Isolation of a Lentivirus from a Macaque with Lymphoma: Comparison with HTLV-III/LAV and Other Lentiviruses

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A retrovirus has been isolated on the human T-cell line HuT 78 after cocultivation of a lymph node from a pig-tailed macaque (*Macaca nemestrina*) that had died with malignant lymphoma in 1982 at the University of Washington primate center. This isolate, designated MnIV (WPRC-1) (*M. nemestrina* immunodeficiency virus, Washington Primate Research Center) shows the characteristic morphology of a lentivirus and replicates to high titers in various lymphocyte lines of human and primate origin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified MnIV revealed multiple bands of structural proteins, including a major viral gag protein of 28 kilodaltons, that did not comigrate with the viral proteins of a human immunodeficiency virus (HIV [FRE-1]) that was also isolated on HuT 78 cells. The relatedness of MnIV to other lentiviruses (HTLV-III/LAV, EIAV, and visna) was examined in radioimmunoassays, by immunoblot techniques, and by N-terminal amino acid sequence analysis of the viral p28 gag protein. The immunoassays revealed cross-reactivity only between MnIV p28 and HTLV-III/LAV p24, and sequence analysis showed that 14 of the 24 N-terminal residues of MnIV p28 and HTLV-III/LAV p24 are identical. These results indicate that MnIV belongs to the same lentivirus family as HTLV-III/LAV but is only partially related to these human acquired immune deficiency syndrome retroviruses.

The primate colony at the University of Washington Regional Primate Research Center (RPRC) consists of approximately 1,300 macaques, primarily pig-tailed macaques (*Macaca nemestrina*). An immune deficiency syndrome associated with retroperitoneal fibromatosis (RF) has been observed in approximately 120 macaques continuously since 1976 (9, 40). Since the original isolation of a type D retrovirus (retrovirus-D/Washington [R-D/W]) from a rhesus monkey with RF (37), multiple isolates have been obtained from various species of macaques with immune deficiency disorders (3). In addition, six cases of poorly differentiated malignant lymphoma have been observed in macaques at this primate center, also beginning in 1976. We describe here the isolation of a lentivirus from one of these animals (T76321), a 6-year old male *M. nemestrina* that had died in 1982 with a poorly differentiated lymphoblastic lymphoma. Characterization of this isolate, named MnIV (WPRC-1) (*M. nemestrina* immunodeficiency virus, Washington Primate Research Center), shows it to be a lentivirus that is partially related to the HTLV-III/LAV class of lentiviruses that have been isolated from patients with acquired immune deficiency syndrome (AIDS) (2, 29).

At the New England RPRC, a retrovirus called STLIV-III has been isolated from four rhesus monkeys with lymphoma, splenomegaly, or opportunistic infections (6). Preliminary immunological characterization showed that STLIV-III is closely related to HTLV-III, and macaques that were inoculated with this virus developed an immune deficiency syndrome characterized by weight loss, opportunistic infections, and a decrease in T4+ peripheral blood lymphocytes (17, 20). A virus has also been isolated from apparently healthy African green monkeys (STLV-III AGM) that contains viral proteins that are immunologically related to and of

the same size as the major viral proteins of HTLV-III/LAV (15). The results described here show that the viral proteins of MnIV are of different molecular weights than those described for HTLV-III/LAV and that only one of these, the major gag protein, shows an immunological cross-reactivity.

MATERIALS AND METHODS

Cells and culture conditions. Indicator cell lines used to test for replication of retroviruses were A549 (22) and DBS, Cf2Th, HOS, MOLT-3, NC37, Raji, and 26CB-1, obtained from the American Type Culture Collection, Rockville, Md. Monolayer cell lines were grown in plastic tissue culture flasks with Dulbecco modified Eagle medium supplemented with 10% fetal calf serum; lymphocytes were grown in RPMI 1640 medium with 10% fetal calf serum.

Infection of host cells with viruses. Approximately 10^7 host cells were seeded 24 h before infection into 25-cm² plastic tissue culture flasks in complete medium containing 2 µg of polybrene per ml. The cells were infected for 3 h with 1.0 ml of undiluted, filtered (pore size, 0.45 µm; Millipore Corp., Bedford, Mass.) medium from virus-producing cell cultures and then cultured for 3 days in a total volume of 7 ml. Virus-containing medium was removed, and the cells were subcultured weekly. Culture fluids were assayed for reverse transcriptase activity every 3 weeks.

Reverse transcriptase assay. The presence of retroviruses was detected by assaying for reverse transcriptase activity. Culture fluid (15 ml) from a T-75 flask containing confluent monolayer cell lines or the supernatant fluid from 10^7 lymphocyte cells was clarified by centrifugation at $12,000 \times g$ for 10 min. The supernatant was then pelleted through a cushion consisting of 20% glycerol, 0.05 M Tris-hydrochloride (pH 7.8), and 0.10 M KCl at $105,000 \times g$ for 90 min. The pellet was suspended in 0.1 ml of 0.05 M Tris-hydrochloride (pH 7.8)–0.10 M NaCl– 10^{-3} M dithiothreitol–0.1% Triton

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FIG. 1. Electron microscopy (magnification, $\times 72,000$) of HuT 78 cells infected with MnIV. Infected cells were fixed in glutaraldehyde and embedded in epoxy resins. (a and b) Bud formation of virus particle at cell membrane; (c) immature, extracellular virus particle; (d and e) mature, extracellular virus particle.

X-100. A 0.01-ml portion of this virus suspension was added to a 0.04-ml reaction mixture containing 0.04 M Tris hydrochloride (pH 7.8), 0.06 M KCl, 2×10^{-3} M dithiothreitol, 0.01 M magnesium acetate, 0.8 A_{260} units of poly(rA) template-oligo(dT₁₂₋₁₈) primer (Pharmacia Fine Chemicals, Piscataway, N.J.), and 6×10^{-5} M [³H]TTP (80,000 cpm/pmol; New England Nuclear Corp., Boston, Mass.). [³H]TMP incorporation into radioactive poly(dT) was measured after a 60-min incubation at 37°C.

Virus production. MnIV (WPRC-1) and two independent isolates of HIV (FRE-1 and FRE-3) were grown in HuT 78 cells, and R-D/W was grown in a human lung adenocarcinoma cell line (A549) by Advanced Biotechnologies, Inc., Silver Spring, Md., and harvested by centrifugation. Virus was layered on a linear density gradient consisting of 15 to 60% (wt/vol) sucrose in 0.05 M Tris-hydrochloride (pH 7.8) and centrifuged at 23,000 rpm for 16 h at 4°C in an SW27 rotor (Beckman Instruments, Inc., Palo Alto, Calif.). Fractions with reverse transcriptase activity that banded at 1.15 to 1.17 g/cm³ were pooled and used as a source of virus for raising antisera in rabbits or for protein purification by reversed-phase high-pressure liquid chromatography (HPLC).

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 10 to 20% gradient gels by the method of Laemmli (19). Proteins were visualized by staining with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.).

Separation of viral proteins by reversed-phase HPLC. Purified virus was disrupted in saturated guanidine hydrochloride–10% mercaptoethanol, adjusted to pH 2 by addition of trifluoroacetic acid, and injected into a high-pressure liquid chromatograph, and the viral proteins were separated on a μ Bondapak C₁₈ column (Waters Associates, Inc., Milford, Mass.) by using an acetonitrile gradient as previously described (13).

Liquid-phase amino acid sequencing. Semiautomated microsequencing was performed with a model 890 C sequencer (Beckman Instruments, Inc., Fullerton, Calif.) after Edman degradation of 3 to 6 nmol of purified protein as previously described (12). Phenylthiohydantoin amino acid derivatives obtained at each Edman cycle were identified by HPLC (11).

RESULTS

Lymph node tissue that had been stored frozen since 1982 from a pig-tailed macaque with lymphoma (*M. nemestrina* T76321) was minced and cocultivated with various cell lines known to support the replication of primate retrovirus. Cell culture supernatant fluids were assayed periodically for magnesium- and manganese-dependent reverse transcriptase

activity. A type D retrovirus was isolated on A549, a human lung adenocarcinoma cell line; this virus was related to those previously isolated at this primate center (3, 37). In addition, after 3 weeks of cultivation, a magnesium-dependent reverse transcriptase activity was noted on HuT 78, a human T-cell line (8). This retrovirus was endpoint diluted on HuT 78 cells and examined by electron microscopy (Fig. 1). The morphology of the budding particles (Fig. 1A and B), the extracellular immature particles (Fig. 1C), the mature particles with bar-shaped nucleotides (Fig. 1D), which, in cross-sections, appeared smaller and circular (Fig. 1E), and the lack of intracytoplasmic type A particles are all features characteristic of other lentiviruses such as HTLV-III and visna (10).

This isolate was designated MnIV (WPRC-1), for *M. nemestrina* immunodeficiency virus, Washington Primate Research Center isolate, as recommended by the International Committee on the Taxonomy of Viruses (Science 232:697, 1986). The host range of MnIV was compared with that of an HTLV-III isolate (HIV [FRE-1]) obtained in our laboratory after cocultivating HuT 78 cells with peripheral blood lymphocytes from a patient with AIDS and also compared with a type D isolate (R-D/W) obtained from a *M. nemestrina* with immunodeficiency and RF at the Washington RPRC. All three viruses replicated in the continuous human T-cell line HuT 78, although the type D isolate grew to lower titers (Table 1). These results differ from those obtained with the New England RPRC type D isolate, which does not replicate in HuT 78 cells (6). The MOLT-3 human T-cell line, which has been reported to grow the human AIDS retrovirus isolate ARV (21), did not support the growth of MnIV. The MOLT-3 cell line can therefore be used to distinguish the growth of ARV and related human AIDS retroviruses from that of MnIV. R-D/W replicates well in the Raji human lymphoblast cell line, as well as in a variety of monolayer cell lines of human, macaque, and canine origin. All three viruses replicate in baboon and macaque peripheral blood lymphocytes. ARV has previously been shown to replicate poorly in these primate lymphocytes (21).

Even though very high levels of MnIV virus particles are present in tissue culture fluid samples, as indicated by reverse transcriptase activity, the number of particles seen by electron microscopy, and quantitation of viral antigen by radioimmunoassay, the infectivity of the virus as measured in cell culture was less than 0.1% of the level normally seen with other retroviruses. This low level of infectivity was substantiated by the lack of readily detectable high-molecular-weight 35S or 70S viral RNA in disrupted endpoint-diluted fresh virus preparations. The molecular basis for this unusual viral characteristic is currently under investigation.

MnIV was purified by sucrose gradient density centrifugation; the viral particles banding at 1.16 to 1.18 g/cm³ were disrupted, and the proteins were separated by SDS-PAGE. The proteins associated with purified MnIV, with an HTLV-III/LAV isolate obtained in our laboratory (HIV [FRE-3]), with equine infectious anemia virus (EIAV), and with R-D/W were compared (Fig. 2). MnIV (lane 1) contains prominent bands at approximately 28, 16, and 14 kilodaltons (kDa). These major bands are distinct from those seen with HTLV-III/LAV (lane 2), for which p24, p18, and p16 gag proteins have been described (30, 32, 41). This particular HTLV-III/LAV retroviral isolate (HIV [FRE-3]) is unusual in containing large amounts of the Pr55 gag precursor (R. Benveniste, K. Nagashima, L. Eron, and M. Gonda, manuscript in preparation). EIAV (lane 3) shows characteristic gag proteins at p26, p14, and p9 (36). The gag proteins of R-D/W have been purified, and the N-terminal sequence has been determined; lane 4 reveals the p27, p20 phosphoprotein (pp20), p14, p12, and p10 proteins that have been described (13), as well as the p22 transmembrane protein. In the low molecular mass region of the gel (less than 30 kDa), the proteins associated with MnIV had a different mobility from those of the other retroviruses (Fig. 2).

The antigenic relatedness of MnIV, HTLV-III/LAV (HIV-FRE-3), and R-D/W was compared by using Western blot techniques. Retroviral proteins were separated by SDS-PAGE, transferred to aminobenzylloxymethylcellulose paper (4), and reacted with antisera raised against viral proteins. The presence of antibodies was detected with ¹²⁵I-labeled protein A from *Staphylococcus aureus*. Figure 3 shows representative results obtained with various antisera on the proteins of MnIV (lane 1), R-D/W (lane 2), and our HIV

FIG. 2. SDS-PAGE analysis of proteins associated with purified MnIV (lane 1), HIV (FRE-3) (lane 2), the cell-adapted Wyoming strain of EIAV (lane 3) (23), and R-D/W (lane 4). Proteins were separated by electrophoresis through a 10 to 20% gradient slab gel prepared by the method of Laemmli (19). Each lane contains approximately 20 μ g of viral protein. The positions of standard molecular weight marker proteins are indicated on the right, as are the gag proteins purified from R-D/W (p10, p12, p14, pp20, and p27), as well as the transmembrane protein (p22) (13).

TABLE 1. Host range of MnIV, HIV (FRE-1), and R-D/W

Cells ^a	Origin	Supernatant polymerase activity (10 ³ cpm of [³ H]TMP incorporated at 6 wk) ^b		
		MnIV	HIV (FRE-1)	R-D/W
Lymphocytes				
HuT 78	Human T cell	3,400.0	2,600.0	465.7
MOLT-3	Human T cell	9.4	666.7	96.7
NC 37	Human lymphoblast	12.2	12.9	236.6
Raji	Human lymphoblast	10.6	12.4	374.8
26CB-1	Baboon B cell	8.2	10.2	3.3
Baboon	PBL (primary)	149.7	68.6	38.1
Macaque	PBL (primary)	334.7	84.5	45.2
Monolayer				
A549	Human lung adenocarcinoma	7.2	6.8	1,102.7
HOS	Human osteosarcoma	4.6	5.6	65.8
DBS	Rhesus fibroblasts	8.7	8.9	120.2
Cf2Th	Canine thymus	5.9	4.9	155.8

^a All cell lines except A549 (22) were obtained from the American Type Culture Collection and cultured as described in Materials and Methods.

^b 1.0 ml of filtered (pore size, 0.45 μ m) supernatant fluid from virus-producing cell lines was infected onto each of the cell lines or primary cultures listed. The source of MnIV was a HuT 78 cell line producing a biologically endpoint-diluted virus, the source of HIV (FRE-1) was a HuT 78 cell line producing an endpoint-diluted virus isolated from a patient with AIDS, and the source of R-D/W was the A549 cell line producing an endpoint-diluted isolate obtained from a pig-tailed macaque with simian AIDS and RF (3). Cells were subcultured weekly in the presence of polybrene (2 μ g/ml). Reverse transcriptase assays were performed as described in Materials and Methods; results are expressed as counts per minute of [³H]TMP incorporated into radioactive poly(dT) in a 60-min incubation at 37°C; 10³ cpm represents 1.2 pmol of TMP incorporated. The polymerase activities in the uninfected cell lines varied from 3,500 to 15,000 cpm in this assay.

(FRE-3) isolate (lane 3). The proteins present on strip A were incubated with rabbit antiserum to whole disrupted MnIV; the presence of ¹²⁵I radioactivity is noted corresponding to proteins of 28, 16, 14, and 5 kDa. This serum also cross-reacts with HIV (FRE-3) p24 and the Pr55 gag precursor that is so prominent in this human AIDS isolate (Fig. 2). The differences in the mobilities of the major gag proteins of MnIV and HIV (FRE-3) are clearly evident. No cross-reactivity is noted with any R-D/W proteins. Strip B contains protein from the same three viruses reacted with sera from patients with AIDS. Seven sera from patients with AIDS were tested; all gave essentially similar results. The HIV (FRE-3) proteins that are detected by the AIDS sera include the major gag protein p24, as well as p18, a band at 41 kDa (gp 41), and the Pr55 gag precursor. Cross-reactivity was noted only with the p28 protein of MnIV; none was observed with R-D/W proteins. Strip C was reacted with rabbit sera raised against the purified R-D/W proteins (13) shown. No cross-reactivity with MnIV or HIV (FRE-3) was observed. Similar results have also been obtained by transferring viral proteins to nitrocellulose paper (39), treating them first with the sera described above and then with peroxidase-

FIG. 3. Immunoradiographic analysis of cross-reactivities between MnIV (lane 1), R-D/W (lane 2), and HIV (FRE-3) (lane 3) all grown on HuT 78 cells. Purified virus was disrupted, and 20 μ g of protein was separated by electrophoresis through a 10 to 20% gel as described in the legend to Fig. 2. The proteins were transferred to ABM paper (Schleicher & Schuell, Inc., Keene, N.H.) and incubated with antibody, and 125 I-labeled staphylococcal protein A was added as previously described (4). (A) Rabbit antibody raised against whole, disrupted MnIV virus grown in HuT 78 cells (1:500 dilution); (B) serum from a patient with AIDS (provided by L. Eron, Infectious Diseases Physicians, Fairfax, Va.) (1:300 dilution); (C) rabbit sera raised against purified R-D/W p27, pp20, p14, p12, and p10 proteins were mixed (final dilution 1:200).

conjugated antiserum, and developing them with chloronaphthol. Taken together, these data indicate that only one of the gag proteins of HTLV-III/LAV (p24) (and its precursor Pr55) shows an immunological cross-reactivity with MnIV p28. The other viral proteins of MnIV (16 and 14 kDa) have no detectable antigenic relatedness to the HTLV-III/LAV class of viruses as determined by these immunoblot techniques.

The relatedness of the major core antigen of MnIV to the core antigen of other retroviruses was examined further by competition radioimmunoassays. Sucrose gradient-purified MnIV was lysed and tested for its ability to compete for the binding of selected antisera to iodinated purified retroviral core antigens. MnIV did not compete in assays for HTLV-I p24 (Fig. 4C), or Mason-Pfizer monkey virus (the prototype type D virus) p27 (Fig. 4B) or in a broadly specific colobus monkey type C virus (CPC-1) p28 assay which detects all mammalian type C retroviruses (Fig. 4D). The lack of competition in these assays indicates that the major gag protein of MnIV is not immunologically related to either primate type C or type D virus or to HTLV-I, the human isolate obtained from patients with certain T-cell malignancies (28). HTLV-III grown in H9 cells and HIV (FRE-1) grown in HuT-78 cells competed completely in the HTLV-III p24 assay (Fig. 4A), whereas MnIV competed only partially. The slope of the competition curve suggests that the major gag proteins of MnIV and HTLV-III are only distantly related. This partial competition was not due to low levels of MnIV antigen, since in a heterologous assay in which anti-MnIV sera was used to precipitate 125 I-labeled HTLV-III p24, MnIV gave complete competition (data not

shown). The other lentiviruses (EIAV, caprine arthritis encephalitis virus, and visna virus, a pathogenic lentivirus isolated from sheep), did not compete in any of the four immunological assays (data not shown).

To study the proteins of MnIV in greater detail, sucrose gradient-purified virus was disrupted with guanidine hydrochloride and mercaptoethanol, and the viral proteins were separated with an acetonitrile gradient by using the HPLC techniques that have previously been used to purify the structural proteins of other retroviruses (12). The details of the purification of the MnIV proteins are described elsewhere (L. Henderson, R. Sowder, T. Copeland, R. Benveniste, and S. Oroszlan, manuscript in preparation). The N-terminal amino acid sequence of the purified p28 protein of MnIV was determined by Edman degradation and compared with the N-terminal sequences of the major gag proteins of other lentiviruses (HTLV-III/LAV, EIAV, and visna virus) and the primate oncornaviruses R-D/W and the endogenous baboon isolate M7 as well as to Moloney murine type C virus (see Fig. 5). The greatest degree of sequence homology is seen between MnIV and HTLV-III/LAV, in which 14 of 24 compared positions have identical residues. Lower degrees of homology were seen with the gag proteins of the other lentiviruses, EIAV and visna virus (nine and three identities, respectively). The N-terminal amino acid sequences of the major gag proteins of type D and type C primate oncornavirus are also shown in Fig. 5; they show little amino acid sequence homology to the N-terminal region of MnIV p28. Computer-assisted comparisons of these sequences by the Dayhoff Align program (7), which takes into account homologous replacements, gave Align

FIG. 4. Immunologic relatedness of the major core protein of retroviruses. Competitive radioimmunoassay for the major gag protein of HTLV-III (A), Mason-Pfizer monkey virus (B), HTLV-I (C), and colobus type C virus (CPC-1) (D). Sucrose gradient-purified viruses were disrupted in detergent and tested for their ability to compete for the binding of 125 I-labeled viral core proteins to limiting amounts of antisera (1). (A) 125 I-labeled HTLV-III p24 versus anti-HTLV-III rabbit serum. (B) 125 I-labeled Mason-Pfizer monkey virus p27 versus anti-MPMV rabbit serum. (C) 125 I-labeled HTLV-I versus anti-HTLV-I rabbit serum. (D) 125 I-labeled colobus virus p28 versus antisera prepared in a goat by sequential inoculations of p30 core antigens purified from the Rauscher strain of murine leukemia virus and feline leukemia virus. Viruses used as competitors in the assay included MnIV (■), HTLV-III propagated in H9 (○) (29), or an isolate (HIV [FRE-1]) obtained on HuT 78 cells (●), R-D/W (△) (3, 37), HTLV-I (□) (28), a macaque type C virus (MAC-1) (▽) (38), a colobus type C virus (▲) (34), and Rauscher murine type C virus (▼) (31). Other controls (not shown) which did not compete included uninfected HuT 78 cells, EIAV, visna virus, and caprine arthritis encephalitis virus.

Lentiviruses

MnIV	P-V-Q-Q-V-G-C-N-Y-T-H-L-P-L-S-P-R-T-L-N-A-W-V
HTLV-III/LAV	P-I-V-Q-N-I-Q-G-Q-M-V-H-Q-A-I-S-P-R-T-L-N-A-W-V
EIAV	P-I-M-I-D-G-A-G-N-R-N-F-R-P-L-T-P-R-G-Y-T-T-W-V
Visna	P-I-V-N-L-Q-A-G-G-R-S-W-K-A-V-E-S-V-V-F-Q-Q-L-Q-

OncornavirusesPrimate

R-D/W	P-V-T-E-T-M-D-G-Q-G-Q-A-W-R-H-N-G-F-D-F-T-V-I-
Baboon (M7)	P-L-R-T-V-N-R-T-I-Q-Y-W-P-F-S-A-S-D-L-Y-N-W-K-T-

Murine

Moloney-MuLV	P-L-R-A-G-G-N-G-Q-L-Q-Y-W-P-F-S-S-S-D-L-Y-N-W-K-
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FIG. 5. Comparison of the N-terminal amino acid sequence of the major gag protein of MnIV (p28) to that of other lentiviruses and to two primate and one murine oncornavirus. MnIV virus was sucrose gradient purified, and the viral proteins were separated on a μ Bondapak C₁₈ column by using an acetonitrile gradient as described in Materials and Methods. The purified p28 gag protein gave a single band by SDS-PAGE and was judged greater than 95% homogeneous; the amino acid sequence was determined as described in Materials and Methods. A gap (*) was artificially introduced into the MnIV sequence to maximize homology with other compared sequences; residues that are homologous among the lentiviruses are boxed. The N-terminal sequences of the major gag protein of HTLV-III/LAV (30, 32, 41), EIAV (36), visna virus (35), the infectious macaque type D virus (R-D/W) (13), baboon type C virus, and Moloney murine type C virus (27) have been previously published.

scores of 7.8 (MnIV versus HTLV-III/LAV), 5.6 (MnIV versus EIAV), 0.4 (MnIV versus visna), 6.1 (HTLV-III/LAV versus EIAV), 1.6 (HTLV-III/LAV versus visna virus), and 3.3 (EIAV versus visna virus). Although significant, the alignment score between MnIV and HTLV-III/LAV was less than that observed between the N-terminal amino acid sequence of the baboon and murine type-C virus gag proteins (Align score, 9.2). By these comparisons, therefore, MnIV appears more closely related to HTLV-III/LAV than to the other lentiviruses, EIAV and visna.

The N-terminal amino acid sequence of the p28 protein of MnIV (WPRC-1) leaves little doubt as to its assignment as the major gag protein of the virus. The amino acid sequence comparison data further support the classification of MnIV as a member of the HTLV-III/LAV class of retroviruses, although its relationship to EIAV and visna is not clear from this sequence. The extent of homology between the major gag proteins of MnIV and HTLV-III/LAV is consistent with the immunological cross-reactivity observed in immunoblots (Fig. 3) as well as with the partial immunological competition seen in the radioimmunoassay (Fig. 4).

DISCUSSION

Primate retroviruses have been isolated that are reported as being closely related to HTLV-III/LAV. The first isolates were obtained from four rhesus monkeys at the New England RPRC (6). These isolates, called STLV-III, contain viral proteins that, by immunoprecipitation, were of the same molecular size as those seen for HTLV-III (160, 120, 55, and 24 kDa) (17). In addition, various isolates from apparently healthy African green monkeys also contain proteins that are similar to those of HTLV-III both in molecular weight and immunological relatedness (15). The results shown here appear to be distinct from those described for the STLV-III isolates from rhesus and African

green monkeys. The MnIV viral proteins differ from those of HTLV-III/LAV with respect to molecular weight, antigenic cross-reaction, and amino acid sequence. Although the data do show that MnIV is a lentivirus that is partially related to HTLV-III/LAV, it is not clear whether the degree of antigenic relatedness and the degree of amino acid sequence homology in the N-terminal region of the major gag protein (58%) are consistent with a recent infection of humans by this particular viral isolate. The recent isolation of a T-lymphotropic retrovirus that is partially related to STLV-III AGM from apparently healthy people in West Africa suggests however that humans may harbor viruses more closely related to these primate lentiviruses (16). The relatedness of MnIV to the STLV-III isolates from rhesus monkeys and African green monkeys that are more closely related to HTLV-III remains to be determined. An STLV-III virus recently isolated from mangabeys (26) appears to contain proteins that are more similar in molecular weight to those described in this report for MnIV.

Type D retroviruses have been isolated at various primate research centers (5, 24, 37). In addition, antibodies to HTLV-I have been described in macaques with lymphoma at both the New England RPRC and the primate colony in Sukhumi, USSR (14, 33). Primate retroviruses closely related to HTLV-I have been identified in macaques, African green monkeys, and chimpanzees (18, 25, 42). The primate colony at the University of Washington consists of macaques that are infected with type D retroviruses (3, 37). HTLV-I related retroviruses (R. Benveniste, manuscript in preparation), and lentiviruses (MnIV). The role that each of these viruses plays in primate disease is not clear; both a type D virus and MnIV were isolated from macaque T76321 (the animal with lymphoma). Preliminary serologic surveys for naturally occurring antibodies to MnIV indicate that baboons (*P. cynocephalus*) (also housed at the Washington RPRC), and various species of macaques are seronegative. The source of infection of the macaque from which MnIV was isolated is currently unknown; siblings and parents of that animal are also seronegative. MnIV and STLV-III have been isolated from pig-tailed macaques and rhesus monkeys, respectively (Asian Old World monkey species), with lymphoma, whereas STLV-III AGM and STLV-III/Delta were isolated from healthy African Old World monkeys (African green monkeys and mangabeys). We have inoculated both species of macaques, as well as baboons (another African primate species), with biologically endpoint diluted MnIV to study the pathogenicity of this isolate and to examine any effect of the host species on disease progression.

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