Importance of Membrane-Bound Antigens of Toxoplasma gondii and Their Fixation for Serodiagnosis of Toxoplastic Encephalitis in Patients with Acquired Immunodeficiency Syndrome

YASUHIRO SUZUKI and JACK S. REMINGTON

Department of Parasitology, Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo, Japan, and Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, California 94301

Received 10 May 1990/Accepted 5 July 1990

Titers in a direct agglutination test using Formalin-fixed toxoplasma tachyzoites were significantly higher in patients with acquired immunodeficiency syndrome with toxoplastic encephalitis (TE) than in those without TE. In contrast, when fresh (not fixed) tachyzoites were used, the titers did not differ from those in patients without TE. The direct agglutination test using Formalin-fixed tachyzoites better distinguished between patients with TE and those without TE than an agglutination test using latex particles coated with Formalin-fixed sonicated-tachyzoite antigens. Thus, both selective use of membrane-bound antigens and their fixation are important for serodiagnosis of TE in patients with acquired immunodeficiency syndrome.

Toxoplastic encephalitis (TE) is one of the most commonly recognized opportunistic infectious diseases of the central nervous system in patients with the acquired immunodeficiency syndrome (AIDS) (2-4). It has been estimated that 30% of AIDS patients who have preexisting toxoplasma antibodies will ultimately develop TE (I. H. Grant, J. M. W. Gold, and D. Armstrong, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 441, 1986). Approximately 16 to 38% of adults with AIDS in different geographic locales in the United States have toxoplasma antibodies (5) and thus are at risk for developing TE. In some Western European countries where latent toxoplasma infection is even more common, the incidence of TE affects 25 to 50% of patients with AIDS.

The only definitive method for diagnosis of TE in patients with AIDS has been the histologic demonstration or isolation of organisms from brain biopsy material. Thus, there is a great need to develop noninvasive, sensitive, and specific methods for early diagnosis of TE in this population of patients. We recently found that the Formalin- and acetone-fixed organisms used in agglutination tests can diagnose 70 to 80% of AIDS patients with TE (6). In contrast, an agglutination test using latex particles coated with toxoplasma lysate antigens (LA) could not distinguish AIDS patients with the encephalitis from those without it (6a). The difference between the direct agglutination test and the LA test is in the selection and fixation of toxoplasma antigens used for detection of immunoglobulin G (IgG) antibodies. Only membrane-bound antigens of tachyzoites are responsible for reaction with antibodies in the direct agglutination tests, whereas both membrane-bound and cytoplasmic antigens are responsible for the reaction in the LA (9). In addition, the tachyzoites used for the direct agglutination tests are fixed with either Formalin, acetone, or methanol, whereas fresh (not fixed) antigens are used for sensitization of latex particles in the LA test. The present study was performed to determine whether it is the selective use of membrane-bound antigens or fixation of the antigens that is more important for serodiagnosis of TE in patients with AIDS.

Serum specimens were taken from 30 AIDS patients from the United States: a control group of 15 patients with IgG toxoplasma antibodies who were ambulatory and asymptomatic for central nervous system diseases, and a group of 15 patients with IgG toxoplasma antibodies from whose brains biopsy or aspirate toxoplasma was isolated. The agglutination test using Formalin-fixed tachyzoites was performed as described previously (1). Briefly, Formalin-fixed tachyzoites were suspended in alkaline buffer (0.05 M H3BO3, 0.024 M NaOH; pH 8.7) containing 1% bovine serum albumin at a concentration of $3 \times 10^7$ organisms per ml. A 50-µl volume of the suspension was added to 50 µl of twofold dilutions of sera in microtiter plates. Sera were diluted in phosphate-buffered saline (0.01 M, pH 7.2) containing 0.2 M 2-mercaptoethanol. Plates were read after overnight incubation at room temperature. The agglutination test with fresh tachyzoites was performed as described above for Formalin-fixed tachyzoites. A latex agglutination kit (Toxo-test-MT) kindly provided by Eiken Chemicals Co. Ltd. (Tokyo, Japan) was used as described in the package insert. Latex particles had been sensitized with a preparation of sonicated tachyzoites containing both cytoplasmic and membrane-bound antigens. For fixation of the latex particles sensitized with toxoplasma antigens, the particles were suspended in 20% Formalin in phosphate-buffered saline at 4°C overnight. This is the same fixation procedure used for tachyzoites for the direct agglutination test. Levels of significance for comparisons of antibody titers between groups of patients were determined by using the Student t test distribution. Agglutination tests were performed twice. The results of each test were read by two persons who had no prior knowledge of the experimental design. The results obtained were identical.

Figure 1 shows the direct agglutination test results obtained with Formalin-fixed and fresh tachyzoites and sera of AIDS patients with and without TE. Patients with TE had remarkably higher antibody titers in the agglutination test with Formalin-fixed organisms than did those without the encephalitis ($P < 0.001$). We previously reported that the...
patients with biopsy-proven TE had titers of ≥10,240. In the present study, 87% (13 of 15) of the patients with biopsy-proven TE had titers of ≥10,240. In marked contrast, there was no significant difference in antibody titers between the two groups of patients when the agglutination test with fresh tachyzoites was used. These results indicate that a modification of membrane-bound antigens of tachyzoites with Formalin is critically important for their ability to react with IgG antibodies in AIDS patients to distinguish those with TE from those without TE.

Next, we examined whether selective use of membrane-bound antigens of tachyzoites is important for the serodiagnosis of TE in patients with AIDS. Figure 2 shows the results of the agglutination test using latex particles coated with either Formalin-fixed or nonfixed sonicated-tachyzoite antigens. There was no significant difference in the antibody titers in the agglutination test using latex particles coated with fresh antigens. This result is consistent with our previous finding of the insensitivity of the latex agglutination test for serodiagnosis of TE in patients with AIDS (6a). In contrast, in the agglutination test in which latex particles were sensitized with Formalin-fixed sonicated-tachyzoite antigens, the antibody titers were significantly higher in the AIDS patients with TE than in those without TE (P < 0.05). The difference in antibody titers between the two groups of patients was more remarkable when the direct agglutination test with Formalin-fixed tachyzoites was used (Fig. 1) than when the agglutination test with Formalin-fixed sonicated-tachyzoite antigens was used (Fig. 2). In the former test, 67% (10 of 15) of the patients with TE showed titers higher than the mean plus 1 standard deviation of the titers for the control patients without TE, whereas in the latter test, 33% (5 of 15) of the patients with TE showed titers higher than the mean value for the control patients. These results indicate that both the selective use of membrane-bound antigens and their fixation are important for serodiagnosis of TE in patients with AIDS.

We previously reported that fixation of tachyzoites with either Formalin or acetone makes a remarkable difference in their ability to react with IgG antibody of AIDS (6) and non-AIDS (7, 8) patients and that this difference is the value for diagnosis of acute toxoplasmosis (6–8).

Verhofstede et al. (10) reported that an enzyme-linked immunosorbent assay which was used to measure IgG toxoplasma antibodies and in which Formalin-fixed tachyzoites were used to coat wells of microtiter plates was more sensitive for distinguishing acute from chronic toxoplasma infection in non-AIDS patients than an assay in which fresh tachyzoites were used. A combination of more effective selection of antigens among the membrane-bound antigens and modification of their antigenicity by more suitable fixation should provide more sensitive and specific methods for serodiagnosis of TE in AIDS patients.

We thank Pamela Stepick-Biek and Dorothy Gibbons for technical assistance.

This work was supported by a grant from Ohyama Health Foundation of Japan and by Public Health Service grant A104717 from the National Institutes of Health.

LITERATURE CITED

FIG. 1. Comparison of toxoplasma antibody titers in AIDS patients with and without TE in the direct agglutination tests using Formalin-fixed and fresh (not fixed) tachyzoites. Titers are expressed as the reciprocal of the highest positive dilution of serum. Each open circle and bar indicates the mean value plus 1 standard deviation of titers in each group.

FIG. 2. Comparison of toxoplasma antibody titers in AIDS patients with and without TE in the latex agglutination tests using Formalin-fixed and fresh (not fixed) sonicated-tachyzoite antigens. Titers are expressed as the reciprocal of the highest positive dilution of serum. Each open circle and bar indicates the mean value plus 1 standard deviation of titers in each group.

