Recombinant p51 as Antigen in an Immune Complex Transfer Enzyme Immunoassay of Immunoglobulin G Antibody to Human Immunodeficiency Virus Type 1

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An ultrasensitive enzyme immunoassay (immune complex transfer enzyme immunoassay) of antibody immunoglobulin G (IgG) to human immunodeficiency virus type 1 (HIV-1) has been developed using recombinant HIV-1 reverse transcriptase (rRT) as antigen. However, some disadvantages were noted in the use of rRT as antigen: rRT was produced only with low efficiency in widely used strains of Escherichia coli using a rather long DNA fragment (3,012 bp) of the whole HIV-1 pol gene, and it was impossible to produce fusion proteins of RT for simple purification, since rRT is a heterodimer of p66 and p51. In this study, recombinant HIV-1 p51 and p66 with Ser-Ser at the N termini (Ser-Ser-rp51 and Ser-Ser-rp66) were produced in E. coli as fusion proteins with maltose binding protein containing a factor Xa site between the two proteins and were purified after digestion with factor Xa. Ser-Ser-rp51 was produced in larger amounts and purified in higher yields with less polymerization than Ser-Ser-rp66. Polymerized Ser-Ser-rp66 tended to be precipitated on mercaptoacetylitation for conjugation to β-N-galactosidase (used as a label) and showed higher nonspecific and lower specific signals in an immune complex transfer enzyme immunoassay of antibody IgG to HIV-1 than Ser-Ser-rp51. The signals for serum samples of HIV-1-seropositive subjects by immune complex transfer enzyme immunoassay of antibody IgG to HIV-1 using Ser-Ser-rp51 as antigen (Y) were well correlated to those obtained using rRT as antigen (X) (log Y = 0.99 log X + 0.23; r = 0.99). Thus, the use of rp51 as antigen was advantageous over that of rp66 and rRT in an immune complex transfer enzyme immunoassay of antibody IgG to HIV-1.

Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), a heterodimer of p66 and p51 (p66 devoid of C-terminal amino acids) (6, 28), has been reported to be highly reactive with serum samples from HIV-1-seropositive subjects. By conventional enzyme-linked immunosorbent assay (ELISA) (5) and Western blotting (3), the seropositivity rate of sera from subjects infected with either HIV-2 or hepatitis B virus (25). RT of HIV has been reported to be antigenically distinct from those of human T-lymphotropic virus type I (HTLV-I) and HTLV-II (4), and no significant reaction has been observed with serum samples from HTLV-I-infected subjects by the immune complex transfer enzyme immunoassay (13).

Recently, an ultrasensitive enzyme immunoassay (immune complex transfer enzyme immunoassay) for antibody immunoglobulin G (IgG) to HIV-1 using rRT as antigen and β-N-galactosidase from Escherichia coli as label has been developed (13). Antibody IgG to RT was allowed to react simultaneously with 2,4-dinitrophenyl-bovine serum albumin-rRT conjugate and rRT-β-N-galactosidase conjugate, and the immune complex of the three components formed was trapped on polystyrene beads coated with (anti-2,4-dinitrophenyl group) IgG. After washing, the immune complex was transferred to polystyrene beads coated with (anti-human IgG γ-chain) IgG in the presence of 2,4-dinitrophenyl-l-lysine. By this enzyme immunoassay, which was 300- to 1,000-fold more sensitive than Western blotting for p66 band, diagnosis and confirmation of HIV-1 infection using urine (7, 8, 13), whole saliva (19, 20), and serum (9) have become more reliable than by previous methods. Notably, diagnosis of HIV-1 infection was possible using even 1 μl of whole saliva (19). However, the following disadvantages were noted in the use of rRT as antigen. rRT had to be produced using a rather long (3,012-bp) DNA fragment of the whole HIV-1 pol gene (1, 26) and was not efficiently produced in widely used strains of E. coli. In addition, it was impossible to produce fusion proteins of RT for simple purification since rRT is a heterodimer of p66 and p51, as described above.

This report describes the preparation of recombinant HIV-1 p51 and its use as antigen in an immune complex transfer

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enzyme immunoassay of antibody IgG to HIV-1 in comparison with those of recombinant HIV-1 p66 and rRT.

**MATERIALS AND METHODS**

Enzymes and competent **E. coli** cells. Recombinant **Tqag** DNA polymerase (TaKaRa Taq) and ligase were obtained from Takara Shuzo Co., Kyoto, Japan. Restriction enzymes were obtained from New England Biolabs, Inc., Beverly, Mass. Competent cells of **E. coli** DH5α and BL21 were obtained from Life Technologies, Rockville, Md., and Novagen Inc., Madison, Wis., respectively.

Construction of plasmids. Plasmids for the production of recombinant HIV-1 p66 (rp66) and p51 (rp51) were produced using HIV-1 p66 and p51 DNAs of pNL4-3, a plasmid derived from pNL4-3, by PCR amplified from DNA constructed from pNL4-3 and pNL4-3. The sequences of p66 and p51 derived from Ncol. Glycine oxidase tetrandecin (Ncol). Glycine oxidase tetrandecin (Ncol) was inserted into a vector derived from pMAL-c2 (New England Biolabs) after digestion with **Sal** and **Hind** III (pMALMBPSP1102). Second, HIV-1 p66 DNA was produced by PCR using pNL4-3 as template and two primers (5′-GCACCGTGCAATGGATAGTCC TATTAGA and 5′-GGCTTACTATCATGATTTCGTTCC) so as to have **Sal** and **Hind** III split sequences at its 5′ and 3′ ends, respectively. Purification of the recombinant protein was carried out at 4°C throughout, except for the final chromatography with Ultrigel AAc44 (Bioseps, Villevenne la Garene, France) at room temperature. Protein concentrations were determined with a bichoninic acid protein assay reagent kit (Pierce, Rockford, Ill.).

**MBP-Ser-rp66.** The cells from 18 liters of culture medium were suspended in 900 ml of buffer A, sonicated, and centrifuged at 25,000 × g for 20 min. The supernatant fluid (900 ml) was brought to 35% saturation and dialyzed against 2 liters of buffer B. The precipitate was dissolved in 17 ml of buffer C and dialyzed three times against 2 liters of buffer F overnight. The dialysate (17 ml) was applied to a column (2.5 by 4.1 cm) of SP Sepharose FF (Amersham Pharmacia Biotech, Buckinghamshire, England) in buffer B. After washing, MBP-Ser-Ser-rp66 was eluted with a linear gradient of 0 to 0.3 M NaCl in 20 ml of buffer F. The fractions from 0.11 to 0.16 M NaCl were combined and dialyzed twice against 2 liters of buffer F overnight. The dialysate (14 ml) was applied to a column (5.0 by 12.0 cm) of DEAE Sepharose FF (Amersham Pharmacia Biotech) in buffer D and, after washing with buffer D, Ser-Ser-rp66 was eluted with a linear gradient of 0 to 0.5 M NaCl in 200 ml of buffer D. The fractions from 0.08 to 0.4 M NaCl (140 ml, 14 mg of protein) were combined and dialyzed twice against 2 liters of buffer E overnight. The dialysate (141 ml) was applied to a column (1.5 by 2.8 cm) of rabbit (anti-MBP) IgG-Sepharose 4B in buffer E. The flowthrough fractions (151 ml, 13 mg of protein) were applied to a column (2.5 by 2.0 cm) of Affigel heparin (Bio-Rad Laboratories, Hercules, Calif.) in buffer E. After washing with buffer E, Ser-Ser-rp66 was eluted with a linear gradient of 0.05 to 1.0 M NaCl in 100 ml of buffer E. The fractions from 0.1 to 0.6 M NaCl (54 ml, 7.6 mg of protein) were dialyzed twice against 2 liters of buffer F overnight. The dialysate (50 ml) was applied to a column (1.5 by 2.8 cm) of Macro-Prep ceramic hydroxyapatite (type II, 20 μm; Bio-Rad Laboratories) in buffer F and, after washing with buffer F, Ser-Ser-rp66 was eluted with a linear gradient of 0.02 to 0.3 M phosphate in 50 ml of buffer F. The fractions from 0.30 to 0.35 M phosphate (52 ml, 2.4 mg of protein) were combined and dialyzed against 2 liters of buffer F overnight. The dialysate (189 ml) was applied to a column (5.0 by 18.3 cm) of DEAE-Sepharose FF in buffer B. After washing...
FIG. 1. Construction of plasmids for the production of MBP-Ser-Ser-rp66.
FIG. 2. Construction of pMALMBPSP2SP1Ser662121 from pMALMBPSP2SP1p662101.
with buffer B, MBP-Ser-Ser-rp51 was eluted with 450 ml of buffer B containing 0.1 M NaCl. The eluate (125 ml) was applied to a column (5.0 by 6.0 cm) of Amylose resin in buffer B. After washing with buffer B containing 0.1 M NaCl, MBP-Ser-Ser-rp51 was eluted with buffer B containing 10 mM maltose. The eluate (41 ml) was adjusted to a concentration of 0.8 M (NH₄)₂SO₄ by addition of 440 µl of 10% Triton X-100 in water and 500 µl of a 1-mg/ml factor Xa solution at 23°C for 20 h. The reaction mixture was dialyzed against 2 liters of buffer H overnight, and the dialysate (59 ml, 39 mg of protein) was applied to a column (2.5 by 6.0 cm) of SP Sepharose FF in buffer H. After washing with buffer H, Ser-Ser-rp51 was eluted with 150 ml of buffer H containing 0.3 M NaCl. The eluate (36 mg of protein) was applied to a column (2.5 by 3.1 cm) of Macro-Prep ceramic hydroxyapatite (type II, 20 µm) in buffer H. After washing with buffer H, Ser-Ser-rp51 was eluted with 150 ml of 0.2 M sodium phosphate buffer, pH 6.8, containing 0.1% Triton X-100 and 150 ml of 0.4 M sodium phosphate buffer, pH 6.8, containing 0.1% Triton X-100. The eluate fractions (18 and 23 ml, 17 mg of protein) were combined and concentrated with Centricon 30. The concentrated fractions (1.1 ml) were applied to a column (1.5 by 56 cm) of Ultrogel AcA44 in buffer G. Fractions 61 to 71 (11 ml) were concentrated to 1.7 ml containing 7.2 mg of protein with Centricon 30 and were used for labeling with 2,4-dinitrophenyl groups and α-D-galactosidase.

**Antibodies.** Rabbit (anti-2,4-dinitrophenyl–bovine serum albumin) serum and rabbit anti-MBP serum were obtained from Shibayagi, Gumma, Japan. Rabbit (anti-human IgG γ-chain) IgG was obtained from Medical and Biological Laboratories, Nagoya, Japan. IgG was prepared from serum by fractionation with Na₂SO₄ followed by passage through a column of diethylaminoethyl cellulose (17).

**Coupling of proteins to Sepharose 4B.** Rabbit (anti-MBP) IgG (10 mg), 2,4-dinitrophenyl–bovine serum albumin (10 mg), and human IgG (10 mg) were coupled to 1.0 g of CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech) in accordance with the manufacturer's instructions.

**Affinity purification of antibodies.** Affinity-purified (anti-2,4-dinitrophenyl–bovine serum albumin) IgG (22) and (anti-human IgG γ-chain) IgG (23) were prepared by elution at pH 2.5 from columns of 2,4-dinitrophenyl–bovine serum albumin–Sepharose 4B and human IgG-Sepharose 4B, respectively.

**Coating of polystyrene beads with proteins.** Colored and white polystyrene beads (3.2 mm in diameter; Immuno Chemical, Okayama, Japan) were coated by physical adsorption with affinity-purified (anti-2,4-dinitrophenyl–bovine serum albumin) IgG (0.1 g/liter) and affinity-purified (anti-human IgG γ-chain) IgG (0.1 g/liter), respectively (18).
Labeling of recombinant proteins with 2,4-dinitrophenyl groups. Mercaptoacetyl-Ser-Ser-rp51. Ser-Ser-rp51 (0.84 mg, 16 nmol) in 1 ml of buffer G was incubated with 30 μl of 7.2 mM N-succinimidyl-3-acylmercaptoacetate (Pierce) in N,N-dimethylformamide at 30°C for 10 min, and was processed as described previously (14, 17). The average number of thiol groups introduced per Ser–Ser–rp51 molecule was 4.0 (17).

(i) 2,4-Dinitrophenyl–Ser–Ser–rp51 conjugate. Mercaptoacetyl-Ser-Ser-rp51 (0.08 mg, 0.5 nmol) in 1 ml of buffer J was incubated at 4°C for 20 h with N-6-maleimidohexanoyl-ε-N,2,4-dinitrophenyl-L-lysine solution, which had been prepared by incubation of ε-N,2,4-dinitrophenyl-L-lysine hydrochloride (Sigma) (0.18 mg, 0.46 μmol) in 91 μl of N,N-dimethylformamide with 0.25 μl of 0.1 M sodium phosphate buffer, pH 7.0, and N-succinimidyl-6-maleimidohexanoate (Dojindo Laboratories, Kumamoto, Japan) (0.12 mg, 0.38 μmol) in 38 μl of N,N-dimethylformamide at 30°C for 30 min. The reaction mixture was subjected to gel filtration on a Sephadex G-25 medium (Amersham Pharmacia Biotech) column (1.0 by 30 cm) in buffer G containing 0.1 M NaCl. The average number of 2,4-dinitrophenyl groups introduced per Ser-Ser-rp51 molecule was 3.1 (10). Fractions containing the conjugate were stored at 4°C after addition of bovine serum albumin (0.1 μl/g) and NaN3 (1 μl/g) until use.

(ii) 2,4-Dinitrophenyl–Ser–Ser–rp66 conjugate. 2,4-Dinitrophenyl–Ser–Ser–rp66 conjugate was prepared using mercaptoacetyl-Ser-Ser-rp66 (0.24 mg, 3.7 nmol) and ε-N-6-maleimidohexanoyl-ε-N,2,4-dinitrophenyl-L-lysine solution (71 μg, 109 nmol) as described above. HIV-1 rRT, prepared as described previously (26), was 2,4-dinitrophenylated using mercaptoacetyl-rRT (0.24 mg, 3.7 nmol) and ε-N-6-maleimidohexanoyl-ε-N,2,4-dinitrophenyl-L-lysine solution (48 μg, 74 nmol) as described above. The average number of maleimide groups introduced per β-ogalactosidase molecule was 2.7 (17).

(iii) Ser–Ser–rp51–β-ogalactosidase conjugate. The reaction mixture was incubated with 2,4-dinitrophenyl–bovine serum albumin (4 mg/ml) in 0.1 ml of buffer G at 4°C for 20 h. After incubation, the reaction mixture was incubated with 22 μl of 0.1 M NaCl and 0.05% Triton X-100. Fractions containing the conjugate were stored at 4°C until use. The average number of Ser-Ser-rp51 molecules conjugated per β-ogalactosidase molecule was 1.7 as calculated from the decrease in the number of maleimide groups (17). The amount of conjugate was calculated by β-ogalactosidase activity.

(iv) Ser–Ser–rp66–β-ogalactosidase and rRT–β-ogalactosidase conjugates. Ser-Ser-rp66–β-ogalactosidase and rRT–β-ogalactosidase conjugates were prepared using mercaptoacetyl-Ser–Ser–rp66 (0.15 mg, 2.3 nmol), maleimide–β-ogalactosidase (0.27 mg, 0.5 nmol), mercaptoacetyl-rRT (0.44 mg, 7.0 nmol), and mercaptoacetyl-β-ogalactosidase (0.094 mg, 1.8 nmol) as described above for Ser-Ser–rp51–β-ogalactosidase conjugate.

Immune complex transfer enzyme immunoassay of antibody IgG to HIV-1. Immune complex transfer enzyme immunoassay of antibody IgG to HIV-1 was performed essentially in the same ways as described previously (12, 17). An aliquot (10 μl) of serum samples was incubated with 140 μl of buffer J containing 0.4 M NaCl, 50 μg of inactive β-ogalactosidase (β-galactosidase–mutinein; Boehringer Mannheim), 100 fmol each of 2,4-dinitrophenyl-antigen and antigen–β-ogalactosidase conjugate at room temperature for 2 h. Inactive β-ogalactosidase was used to eliminate interference by anti-β-ogalactosidase antibodies (24). The reaction mixture was incubated for 1 h with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG. The colored polystyrene beads were washed twice by addition and aspiration of 2 ml of buffer J containing 0.1 M NaCl and were incubated for 1 h with two white polystyrene beads coated with affinity-purified (anti-human IgG γ-chain) IgG in 150 μl of buffer J containing 0.1 M NaCl and 1.0 mM ε-N,2,4-dinitrophenyl-L-lysine. The incubations with polystyrene beads were performed with shaking at 180 rpm at room temperature throughout. After washing as described above, β-ogalactosidase activity bound to the white polystyrene beads was assayed at 30°C for 1 h by fluorometry using 4-methylumbelliferyl-β-o-galactoside as substrate (16). The fluorescence intensity was measured relative to 100% 4-methylumbelliferyl-β-o-galactoside as described in 0.1 M glycine-NaOH, pH 10.3, using 360 nm for excitation and 450 nm for emission analysis with a spectrofluorophotometer (F-3100; Hitachi, Tokyo, Japan).

Western blotting. Western blotting was performed with a commercial kit (Serodia-HIV; Fujirebio Inc., Tokyo, Japan) as described previously (12), and seropositivity was confirmed by Western blotting using a commercial kit (Ortho kit) (7).

RESULTS

Production and purification of recombinant HIV-1 proteins. In order to detect antibody IgG to HIV-1 RT (a heterodimer of p51 and p66), recombinant HIV-1 p51 and p66 with Ser-Ser at the N termini (Ser-Ser-rp51 and Ser-Ser-rp66) were produced as fusion proteins with MBP containing a factor Xa site between the two proteins in E. coli transformed with plasmids containing the corresponding DNAs and were purified from sonic extracts of the cells by successive processes of column chromatographies with DEAE Sepharose and Amynlose resin, digestion with factor Xa, and column chromatographies with SF Sepharose, (anti-MBP) IgG-Sepharose, Affi-Gel heparin, hydroxyapatite, and Ultrogel ACA4.

Electrophoresis of sonic extracts indicated that MBP-Ser-Ser-rp51 was produced in much larger amounts per unit of culture medium volume than MBP-Ser-rp66. This was reflected in the fact that the amounts of MBP-Ser-Ser-rp51 and MBP-Ser-Ser-rp66 obtained from a column of Amynlose resin were 215 and 44 mg, respectively (Table 1). The final yields of Ser-Ser-rp51 and Ser-Ser-rp66 from a culture medium volume of 18 liters which could be used for labeling with 2,4-dinitrophenyl groups and β-ogalactosidase were 7.2 and 1.0 mg (Peak II), respectively (Table 1). In addition, Ser-Ser-rp66 was converted to a mixture of almost equal amounts of Ser-Ser-rp66 and a smaller molecule, probably Ser-Ser-rp51, during purification and was polymerized to various extents in the eluate from a column of hydroxyapatite (Fig. 4). Polymerized Ser-Ser-rp66 tended to be precipitated on mercaptoacetylation for conjugation to β-ogalactosidase.

Comparison of Ser-Ser-rp51 with Ser-Ser-rp66 and rRT as antigens by immune complex transfer enzyme immunoassay. Ser-Ser-rp51 was compared with Ser-Ser-rp66 and rRT as antigens by immune complex transfer enzyme immunoassay of antibody IgG to HIV-1 using β-ogalactosidase from E. coli as label. rRT was produced in E. coli transformed with a plasmid containing the HIV-1 pol gene for protease, RT, and integrase and was purified as described previously (26). Serum samples (10 μl) were incubated with 2,4-dinitrophenyl–bovine serum albumin–antigen conjugate and antigen–β-ogalactosidase conjugate and subsequently with colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG to trap the immune complex of the three components formed. After washing, the colored polystyrene beads were incubated with 2,4-dinitrophenyl-L-lysine and white polystyrene beads coated with affinity-purified (anti-human IgG γ-chain) IgG to transfer the immune complex from the colored polystyrene beads to the white ones. β-ogalactosidase activity bound to the white polystyrene beads was assayed by fluorometry.

(i) Dilution curves of two positive serum samples. When two serum samples from HIV-1-seropositive subjects serially diluted up to 100-fold with serum from an HIV-1-seronegative subject were tested, the dilution curves obtained by using Ser-Ser-rp51 as antigen were almost completely parallel with those obtained by using rRT. However, Ser-Ser-rp66 showed much...
higher nonspecific and lower specific signals than Ser-Ser-rp51. Therefore, Ser-Ser-rp66 was not used in the following experiments.

(ii) Positive and negative signals for more serum samples. The positive and negative signals were examined using serum samples from 79 HIV-1-seropositive subjects (50 asymptomatic carriers, 9 patients with ARC, and 20 patients with AIDS) and 200 HIV-1-seronegative subjects, respectively.

The positive signals obtained with Ser-Ser-rp51 as antigen (Y) were well correlated to those obtained with rRT as antigen (X) \((\log Y = 0.99 \log X + 0.23; r = 0.99)\) (Fig. 5) and were up to 2.4-fold (1.6-fold, on average) higher than those obtained with rRT as antigen for 76 (96%) of the 79 seropositive subjects but equal or 1.2- to 1.7-fold lower for 3 seropositive subjects (4%) (Fig. 5 and 6). The negative signals obtained with Ser-Ser-rp51 \((0.46 \pm 0.34 \text{ [standard deviation]; range, 0 to 2.0})\) were not significantly different from those obtained with rRT \((0.44 \pm 0.34 \text{ [standard deviation]; range, 0 to 5.2})\). The lowest positive signals for the asymptomatic carriers and the patients with ARC and AIDS were 124,000-, 172,000-, and 13,000-fold, respectively, higher than the highest negative signal (Fig. 6). Namely, the sensitivity, specificity and, positive and negative predictive values were all 100% (Table 2).

Comparison of immune complex transfer enzyme immunoassays using Ser-Ser-rp51 and rRT as antigens with Western blotting. Two serum samples from HIV-1-seropositive subjects serially diluted with serum from an HIV-1-seronegative subject were tested by immune complex transfer enzyme immunoassay using Ser-Ser-rp51 as antigen and Western blotting (Ortho HIV Western Blot Kit) for p51 and p66 bands. The former was 1,000- to 6,000-fold more sensitive than the latter.

![FIG. 4. Elution profiles of Ser-Ser-rp66 (A) and Ser-Ser-rp51 (B) from a column of Ultrogel AcA44. The flow rate was 0.4 ml/min, and the fraction volume was 1.0 ml. See Materials and Methods for other conditions.](image1)

![FIG. 5. Correlation between signals by immune complex transfer enzyme immunoassays using Ser-Ser-rp51 and rRT as antigens. Circles, triangles, and squares indicate serum samples from HIV-1-asymptomatic carriers and patients with ARC and AIDS, respectively. The regression equation was \(\log Y\) (signals with Ser-Ser-rp51) = 0.99 \(\log X\) (signals with rRT) + 0.23, and the correlation coefficient was 0.99.](image2)
In addition, immune complex transfer enzyme immunoassays using Ser-Ser-rp51 and rRT as antigens were compared with Western blotting using the 79 serum samples from HIV-1-seropositive subjects and the 100 to 200 serum samples from HIV-1-seronegative subjects described above (Fig. 6 and Table 2). The detection rates of antibody IgG to gp160, gp41, p66, p51, p24, and p17 by Western blotting were 100, 99, 96, 90, 87, and 71%, respectively. The specificities and predictive values of Western blotting were less than 100% (82 to 99%), except that the specificity and positive predictive value for p66 band and the negative predictive values for gp160 and gp41 bands were 100%. In contrast, the detection rates of antibody IgG to HIV-1 by immune complex transfer enzyme immunoassays using Ser-Ser-rp51 and rRT as antigens and the specificities and predictive values of immune complex transfer enzyme immunoassays using Ser-Ser-rp51 and rRT were all 100%.

Comparison of immune complex transfer enzyme immunoassay using Ser-Ser-rp51 as antigen with the conventional ELISA using five recombinant proteins as antigen. Immune complex transfer enzyme immunoassay with Ser-Ser-rp51 as antigen was compared with the conventional ELISA using five recombinant proteins (gp120, gp41, p24, p17, and p15) as antigens using the 79 serum samples from HIV-1-seropositive subjects and the 131 to 200 serum samples from HIV-1-seronegative subjects described above (Table 2). The sensitivity, specificity, and predictive values of the immune complex transfer enzyme immunoassay were all 100%. However, the specificity and positive predictive value of the conventional ELISA were 99%. Furthermore, the lowest signals for the seropositive subjects by immune complex transfer enzyme immunoassay and the conventional ELISA were 13,000- and 124-fold, respectively, higher than the highest signals for the seronegative subjects.

**DISCUSSION**

Both Ser-Ser-rp51 and Ser-Ser-rp66 were readily produced as fusion proteins with MBP for easy purification using short

**TABLE 2. Sensitivity, specificity, and predictive values of various methods**

| Method and antigen(s) used | Sensitivity (%) (no. positive/no. tested) | Specificity (%) (no. negative/no. tested) | Predictive value |
|----------------------------|------------------------------------------|------------------------------------------|-----------------|
|                            |                                          |                                          | Positive (%)    | Negative (%) |
| Immune complex transfer enzyme immunoassay |                                          |                                          |                 |
| rRT                       | 100 (79/79)                              | 100 (200/200)                           | 100             | 100          |
| Ser-Ser-rp51              | 100 (79/79)                              | 100 (200/200)                           | 100             | 100          |
| Western blotting          |                                          |                                          |                 |
| gp160                     | 100 (79/79)                              | 99 (99/100)                             | 99              | 100          |
| gp41                      | 99 (78/79)                               | 98 (98/100)                             | 98              | 100          |
| p66                       | 96 (76/79)                               | 100 (100/100)                           | 100             | 99           |
| p51                       | 90 (71/79)                               | 97 (97/100)                             | 96              | 95           |
| p24                       | 87 (69/79)                               | 85 (85/100)                             | 82              | 90           |
| p17                       | 71 (56/79)                               | 97 (97/100)                             | 95              | 82           |
| ELISA; gp120, gp41, p24, p17, p15 | 100 (79/79)                              | 99 (130/131)                           | 99              | 100          |
DNA fragments with 1,320 and 1,680 bp, respectively, while rRT had to be produced using a rather long DNA with 3,012 bp of the whole HIV-1 pol gene and could not be produced as a fusion protein with MBP due to the fact that RT is a heterodimer of p51 and p66 (1, 26). However, the use of Ser-Ser-rp51 as antigen seemed to be more competent for than in the conventional ELISA. Ser-Ser-rp51 showed higher specific and lower nonspecific signals than those of Ser-Ser-rp66 and specific signals as high as those of rRT (Fig. 5 and 6).

The detection rate of antibody IgG to HIV-1 in 79 HIV-1-seropositive subjects by immune complex transfer enzyme immunoassay using Ser-Ser-rp51 as antigen was 100% (Fig. 6), while the detection rates of antibody IgGs to p51 and p66 by Western blotting in 79 HIV-1-seropositive subjects were 90 to 96% (both 96%) in 50 asymptomatic carriers, both 100% in 9 patients with ARC, and 70% and 95%, respectively, in 20 patients with AIDS (Table 2). This was consistent with a previous report that the detection rates of antibody IgG to HIV-1 by Western blotting for p51 and p66 bands were both 96% in asymptomatic carriers, 30 and 100%, respectively, in patients with ARC, and both 86% in patients with AIDS (15) and with the finding described above that the immune complex transfer enzyme immunoassay for antibody IgG to HIV-1 using Ser-Ser-rp51 as antigen was 1,000- to 6,000-fold more sensitive than Western blotting for p66 and p51 bands when two serially diluted serum samples from HIV-1-seropositive subjects were tested.

In the immune complex transfer enzyme immunoassay using Ser-Ser-rp51 alone as antigen, the difference between the lowest signal for 79 HIV-1-seropositive subjects and the highest signal for 200 HIV-1-seronegative subjects was 13,000-fold (Fig. 6), while the difference was only 124-fold in the conventional ELISA using Ser-Ser-rp51 alone as antigen, the difference between the lowest signal for 200 HIV-1-seronegative subjects and the highest signal for 79 HIV-1-seropositive subjects was 13,000-fold, respectively, in 5 patients with ARC, and both 86% in patients with AIDS (15) and with the finding described above that the immune complex transfer enzyme immunoassay for antibody IgG to HIV-1 using Ser-Ser-rp51 as antigen was 1,000- to 6,000-fold more sensitive than Western blotting for p66 and p51 bands when two serially diluted serum samples from HIV-1-seropositive subjects were tested.

In the immune complex transfer enzyme immunoassay using Ser-Ser-rp51 alone as antigen, the difference between the lowest signal for 79 HIV-1-seropositive subjects and the highest signal for 200 HIV-1-seronegative subjects was 13,000-fold (Fig. 6), while the difference was only 124-fold in the conventional ELISA using Ser-Ser-rp51 alone as antigen, the difference between the lowest signal for 200 HIV-1-seronegative subjects and the highest signal for 79 HIV-1-seropositive subjects was 13,000-fold, respectively, in 5 patients with ARC, and both 86% in patients with AIDS (15) and with the finding described above that the immune complex transfer enzyme immunoassay for antibody IgG to HIV-1 using Ser-Ser-rp51 as antigen was 1,000- to 6,000-fold more sensitive than Western blotting for p66 and p51 bands when two serially diluted serum samples from HIV-1-seropositive subjects were tested.
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