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Optimal Conditions of Immune Complex Transfer Enzyme Immunoassays for Antibody IgGs to HIV-1 Using Recombinant p17, p24, and Reverse Transcriptase as Antigens

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The immune complex transfer enzyme immunoassays for antibody IgGs to p17, p24, and reverse transcriptase (RT) of HIV-1 were tested under various conditions. Antibody IgGs to HIV-1 were reacted for up to 20 hr with 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates and recombinant HIV-1 protein-β-D-galactosidase conjugates, and the immune complexes formed, comprising the three components, were trapped onto polystyrene beads coated with (anti-2,4-dinitrophenyl group) IgG by incubation at 4–30°C for up to 2 hr with shaking and were transferred onto polystyrene beads coated with (antihuman IgG γ-chain) IgG in the presence of excess of *εN*-2,4-dinitrophenyl-L-lysine by incubation at 4–30°C for up to 2 hr with shaking. When serum randomly collected from an HIV-1 seropositive subject and serum included in an Western blot kit were tested, the formation of the immune complex was almost completed within 1 hr for antibody IgG to p17, within 1–2 hr for antibody IgG to p24 and within 4 hr for antibody IgG to RT. Even for antibody IgG to p17, however, the immune complex continued to be formed for at least 2 hr, when serum samples at early stages of HIV-1 infection were tested. Trapping and transferring of the immune complexes were faster at higher temperatures and were almost completed within 0.5–1.5 hr, although the amount of the immune complexes trapped and transferred at 25 and/or 30°C increased for 0.5–1 hr, but subsequently tended to decline. When the formation, trapping, and transferring of the immune complexes were performed for 0.5, 1, and 1 hr, respectively, with shaking followed by 1 hr assay of bound β-D-galactosidase activity, the sensitivities for antibody IgGs to p17, p24, and RT using 10 µl of serum samples were similar to or significantly higher than those of the corresponding previous immune complex transfer enzyme immunoassays using 10 µl of serum samples, in which the formation, trapping, and transferring of the immune complexes were performed for 3, 16, and 3 hr, respectively, without shaking, followed by 2.5 hr assay of bound β-D-galactosidase activity, and the sensitivities for antibody IgGs to p17, p24, and RT using 100 µl of serum samples were 21–22-fold, 5.5–6.3-fold, and 5.3–6.0-fold, respectively, higher. When each period of time for the formation, trapping, and transferring of the immune complexes was prolonged to up to 4 hr, the sensitivities for antibody IgGs to p17, p24, and RT using 100 µl of serum samples were improved 88–93-fold, 15–17-fold and 20–24-fold, respectively, as compared with those of the previous ones. J. Clin. Lab. Anal. 12:98–107, 1998.
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INTRODUCTION

Ultrasensitive enzyme immunoassays (immune complex transfer enzyme immunoassays) for antibody IgGs to p17, p24, and reverse transcriptase (RT) of HIV-1 have been developed using recombinant p17, p24, and RT (rp17, rp24, and rRT) as antigens (1–7). The immune complexes, comprising 2,4-

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dinitrophenyl-recombinant protein conjugates, antibody IgGs to HIV-1 and recombinant protein-β-D-galactosidase conjugates, were formed by 3 hr incubation and were trapped onto polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG by overnight incubation. After washing, the immune complexes were eluted from the polystyrene beads with *εN*-2,4-dinitrophenyl-L-lysine and were transferred to polystyrene beads coated with affinity-purified (antihuman IgG γ-chain) IgG by 3 hr incubation. Bound β-D-galactosidase activity was assayed by fluorometry for 2.5 hr. The volume of serum samples used was 10 µl. These immunoassays have made possible diagnosis of HIV-1 infection with urine (1-4) and whole saliva samples (5,6) and have been shown to be more useful as a confirmatory test with higher sensitivities and specificities than Western blotting (7). Notably, antibody IgG to p17 of HIV-1 could be detected as early as or even earlier than antibodies to HIV-1 by conventional methods (7,8). In addition, an ultrasensitive enzyme immunoassay based on a similar principle for p24 antigen of HIV-1 has also been developed (9,10). On the basis of these results, the window period after HIV-1 infection, during which diagnosis of HIV-1 infection is not possible due to the absence of detectable antibodies to HIV-1, has been shortened by simultaneous detection of both p24 antigen and antibody IgGs to p17 and RT (8). In these immunoassays, however, the period of time used for the immunoreactions involved was considerably long as described above. Recently, it has been made possible to perform more sensitive immune complex transfer enzyme immunoassays for antibody IgG to p17 within shorter periods of time (15–60 min each for the formation, trapping, and transferring of the immune complex) by incubations for the immunoreactions with shaking using a larger volume of serum samples (100 µl) and solid phases with larger surface areas (11).

This report describes optimal conditions of the immune complex transfer enzyme immunoassays for antibody IgGs to p17, p24, and reverse transcriptase of HIV-1.

MATERIALS AND METHODS

Buffer

The regularly used buffer was 10 mmol/L sodium phosphate buffer, pH 7.0, containing 1.0 g/L bovine serum albumin (fraction V, Intergen, Purchase, NY), 1.0 mmol/L $MgCl_2$ and 1.0 g/L NaN_3 (buffer A).

Recombinant Proteins of HIV-1

Recombinant reverse transcriptase (rRT), recombinant p17 (rp17) and recombinant p24 (rp24) were produced in *Escherichia coli* transformed with expression plasmids carrying the corresponding cDNAs and were purified as described previously (1,12–14). The recombinant proviral clone used was pNL4-3 (15), which contained DNA from HIV-1 isolates NY5 (GenBank accession number HIVNL43) and LAV (16), and the sequences for RT, p17, and p24 derived from NY5.

Previous Immune Complex Transfer Enzyme Immunoassays for Antibody IgGs to HIV-1

Antibody IgGs to p17, p24, and RT of HIV-1 were measured essentially in the same way as described previously (7). An aliquot (10 µl) of serum was mixed with 90 µl of buffer A containing 0.4 mol/L NaCl and was incubated for 3 hr with 50 µl of buffer A containing 0.4 mol/L NaCl, inactive β-D-galactosidase (50 µg), and 100 fmol each of 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugate and recombinant HIV-1 protein-β-D-galactosidase conjugate. To the reaction mixture, two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG were added, and the incubation was continued for 16 hr. The colored polystyrene beads were washed and incubated for 1 hr with two white polystyrene beads coated with affinity-purified (antihuman IgG γ-chain) IgG in 150 µl of buffer A containing 0.1 mol/L NaCl and 1 mmol/L *εN*-2,4-dinitrophenyl-L-lysine. The colored polystyrene beads were removed, and the incubation was continued for 2 hr. The incubations were performed at room temperature without shaking. The white polystyrene beads were washed and β-D-galactosidase activity bound was assayed at 30°C for 2.5 hr by fluorometry using 4-methylumbelliferyl-β-D-galactoside as substrate (17). The fluorescence intensity was measured by adjusting that of 1×10^{-8} mol/L 4-methylumbelliferone in 0.1 mol/L glycine-NaOH buffer, pH 10.3 to 100 using 360 nm for excitation and 450 nm for emission analysis with a spectrofluorophotometer (F-3010, Hitachi, Tokyo, Japan).

Present Immune Complex Transfer Enzyme Immunoassays for Antibody IgGs to HIV-1

The present immune complex transfer enzyme immunoassays were performed in the same way as the previous ones described above except for the following modifications. Serum samples (10 µl or 100 µl) were incubated with the two conjugates and inactive β-D-galactosidase for up to 20 hr and subsequently with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for up to 2 hr. When 100 µl of serum samples was used, the concentration of NaCl in the final mixture of serum samples and the two conjugates was adjusted to 0.4 mol/L assuming that in serum to be 0.15 mol/L. The colored polystyrene beads after washing were incubated for up to 2 hr with affinity-purified (antihuman IgG γ-chain) IgG-coated polystyrene beads in the presence of *εN*-2,4-dinitrophenyl-L-lysine. The incubations were performed at 4, 25, and 30°C with 180 shakings per min throughout. Bound β-D-galactosidase activity was assayed at 30°C for up to 4 hr.

Other Immunological Methods

The gelatin particle agglutination test for antibodies to HIV-1 was performed using a commercial kit with a lysate of

HIV-1 as antigen (SERODIA-HIV, Fujirebio, Tokyo, Japan). Western blotting for antibody IgGs to HIV-1 was performed using a commercial kit preblotted with nine proteins of HIV-1 (gp160, gp120, p66, p55, p51, gp41, p31, p24, and p17) (Ortho HIV Western Blot Kit, Ortho Diagnostic Systems, Raritan, NJ).

Seroconversion Serum Panels

Two seroconversion serum panels, SV-0241 and Panel Z, were obtained from North American Biologicals (Miami, FL) and Boston Biomedica (West Bridgewater, MA), respectively.

Serum Samples Randomly Collected From HIV-1 Seronegative and Seropositive Subjects

Serum samples were randomly collected from 50 HIV-1 seronegative subjects (23 males, ages 22–59 yr and 27 females, ages 27–73 yr) and two HIV-1 seropositive subjects (male and female asymptomatic carriers (AC), ages 22 and 39 years) and were stored at –20°C until use. The seronegativity and seropositivity were tested by the gelatin particle agglutination test. The seropositivity was confirmed by Western blotting.

RESULTS AND DISCUSSION

In the present immune complex transfer enzyme immunoassays for antibody IgGs to HIV-1, the immune complexes comprising 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates, antibody IgGs to HIV-1, and recombinant HIV-1 protein-β-D-galactosidase conjugates were trapped onto colored polystyrene beads coated with (anti-2,4-dinitrophenyl group) IgG and were transferred to white polystyrene beads coated with (antihuman IgG γ-chain) IgG in the presence of excess of *εN*-2,4-dinitrophenyl-L-lysine (Fig. 1). Incubations for the immunoreactions were performed at 4–30°C with shaking throughout, and β-D-galactosidase activity bound to the white polystyrene beads was assayed by fluorometry at 30°C. The period of time required for each immunoreaction and the sensitivity were examined.

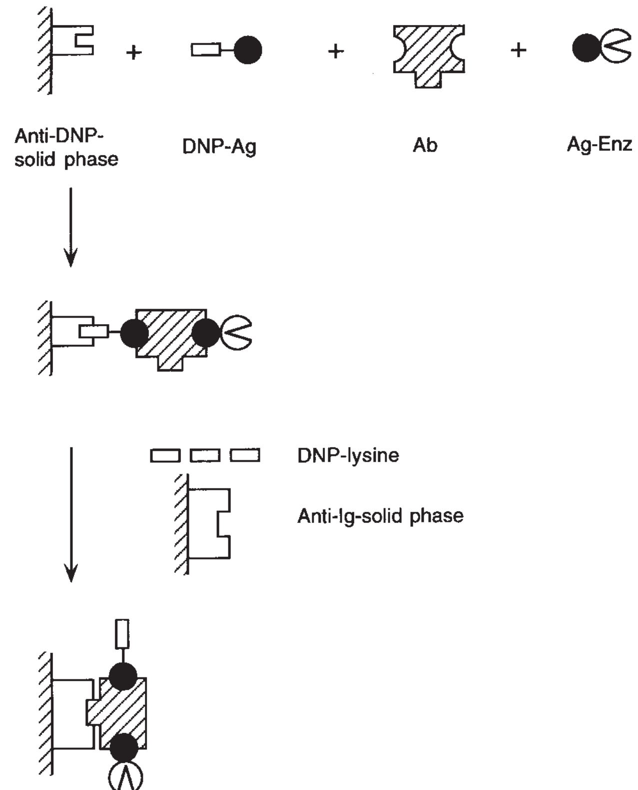

Fig. 1. Immune complex transfer enzyme immunoassay for antibody IgG. DNP: 2,4-dinitrophenyl group. Ag: antigen. Enz: enzyme.

Time Courses for the Formation of the Immune Complexes Comprising Antibody IgGs to HIV-1 and the Two Conjugates

In order to determine the period of time required for the formation of the immune complexes, serum samples containing antibody IgGs to HIV-1 were incubated at room temperature with 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates and recombinant HIV-1 protein-β-D-galactosidase conjugates at room temperature for up to 20 hr and subsequently with the colored polystyrene beads for 20 min with shaking (Fig. 2). When serum from an HIV-1 seropositive subject and serum included in an Western blot kit for HIV antibody test (Ortho Diagnostic Systems) were tested, β-D-galactosidase activities bound to the colored polystyrene beads reached values close to the maximum within 1 hr for antibody IgG to p17, within 1–2 hr for antibody IgG to p24, and within 4 hr for antibody IgG to RT. Even for antibody IgG to p17, however, bound β-D-galactosidase activity continued to increase for 1 hr, when serum samples in seroconversion serum panels, namely, at early stages of the infection, were tested. Thus the formation of the immune complex was almost completed within 1 hr for antibody IgG to p17, within 1–2 hr for antibody IgG to p24 and within 4 hr for antibody IgG to RT, although longer periods of time were required with some serum samples at early stages of HIV-1 infection.

Time Courses of Trapping the Immune Complexes Comprising Antibody IgGs to HIV-1 and the Two Conjugates

On the basis of the above results, serum samples included in the Ortho Western blot kit described above after dilution with serum from an HIV-1 seronegative subject were incubated at room temperature with the two rp17 and rp24 conjugates for 1.5 hr and with the two rRT conjugates for 4 hr and subsequently with the colored polystyrene beads at 4, 25 or 30°C for up to 2 hr with shaking (Fig. 3). β-D-Galactosidase activity bound to the colored polystyrene beads at 25°C reached

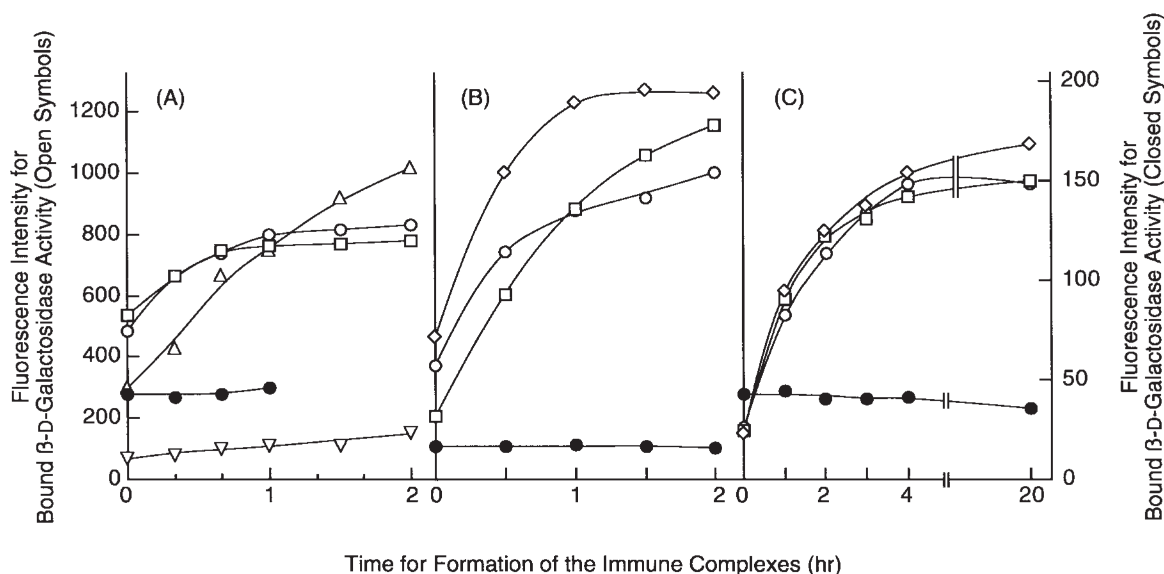

Fig. 2. Time course for the formation of the immune complexes comprising 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates, antibodies to HIV-1 proteins and recombinant HIV-1 protein-β-D-galactosidase conjugates. **A.** Antibody IgG to p17. **B.** Antibody IgG to p24. **C.** Antibody IgG to RT. An aliquot (10 µl) of serum samples was incubated at room temperature with 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugate and recombinant HIV-1 protein-β-D-galactosidase conjugate for up to 20 hr and subsequently with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 20 min with shaking. β-D-Galactosidase activity bound to the colored polystyrene beads was assayed at 30°C for 1 hr. Open circles: Serum included in a Western blot kit for HIV antibody test (Ortho Diagnostic Systems) was diluted 5,000-fold (A), 2,000-fold (B), and 400-fold (C) with serum from an HIV-1 seronegative subject. Open squares: Serum from an HIV-1 seropositive subject was diluted 10,000-fold (A), 5,000-fold (B), and 2,500-fold (C) with serum from an HIV-1 seronegative subject. Open rhombuses: Serum from another HIV-1 seropositive subject was diluted 2,000-fold (B and C) with serum from an HIV-1 seronegative subject. Open triangles: Serum on the day of 27 in panel Z, Boston Biomedica, was used without dilution. Open counter triangles: Serum on the day of 14 in panel SV-0241, North American Biologicals, was used without dilution. Closed circles: Nonspecific rabbit serum was substituted for human serum samples.

values close to the maximum within 1–1.5 hr for all the three antibody IgGs. With the rp17 conjugates, β-D-galactosidase activity bound at 4°C increased more slowly and reached values close to the maximum within 2 hr, and that bound at 30°C increased faster but decreased after 1 hr. Thus trapping of the immune complexes at 25°C was completed within 1–1.5 hr for all the three antibody IgGs. Trapping of the immune complex for antibody IgG to p17 was slower at 4°C and faster at 30°C, and the immune complex trapped at 25°C and 30°C was lost slowly after 1 hr and 0.5 hr, respectively. β-D-Galactosidase activities bound nonspecifically in the absence of antibody IgGs to HIV-1 were higher at higher temperatures and reached the maximal values within 0.5–1 hr.

Time Courses of Transferring the Immune Complexes Comprising Antibody IgGs to HIV-1 and the Two Conjugates

On the basis of the above results, serum samples included in the Ortho Western blot kit described above after dilution with serum from an HIV-1 seronegative subject were incubated at room temperature with the two rp17 and rp24 conjugates for 1.5 hr and with the two rRT conjugates for 4 hr and subsequently with the colored polystyrene beads for 1 hr with shaking. The colored polystyrene beads after washing were incubated with the white polystyrene beads in the presence of *εN*-2,4-dinitrophenyl-L-lysine at 4, 25 and 30°C for up to 2 hr with shaking (Fig. 4). Bound β-D-galactosidase activities increased faster at higher temperatures, reached values close to the maximum within 1–1.5 hr at 4 to 30°C, and decreased after 1 hr at 30°C for antibody IgG to p17 and after 1.5 hr for antibody IgG to RT. Thus transferring of the immune complexes was completed within 1–1.5 hr for all the three antibody IgGs, although faster at higher temperatures. The immune complexes trapped at 25–30°C were lost after 1–1.5 hr for antibody IgGs to p17 and RT. β-D-Galactosidase activities bound nonspecifically in the absence of antibody IgGs to HIV-1 were very low.

Sensitivities of the Present Immune Complex Transfer Enzyme Immunoassays for Antibody IgGs to HIV-1

Serum randomly collected from an HIV-1 seropositive subject was serially diluted with serum from an HIV-1 seronegative subject, and the diluted serum samples were tested by the present immune complex transfer enzyme immunoassays under various conditions. The period of time for each immunoreaction

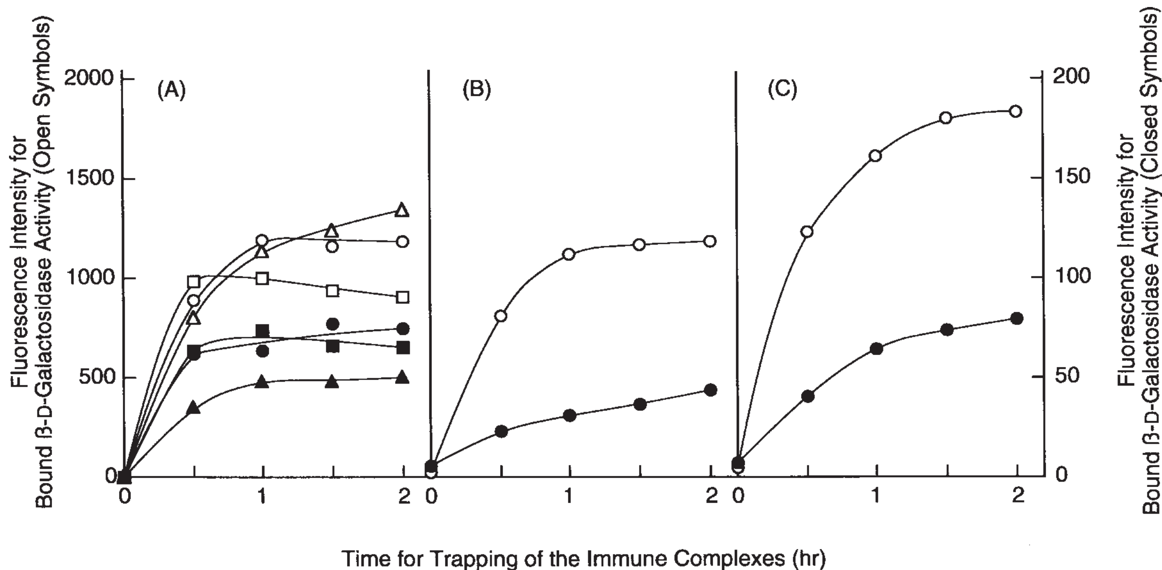

Fig. 3. Time course for trapping of the immune complexes. **A.** Antibody IgG to p17. **B.** Antibody IgG to p24. **C.** Antibody IgG to RT. An aliquot (10 μl) of serum samples was incubated at room temperature with 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates and recombinant HIV-1 protein-β-D-galactosidase conjugates for 1.5 hr (A and B) and 4 hr (C) and subsequently with colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG at 4°C (triangles), 25°C (circles) and 30°C (squares) for up to 2 hr. β-D-Galactosidase activity bound to the colored polystyrene beads was assayed at 30°C for 1 hr. Open symbols: Serum included in the Ortho Western blot kit used in Figure 2 was diluted 5,000-fold (A), 2,000-fold (B), and 400-fold (C) with serum from an HIV-1 seronegative subject. Closed symbols: Nonspecific rabbit serum was substituted for human serum samples.

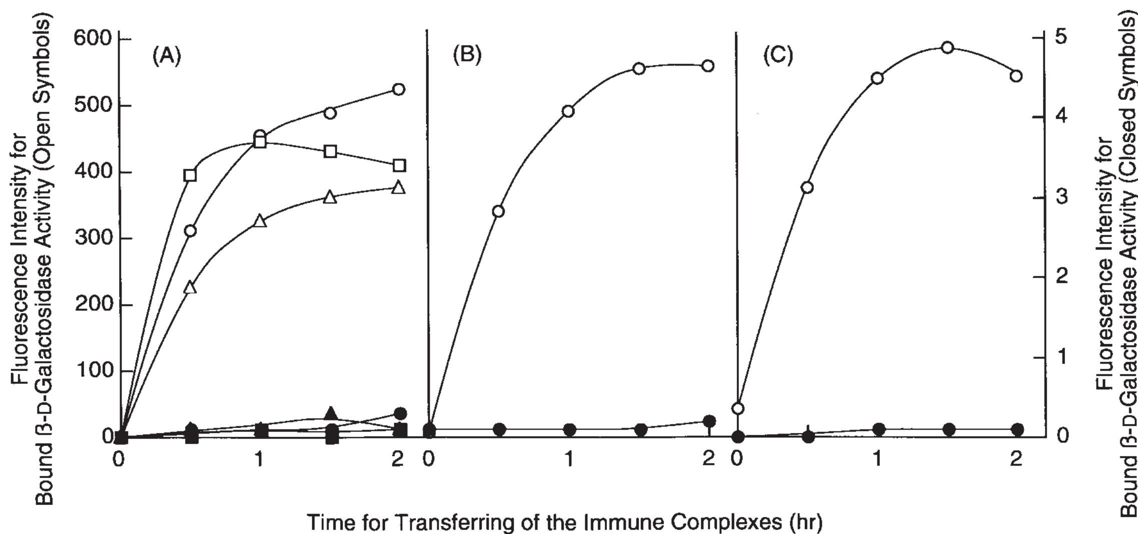

Fig. 4. Time course for transferring of the immune complexes. **A.** Antibody IgG to p17. **B.** Antibody IgG to p24. **C.** Antibody IgG to RT. An aliquot (10 μl) of serum samples was incubated at room temperature with 2,4-dinitrophenyl-bovine serum albumin-recombinant HIV-1 protein conjugates and recombinant HIV-1 protein-β-D-galactosidase conjugates for 1.5 hr (A and B) and 4 hr (C) and subsequently with colored polystyrene beads coated with (anti-2,4-dinitrophenyl group) IgG for 1 hr. The colored polystyrene beads after washing were incubated with white polystyrene beads coated with (antihuman IgG γ-chain) IgG in the presence of *εN*-2,4-dinitrophenyl-L-lysine at 4°C (triangles), 25°C (circles), and 30°C (squares) for up to 2 hr. β-D-Galactosidase activity bound to the white polystyrene beads was assayed at 30°C for 1 hr. Open symbols: Serum included in the Ortho Western blot kit used in Figure 2 was diluted 5,000-fold (A), 2,000-fold (B), and 400-fold (C) with serum from an HIV-1 seronegative subject. Closed symbols: Nonspecific rabbit serum was substituted for human serum samples.

TABLE 1. Signal by Previous and Present Immune Complex Transfer Enzyme Immunoassays for Antibody IgG to p17[a]

Immune complex transfer enzyme immuno-assay	Condition of immunoassay					Signal (fluorescence intensity of bound β-D-galactosidase activity)									
	Volume of diluted serum samples	Time for				Serum from HIV-1 seronegative subjects (n=50)	Dilution of serum from an HIV-1 seropositive subject with serum from an HIV-1 seronegative subject (-fold)								
		formation of the immune complex	trapping of the immune complex	transfer-ring of the immune complex	assay of bound enzyme activity		10^8	3×10^7	10^7	3×10^6	10^6	3×10^5	10^5	10^3	3×10^2
	µl	hr	hr	hr	hr	mean±SD (range)									
Previous, without shaking	10	3	16	3	2.5	0.3±0.1 (0.1–0.9)	0.2	0.5	0.6	0.7	1.6	4.2 (1.0)	14 (1.0)	NT	NT
Present, with shaking	10	0.5	1	1	1	0.1±0.1 (0.1–0.4)	0.1	0.2	0.6	1.6	4.8	14 (3.6)	44 (3.2)	NT	NT
	10	0.5	1.5	1.5	2	–	0.3	0.4	1.1	2.8	9.6	27 (6.9)	90 (6.6)	NT	NT
	100	0.5	1	1	1	0.2±0.1 (0.0–0.5)	0.4	0.9	2.7	8.0	31	83 (21)	302 (22)	NT	NT
	100	0.5	1.5	1.5	2	0.3±0.1 (0.1–0.7)	1.6	2.5	7.4	19	66	201 (51)	621 (45)	NT	NT
	100	0.5	1.5	1.5	4	0.4±0.2 (0.1–0.9)	1.5	3.8	13	36	118	364 (93)	1212 (88)	NT	NT
Western blotting for p17 band	–	–	–	–	–	NT	NT	NT	NT	NT	–	–	–	–	+

[a]Values in parentheses are the ratios of the signals by the present immune complex transfer enzyme immunoassays to those by the previous one.
NT: not tested.

TABLE 2. Signal by Previous and Present Immune Complex Transfer Enzyme Immunoassays for Antibody IgG to p24[a]

Immune complex transfer enzyme immuno-assay	Condition of immunoassay					Signal (fluorescence intensity of bound β-D-galactosidase activity)									
	Volume of diluted serum samples	Time for				Serum from HIV-1 seronegative subjects (n=50)	Dilution of serum from an HIV-1 seropositive subject with serum from an HIV-1 seronegative subject (-fold)								
		formation of the immune complex	trapping of the immune complex	transfer-ring of the immune complex	assay of bound enzyme activity		10^8	3×10^7	10^7	3×10^6	10^6	3×10^5	10^5	3×10^3
	µl	hr	hr	hr	hr	mean±SD (range)								
Previous, without shaking	10	3	16	3	2.5	0.3±0.1 (0.1–0.9)	0.5	0.7	1.4	2.5	9.0 (1.0)	25 (1.0)	76 (1.0)	NT
Present, with shaking	10	0.5	1	1	1	0.3±0.1 (0.1–0.6)	0.3	0.5	1.2	3.1	9.6 (1.1)	29 (1.1)	96 (1.3)	NT
	10	1.5	1.5	1.5	2	0.5±0.1 (0.3–0.7)	1.0	1.6	3.2	8.0	25 (2.8)	73 (2.9)	241 (3.2)	NT
	100	0.5	1	1	1	0.2±0.1 (0–0.6)	1.2	2.2	5.6	15	49 (5.5)	142 (5.7)	479 (6.3)	NT
	100	1.5	1.5	1.5	2	0.5±0.2 (0–1.1)	3.5	6.4	15	42	133 (15)	391 (16)	1259 (17)	NT
Western blotting for p24 band	–	–	–	–	–	NT	NT	NT	NT	NT	–	–	–	+

[a]Values in parentheses are the ratios of the signals by the present immune complex transfer enzyme immunoassays to those by the previous one.
NT: not tested.

TABLE 3. Signal by Previous and Present Immune Complex Transfer Enzyme Immunoassays for Antibody IgG to RT[a]

Immune complex transfer enzyme immuno-assay	Condition of immunoassay					Signal (fluorescence intensity of bound β-D-galactosidase activity)									
		Time for					Dilution of serum from an HIV-1 seropositive subject with serum from an HIV-1 seronegative subject (-fold)								
	Volume of diluted serum samples	formation of the immune complex	trapping of the immune complex	transfer-ring of the immune complex	assay of bound enzyme activity	Serum from HIV-1 seronegative subjects (n=50)	10^7	3×10^6	10^6	3×10^5	10^5	3×10^4	10^4	10^3	3×10^2
	μl	hr	hr	hr	hr	mean±SD (range)									
Previous, without shaking	10	3	16	3	2.5	0.8±0.2 (0.5–1.4)	0.9	1.1	1.4	2.3	4.3	12 (1.0)	33 (1.0)	NT	NT
Present, with shaking	10	0.5	1	1	1	0.6±0.1 (0.3–0.8)	0.8	0.9	1.2	2.0	5.0	15 (1.3)	43 (1.3)	NT	NT
	10	4	1.5	1.5	2	0.9±0.2 (0.5–1.2)	1.6	1.9	3.3	6.0	16	46 (3.9)	146 (4.4)	NT	NT
	100	0.5	1	1	1	0.6±0.1 (0.4–0.9)	0.8	1.1	2.4	6.5	19	60 (5.3)	196 (6.0)	NT	NT
	100	4	1.5	1.5	2	0.9±0.2 (0.6–1.4)	2.1	3.5	9.2	24	78	229 (20)	788 (24)	NT	NT
Western blotting for p66 band	–	–	–	–	–	NT	NT	NT	–	–	–	–	–	±	+

[a]Values in parentheses are the ratios of the signals by the present immune complex transfer enzyme immunoassays to those by the previous one.
NT: not tested.

and the assay of bound β-D-galactosidase activity and the volume of the diluted serum samples were varied. However, the incubation for each immunoreaction was performed at room temperature with shaking and bound β-D-galactosidase activity was assayed at 30°C throughout. The signal (the fluorescence intensity for β-D-galactosidase activity bound to white polystyrene beads coated with affinity-purified (anti-human IgG γ-chain) IgG) and the sensitivity were compared with those by the previous immune complex transfer enzyme immunoassays using 10 μl of the diluted serum samples, in which the immune complexes were formed, trapped, and transferred at room temperature for 3 hr, 16 hr, and 3 hr, respectively, without shaking and bound β-D-galactosidase activity was assayed at 30°C for 2.5 hr (Tables 1, 2, and 3).

With 10 μl of serum samples, the signals for the diluted serum samples from an HIV-1 seropositive subject by the present immune complex transfer enzyme immunoassays of antibody IgGs to p17, p24, and RT were 3.2–3.6-fold, 1.1–1.3-fold, and 1.3-fold, respectively, higher than those by the corresponding previous immune complex transfer enzyme immunoassays, even when the formation, trapping, and transferring of the immune complexes were performed for 0.5, 1 and 1 hr, respectively, with 1 hr assay of bound β-D-galactosidase activity, and were 6.6–6.9-fold, 2.8–3.2-fold, and 3.9–4.4-fold, respectively, higher, when the periods of time for the formation, trapping, and transferring of the immune complexes and the assay of bound β-D-galactosidase activity were prolonged to up to 4 hr. The signals for serum samples from HIV-1 seronegative subjects were even lower than those by the previous immune complex transfer enzyme immunoassays. As a result, the sensitivities for antibody IgGs to p17, p24, and RT were improved almost to the same extents as the signals were enhanced and were 10,000-fold, 3,000–10,000-fold, and 1,000–3,000-fold, respectively, higher than those of Western blotting for p17, p24, and p66 (a subunit of RT) bands.

With 100 μl of serum samples, the signals for the diluted serum samples from an HIV-1 seropositive subject by the present immune complex transfer enzyme immunoassays of antibody IgGs to p17, p24, and RT, were 21–22-fold, 5.5–6.3-fold, and 5.3–6.0-fold, respectively, higher than those by the corresponding previous immune complex transfer enzyme immunoassays, even when the formation, trapping, and transferring of the immune complexes were performed for 0.5, 1 and 1 hr, respectively, with 1 hr assay of bound β-D-galactosidase activity and were 88–93-fold, 15–17-fold, and 20–24-fold, respectively, higher, when the periods of time for the formation, trapping, and transferring of the immune complexes and the assay of bound β-D-galactosidase activity were prolonged to up to 4 hr. The signals for serum samples from HIV-1 seronegative subjects were even lower than those by the previous immune complex transfer enzyme immunoassays. As a result, the sensitivities for antibody IgGs to p17, p24, and RT were improved almost to the same extents as the signals were enhanced and were 30,000–100,000-fold, 10,000–30,000-fold, and 3,000–10,000-fold, respectively, higher than those of Western blotting for p17, p24, and p66 bands.

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