INFECTION AND IMMUNITY, Oct. 1992, p. 4051–4058
0019-9567/92/104051-08$02.00/0

Vol. 60, No. 10

Adhesion to and Invasion of Cultured Human Cells by *Bartonella bacilliformis*

E. McGINNIS HILL,[1]* A. RAJI,[1] M. S. VALENZUELA,[2] F. GARCIA,[3] AND R. HOOVER[4]

Department of Microbiology[1] and Division of Biomedical Sciences,[2] Meharry Medical College, Nashville, Tennessee 37208; Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, Kansas 66103[3]; and Department of Pathology, Vanderbilt University, Nashville, Tennessee 37232[4]

Received 27 April 1992/Accepted 9 July 1992

Bartonella bacilliformis **was tested for its ability to adhere to and invade tissue culture cell monolayers. The parasite was able to efficiently bind and penetrate human dermal fibroblasts, human laryngeal epithelium, and human umbilical vein endothelial cells. Exposure of the organism to immune serum prepared against a crude *Bartonella* extract containing cell wall and membranous material resulted in decreased ability of the parasite to invade host cells. There was also an overall reduction in the invasiveness of bartonellae and total host cell association when human laryngeal epithelial cells and human umbilical vein endothelial cells were preexposed to cytochalasin D, indicating an active involvement of host cells in the uptake of bartonellae. Transmission electron microscopy revealed the presence of bartonellae inside and outside intracellular vacuoles. These data suggest that a surface-associated factor is involved in the invasion process and that internalization of the parasite by host cells involves a microfilament-dependent process similar to phagocytosis.**

Bartonella bacilliformis is a facultative intracellular bacterium known for its predilection toward erythrocytes and vascular endothelial cells (4, 5, 16, 18, 22). Penetration of these cells results in two distinct clinical syndromes, Oroya fever and verruga peruana. The Oroya fever syndrome is characterized by a severe hemolytic anemia caused by the parasitization of nearly all of the erythrocytes. Verruga peruana is characterized by the appearance of hemangiomalike papules or nodules on the skin as a result of the penetration of capillary endothelial cells and their subsequent proliferation. Yet to be understood, however, is the mechanism of invasion employed by this highly motile parasite with polar flagella. It was suggested by Benson et al. that binding of *B. bacilliformis* to erythrocytes leads to indentations and deformations of the membranes which eventually result in the internalization of the organism within vacuoles (1). Recently, an extracellular protein that deforms erythrocyte membranes in *B. bacilliformis* was identified (14). Thus, invasion of the erythrocyte by the parasite may be considered a kind of induced phagocytosis. Receptor-mediated phagocytosis is utilized by eucaryotes to internalize macromolecules and viruses and has also been implicated in the penetration into epithelial cells by members of the family *Enterobacteriaceae* (2, 3, 7, 8, 10, 13). In order to understand the pathogenicity of *B. bacilliformis* infection, elucidation of the mechanisms utilized by the organism to invade host cells is important and provides the rationale for this study. We report here the ability of *B. bacilliformis* to penetrate different cultured human cells by induced phagocytosis and suggest the involvement of a surface-associated factor in the invasion process.

(This work was presented in part at the 91st General Meeting of the American Society for Microbiology, Dallas, Tex., 5 to 9 May, 1991 [13a].)

* Corresponding author.

MATERIALS AND METHODS

Bacteria and culture conditions. A strain of *B. bacilliformis*, designated ATCC 35686, was obtained from the American Type Culture Collection, Rockville, Md. For growth of the bacterium on solid medium, a modified procedure of Benson et al. was followed (1). Upon receipt in the laboratory, the strain was placed on brain heart infusion agar plates containing a final concentration of 0.5% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), 2.0% Casamino acids (Difco), 0.2% glucose, and 10% (vol/vol) human blood erythrocyte lysate, which was prepared by the addition of 3:1 (vol/vol) sterile distilled water–packed outdated erythrocytes (American Red Cross, Nashville, Tenn.). The plates were overlaid with 3.0 ml of phosphate-buffered saline (PBS) at pH 7.5 and incubated at 26°C in a water-saturated atmosphere.

Radiolabeling of *B. bacilliformis*. Late-log-phase cultures were harvested and placed in 5.0 ml of methionine-deficient RPMI 1640 tissue culture medium containing 0.2% glucose. Radiolabeling with 50 μCi of [^{35}S]methionine (specific activity, 1,000 Ci/mmol) per ml was allowed to proceed for 3 h, with bartonellae incorporating an average of approximately 5×10^{-5} cpm per organism.

Tissue culture cells. Human diploid fibroblasts were provided by Shirley Russell, Department of Microbiology, Meharry Medical College, Nashville, Tenn., and were maintained in F10 culture medium supplemented with 10% fetal calf serum. HeLa cells were obtained from Jhaswant Bhorjee, Division of Biomedical Sciences, Meharry Medical College, and maintained in minimal essential medium supplemented with 10% fetal bovine serum. Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical veins by collagenase digestion as described by Jaffe et al. (12). They were identified as endothelial cells on the basis of their cobblestone morphology, uptake of acetylated low-density lipoprotein, and positive staining for factor VIII antigen. They were maintained in M199 growth medium supplemented with 10% neonatal calf serum, heparin, and endothelial cell growth supplement. Human laryngeal epi-

TABLE 1. Association of *B. bacilliformis* with cultured human cells

Host	Length of incubation (h)	Added cpm[a]	Recovered cpm[b,c]	% of added cpm recovered[c,d]
HEp-2 cells	1	5,896	1,216 ± 174	20.63 ± 3.15
	3	5,896	1,631 ± 103	27.66 ± 1.87[e]
HUVECs	1	3,250	672 ± 45	20.68 ± 1.53
	3	3,250	927 ± 78	28.52 ± 2.67[e]

[a] A 100-μl volume of a 1.0-ml suspension (5.89 × 10^4 cpm/10^9 cells) of 7-day-old bartonellae was added to monolayers of HEp-2 cells, 50 μl was added to HUVECs, and incubation was allowed to proceed for 1 or 3 h at 37°C.

[b] Values represent bartonellae adhering to and invading monolayers after six washes with PBS.

[c] Data are means and standard deviations of eight determinations.

[d] Values represent the percentages of added counts per minute adhering to and invading monolayers, calculated as (recovered cpm/added cpm) × 100.

[e] Significantly different from value at 1 h of incubation ($P < 0.001$).

TABLE 2. Resistance of cell-associated bartonellae to mild trypsin treatment

Host	Length of incubation (h)	Added cpm[a]	Recovered cpm[b,c]	% of added cpm recovered[c,d]
HEp-2 cells	1	5,896	594 ± 61	10.07 ± 1.1
	3	5,896	1,408 ± 124	23.89 ± 2.3[e]
HUVECs	1	3,500	395 ± 35	11.27 ± 1.1
	3	3,500	736 ± 31	21.03 ± 1.0[e]

[a] Monolayers of host cells were infected with [^{35}S]methionine-labeled *B. bacilliformis* cells for 1 or 3 h at 37°C as described above.

[b] Values represent added counts per minute remaining associated with host cells following incubation of monolayers in the presence of 0.08% trypsin in PBS at 37°C and differential centrifugation of cells for 5 min at 1,000 rpm in an IEC HN-SII bench top centrifuge.

[c] Data are means and standard deviations of six determinations.

[d] Values represent the percentages of added counts per minute remaining associated with host cells following trypsin treatment.

[e] Significantly different from value at 1 h of incubation ($P < 0.001$).

thelial cells, HEp-2 (ATCC CCL 23), were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. Cells were grown in 60-mm-diameter tissue culture dishes at 37°C in the presence of 5% CO_2 and 95% air in a humidified atmosphere.

Radioactive binding assay. [^{35}S]methionine-radiolabeled bartonellae were washed to remove unincorporated radioactivity and placed in 1.0 ml of PBS containing 10 mM glucose. Bartonellae were mixed with target cell monolayers containing 2.0 ml of PBS at a multiplicity of infection of 100:1 for 30 min at 37°C. At the end of the incubation period, the monolayers were washed at least six times with warm PBS. Cold 10% trichloroacetic acid was added to the monolayers, and precipitable radioactivity was measured with a Packard scintillation spectrometer. The percentage of bartonellae bound to monolayers was calculated as the percentage of added radioactivity that remained associated with the monolayer after the monolayer was washed with PBS.

Radioactive adherence and invasion assay. To determine the total number of target cell-associated bartonellae, a modification of the radioactive technique of Walker and Winkler (21) was used. Monolayers of HUVECs (10^5) and HEp-2 cells or dermal fibroblasts (10^6) were usually infected with about 10^7 or 10^8 radiolabeled bartonellae with incubation for 1 or 3 h at 37°C. At the end of the incubation period, monolayers were washed at least six times to remove nonadherent parasites and lysed with 0.5 mM EDTA–0.05% trypsin, and trichloroacetic acid-precipitable radioactivity was measured. Results are expressed as radioactivity recovered from the monolayers and represent bartonellae adhering to and invading host cells. To determine the number of intracellular bacteria, infected monolayers were washed with PBS and incubated in the presence of 0.08% trypsin for 20 min at 37°C to remove externally bound parasites. The detached monolayer and released extracellular parasites were then subjected to differential centrifugation at 1,000 rpm for 5 min in an IEC HN-SII bench top centrifuge. Pelleted target cells containing intracellular bartonellae were washed at least three times and lysed with 0.5 mM EDTA, and trichloroacetic acid-precipitable radioactivity was measured. Results are expressed as trypsin-resistant radioactivity remaining associated with the cell pellet and represent internalized bacteria.

In experiments determining the effects of cytochalasin D on invasion- and cell-associated bartonellae, a 1-mg/ml solution was prepared in dimethyl sulfoxide (Sigma). The solution was diluted in either RPMI 1640 or M199 growth medium to give final concentrations ranging from 0.5 to 5 μg/ml. Approximately 2.0 ml of each was added to confluent monolayers of HEp-2 cells and endothelial cells 1 h prior to the addition of bartonellae and during the incubation of bartonellae with these cells. Plates were incubated for 1 or 3 h at 37°C, and the numbers of cell-associated and intracellular parasites were determined as described above.

Preparation of *Bartonella* extract and antiserum. *B. bacilliformis* cells were collected from blood agar plates, washed in PBS, and pelleted by centrifugation at 10,000 × *g* for 10 min. The cell pellet was resuspended in PBS, sonicated for 3.5 min on ice, and then centrifuged for 15 min at 15,000 × *g* in a microcentrifuge. In the preparation of anti-*Bartonella* serum, the pellet containing cell wall and membranous material in PBS was emulsified 1:1 in complete Freund's

TABLE 3. Reduction of trypsin-resistant radioactivity recovered from monolayers by treatment of host cells with cytochalasin D or bartonellae with anti-*Bartonella* immune serum

Host and treatment	Added cpm[a]	Recovered cpm[b]	% Inhibition
HEp-2 cells			
− cyto D	5,896	594 ± 61	
+ cyto D	5,896	172 ± 12[c]	71
HUVECs			
− cyto D	3,500	395 ± 35	
+ cyto D	3,500	107 ± 22[c]	73
Bartonellae treated with preimmune serum	3,500	400 ± 30	
Bartonellae treated with anti-*Bartonella* immune serum	3,500	202 ± 10[c]	50

[a] Monolayers of host cells previously incubated for 1 h in the presence (+) or absence (−) of cytochalasin D (cyto D) were infected with 7-day-old [^{35}S]methionine-labeled *B. bacilliformis* cells and incubated for 1 h at 37°C as described above. Bartonellae were pretreated with preimmune or immune serum for 1 h at room temperature prior to infection of HUVEC monolayers.

[b] Values represent added counts per minute remaining associated with host cells following 30 min of mild trypsin treatment after incubation of coculture either in the presence and absence of 5 μg of cytochalasin D per ml or with bartonellae pretreated with preimmune and immune sera. Data are means and standard deviations of 4 to 6 determinations.

[c] Significantly different from control (preceding) value ($P < 0.001$).

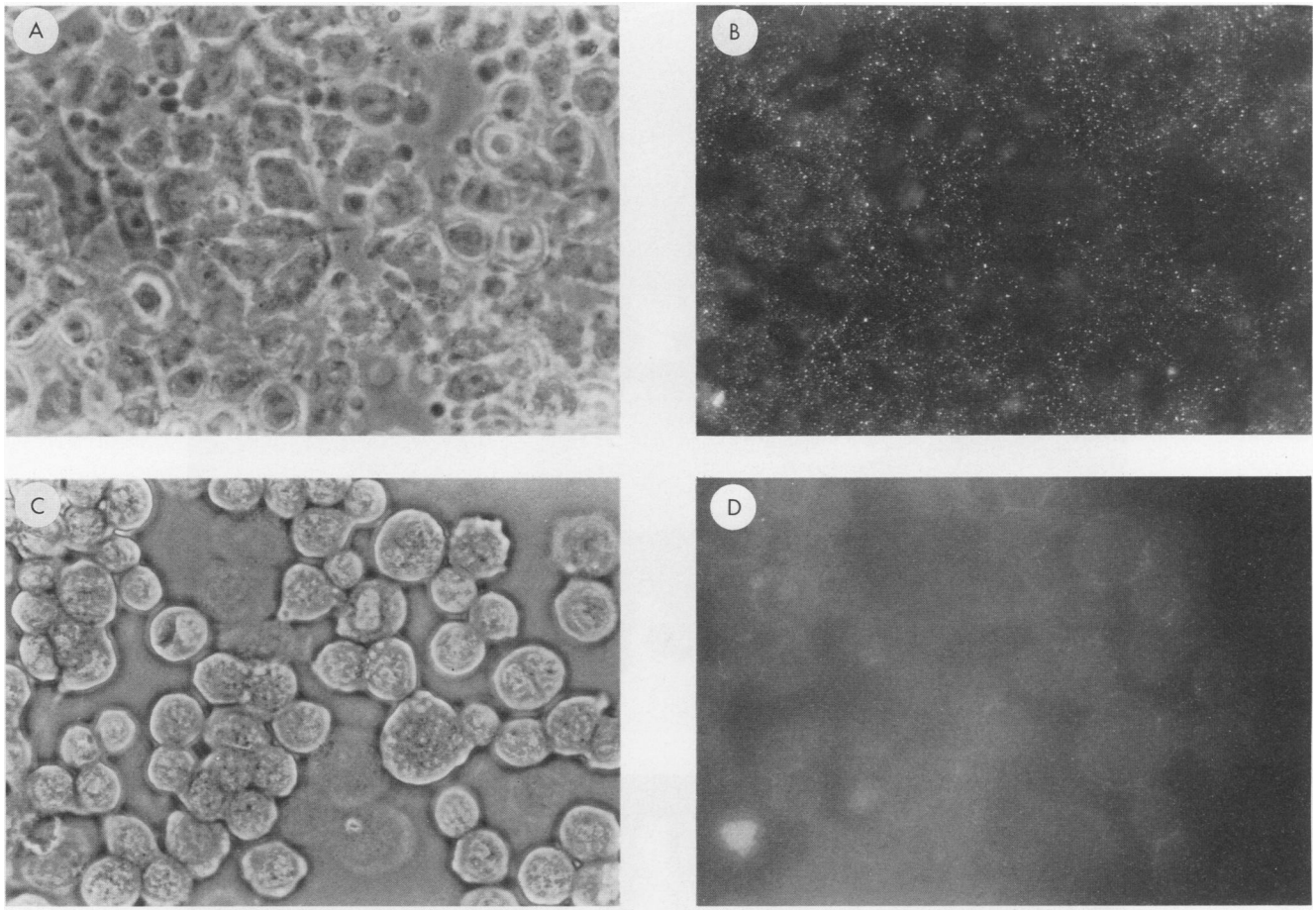

FIG. 1. Immunofluorescence microscopy showing attachment of *B. bacilliformis* cells to HEp-2 cells. Bartonellae were allowed to attach to HEp-2 monolayers for 1 h at 0°C to avoid internalization as described in Materials and Methods. Trypsin-treated cultures and untreated controls were exposed to anti-*Bartonella* immune serum for 1 h at 4°C, washed, and stained with fluorescein-conjugated second antibody to detect extracellular organisms. Reduced fluorescence was obtained for trypsin-treated cultures compared with that for untreated controls. Fluorescence (B and D) and corresponding phase-contrast (A and C) micrographs show untreated control monolayer with abundant fluorescein-stained extracellular bartonellae (B) and trypsin-treated monolayer with reduced fluorescence due to the detachment of bartonellae from the HEp-2 cell surface (D). Magnification, ×192.

adjuvant for the initial subcutaneous injection into the abdomens of rabbits. Three subsequent injections at 2-week intervals consisted of a 1:1 emulsion of *Bartonella* extract and incomplete Freund's adjuvant.

Immunofluorescence microscopy. Immunofluorescence was used to determine whether mild trypsin treatment of *Bartonella*-infected host cells could effectively remove externally bound parasites, thus allowing one to distinguish intracellular organisms. HEp-2 cells were seeded onto coverslips, and bartonellae were allowed to attach at 0°C for 1 h, during which approximately 16% of the added radioactivity became bound. Cultures were incubated in the presence of 0.08% trypsin for 5 to 7 min at 37°C to dissociate externally bound parasites along with untreated controls. Under these conditions, the HEp-2 cells remained attached to the coverslips. Anti-*Bartonella* immune serum (1:100 dilution) was added to untreated controls and trypsin-treated cultures which were then incubated for 1 h at 4°C and washed twice with PBS. A 1:10 dilution of the second antibody, fluorescein-conjugated goat anti-rabbit immunoglobulin G (heavy and light chains) (GIBCO-BRL), was then added, and incubation was carried out for 30 min at room temperature. The coverslips were washed and inverted onto glass slides and sealed. Cells were visualized with an Olympus BH-2 fluorescence microscope.

Electron microscopy. Following invasion of host cells by bartonellae, monolayers were washed and fixed in 4.0% paraformaldehyde–2.0% glutaraldehyde. Postfixation was with 2.0% osmium tetroxide and was followed by dehydration through a series of ethanol solutions and embedment in Epon. Sections were cut with a diatome diamond knife mounted on copper grids, stained with uranyl acetate and lead acetate, and examined with a Philips 301 electron microscope.

RESULTS

Bartonella association with host cells. Bartonellae were incubated with HEp-2 cells and HUVECs at a multiplicity of infection of about 100:1 for 1 or 3 h, and the numbers of adherent and intracellular bartonellae were determined as described above. Table 1 shows that the parasite adhered to

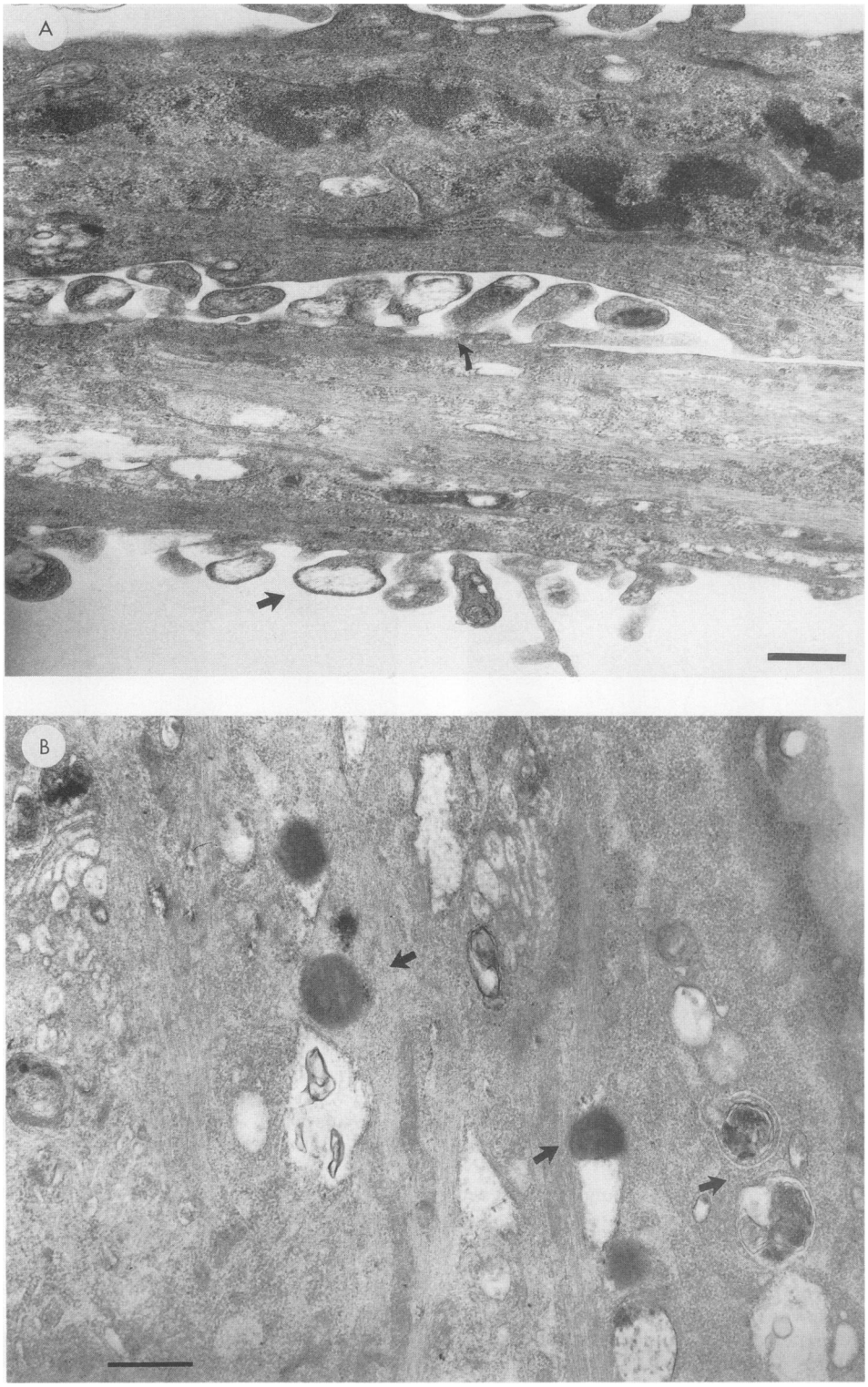

FIG. 2. Electron microscopy of *B. bacilliformis*-host cell interactions. *B. bacilliformis* cells were grown at 26°C in a humidified atmosphere. Late-log-phase cells were added to monolayers of human dermal fibroblasts, HeLa cells, and endothelial cells to allow binding and uptake as described in Materials and Methods. Following incubation, the monolayers were washed, fixed, and prepared for electron microscopy as described in Materials and Methods. (A) Binding of bartonellae to fibroblasts after a 30-min incubation at 37°C, showing material extending from the surface of the fibroblast (arrows) that mediates contact between the two cell surfaces (magnification, ×20,900). (B) Electron micrograph of thin sections of human dermal fibroblasts with intracellular bartonellae, demonstrating bartonellae inside vacuoles (arrows) (magnification, ×18,150). (C) HeLa cells with intracellular bartonellae (arrow) (magnification, ×28,500). (D) HUVECs with intracellular bartonellae (arrow) (magnification, ×18,150). (E) Human laryngeal epithelial cells with intracellular bartonellae (arrows) (magnification, ×8,550). The bar in each panel represents approximately 0.5 μm.

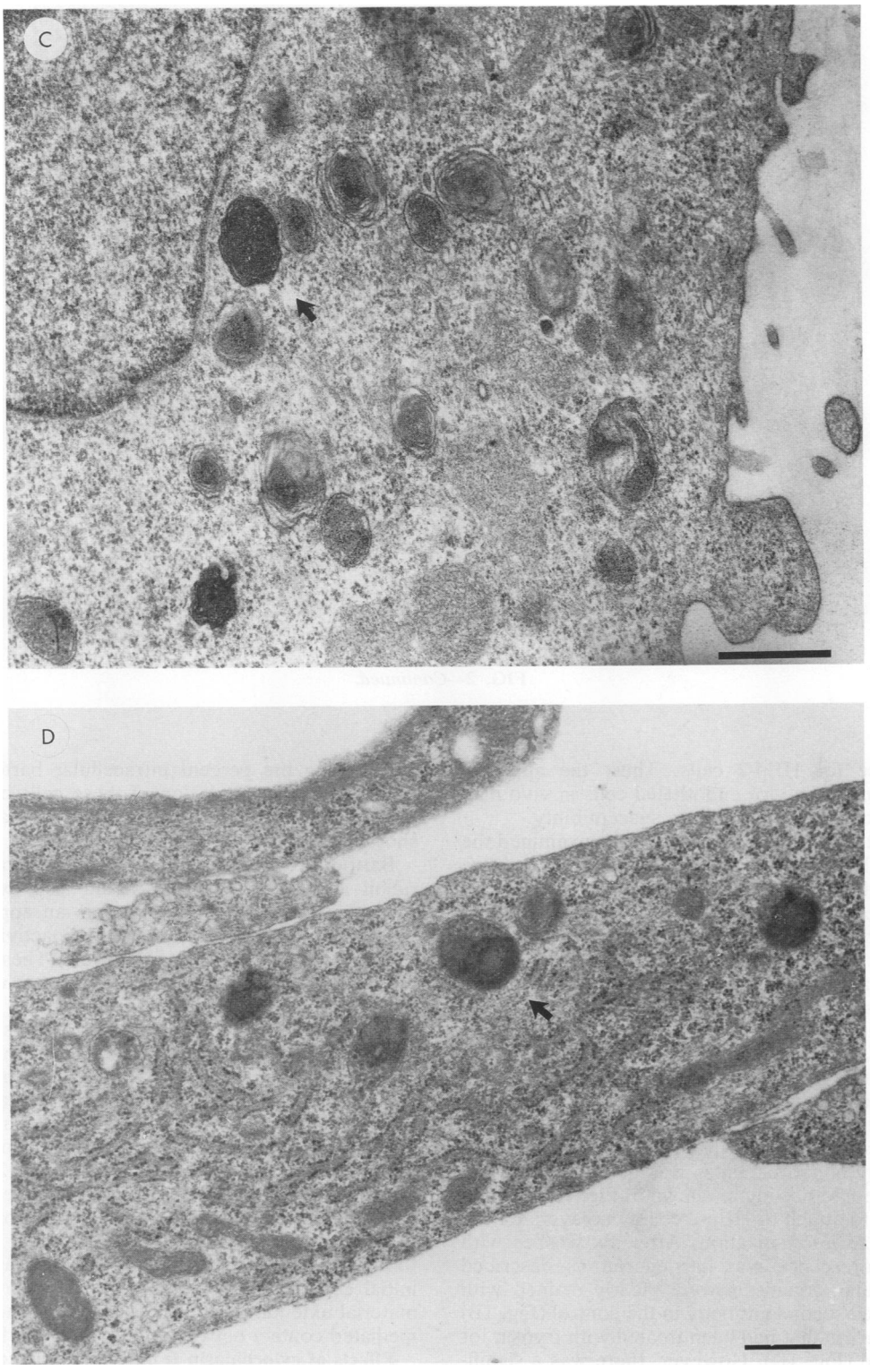

FIG. 2—*Continued.*

and entered the two different cell types with the same degree of efficiency. After 1 h, approximately 21% of the added radiolabeled bartonellae had become intracellularly and extracellularly associated with HEp-2 cell monolayers compared with approximately 28% after 3 h, which is a statistically significant increase. The percentages of added radiolabeled bartonellae remaining associated with HUVEC monolayers after 1 and 3 h of incubation were almost

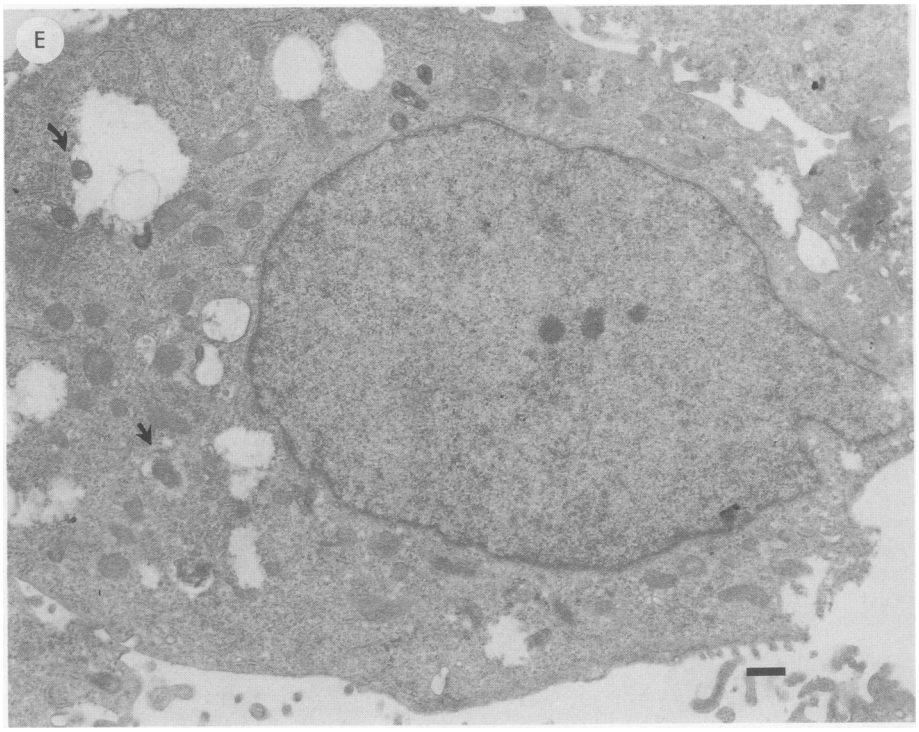

FIG. 2—*Continued.*

identical to those for HEp-2 cells. Thus, the apparent predilection of bartonellae for endothelial cells in vivo may not necessarily be due to their greater susceptibility.

Invasion of host cells by *B. bacilliformis*. We examined the penetration of cultured cells by *B. bacilliformis* by using a radioactive invasion assay and mild trypsin treatment of *Bartonella*-infected cells to remove extracellularly bound organisms. The trypsin-resistant organisms are presumed to be intracellularly localized. As shown in Table 2, when [^{35}S]methionine-radiolabeled bartonellae were added to confluent monolayers of HEp-2 cells, approximately 10% of the added radioactivity was recovered after 1 h of incubation and exposure to 0.08% trypsin compared with approximately 24% after 3 h. Similarly, in HUVECs, approximately 11% of the added radioactivity remained after 1 h compared with approximately 21% after 3 h. Whether exposure of *Bartonella*-infected cells to mild trypsin for 20 min effectively releases externally bound parasites was determined by immunofluorescence microscopy as shown in Fig. 1. Bartonellae were allowed to attach to HEp-2 cell monolayers for 1 h at 0°C to minimize internalization. After six washes with PBS, immunofluorescence was carried out as described above. Extracellular organisms were clearly stained with fluorescein-coupled second antibody in the control (Fig. 1B) as well as monolayers that had been treated with trypsin for 5 min and washed (Fig. 1D). However, there was a significant reduction in the amount of fluorescence observed in trypsin-treated *Bartonella*-infected monolayers compared with the amount in the untreated control, suggesting that many of the externally bound bartonellae had been effectively removed by trypsin within 5 min. Further incubation in the presence of trypsin for 15 to 40 min gave similar quantitative results for invasion efficiency. Thus, the data provide support for this method as a reliable means of determining the percent intracellular bartonellae. In contrast, the percent invasion of these cells by a noninvasive strain of *Escherichia coli*, HBT, was less than 0.1% (data not shown).

Bartonellae preincubated at room temperature for 1 h with rabbit anti-*B. bacilliformis* serum also exhibited reduced invasiveness (Table 3). There was an approximately 50% reduction in the trypsin-resistant radioactivity that remained associated with HUVEC monolayers. These results suggest the presence of a surface-associated factor which is required for the intracellular invasion of host cells.

Transmission electron microscopy. Transmission electron microscopy was used to further evaluate and understand the interaction of bartonellae with host cells and also to corroborate the data obtained with the quantitative radioactive invasion assays. In Fig. 2, a transmission electron micrograph clearly demonstrates the presence of bartonellae inside dermal fibroblasts, HeLa cells, HEp-2 cells, and HUVECs within 1 h of exposure to the parasite. In addition, the bartonellae would occasionally be found inside intracellular vacuoles in fibroblasts, HEp-2 cells, and endothelial cells following 1 h of coculture. It was also apparent that the initial contact of the bartonellae with fibroblasts involved material extending from the surfaces of the fibroblasts that mediated contact between the two cell surfaces.

Effects of cytochalasin D on cell-associated bartonellae and invasion. Since most bacteria enter eucaryotic cells via a microfilament-dependent process, we examined the effects of cytochalasin D on the number of cell-associated bartonellae and on the process of invasion (Tables 3 and 4). Exposure of HEp-2 cells to 5 μg of cytochalasin D per ml for 1 h prior to and during incubation with the parasites resulted in a 70% reduction in the total numbers of extra- and intracellular parasites and a 71% reduction in the number of trypsin-

TABLE 4. Effects of cytochalasin D on the association of *B. bacilliformis* with host cell monolayers

Host	Added cpm[a]	Recovered cpm[b]		% Inhibition
		− cyto D	+ cyto D	
HEp-2 cells	5,896	1,216 ± 174	368 ± 74[c]	70
HUVECs	3,250	672 ± 45	165 ± 19[c]	75

[a] Monolayers of HEp-2 cells and HUVECs were infected with cells from 7-day-old cultures of [^{35}S]methionine-labeled *B. bacilliformis* for 1 h at 37°C as described above.

[b] Values represent added counts per minute adhering to and invading monolayers in 1 h in the presence (+) and absence (−) of 5 μg of cytochalasin D (cyto D) per ml. Data are means and standard deviations of eight determinations.

[c] Significantly different from value obtained in the absence of cytochalasin D ($P < 0.001$).

resistant cell-associated parasites. Exposure of HUVECs to a similar concentration resulted in a 75% reduction in the number of cell-associated bartonellae and a 73% reduction in the number of trypsin-resistant cell-associated bartonellae. Similar inhibitory effects were observed with cytochalasin B (data not shown). The data support the notion that bartonellae gain entry into cells by a microfilament-dependent mechanism. However, additional factors, perhaps parasite derived, may be required since complete inhibition was not observed.

DISCUSSION

It is apparent from our results that *B. bacilliformis* can invade a variety of different cell types in vitro, including human dermal fibroblasts, HEp-2 cells, HeLa cells, and HUVECs. During the invasion process, there were no apparent cytotoxic effects on the host cells, such as cell rounding or loss of adherence to the substratum. The reduced ability of bartonellae exposed to anti-*Bartonella* immune serum to invade HUVECs suggests the presence of a factor on the surface of bartonellae that mediates invasion of host cells. The fact that *B. bacilliformis* invaded both HEp-2 cells and endothelial cells with similar degrees of efficiency may suggest that its apparent predilection toward endothelial cells in vivo is related to the route of entry into the body rather than some kind of tissue avidity. Exposure of HEp-2 cells and HUVECs to cytochalasin D had an inhibitory effect on the total number of bartonellae associated with these cells as well as on penetration, suggesting the involvement of a microfilament-dependent process and active participation by the host cell in the uptake of the parasite. Cytochalasin-induced inhibition of typhus rickettsia entry into fibroblasts led Walker and Winkler (21) to propose that internalization of the parasites was a form of induced phagocytosis. However, since a complete inhibition of *Bartonella* uptake by cytochalasin D was not observed, additional factors that are parasite derived may be required for the internalization of bartonellae inside host cells. The recent revelation concerning an extracellular *Bartonella* protein that deforms erythrocyte membranes is interesting (14). Such a factor could conceivably work in conjunction with host microfilaments to facilitate internalization of the parasite. Thus, these observations combined with transmission electron microscopic data demonstrating the presence of parasites inside and outside endocytic vacuoles suggest that the primary mechanism for internalization of bartonellae is also induced phagocytosis. A second mechanism is perhaps bacterium mediated.

Genes that encode cell adhesion and invasion proteins have been identified in *Yersinia*, *Salmonella*, and *Shigella* spp. and enteroinvasive *E. coli* (2, 6, 9, 11, 15, 19, 20). A single locus in the *Yersinia pseudotuberculosis* chromosome confers on noninvasive *E. coli* the ability to bind and invade cultured cells and was designated *inv* (11). Its homolog was isolated from *Yersinia enterocolitica* along with another locus, designated *ail*, which also confers on noninvasive *E. coli* the ability to invade cultured epithelial cells (15). However, DNA sequences homologous to *inv* that do not confer on noninvasive *E. coli* the ability to invade cultured cells (17) have been isolated from *Y. enterocolitica*. Interestingly, it appears that *inv* mRNA is not expressed in these avirulent strains, suggesting that the expression of *inv* DNA sequences is being regulated at the level of transcription.

Currently, we are particularly interested in the identification of genetic determinants in *Bartonella* spp. that are responsible for the invasion phenotype. A preliminary report from our laboratory (17a) indicated that a *Bam*HI fragment from the *Bartonella* chromosome could confer the invasion phenotype on noninvasive *E. coli*.

ACKNOWLEDGMENTS

We thank Shirley Russell and Kathy Trupin for the growth and maintenance of the human dermal fibroblasts and Rhoda Jones for the maintenance of endothelial cells. We also thank Howard Wynder for the electron microscopy and Frank Hatcher of the Hybridoma Research Facility (supported by NSF grant RII 8804780) for assistance with the fluorescence microscopy.

E. McGinnis Hill is the recipient of a Faculty Development Award from the National, Heart, Lung and Blood Institute of NIH (grant K14 HLO2334), and A. Raji is a postdoctoral fellow supported by NIH grant G12 RR03032. Part of this work was supported by seed funds awarded to E.M.H. and M.S.V. from NSF-Minority Research Center of Excellence in Cell and Molecular Biology grant RII8714805.

REFERENCES

1. **Benson, L. A., S. Kar, G. McLaughlin, and G. M. Ihler.** 1986. Entry of *Bartonella bacilliformis* into erythrocytes. Infect. Immun. **54**:347–353.
2. **Clerc, P., B. Baudry, and P. J. Sansonetti.** 1988. Molecular mechanisms of entry, intracellular multiplication and killing of host cells by shigellae. Curr. Top. Microbiol. Immunol. **138**:3–13.
3. **Clerc, P., and P. J. Sansonetti.** 1987. Entry of *Shigella flexneri* into HeLa cells: evidence for directed phagocytosis involving actin polymerization and myosin accumulation. Infect. Immun. **55**:2681–2688.
4. **Cuadra, M.** 1957. Mecanismo de destruccion de los eritrocitos. La hemolisis intravascular. Anal. Fac. Med. Lima **40**:872.
5. **Cuadra, M., and J. Takano.** 1969. The relationship of *Bartonella bacilliformis* to the red blood cell as revealed by electron microscopy. Blood **33**:708–716.
6. **Elsinghorst, E. A., L. S. Baron, and D. J. Kopecko.** 1989. Penetration of human intestinal epithelial cells by *Salmonella*: molecular cloning and expression of *Salmonella typhi* invasion determinants in *E. coli*. Proc. Natl. Acad. Sci. USA **86**:5173–5177.
7. **Finlay, B. B., and S. Falkow.** 1988. Comparison of the invasion strategies used by *Salmonella cholerae-suis*, *Shigella flexneri* and *Yersinia enterocolitica* to enter cultured animal cells: endosome acidification is not required for bacterial invasion or intracellular replication. Biochimie **70**:1089–1099.
8. **Finlay, B. B., and S. Falkow.** 1989. Common themes in microbial pathogenicity. Microbiol. Rev. **53**:210–230.
9. **Galan, J. E., and R. Curtiss III.** 1989. Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. Proc. Natl. Acad. Sci. USA **86**:6383–6387.

10. **Hale, T. L., R. E. Morris, and P. F. Bonventre.** 1979. Shigella infection of Henle intestinal epithelial cells: role of the host cell. Infect. Immun. **24:**887–894.
11. **Isberg, R. R., and S. Falkow.** 1985. A single genetic locus encoded by *Yersinia pseudotuberculosis* permits invasion of cultured animal cells by *E. coli* K-12. Nature (London) **317:**262–264.
12. **Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minich.** 1973. Culture of human endothelial cells derived from umbilical cord veins. Identification by morphologic and immunologic criteria. J. Clin. Invest. **52:**2745–2756.
13. **Kihlstrom, E., and L. Nilsson.** 1977. Endocytosis of *Salmonella typhimurium* 395 MS and MR10 by HeLa cells. Acta Pathol. Microbiol. Scand. Sect. B **85:**322–328.
13a. **McGinnis, E., A. Raji, R. Hoover, and M. S. Valenzuela.** 1991. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991, D-211, p. 113. American Society for Microbiology, Washington, D.C.
14. **Mernaugh, G., and G. M. Ihler.** 1992. Deformation factor: an extracellular protein synthesized by *Bartonella bacilliformis* that deforms erythrocyte membranes. Infect. Immun. **60:**937–943.
15. **Miller, V. L., and S. Falkow.** 1988. Evidence for two genetic loci in *Yersinia enterocolitica* that can promote invasion of epithelial cells. Infect. Immun. **56:**1242–1248.
16. **Noguchi, H.** 1926. The etiology of Oroya Fever. J. Exp. Med. **43:**851–864.
17. **Pierson, D. E., and S. Falkow.** 1990. Nonpathogenic isolates of *Yersinia enterocolitica* do not contain functional *inv*-homologous sequences. Infect. Immun. **58:**1059–1064.
17a. **Raji, A., M. S. Valenzuela, R. Hoover, R. Holt, and E. McGinnis.** 1991. Microbiol. 1991, D-210, p. 113. Abstr. 91st Gen. Meet. Am. Soc. American Society for Microbiology, Washington, D.C.
18. **Reynafarje, C., and J. Ramos.** 1961. The hemolytic anemia of human bartonellosis. Blood **17:**562–578.
19. **Sansonetti, P. J., D. J. Kopecko, and S. B. Formal.** 1982. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. Infect. Immun. **35:**852–860.
20. **Small, P. L. C., and S. Falkow.** 1988. Identification of regions on a 230-kilobase plasmid from enteroinvasive *Escherichia coli* that are required for entry into HEp-2 cells. Infect. Immun. **56:**225–229.
21. **Walker, T. S., and H. H. Winkler.** 1978. Penetration of cultured mouse fibroblasts (L cells) by *Rickettsia prowazeki*. Infect. Immun. **22:**200–208.
22. **Walker, T. S., and H. H. Winkler.** 1981. *Bartonella bacilliformis*: colonial types and erythrocyte adherence. Infect. Immun. **31:**480–486.