A NEW AGGLUTINATION TEST FOR SERUM ANTIBODIES TO ADULT T-CELL LEUKEMIA VIRUS

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A new gelatin particle agglutination test was developed for assay of natural antibodies to adult T-cell leukemia virus (ATLV/HTLV-I). Partially purified viral antigen from culture fluid of a virus-producer cell line was coated on artificial gelatin particle carriers. A high correlation was observed between the titers of antibodies determined by the agglutination test and by indirect immunofluorescence assay. The agglutination test is simple, sensitive and specific, and should be useful for mass screening of human sera for viral antibodies.

Key words: Agglutination test — Antibody assay for ATLV/HTLV-I — Adult T-cell leukemia

The human retrovirus adult T-cell leukemia virus (ATLV), which is similar to HTLV-I isolated in the U.S.A., may be etiologically related to adult T-cell leukemia (ATL), a disease that is endemic in Japan. Natural antibodies to ATLA (ATL-associated antigen), which corresponds to ATLV-specific antigen complex, were found in the sera of almost all patients with ATL tested and also in a considerable number of healthy adults in ATL-endemic areas. Subsequent studies showed that healthy adults having anti-ATLV antibodies were carriers of the virus in their peripheral lymphocytes. The virus may be transmitted from mother to child and between husband and wife. Further studies have shown that ATLV can be transmitted by blood transfusion. To avoid such transmission, a method is required for detection of seropositive donors. Therefore, we developed a simple and sensitive gelatin-particle agglutination (PA) procedure for mass screening of the anti-ATLV antibody in human sera.

The viral antigen was prepared from culture fluid of the virus-producing cell line TCL-Kan as follows. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum. Virus particles were concentrated and purified as described previously. Briefly, virus particles in the culture fluid were concentrated by zonal centrifugation on a linear 0-55% sucrose gradient in 10mM Tris-HCl, pH 7.4, 100mM NaCl, 10mM EDTA at 30,000 rpm. The fractions corresponding to a density of about 1.16 g/cm³ were collected. The virus concentrate was purified further by recentrifugation on a 20-65% sucrose gradient at 30,000 rpm for 18 hr.

The virus was disrupted with detergent, 0.5% Nonidet p-40. The resulting virus antigen was coated on Fuji particles (Ikeda et al., in preparation), which are novel artificial spherical particles of about 3 μm diameter made of gelatin and gum arabic. The solution of viral protein (10 μg/ml) was mixed with an equal volume of gelatin particle suspension (2 × 10⁹/ml). The mixture was allowed to stand for 1 hr at 37° and the particles were washed with physiologic saline. The antigen-coated particles were suspended in phosphate-buffered saline, pH 7.4, and lyophilized. From this lyoplysate, a 1% suspension containing 1 μg protein/5 × 10⁸ particles/ml was prepared. In the assay, 25 μl aliquots of the particle suspension were mixed with equal volumes of ser-
diluted serum in U-bottomed wells of a plastic microtiter plate, and the mixtures were allowed to stand for 2 hr at room temperature. The agglutination patterns, shown in Fig. 1, were read and the titer of antibody was expressed as the highest dilution of serum giving definite agglutination. Final serum dilution of 1:16 or higher showing agglutination by PA was interpreted as positive. Anti-ATLA was determined by an indirect immunofluorescence (IF) test using acetone-fixed MT-1 cells on slides as ATLA. The ATLA was reacted with serially diluted serum followed by reaction with anti-human IgG antibody labeled with fluorescein isothiocyanate. The procedure was described fully in a previous paper. When IF dilution of serum of 1:5 or greater gave a positive immunofluorescence, the serum was judged to be IgG anti-ATLA-positive. Titer of antibody in these tests was expressed as the reciprocal of the greatest serum dilution which gave a positive reaction.

As shown in Fig. 2, a significant correlation was observed between the results of the IF and PA tests ($P<0.01$). Of 265 sera, 155 gave negative reactions in both the IF and the PA test, and 105 gave positive reactions in both tests. No serum gave a positive reaction in the IF test but a negative one in the PA test. However, 5 sera gave a positive reaction only in the PA test. One of these showed a high titer (1:256) and contained IgM antibody, while the other 4 sera showed low titers (1:16) in the PA test and their antibody was not IgM. The antibody activity in each of these sera was completely absorbed with purified viral antigen, suggesting that it was virus-specific. Thus the titers of antibody determined by the PA test seem to be higher than those determined by the IF test.

The present PA test is as specific as the IF test, and its sensitivity is comparable with, or higher than that of the latter. The procedure in the PA test is much simpler than that in the IF test or in enzyme-linked immunosorbent assays (ELISA) because it involves only a one-step reaction of antigen and antibody, whereas more steps are required in the IF test and ELISA.

![Fig. 1. Particle agglutination patterns of anti-ATLV positive and negative sera. Arrows indicate the highest dilution giving definite agglutination, which appears as a definite large ring pattern with a rough multiform outer margin and peripheral agglutination. Titers of sera Nos. 1, 2 and 3 were 16, 256 and less than 16, respectively.](image)
AGGLUTINATION TEST FOR ANTI-HUMAN RETROVIRUS

The time in the PA test is also much shorter than in the other tests. Thus the PA test should be suitable for mass screening of sera from blood donors.

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Fig. 2. Correlation of antibody titers determined by the PA and IF tests.

REFERENCES


