Immune Complex Transfer Enzyme Immunoassay for Antibody IgM to HIV-1 p17 Antigen

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The immune complex transfer enzyme immunoassay for antibody IgM to HIV-1 p17 antigen is described. Serum samples containing antibody IgM to HIV-1 p17 antigen were incubated simultaneously with 2,4-dinitrophenyl-bovine serum albumin-recombinant p17 (rp17) conjugate and rp17- β -D-galactosidase conjugate, and the immune complex formed comprising the three components was trapped onto colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG. Subsequently, the immune complex was transferred to white polystyrene beads coated with monoclonal mouse (antihuman IgM) IgG in the presence of excess of ϵ N-2,4-dinitrophenyl-L-lysine. The signal for antibody IgM to p17 antigen was the fluorescence intensity by fluorometric assay of β -D-galactosidase activity bound to the white polystyrene beads. The periods of time required for the formation, trapping, and transferring of the immune complex comprising the

three components were more than 4 hr, 2 hr, and 3 hr, respectively. The immunoassay developed was shown to be specific by inhibition of transferring the immune complex in the presence of excess of nonspecific IgM but not IgG. Signals for antibody IgM to p17 antigen with serum samples of HIV-1 seroconversion serum panels,—that is, with serum samples in early stages of the infection—tended to be higher than those with serum samples from HIV-1 asymptomatic carriers probably long after the infection and patients with ARC and AIDS. In contrast, signals for antibody IgG to p17 antigen with serum samples of HIV-1 seroconversion serum panels tended to be higher than signals for antibody IgM to p17 antigen but were much lower than signals for antibody IgG to p17 antigen with serum samples from HIV-1 asymptomatic carriers and patients with ARC and AIDS. *J. Clin. Lab. Anal.* 12:329–336, 1998. © 1998 Wiley-Liss, Inc.

Key words: antibody; human immunodeficiency virus type 1; p17 antigen; β -D-galactosidase

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-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 ϵ N-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 μ L. 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). Recently, it has been made possible to per-

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form more sensitive immune complex transfer enzyme immunoassays for antibody IgG to p17 antigen within shorter periods of time (15 to 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 μ L) and solid phases with larger surface areas (11).

This report describes the immune complex transfer enzyme immunoassay for antibody IgM to HIV-1, which has been reported to be detected in early stages of HIV-1 infection (12,13), in comparison with that for antibody IgG to HIV-1.

MATERIALS AND METHODS

Buffers

The regularly used buffer was 10 mmol/L sodium phosphate buffer, pH 7.0, containing 1.0 g/L bovine serum albumin (fraction V, Interger Company, Purchase, NY), 0.1 mol/L NaCl, 1.0 mmol/L MgCl_2 and 1.0 g/L NaN_3 (buffer A). In other buffers, the concentration of bovine serum albumin was 0.1 g/L (buffer B) and the concentration of NaCl was 0.4 mol/L (buffer C).

Antibodies

Rabbit (antihuman IgG γ -chain) IgG and rabbit (antihuman IgM μ -chain) IgG were obtained from Medical and Biological Laboratories Co., Ltd., Nagoya, Japan. Monoclonal mouse (antihuman IgM) IgG₁ (Product No. 7408) was obtained from Oy Medix Biochemica Ab, Kauniainen, Finland.

Polystyrene Beads Coated With Affinity-Purified (Anti-2,4-Dinitrophenyl Group) IgG, Affinity-Purified (Antihuman IgG γ -Chain) IgG and Monoclonal Mouse (Antihuman IgM) IgG₁

Colored polystyrene beads (3.2 mm in diameter, Immuno Chemical Inc., Okayama, Japan) were coated by physical adsorption with affinity-purified (anti-2,4-dinitrophenyl-bovine serum albumin) IgG (0.05 g/L) (14). White polystyrene beads (3.2 mm in diameter, Immuno Chemical Inc.) were coated by physical adsorption with affinity-purified rabbit (antihuman IgG γ -chain) IgG (3 to 100 mg/L) and monoclonal mouse (antihuman IgM) IgG₁ (3 to 30 mg/L), respectively (14).

Rabbit (Antihuman IgG γ -Chain) Fab'- and Rabbit (Antihuman IgM μ -Chain) Fab'- β -D-Galactosidase Conjugates

Rabbit (antihuman IgG γ -chain) F(ab')₂ and rabbit (antihuman IgM μ -chain) F(ab')₂ were prepared by digestion of IgG with pepsin, and Fab' was prepared by reduction of F(ab')₂ with 2-mercaptoethylamine (15). Fab' was conjugated to β -D-galactosidase (EC 3.2.1.23) from *Escherichia coli* using *N,N'*-o-phenylenedimaleimide (15).

Two-Site Enzyme Immunoassays for Human IgG and IgM

In two-site enzyme immunoassay for IgG, a sample mixed with buffer A to 150 μ L was incubated with one polystyrene bead coated with affinity-purified rabbit (antihuman IgG γ -chain) IgG (100 μ g/ml) for 1 hr. The polystyrene bead was washed twice with 2 mL of buffer B and was incubated with 150 μ L of buffer A containing rabbit (antihuman IgG γ -chain) Fab'- β -D-galactosidase conjugate (100 fmol) and nonspecific rabbit F(ab')₂ (0.1 mg) for 1 hr. The incubations were performed at room temperature with shaking. The polystyrene bead was washed twice with 2 mL of buffer B, and bound β -D-galactosidase activity was assayed at 30°C for 1 hr by fluorometry using 4-methylumbelliferyl- β -D-galactoside as substrate (16).

In two-site enzyme immunoassay for IgM, polystyrene beads coated with monoclonal mouse (antihuman IgM) IgG₁ (10 μ g/ml) and rabbit (antihuman IgM μ -chain) Fab'- β -D-galactosidase conjugate were substituted for polystyrene beads coated with affinity-purified rabbit (antihuman IgG γ -chain) IgG and rabbit (antihuman IgG γ -chain) Fab'- β -D-galactosidase conjugate, respectively.

2,4-Dinitrophenyl-Bovine Serum Albumin-Recombinant HIV-1 p17 (rp17) Conjugate

Thiol groups introduced into rp17 molecules were reacted with 6-maleimidohexanoyl-2,4-dinitrophenyl-bovine serum albumin (1).

HIV-1 rp17- β -D-Galactosidase Conjugate

Thiol groups introduced into rp17 molecules were reacted with maleimide- β -D-galactosidase (EC 3.2.1.23) (1).

Immune Complex Transfer Enzyme Immunoassays for Antibody IgG to HIV-1 p17 Antigen

Antibody IgG to HIV-1 p17 antigen was measured essentially in the same way as described previously (7,17). An aliquot (10 μ L) of serum was mixed with 90 μ L of buffer C and was incubated for up to 20 hr with 50 μ L of buffer C containing inactive β -D-galactosidase (50 μ g) (Mutein, Boehringer Mannheim, Mannheim, Germany) and 100 fmol each of 2,4-dinitrophenyl-bovine serum albumin-rp17 conjugate and rp17- β -D-galactosidase conjugate. Subsequently, the reaction mixture was incubated with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for up to 2 hr. The colored polystyrene beads were washed and incubated for up to 4 hr with two white polystyrene beads coated with affinity-purified (antihuman IgG γ -chain) IgG (100 μ g/ml) in 150 μ L of buffer A containing 1 mmol/L ϵ N-2,4-dinitrophenyl-L-lysine. The incubations were performed at room temperature with shaking. The white polystyrene

beads were washed, and β -D-galactosidase activity bound was assayed at 30°C for up to 20 hr by fluorometry using 4-methylumbelliferyl- β -D-galactoside as substrate (16). The fluorescence intensity was measured with a spectrofluorophotometer (F-3010, Hitachi, Ltd., Tokyo, Japan) using 360 nm for excitation and 450 nm for emission analysis. The fluorescence intensity of 1×10^{-8} mol/L 4-methylumbelliferone in 0.1 mol/L glycine-NaOH buffer, pH 10.3 was adjusted to 100.

Immune Complex Transfer Enzyme Immunoassays for Antibody IgM to HIV-1 p17 Antigen

White polystyrene beads coated with monoclonal mouse (antihuman IgM) IgG₁ (10 μ g/ml) were substituted for those coated with affinity-purified (antihuman IgG γ -chain) IgG in the immune complex transfer enzyme immunoassay for antibody IgG to HIV-1 p17 antigen described above.

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 Inc., Tokyo, Japan). Western blotting for antibody IgG 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 Inc., Raritan, NJ).

Seroconversion Serum Panels

One seroconversion serum panel (Panel E) was obtained from Boston Biomedica, West Bridgewater, MA. Two seroconversion serum panels (SV-0051 and SV-0241) were obtained from North American Biologicals, Inc., Miami, FL.

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

Serum samples were randomly collected from 50 HIV-1 seronegative subjects (23 males aged 22–59 yr and 27 females aged 27–73 yr) and 15 HIV-1 seropositive subjects (3 male asymptomatic carriers (AC) aged 17–22 years, 2 female asymptomatic carriers aged 22 and 39 years, 4 male patients aged 10–52 years with AIDS-related complex (ARC), one female patient aged 18 years with AIDS-related complex, 5 male patients aged 14–59 years with AIDS) 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 immune complex transfer enzyme immunoassays for antibody IgG and IgM to HIV-1 p17 antigen, serum

samples were incubated simultaneously with 2,4-dinitrophenyl-bovine serum albumin-recombinant p17 (rp17) conjugate and rp17- β -D-galactosidase conjugate, and the immune complexes formed comprising the three components were trapped onto colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG. Subsequently, the immune complexes were transferred to white polystyrene beads coated with affinity-purified (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁ in the presence of excess of ϵ N-2,4-dinitrophenyl-L-lysine (Fig. 1). Incubations for the immunoreactions were performed at room temperature with shaking throughout.

In order to develop the immune complex transfer enzyme immunoassay for antibody IgM to p17 antigen, which is as sensitive as that for antibody IgG to p17 antigen, the following experiments were performed.

Amount of IgG and IgM Released From Colored Polystyrene Beads Incubated With Serum Samples

As in the immune complex transfer enzyme immunoassays for antibody IgG and IgM to p17 antigen, colored polystyrene beads (two beads/assay) coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG were incubated with 10 and 100 μ L of serum samples in a total volume of 150 μ L for

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

1 hr and, after washing, with 150 μ L of ϵ N-2,4-dinitrophenyl-L-lysine solution for 1 hr. During incubation with serum samples, IgG and IgM were nonspecifically adsorbed onto the colored polystyrene beads and were partially released during incubation in the presence of ϵ N-2,4-dinitrophenyl-L-lysine. The amounts of IgG and IgM released into the ϵ N-2,4-dinitrophenyl-L-lysine solution were from 3.7 to 56 fmol/assay and from 0.2 to 11 fmol/assay, respectively, which were measured by two-site enzyme immunoassays for IgG and IgM (Table 1). The specificity of the two-site enzyme immunoassays used was satisfactory for the above experiments. Namely, the detection limits of IgG and IgM by the two-site enzyme immunoassay for IgG were 0.1 fmol/assay and 20 fmol/assay, respectively, and those by the two-site enzyme immunoassay for IgM were $>10^4$ fmol/assay and 0.01 fmol/assay, respectively.

Capacity of White Polystyrene Beads Coated With Affinity-Purified (Antihuman IgG γ -Chain) IgG and Monoclonal Mouse (Anti-Human IgM) IgG₁ to Trap IgG and IgM

In order to test the capacity of white polystyrene beads coated with affinity-purified (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁ to trap IgG and IgM, various amounts (1–1000 fmol) of nonspecific human IgG and IgM in the ϵ N-2,4-dinitrophenyl-L-lysine solution were incubated with two white polystyrene beads coated with increasing concentrations (from 3 to 100 μ g/mL) of the anti-immunoglobulin IgGs for 1, 2, and 3 hr (Table 2). The fractions of IgG and IgM trapped were larger when coated with 10 to 100 μ g/mL than when coated with 3 μ g/mL, and the fractions of IgG trapped were larger than those of IgM trapped. The fractions of IgG trapped differed only slightly when the white polystyrene beads coated with 10 to 100 μ g/mL were incubated with 1 to 300 fmol of IgG and significantly de-

creased when incubated with 1,000 fmol. The fractions of IgM trapped differed only slightly when the white polystyrene beads coated with 10 to 100 μ g/mL were incubated with 1 to 30 fmol of IgM and significantly decreased when incubated with 300 fmol or more.

From these results, transferring of the immune complexes comprising the three components to the white polystyrene beads was indicated not to be intolerably inhibited by the amounts of nonspecific IgG (3.7–56 fmol) and IgM (0.2–11 fmol) released from the colored polystyrene beads incubated with 10 to 100 μ L of serum samples described above, and the amounts of nonspecific human IgG and IgM to be added for test of the specificity of the immune complex transfer enzyme immunoassays for antibody IgG and IgM to p17 antigen described below were indicated to be 1,000 fmol (0.15 μ g) or more of IgG and 300 fmol (0.27 μ g) or more of IgM.

Time Courses of the Immunoreactions Involved in the Immune Complex Transfer Enzyme Immunoassay

In order to perform the immune complex transfer enzyme immunoassays for antibody IgG and IgM to HIV-1 p17 antigen within as short a period of time as possible, the time courses of the formation, trapping and transferring of the immune complexes were examined (Fig. 2).

First, in order to determine the period of time required for the formation of the immune complexes comprising the three components, two serum samples (10 μ L) (one taken from an HIV-1 seroconversion serum panel and the other collected randomly from an HIV-1 asymptomatic carrier) were incubated with the two rp17 conjugates for up to 20 hr. The immune complexes formed comprising the three components were trapped onto the colored polystyrene beads for 1 hr and were transferred to the white polystyrene beads for 1 hr. The signals (fluorescence intensities for bound β -D-galactosidase activity) with serum from the seroconversion serum panel increased almost linearly for 4 hr of incubation with the two rp17 conjugates, and those at 20 hr incubation were 4-fold higher than those at 4-hr incubation. By contrast, the signal with serum from the asymptomatic carrier reached the maximum within 0.5 to 1 hr, although the data are not shown.

Second, in order to determine the period of time required for trapping the immune complexes comprising the three components on the basis of the above result, serum from the seroconversion serum panel described above was incubated with the two rp17 conjugates for 20 hr. The immune complexes formed comprising the three components were trapped onto the colored polystyrene beads for up to 2 hr and were transferred to the white polystyrene beads for 1 hr. The signal with affinity-purified (antihuman IgG γ -chain) IgG-coated white polystyrene beads reached the maximum within 1 hr of incubation with the colored polystyrene beads, and that with monoclonal mouse

TABLE 1. Amount of IgG and IgM Released From Colored Polystyrene Beads Incubated With Serum Samples^a

Serum no.	Amount of IgG released		Amount of IgM released	
	Volume of serum incubated (μ L)		Volume of serum incubated (μ L)	
	10	100	10	100
	fmol/tube			
1	3.7	56	2.0	11
2	16	47	0.5	2.4
3	9.7	51	0.2	0.7
4	3.7	36	0.9	4.9
5	15	56	0.7	2.6

^aTwo colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG were incubated with indicated volumes of serum samples in a total volume of 150 μ L for 1 hr and, after washing, with 150 μ L of buffer A containing 1 mmol/L ϵ N-2,4-dinitrophenyl-L-lysine for 1 hr. The incubations were performed at room temperature with shaking. The amount of IgG and IgM released into the ϵ N-2,4-dinitrophenyl-L-lysine solution was measured by two-site enzyme immunoassays.

TABLE 2. Fraction of Human IgG and IgM Trapped Onto White Polystyrene Beads Coated With Affinity-Purified Rabbit (Antihuman IgG γ -Chain) IgG and Monoclonal Mouse (Antihuman IgM) IgG₁^a

Concentration of antibody IgG used for coating polystyrene beads (μg/mL)	Incubation time for trapping (hr)	Fraction of IgG trapped				Fraction of IgM trapped			
		Amount of IgG incubated (fmol)				Amount of IgM incubated (fmol)			
		1	30	300	1000	1	30	300	1000
		%							
3	1	85	87	40	15	18	19	8	0
3	2	100	96	32	12	38	21	10	0
3	3	100	98	52	12	43	27	6	0
10	1	95	92	88	49	61	63	44	14
10	2	100	99	96	48	83	84	46	19
10	3	100	100	99	47	89	94	46	10
30	1	94	—	—	57	72	65	56	23
30	2	100	—	—	58	84	85	63	28
30	3	100	—	—	58	90	93	71	39
100	1	95	91	87	55	—	—	—	—
100	2	100	99	97	58	—	—	—	—
100	3	100	100	99	59	—	—	—	—

^aTwo white polystyrene beads coated with indicated concentrations of affinity-purified rabbit (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁ were incubated with indicated amounts of human IgG and IgM, respectively, in 150 μL of buffer A containing 1 mmol/L ϵN -2,4-dinitrophenyl-L-lysine at room temperature with shaking for indicated periods of time, and the amounts of human IgG and IgM in the ϵN -2,4-dinitrophenyl-L-lysine solution were measured by two-site enzyme immunoassay.

Fig. 2. Time courses for the formation, trapping, and transferring of the immune complexes comprising 2,4-dinitrophenyl-bovine serum albumin-HIV-1 rp17 conjugate, antibody IgG and IgM to HIV-1 p17 antigen and HIV-1 rp17- β -D-galactosidase conjugate. Samples from serum of an HIV-1 seronegative subject and serum of an HIV-1 seroconversion serum panel (SV-0241, G, 23 days after the initial blood collection) diluted 10-fold with the negative serum were used. An aliquot (10 μL) of the serum samples was incubated at room temperature with 2,4-dinitrophenyl-bovine serum albumin-HIV-1 rp17 conjugate and HIV-1 rp17- β -D-galactosidase conjugate (formation of the immune complex) and subsequently with colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG (trapping of the

immune complex). The colored polystyrene beads after washing were incubated with white polystyrene beads coated with affinity-purified rabbit (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁ in the presence of ϵN -2,4-dinitrophenyl-L-lysine at room temperature (transferring of the immune complex). β -D-Galactosidase activity bound to the white polystyrene beads was assayed at 30°C for 20 hr. The periods of time for the formation, trapping, and transferring of the immune complex were 0 to 20 hr, 1 hr, and 1 hr, respectively (A), 20 hr, 0 to 2 hr and 1 hr, respectively (B), and 20 hr, 1 hr and 0 to 4 hr, respectively (C). Circles and squares indicate results for antibody IgG and IgM, respectively. Open and closed symbols indicate results with the diluted serum and the negative serum, respectively.

TABLE 3. Specificity of the Immune Complex Transfer Enzyme Immunoassays for Antibody IgM and IgG to p17 Antigen^a

Immune complex transfer enzyme immunoassay for		Serum or panel no.	Days after the initial blood collection	Dilution with serum from an HIV-1 seronegative subject	Signal (fluorescence intensity for bound β -D-galactosidase activity)						
					None	Addition (μ g/tube)					
						IgG			IgM		
						0.1	1 (667 fmol)	10	0.1	1 (1,111 fmol)	10
Antibody IgG to p17	HIV-1 seronegative			–	0.8			0.7			0.8
	Sera from HIV-1 asymptomatic carriers	T-28	–	10,000	2,553	2,008	159	16			2,467
		T-64	–	10,000	3,282	1,877	58	9.8			3,540
	Sera from HIV-1 seroconversion serum panels	SV-0241	23 days	10	3,320	1,841	109	15			3,335
		SV-0051	27 days	10	10,190	6,266	311	39			10,730
Antibody IgM to p17	HIV-1 seronegative			–	0.3			0.4			0.3
	Sera from HIV-1 asymptomatic carriers	T-28	–	10	264			261			4.8
		T-64	–	10	48			43			4.2
	Sera from HIV-1 seroconversion serum panels	SV-0241	23 days	10	1,705			1,699	1,451	99	13
		SV-0051	27 days	10	1,740			1,658	1,338	132	23

^aSerum samples (10 μ L) were incubated with 100 fmol each of 2,4-dinitrophenyl-bovine serum albumin-rp17 conjugate and rp17- β -D-galactosidase conjugate for 16 hr and subsequently with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr. For antibody IgG and IgM to p17 antigen, the two colored polystyrene beads after washing were incubated with two white polystyrene beads coated with affinity-purified (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁, respectively, in the presence of *ε*N-2,4-dinitrophenyl-L-lysine with and without addition of indicated amounts of IgG and IgM for 1 hr. β -D-Galactosidase activity bound to the white polystyrene beads was assayed for 20 hr.

(antihuman IgM) IgG₁-coated white polystyrene beads more slowly reached the maximum (within 2 hr).

Third, in order to determine the period of time required for transferring the immune complexes comprising the three components on the basis of the above results, the serum sample was incubated with the two conjugates for 20 hr. The immune complexes comprising the three components were trapped for 1 hr and were transferred for up to 4 hr. The signal reached the maximum within 2 hr of the transference with affinity-purified (antihuman IgG γ -chain) IgG-coated white polystyrene beads and more slowly within 3 hr with monoclonal mouse (antihuman IgM) IgG₁-coated white polystyrene beads. Namely, transferring of the immune complexes was almost completed within 2–3 hr.

On the basis of these results, in the following experiments, serum samples (10 μ L) were incubated with the two conjugates for 20 hr, and the immune complexes formed were trapped onto the colored polystyrene beads for 1 hr and were transferred to the white polystyrene beads for 1 hr.

Specificity of the Immune Complex Transfer Enzyme Immunoassays for Antibody IgG and IgM to p17 Antigen

In order to test the specificity of the immune complex transfer enzyme immunoassays for antibody IgG and IgM to p17

antigen, the immune complexes comprising the three components were transferred from the colored polystyrene beads to the white polystyrene beads with and without addition of excess of nonspecific human IgG and IgM.

Two serum samples were randomly collected from two HIV-1 asymptomatic carriers and were diluted 10-fold and 10,000-fold with serum from an HIV-1 seronegative subject for antibody IgM and IgG to p17 antigen, respectively. Two serum samples were taken from two HIV-1 seroconversion serum panels (SV-0241 and SV-0051) and were diluted 10-fold with the negative serum.

The diluted serum samples (10 μ L) were incubated simultaneously with 2,4-dinitrophenyl-bovine serum albumin-rp17 conjugate and rp17- β -D-galactosidase conjugate for 20 hr and subsequently with colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr. After washing, the colored polystyrene beads were incubated for 1 hr with white polystyrene beads coated with affinity-purified (antihuman IgG γ -chain) IgG and monoclonal mouse (antihuman IgM) IgG₁ in the presence of ϵ N-2,4-dinitrophenyl-L-lysine with and without addition of IgG and IgM (Table 3). The amounts of IgG added were 0.1 (667 fmol), 1 and 10 μ g/assay, and the amounts of IgM added were 0.1, 1 (1,111 fmol), and 10 μ g/assay on the basis of the results described above. β -D-Galactosidase activity bound to the white polystyrene beads was assayed by fluorometry. When affin-

TABLE 4. Signals for Antibody IgG and IgM to p17 Antigen^a in Serum Samples of HIV-1-Infected Subjects at Different Stages of the Infection

Sources of serum samples	Days after the initial blood collection or serum no.	Signal (fluorescence intensity for bound β -D-galactosidase activity)		Ratio of signals for anti-p17 IgG to those for anti-p17 IgM
		Anti-p17 IgG	Anti-p17 IgM	
Seroconversion serum panel				
Panel E	126 days	779	42	19
SV-0241	14 days	79	12	6.4
SV-0241	23 days	1,660	853	1.9
SV-0051	15 days	709	106	6.7
SV-0051	27 days	5,095	870	5.9
Asymptomatic carriers				
	T-28	1,276,500	132	9,670
	T-64	1,641,000	24	68,375
	T-76	9,482,900	21	457,007
	T-17	16,235,400	50	325,685
	T-61	21,957,900	18	1,240,559
Patients with ARC				
	T-15	48,620	93	523
	T-62	219,520	59	3,737
	T-79	282,045	185	1,527
	T-23	311,895	92	3,403
	T-42	531,420	84	6,296
Patients with AIDS				
	T-74	30,670	13	2,415
	T-36	36,820	15	2,480
	T-11	39,470	29	1,385
	T-4	66,745	38	1,775
	T-53	330,695	173	1,910

^aAntibody IgG and IgM to p17 antigen were measured as described in Table 3, except that β -D-galactosidase activity bound was assayed for 1 hr.

ity-purified (antihuman IgG γ -chain) IgG-coated white polystyrene beads were used, signals (fluorescence intensities for bound β -D-galactosidase activity) with serum samples from the asymptomatic carriers and the seroconversion serum panels decreased significantly (21–45 %) by addition of 0.1 μ g (667 fmol) of IgG and markedly (99–100 %) by addition of 10 μ g of IgG but not IgM. When white polystyrene beads coated with monoclonal mouse (antihuman IgM) IgG₁ were used, signals with serum samples from the asymptomatic carriers and the seroconversion serum panels were markedly reduced (from 91 to 99 %) by addition of 1 μ g (1,111 fmol) and 10 μ g of IgM but not IgG.

These results were consistent with the results described above for the capacity of the white polystyrene beads to trap IgG and IgM, and indicated that the immune complex transfer enzyme immunoassays for antibody IgG and IgM to p17 antigen were both highly specific.

Presence of Antibody IgM to p17 Antigen at Higher Concentrations in Early Stages of HIV-1 Infection

Serum samples from three HIV-1 seroconversion serum panels, five HIV-1 asymptomatic carriers, and ten patients with ARC and AIDS were tested by the immune complex transfer enzyme immunoassays for antibody IgG and IgM to p17 antigen. As shown in Table 4, signals (fluorescence intensities for bound β -D-galactosidase activity) for antibody IgG to p17 antigen with serum samples from the seroconversion serum panels, that is, with serum samples in early stages of HIV-1 infection were much lower (from 6.0- to 278,000-fold) than those with serum samples randomly collected from the asymptomatic carriers probably long after the infection and the patients with ARC and AIDS. By contrast, signals for antibody IgM to p17 antigen with serum samples of the seroconversion serum samples tended to be higher than those with serum samples from the asymptomatic carriers and the patients with ARC and AIDS, although much lower than signals for antibody IgG to p17 antigen. Therefore, the ratios of signals for antibody IgG against p17 antigen to signals for antibody IgM against p17 antigen with serum samples from the seroconversion serum panels were much lower (from 28 to 653,000-fold) than those with serum samples from the asymptomatic carriers and the patients with ARC and AIDS.

It remains to be carefully examined using HIV-1 seroconversion serum panels whether the detection of antibody IgM to p17 antigen is useful for shortening the window period after the infection, during which the diagnosis of HIV-1 infection is not possible due to the absence of detectable antibodies to HIV-1.

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