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Monoclonal Antibodies That Recognize a Specific Surface Antigen of *Treponema denticola*

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Spirochetes have been implicated as potential etiologic agents of periodontitis in humans. Murine monoclonal antibodies (MAbs) specific for a serogroup of *Treponema denticola*, an oral spirochete, were developed and characterized in this study. Antibodies secreted by clone IAA11 were judged to be the most useful, since they were able to detect 8 of 15 *T. denticola* strains. This MAb consisted of an immunoglobulin G3 heavy chain and a kappa light chain. MAb IAA11 was found to react with an epitope target located on the outer sheath of the cell wall. This MAb should be of diagnostic and scientific value in the study of *T. denticola* populations in human periodontitis.

Recent studies have shown that an increase in oral spirochete populations is correlated with increased periodontal disease severity (1, 6, 7). It is possible that certain spirochetes may be etiologic agents of severe periodontitis in adults or may serve as diagnostic indicators of this disease. Moore et al. (10) isolated 10 *Treponema* species from plaque samples of patients with severe periodontitis. One of the treponemes more frequently isolated from diseased sites was identified as *Treponema denticola*. *T. denticola* and four other oral treponemes were also found to be among the most likely causative agents of moderate periodontitis (9). The role of specific spirochetes in periodontitis is unclear, since it is difficult and time-consuming to culture, purify, and positively identify *T. denticola* from clinical specimens. Immunochemical assays with specific monoclonal antibodies (MAbs) would greatly facilitate the detection and evaluation of this spirochete in human clinical studies. Here we report the production and characterization of MAbs to *T. denticola* and the extension of knowledge regarding the serological relationships among *T. denticola* strains.

MATERIALS AND METHODS

Antigen preparation. The spirochete isolates studied and their sources are listed in Table 1. The treponemes were routinely grown at 37°C in a Coy anaerobic chamber with an atmosphere of 10% hydrogen–10% carbon dioxide–80% nitrogen. The spirochetes were cultured in mycoplasma broth base (BBL Microbiology Systems) supplemented with 10% rabbit serum and cocarboxylase as described by Jacob et al. (4). Short-chain volatile fatty acids, glucose, and pectin were supplied for *T. socranskii* and *T. pectinovorum* isolates (13, 14). Other antigens were prepared as described previously (12). The spirochete whole-cell antigens were washed in 0.02 M $MgCl_2$–phosphate-buffered saline prior to fixation in formalinized saline.

Immunization and hybridoma fusion. BALB/c mice were immunized with whole-cell antigen preparations from *T. denticola* ATCC 33521 and hybridized according to fusion protocol II as described previously (12). The mice were approximately 14 months old at the time of fusion. Clone supernatants were assayed by an enzyme-linked immunosorbent assay (ELISA) as described previously, except that *T. denticola* ATCC 33521 whole-cell antigens were immobilized on the polystyrene assay plates and alkaline phosphatase-labeled rabbit anti-mouse immunoglobulin G (IgG) (Miles Laboratories, Inc.) was used as the second antibody. The specificity of the MAbs was demonstrated by the ELISA. Isotyping was performed by immunodiffusion and the ELISA as described previously.

Immunoelectron microscopy. The location of the antigenic target of the MAbs was determined by transmission electron microscopy (11). In these studies, *T. denticola* ATCC 33521 was harvested by centrifugation and washed with 0.02 M $MgCl_2$–phosphate-buffered saline as described above. The cells were fixed in Karnovsky fixative for 2 h, washed, and suspended in 0.1 M sodium cacodylate buffer (pH 7.2). The fixed cells were divided into two equal portions, centrifuged, and suspended with either uninoculated Iscove Dulbecco modified Eagle tissue culture medium (negative control) or MAb IAA11 cellular supernatants produced in the same medium. The cells were incubated overnight at 4°C, washed three times with 0.02 M $MgCl_2$–phosphate-buffered saline, and reacted with Auroprobe-EM goat anti-mouse IgG G10 probe reagent labeled with 10.7-nm-mean-diameter gold spheres (Janssen Pharmaceutica, N.V.). Following incubation at 25°C for 2 h, the suspensions were washed three times. Both negative-stain and thin-section procedures were performed on each of the two sample preparations. Thin-section preparations were made subsequent to ethanol dehydration and embedding in Epon. Ultrathin sections were cut, transferred onto 300 mesh nickel grids, and stained with conventional uranyl acetate and lead citrate. Negative-stain preparations were made on the surfaces of Formvar-coated grids followed by staining with 1% sodium phosphotungstate. Both thin-section and negative-stain preparations were examined in an RCA EMU-3G transmission electron microscope operated at 100 kV.

RESULTS

The hybridoma fusion carried out with a 2:1 splenocyte/myeloma (X63Ag8.653) ratio resulted in the successful creation of antibody-secreting hybridomas. Macroscopic growth was observed in 38 of the 1,152 culture wells 13 days after the fusion. A screening ELISA was performed on all of the well supernatants. Based on the strength of ELISA reactions, 18 clones were selected for further study. Seven

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TABLE 1. Specificities of MAb ELISA reactions with antigens from oral spirochetes

Species and strain	Mean ELISA optical density at 405 nm ± SD (% reactivity) of MAb:	
	IAA11	IAC11
T. denticola		
ATCC 33521[a]	2.12 ± 0.09 (100)	2.01 ± 0.05 (100)
Ichelson[b]	2.22 ± 0.02 (105)	2.12 ± 0.01 (105)
D39DP1[b]	1.25 ± 0.19 (59)	1.25 ± 0.00 (62)
N39[b]	1.04 ± 0.02 (49)	0.92 ± 0.01 (46)
FM[b]	0.55 ± 0.03 (26)	0.32 ± 0.01 (16)
Ambigua[b]	0.11 ± 0.00 (5)	0.00 ± 0.00 (0)
TRRD[b]	0.09 ± 0.01 (4)	0.00 ± 0.00 (0)
IPP[b]	0.03 ± 0.02 (1)	0.00 ± 0.00 (0)
ATCC 33520[a]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
ATCC 35404[a]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
ATCC 35405[a]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T32A[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
ST10[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
TD2[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
D65BR1[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. vincentii		
D3A1[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
N9[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. scoliodontum MNII[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. socranskii subsp. *socranskii*		
D34BR1[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
D56BRIII6[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. socranskii subsp. *buccale*		
D11A1[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
D2B8[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. socranskii subsp. *paredis*		
D28C3[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
D46CPE1[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
T. pectinovorum D36DR2[b]	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)

[a] From the American Type Culture Collection.
[b] From the Virginia Polytechnic Institute and State University.

clones producing antibodies with strong ELISA reactivities were detected with an enzyme-labeled anti-mouse IgG reagent. These clones were selected for further study and recloned by limiting dilution. Two of the seven clones were slow growing and were abandoned.

The antibody isotypes were determined for the five remaining clones by double immunodiffusion and by ELISA. All five clones were found to secrete antibodies with IgG3 heavy chains and kappa light chains. However, one clone was discovered to be a polyclone, since it also secreted a mu heavy chain. The two remaining clones showing the greatest ELISA reactivities were then evaluated for specificities against a panel of 50 Formalin-fixed bacterial whole-cell antigens. The specific ELISA reactions with spirochete antigens for the MAbs from clones IAA11 and IAC11 are shown in Table 1. MAb IAA11 and MAb IAC11 reacted with 8 of 15 and 5 of 15 *T. denticola* isolates, respectively. The only nonspecific reaction observed involved a trace reaction with *T. vincentii* D3A1. However, subsequent antigen preparations tested negative. Positive reactions were never observed for any of the other treponemes. Cross-reactions with 25 strains representing nine nontreponemal oral bacteria tested were never observed (Table 2). Figure 1 indicates the titers of MAbs produced by each of the hybridomas.

TABLE 2. MAb specificities for various oral bacteria in the ELISA

Antigen (no. tested)	No. positive for MAb:	
	IAA11	IAC11
Treponema denticola (15)	8	5
T. vincentii (2)	0	0
T. scoliodontum (1)	0	0
T. socranskii (6)	0	0
T. pectinovorum (1)	0	0
Bacteroides gingivalis (9)	0	0
B. intermedius (4)	0	0
B. asaccharolyticus (1)	0	0
B. melaninogenicus (1)	0	0
B. macacae (1)	0	0
Fusobacterium nucleatum (1)	0	0
Streptococcus mutans (2)	0	0
Capnocytophaga ochracea (2)	0	0
Haemophilus actinomycetemcomitans (4)	0	0

The location of the epitope target of MAb IAA11 was determined by immunoelectron microscopy (Fig. 2). Fixed cells of *T. denticola* ATCC 33521 were reacted with either a medium control or with MAb IAA11. The gold-labeled anti-mouse IgG probe label was seen in both negative-stain and thin-section preparations. The location of the gold sphere indirect label was specifically associated with the outer sheath portion of the spirochete cell. The negative control preparation was not reactive.

DISCUSSION

The present study describes the first production of hybridoma cell lines which secrete MAbs specific for an oral spirochete. The MAb secreted by clone IAA11 was judged to be the most valuable, since it was able to detect 8 of 15 *T. denticola* strains. Our evidence of serovarieties among strains of *T. denticola* confirms the observations of earlier studies (2–4, 8). The MAbs appear to be specific for a serotype of *T. denticola* which includes strains ATCC 33521 (originally designated as strain 11 by Jacob and Nauman [5]), Ichelson,

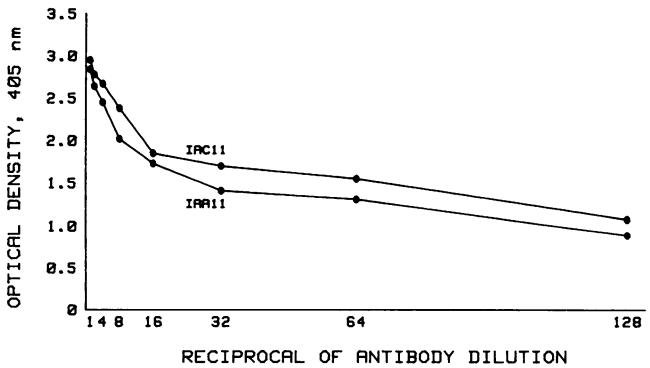

FIG. 1. Titers of MAbs to *T. denticola*, as determined by the ELISA. Microtiter wells coated with 400 μg of *T. denticola* ATCC 33521 were incubated with cellular hybridoma culture supernatants, and the ELISA was performed as described in the text.

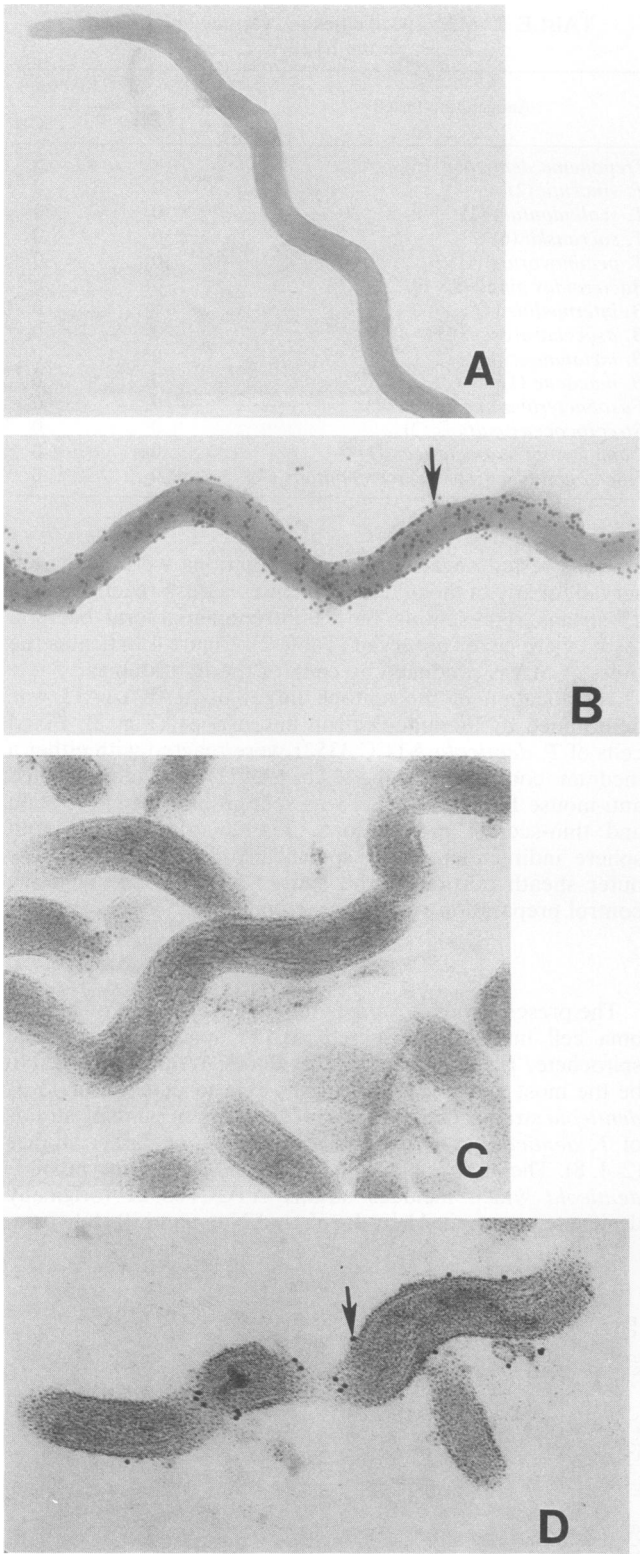

FIG. 2. Electron micrographs showing immunogold-labeled MAb IAA11 located (arrows) on the surface of *T. denticola* ATCC 33521. (A and B) Negative-stain preparations of culture medium-treated cells (control) (A) and MAb IAA11-treated cells (B). The preparations were then treated with a goat anti-mouse IgG colloidal gold spherical probe (10.7-nm diameter). (C and D) Thin-section preparations of samples A and B, respectively. Note the attachment of gold spheres (arrow) to the outer sheath of the spirochete. Magnifications, panels A, C, and D, ×11, 960; panel B, ×11, 730.

D39DP1, N39, and FM. MAb IAA11 showed weak reactivity with strains Ambigua, TRRD, and IPP. The only potential cross-reactions of MAb IAA11 and MAb IAC11 with the 50 bacterial antigens initially studied involved a weak reaction with *T. vincentii* D3A1. This reaction disappeared with subsequent antigen preparations, possibly indicating a trace contaminant in the original antigen preparation. The two MAbs described never reacted with other treponeme antigens or other oral bacterial isolates. The MAbs in this study did not react with strains ATCC 35404 or ATCC 35405, serotype A and C isolates, respectively, as designated by Cheng and Chan (2). Such a reaction would indicate a specificity for a different serogroup or possibly an additional serogroup. The MAbs did react with strain FM, a serogroup III isolate, as designated by Meyer and Hunter (8), but they did not react with *T. vincentii* N9, a member of their serogroup IV.

The antibody isotypes were determined for each of the MAbs selected for study. The observation that only single isotypes were found for each of the antibodies supports the concept that each antibody was derived from a single clone. Our use of an alkaline phosphatase-labeled anti-mouse IgG reagent precluded the selection of non-IgG antibodies.

Immunoelectron microscopy revealed the location of the epitope target of the MAbs. Both negative-stain and thin-section photomicrographs showed that the MAbs reacted with the outer sheath portion of the *T. denticola* cells studied. This finding was expected, since the mice were challenged with an immunogen preparation consisting of washed, Formalin-fixed *T. denticola* whole cells and not intracellular constituents.

Clinical studies involving the quantitative detection of *T. denticola* in plaque samples from periodontitis patients with the MAbs described here will be reported separately.

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