(substructure D) (*14*). For *T. aestivum*, the resonance at 174.9 ppm corresponds to bonded (esterified) ferulic (or sinapic) acid and the signal at 169.6 ppm corresponds to either free ferulic acid (or sinapic) acid. We made this assignment because the carbonyl resonance shifts downfield by 7 ppm when ferulic acid is ester-bonded to a trisaccharide (*13*). The smaller resonances at 60 to 72 ppm can be attributed to a hydroxymethylenic functionality in substructures A and C through F.

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Synthetic Peptide Immunoassay Distinguishes HIV Type 1 and HIV Type 2 Infections

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Efforts to solve the epidemiologic puzzle of AIDS in Africa are complicated by the presence of multiple human retroviruses. Simple serologic tests that unambiguously distinguish among infections by these retroviruses are essential. To that end, a partially conserved immunoreactive epitope was identified in the transmembrane glycoproteins of human immunodeficiency viruses (HIV) types 1 and 2. Synthetic peptides derived from these conserved domains were used in sensitive and specific immunoassays that detect antibodies in sera from patients infected with HIV-1 or HIV-2. By making single amino acid substitutions in the HIV-1 peptide, it was possible to demonstrate HIV-1 strain-specific antibody responses to this epitope. Such custom-designed peptides synthesized from this domain are likely to detect newly discovered HIV types, define infection with specific HIV strains, and allow detection of group-common antibodies.

ALTHOUGH THE ACQUIRED IMMUNE deficiency syndrome (AIDS) is now recognized as an epidemic of global dimensions, Africa has the highest prevalence of this disease (*1*). Infection with human immunodeficiency virus type 1 (HIV-1; also known as HTLV-III, LAV-1, and ARV), the retrovirus first shown to cause AIDS (*2*), is widespread in Central Africa (*3*). Serologic surveys have also confirmed the presence of HIV-1 in East Africa (*4*) and West Africa (*5, 6*), although the number of AIDS cases in these regions is thus far much smaller than in Central Africa.

Recently another pathogenic human retrovirus, termed HIV type 2 (formerly LAV-2), was recovered from West African patients with AIDS (*7*). HIV-2 infection is associated with an immunodeficiency syndrome clinically indistinguishable from that caused by the prototype AIDS virus, HIV-1 (*8, 9*). HIV-2 is related to but distinct from HIV-1 and from simian immunodeficiency virus (SIV or STLV-III), the etiologic agent of simian AIDS (*7*). Extensive serologic cross-reactivity exists between HIV-2 and SIV, whereas cross-reactivity between HIV-2 and HIV-1 is limited to core antigens (*7, 9*). Furthermore, numerous serologic surveys, in which a retroviral isolate termed HTLV-IV was used as antigen, have confirmed human infection in several West African countries (*5, 6, 10*). However, HTLV-IV is not genetically distinguishable from SIV (*11*) or antigenically distinguishable from HIV-2 (*7, 10*). The precise taxonomic relationships among these viruses have not yet been established.

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Table 1. Sequences of synthetic peptides derived from transmembrane glycoproteins of HIV-1 and HIV-2. The amino acid position numbers for HIV-1 are based on the sequence published by Wain-Hobson *et al.* (*21*); those for HIV-2 are based on the sequence published by Guyader *et al.* (*20*). Residues in the HIV-1 peptides that differ from the sequence of the prototype isolate (peptide 2) are underlined. The boxed areas show residues conserved between HIV-1 and HIV-2.

Peptide number	Isolate name	Source	Amino acid sequence*	Reference
			598 609	
1	HIV-1 (Z-3)	Zaire	Leu-Gly-Leu-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys	(14)
2	HIV-1 (LAV_{BRU}; $HTLV\text{-}III_B$; $HTLV\text{-}III_{RF}$; WMJ-1; ARV-2)	France; United States	Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys	(21,29)
3	HIV-1 (LAV-ELI)	Zaire	Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-His-Ile-Cys	(19)
4	HIV-1 (LAV-MAL)	Zaire	Leu-Gly-Met-Trp-Gly-Cys-Ser-Gly-Lys-His-Ile-Cys	(19)
			592 603	
5	$HIV\text{-}2_{ROD}$	Cape Verde Islands	Leu-Asn-Ser-Trp-Gly-Cys-Ala-Phe-Arg-Gln-Val-Cys	(20)

*The three-letter and single-letter abbreviations for the amino acids are: Ala (A), alanine; Arg (R), arginine; Asn (N), asparagine; Asp (D), aspartic acid; Cys (C), cysteine; Gln (Q), glutamine; Glu (E), glutamic acid; Gly (G), glycine; His (H), histidine; Ile (I), isoleucine; Leu (L), leucine; Lys (K), lysine; Met (M), methionine; Phe (F), phenylalanine; Pro (P), proline; Ser (S), serine; Thr (T), threonine; Trp (W), tryptophan; Tyr (Y), tyrosine; and Val (V), valine.

The existence in Africa of multiple human immunodeficiency viruses presents a complex epidemiologic picture. Simple serologic tests that unambiguously distinguish among these retroviruses are essential for sorting out their patterns of transmission and pathogenesis. Immunoassays in which whole virus lysates are used as antigens have poor specificity due to partial cross-reactivity against conserved core antigens (*6, 8*). As an alternative, we devised immunoassays using synthetic peptide antigens that have the inherent advantage of high specificity. We have synthesized a 12–amino acid peptide derived from gp41, the transmembrane glycoprotein of HIV-1, that reacts in an enzyme-linked immunosorbent assay (ELISA) with sera from >99% of HIV-1–infected patients from the United States (*12*). The minimal epitope for immune recognition is a 7–amino acid sequence (*env* amino acid 603–609) containing two essential cysteine residues linked by a disulfide bond (*13*). Another synthetic peptide derived from the homologous location on the transmembrane glycoprotein of HIV-2 is highly reactive with sera from HIV-2–infected patients. Thus, this sequence is a common immunogenic domain among the human immunodeficiency viruses. In addition, by making single amino acid substitutions, we have modified the HIV-1 peptide to enhance its ability to detect nearly all patients infected with African strains of HIV-1.

The reactive peptide (peptide 1) derived from gp41 of HIV-1 (*env* amino acid 598–609; Leu-Gly-Leu-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys) was originally selected on the basis of hydrophilicity and sequence conservation (*12*) and corresponds to isolate HIV-1_{Z-3} (*14*). Additional peptides containing amino acid substitutions based on the published sequences of various HIV-1 and HIV-2 isolates were synthesized (Table 1). All peptides were made by solid-phase methods (*15*) with an automated peptide synthesizer and analyzed for purity by high-pressure liquid chromatography. The peptides were then used as solid-phase antigens in an ELISA (*12, 16*).

Sera were collected from HIV-infected and uninfected individuals in the United States, Zaire, and West Africa (*17*). The sera from Zairian patients were cultured for HIV (*18*) and tested with a commercial ELISA (Litton) based on the use of whole-virus antigens. Selected sera from the Zairian patients were analyzed for antibodies to specific HIV-1 proteins by Western immunoblotting with the use of CDC strain 451 as an antigen source.

Peptide 1 (HIV-1) reacted with sera from 162 of 163 HIV-infected patients from the United States but with 0 of 90 control sera from uninfected individuals (Table 2). However, peptide 1 was bound by sera from only 33 of 38 Zairian HIV-1–infected patients (Table 2). We speculated that this lower sensitivity of the assay for the sera from Zairian patients might be attributable to infection of these patients with strains of HIV-1 having significant amino acid substitutions in the area of gp41 spanned by peptide 1.

We tested this hypothesis by synthesizing three additional peptides containing amino acid substitutions that have been reported to occur between amino acids 598 and 609 of gp41 (Table 1). Peptide 2 (which contains an isoleucine for leucine substitution at position 600) was essentially equivalent in reactivity to peptide 1 (Table 2). The more significant amino acid substitutions made in peptides 3 and 4 slightly reduced their reactivity with sera from American HIV-infected patients but enhanced reactivity with Zairian sera (Table 2).

To analyze further the sera from the Zairian AIDS patients, we used the five samples that did not react with peptide 1, plus nine of the sera that did react with this peptide. All these sera were positive for antibody to HIV-1 in a commercial ELISA that used whole-virus antigen; all of the sera tested had positive Western immunoblots, and most were positive for HIV when cultured (Table 3). Four of the five sera from Zairian patients that failed to react with peptides 1 or 2 had negative or equivocal p41 bands on Western blotting. However, peptide 3 and peptide 4, both of which contain a histidine for leucine substitution at position 607, reacted with all five of the sera from the Zairian patients that were nonreactive with peptide 1 (Table 3). Peptides 3 and 4 both represent sequences from HIV-1 strains isolated in Zaire (*19*). It is interesting that peptides 3 and 4 reacted positively even with those sera that were gp41 negative by immunoblotting, indicating that the peptide ELISA may be a more sensitive test under some circumstances.

We next synthesized a peptide derived from the corresponding region of the transmembrane glycoprotein of HIV-2. The transmembrane glycoproteins of HIV-1 and HIV-2 are distantly related and are homologous at approximately 44% of amino acid positions (*20*). When compared with amino acid sequence 598–609 of HIV-1 (LAV_{BRU}), amino acid sequence 592–603 of HIV-2 ($HIV\text{-}2_{ROD}$) has identical residues at 5 of the 12 positions (Table 1) (*20, 21*). The two cysteine residues that we have shown to be essential for antigenicity of the HIV-1 peptide (*13*) are conserved in HIV-2 (Table 1). Peptide 5, an 11–amino acid peptide based on $HIV\text{-}2_{ROD}$, reacted with five of five sera from HIV-2–infected West Africans. Peptide 5 gave a weakly positive ELISA result with sera from 1 of 40 American HIV-infected patients and 0 of 48 uninfected controls (*22*).

Specimens from two Zairian HIV-1–infected patients (Z-46 and Z-51) reacted with HIV-1 peptides and also less strongly with HIV-2 peptide 5. This may represent cross-reactivity, dual infection, or infection with an unidentified intermediate virus. The single specimen from patient Z-51 was cul-

Table 2. Reactivity by ELISA of synthetic HIV-1 and HIV-2 peptides with serum samples from U.S. and African patients. The data show the number of sera positive out of the number of sera tested and, in parentheses, the percentage positive. ND, not done, insufficient quantity of sera available.

Sera	Peptide 1 (LGLWGCSGKLIC) HIV-1 (Zaire)	Peptide 2 (LGIWGCSGKLIC) HIV-1 (France, United States)	Peptide 3 (LGIWGCSGKHIC) HIV-1 (Zaire)	Peptide 4 (LGMWGCSGKHIC) HIV-1 (Zaire)	Peptide 5 (NSWGCAFRQVC) HIV-2 (Cape Verde Islands)
HIV-1–infected, United States	162/163 (99.4)	40/40 (100)	39/40 (97.5)	35/40 (87.5)	1/40 (2.5)
Uninfected, United States	0/90 (0)	0/48 (0)	0/48 (0)	0/48 (0)	0/48 (0)
HIV-1–infected, Zaire	33/38 (86.8)	30/34 (88.2)	32/34 (94.1)	33/34 (97.1)	2/33 (6.1)
Uninfected, Zaire	0/21 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)
HIV-2–infected, West Africa	0/5 (0)	ND	ND	ND	5/5 (100)

Table 3. Characterization of sera from Zairian AIDS patients. The sera were described as positive (+) or negative (−) as described (*16*). ND, not done, insufficient quantity of sera available.

Specimen	HIV culture	Western blot	Enzyme-linked immunosorbent assay					
			Whole virus HIV-1	Peptide 1 (LGLWGCSGKLIC) HIV-1(Zaire)	Peptide 2 (LGIWGCSGKLIC) HIV-1(France, United States)	Peptide 3 (LGIWGCSGKHIC) HIV-1(Zaire)	Peptide 4 (LGMWGCSGKHIC) HIV-1(Zaire)	Peptide 5 (NSWGCAFRQVC) HIV-2 (Cape Verde Islands)
Z-9	+	p24+,p41+/−	+	−	+	+	+	−
Z-23	+	p24+,p41+/−	+	−	−	+	+	−
Z-24	+	p25+	+	+	+	+	+	−
Z-29	+	ND	+	+	+	−	−	−
Z-31	+	p24+,p41+	+	+	+	+	+	−
Z-34	+	p24+,p41−	+	−	−	+	+	−
Z-47	+	p24+,p41−	+	−	−	+	+	−
Z-50	−	p24+,p41+	+	−	−	+	+	ND
Z-51	−	p41+	+	+	+	+	+	+
Z-52	+	p25+,p41+, p60+	+	+	+	+	+	−
Z-68	+	ND	+	+	+	−	+	−
Z-72	+	p25+	+	+	+	+	+	−
Z-75	+	p18+,p25+, p41+,p60+	+	+	+	+	+	−
Z-81	+	p18+p25+, p41+,p60+	+	+	+	+	+	−

ture-negative; the HIV isolate from Z-46 is being further analyzed.

HIV-2 is closely related to SIV, as shown by both serologic assays and nucleic acid hybridization studies (*7, 23*). The transmembrane glycoprotein of SIV (STLV-III_{AGM}) contains a 12–amino acid sequence [*env* amino acids 608–619; (*24*)] identical at 11 of 12 residues to the sequence of HIV-2_{ROD} from which our HIV-2 peptide (see Table 1, peptide 5) was derived. (STLV-III_{AGM} contains an alanine for serine substitution at the third position.) HIV-2 peptide 5 was reactive with sera from three of three SIV-infected rhesus monkeys but with 0 of 5 sera from monkeys infected with STLV-I or type D simian retrovirus.

Two principal conclusions can be drawn from these data. First, we have identified a highly immunoreactive epitope on the transmembrane glycoproteins of all of the HIV-like viruses (HIV-1, HIV-2, and STLV-III) sequenced to date. The epitope is located in an analogous region of each virus and shares the common amino acid sequence Trp-Gly-Cys-X-X-X-X-X-Cys. By using synthetic peptide antigens derived from these proteins of HIV-1 and HIV-2 in an ELISA, we can detect specific antibodies in sera from patients infected with HIV-1 or HIV-2 or both viruses. As other related retroviruses are isolated and sequenced, peptides derived from this region of the transmembrane glycoprotein should prove to be ready immunodiagnostic reagents.

Second, single amino acid substitutions can be made in this reactive domain of HIV-1 to create immunodiagnostic antigens that are increasingly or decreasingly strain-specific. Genetic studies of HIV-1 isolates have demonstrated geographic variability, especially in proteins encoded by the *env* gene (*19, 25*). This genetic variability has not, however, resulted in significant antigenic variability as measured by ELISA and immunoblotting, indicating that important antigenic regions are conserved among these isolates (*19, 25, 26*). However, when we assay for antibody responses at the level of small peptide antigens, we can detect strain-specific antibodies. For example, peptides 1 and 2 were highly sensitive and specific when used to test sera from American patients positive or negative for HIV-1, but were less sensitive for testing sera from Zairian patients. Substitution of a histidine residue for leucine at *env* position 607 produced a peptide (Table 1, peptide 3) that detected antibodies in Zairian patients not detected by peptides 1 or 2. Therefore, this approach enables one to custom-design highly specific peptide antigens that detect different strains of HIV (for example, for epidemiologic studies) or type-common peptides that detect all strains (for example, for screening blood donors).

A whole-virus ELISA designed to detect infection with HIV-1 will not reliably detect HIV-2 infection. The envelope glycoproteins of HIV-1 and HIV-2 are only about 40% homologous, with little serologic cross-reactivity against the envelope antigens (*8, 9, 20*). The degree of amino acid homology between constitutive core proteins is higher, but serologic cross-reactivity between the core proteins (as measured by ELISA) is partial and inconsistent (*7–9*). Synthetic peptides such as those described in this report provide the basis for more specific immunodiagnostic assays that are needed to clarify the roles of HIV-1 and HIV- in the AIDS epidemic.

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16. Peptide solutions in phosphate-buffered saline were air-dried in polyvinyl 96-well microtiter plates to yield 1 μg of peptide per well. The plates were sequentially incubated with test sera, horseradish peroxidase–conjugated goat antiserum to human immunoglobulin G, and *O*-phenylenediamine substrate solution. Optical densities were analyzed on an automated scanner at 492 nm. The cutoff for positivity was defined as the mean OD_{492} plus 3 standard deviations for a panel of 24 negative control sera. Specimens were scored as positive or negative at a serum dilution of 1:64. All sera were tested at least three times.
17. Sera from HIV-1–infected American patients were collected in San Diego, California (n = 84), and by the Centers for Disease Control from locations

throughout the United States (n = 79). The test panel of 40 HIV-1–positive sera included specimens from 12 asymptomatic seropositive patients (CDC group II), 13 patients with generalized lymphadenopathy (CDC group III) or AIDS-related complex (ARC; CDC group IV-A), and 15 patients with overt AIDS [CDC group IV-C or IV-D; (*27*)]. The sera from Zairian HIV-1–infected patients and healthy Zairian controls were collected in Kinshasa in 1983 and have been all characterized (*26*). The HIV-2–positive sera were collected in Guinea Bissau in 1980 and characterized by immunoblotting and immunofluorescence against HIV-1, HIV-2, and SIV antigens (*28*). The panel of 48 control sera from Americans included 24 healthy laboratory personnel and 24 healthy homosexual men. Specimens were coded, divided into aliquots, and stored at −20°C until tested. Using HIV-1 gp41 (peptide 1 in Table 1) investigators at WHO found the ELISA to be as sensitive as three commercial kits when they tested 22 HIV-1–positive sera from patients in Zambia. No false positives were observed in tests of 411 sera from normal patients, including 32 pregnant women, from Nigeria.

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22. The single serum sample from an American that was reactive with HIV-2 peptide 5 was collected in San Diego in 1985 from a 35-year-old white male homosexual ARC patient. He had no history of travel to Africa or contact with Africans. Another sample collected in 1987 from the patient (now with overt AIDS) was still reactive with HIV-1 peptide 1 but nonreactive with HIV-2 peptide 5.
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The Surface Composition of Charon: Tentative Identification of Water Ice

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The 3 March 1987 Charon occultation by Pluto was observed in the infrared at 1.5, 1.7, 2.0, and 2.35 micrometers. Subtraction of fluxes measured between second and third contacts from measurements made before and after the event has yielded individual spectral signatures for each body at these wavelengths. Charon's surface appears depleted in methane relative to Pluto. Constancy of flux at 2.0 micrometers throughout the event shows that Charon is effectively black at this wavelength, which is centered on a very strong water absorption band. Thus, the measurements suggest the existence of water ice on Pluto's moon.

THE CURRENT SEASON OF MUTUAL events between Pluto and its satellite Charon presents the opportunity for many unique experiments. For example, monitoring of these events allows the determination of absolute sizes and bulk density of these bodies with unprecedented precision. It is also possible to separate the contributions of both bodies to the total observed light. A spectrum obtained during totality may be subtracted from the mean of spectra obtained just before and just after an event. The remainder is a spectrum of the Pluto-facing hemisphere of Charon alone. Both bodies rotate synchronously, which during a central event amounts to only about 2°. Any color variation therefore must arise from compositional differences between the two bodies, rather than a regional variation on the surface of an individual body.

Near-infrared spectrophotometry is a powerful diagnostic tool for the identification of ices on outer solar system bodies because of the strength of molecular transitions in the 1.0- to 2.5-μm region. On the basis of elemental abundances and the stability of ices, the candidate materials for solid surfaces are relatively few, and even filter photometry in the 1.0- to 2.5-μm region is highly diagnostic of surface composition. We report here observations of an occultation of Charon by Pluto with a near-infrared filter set selected to distinguish the most likely surface constituents.

The observations reported here were made with the Infrared Photometer and the Multiple Mirror Telescope Observatory (MMT) at Mount Hopkins, Arizona. This photometer uses an InSb detector cooled with liquid helium. Measurements were made through an aperture 8.7 arc sec in diameter, and relative to sky reference areas 10 arc sec above and below Pluto in elevation. The stars SAO 120107, HD 105601, and HD 129655 were used for absolute flux calibration.

Data on Pluto were recorded between 0730 and 1315 UT on 3 March 1987. The approximate geometry of the Pluto-Charon system for these times is indicated in Fig. 1. According to the ephemeris of Tholen *et al.* (*1*), this interval spanned the times from roughly 1 hour before first contact until fourth contact. Observations were terminated at approximately 1315 due to brightening of the sky. The sky was clear all night.

Because of the faintness of Pluto, it is currently impossible to obtain a continuous infrared spectrum of Pluto at reasonable signal-to-noise during the few short hours of a single eclipse event. We therefore observed with four filters, each of which has a spectral resolution of about 5%, or 0.1 μm (*2*). From previous work (*3*) methane was known to be the dominant absorber for the combined Pluto-Charon system. The two strongest bands for methane in the near-infrared are at 1.7 and 2.35 μm; two additional filters were used to measure the nearby continuum at 1.5 and 2.0 μm. The infrared photometer was set to cycle automatically through these filters; each cycle took approximately 15 minutes. After every two cycles a set of similar measurements was obtained on SAO 120599, a nearby G0V star expected to have colors identical to those of the sun. Our observations are summarized in Table 1 and depicted graphically in Fig. 2.

It should be noticed that the out-of-eclipse light curve slope persists during totality. This is in itself proof that the albedo "spots" first proposed by Marcialis (*4, 5*) do in fact reside on Pluto, and not on the satellite.

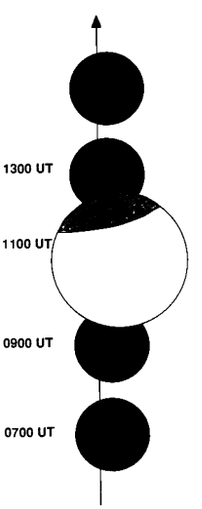

Fig. 1. Approximate geometry of the 3 March 1987 occultation of Charon by Pluto. The satellite was completely hidden for about 2 hours.

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