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Detection and Differentiation of Antibodies to Human T-Cell Lymphotropic Virus Types I and II by the Immunofluorescence Method

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We compared the sensitivities of the prototype human T-cell lymphotropic virus type I (HTLV-I)- and HTLV-II-transformed cell lines, MT2 and Mo-T, with that of an HTLV-II-infected cell line, clone 19, established in our laboratory, in the immunofluorescence (IF) test for detection of antibody to HTLV-I and HTLV-II. In addition, IF antibody titers with the three antigens were determined, and the results were compared with HTLV-I and HTLV-II typing by polymerase chain reaction (PCR). The MT2 cell line was more sensitive than the two HTLV-II cell lines for detecting HTLV-I antibody by IF, and clone 19 was more sensitive than Mo-T or MT2 for measuring HTLV-II antibody. In the titration study, the antigen that gave the highest titer correlated completely with the HTLV type determined by PCR, indicating that the relatively simple IF titration method can be used for differentiating HTLV-I and HTLV-II antibody in sera and plasmas.

We use the enzyme immunoassay (EIA) and the indirect immunofluorescence (IF) test to screen sera for antibody to human T-cell lymphotropic virus type I and type II (HTLV-I and HTLV-II). The Western immunoblot (WB) and the radioimmunoprecipitation assay (RIPA) are employed when the results of the EIA and IF screening methods do not agree (4). Because HTLV-I and HTLV-II have approximately 60% genetic homology, these serologic methods detect antibody to both types.

In the IF test, the specimens are screened on both HTLV-I- and HTLV-II-infected cells by using the prototype cell strains, MT2 and Mo-T. Sera with high antibody titers fluoresce brightly with both antigens, regardless of the infecting HTLV type. However, low-titered sera usually react more strongly with the homologous antigen, and very weakly reactive sera may fluoresce only with the homologous antigen (3). Although we have found the IF test to be more sensitive than the currently licensed EIA kits (2), we have encountered one sample identified by the polymerase chain reaction (PCR) as HTLV-II that was negative by EIA and did not react in the IF test with either MT2 or Mo-T (5).

Most HTLV-seropositive individuals in California are intravenous drug users, and the antibodies in their sera appear to be mainly due to HTLV-II infection (5). In this study, we compared the antigenicity of MT2 and Mo-T with that of clone 19, an HTLV-II-infected cell line established in our laboratory from the peripheral blood mononuclear cells of an intravenous drug user from California, to determine whether clone 19 could serve as a more sensitive antigen for detecting antibody to HTLV-II by IF. In addition, titers of antibody on the three antigens were compared with PCR typing results to investigate the possibility that HTLV typing can be accomplished by IF titration on HTLV-I- and HTLV-II-infected cells.

The sera or unclotted blood specimens selected for this study were from blood donors and from patients attending drug treatment centers and were submitted for HTLV-I and HTLV-II serology without patient identifiers. The specimens were screened by EIA (Abbott Laboratories, North Chicago, Ill.) according to the directions of the manufacturer and by IF as previously described (4).

Briefly, slides were prepared by mixing 1 part MT2, Mo-T, or clone 19 cells with 3 parts uninfected H9 cells, and 10 cell spots were made on each slide. One hundred percent of the cells in these three lines are infected, and the H9 cells served as a control for nonspecific staining. The slides were fixed in acetone for 10 min and stored at −70°C. Before use, each batch of slides was tested for sensitivity with a panel of HTLV-I- and HTLV-II-positive sera with well-characterized IF reactivities. For the study, a 1:10 dilution of test specimen was reacted with each antigen for 20 min at 36°C. The slides were washed in phosphate-buffered saline for 5 min, reacted with the working dilution of anti-human globulin conjugate for 20 min, washed, and mounted with coverslips.

Specimens that yielded discrepant results between EIA and IF in the screening tests were tested by WB with an HTLV-I lysate (Hillcrest Biologicals, Inc., Cypress, Calif.) spiked with a recombinant *env* protein (Hoffmann-La Roche, Inc., Nutley, N.J.), and some specimens were also tested by RIPA, as previously described (3). Samples for IF endpoint determinations were titrated in fourfold dilutions, and the endpoint was the highest dilution exhibiting 1+ fluorescence, on the basis of a subjective brightness scale of 1+ to 4+. Specimens displaying similar endpoints with the HTLV-I and HTLV-II antigens were retested in twofold dilutions. The PCR was performed as previously described (5) with primer pair SK110-SK111 and probes that were specific for HTLV-I (SK112) and HTLV-II (SK188).

Over a period of 3 months, all diagnostic specimens submitted for HTLV-I and HTLV-II serology were screened by EIA and by IF using MT2, Mo-T, and clone 19 cells. Thirty serum specimens were reactive by both EIA and IF. Four others, which were confirmed as positive by WB and RIPA, were IF positive and EIA negative. Twenty-nine specimens reacted with all three antigens, four reacted with MT2 and clone 19 but not with Mo-T, and one was nonreactive on MT2 but was positive on both Mo-T and clone 19

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TABLE 1. Antibody determinations by IF on PCR-identified specimens with HTLV-I (MT2) and HTLV-II (Mo-T and clone 19) antigens

Specimen	HTLV type[a]	IF results						EIA result
		Screening[b]			Titer[c]			
		MT2	Mo-T	Clone 19	MT2	Mo-T	Clone 19	
1	I	4+	4+	4+	65,536	1,024	1,024	+
2	I	4+	2+	4+	65,536	64	256	+
3	I	4+	4+	4+	4,096	256	1,024	+
4	I	4+	3+	4+	1,024	256	256	+
5	I	3+	2+	3+	1,024	64	256	+
6	I	4+	1+	2+	256	16	64	+
7	I	3+	2+	3+	256	16	64	+
8	I	2+	1+	1+	64	16	16	+
9	I	3+	1+	1+	64	8	8	+
10	I	3+	1+	1+	64	4	4	+
11	I	2+	1+	1+	32	4	16	+
12	II	4+	4+	4+	1,024	4,096	4,096	+
13	II	4+	4+	4+	1,024	2,048	4,096	+
14	II	2+	2+	3+	256	4,096	4,096	+
15	II	4+	4+	4+	256	1,024	4,096	+
16	II	4+	4+	4+	256	1,024	4,096	+
17	II	2+	3+	4+	256	1,024	4,096	+
18	II	3+	4+	4+	256	1,024	1,024	+
19	II	3+	3+	3+	256	1,024	1,024	+
20	II	3+	4+	4+	256	1,024	1,024	+
21	II	3+	3+	4+	256	256	1,024	+
22	II	2+	3+	3+	64	4,096	4,096	+
23	II	3+	4+	4+	64	1,024	4,096	+
24	II	2+	3+	3+	64	1,024	4,096	+
25	II	2+	4+	4+	64	256	4,096	+
26	II	2+	3+	4+	64	256	1,024	+
27	II	1+	2+	3+	64	256	1,024	+
28	II	1+	2+	4+	16	64	1,024	+
29	II	1+	1+	1+	8	8	16	−
30	II	−	1+	1+	<8	8	16	−
31	II	−	−	1+	<8	<8	16	−

[a] As determined by PCR.
[b] Degree of fluorescence (1+ to 4+) at 1:10 dilution.
[c] Reciprocal of highest dilution exhibiting 1+ fluorescence.

substrates. These specimens were submitted either as sera or clotted whole blood, so peripheral blood mononuclear cells were not available for PCR typing.

The plasmas from a separate group of heparinized whole blood specimens were screened for HTLV-I and HTLV-II antibody, and PCR was performed with the peripheral blood mononuclear cells of the reactive samples. Plasmas from 11 HTLV-I- and 20 HTLV-II-infected individuals, as determined by PCR typing, were titrated against the three antigens (Table 1). Sample 30 was negative on the MT2 substrate, and sample 31 was nonreactive with both MT2 and Mo-T antigens. Specimens 29 to 31, which possessed low levels of antibody to HTLV-II, were negative by EIA.

When differences in fluorescence intensity between the antigens were observed at the screening dilution, the brightest staining was indicative of the HTLV type, and in all instances, at least a twofold-higher titer was demonstrated with the homologous antigen in the titration study. The titers with the clone 19 antigen were equal to or greater than the titers with Mo-T, regardless of whether the antibody was due to infection with HTLV-I or with HTLV-II, indicating that clone 19 was more sensitive than Mo-T in detecting antibody to both viruses.

Ideally, one would like to use a single antigen when testing sera for antibody to HTLV-I and HTLV-II. However, it appears that for maximum sensitivity, both an HTLV-I and an HTLV-II antigen should be employed in the IF test when a population in which both these agents are expected to be present is screened. Because we depend upon the IF test as our most reliable screening tool for detection of HTLV-I and HTLV-II antibody, the establishment of an HTLV-II-infected cell line that is more sensitive than Mo-T is most useful.

For the last 1 1/2 years, PCR typing and IF endpoint titrations on MT2 and clone 19 cells have been performed on all unclotted blood specimens submitted to our laboratory that are positive for HTLV-I or HTLV-II antibody. Seventeen HTLV-I-positive and 139 HTLV-II-positive specimens have been identified by PCR, and all 156 of these have been correctly identified by IF titration. To date, no dual infections have been detected by PCR. Three different batches of MT2 and clone 19 slides have been used during this 1 1/2-year period, which indicates that these cell lines yield consistent IF typing results.

WB assays for the differentiation of HTLV-I and HTLV-II antibody that utilize recombinant antigens specific for HTLV-I have been developed (1, 6). Other laboratories have differentiated the two antibodies on the basis of either differences in intensity of the reaction with the two core proteins, p19 and p24 (7), or the presence or absence of a reaction to gp46 (3) in the WB. We have found that titration of a specimen by IF on HTLV-I- and HTLV-II-infected

cells, which is a relatively easy procedure for most laboratories to perform, is also a reliable method for determining HTLV type.

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