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ELISA Inhibition Test Using Monoclonal Antibody Specific for *Treponema pallidum* as the Serologic Test for Syphilis

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Monoclonal antibodies (Mabs) specific for *Treponema pallidum* were produced using hybridoma technology. In this study an ELISA inhibition test based on competitive inhibition by antibodies from human test sera of the binding of the enzyme-labelled Mabs to *T. pallidum* was performed. Inhibition of the seroreactivity of Mabs was decreased according to the dilution of rabbit antiserum to *T. pallidum*. Seropositivity was found in 100% of secondary and early latent syphilis patients, 75% of primary syphilis patients, and 80% of late latent syphilis patients. The mean percentage inhibition was significantly higher in each syphilis group than in the controls, and statistically significant differences were shown between all the syphilis groups. VDRL and TPHA titers were correlated with the percentage inhibitions. Therefore the ELISA inhibition test using Mab specific for *T. pallidum* might well be a suitable tool as a new serologic test for syphilis.

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A serodiagnostic test could have great value as an epidemiological tool at the community level. Various workers have developed serologic tests for syphilis based on antibody detection. The specificity of a serodiagnostic test would be improved (1) with the use of specific monoclonal antibodies (Mabs).

As ELISA is safer than radioimmunoassay and shows a high sensitivity and specificity, some reseachers have become interested in serologic tests using ELISA (2–6). An ELISA inhibition test based on competitive inhibition by antibodies from human test sera of the binding of the enzyme-labelled Mab to *M. leprae* was developed and was reported as a suitable tool for the diagnosis of leprosy (7). Since many samples can be tested at one time, this test is more cost-effective and less time-consuming.

Mabs specific for *Treponema pallidum* were produced using hybridoma technology and were characterized (8). Those being radiolabelled were employed in the scintigraphic diagnosis of syphilitic lesions in rabbits (9).

Here we performed an ELISA inhibition test with enzyme-labelled Mabs. The results of the screening of various sera in this ELISA inhibition test are presented.

MATERIALS AND METHODS

Organisms

T. pallidum (Nichols) and *T. phagedenis*, biotype Reiter, as antigens, were provided by the Center for Disease Control, Atlanta, Georgia, and were inoculated into the testes of white rabbits. Organisms in the supernatant were purified by Percoll density gradient centifugation (10).

Serum samples

Serum samples from 61 patients and 158 non-syphilitic controls were obtained. Sera were classified clinically and serologically using the VDRL, TPHA and 19s (IgM)-FTA. Non-syphilitic sera were confirmed as negative using the above-mentioned serologic tests. Serum samples had been stored at −70°C.

Hybridoma techniques

Female BALB/c mice were immunized by subcutaneous injection boostered by the intraperitoneal route. The methods of hybridization, cloning, bulk culture of selected hybridomas and immunoglobulin class determination have previously been described (8).

ELISA inhibition test

ELISA was performed as described previously (11). Polystyrene ELISA microtiter plates were coated with antigens of *T. pallidum*. To each well was added at the same time 20 μl of serum (1:5 diluted) and 80 μl of peroxidase (HRPO)-labelled Mab diluted to 1:1000 in PBS. After washing three times with T-PBS, horse-radish peroxidase (HRPO)-labelled anti-mouse immunoglobulins (DAKO Immunochemicals, Inc., Copenhagen, Denmark) diluted with 1% normal rabbit serum were incubated for 1 h. After three washes with T-PBS, 50 ml of substrate-dye mixture (0.018% H_2O_2 and 0.1 mg/ml *o*-phenylene diamine in T-PBS) were added to each well. Incubation was continued in the dark at room

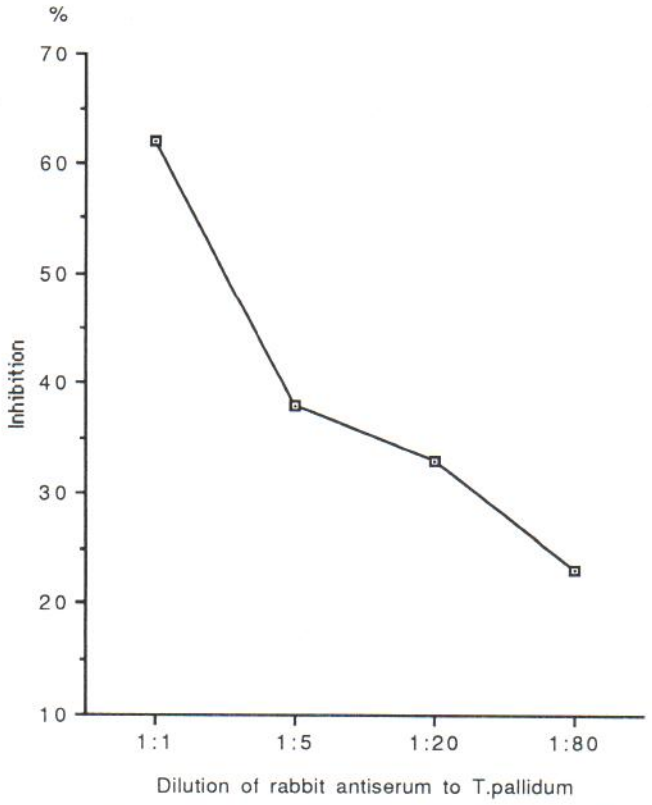

Fig. 1. Inhibition of the seroreactivity of monoclonal antibody (YS3) with rabbit antiserum to *T. pallidum*.

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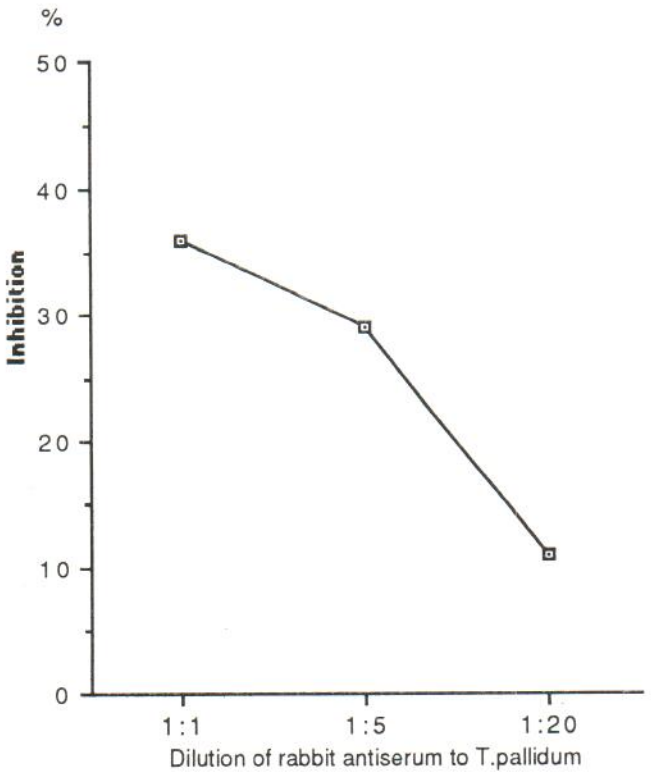

Fig. 2. Inhibition of the seroreactivity of monoclonal antibody (YS307) with rabbit antiserum to *T. pallidum.*

temperature for 30 min. The reaction was stopped by the addition of 25 ml of 8N H_2SO_4 per well. The optical density (OD_{490nm}) of each well was determined on an ELISA autoreader (Dynatech Laboratories, Inc., Alexandria, VA). *T. pallidum*-immunized polyclonal mouse serum was used as positive control serum, normal mouse serum being used as negative. Sera which gave more than a percentage inhibiton of 2 S.D. were scored as positive.

The percentage inhibition of the test sera was found using the formula:

$$\text{Inhibition (\%)} = 1 - \left[\frac{\text{O.D. sample}}{\text{O.D. neg.}}\right] \times 100$$

RESULTS

Inhibition percentages of Mabs (YS3) were 62% in 1:1 dilution of antiserum to *T. pallidum*, 38% in 1:5, 33% in 1:20 and 23% in 1:80, and those of Mabs (YS307) were 36% in 1:1, 29% in 1:5 and 11% in 1:20, respectively. Inhibition of the seroreactivity of Mabs was decreased according to the dilution of rabbit antiserum to *T. pallidum* (Figs 1, 2).

Seropositive sera by Mab (YS307) were 13 of 13(100%), 20 of 20(100%) in secondary and early latent syphilis, 6 of 8(75%) in primary syphilis, and 16 of 20(80%) in late latent syphilis. The mean percentage inhibition was 32.9 ± 24.0% in primary syphilis, 67.2 ± 19.7% in secondary syphilis, 78.8 ± 12.9% in early latent syphilis, and 34.2 ± 23.9% in late latent syphilis, which was significantly higher than 0 ± 7.6% in non-syphilitic controls (Student's *t*-test, multiple range test: Scheffe, $p < 0.05$), and statistically significant differences were shown between all the syphilis groups (ANOVA test) (Table I, Fig. 3).

Table I. *Number of seropositive sera and mean inhibition values of monoclonal antibody (YS307) by an ELISA inhibition test in 61 syphilitic patients and 158 non-syphilitic controls*

Diagnosis	No. positive/ No. tested (%)	% Mean inhibition ± SD
Non-syphilitic control	3/158 (1.9)	0 ± 7.6
Primary	6/8 (75)	32.9±24.0*
Secondary	13/13 (100)	67.2±19.7*
Early latent	20/20 (100)	78.8±12.9*
Late latent	16/20 (80)	34.2±23.9*

Positive: > Mean inhibition + 2 SD value of non-syphilitic controls
*$p < 0.05$: Compared to non-syphilitic controls

VDRL and TPHA titers were correlated with the percentage inhibitions (regression analysis, data not shown).

DISCUSSION

If a syphilitic patient's serum containing antibodies against the same antigenic determinant as the Mab is incubated together with labelled Mab, this will result in competition of both for that same antigenic determinant. When the patient's serum contains enough of these antibodies, they will win this competition, as a result of which the labelled Mab will be washed from the plate and will not change the substrate into a coloured product.

The ELISA inhibition test described here makes use of the specificity of a Mab recognizing a single antigenic determinant

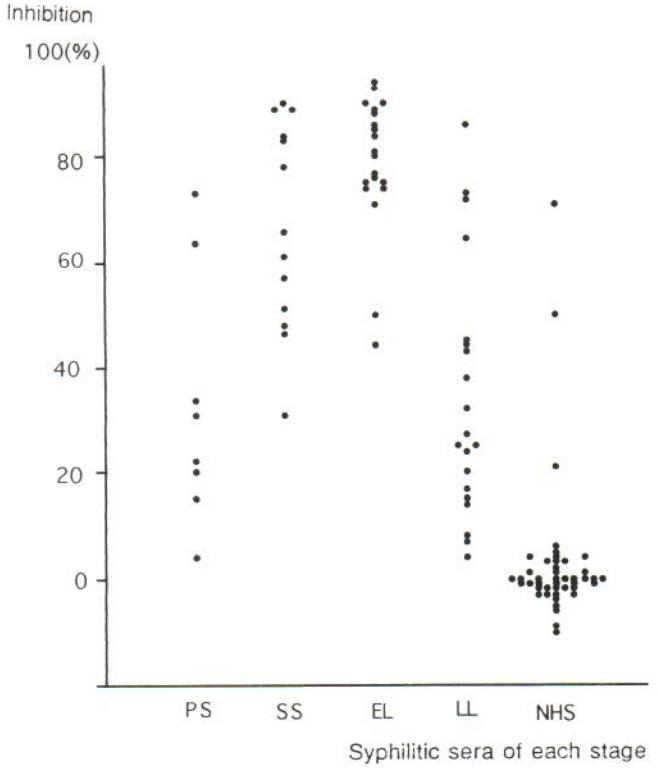

Fig. 3. Inhibition of the seroreactivity of monoclonal antibody (YS307) by sera from each stage of syphilis and controls. *PS:* primary syphilis; *SS:* secondary syphilis; *EL:* early latent syphilis; *LL:* late latent syphilis; *NHS:* normal human serum.

on a 47 kDa of treponemal antigen. Treponemal antigen of 47 kDa is an immunodominant surface-associated polypeptide (12), which shows strong immunogenicity in *T. pallidum*-infected humans and rabbits.

IJsselmuiden et al. (6) reported that the inhibition ELISA had been developed and offered the potential for additional confirmation of early untreated syphilis. Their results showed sensitivity of 97% and specificity of 100%, comparable to TPHA and FTA-ABS tests for diagnosing early untreated syphilis, but they considered samples showing an increase of extinction of at least 0.5 between the lowest and highest dilution as positive. Thus samples need to be diluted several times and therefore the procedures tend to be more complex.

The sensitivity and the specificity of our results are comparable to other serologic tests and are well correlated with VDRL and TPHA statistically, and the several negative sera at the ELISA inhibition test, which were present at the stages of primary and late latent syphilis, showed weakly reactive at the VDRL test.

Therefore the ELISA inhibition test using Mabs specific for *T. pallidum* might well be a suitable tool as a new serologic test for syphilis.

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