Intracellular Localization of *Borrelia burgdorferi* within Human Endothelial Cells

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The later stages of infection by the Lyme disease pathogen, *Borrelia burgdorferi*, are characterized by the persistence of the organism in individuals possessing a strong anti-*Borrelia* immune response. This suggests that the organism is sequestered in a tissue protected from the immune system of the host or there is a reservoir of the organism residing within the cells of the host. In this report, the ability of *B. burgdorferi* to gain entrance into human umbilical vein endothelial cells was explored as a model for invasion. Incubation of *B. burgdorferi* with human umbilical vein endothelial cells at ratios ranging from 200:1 to 5,000:1 resulted in the intracellular localization of 0 to 25% of *B. burgdorferi* in 24 h. The intracellular location of the spirochetes was demonstrated by the incorporation of radiolabeled *B. burgdorferi* into a trypsin-resistant compartment and was confirmed by double-immunofluorescence staining which differentiated intracellular from extracellular organisms. Actin-containing microfilaments were required for the intracellular localization, indicating that the host cell participates in the internalization process. Activation of endothelial cells by agents known to increase the expression of several adhesion molecules had no effect on the interaction of *B. burgdorferi* with the endothelial monolayer. This indicates that the endothelial receptor for *B. burgdorferi* is constitutively expressed and that internalization is not dependent upon adhesion molecules whose expression is induced by inflammatory mediators. The demonstration of *B. burgdorferi* within endothelial cells suggests that intracellular localization may be a potential mechanism by which the organism escapes from the immune response of the host and may contribute to persistence of the organism during the later stages of Lyme disease.

Lyme disease is caused by the tick-borne spirochete *Borrelia burgdorferi* (8, 19). Infection by this organism causes multiple and varied symptoms which have been categorized into three stages (17, 27, 29). The first stage involves the initial symptoms of the disease including erythema chronicum migrans at the site of the tick bite and more generalized flu-like symptoms. The second and third stages of disease occur weeks to months after the first stage and can involve several different organs including cardiac tissue, the central nervous system, and joints. In general, infected humans and animals experiencing symptoms of stage II and III disease have an active anti-*Borrelia* immune response including both humoral and cellular immunity. Antibiotic therapy resolves stage II and III symptoms in most individuals, indicating that these later stages of disease are due to persistence of the organism. The ability of the spirochete to maintain infection in the presence of a specific immune response suggests that the organism may hide from the defenses of the host in the later stages of infection. The neurological involvement seen in some individuals and the improved success in treatment of patients with stage II and III disease with ceftriaxone, an antibiotic which crosses the blood-brain barrier, implies that the central nervous system may serve as a protective niche (11). Other investigators have shown that *B. burgdorferi* can transcytose an endothelial monolayer, providing a mechanism by which these spirochetes could invade a variety of tissues (10, 30). It has also been speculated that *B. burgdorferi* resides within cells of certain tissues during or between stages of disease, which could provide a second mechanism contributing to persistence by the organism (5, 26, 27).

In this study we have addressed the possibility of *B. burgdorferi* localization within mammalian cells by determining if the spirochete could be demonstrated within human endothelial cells. Endothelial cells were chosen because spirochetes can penetrate endothelial monolayers and adhere to endothelial cells in vitro (10, 30, 31) and because damage to the endothelium has been observed in animals and patients infected with *B. burgdorferi* (12, 28). Spirochete internalization by endothelial cells was demonstrated by the uptake of radiolabeled bacteria into a trypsin-resistant endothelial cell compartment. This was verified by visualization of the bacterium within the endothelial cell.

MATERIALS AND METHODS

**Bacteria.** Low-passage frozen stocks of the JD-1 strain of *B. burgdorferi*, provided by Sam Telford and Jose Ribeiro of the Harvard School of Public Health, were maintained at −70°C (23). Frozen stocks were seeded in 16 ml of BSK-II (1) containing 40 µCi of [3H]adenine and cultured for 3 to 4 days at 32°C until reaching a density of approximately 10⁷ spirochetes per ml. This gave optimal labeling of spirochetes, with approximately 5,000 cpm incorporated per 10⁷ spirochetes. A derivative of *B. burgdorferi* SH2-82 that has been passaged 300 times in the laboratory and is no longer infective for mice was obtained from Tom Schwan of the Rocky Mountain Laboratories (26).

**Endothelial cells.** Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical cords by the standard procedure that uses collagenase digestion (18) and were cultured in endothelial growth medium (Clonetics) on tissue culture flasks coated with 2% gelatin. HUVECs up to passage six were used in internalization experiments. Virtually 100% of HUVECs were able to internalize fluorescein-. * Corresponding author.
ated, acetylated low-density lipoprotein (LDL), a ligand bound specifically by the scavenger receptor of endothelial cells and macrophages (33), thus ensuring that the HUVEC cultures were free from smooth-muscle or other contaminating cell types.

**Internalization assay.** Internalization of *B. burgdorferi* by endothelial cells was measured in an assay that allows discrimination between spirochetes that are tightly adherent to the extracellular surface of the HUVECs from those inside the HUVECs. The low-passage JD-1 strain of *B. burgdorferi* was labeled by growth for 3 to 5 days in BSK-II containing 2.5 μCi of [3H]adenine per ml and washed twice with RPMI 1640 containing 20% heat-inactivated fetal calf serum (RPMI-FCS). Under optimal conditions, the spirochetes were labeled to a specific activity of $2 \times 10^5$ bacteria per cpm. The spirochetes were counted, and the indicated number was added to the cultured HUVECs. HUVECs were seeded at $2 \times 10^5$ cells per 2-cm² well in a 24-well cluster dish and allowed to form a confluent monolayer overnight. The indicated number of spirochetes was added to each well, and samples were incubated at 37°C for the indicated times. Four wells were prepared for each sample. Supernatant and cells from two wells for each sample were pooled to have adequate cells for a pellet. This allowed duplicate data points to be collected for each sample. Samples were harvested by removing the supernatants, rinsing the surface of cells with RPMI 1640 lacking fetal calf serum, and pooling the supernatant and washes for determination of counts per minute of the supernatant. Trypsin was added to the HUVEC monolayers to loosen the cells from the dish and to release adherent extracellular bacteria from the HUVECs. The wells were washed with RPMI-FCS, and the washes were pooled with the trypsin solution to inactivate the trypsin. The HUVECs were then washed twice with RPMI-FCS by centrifugation at $20 \times g$, conditions which pellet HUVECs but not spirochetes. The supernatants from these centrifugations were pooled and counted for the determination of trypsin-sensitive counts representing extracellular spirochetes. The HUVEC cell pellets were also counted for determination of trypsin-resistant counts representing intracellular spirochetes. The sum of trypsin-sensitive and trypsin-resistant counts per minute represent the total counts of cell-associated spirochetes.

**Antiserum.** Immune rabbit sera was raised by two immunizations, separated by 7 days, with $2 \times 10^8 B. burgdorferi$ that had been killed by heating at 56°C for 30 min. After 10 days, the rabit was bled at weekly intervals for 4 weeks and the titer for immunofluorescence staining was determined to be at least 1:250. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin G(IgG) and rhodamine-conjugated goat anti-rabbit IgG were purchased from TAGO (Burlingame, Calif.). The monoclonal antibodies H5352 (recognizing OspA [5]), HSTS (recognizing OspB [4]), and H9724 (recognizing flagellin [3]) were obtained from Tom Schwan.

**Microscopic demonstration of intracellular organisms.** Double-staining experiments were designed to discriminate intracellular from extracellular bacteria as described previously for invasion of mammalian cells by *Listeria monocytogenes* (25). HUVECs were cultured on gelatin-coated glass coverslips placed in six-well cluster dishes, and *B. burgdorferi* was added at a concentration of $10^7$/ml and allowed to bind and infect during a 24-h incubation period at 37°C. Coverslips were washed with phosphate-buffered saline (PBS) to remove loosely adherent bacteria and fixed with 4% Formalin for 20 min at room temperature. Coverslips were incubated in a 1:250 dilution of rabbit anti-*B. burgdorferi* antiserum for 30 min. All antibody incubations were for 30 min at room temperature, and all antibodies were diluted 1:250 with 20% normal human serum in PBS. Coverslips were washed in PBS and incubated in FITC-conjugated goat anti-rabbit IgG for 30 min. Coverslips were washed in PBS, and plasma membranes of the endothelial cells were permeabilized by incubation with methanol for 2 min. After an additional PBS rinse, coverslips were stained with the same rabbit anti-*B. burgdorferi* antiserum used in the first step, washed, and stained with rhodamine-conjugated goat anti-rabbit IgG. In this protocol, any spirochetes adherent to the extracellular surface of the HUVECs will stain with both antibodies, whereas those bacteria within the cell are not stained prior to permeabilization and only stain with rhodamine. Samples were visualized with a Zeiss fluorescent microscope equipped with Epi fluorescence.

**RESULTS**

**Demonstration of intracellular localization within HUVECs by *B. burgdorferi*.** Several experiments have been performed which demonstrate that *B. burgdorferi* adhere and invade human endothelial cells. The results shown in Fig. 1 are from a typical experiment and demonstrate that both cell association and internalization continue to increase until 48 h, when both are maximal. In the experiment shown in Fig. 1, 14% of the added spirochetes were adherent at 48 h and 11% were intracellular by this time. After a 24-h incubation period, 50 to 75% of spirochetes associated with HUVECs had reached a trypsin-resistant, intracellular location.

An experiment was performed to establish that spirochetes incubated in tissue culture medium were not clumping together and forming large complexes that would pellet under the conditions used to separate spirochetes from HUVECs. In this experiment, spirochetes were incubated for 24 h in wells containing endothelial growth medium but
no HUVECs, centrifuged, and washed alone or mixed with HUVECs prior to centrifugation and washes. Of the spirochete counts, 3% was found in the pellet fraction when spun by themselves and 4% was found when the spirochetes were spun with HUVECs. In the experimental samples (Fig. 1 to 5), the nonassociated spirochetes were removed first and counted as the supernatant fraction. Of the remaining cell-associated spirochetes, only 4% could be placed in the trypsin-resistant fraction because of trapping of spirochetes by the larger HUVEC cells or pelleting of spirochetes during the low-speed centrifugation. This is between 0.5 and 2% of the total spirochete counts added to the wells in the samples in Fig. 1 to 5.

Effect of agents that alter endothelial cell function on internalization of B. burgdorferi. Further experiments were performed to characterize the invasion of HUVECs by spirochetes. Tumor necrosis factor (TNF) is known to enhance the binding of leukocytes to endothelial cells by its ability to rapidly increase endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) and to induce synthesis and expression of endothelial cellular adhesion molecule 1 (6) and endothelial adhesion molecule 1 (21) with a time optimum of 2 to 4 h. Since leukocyte binding to intercellular adhesion molecule 1 and endothelial adhesion molecule 1 on endothelial cells is followed by penetration of the endothelium, it is possible that spirochetes might follow a similar mechanism involving the same endothelial cell receptors. HUVECs were incubated with 100 U of TNF (Genentech) per ml or with media alone for 3 h at 37°C. Radiolabeled B. burgdorferi were then added to the HUVEC monolayers, and the monolayers were incubated for 30 min, 4 h, and 24 h to determine if increased expression of either of these receptors could enhance the endothelial cell binding and/or internalization of B. burgdorferi. In a parallel experiment performed with the same batch of HUVECs and TNF, the percentage of neutrophils bound by endothelial cells increased from 14 to 79% with TNF treatment, indicating that receptors for neutrophil adhesion had been induced. At early time points, TNF had no effect on the binding or internalization by HUVECs of B. burgdorferi, while at 24 h the internalization was reduced by about one third (Fig. 2). The effect at 24 h is probably not the result of reduced expression of receptors but rather the result of destabilizing effects of TNF on cytoskeletal structures, as has been reported for bovine aortic endothelial cells (7). This is consistent with findings presented in Fig. 3 (discussed below) in which disruption of the cytoskeleton prevented internalization of the spirochetes. The experiment presented in Fig. 2 provides strong evidence that none of the TNF-inducible adhesion molecules on endothelial cells serve as receptors for B. burgdorferi binding or internalization.

Stimulation of endothelial cells with histamine or with phorbol esters induces the rapid expression of the neutrophil adhesion molecule GMP-140 from preformed intracellular pools (16). Incubation of endothelial cells with 60 ng of phorbol myristate acetate (Sigma) per ml for 30 min in Hanks balanced salt solution containing 0.5% albumin, conditions which stimulate endothelial cell binding of neutrophils, had no effect on the binding or internalization of B. burgdorferi (data not shown), suggesting that GMP-140 is not a receptor for this organism.

We next determined if inhibition of actin polymerization with cytochalasin D could inhibit HUVEC internalization of B. burgdorferi. A 12-h pretreatment of HUVECs with 1 μg of cytochalasin D (Sigma) per ml completely blocked internalization of spirochetes (Fig. 3). Cytochalasin D also greatly reduced the binding of spirochetes to HUVECs, suggesting either that normal binding and internalization involves receptor recycling which cannot occur in treated cells or that clustering of receptors on the plasma membrane is required for optimal spirochete binding and that this is inhibited by cytochalasin D. The complete inhibition of localization of bacteria to a trypsin-resistant compartment by cytochalasin D provides further evidence that these bacteria were indeed intracellular rather than attached to the plasma membrane via a trypsin-insensitive structure. These

![FIG. 2. Effect of TNF on the binding and internalization of B. burgdorferi by HUVECs. Adherent HUVECs were treated with 100 U of TNF per ml for 2 h (○, cell associated; □, trypsin resistant) or with medium alone (●, cell associated; ■, trypsin resistant) prior to the addition of 2 × 10⁴ spirochetes per well. Duplicate samples were included for each time point, and values were determined as described in Materials and Methods.](http://iai.asm.org/)
findings also indicate that the host is an active participant in the internalization process and that the spirochetes may make use of the actin polymers of the host in their uptake. Many invasive bacteria do require intact microfilaments for invasion, and, thus, this finding is consistent with invasion of mammalian cells by other pathogenic organisms (14, 32).

The ability of trypsin to completely remove cell-associated bacteria from the cytochalasin D-treated endothelial cells (Fig. 3, bottom line) demonstrates that this protocol successfully removes extracellular bacteria from endothelial cells and validates its usefulness in quantification of intracellular organisms. These findings are further supported by the fluorescent micrographs in Fig. 6E and F and in Table 1, in which all spirochetes were extracellular in slides prepared from cytochalasin D-treated endothelial cells. Cytochalasin D appears to have no effect on the viability of the spirochetes, as the addition of 1 μg of cytochalasin D per ml to bacterial cultures for 24 h had no effect on the percentage of spirochetes that were motile (>95%).

**Inhibition of internalization by rabbit anti-*B. burgdorferi* antiserum.** The specificity of the interaction between *B. burgdorferi* and HUVECs was analyzed by testing for the ability of rabbit polyclonal antibody to *B. burgdorferi* to block binding and internalization. The addition of rabbit anti-*B. burgdorferi* antiserum reduced binding by 67% of the control, incubated with normal rabbit serum, and reduced internalization to 34% of the control at the 4-h time point (Fig. 4). At the 24-h time point, no difference was detected between antibody-treated samples and controls in the experiment shown in Fig. 4, while in some experiments slight enhancement in the antibody-treated samples was observed at this time. This could be the result of aggregation of the bacteria by the antibody, leading to enhanced uptake and masking of the inhibitory effect seen at the earlier time points. Monoclonal antibodies directed against OspA (5), OspB (4), and flagellin (3) had no effect on binding or internalization (data not shown); however, others have shown that two different monoclonal antibodies to OspB can partially block adherence to HUVECs (31).

**Cell specificity of internalization.** The specificity of uptake was examined by analysis of different host cells. Two dose-response experiments are shown in Fig. 5 in which the binding and internalization by HUVECs and HeLa cells of increasing numbers of *B. burgdorferi* were measured. Greater that 60% of those spirochetes associated with HUVECs were intracellular for each dose of spirochetes in Fig. 5A. This is in contrast to the binding to HeLa cells (Fig. 5B), where *B. burgdorferi* bound very well, but only 10 to 15% of spirochetes that bound to HeLa cells were able to achieve an intracellular location. This represented 2 to 5% of the total number of spirochetes added to the cultures. This suggests that *B. burgdorferi* may bind to distinct receptors on different cell types, only some of which are involved in internalization, or that HeLa cells do not have the cytoskeletal structures required for the spirochete to invade. Other invasive bacteria can invade HeLa cells (13, 34), indicating that this cell type can serve as a host for intracellular localization by some bacteria.

**Strain specificity of bacterial uptake.** A derivative of *B. burgdorferi* Sh-2-82, which has been passaged 300 times in the laboratory and shown to have lost the ability to infect white-footed mice, was tested for the ability to achieve an intracellular localization (26). After a 24-h incubation period with HUVECs, 23% ± 0.2% of labeled spirochetes were associated with endothelial cells and 15% ± 0.1% of the organisms were intracellular. Therefore, the ability to achieve an intracellular localization is a property shared by both virulent and avirulent strains of *B. burgdorferi*. This is in further agreement with the endocytic nature of the internalization process.

**Fluorescent-antibody staining of intracellular spirochetes.** Double-staining techniques were used in which spirochete-infected endothelial cells were stained before and after permeabilization with methanol (25). Apparently intact spirochetes staining with both FITC- and rhodamine-conjugated goat anti-rabbit IgG were observed (Fig. 6A to D), as were spirochetes staining only with rhodamine-conjugated second antibody (Fig. 6B and D, arrows). Brightly stained intracellular debris was occasionally observed and was presumed to represent fragments of intracellular spirochetes. Neither intact spirochetes nor brightly staining speckles were identified in HUVECs not exposed to spirochetes (data not shown), confirming that the intracellular speckles were due to specific staining of spirochetal antigens rather than trapping of conjugated antibody by the HUVECs. Many spirochetes were found adhering to cytochalasin D-treated HUVECs; however, they always stained with both antibodies (Fig. 6E and F). This is consistent with the finding that all radiolabeled spirochetes adherent to cytochalasin D-treated endothelial cells could be released by trypsin. The data from a representative experiment are presented in tabular form in Table 1. The fluorescent antibody studies permitted a much lower ratio of spirochetes to endothelial cells (approximately 30:1) than did the experiments assessing uptake of radiolabeled bacteria. This difference and differences in the integrity of the monolayer obtained with endothelial cells plated on glass coverslips rather than plastic are probably responsible for the lower ratio of spirochetes to endothelial cells detected in the stained samples relative to the results with uptake of radiolabeled spirochetes. The results of the fluo-
rescent antibody staining (Fig. 6 and Table 1) provide confirmation of the intracellular localization of *B. burgdorferi* within normal endothelial cells and the requirement for host microfilaments for this localization.

**DISCUSSION**

The achievement of an intracellular location within mammalian cells is a potent virulence factor for many bacterial species, including many which grow well in the extracellular environment. These include *Yersinia* spp., *Salmonella* spp., *Shigella* spp., invasive *Escherichia coli*, and *L. monocytogenes* (15). Invasion by many of these organisms is associated with intracellular multiplication and cell-to-cell spreading. This may also result in invasion of other tissues (*Salmonella* spp. and *L. monocytogenes*) (14, 32) or may enhance survival in the localized site of infection (*Shigella* spp.) (9). Other organisms appear to invade without replicating (*Yersinia* and *Bordetella* spp.) within the invaded cell and may use invasion as a mechanism of passing from one tissue to another (13–15). The ability to invade cells provides the bacterium with a mechanism by which to escape destruction by the innate and specific aspects of the immune response of the host. An intracellular location may also provide protection from antibiotics because the concentration of many antibiotics within cells will be less than that in the extracellular milieu (15, 25).

Because of the virulence potential of host cell invasion, we wanted to know if *B. burgdorferi* could reside within a mammalian cell. We wished to demonstrate this as a property distinct from the ability to transcytose an endothelial cell monolayer. Our results demonstrate that *B. burgdorferi* can achieve an intracellular localization in vitro and suggests that intracellular localization may occur in vivo. The ability to localize within tissue cells may allow the spirochete, which has a very slow division time (10 to 12 h under optimal conditions), a real opportunity to avoid killing by antibiotics, antibody-plus-complement-mediated lysis, or antibody-mediated phagocytosis, and allow survival of low levels of organisms for prolonged periods of time. One characteristic of the internalization process is a requirement for intact host cell microfilaments. This is consistent with the requirements for invasion by several other pathogenic bacteria and suggests that the host cell is actively participating in the invasion process (15). Others have referred to this type of event as "parasite directed endocytosis," reflecting the participation of both the microbe and the host cell in the internalization process (20). The tissue normally serving as a reservoir in vivo is not known, but the contrast in efficiency between the HeLa cell line and HUVECs suggests that some cell specificity will exist.

In the experiments presented in Fig. 1 to 5, the low specific activity of the metabolically labeled bacteria required a relatively high ratio of spirochetes to endothelial cells to measure internalization. The validity of these findings was confirmed by immunofluorescent analysis (Fig. 6 and Table 1) in which the binding and internalization of far fewer spirochetes could be measured. In both types of analysis, 50 to 75% of spirochetes associated with endothelial cells were intracellular and treatment with cytochalasin D completely prevented the intracellular localization of bacteria. A further indication of the importance of the host in mediating the uptake of this organism comes from the finding that noninfective organisms are internalized and that heat-killed spirochetes which form large clumps are also readily internalized.

The existence of an intracellular reservoir of viable *B. burgdorferi* could explain the clinical observations of Lyme disease, in which the bacteria can be demonstrated in low numbers in infected tissues and synovial fluids but are difficult for the host to eliminate (17, 27, 29). The slow growth of the organism further suggests that a small number of viable spirochetes can "innoculate" the host in the tick-borne stage and cause disease. The persistence of the spirochetes in the host, if not eliminated by the host's immune system, could possibly lead to an inflammatory reaction which, if not controlled, could cause the host's death.

**FIG. 5.** Effect of spirochete number on the binding and internalization of *B. burgdorferi* by HUVECs and HeLa cells. Binding of the indicated numbers of labeled spirochetes to HUVECs (A) and HeLa cells (B). Cell-associated (solid bars) and trypsin-resistant (hatched bars) counts per minute are presented as described in Materials and Methods. Values represent the average of duplicates, and the variation between the samples is indicated by the error bars.
of bacteria might survive for some time within a cell before accumulating sufficient density of spirochetal peptides within class I or class II antigen, presenting structures to cause the host cell to be recognized as foreign. Thus, the demonstration of *B. burgdorferi* within human cells would provide a model for studying the role of intracellular localization on the virulence of this organism. A further indication that the organism might be invading and surviving within cells of infected tissue comes from the recent demonstration of viable *B. burgdorferi* in cardiac tissue from a
TABLE 1. Quantification of intracellular B. burgdorferi by indirect immunofluorescence microscopy

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total no. of spirochetes</th>
<th>% Spirochete intracellular&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirochetes + HUVECs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>586</td>
<td>125</td>
</tr>
<tr>
<td>Spirochetes stained with FITC-conjugated anti-rabbit IgG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300</td>
<td>58</td>
</tr>
<tr>
<td>Spirochetes stained with rhodamine-conjugated anti-rabbit IgG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>183</td>
<td>0.5</td>
</tr>
<tr>
<td>Spirochetes + cytochalasin D-treated HUVECs</td>
<td>466</td>
<td>184</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total number of endothelial cells in 20 distinct microscopic fields. Spirochete counts were made from the same 20 fields.

<sup>b</sup> Total number of spirochetes that stained prior to endothelial cell permeabilization with rabbit anti-B. burgdorferi antiserum and FITC-conjugated goat anti-rabbit IgG (detects extracellular bacteria).

<sup>c</sup> Total number of spirochetes that stained after endothelial cell permeabilization with rabbit anti-B. burgdorferi antiserum and rhodamine-conjugated goat anti-rabbit IgG (detects both intracellular and extracellular bacteria).

<sup>d</sup> [(Spirochetes stained with rhodamine-conjugated anti-rabbit IgG) – (Spirochetes stained with FITC-conjugated anti-rabbit IgG)] / [(Spirochetes stained with rhodamine-conjugated anti-rabbit IgG)] × 100.

patient with dilated cardiomyopathy and the apparent localization of at least one spirochete within a myocardial cell (12, 28).

There are two reports documenting another important interaction between B. burgdorferi and human endothelial cells, i.e., the ability to transcytose monolayers of endothelial cells (10, 30). These findings are important as they provide a mechanism by which the spirochete can escape from the blood and move into a variety of tissues in the infected animal. In one report, it was suggested that B. burgdorferi interacts with fibronectin in the subendothelial matrix (30); however, the bacteria used in that study were labeled with [³H]thymidine, which others have shown not to be incorporated into B. burgdorferi (22). In that study it is possible that much of the [³H]thymidine was associated with the surface of the bacterial cell instead of metabolically incorporated into bacterial DNA; therefore, it is difficult to interpret those antibody inhibition studies (30).

We were not able to identify the cellular receptor for B. burgdorferi involved in endothelial cell uptake of this organism. Our results suggest that the expression of the endothelial cell receptor was not induced by incubation with the spirochetes because the slow kinetics did not reveal a lag time for binding or internalization (Fig. 1 to 3). Additionally, two agents which induce inflammatory signals, TNF and phorbol myristate acetate, had no effect on binding or internalization, indicating that inflammatory stimulus was not required for expression of receptor or endocytic activity by the endothelial cells. In our studies, binding and internalization were seen with one noninfectious strain of B. burgdorferi, suggesting this is not the only determinant of infectivity. It will be interesting to compare the intracellular survival of infectious and noninfectious strains of B. burgdorferi.

The experiments described in this paper provide a model by which to study the role of intracellular survival in persistence of the spirochete during infection of the host. By studying this interaction in vitro, it should be possible to determine how long spirochetes can persist within endothelial cells and whether viable spirochetes can be recovered from endothelial cells. This model should allow characterization of the spirochete and endothelial cell molecules involved in infection. Further analysis of this interaction may provide information on the type of intracellular compartment in which the spirochetes reside and whether intact spirochetes and degraded spirochetes are associated with different types of cellular vacuoles. Information from these studies may also provide insight into the involvement of endothelial cell localization in the pathological aspects of Lyme disease.

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