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Clustering of Fibronectin Adhesins toward *Treponema denticola* Tips upon Contact with Immobilized Fibronectin

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***Treponema denticola* has been shown to bind to immobilized fibronectin (Fn) by its tips. Yet labeling of cells in suspension with an Fn-gold conjugate to localize the Fn adhesins shows that they are distributed in patches along the entire cell length. Subsequent experiments have shown that the number and proportion of tip-oriented cells increase with time, suggesting that Fn contact stimulates *T. denticola* to rearrange adhesins toward its tips. To test this hypothesis, *T. denticola* cells were allowed to migrate in a 2% methylcellulose column toward nitrocellulose filters coated with Fn, laminin, bovine serum albumin, or phosphate-buffered saline. Cells close to and distant from the filters were collected, labeled with Fn-gold probes, and examined by transmission electron microscopy. The number of gold particles on each of 20 cells was counted, dividing each cell into thirds along its length: the end third with the most label (end 1), the middle third, and the end with the least label (end 2). The mean number (± standard deviation) of gold probes per third was calculated. Fn-gold probes clustered toward one end of *T. denticola* cells when in contact with Fn-coated nitrocellulose, with >55% of probes in end 1. In contrast, no clustering toward *T. denticola* ends occurred with laminin-, bovine serum albumin, or phosphate-buffered saline-coated filters or in the absence of a filter. Blocking access of the *T. denticola* cells to the Fn-coated nitrocellulose filter by placing an uncoated filter between them prevented clustering of Fn-gold. Removal of *T. denticola* cells from direct contact with the Fn-coated filter did not promote redistribution of clustered probes. These data suggest that *T. denticola* is stimulated to cluster Fn adhesins irreversibly toward its tips when it migrates into contact with immobilized Fn. This might be significant for establishing multiple adhesive interactions with host cells and ligands.**

Treponema denticola is a small, readily cultivable spirochete that has been implicated in the etiology of periodontitis (22, 23, 26, 33, 34). It has many potentially pathogenic properties, including the ability to elaborate a variety of proteolytic enzymes with chymotrypsin- and trypsin-like activities (28, 38). *T. denticola* can also suppress T lymphocytes, inhibit oxidative bursts in polymorphonuclear leukocytes, degrade matrix proteins, and elaborate various cytotoxic factors and endotoxin-like molecules (24, 31, 32). In vitro, *T. denticola* stimulates a variety of cytopathic responses in cultured cells. These include cell shrinkage, loss of volume regulation, depolymerization of F-actin, and decreased expression of desmoplakin II and cytokeratins in oral epithelial cells, as well as membrane deformation due to cytoskeletal rearrangement in gingival fibroblasts (2, 3, 6, 10, 30, 39, 43). Both cell types detach from their substratum matrix in response to *T. denticola* challenge.

To stimulate such responses, *T. denticola* cells would likely have to adhere to host cells and substrates in the extracellular matrix. Spirochetes do not have specialized structures, like fimbriae, that serve to bear multiple adhesins in a peripheral location that is accessible to substrates. Therefore, they have likely evolved alternate means to achieve avid adhesion. Spirochetes such as *T. denticola*, *Treponema pallidum*, *Leptospira* spp., and intestinal spirochetes adhere in a polar orientation to host cells and in some instances to ligands such as immobilized fibronectin (Fn) and laminin (9, 20, 21, 36, 42). We have quantified *T. denticola* adhesion to Fn and laminin and have shown that strains that adhere well frequently bind by their tips (9). This suggests that specific adhesins cluster near the tips. Recent studies using transblots of polyacrylamide gel electrophoresis gels have demonstrated the presence of Fn-binding proteins in the outer membrane of *T. denticola* (18, 40) and other treponemes like *T. pallidum* (29), but none so far has reported the localization of these adhesins at or near the poles of the bacteria. Rather, it is assumed that they are distributed randomly along the cell length. The aim of this study was to locate Fn adhesins on *T. denticola* and to determine whether these might be induced to cluster toward the tips. Our test hypothesis was that migration toward or contact with immobilized Fn stimulates clustering of Fn-binding adhesins near the tips of *T. denticola*.

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MATERIALS AND METHODS

Treponema strain, culture conditions, and reagents. *T. denticola* ATCC 33520 was provided by E. C. S. Chan, McGill University, Montreal, Canada. Stocks were maintained in spirochete medium (described below) supplemented with 0.3% Noble agar and subcultured once every 3 weeks. Cultures for experiments were grown as described previously (9). Briefly, the bacteria were grown in spirochete medium (containing, per liter, 12.5 g of brain heart infusion broth, 10.0 g of Trypticase, 2.5 g of yeast extract, 0.5 g of sodium thioglycolate, 1.0 g of L-cysteine hydrochloride, 0.25 g of L-asparagine, 2.0 g of glucose, 2.0 ml of volatile fatty acid mixture, 20.0 ml of rabbit serum, 20.0 ml of 10% sodium bicarbonate, and 3.0 ml of 0.2% cocarboxylase) for 72 h (late log phase) at 37°C in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) in an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Cells were harvested by centrifugation for 10 min at 800 × *g*, washed three times, and resuspended in phosphate-buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 4.6 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.2) to a

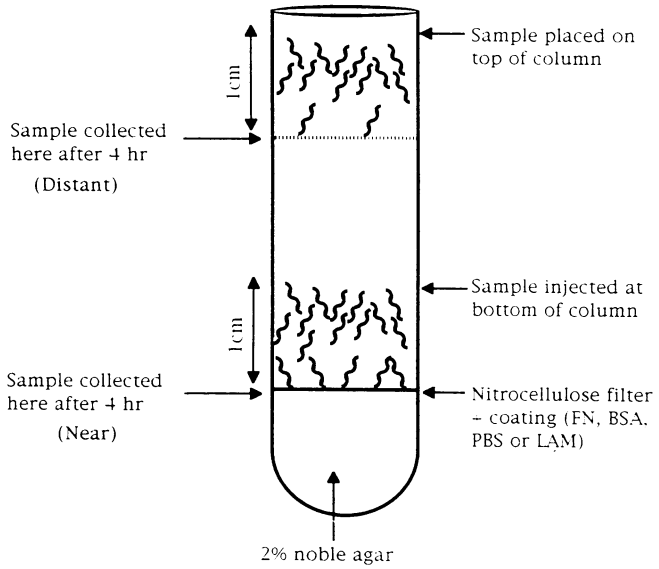

FIG. 1. Migration column with 2% methylcellulose. Lam, laminin.

concentration of approximately 1.2×10^{10} bacteria per ml except where indicated. Human Fn, laminin, and bovine serum albumin (BSA) were obtained from Sigma Chemical Co., St. Louis, Mo. Methylcellulose was obtained from BDH Chemicals Ltd., Poole, England; Noble agar was from Difco Laboratories, Detroit, Mich.; and nitrocellulose membranes (12-mm diameter) were obtained from Millipore Corp., Bedford, Mass.

Migration of *T. denticola* toward protein-coated nitrocellulose membranes. To test the hypothesis that migration toward or contact with immobilized Fn stimulates clustering of adhesins, a migration column was set up as illustrated (Fig. 1). All manipulations were done in the anaerobic chamber unless stated otherwise. Noble agar (2%) was placed at the bottom of a clear plastic centrifuge tube (Ultra-Clear; 14 by 89 mm; Beckman Instruments, Palo Alto, Calif.). On top of this was a nitrocellulose membrane which had previously been coated with either 40 μl of PBS (pH 7.2) or one of the following proteins: Fn (1 mg/ml), laminin (1 mg/ml), or BSA (3% [wt/vol]). The laminin- and Fn-coated filters were also blocked with 80 μl of BSA (3%). Methylcellulose (2%; high viscosity—4,000 cP) was gently added to the tube to a depth of 6 cm. The column was allowed to equilibrate in the anaerobic chamber for 48 h, after which it was removed and the bacterial suspension was added. Approximately 1.0 ml was injected with a syringe and 26-gauge needle through the side of the tube 1 cm above the coated filter. The needle was removed, and the hole was sealed with tape. One milliliter of suspension was also placed on the top of the column, which was then left at room temperature in air. After 4 to 5 h, samples of bacteria were withdrawn with a syringe and 18-gauge needle from (i) 1 cm from the top of the column but still distant from the nitrocellulose filter and (ii) near the filter at the bottom of the column, in both cases collecting bacteria which had migrated 1 cm from the original points of sample injection. The bacterial samples were washed with PBS to remove residual methylcellulose and then labeled with Fn-gold conjugate. A series of preliminary experiments comparing live and killed treponemes confirmed that the bacteria were actively motile in the column and had not moved 1 cm because of sedimentation.

To determine whether direct contact with Fn was necessary for adhesins to cluster, an uncoated nitrocellulose filter was placed on top of the Fn-coated filter before the methylcellulose was added. This would block direct access of bacteria to the immobilized Fn.

Experiments were conducted to determine whether clustered Fn-gold probes would redistribute after the bacteria were removed from contact with Fn-coated filters. *T. denticola* migration assays were done as described above but instead of being labeled immediately after removal from the Fn-coated nitrocellulose membrane, the cells were left at either 4 or 37°C for 3 and 24 h.

To test the effect of a protein synthesis inhibitor on Fn-adhesin distribution, the migration assay was run as described above, but the washed bacterial suspension was preincubated with 40 μg of chloramphenicol per ml for 2 h at 37°C before injection into the migration column.

Fn-gold preparation and the protocol for ligand-gold labeling of *T. denticola*. Fn-gold conjugate was prepared according to the method of Slot and Geuze (35). Briefly, colloidal gold particles with a diameter of approximately 10 nm were made by combining tetrachloroauric acid with tannic acid. Fn was conjugated to the gold particles at a pH of 6.9, 0.5 pH unit on the basic side of the isoelectric point of Fn. The Fn-gold conjugate was centrifuged at 82,700 × *g* for 30 min to remove unbound protein and then stored at 4°C for up to 2 months. Before each labeling experiment, the conjugate was centrifuged at 11,750 × *g* to remove aggregates and at 82,700 × *g* to remove dissociated protein. The bacterial cells that were washed free of methylcellulose were labeled by adding 15 μl of Fn-gold conjugate or, in some cases, polyethylene glycol bound to gold as a control for nonspecific binding (13). These were mixed, incubated for 2 h at 37°C or overnight at 4°C, and then washed with PBS to remove unbound gold conjugate. A 10-μl aliquot of the samples was mounted on a 300-mesh nickel grid coated with Formvar and carbon. The samples were stained with 1% methylamine tungstate and examined with the transmission electron microscope (TEM) (Philips 400T) at 80 kV.

Determination of the distribution and number of Fn-gold probes on *T. denticola*. The distribution and number of gold probes per bacterium were determined by TEM. For each sample, 20 bacteria were counted. Each bacterium was divided into thirds along its length by using a marker in the viewing field of the TEM. The end third with the most gold particles was assigned to data set end 1, and the end with the fewest gold particles was assigned to data set end 2. The first TEM field was chosen at random; then subsequent fields were examined in a zigzag pattern until the 20 bacteria were counted. Bacteria with large aggregates of Fn were excluded from the data sets. The mean number (± standard deviation) of gold particles in each end third and the middle third was calculated.

To control for subjectivity, a blind study was conducted. By the method described above for bacterial selection, photographs of 20 bacteria from each set of those that had migrated (i) near the Fn-coated filter and (ii) distant from the filter were taken by laboratory personnel other than the primary investigators. The number of gold probes in each third was counted by two independent investigators, and the means and standard deviations were calculated for both sample sets. The data obtained by the principal investigator and each of the independent investigators were comparable and showed strong correlations ($R = 0.97$ and 0.94). Data were analyzed by Student's *t* test for unpaired data to determine statistically significant differences.

Attachment of *T. denticola* to Fn-coated latex spheres. Seventy-five microliters of latex beads with a diameter of 0.97 μm (Polysciences Inc., Warrington, Pa.) was placed in a 1.5-ml

Eppendorf tube, washed three times with PBS, resuspended in 100 μl of Fn (1 mg/ml), and then incubated at 37°C for 2 h. The beads were washed to remove unbound Fn, and 200 μl of 3% (wt/vol) BSA was added. The conditioned beads were incubated at 37°C for 1 h and then washed in PBS. Two hundred microliters of a *T. denticola* suspension (4×10^9 bacteria per ml) was mixed with the coated latex beads and incubated at 37°C for 2 h. After incubation, the mixture was washed in PBS to remove unbound bacteria and prepared for scanning electron microscopy (SEM). The beads with attached bacteria were fixed in 2.5% glutaraldehyde in PBS for 2 h, washed in PBS, and dehydrated in a graded series of ethanol. Samples were air dried, coated with either carbon or 15-nm-diameter gold film, and examined with a Hitachi S-2500 SEM.

Effect of incubation time on tip binding. *T. denticola* cells were allowed to attach to Fn-coated coverslips as previously described (9). Briefly, plastic coverslips (Dispo; American Scientific Products, McGraw Park, Ill.; 22 by 22 mm) were incubated at 37°C for periods ranging from 30 min to 4 h. Following incubation, the unbound bacteria were washed away with PBS and the number of attached bacteria was counted by dark-field microscopy. The orientation of the bacteria was also noted. For each sample three microscopic fields were counted, and the samples were run in triplicate. The mean number (± standard deviation) of attached bacteria and the percentage of tip-oriented bacteria were calculated for each time interval.

Effect of inhibitors on *T. denticola* adhesion to Fn. *T. denticola* suspensions were pretreated overnight with the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) at 170 μg/ml or Na-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) at 150 μg/ml (3) or incubated anaerobically overnight with 1 mg of metronidazole (Sigma Chemical Co.) per ml. The treated bacterial suspensions were washed and then exposed to Fn- or BSA-coated coverslips at 37°C for 2 h. The mean number of tip-attached bacteria was determined as described previously (9). Untreated suspensions served as controls. Propidium iodide at a concentration of 10 μg/ml was used to stain a portion of each sample of the treated bacteria to determine the percentage of viable cells remaining in the population. Viable cells excluded the dye and remained unstained, whereas dead cells fluoresced red (3). Heat killing experiments in our laboratory have found that *T. denticola* suspensions contain a progressively greater percentage of propidium iodide-staining cells with increased heating time.

RESULTS

Confirmation of tip adhesion—effect of incubation time on *T. denticola* attachment to immobilized Fn. *T. denticola* adhesion to Fn-coated coverslips was time dependent (Table 1). Tip-oriented binding also increased with time. Whereas at 30 min only 7% of attached bacteria were bound by their tips, the number rose to 70% after 4 h ($P < 0.002$). The bacteria were observed to contact the Fn-coated coverslip at first along their lengths, but with time they became reoriented into an end-on position.

PMSF and TLCK both affected the adhesion of *T. denticola* to Fn. Whereas TLCK decreased the number bound by only 16%, pretreatment with PMSF resulted in a decrease of 71% (data not shown), suggesting that adhesion was affected more by an inhibitor of chymotrypsin-like than trypsin-like proteases. Pretreatment with metronidazole decreased the number of *T. denticola* cells bound by 59% even though an estimate of individual cell death by propidium iodide exclusion assay indicated that 86% of cells remained intact.

Distribution of labeled Fn adhesins. *T. denticola* cells were able to migrate at least 1 cm in 4 h in the methylcellulose column. When the bacteria that migrated near the Fn-coated filter were compared with those distant from the filter near the top of the column, there was a difference in the distribution of labeled adhesins, depending on the protein used to coat the filter. Bacteria in samples distant from the filters were labeled with Fn-gold probes randomly along their entire length. No noteworthy clustering of Fn adhesins was observed. Similarly, the bacteria sampled near the filters in the columns containing filters coated with BSA, laminin, or PBS absorbed Fn-gold probes with no particular distribution ($P \geq 0.95$) (Fig. 2 and 3). The only bacteria that had labeled adhesins more densely concentrated toward one end were those taken near or on the Fn-coated filter. For these, a mean of greater than 55% of the total gold probe count was found in end 1, with fewer probes in the middle and end 2 ($P < 0.002$). For bacteria that were in the same column and had migrated the same distance but were not in contact with the Fn-coated filter, the Fn-gold probes were evenly distributed along the length of the cell (Fig. 3 and 4). The distribution of gold probes followed an identical pattern when experiments were done under anaerobic conditions. Whereas the Fn-gold probe bound specifically only to the bacteria, the polyethylene glycol-gold control bound nonspecifically to the Formvar-coated grid and to a lesser extent to the bacteria.

TABLE 1. Adherence of *T. denticola* ATCC 33520 to Fn with time

Time (h)	Filter coating	Mean no. of cells attached		% Attached by tips
		Per field	By tips	
0.5	Fn	14 ± 4.0[a]	1	7
	BSA	0	0	
1.0	Fn	46 ± 8.2	19	40
	BSA	3	1	
2.0	Fn	92 ± 8.9	58	64
	BSA	3	2	
3.0	Fn	145 ± 12.0	97	67
	BSA	3	2	
4.0	Fn	182 ± 7.6	127	70
	BSA	5	3	

[a] Mean ± standard deviation.

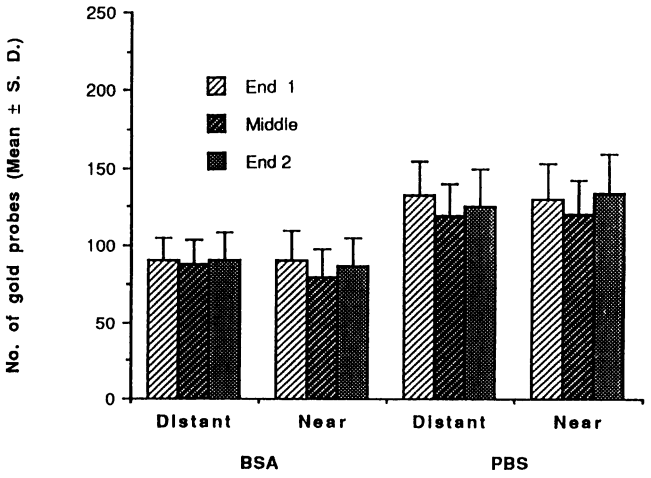

FIG. 2. Mean number of gold probes per third of 20 *T. denticola* cells counted after they had migrated distant from and near BSA- and PBS-coated filters in methylcellulose columns.

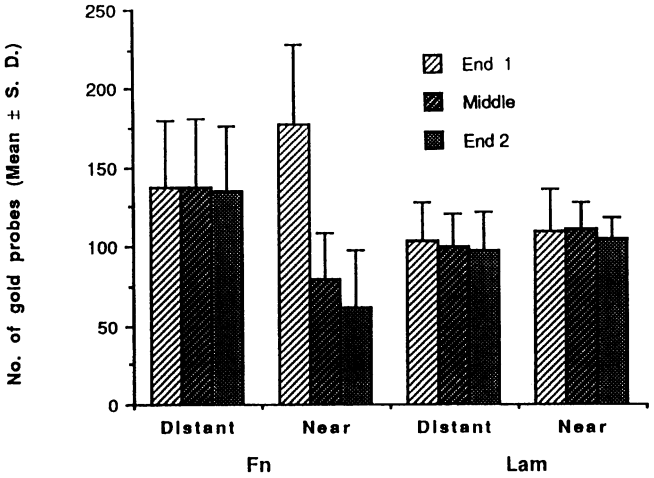

FIG. 3. Mean number of gold probes per third of 20 *T. denticola* cells counted after they had migrated distant from and near Fn- and laminin (Lam)-coated filters in methylcellulose columns.

The most concentrated Fn-gold labeling near one end versus the other was seen in the experiments in which *T. denticola* had direct access to the Fn-coated filters ($P < 0.001$). In the column in which access was blocked by an uncoated filter, the bacteria did not show any greater accumulation of gold probes at one end or the other ($P \geq 0.95$) (Fig. 5).

Preincubation of *T. denticola* with chloramphenicol did not alter the Fn probe clustering effect. Both chloramphenicol-treated and untreated *T. denticola* cells had Fn-gold probes enriched at one end after migrating near the Fn-coated filter ($P \geq 0.95$). For replicate experiments, the number of gold probes for end 1 ranged from 50 to 58%, that for the middle third ranged from 25 to 30%, and that for end 2 ranged from 5 to 23%.

Determination of redistribution of Fn adhesins. The bacteria that had migrated near the Fn-coated filter and then were removed from the filter, washed, and left for 3 to 24 h at 4 and 37°C before being labeled all had gold probes clustered toward one end ($P < 0.002$). Even after 24 h at 37°C, the labeled adhesins did not redistribute along the entire cell length (data not shown). The distribution of gold probes was similar to that observed with cells that were labeled immediately after removal from the Fn-coated filter.

Attachment of *T. denticola* to Fn-coated latex beads. *T. denticola* attached readily to Fn-coated latex beads. Usually the bacteria were tethered only by one end, not by their middle third (Fig. 6). Only occasionally were latex beads observed attached to both ends of a single *T. denticola* cell.

DISCUSSION

In this study we have tested the hypothesis that contact with a suitable ligand stimulates the reorientation of *T. denticola* adhesins. We have demonstrated that Fn adhesins concentrate toward one end of *T. denticola* cells when the cells migrate into contact with immobilized Fn. Neither BSA nor laminin elicited clustering of labeled Fn adhesins when the bacteria migrated toward filters coated with them. This suggests that the response of Fn adhesin clustering is specific for Fn as the target substrate. Likewise the clustering was not due to autoaggregation of the gold probes, since with the polyethylene glycol-gold no clustering was observed.

It has been shown by Charon et al. that the outer sheath of the spirochete *Leptospira interrogans* is very fluid (7). When antibody-coated latex beads were attached to antigens in the outer sheath, lateral movement of the beads at rates as high as 11 μm/s was detected. The movement was thought to be due to the drag created during forward motion of the cells in a viscous medium. In our study the bacteria that had migrated the same distance in the same column but had been sampled distant from the immobilized Fn or that had not established contact with Fn did not exhibit clustering of labeled Fn adhesins. Thus, motility alone seems insufficient to stimulate redistribution of surface adhesins.

Furthermore, the observed increase in tip attachment with prolonged incubation suggests that time is required for rearrangement of adhesins toward the tips, thus effecting a change in the attachment of *T. denticola* from a lateral to an end-on position when in contact with immobilized Fn. Moreover, metronidazole inhibited attachment to Fn while only marginally affecting bacterial vitality as defined by propidium iodide exclusion. One of the reported modes of action of metronidazole is that it affects low-redox-potential electron transport proteins in anaerobes, thereby altering energy generation by the cell (16, 27). Thus, metronidazole might have had a deleterious effect by restricting the energy available for motility or adhesin translocation, indirectly affecting attachment avidity.

The marked inhibition of attachment of *T. denticola* by PMSF might indicate that the chymotrypsin-like enzyme, which is surface bound and has the potential to bind to and degrade Fn and other extracellular matrix proteins (17), is involved in bacterial attachment to Fn. Although this might not be the primary mode of *T. denticola*'s attachment, it could conceivably account for some specificity of substrate recognition initially and perhaps for release of the free end as the cell passes from a lateral to a polar orientation.

The findings also do not support the idea that the observed clustering is due to migration through a chemotactic gradient, although this cannot be ruled out entirely. The bacteria would most likely have contacted a significant concentration of Fn only when they attached directly to the Fn-coated nitrocellulose filter. This is supported by experiments in which an uncoated filter placed on top of the Fn-coated filter, blocking direct access of the bacteria to Fn, prevented Fn adhesin clustering.

Clustering of labeled Fn adhesins was not readily reversed, suggesting that the outer sheath of the spirochetes had undergone some irreversible changes, perhaps adhesin cross-linking by the multivalent Fn-gold probes. This contention is supported by data from our previous investigation (9). When *T. denticola* cells were incubated with Fn-coated coverslips for 2 h at 4°C, fewer cells were bound by their tips compared with controls. After prolonged incubation (24 h), the number of attached cells was similar to that of the control (37°C for 2 h), but the percentage of tip-oriented treponemes was still significantly lower. If clustering of adhesins through a more fluid outer sheath is promoted at body temperature, such functional adaptation would be expected to foster a more avid attachment to tissues. As densely packed clumps of gold probes were seen only on bacteria from Fn-coated filters, the Fn probes were evidently not aggregated prior to use.

Chloramphenicol, an inhibitor of protein synthesis to which growth of *T. denticola* is sensitive at the concentration used, did not adversely affect Fn adhesin clustering. This suggests that if there was any de novo Fn adhesin synthesis induced during migration into contact with Fn, it was responsible for such a

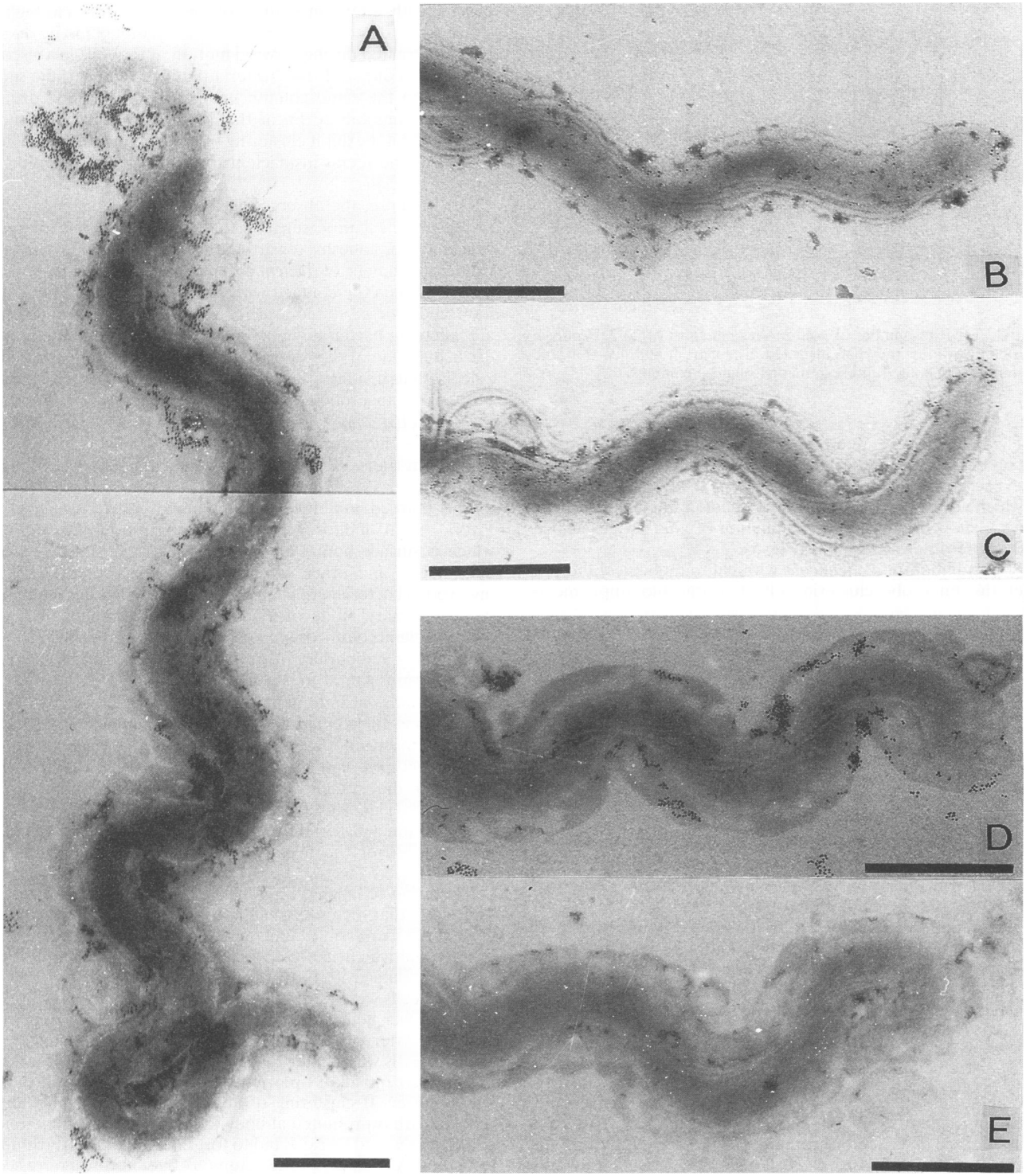

FIG. 4. TEM of Fn-gold-labeled *T. denticola* cells from methylcellulose columns containing Fn-coated filters. (A) Bacterium that migrated near Fn-coated filter, showing concentration of gold probes in end 1; (B to E) higher magnification of spirochete ends showing end 1 (B) and end 2 (C) of a spirochete distant from the filter with similar distribution of gold probes and end 1 (D) and end 2 (E) of a spirochete near the filter with clustering of gold probes only in end 1. (Bars represent 0.5 μm.)

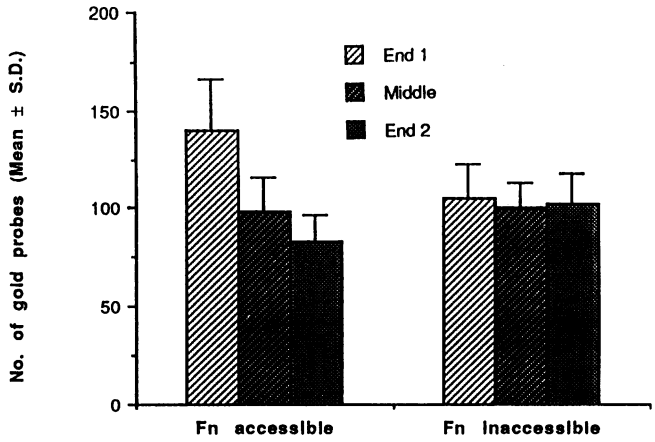

FIG. 5. Mean number of gold probes per third of 20 *T. denticola* cells counted after migrating in methylcellulose columns. (Right) Access to the Fn filter blocked by uncoated filter; (left) free access for direct contact with Fn-coated filter.

small proportion of the Fn adhesin localization that it did not significantly affect the outcome of the experiment.

The SEM findings provide supportive evidence for the clustering of adhesins near one end of the *T. denticola* cell. In no instance was an Fn-coated latex bead attached to the middle of the bacterium, with both ends of the spirochete free. Moreover, the Fn-coated latex beads adhered exclusively to one end in most cases. This suggests that after the 2-h incubation period, only one end of the bacterium has the ability to form an avid attachment to a ligand immobilized on such a large support, which is consistent with the time dependence for tip orientation. This end would presumably be the one toward which more adhesins had clustered.

T. denticola has been reported to attach to several host proteins, including laminin, collagen types I and IV, fibrinogen, gelatin, and Fn, which all might mediate attachment to oral tissues (9, 17, 38, 40). Although the precise molecular interactions have not been characterized, they may involve sulfhydryl-group-containing proteins or carbohydrate residues on the bacterial surfaces (19). There are several *T. denticola* polypeptides that absorb Fn from solution. Umemoto et al. identified three Fn-binding proteins, 53- and 72-kDa outer membrane proteins and a 38-kDa axial flagellar protein (40, 41). Haapasalo et al. also reported a major 53-kDa antigen as well as an obvious 72-kDa protein and several other minor proteins, including a 45-kDa outer sheath protein, which all bind Fn. Some of these proteins have been shown to be distributed along the entire *T. denticola* cell (18). We do not yet know if any of these are the adhesins that are stimulated to cluster upon contact with immobilized Fn.

It is unlikely that the 53-kDa protein fits the description of a freely fluid adhesin. Egli and coworkers recently reported that it appears to be a very large porin that is distributed rather evenly in a regularly patterned array over the *T. denticola* surface (12). They hypothesized that its Fn-binding function would retain a readily digested source of peptides that might be cleaved near the porin orifice by surface-associated proteases. Although it might not cluster toward the cell tips and contribute to avid adhesion to immobilized ligands, tip-oriented adhesion of *T. denticola* would allow the free cell end to swirl through suspending fluids, fostering the putative sieving functions of the 53-kDa porin that Egli and coworkers hypothesized.

Clustering of adhesins by treponemes in response to contact with specific ligands would likely promote more avid adhesion

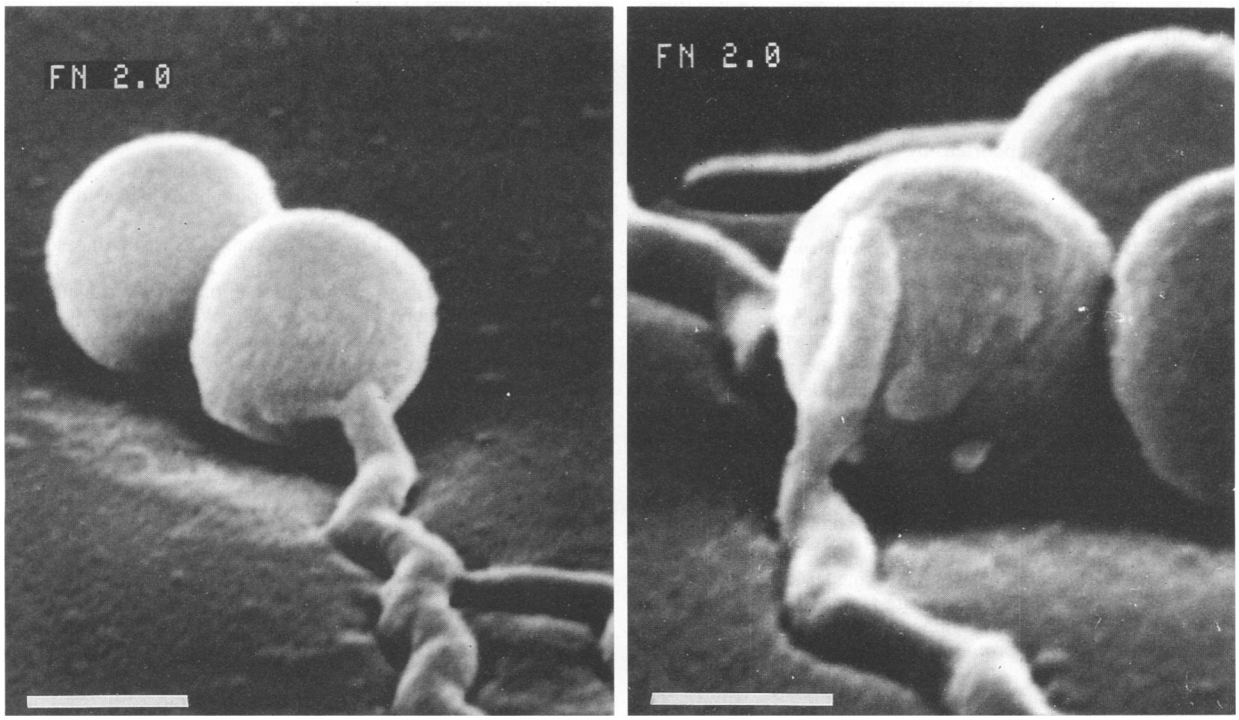

FIG. 6. SEM of *T. denticola* showing two examples of end-on attachment to Fn-coated latex beads. (Bars represent 0.5 μm.)

than if single, scattered adhesins were to establish contact. This is supported by our findings that tip orientation increases with time. There are many examples of localization of receptor functions to specific regions within a bacterium. These include the large crystalline array of bacteriorhodopsin within the invaginated cytoplasmic membrane of the photosynthetic *Hallobacterium halobium* (11), a guanosine triphosphate-binding protein that occurs only at the annular ring at the septation site in *Escherichia coli* (5, 15), and the chemoreceptors localized at the flagellated pole of *Caulobacter cresentus* during a portion of its life cycle (1). Maddock and Shapiro also showed clustering of up to 80% of membrane-bound chemoreceptors at the poles in *E. coli* (25). Yet no evidence was presented to suggest that clustering was induced in response to the presence of an attractant. This was not the case with *T. denticola*, in which the appropriate ligand had to be contacted for clustering to occur.

Perhaps cytadhesin localization in *Mycoplasma pneumoniae* provides a closer analogy to the situation that we have observed in *T. denticola*. Virulent, hemagglutinating *M. pneumoniae* cells bear concentrated clusters of P1 adhesin at the termini of tip structures that mediate adhesion (14). Although there is evidence that P1 adhesins are also synthesized by nonhemagglutinating mutants, Baseman and coworkers have found them devoid of P1 clusters in the cell termini (4, 8). It is interesting that their investigations into cytadhesins and Fn adhesins of *T. pallidum* have not yet yielded a pattern of polar adhesin concentration (37).

To our knowledge, this is the first report of treponemes concentrating their adhesins to a specific location to promote adhesion. Contact-stimulated clustering of specific adhesins might foster both colonization and substrate degradation, functions which would be highly significant for survival within the environment of periodontal pockets. The broader implication of this work is that stimulated outer membrane adhesin reorganization might be an example of a mechanism by which bacteria compensate for the restrictions of a simple architecture to function more effectively in colonization. It is possible that adhesin clustering will be found to be a common function among motile bacteria that require adhesion for retention on bathed surfaces.

ACKNOWLEDGMENT

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