RAPID COMMUNICATION Jpn. J. Cancer Res. 82, 367–370, April 1991

Improvement of Gelatin Particle Agglutination Test for Detection of Anti-HTLV-I Antibody

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Partial modifications of antigen components were made to improve the gelatin particle agglutination (PA) test for the detection of antibodies against human T cell leukemia virus type-I. Envelope glycoproteins prepared by lentil lectin affinity chromatography were further added to the purified viral antigens to be coated on the gelatin particles. Comparative studies with a conventional PA test kit (Serodia ATLA) and indirect immunofluorescence assay showed that the specificity and sensitivity of the new PA test were increased and that abnormal agglutination such as the prozone phenomenon was abolished by this improvement.

Key words: Adult T cell leukemia — Human T cell leukemia virus type-I — Antibody detection — Particle agglutination

Human T cell leukemia virus type-I (HTLV-I[4]),[1,2] the causative virus of adult T cell leukemia (ATL), HTLV-I-associated myelopathy and/or tropical spastic paraparesis, is transmitted by blood transfusion,[3] from male to female by sexual contact[4,5] and from mother to child by breast-feeding.[6–8] To interrupt the viral infection through transfusion, the Japanese Red Cross Blood Centers started the screening of antibodies to HTLV-I by using the gelatin particle agglutination (PA) test[9] in 1986. After the initiation of blood screening, a remarkable reduction of transfusion-associated seroconversion was observed in seroepidemiological studies.[10,11] However, it has been reported that the majority of PA-positive specimens carrying relatively low antibody titers did not show positive results by testing with other methods such as indirect immunofluorescence (IF) assay,[1] western blot (WB) and/or enzyme-linked immunosorbent assay (ELISA),[12] and that this dissociation may be caused by false-positive reaction in the PA test.[11,13–15] Recently, the antibody screening of pregnant women has been implemented to prevent mother-to-child transmission by breast-feeding, and the nonspecific reaction of the PA test has also been discussed in this connection. Detailed analyses indicate that false-positive reactions may be derived from contaminating cellular components in the purified viral antigens and from cross reaction of HTLV-I core antigens, especially p19, with some cellular proteins.[16–18] Homology analysis of the amino acid sequence of p19 by using the Protein Research Foundation database (Osaka) showed that C-terminal (amino acids 1–13) and N-terminal (amino acids 101–115 and 120–130) regions which are reported to be possible epitopes of p19[19] have sequences partially homologous to those of human infectious viruses such as cytomegalovirus, herpes simplex virus (HSV) type-1 and -2, varicella zoster virus (VZV), Epstein-Barr virus and rubella virus. Additionally, sera obtained from patients with VZV and/or HSV type-1 showed positive reactions in the PA test and to p19 in the WB method, and these reactions were abolished after the absorption of sera with purified VZV and/or HSV type-1 antigens (manuscript in preparation). Therefore, antibodies against these viruses may be one of the causative factors of nonspecific reaction in the PA test through cross reactivity with HTLV-I p19.

On the other hand, it has been reported that the prozone effect and false-negative results were observed with the conventional PA test.[20] This phenomenon seemed to be caused by an unbalanced combination of env antigen coated on the particles and anti-env antibody in the specimen, resulting in no particle agglutination.

In view of this, we attempted improve the specificity and the prozone phenomenon of the PA test by reducing core proteins and increasing envelope glycoproteins. HTLV-I envelope proteins may dissociate from viral particles during the cultivation and the purification by sucrose density gradient centrifugation,[21] and then core proteins would be relatively enriched in virus antigens. Since envelope proteins are glycated,[22,23] lentil lectin

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[4] Abbreviations: HTLV-I, human T cell leukemia virus type-I; ATL, adult T cell leukemia; ELISA, enzyme-linked immunosorbent assay; IF, indirect immunofluorescence; PA, particle agglutination; WB, western blot.

and/or concanavalin A affinity chromatography may be utilized for removal of envelope proteins from purified HTLV-I.

Viral antigens were prepared from culture fluid of HTLV-I-producing TCL-Kan cells[24] by sucrose density gradient ultracentrifugation and by successive disruption with 1.0% Nonidet P-40.[9] Lentil lectin affinity chromatography was used for purification of envelope glycoproteins. Briefly, disrupted viral antigens (460 μg/ml, 150 ml) were loaded on a 150 ml column of lentil lectin-Sepharose 4B (Pharmacia Fine Chemicals Co. Ltd., Uppsala, AB) and washed with 10 m*M* tris HCl buffer solution (pH 8.3) supplemented with 0.5% sodium deoxycholate. Lectin-bound envelope glycoproteins were eluted with 5% α-methyl D-mannoside and dialyzed against 0.1 *M* bicarbonate beffer solution (pH 9.6). In the new PA test, these affinity-purified env antigens were mixed with disrupted HTLV-I antigens, and coupled with gelatin particles.

The specificity of the new PA test was evaluated using 302 serum samples obtained from healthy individuals having low antibody titer and/or false-positive reaction in the conventional PA test. Fig. 1 shows the results of comparative studies of the new PA test with both the conventional PA test kit and IF using MT-1, MT-2 and TCL-Kan cells as antigens. All IF-positive sera reacted in the new PA test. A positive reaction, however, was detected in 36 (19.4%) and 140 (75.3%) out of 186 IF-negative specimens by the new and conventional PA tests, respectively. In other words, almost all of the IF-negative specimens having antibody titers of 1:16, 1:32 and 1:64 in the conventional PA test were converted to negative results in the new test. The reason for this effect may be both a decrease of core proteins and an increase of glycoproteins.

Conventional PA tests showed a prozone phenomenon which risks giving false-negative results. To examine whether the new PA test is free from the prozone phenomenon, 4 sera were studied at 1:16 (Fig. 2). All sera showed a positive reaction when new, non-conventional PA tests were used, suggesting that the increase of envelope proteins on gelatin particles is useful for cancelling the prozone phenomenon.

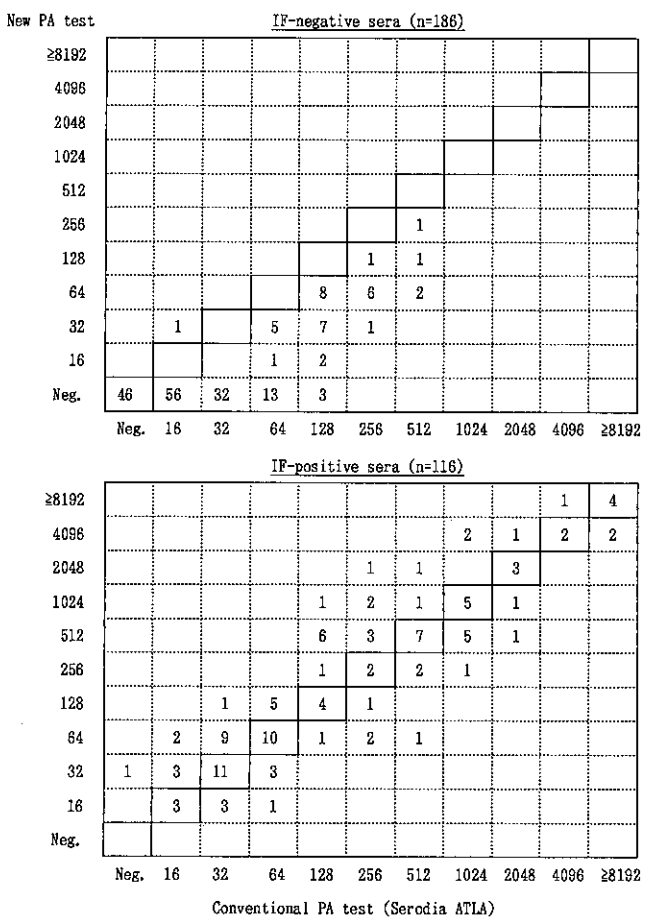

Fig. 1. Distribution of anti-HTLV-I antibody titers measured by new and conventional PA tests. Sera obtained from healthy individuals were assayed, and the results were expressed as the reciprocal number of serum dilution showing a positive reaction.

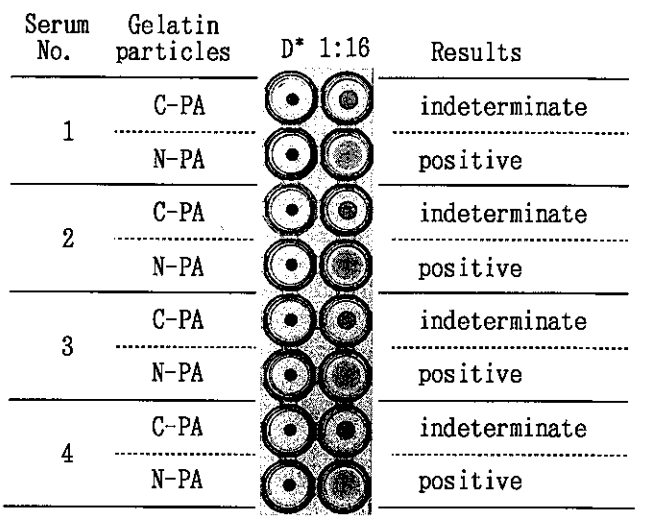

Fig. 2. Assay of 4 sera showing the prozone phenomenon. Sixteen-fold-diluted sera were incubated with purified HTLV-I-coated gelatin particles (conventional PA test, C-PA) or envelope protein-enriched HTLV-I-coated gelatin particles (new PA test, N-PA), and the results were determined after 2 h. As the control, unsensitized gelatin particles were mixed with 8-fold-diluted sera (D*). The protocol mentioned above is the usual method for mass antibody screening.

Several reports indicate that recombinant envelope proteins and/or synthetic peptides of the envelope region can be utilized to detect the anti-envelope proteins by WB and/or ELISA.[25-28] Some sera from HTLV-I-positive individuals, however, did not react to these antigens, and this false-negativity may be caused by conformational changes of the antigenic determinant. Accordingly, we consider that native envelope glycoproteins are more useful for mass antibody screening, and that the new PA test will be very effective in serological analysis, especially for sera showing low antibody titers and the prozone phenomenon.

(Received November 28, 1990/Accepted February 18, 1991)

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