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Development of Monoclonal Antibodies that Recognize *Treponema pallidum*

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We developed a panel of monoclonal antibodies to *Treponema pallidum* (Nichols) antigens, some of which recognize treponemal antigens on *T. pallidum* (Nichols), *T. pallidum* strain 14, and *Treponema phagedenis* biotype Reiter. The antibodies were detected by either an enzyme-linked immunosorbent assay or a radioimmunoassay.

Until recently, little specific information about the mechanisms of resistance in syphilis or the antigenic structure of *Treponema pallidum* have been available. Both the humoral and cell-mediated immune responses have been implicated in acquired or protective immunity (or both) to syphilis; however, their roles have not been fully delineated. Detailed knowledge about the antigenic structure of the organism and its pathogenicity have been limited, partly due to technical problems such as the inability to grow *T. pallidum* in vitro for sustained periods of time. Recently, several new techniques have been utilized to characterize *T. pallidum* protein antigens. Alderete and Baseman (2) utilized radioimmunoprecipitation to assess *T. pallidum* surface proteins, whereas Lukehart et al. (5) and Hanff et al. (4) used a Western blotting technique (11) to study *T. pallidum* antigens. These reports indicate that *T. pallidum* generates a complex humoral response to a number of treponemal antigens during syphilitic infection. Robertson et al. (10) reported the development of hybridomas producing antibodies to *T. pallidum*. In this report, we describe additional monoclonal antibodies, detected by an enzyme-linked immunosorbent assay (ELISA), which recognize treponemal surface components.

T. pallidum (Nichols) (obtained from J. Baseman) was maintained by intratesticular passage in rabbits treated with cortisone as previously described (3, 6). A street strain of *T. pallidum* (strain 14; a more recent human isolate, obtained from Sandra Larsen of the Centers for Disease Control, Atlanta, Ga.) was grown in rabbits that did not receive cortisone.

T. pallidum organisms were extracted in phosphate-buffered saline–10% heat-inactivated normal rabbit serum as previously described (9). *Treponema phagedenis* biotype Reiter was grown in spirolate broth supplemented with 10% heat inactivated normal rabbit serum (5). *T. pallidum* antigens used for the ELISA were either phosphate-buffered saline-washed organisms or organisms purified by Percoll density gradients (4, P. A. Hanff, S. J. Norris, M. A. Lovett, and J. N. Miller, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, 130, p. 176).

The *T. pallidum*, *T. phagedenis* biotype Reiter, and normal testicular extracts were sonicated as previously described (J. D. Folds, A. S. Rauchbach, E. Shores, and J. M. Saunders, submitted for publication). The ELISA procedure was performed as described by Folds et al. (submitted for publication). The radioimmunoassay (RIA) procedure was similar to the method described by Zeltzer et al. (12) except that ^{125}I-labeled goat anti-mouse immunoglobulin (New England Nuclear) at 2.5 $\times 10^5$ cpm per well was used to detect antibody. Antigens used to prepare RIA plates were either sonicated organisms as used for the ELISA or whole organisms (10^6 cells per well).

BALB/c mouse spleen cells were sensitized to *T. pallidum* (Nichols) by using a 6-week immunization schedule. Mice were initially injected intradermally with virulent *T. pallidum* (10^7 organisms) in complete Freund adjuvant. Additional injections made intraperitoneally with 10^7 *T. pallidum*, and a final intraperitoneal booster injection of *T. pallidum* antigen (100 μg of protein) was given 3 days before cell fusion. The cell fusion method was similar to previously described procedures (8). Briefly, washed, sensitized mouse spleen cells and myeloma cells (either P3×63-Ag 8.653 or Sp2/0-Ag 14) were combined and then copelleted and exposed to 30% polyethylene glycol (PEG 1500) to effect cell fusion. The cells were washed and suspended to approximately 2.5×10^6 cells per ml and portioned to microtiter wells. The cultures were fed Dulbecco modified Eagle medium–hypoxan-

TABLE 1. ELISA for eight representative hybridoma culture supernatants and controls

Prepn	Antigen attached (optical density at 492 nm)[a]				
	T. pallidum (Nichols)	*T. pallidum* (Nichols) (Percoll purified)	*T. pallidum* strain 14 (Percoll purified)	*T. phagedenis* biotype Reiter	Normal testicular extract
Clones					
IV	0.2	0.3	0.5	—[b]	—
VI	0.3	0.6	0.9	—	—
X	0.5	0.7	1.0	—	—
XVI	0.3	0.7	1.2	—	—
III	0.4	0.6	1.0	0.2	0.3
XIV	0.3	0.5	0.7	0.2	0.2
II	0.3	0.5	0.8	—	0.2
VII	0.4	0.8	0.8	—	0.2
Controls					
Syphilitic mouse sera (1:50)[c]	0.65	0.9	0.8	0.6	0.45
Normal mouse serum (1:50)	0.04	0.06	0.05	0.07	0.08
P3X-Ag8.653 culture supernatant	0.02	0.01	0.02	0.01	0.03

[a] Measured in a Titertek ELISA reader.
[b] —, Not reactive, optical density of less than 0.20.
[c] Serum taken from BALB/c mice at least 45 days after inoculation with *T. pallidum* (Nichols).

thine-aminopterin-thymidine as the selection medium. When the hybrids exhibited good growth, the culture supernatants were screened for anti-*T. pallidum* activity by using an ELISA technique. Cultures which were identified as positive were expanded, and those which continued to produce relevant antibody were cloned by using a soft agar method. Hybrid cell clones were selected and again tested for antibody directed against *T. pallidum*, and those cell cultures positive for antibody activity were further expanded.

The hybridoma cell lines were selected by using the ELISA and sonicated *T. pallidum* (Nichols) antigen. To evaluate the specificity of the monoclonal antibodies, ELISAs were performed with different antigens. *T. pallidum* strain 14, *T. phagedenis* biotype Reiter, and an extract of normal rabbit testicular tissue were used as antigens. Table 1 illustrates several representative hybridoma culture supernatants which react with both *T. pallidum* (Nichols) and *T. pallidum* street strain 14; however, the Percoll-purified organisms displayed greater reactivity than did the non-Percoll-purified organisms. Some culture supernatants showed cross-reactions with *T. phagedenis* biotype Reiter or normal testicular extract (or both).

The monoclonal antibodies were also characterized by using the RIA procedures to evaluate the specificity of the hybridomas. Table 2 indicates that the hybridoma culture supernatants were reactive in the RIA with either sonicated or intact *T. pallidum*; this correlated well with the ELISA results. When sonicated or whole *T. phagedenis* biotype Reiter was used as the antigen in the RIA, several culture supernatants that were not reactive by the ELISA were either not reactive or weakly reactive by the RIA, whereas some monoclonal antibodies displayed significant reactivity toward *T. phagedenis* biotype Reiter.

The isotypes of the monoclonal antibodies were determined via immunodiffusion and most were immunogobulin G1 (IgG1); however, one was IgG2B and four were not typable by standard sera against IgG subclasses, IgM, or IgA.

By using an ELISA and RIA method, the monoclonal antibodies strongly reacted with both *T. pallidum* strains, suggesting that they possess common antigens. This is an important observation since *T. pallidum* (Nichols) has been maintained in cortisone-treated rabbits and may have altered antigens. It is also interesting to note that Percoll purification of *T. pallidum* resulted in greater reactivity with the monoclonal antibodies. This may be due to the concentration of *T. pallidum*-specific components in the antigenic suspension used for testing or perhaps to the decreased rabbit testicular tissue contamination, which may interfere with the immunoassays. For the most part, there was good correlation between the ELISA and RIA results; however, there were several clones whose antibody showed greater reactivity by RIA than in the ELISA.

Some monoclonal antibodies showed reactivity against *T. phagedenis* biotype Reiter by RIA;

TABLE 2. RIA for eight representative hybridoma culture supernatants and controls

Prepn	Antigen attached (cpm × 10^3)[a]			
	Sonicated *T. pallidum* (Nichols)	Whole *T. pallidum* (Nichols)	Sonicated *T. pallidum* strain 14	Whole *T. pallidum* strain 14
Clones				
IV	10.7	9.3	11.0	8.6
VI	31.1	28.8	31.4	24.4
X	23.4	20.2	24.3	15.3
XVI	27.2	24.2	29.8	23.7
III	30.1	24.9	28.7	21.9
XIV	17.3	15.5	19.4	14.7
II	15.6	12.2	17.6	14.1
VII	28.0	24.4	29.0	16.1
Controls				
Syphilitic mouse sera (1:50)[b]	43.6	42.2	46.0	37.7
Normal mouse serum (1:50)	4.4	4.4	4.5	4.3
P3X-Ag8.653 culture supernatant	3.6	3.7	3.9	3.3

[a] All antigens were Percoll purified.
[b] Serum taken from BALB/c mice at least 45 days after inoculation with *T. pallidum* (Nichols).

however, in the ELISA there was minimal activity. This is not unusual since *T. pallidum* and *T. phagedenis* biotype Reiter are known to share some antigenic determinants. This is also consistent with the results of Robertson et al. (10) who used slightly different methods to develop monoclonal antibodies to *T. pallidum* and found that some monoclonal antibodies cross-reacted with *T. phagedenis* biotype Reiter. Several of our monoclonal antibodies showed reactivity with rabbit normal testicular extract. Once again this might be expected since the treponemes used for sensitization of the mice were grown in rabbit testes, and the treponemes were not highly purified. Furthermore, Miller et al. (7) showed that there is cross-reactivity with treponemes and rabbit testicular tissue, and Alderete and Baseman (1) have demonstrated that host proteins avidly bind to *T. pallidum*.

We have generated a number of hybridoma lines which produce monoclonal antibodies directed against *T. pallidum* (Nichols) as well as a more recently established strain, *T. pallidum* strain 14. This indicated that the antibodies detect antigens on more than one strain of *T. pallidum* and not antigens that are unique to the Nichols strain. These monoclonal antibodies will be further characterized via Western blotting, radioimmunoprecipitation, or other procedures to identify the specific antigens recognized. It may then be possible to utilize the monoclonal antibodies to purify and identify specific *T. pallidum* surface components.

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