Serologic Confirmation of Simian T-Lymphotropic Virus Type I Infection by Using Immunoassays Developed for Human T-Lymphotropic Virus Antibody Detection

DONNA L. RUDOLPH,¹ JOANN YEE,² JAY MONE,³ STEVEN K. H. FOUNG,⁴ JAMES J. LIPKA,⁴
GREGORY R. REYES,⁵ KENNETH HADLOCK,⁵ LILY CHAN,⁶ FRANCOIS VILLINGER,⁷
MICHAEL D. LAIRMORE,⁸ SASWATI SINHA,¹ AND RENU B. LAL^{1*}

Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, National Centers for Infectious Diseases, Centers for Disease Control,¹ and Yerkes Regional Primate Research Center,⁷ Atlanta, Georgia 30333; Simian Retrovirus Reference Laboratory, California Primate Research Center, University of California, Davis, California 95616²; Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas 78228³; Department of Pathology, Stanford University School of Medicine, Stanford, California 94305⁴; Department of Molecular Virology, Genelabs Inc., Redwood City, California 94063⁵; Diagnostic Biotechnology, Singapore Science Park, Singapore⁶; and Department of Veterinary Pathobiology, Ohio State University, Columbus, Ohio 43210⁸

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Serum specimens from diverse species of Old World monkeys, categorized as seropositive ($n = 97$) or seronegative ($n = 23$) for human T-lymphotropic virus (HTLV) infection, were tested by using recombinant *env*-spiked Western immunoblot assays and synthetic peptide assays for simultaneous detection and discrimination of simian T-lymphotropic virus (STLV) infection. Of the 97 seropositive specimens, 93 reacted with the recombinant transmembrane (r21^{env}) protein and 90 reacted with a recombinant, MTA-1, derived from the central region of the external glycoprotein of HTLV-I (rgp46^{env}), thus yielding test sensitivities of 96 and 93%, respectively. While 1 of the 23 negative monkey specimens reacted with r21^{env}, none reacted with rgp46^{env}, for overall specificities of 96 and 100%, respectively. Analysis of synthetic peptide-based immunoassays demonstrated that while 85 of 97 (88%) seropositive specimens reacted with HTLV-I-specific epitope (p19^{gag}), none of the specimens reacted with HTLV-II-specific epitope (gp52^{env}). These results show that recombinant envelope-spiked Western blots provide a simple means for serologic confirmation of STLV-I infection and that type-specific synthetic peptides can be used to confirm the virus type in seropositive monkey specimens.

Simian T-cell lymphotropic virus type I (STLV-I) is closely related to human T-lymphotropic virus type I (HTLV-I) (11, 25) and is etiologically associated with leukemia and lymphoma (3, 10, 20, 21, 23, 24). Because of the extensive homology between the structural proteins of these viruses (25), serologic confirmation of STLV-I infection has mainly relied upon antibodies cross-reacting with HTLV-I/II antigens. Unlike the U.S. Public Health Service seropositivity criteria for HTLV-I/II, which requires antibody reactivity to both *gag* and *env* products (1), no specific guidelines have been defined for seropositivity in STLV-I infection. Thus, the seropositive rates can vary from none to as high as 60% depending upon the test algorithm used and the species of the monkey tested (2, 6, 7, 12, 14). Since the occurrence of *gag* reactive antibodies to HTLV often represents a false-positive reaction (13), we believe that it is important to include *env* reactive antibodies in the STLV-I seropositivity algorithm.

Recent studies have demonstrated that while Western blot (immunoblot) assay (WB) can easily detect antibodies to *gag* products, it often fails to detect *env* antibodies (1, 8). In the absence of clear *env* reactivity on WB, a specimen should be further analyzed by radioimmunoprecipitation. While radioimmunoprecipitation can easily detect *env* reactivity, the technique is cumbersome and requires radiolabelled viral proteins. More recently, WB assays containing purified viral lysate spiked with recombinant *env* proteins have been

developed which are highly sensitive and specific for the detection of *env* antibodies in HTLV-infected serum specimens (18, 19). In addition, synthetic peptide and recombinant protein-based assays have provided a simple tool to increase the specificity of *env* detection and serologically distinguish between HTLV-I and HTLV-II (16, 19). Because of significant conservation of primary amino acids between HTLV-I and STLV-I (15, 25), we sought to determine the usefulness of these recombinant constructs to detect *env* antibodies in STLV-I-infected monkey serum specimens. In addition, we have analyzed the immune responses to the HTLV type-specific epitopes in these specimens.

MATERIALS AND METHODS

Serum specimens. Serum specimens from Old World monkeys ($n = 120$), including baboons (*Papio cynocephalus*; $n = 38$), rhesus monkeys (*Macaca mulatta*; $n = 48$), African green monkeys (*Cercopithecus aethiops*; $n = 18$), sooty mangabeys (*Cercocebus atys*; $n = 10$) and cynomolgus monkeys (*Macaca fascicularis*; $n = 6$), were chosen for the study. All of the serum specimens were initially tested for HTLV-I/II antibodies by using a commercial ELISA (HTLV-I ELISA; Dupont, Wilmington, Del.), and initial reactive specimens were further tested by WB (Dupont). Sera containing antibodies to at least two HTLV structural gene products (*gag* p24 and/or p19 and *env* gp46 and/or gp68) by WB were considered positive (HTLV^{POS}), while sera reacting with only *gag* gene products were considered indeterminate and were not included in the present study.

* Corresponding author.

TABLE 1. Analysis of HTLV serologic tests for confirmation of STLV infection

Species	No. (%) positive by recombinant-spiked WB ^a		No. (%) positive by select-HTLV ^b	
	r21 ^{env}	rgp46 ^{env}	HTLV-I ^{gag}	HTLV-II ^{env}
HTLV^{pos}				
Baboons (<i>n</i> = 30)	30 (100)	29 (97)	26 (87)	0 (0)
Rhesus monkeys (<i>n</i> = 40)	36 (90)	35 (87)	35 (88)	0 (0)
African green monkeys (<i>n</i> = 15)	15 (100)	15 (100)	15 (100)	0 (0)
Mangabeys (<i>n</i> = 8)	8 (100)	7 (87)	6 (75)	0 (0)
Cynomolgus monkeys (<i>n</i> = 4)	4 (100)	4 (100)	3 (75)	0 (0)
HTLV^{neg}				
Baboons (<i>n</i> = 8)	1 (12)	0 (0)	0 (0)	0 (0)
Rhesus monkeys (<i>n</i> = 8)	0 (0)	0 (0)	0 (0)	0 (0)
African green monkeys (<i>n</i> = 3)	0 (0)	0 (0)	0 (0)	0 (0)
Mangabeys (<i>n</i> = 2)	0 (0)	0 (0)	0 (0)	0 (0)
Cynomolgus monkeys (<i>n</i> = 2)	0 (0)	0 (0)	0 (0)	0 (0)

^a Recombinant-spiked WB assays include a transmembrane protein (r21^{env}) and central region of external glycoprotein (MTA-1; rgp46^{env}).

^b Select-HTLV assays include HTLV-I and HTLV-II type-specific synthetic peptides.

Sera exhibiting no reactivity to any HTLV gene product were considered negative (HTLV^{neg}).

Recombinant env protein-spiked WB. Two recombinant env constructs were used to spike WB strips containing purified HTLV viral lysate. The first construct, r21^{env} (Cambridge Biotech, Rockville, Md.), is a 14-kDa protein derived from the transmembrane envelope protein of HTLV-I (18). The second construct, MTA-1, is derived from the central region of the external gp46 of HTLV-I and was slot blotted onto WB strips containing both purified HTLV lysate and r21^{env} (Diagnostic Biotechnology, Singapore) (19). Both assays were run according to the manufacturer's recommendations. Briefly, the spiked WB strips were washed, excess reactive sites were blocked by blocking buffer, and the strips were incubated with a 1:100 dilution of test serum in Tris-buffered saline. After several washes, alkaline phosphatase-conjugated anti-human immunoglobulin G (IgG) was added for 1 h, followed by several washes. The color was developed by adding substrate solution containing 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium. The bands were visually scored on a scale of 0 to 3, with 0 denoting no reactivity and 3 denoting strong reactivity.

Synthetic-peptide-based immunoassay. A synthetic-peptide-based immunoassay (Select-HTLV; Coulter Immunology) which discriminates between HTLV-I and HTLV-II infection was performed as previously described (16). Briefly, wells coated with synthetic peptide (HTLV-I-specific p19^{gag} and HTLV-II-specific gp52^{env}) were incubated with 100 µl of test serum at a 1:50 dilution and incubated at room temperature for 30 min. The wells were washed, 100 µl of peroxidase-conjugated goat anti-human IgG was added, then the wells were washed again, and an appropriate substrate was added. The plates were read with an ELISA reader (SLT Labinstruments, Ronkonkome, N.Y.) at 450 nm. The cutoff value was calculated by adding 0.1 to the mean optical density of negative control sera on respective peptides. Specimens with an optical density less than the cutoff value were considered nonreactive for antibodies to HTLV-I/II. If the absorbance value was higher than the cutoff value in either the HTLV-I or HTLV-II peptide assay, the specimen was considered HTLV-I or HTLV-II positive, depending on the specific peptide reactivity.

RESULTS

Analysis of recombinant env protein-spiked WB. Serum specimens from Old World monkeys, previously shown to be seropositive for antibodies to HTLV structural proteins (HTLV^{pos}; *n* = 97), were tested on two recombinant env protein-spiked Western blot assays to determine reactivity to HTLV-I envelope proteins. Serum specimens from 30 of 30 (100%) baboons, 36 of 40 (90%) rhesus monkeys, 15 of 15 (100%) African green monkeys, 8 of 8 (100%) mangabeys, and 4 of 4 (100%) cynomolgus monkeys reacted with r21^{env}, in addition to reacting with p24^{gag} and p19^{gag} proteins (Table 1). A representative WB pattern (Fig. 1A) demonstrates strong bands at 24, 19, and 14 kDa, corresponding to p24^{gag}, p19^{gag}, and r21^{env}, respectively. Some additional bands at 28, 32, and 53 kDa all represent intermediary processed products of gag gene products.

A similar analysis of a recombinant construct (MTA-1), derived from the external glycoprotein of HTLV-I (19), demonstrated that the serum specimens of 29 of 30 (97%) baboons, 35 of 40 (87%) rhesus monkeys, 15 of 15 (100%) African green monkeys, 7 of 8 (87%) mangabeys, and 4 of 4 (100%) cynomolgus monkeys reacted with rgp46^{env} protein. A representative WB (Fig. 1B) demonstrates a high-molecular-weight band (MTA-1, which is slot blotted on the WB), in addition to bands of 24, 19, and 14 kDa; these bands represent MTA (rgp46^{env}), p24^{gag}, p19^{gag}, and r21^{env} proteins, respectively. Thus, MTA-1 blots allow detection of two env gene products, one generic for HTLV-I and HTLV-II (r21^{env}) and one HTLV-I specific (MTA-1), both in one strip. Neither the native external glycoprotein (gp46) or the env precursor (gp61/68) was detectable in any of these WBs.

The specificity of r21^{env} and rgp46^{env} was next determined by testing specimens from HTLV^{neg} specimens. Of the 8 seronegative baboon specimens, 1 reacted with r21^{env}; none of the other 15 seronegative monkey specimens reacted with r21^{env}, thus yielding an overall specificity of 96% (1 of 23). None of the 23 seronegative monkey specimens reacted with rgp46^{env} protein, thus yielding 100% specificity. The overall sensitivity and specificity for r21^{env} was 96%, whereas for rgp46^{env} they were 93 and 100%, respectively.

FIG. 1. Representative WB patterns for r21^{env} (A) and MTA-1 (B). Lane 1 in each panel represents an HTLV-I-positive control, lanes 2 and 3 are representative serum specimens from monkeys who tested positive for antibodies to HTLV-I, and lane 4 is a serum specimen from a monkey who tested negative for antibodies to HTLV-I. Molecular mass markers (in kilodaltons) for *gag* proteins and recombinant *env* proteins (r21 and MTA-1) are shown at left.

Synthetic-peptide immunoassays. Antibody responses to synthetic epitopes derived from the p19^{gag} protein of HTLV-I and the gp52^{env} protein of HTLV-II were next determined. While serum specimens from 26 of 30 (87%) baboons, 35 of 40 (88%) rhesus monkeys, 15 of 15 (100%) African green monkeys, 6 of 8 (75%) mangabeys, and 3 of 4 (75%) cynomolgus monkeys reacted with the p19^{gag} epitope of HTLV-I, none of the serum specimens reacted with the gp52 epitope of HTLV-II. This further substantiates our previous finding that Old World monkeys contain STLVI that is more closely associated with HTLV-I than HTLV-II (22). None of the 23 seronegative specimens reacted with either the HTLV-I-specific or HTLV-II-specific epitope.

DISCUSSION

Nonhuman primates have recently been shown to have serum antibodies that cross-react with HTLV-I antigens in most serologic assays. The antibodies to HTLV-I/II in Old World monkeys have been reported in several different species, including rhesus monkeys, baboons, chimpanzees, African green monkeys, and cynomolgus monkeys, with rates of infection varying depending upon the species tested (6, 7, 12, 14). While some of the seroprevalence rates are based on antibody reactivity to both *gag* and *env*, others only account for reactivity to *gag* (2, 6, 7); thus, more reliable assays are needed to define the true seroprevalence of STLVI infection.

Recently, recombinant constructs derived either from the transmembrane glycoprotein of HTLV-I/II (r21^{env} [18]) or from the external glycoprotein of HTLV-I (MTA-1 [19]) have been developed, both of which provide an increased

sensitivity of *env* detection in HTLV-I-infected individuals. In concordance with these studies, serum specimens from STLVI-infected monkeys also reacted with both r21^{env} (93 of 97 [96%]) and MTA-1 (90 of 97 [93%]) proteins. The 96 and 93% seroreactivities to r21^{env} and rgp46^{env}, respectively, in STLVI-infected monkeys are lower than those seen in humans and may represent lower antibody titers in these monkeys. Alternatively, variation in the envelope sequences between HTLV-I and STLVI may account for our inability to detect antibodies in 100% of STLVI-infected monkeys. Indeed, minor variations in the amino acid sequences of both the central region of gp46 and the transmembrane protein of HTLV-I and STLVI have been documented (15). Finally, the assay systems investigated here were optimized for the detection of human antibodies to HTLV-I, and the detection of simian IgG by a goat anti-human IgG may result in lower detection limits than a simian-specific reagent would allow.

The specificity of MTA-1 detection was 100%, whereas that of r21^{env} was 92%. Recent studies have demonstrated some false positivity of r21^{env} in the HTLV testing algorithm for humans (9, 19). Significant homologies of the transmembrane proteins of all known retroviruses may account for the false-positive rates. On the other hand, the r21^{env} reactivity may represent early seroconversion that is not yet detectable by the MTA-1 construct.

The immunodominance of the transmembrane protein and the central region of the HTLV-I external glycoprotein is well documented (4, 5, 17, 19). Several recombinant envelope glycoproteins have recently been demonstrated to have differential reactivities with specimens from HTLV-I/II-infected individuals. For example, a plasmid (RP-D³¹³⁻⁴⁸⁸) encoding a 30- to 32-kDa protein reacts with 74% of HTLV-I-infected specimens (4), while a plasmid (p21e/gp46) encoding a 38-kDa protein which encompasses 50% of the gp46 region and the full gp21e region reacts with 95% of HTLV-I/II-infected specimens (5). Similarly, synthetic peptides have also demonstrated several immunodominant epitopes in the structural proteins of HTLVs, some of which have recently been utilized for serologic differentiation of HTLV-I from HTLV-II (16, 17). Our analysis of these STLVI-infected specimens on type-specific synthetic peptides demonstrated that 88% (85 of 97) of the specimens reacted with HTLV-I-specific p19^{gag} epitope; none of the specimens demonstrated any reactivity to the HTLV-II-specific gp52^{env} epitope. These results corroborate recent reports in which no HTLV-II-specific reactivity could be demonstrated either by polymerase chain reaction (13a) or by synthetic-peptide assays (22).

Although a homologous STLVI antigen was not used in the present study for serologic confirmation of STLVI, our results demonstrate that the commercial assays developed for HTLV detection can be reliably used for STLVI confirmation. In addition, type-specific assays can be utilized to initially screen for "STLV-II-like" virus in those specimens that test seropositive for STLVI. These reagents will provide a uniform test system to confirm true STLVI infections in natural and captive monkey colonies.

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