

two white-tip reef sharks, *Trienodon obesus*. When 100 mg of this material dissolved in 5 ml of sea water was injected into the mouth of the docile shark through an extension tube attached to a syringe, the shark displayed a clear escape behavior; control experiments elicited no response. This observation suggests that the pardaxins, as well as the previously described pavoninins, are shark-repelling factors of the defense secretion.

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8. Typically with an HPLC column (10 by 250 mm) of Hypersil WP-300-5C<sub>8</sub>, eluted with 30 minutes linear gradient from 36 to 72% aqueous acetonitrile with 0.1% trifluoroacetic acid at 3 ml/min, monitored by absorption at 220 nm.
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## Isolation of a New Human Retrovirus from West African Patients with AIDS

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The etiological agent of AIDS, LAV/HTLV-III, is common in Central Africa but is not endemic in other areas of that continent. A novel human retrovirus, distinct from LAV/HTLV-III, has now been isolated from two AIDS patients from West Africa. Partial characterization of this virus revealed that it has biological and morphological properties very similar to LAV but that it differs in some of its antigenic components. Although the core antigens may share some common epitopes, the West African AIDS retrovirus and LAV differ substantially in their envelope glycoproteins. The envelope antigen of the West African virus can be recognized by serum from a macaque with simian AIDS infected by the simian retrovirus termed STLTV-III<sub>mac</sub>, suggesting that the West African AIDS virus may be more closely related to this simian virus than to LAV. Hybridization experiments with LAV subgenomic probes further established that this new retrovirus, here referred to as LAV-II, is distantly related to LAV and distinct from STLTV-III<sub>mac</sub>.

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) has been etiologically linked to infection by the human retrovirus termed lymphadenopathy-AIDS-virus (LAV), which is also known as human T-lymphotropic virus type III (HTLV-III) or AIDS-related virus (ARV) (1–4). This virus is closely related by many characteristics to the lentiviruses (5, 6). Retrospective clinical and seroepidemiological data indicate a recent emergence of this viral infection in the West, where the AIDS epidemic apparently began in 1981 (7), and

it is frequently assumed that the virus was imported from other parts of the world.

Several studies have indicated that LAV infection is common and may be endemic in certain Central African countries (8–10). Although AIDS patients in these countries do not belong to the classical high-risk groups recognized in the West, transmission of the virus is likely to be the same, that is, by sexual or blood contact. Retrospective studies indicate that the AIDS virus was present in Central Africa in the 1970's, and there is serological evidence that LAV infec-

tion appeared earlier in Africa than in the West (11, 12). It has therefore been suggested that the AIDS epidemic originated in Central Africa.

Numerous LAV isolates have been obtained from African patients with AIDS or related syndromes, as well as from healthy LAV carriers. These isolates are not distinguishable from the American or European isolates by their biological and serological properties (8, 10), indicating that, despite their somewhat higher genetic variability compared to isolates in the West (13, 14), their antigenic regions are highly conserved. Some African patients with AIDS, however, show repeatedly negative tests for serum antibodies to LAV/HTLV-III. We have studied two such patients from West Africa, where the number of AIDS cases is low and where LAV infection seems not to be endemic (15). We report here the isolation, from these two patients, of a new human retrovirus (16) that is related to but distinct from both LAV/HTLV-III and the recently described simian retrovirus termed STLTV-III<sub>mac</sub>, the putative etiological agent of simian AIDS in captive macaques (17–19).

The first patient is a 29-year-old man from Guinea Bissau (adjoining the southern

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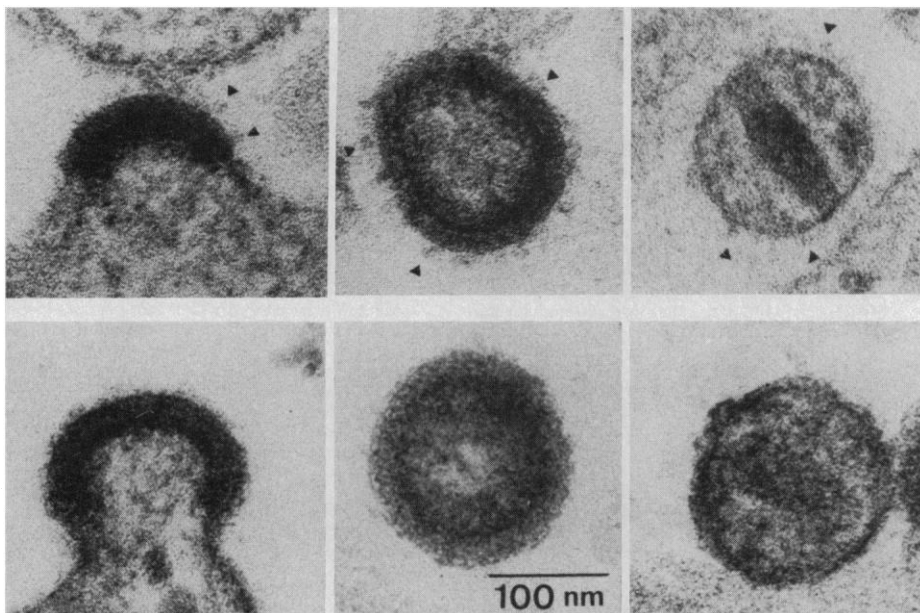


Fig. 1. Electron micrographs of ultrathin sections of lymphocytes that were obtained from a healthy donor and infected in vitro with virus isolated from patient 1 (top panel) or with LAV/HTLV-III (bottom panel). (Left) Budding particle; (center) immature particle; (right) mature virion. Arrowheads indicate the presence of surface spikes.

border of Senegal), who does not belong to any risk group for AIDS. His symptoms began in 1983, with diarrhea, weight loss, and chronic lymphadenopathy. Later on, esophageal candidiasis and digestive cryptosporidiosis developed. This patient was hospitalized in Portugal (Hospital de Egas Moniz), where the diagnosis of AIDS was proposed. The number of circulating T4 lymphocytes was 85 per microliter of blood, with a T4/T8 ratio of 0.15. An enzyme-linked immunosorbent assay (ELISA) for antibodies to LAV was repeatedly negative.

The second patient is a 32-year-old man, born and living in Cape Verde (500 km off the coast of Senegal). He was well until

January 1982 when he developed severe diarrhea, fever, and progressive weight loss (20 kg). He was referred to France (Claude Bernard Hospital) in June 1983. AIDS was diagnosed according to the U.S. Centers for Disease Control criteria on: recurrent *Salmonella enteritidis* bacteremia, cryptosporidiosis, *Isospora belli* infection and brain toxoplasmosis. Skin tests were negative and the T4 lymphocyte count was 100 per microliter. None of the known risk factors was identified. At present this outpatient has no new opportunistic infection (11 months of follow-up) and he has gained 15 kg in weight. His T4 cells are still 100 per microliter of blood. Sequential serum samples have

been examined since 1983 and have always been negative for LAV antibodies by ELISA (Elavia), Western blot, and radioimmuno-precipitation assay (RIPA).

Both patients have been treated with suramin, administered intravenously. Virus was isolated from patient 1 both before and after he received a 6-month course of suramin treatment. Virus was isolated from patient 2 after he received a 1-year course of treatment with the drug.

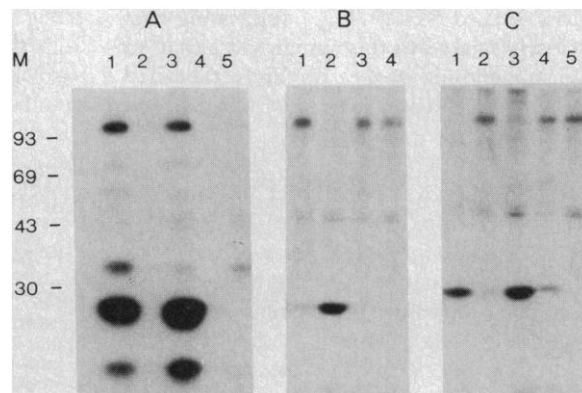
For virus isolation, the patient's peripheral blood lymphocytes were cocultivated with normal human T lymphocytes that had been stimulated with phytohemagglutinin (PHA) (1). T-cell growth factor (IL-2) and goat antiserum to  $\alpha$ -interferon were present in the medium. The cultures were monitored for reverse transcriptase (RT) activity in the supernatant and for the appearance of cytopathic effects. In cultures of the isolates from both patients, RT activity was detectable after 10 days, reached a maximum after 15 days, and then rapidly decreased. The same cells were also cocultivated with the human tumor T-cell line HUT-78 (20), where RT activity was detected after 2 weeks of culture and remained quite stable. In all cultures, a typical cytopathic effect was detected, remarkably similar to that of LAV, with numerous multinucleated giant cells and extensive cell lysis.

Examination of sections of infected cells by electron microscopy revealed the presence of mature virions and of budding viral particles similar to those of LAV. An important difference, however, was that spikes could always be observed at the surface of the West African virus whereas such spikes are not always observed on LAV by the same technique (Fig. 1).

The virus was also examined for its capacity to grow on different subsets of T lymphocytes. Peripheral blood lymphocytes from a healthy donor were separated into T4- and T8-enriched fractions as previously described (21). As with LAV, only the T4-enriched fraction was able to produce the virus. In addition to being grown in the HUT-78 cell line, the isolate from patient 2 was also propagated in CEM cells (another human lymphoid tumor cell line) that had been enriched in T4<sup>+</sup> T cells by adsorption with OKT4 antibody on an affinity chromatography column.

Serum samples from both patients were examined for the presence of antibodies to LAV, antibodies to their own viral isolates, and antibodies to STLV-III<sub>mac</sub>. The STLV-III<sub>mac</sub> had been isolated from a macaque with simian AIDS and was provided as an STLV-III<sub>mac</sub>-infected HUT-78 culture (22). Labeling of infected cell cultures with [<sup>35</sup>S]cysteine, immunoprecipitation of solu-

Fig. 2. Immunoprecipitation of metabolically labeled proteins from LAV/HTLV-III, the new West African retrovirus, and STLV-III<sub>mac</sub> with different sera. Cells were incubated with [<sup>35</sup>S]cysteine (200  $\mu$ Ci/ml) in cysteine-free medium for 16 hours. Clarified supernatant was centrifuged at 60,000g for 90 minutes. The pellet was lysed in RIPA buffer (1), immunoprecipitated with different sera, and then subjected to SDS-PAGE. (A) Antigens from LAV grown in CEM Cl.13 cells. Lane 1, serum positive for LAV/HTLV-III antibodies; lane 2, serum from patient 1; lane 3, serum from a healthy LAV carrier from Central Africa; lane 4, serum from a macaque with simian AIDS infected with STLV-III<sub>mac</sub>; lane 5, serum from patient 2. (B) Antigens from West African retrovirus isolated from patient 1, grown on HUT-78 cells. Lane 1, serum from patient 1; lane 2, serum positive for antibodies to LAV; lane 3, serum from STLV-III<sub>mac</sub>-infected macaque; lane 4, serum from patient 2. (C) Antigens from STLV-III<sub>mac</sub> isolated from a macaque with simian AIDS. Lanes 1 to 5, same as in (A) (M, size markers).



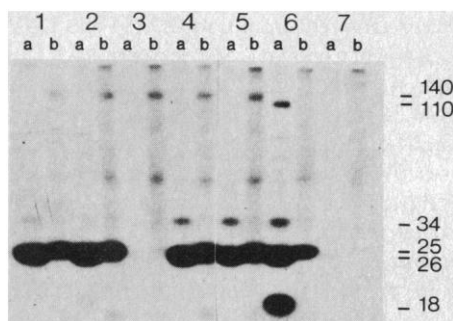


Fig. 3. Immunoprecipitation of [ $^{35}$ S]cysteine-labeled lysates of LAV (lane a) and isolate from patient 2 (lane b) by different sera. Lane 1, serum from a healthy woman living in Guinea Bissau who was positive for LAV antibody in an ELISA test. Lane 2, serum from a healthy man from Guinea Bissau who was positive in an immunofluorescence assay in which we used LAV-II-infected HUT-78 cells. Lane 3, serum from a Guinean woman with AIDS. Lane 4, serum from a Guinean woman with AIDS-related complex. Lane 5, serum from a healthy Senegalese woman (34). Lane 6, serum from a healthy homosexual man infected with LAV. Lane 7, negative serum.

ble virus extracts from these cultures, and subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as well as Western blot analyses, were conducted as already described (23).

The serum of both patients failed to react with extracts of [ $^{35}$ S]cysteine-labeled LAV (Fig. 2A). They did, however, precipitate weakly and inconstantly a protein of 34 kD, termed p34 or p31, which probably corresponds to a *pol* (endonuclease-integrase) gene product (24). When tested with lysates of the virus isolated from patient 1 (Fig. 2B), serum samples from patients 1 and 2 strongly precipitated a high molecular weight protein (130,000 to 140,000) which, by analogy with LAV, can be assumed to be the major envelope glycoprotein (termed gp140). Similar results were obtained with extracts from the isolate of patient 2. It is interesting that the serum from a macaque infected with STL<sub>mac</sub>-III<sub>mac</sub> also reacted with the gp140 that is present in extracts of the virus isolates from the two West African patients, and that the serum of both patients precipitated a protein with similar molecular weight in [ $^{35}$ S]cysteine-labeled STL<sub>mac</sub>-III<sub>mac</sub> virus extracts (Fig. 2C).

When tested with extracts of the three types of virus (LAV, the new isolates from patients 1 and 2, and STL<sub>mac</sub>-III<sub>mac</sub>), the sera from both patients, as well as serum from the STL<sub>mac</sub>-III<sub>mac</sub>-infected macaque, showed only a weak reaction with proteins with a molecular weight consistent with *gag* gene products. This weak reaction with *gag* proteins is common in patients with advanced AIDS caused by LAV. However, reference antisera to LAV, which do not

react with the gp140, precipitated a 26-kD protein (p26) in [ $^{35}$ S]cysteine-labeled extracts of the West African virus isolates (Fig. 2B). These reference antisera also precipitated a protein of 27 kD in extracts of STL<sub>mac</sub>-III<sub>mac</sub> (Fig. 2C).

We also examined sera from four other West African individuals, one with AIDS, one with prodromes, and two with no symptoms, for the presence of antibodies to LAV and to the new West African virus isolates (Fig. 3). When tested with lysed virus from patient 2, all four sera reacted with gp140 and three of them also reacted with p26. When reacted with the same lysate, the reference antisera to LAV, as well as a sheep antibody to LAV *gag* p25, precipitated p26 but did not react with gp140. The three sera from West Africans that reacted with p26 precipitated p25 in lysates of LAV but did not precipitate gp110. We therefore believe that these four individuals were infected by a virus similar to that isolated from patients 1 and 2.

Although they displayed some common antigenic structures, some of the proteins of LAV, the new West African retrovirus, and STL<sub>mac</sub>-III<sub>mac</sub> appeared to differ in gel mobility (Fig. 4A). The major *gag* protein of LAV has an apparent molecular size of 25 kD, whereas it is 27 kD in STL<sub>mac</sub>-III<sub>mac</sub> and seems to be around 26 kD in the West African virus. The large-sized antigen observed in RIPA, which corresponds to the outer membrane portion of the whole glycoprotein molecule, is 110 kD in LAV (25, 26) but 140 kD in both of the West African isolates and STL<sub>mac</sub>-III<sub>mac</sub> (Fig. 4A). The transmembrane portion of this envelope glycoprotein, which is not observed in [ $^{35}$ S]cysteine-labeled virus extracts, but can be identified on Western blots as a broad band, is approximately 41 kD in LAV (26, 27), 36 kD in the West African isolates, and 32 kD in STL<sub>mac</sub>-III<sub>mac</sub> (Fig. 4B). Thus the West African AIDS retrovirus appears to be antigenically related to, but distinct from, both LAV and STL<sub>mac</sub>-III<sub>mac</sub>. The fact that its envelope glycoprotein is precipitated only by sera from West African patients and from an STL<sub>mac</sub>-III<sub>mac</sub>-infected macaque suggests that this virus may be more closely related to STL<sub>mac</sub>-III<sub>mac</sub> than LAV.

To study further the relation between the West African retrovirus and LAV, we performed dot-blot hybridization experiments with their genomic RNA, obtained from purified viral particles, and various LAV/DNA probes. Since such experiments were not intended to give quantitative results, the amount of viral RNA deposited on each dot was estimated according to RT activity in viral pellets. Viral RNA from both isolates did not hybridize under stringent conditions

[50% formamide, 42°C, washing in 0.1× SSC (standard saline citrate), 0.1% SDS, 65°C] with any whole genomic or subgenomic LAV/DNA probe. This hybridization was repeatedly negative, even though viral particles were purified from several highly infectious culture supernatants with a high RT activity. Under low stringency conditions (30% formamide, 42°C, washing in 2× SSC, 0.1% SDS, 50°C), viral RNA from both isolates hybridized with LAV DNA subgenomic probes representing different regions of the genome (Fig. 5). These single-stranded DNA probes were obtained from M13 subclones of LAV genome by the prime-cut method (28). All of these probes, which were complementary to the plus (+) DNA strand, strongly hybridized with genomic RNA from both the LAV standard isolate (LAV<sub>1</sub>) and another isolate from a Zairian patient with AIDS. Two probes obtained from the *gag* region (nucleotides 990–1070 and 990–1260) hybridized weakly with the two West African retrovirus spots, and one (nucleotides 990–1260) also hybridized with the STL<sub>mac</sub>-III<sub>mac</sub>.

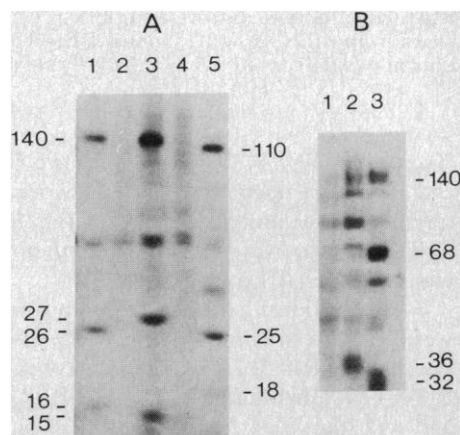
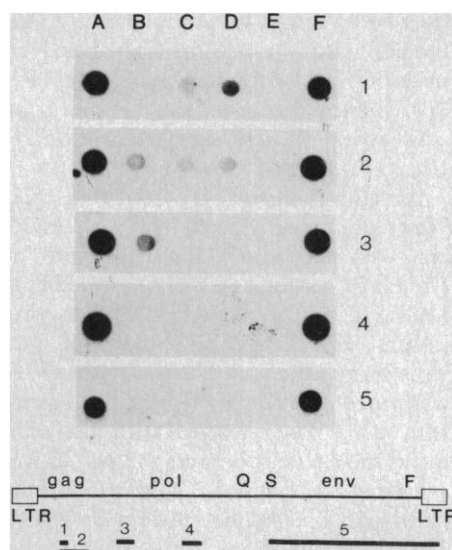


Fig. 4. Comparison of the electrophoretic mobility of the proteins of LAV, the new West African retrovirus, and STL<sub>mac</sub>-III<sub>mac</sub>. (A) SDS-PAGE of immunoprecipitates of extracts of [ $^{35}$ S]cysteine-labeled viruses. Lane 1, virus isolated from patient 1, immunoprecipitated with serum from the same patient; lane 2, same virus as in lane 1, with negative control serum from a healthy laboratory worker; lane 3, STL<sub>mac</sub>-III<sub>mac</sub> immunoprecipitated with serum from a STL<sub>mac</sub>-III-infected macaque; lane 4, same virus as in lane 3, with negative control serum; lane 5, LAV immunoprecipitated by serum from a European AIDS patient. (B) Western blot analysis. Cell lysates from uninfected or infected HUT-78 cells were subjected to SDS-PAGE, electrophoretically transferred onto nitrocellulose as described (36), and reacted with the serum of patient 1 (diluted 1:100). The blot was then washed, and the binding of antibodies revealed by  $^{125}$ I-labeled sheep antiserum to human immunoglobulin G. Lane 1, uninfected HUT-78 cells; lane 2, West African virus-infected HUT-78 cells; lane 3, STL<sub>mac</sub>-III<sub>mac</sub>-infected HUT-78 cells. Numbers in the margins of each panel show the approximate sizes of the most representative viral proteins.

Fig. 5. Dot-blot hybridization of viral genomic RNA from LAV, STLV-III<sub>mac</sub>, and West African virus isolates with various LAV subgenomic DNA probes. Cell culture supernatants (0.5 to 1 ml for each spot) were centrifuged for 20 minutes at 45,000 rev/min, the pellet was resuspended in NTE buffer containing 0.1% SDS, and spotted onto nitrocellulose that had been presoaked in 2× SSC (0.3M NaCl, 0.03 M sodium citrate). After baking (2 hours at 80°C), filters were hybridized with various LAV DNA probes in non-stringent conditions (30% formamide, 5× SSC, 42°C), washed in 2× SSC with 0.1% SDS, at 50°C, and autoradiographed for 48 hours at -70°C with intensifying screens. Probes 1 to 4 are single-stranded LAV DNA probes, obtained by the prime-cut method as described (28). Briefly, M13 single-stranded templates carrying LAV subgenomic inserts (36) were annealed to the 17-mer M13 sequence primer (Biolabs), and the complementary strand was synthesized with Klenow enzyme in TM buffer (tris 10 mM, pH 7.5, MgCl<sub>2</sub> 10 mM), with dATP, dGTP, dTTP, and [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham, 3000 Ci/mmol). DNA was then digested by an appropriate restriction enzyme, heat denatured, and subjected to electrophoresis on a denaturing polyacrylamide gel (6% acrylamide, 8M urea in TBE). The gel was autoradiographed for 5 minutes and the probe was cut off and eluted in 300 mM NaCl with 0.1% SDS. The specific activity (AS) of these single-stranded probes was estimated to 5 × 10<sup>8</sup> to 10<sup>9</sup> cpm/μg. Probe 1, nucleotides 990–1070; probe 2, nucleotides 990–1260; probe 3, nucleotides 2170–2240; probe 4, nucleotides 3370–3640. Probe 5 is a pUC-18 plasmid carrying the Eco RI–Sac I fragment (nucleotides 5290–9130) of the LAV clone λ J19 (37), nick-translated to a specific activity of approximately 10<sup>8</sup> cpm/μg. Spots A, virus from an LAV-infected CEM.c1.13 culture; spots B, virus from STLV-III<sub>mac</sub>-infected HUT-78 cells; spots C and D, virus isolates from patients 2 and 1, respectively; spots E, negative control from uninfected HUT-78 cells; spots F, virus from a Zairian patient with AIDS, grown on normal T lymphocytes with TCGF (IL-2). All spots are made with an amount of virus corresponding to 25,000 cpm of RT activity, except for spots C, 15,000 cpm.



III<sub>mac</sub> spot (Fig. 5). A probe from the 5' end of the *pol* open reading frame, corresponding to the protease region, hybridized neither with the West African retrovirus nor with the STLV-III<sub>mac</sub> spots.

Two additional single-stranded probes were obtained from the *pol* region. The first one, from the RT region (nucleotides 2170–2240), hybridized with STLV-III<sub>mac</sub>, and with the West African retrovirus, but more weakly with the latter. The other one, from the middle of the *pol* region (nucleotides 3370–3640), failed to hybridize with any of these spots.

Finally, a nick-translated probe involving the whole *env* gene and the long terminal repeat (nucleotides 5290–9130) did not hybridize with either STLV-III<sub>mac</sub> or the West African retrovirus. In control experiments, we found no hybridization, with any of the probes used, with spots from supernatants of noninfected cell cultures (Fig. 5). These data further indicate that the isolates from the West African AIDS patients may be only distantly related to LAV/HTLV-III. Their genome seems to cross-hybridize weakly only with domains of the genome of LAV/HTLV-III which are known to be highly conserved among retroviruses of the same group. Although the serological data suggest that the new isolates may be closely related to STLV-III<sub>mac</sub>, particularly in their

envelope antigens, the hybridization experiments show that they may differ from this simian retrovirus in some regions of the *gag* and the *pol* genes.

These two new isolates seem to be indistinguishable from one another. Following the precedent set for the human T-cell leukemia viruses HTLV-I and HTLV-II, which are similar in structure but have appreciably divergent nucleotide sequences (29, 30), we propose that the new West African AIDS retrovirus should be called LAV type II (31). Thus LAV type I will include all the isolates from Central Africa, the United States, and Europe that have common antigenicity and differ by less than 30% in their nucleotide sequences. Although it seems likely that LAV-I and -II have diverged from a common ancestor, together with STLV-III<sub>mac</sub>, the exact extent of the relatedness of these viruses and the possible genetic events leading to their evolutionary divergence will be better assessed by molecular cloning and nucleotide sequence analysis of their genome.

The two AIDS patients from West Africa are thought to have acquired the virus through heterosexual contact, suggesting that AIDS virus transmission occurs in West Africa as well as Central Africa. It will thus be necessary, in further seroepidemiological studies in Africa and perhaps in other areas,

to use antigens from both LAV-I and LAV-II. It is not known whether or not LAV-II is identical to the virus termed HTLV-IV that was isolated from three healthy Senegalese individuals (32) and that induces antibodies reactive with some of the antigens of STLV-III<sub>AGM</sub>, a virus isolated from healthy wild-caught African Green monkeys (33, 34). The serum of one of these Senegalese individuals (35) reacted with the two major antigens of LAV-II (Fig. 3), indicating that HTLV-IV and LAV-II may be closely related. However, HTLV-IV in Senegalese individuals (32), seems not to be associated with disease.

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