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Movement of antibody-coated latex beads attached to the spirochete *Leptospira interrogans*

(motility/membrane fluidity/bacterial adherence)

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ABSTRACT **Antibody-coated latex beads (Ab-beads) were attached to *Leptospira interrogans* serovars *illini* 3055 and *icterohaemorrhagiae* SC1157. The movement of the Ab-beads relative to the motion of the cells was observed by direct darkfield microscopy or was recorded on videotape. When the Ab-beads were attached to the front end of motile cells, the Ab-beads were displaced towards the back end of the cells. When the cells reversed direction, the Ab-beads also reversed direction. A number of hypotheses were proposed and tested to account for this Ab-bead displacement. The one best supported by the evidence states that the Ab-beads are attached to antigens of the outer membrane sheath. These antigens are dragged laterally through the sheath due to the forward motion of the cells and the retarding forces of the medium acting on the beads. The results obtained provide information on the nature of the outer membrane sheath of *L. interrogans*, the basis for certain movements of spirochetes, and insight on how spirochetes attach to eukaryotic cells and tissues. In addition, the results indicate that antigens can move laterally through membranes as rapidly as 11 μm/sec.**

Leptospira interrogans is a spirochete with a well-characterized ultrastructure and a unique mode of motility. Outermost is an outer membrane sheath (OS), and within this sheath is the right-handed helical cell cylinder and two axial filaments (AF) (1–4). Each AF is subterminally attached to the cell cylinder, and genetic evidence suggests that the AFs are directly involved in motility (5). On the basis of this and other evidence, a motility model was proposed which states that rotational motors at the base of the AFs propel the organisms in a manner analogous to the flagella of rod-shaped bacteria (6).

In the course of testing this motility model, we coated latex beads with antibodies directed to whole cells (Ab-beads) and attached these Ab-beads to swimming cells. Our goal was to track the rotational movement of the Ab-beads as the cells swam in a given direction. However, we were surprised to find that when Ab-beads were attached to the front end of the cells they were rapidly displaced along the length of the cells until the Ab-beads reached the back end. The Ab-beads reversed direction as the cells reversed direction. These and related motions of cells and the attached Ab-beads were recorded on videotape, and tracings from these tapes are presented in this communication. The results suggest that the Ab-beads are attached to antigens of the OS. These antigens are dragged laterally through the OS to the back end of the cells due to the forward motion of the cells and the retarding forces of the medium acting on the beads.

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EXPERIMENTAL PROCEDURES

Bacteria. *L. interrogans* serovar *illini* 3055 (referred to as serovar *illini*), and the linear motility mutants DB115, DB218, DB290, and DB340 derived from serovar *illini* have been described (5). These linear mutants lack the hook- and spiral-shaped ends typical of wild-type serovar *illini*; they are also deficient in both translational and nontranslational motility. Serovar *icterohaemorrhagiae* SC1157 was obtained from R. C. Johnson of the University of Minnesota.

Media and Culture Conditions. The cells were maintained in the Tween-80/bovine serum albumin (Scientific Protein Laboratories, Waunakee, WI) complete medium described by Ellinghausen and McCullough and modifed by Johnson and Harris (EMJH complete medium) (7). Cultures were maintained at 30°C on a rotary environmental shaker, and cell growth was monitored by nephelometry, using a Coleman model 7 nephelometer (7).

Preparation of Immunoglobulin Fraction. Cell antigen was prepared by harvesting 20 ml of logarithmic-phase cells (7×10^8 cells per ml) by centrifugation at 17,000 × *g* for 15 min at 5°C, washing the cells three times by alternate cycles of suspending the pellet in 50 ml of cold (5°C) EMJH basal medium (without albumin) (7), and centrifuging as before. Immunization was carried out by injecting 0.25 ml of the washed cell suspension (1.4×10^9 cells per ml) into the marginal ear vein of rabbits three times a week for 3 weeks. On the fourth week, a single injection of a 1-ml cell suspension (1.4×10^9 cells) was administered to each rabbit, and the sera were harvested on the fifth week and stored at −70°C. Microscopic agglutination titers were approximately 1:6000. The immunoglobulin fraction was obtained by three cycles of precipitation with 35% saturated ammonium sulfate and redissolving the resultant precipitate in water (8). The final fraction was desalted by chromatography through a Sephadex G-25 column using saline as the eluent and stored at −70°C (9).

Attachment of Antibodies to Latex Beads. Approximately 0.1 ml of a uniform latex bead suspension (radii *r* were 0.12 μm, 0.25 μm, or 0.40 μm) at a concentration of 2×10^{10} to 2×10^{11} beads per ml (Duke Scientific, Palo Alto, CA, and Difco, Detroit, MI) was added to 4.9 ml of glycine/saline buffer, pH 9.6 (G-buffer) (10). One milliliter of this suspension was mixed with 1.0 ml of the immunoglobulin fraction previously diluted in G-buffer to 65–650 μg of protein per ml. After incubation for 3 hr at 23°C, 12 ml of G-buffer containing 0.5% bovine serum

Abbreviations: Ab-beads, antibody-coated latex beads; AF, axial filament; OS, outer membrane sheath; EMJH complete or basal medium, complete or basal medium of Ellinghausen and McCullough modified by Johnson and Harris; G-buffer, glycine/saline buffer.

albumin was added to the tube. To separate the Ab-beads from antibodies that failed to attach, the Ab-bead suspension was gently layered onto a 4-ml 45% wt/vol sucrose cushion in a nitrocellulose tube. This suspension was centrifuged for 30 min at 42,000 × *g* in a Beckman SW 27 rotor at 20°C. The Ab-beads banded at the G-buffer–sucrose interface and were obtained by piercing the bottom of the tube and collecting the fractions containing the Ab-beads. The Ab-beads were stored in a final volume of 2 ml in G-buffer at 4°C and were stable for at least one month.

Ab-Bead Attachment to Cells and Direct Darkfield Microscopy. To attach Ab-beads to cells, logarithmic-phase cells were diluted to 1×10^8 cells per ml in 0.5% albumin/saline for serovar *illini* [the viscosity was 1.0 centistoke (1 mm^2/sec) as determined with a Cannon–Manning viscometer], or in EMJH basal medium with 0.5% albumin and 0.9% NaCl, for serovar *icterohaemorrhagiae*. Eighty microliters of these cells was mixed with 5 μl of Ab-beads for 2 min at 23°C in a serology tube. Approximately 20 μl of this suspension was placed onto a precleaned slide and sealed with a square 22-mm cover-glass with silicone along its edges. Cells with attached Ab-beads were viewed at ×400 and ×900 under darkfield illumination at 25°C. Greater than 50% of the cells had Ab-beads attached. In experiments using methylcellulose, cell/Ab-bead suspensions were mixed immediately prior to observation with EMJH medium containing methylcellulose [4000 centipoise (4 Pa·sec); Fisher]. The final methylcellulose concentration was 1% (6, 11). Cell lengths and velocities were determined by using a calibrated ocular micrometer. Cell velocity determinations were made on free-swimming cells when the attached Ab-beads were not being displaced. The rates of displacement of Ab-beads with $r = 0.4$ μm on swimming cells, and of Ab-beads fixed to a glass surface, were determined by dividing the length of each cell by the time for the Ab-bead to travel from the front to the back of the cell. Because Ab-beads with $r = 0.12$ μm were displaced more slowly, the cells often reversed direction before the Ab-beads reached the back end of the cells. Accordingly, the rate of displacement for 0.12 μm Ab-beads was based on the time for the Ab-bead to be displaced ½ the length of a cell, divided by ½ the length of that cell.

Videotaping Cells with Attached Ab-Beads. Cells with attached Ab-beads were videotaped by using an RCA TC1000 camera attached to a Reichert Univar microscope under darkfield illumination and a xenon light source. To prevent cell immobilization by this light source, a yellow barrier filter with a 50% cutoff at 530 nm (Fish Schurman, New Rochelle, NY) was placed over the illumination lens (12). Videotapes (UCA, ¾-inch video cassette, 3M, St. Paul, MN) of cells were made with a ×40 or ×100 objective. Tracings from the tapes were made by stopping the playback apparatus field by field on the video monitor (60 fields per second), and tracing the images onto plastic transparency sheets (Write-on film, 3M). Cell lengths and velocities were determined by calibration to a video recording of a substage micrometer.

RESULTS

Displacement of Ab-Beads on Swimming Cells. Ab-beads were attached to swimming cells of serovars *illini* and *icterohaemorrhagiae*. Coating the latex beads with specific antibody was necessary for attachment, because latex beads alone, or latex beads coated with antibody to a different serovar, failed to attach to the cells. Electron microscopy confirmed that the Ab-beads adhered to the outer surface of the cells (unpublished). The movements of Ab-beads and cells were observed by direct darkfield microscopy or were recorded on videotape. The following general trend was observed: When a cell swam in one direction with an Ab-bead of $r = 0.25$ μm or greater attached to the front end of that cell, the Ab-bead was rapidly displaced to the back end of that cell (Fig. 1). The Ab-bead remained at the back end as the cell continued to swim in the same direction. When the cell reversed direction, the Ab-bead was displaced to the opposite end as before (not shown). Because Ab-bead displacement was an unexpected finding, a number of hypotheses were tested to account for this displacement.

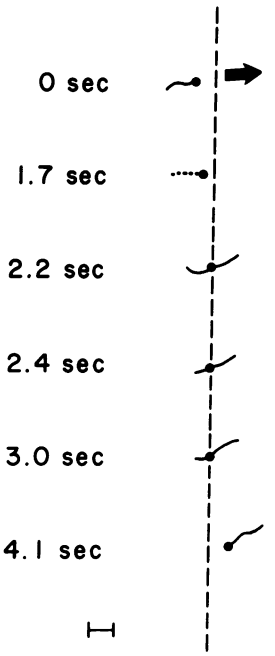

FIG. 1. Displacement of Ab-bead (●, $r = 0.25$ μm) on serovar *illini*. The vertical broken line represents a reference position of the cell in the field. The dotted cell was partially out of focus. Bar represents 5 μm. The arrow denotes the direction of swimming of the cell.

Displacement of Ab-Beads on Tethered Cells. One hypothesis for Ab-bead displacement states that the attached Ab-beads roll along the OS of the cell. Thus, as the cell swims in a given direction, the Ab-bead, which is likely to contain multiple antibodies, rolls backwards along the OS and moves from one antigen to another. To test this hypothesis, cells of both serovars were examined with the Ab-beads attached both to the cells and to either the cover-glass or slide. Such tethered cells occurred spontaneously in cell Ab-bead preparations. We found that these cells rapidly moved back and forth across the surface of the stationary Ab-bead (Fig. 2). The movement of the cells was accomplished without slip—i.e., it was primarily a screwing motion. Analysis of videotapes of five cells undergoing this motion indicated that the rate of Ab-bead displacement from one end of the cell to the other was 11.0 ± 1.1 μm/sec (mean ± SEM, range 6.9–13.6 μm/sec). In addition, when the cells were tethered to more than one Ab-bead and the Ab-beads were attached to different points on a glass surface, cells moved back and forth in a creeping motion (not shown). These results suggest that Ab-bead displacement is not dependent on rolling of the Ab-bead along the cell OS.

Displacement of Multiple Ab-Beads on Swimming Cells. A second hypothesis that could account for Ab-bead displacement is a motion whereby the entire OS is actively revolving as a unit. According to this hypothesis, the Ab-beads are displaced to the back end of the cell due to a revolving motion of a presumably rigid OS lengthwise around the cell cylinder, like a tread of a tank. To test this hypothesis, we examined cells that had more

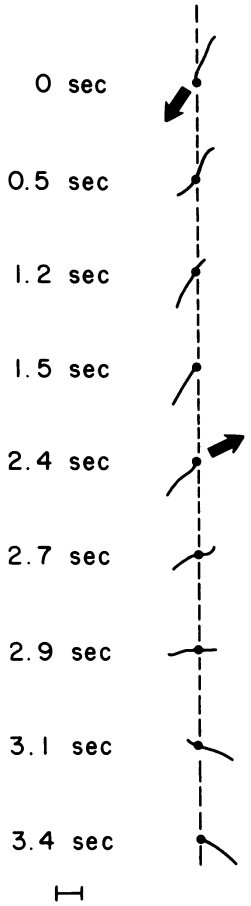

FIG. 2. Displacement of Ab-bead (r = 0.12 μm) on serovar *illini* tethered to the cover-glass. Broken line and bar as in Fig. 1.

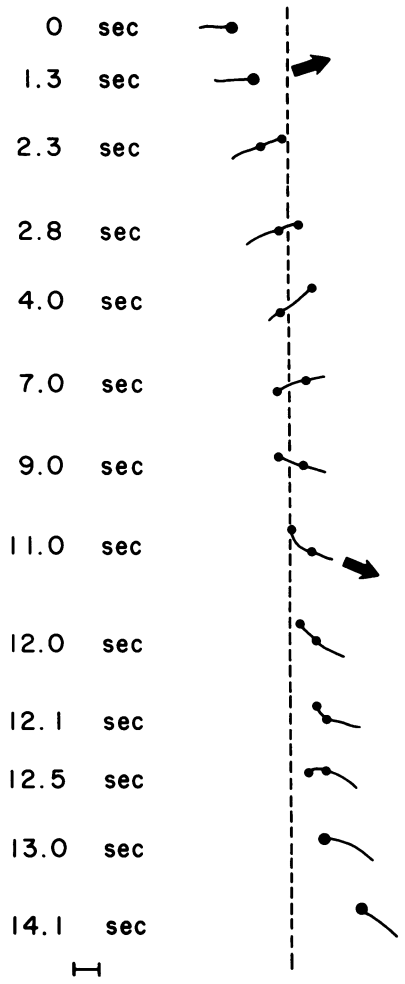

FIG. 3. Separation of a clump of two Ab-beads (r = 0.25 μm) at the front end of a cell of serovar *illini* and their subsequent coming together again at the back end of that cell. Broken line and bar as in Fig. 1.

than one Ab-bead attached to different positions on each cell. If the OS is revolving around the cell cylinder, the Ab-beads attached to the cell should remain equidistant from one another. Contrary to this hypothesis, we found that Ab-beads attached to different sites on a swimming cell came together at the back end (not shown). In addition, cells were occasionally seen with a clump of Ab-beads at the back end of a cell. When these cells reversed direction, the Ab-beads separated and displaced to the opposite end at different rates (Fig. 3). These results taken together suggest that the moving OS hypothesis does not account for Ab-bead displacement.

Failure of Ab-Bead Displacement on Motility Mutants. A third hypothesis to account for Ab-bead displacement is based on the fluid-mosaic model of membrane structure (13). This hypothesis states that the Ab-bead is attached to an antigen (or antigens) of the OS. As the cell swims in one direction, the movement of the antigen that is attached to the Ab-bead is retarded due to viscous forces of the medium acting on the Ab-bead. As a result, the antigen is dragged laterally through the OS. Thus the Ab-bead–antigen complex is displaced from the front to the back end of the cell, and its displacement is related to the retarding forces acting on the Ab-bead and the motility and direction of the swimming of the cell. This hypothesis is consistent with the results found with cells adhering to a glass surface via the Ab-beads and the results with the displacement of multiple Ab-beads attached to a cell (Figs. 2 and 3).

A number of experiments were done to test this hypothesis. First, according to this hypothesis, Ab-bead displacement is dependent on cell motility. To test this, Ab-beads were attached to serovar *illini* motility mutants DB115, DB218, DB290, and DB340, and their movements were recorded on videotape. The attached Ab-beads were not displaced along the length of any of the mutants (Fig. 4 for DB115). When the Ab-beads attached

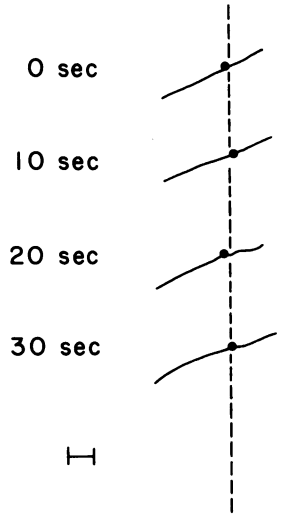

FIG. 4. Failure of Ab-bead (r = 0.25 μm) displacement attached to the motility mutant of serovar *illini* DB115. Broken line and bar as in Fig. 1.

to the cells were also fixed to a glass surface, the Ab-beads remained at the same position on the cells. These results are consistent with this hypothesis, and they indicate that Ab-bead displacement is dependent on cell motility.

Dependence of Displacement on Ab-Bead Size. One prediction of the fluid-mosaic membrane hypothesis states that the rate of Ab-bead displacement is dependent on the viscous retarding force applied to the Ab-bead. This force is primarily related to the size rather than the mass of the Ab-beads because inertial effects, in contrast to viscous effects, are negligible for particles of this size (14). To test this, Ab-beads of two sizes (r = 0.12 and 0.4 μm) were attached to motile serovar *illini*, and the rates of the Ab-bead displacement along the cell length were determined by direct microscopy when the cells reversed direction. Ab-beads with r of 0.4 μm were displaced from one end of the cell to the other at a mean rate (±SEM) of 4.9 ± 1.4 μm/sec for eight cells examined. The maximal rate of displacement was 14 μm/sec, and one cell had an attached Ab-bead with no displacement. On the other hand, Ab-beads of smaller size with an r of 0.12 μm were displaced at a considerably slower rate, if at all. The mean rate of displacement was 0.31 ± 0.1 μm/sec for 10 cells examined. The fastest rate of Ab-bead displacement was 0.9 μm/sec. Four cells showed no Ab-bead displacement. In addition, we observed that when cells swimming with the same velocity and having attached Ab-beads of either size were compared, the large Ab-beads were displaced faster than the small Ab-beads. For example, at a velocity of 2.0 ± 0.5 μm/sec, two cells with attached large Ab-beads had a rate of Ab-bead displacement of 4.5 μm/sec and 6.0 μm/sec. At this same velocity, six cells with attached small Ab-beads had a rate of Ab-bead displacement of 0.36 ± 0.09 μm/sec, with two of the six showing no displacement. These results suggest that the rate of Ab-bead displacement is dependent on Ab-bead size.

Displacement of Ab-Beads on Cells in Methylcellulose. According to the fluid-mosaic membrane hypothesis, the retarding forces that inhibit Ab-bead movement influence the rate of Ab-bead displacement. When small Ab-beads (r = 0.12 μm) were fixed to both cells and slide, the Ab-beads were displaced along the cell length considerably faster than small Ab-beads attached to free-swimming cells (Fig. 2). Furthermore, when cells with attached small Ab-beads were suspended in a gel-like medium (11) consisting of motility medium and 1% methylcellulose, small Ab-bead displacement was also very rapid (Fig. 5). Because the Ab-beads remained relatively stationary during displacement, the Ab-beads were seemingly caught in the matrix of the gel. These results of cells with fixed Ab-beads and cells in methylcellulose indicate that retarding Ab-bead movement enhances Ab-bead displacement.

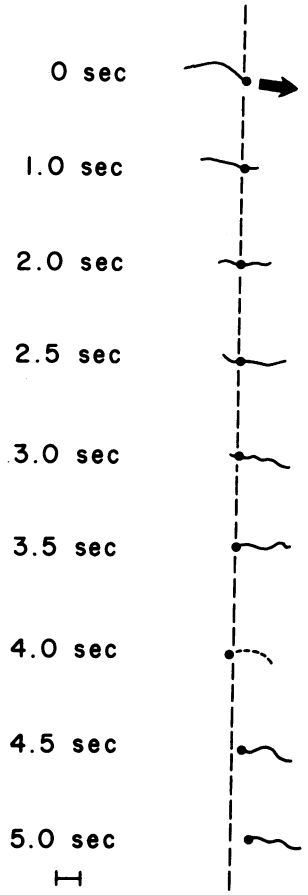

FIG. 5. Displacement of Ab-bead (r = 0.12 μm) attached to serovar *illini* in 1% methylcellulose. Broken line, bar, and dotted cell as in Fig. 1.

DISCUSSION

The present communication reports that Ab-beads attached to two serovars of *L. interrogans* are displaced backwards along the lengths of swimming cells. Because these serovars belong to markedly different DNA hybridization groups (15), the results obtained are likely to be relevant to other serovars of the genus. Jarosch observed a similar phenomenon of India ink particles being displaced along the length of *L. interrogans* (16). Conceivably, these particles could be adhering to the cells in a manner analogous to the Ab-beads attaching to *L. interrogans*. Our results differ from similar studies made on the movement of latex beads on gliding bacteria (17). Pate and Chang found that latex beads alone attach to *Cytophaga johnsonae* and *Flexibacter columnaris*. On the other hand, we found that precoating the latex beads with antibodies is necessary for their attachment to *L. interrogans*. In addition, latex beads that are attached to the gliding bacteria move in many different directions (17). On *L. interrogans*, the Ab-beads consistently move towards the back end of the cells.

Although the results indicate that the rate of displacement of Ab-beads on swimming cells is dependent on Ab-bead size, wide variation was found with respect to the rate of displacement. Thus, for Ab-beads with a radius of 0.4 μm, some Ab-beads were displaced very fast (14 μm/sec), whereas on one cell the Ab-bead was not displaced. Although there are a number of possible explanations to account for this variation, such as the velocity of the cells, the nature and size of the membrane antigens are apparent factors. Because the Ab-beads were prepared with antibodies to whole cells, the Ab-beads are likely to attach to different OS antigens. Some of these antigens could conceivably move laterally through the membrane more readily than others, because different lateral diffusion rates have been observed, for example, for the matrix protein and the lipopolysaccharide of *Escherichia coli* (18). Along these lines, the different rates at which the two Ab-beads move to the back of the cell as shown in Fig. 3 could conceivably be due to their attachment to different OS antigens. In addition, although little is known about the OS antigens of *L. interrogans*, one or more could be analogous to the Braun lipoprotein of *E. coli*, which is anchored to the peptidoglycan and extends into the outer membrane (18, 19). If a large Ab-bead is attached to an antigen of this type, it should not be displaced from one end of the cell to the other. Accordingly, we expect that future studies with antibodies to specific OS antigens will eliminate much of the variation in the rate of displacement of attached Ab-beads.

The displacement of Ab-beads on cells of *L. interrogans* indicates that the lateral movement of antigens through mem-

branes can be very fast. The mean rate of displacement of Ab-beads on tethered cells was 11.0 ± 1.1 μm/sec. This rate is considerably faster than the energy-dependent lateral movement of membrane antigens associated with capping found with eukaryotic cells (20). For example, Schreiner and Unanue (21) found that 4 min were required for 90% of murine lymphocytes (diameter 9–14 μm) to form caps when fluorescently labeled IgG was used. The very high rate of Ab-bead displacement with *L. interrogans* is likely due to the external forces applied to the antigens via attached Ab-bead and the rapid motility of the cells. As a corollary, we wonder if Ab-beads attached to specific lipopolysaccharide and membrane proteins on motile *Escherichia coli* will be displaced as rapidly as observed with *L. interrogans*.

Observations of *L. interrogans* tethered to fixed Ab-beads permits us to interpret certain aspects of the motion of *L. interrogans* and other spirochetes. Cox and Twigg (22) observed that cells of *L. interrogans* that adhere to a glass surface often move back and forth without slip as if the cells were passing through a staple or loop ("staple motion") (22, 23). We found a similar back-and-forth motion of *L. interrogans* attached to Ab-beads fixed to a glass surface (Fig. 2). We propose that staple motion occurs when certain OS components stick to a glass surface, possibly by electrostatic forces, in an analogous manner to cells adhering to fixed Ab-beads. The cells move without slip back and forth, as they push off the Ab-bead (or glass surface) in a mechanism similar to that by which *L. interrogans* pushes off the fibers in the gel of methylcellulose (6, 11). Similarly, the creeping (crawling) motion on a glass surface described for *L. interrogans* (22, 23) and *Spirochaeta plicatilis* (23, 24) may be accounted for by an analogous mechanism. In this case, the OS components are attached to many points on the glass surface. The cells creep back and forth as we observed with cells attached to multiple Ab-beads. Occasionally, they may show a net forward movement when one of the sites on the OS becomes detached from the glass.

L. interrogans attached to fixed Ab-beads may yield some information concerning the nature of the interaction of spirochetes to tissues and to eukaryotic cells. *L. interrogans*, *Treponma pallidum*, and other spirochetes are seen attached in an end-on arrangement to cultured mammalian cells, tissues, and *in vitro* to plastic films coated with lectins (2, 25–27). Hayes *et al.* (26) suggest that for *T. pallidum* the end of the cell serves as a specialized attachment organ. However, we propose that there may not be a receptor specifically at the end of the spirochete for the tissue or cell. Instead, this receptor may be distributed throughout the OS of the spirochete. Because the cells are motile and have a fluid OS, once the spirochetes attach to the tissue or cells, they eventually assume an end-on configuration. In support of this concept, *T. pallidum* are occasionally seen attached to cultured cells along their entire length (25, 26). This result would support multiple attachment sites on the spirochetes for the cultured cell.

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