. Wigand, and A. B. Fischer.** 1974. Oncornavirus-like particles in HeLa cells. I. Fine structure and comparative morphological classification. Int. J. Cancer **13**:246–253.
19. **Hall, W. W., M. H. Kaplan, S. Z. Salahuddin, N. Oyaizu, G. Curado, M. Coronesi, K. Nagashima, and R. C. Gallo.** 1990. Concomitant infections with human T-cell leukemia viruses (HTLVs) and human immunodeficiency virus (HIV): identification of HTLV-II infection in intravenous drug abusers (IVDAs), p. 115–128. *In* W. Blattner (ed.), Human retrovirology: HTLV. Raven Press, New York.
20. **Harlow, E., and D. Lane.** 1988. Immunoblotting, p. 471–510. *In* Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Heberling, R. L., S. T. Barker, S. S. Kalter, G. C. Smith, and R. J. Helmke.** 1977. Oncornavirus: isolation from a squirrel monkey (*Saimiri sciureus*) lung culture. Science **195**:289–292.
22. **Hunsmann, G., R. M. Flugel, and R. Walder.** 1990. Retroviral antibodies in Indians. Nature (London) **345**:120.
23. **Hunt, R. D., B. J. Blake, L. V. Chalifoux, P. K. Sehgal, N. W. King, and N. L. Letvin.** 1983. Transmission of naturally occurring lymphoma in macaque monkeys. Proc. Natl. Acad. Sci. USA **80**:5085–5089.
24. **Ilyin, K. V., A. F. Bykovsky, and V. M. Zhdanov.** 1973. An oncornavirus isolated from human cancer cell line. Cancer **32**:89–96.
25. **Irlin, I. S., K. V. Ilyin, A. F. Bykovsky, G. G. Miller, M. Y. Volkova, T. F. Lozinsky, and V. M. Zhdanov.** 1973/1974. Common antigens in oncornaviruses from continuous human cell lines and Mason-Pfizer virus. Intervirology **2**:95–99.
26. **Kanki, P. J., J. Alroy, and M. Essex.** 1985. Isolation of T-lymphotropic retrovirus related to HTLV-III/LAV from wild-caught African green monkeys. Science **230**:951–954.
27. **King, N. W.** 1986. Simian models of acquired immunodeficiency syndrome (AIDS): a review. Vet. Pathol. **23**:345–353.
28. **Kovacs, J. A., and H. Masur.** 1988. Opportunistic infections, p. 199–226. *In* V. T. De Vita, S. Hellman, and S. A. Rosenberg (ed.), AIDS: etiology, diagnosis, treatment, and prevention, 2nd ed. J. B. Lippincott Co., Philadelphia.
29. **Kozak, M.** 1987. An analysis of 5′ non-coding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. **15**:8125–8148.
30. **Krause, H., V. Wunderlich, and W. Uckert.** 1989. Molecular cloning of a type D retrovirus from human cells (PMFV) and its homology to simian acquired immunodeficiency type D retroviruses. Virology **173**:214–222.
31. **Letvin, N. L., K. A. Eaton, W. R. Aldrich, P. K. Sehgal, B. J. Blake, S. F. Schlossman, N. W. King, and R. D. Hunt.** 1983. Acquired immunodeficiency syndrome in a colony of macaque monkeys. Proc. Natl. Acad. Sci. USA **80**:2718–2722.
32. **Mack, D., and J. Sninsky.** 1988. A sensitive method for the identification of uncharacterized viruses related to known virus groups: hepadnavirus model system. Proc. Natl. Acad. Sci. USA **85**:6977–6981.
33. **Marx, P. A., D. H. Maul, K. G. Osborn, N. W. Lerche, P. Moody, L. J. Lowenstine, R. V. Henrickson, L. O. Arthur, R. V. Gilden, M. Gravell, W. T. London, J. L. Sever, J. A. Levy, R. J. Munn, and M. B. Gardner.** 1984. Simian AIDS: isolation of a type D retrovirus and transmission of the disease. Science **223**:1083–1086.
34. **Morozov, V. A., P. O. Ilyinskii, W. A. Uckert, W. Wunderlich, and K. V. Ilyin.** 1989. Antibodies to structural and nonstructural *gag*-coded proteins of type D retroviruses in humans with lymphadenopathy and AIDS. Int. J. Tissue React. **11**:1–5.
35. **Morozov, V. A., F. Saal, A. Gessain, A. Terrinha, and J. Peries.** 1991. Antibodies to *gag* gene-coded polypeptides of Mason-Pfizer monkey virus in healthy people from Guinea Bissau. Intervirology **32**:253–257.
36. **Oda, T., S. Ikeda, S. Watanabe, M. Hatsushika, K. Akiyama, and F. Mitsunobu.** 1988. Molecular cloning, complete nucleotide sequence, and gene structure of the provirus genome of a retrovirus produced in a human lymphoblastoid cell line. Virology **167**:468–476.
37. **Popovic, M., V. S. Kalyanaraman, M. S. Reitz, and M. G. Sarngadharan.** 1982. Identification of the RPMI 8226 retrovirus and its dissemination as a significant contaminant of some widely used human and marmoset cell lines. Int. J. Cancer **30**:93–99.
38. **Power, M. D., P. A. Marx, M. L. Bryant, M. B. Gardner, P. J. Barr, and P. A. Luciw.** 1986. Nucleotide sequence of SRV-1, a type D simian acquired immune deficiency syndrome retrovirus. Science **231**:1567–1572.
39. **Reed, K. C., and D. A. Mann.** 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res. **13**:7207–7221.
40. **Selik, R. M., E. T. Starcher, and J. W. Curran.** 1987. Opportunistic diseases reported in AIDS patients: frequencies, associations, and trends. AIDS Res. **1**:175–182.
41. **Shih, A., R. Misra, and M. Rush.** 1989. Detection of multiple, novel reverse transcriptase coding sequences in human nucleic acids: relation to primate retroviruses. J. Virol. **63**:64–75.
42. **Sonigo, P., C. Barker, E. Hunter, and S. Wain-Hobson.** 1986. Nucleotide sequence of Mason-Pfizer monkey virus: an immunosuppressive D-type retrovirus. Cell **45**:375–385.
43. **Teich, N.** 1982. Taxonomy of retroviruses, p. 25–208. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Thiry, L., S. Sprecher-Goldberger, M. Bossens, J. Cogniaux-Le Clerc, and P. Vereer-Straeten.** 1978. Neutralization of Mason-Pfizer virus by sera from patients treated for renal disease. J. Gen. Virol. **41**:587–597.
45. **Thiry, L., S. Sprecher-Goldberger, M. Bossens, and F. Neuray.** 1978. Cell-mediated immune response to simian oncornavirus antigens in pregnant women. J. Natl. Cancer Inst. **60**:527–532.
46. **Uckert, W., M. Fleischhacker, and R. Kettman.** 1986. Isolation

and characterization of covalently closed circular proviral DNA molecules of several type D retroviruses isolated from human cell lines. Virology **155**:742–746.

47. **Weiss, R.** 1982. Human RNA tumor viruses, p. 1205–1281. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Wright, D. K., and M. Manos.** 1990. Sample preparation from paraffin-embedded tissues, p. 153–158. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., San Diego.