INFECTION AND IMMUNITY, May 1994, p. 2021–2026
0019-9567/94/$04.00+0

Vol. 62, No. 5

Factors Affecting Invasion of HT-29 and HEp-2 Epithelial Cells by Organisms of the *Mycobacterium avium* Complex

LUIZ E. BERMUDEZ* AND LOWELL S. YOUNG

Kuzell Institute for Arthritis and Infectious Diseases, California Pacific Medical Center Research Institute, San Francisco, California 94115

Received 5 December 1993/Returned for modification 20 January 1994/Accepted 22 February 1994

Organisms of the *Mycobacterium avium* complex cause disseminated blood-borne infection in patients with AIDS, who acquire the infection mainly through the gastrointestinal tract. Prior to causing infection, *M. avium* must colonize and invade the intestinal mucosa. This study examined the ability of several serovars of the *M. avium* complex to bind to and invade the HT-29 intestinal mucosal cell line and the HEp-2 laryngeal cell line. Logarithmic-phase *M. avium* was more efficient in binding and invasion than organisms in the stationary phase of growth. Bacteria incubated at 37 and 40°C adhered to and invaded HT-29 cells more efficiently than bacteria cultured at 30°C. The ability of *M. avium* to invade HT-29 and HEp-2 cells was inhibited when the cells were incubated with cytochasalin B prior to exposure to the bacterium, suggesting active participation of the mammalian cell in the process of internalization. Two protein kinase inhibitors, staurosporin and H7, blocked invasion of *M. avium*, and a specific tyrosine protein kinase inhibitor, genistein, also blocked the internalization but not the binding of bacteria. The findings suggest that *M. avium* binds to a specific receptor(s) on the epithelial cells and uses the cytoskeleton of the mammalian cell to become internalized.

Many pathogenic organisms are capable of entering into a number of cell types that are not considered phagocytic. These organisms (e.g., *Salmonella* spp., *Chlamydia trachomatis*, and *Shigella* spp.) commonly cause systemic or local infections (6, 9, 20). Recent observations have determined that many of the organisms using mucosal membranes as the initial route of infection have the ability to bind and invade epithelial cells (5, 13), usually by utilizing host proteins (soluble or associated with cells) to facilitate adhesion and internalization. Molecular studies on organisms such as *Yersinia pseudotuberculosis* and *Y. enterocolitica* have shown that bacteria utilize at least three mechanisms to invade epithelial cells (13, 22), one of them dependent on the expression of a binding protein, invasin (16).

The onset of AIDS has been associated with a significant increase in the incidence of infectious caused by organisms of the *Mycobacterium avium* complex (4, 10). *M. avium* disease in non-AIDS patients is usually a limited pulmonary infection, whereas patients with AIDS develop bacteremia and disseminated disease (33).

There is substantial epidemiologic and clinical evidence that the main route of *M. avium* infection in patients with AIDS is the gastrointestinal tract (8), although other studies suggest that the respiratory tract can be an alternative route of infection (17). To cause infection through the gastrointestinal route, bacterial pathogens are dependent on their ability to overcome the mucosal barrier. The initial site of *Salmonella* infection is the distal ileum, where the bacteria associate with the epithelium lining and Peyer's patches (20).

Previous studies in our as well as in other laboratories using an animal model in which *M. avium* is administered by gavage had shown that most of the bacteria colonize the terminal ileum (2, 24). Since binding and invasion of the epithelial mucosal cell is the first step toward the establishment of infection, we investigated the ability of *M. avium* strains to bind and invade the HT-29 intestinal cell line and the HEp-2 laryngeal cell line. Furthermore, we examined the involvement of enzymes in transducing signals needed for *M. avium* internalization by epithelial cells.

* Corresponding author. Mailing address: Kuzell Institute for Arthritis and Infectious Diseases, 2200 Webster St., Suite 305, San Francisco, CA 94115. Fax: (415) 441-8548.

MATERIALS AND METHODS

Bacteria. *M. avium* complex strains 101 (serovar 1), 109 (serovar 4), 100 (serovar 8), and CDC 86-2486 (serovar 16) were used for these experiments. Strains 101, 100, and 109 were isolated from the blood of patients with AIDS, while strain CDC 86-2486 was a strain belonging to serotypes never before isolated as causing disease in patients with AIDS and was kindly provided by Robert Good (Centers for Disease Control and Prevention, Atlanta, Ga.). For the experiments, the bacteria either were cultured in Middlebrook 7H10 agar for 10 days and resuspended in Middlebrook 7H9 broth for 5 days (logarithmic control) or were cultured in Middlebrook 7H9 broth for 25 days (stationary phase of growth). Bacteria were subsequently washed in Hanks balanced salt solution (HBSS) and used in the assays.

Cells. Colon carcinoma cell line HT-29, a well-differentiated cell line with marked characteristics of human intestinal cells, was purchased from the American Type Culture Collection (Rockville, Md.) and maintained in McCoy 5A medium without glucose (GIBCO Laboratories, Detroit, Mich.) supplemented with 1% galactose, 2 mM L-glutamine, and 5% fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.). The glucose was replaced by galactose (1%). For the assays, 10^5 HT-29 cells were seeded in each well and left to grow for 24 h to semiconfluence in a 24-well tissue culture plate.

Laryngeal cell line HEp-2, also purchased from the American Type Culture Collection, was maintained in RPMI 1640 supplemented with 5% fetal bovine serum. Monolayers were prepared by seeding $\sim 10^5$ cells in each well of a 24-well tissue culture plate and incubated overnight. Both cell lines were used between passages 50 and 60 in all assays.

Adherence assay. The medium overlying the HT-29 and HEp-2 monolayers was replaced with 1 ml of ice-cold tissue

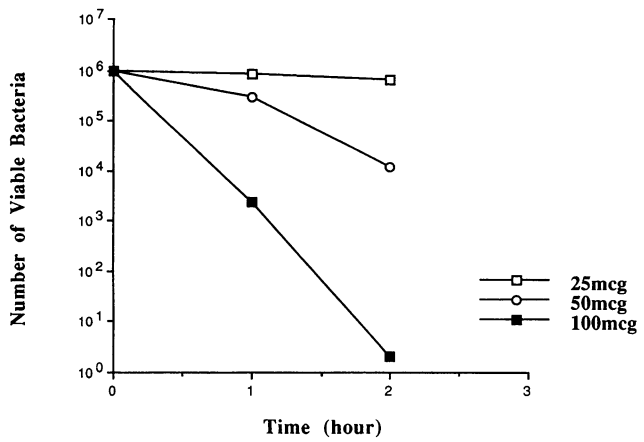

FIG. 1. Effect of treatment of mycobacteria with amikacin. *M. avium* (10^6 bacteria) was cultured in RPMI 1640 in a 24-well tissue culture plate without mammalian cells for 2 h. Then different concentrations of amikacin were added, and the culture was incubated for 2 h at 37°C. After 2 h, the supernatant was removed (medium 1) and 500 μl of HBSS was added to each well. The wells were subsequently scraped with a rubber policeman, and the supernatant (medium 2) was collected. Media 1 and 2 were plated as described in Materials and Methods. The graph (medium 1) represents the effects of different concentrations of amikacin on *M. avium* viability. No bacteria was cultured from medium 2.

culture medium. *M. avium* 101 (10^6 bacteria per well) was then added to HT-29 and HEp-2 monolayers. Control wells without mammalian cells were similarly prepared to quantitate nonspecific bacterial adherence to the plastic. The bacteria were allowed to adhere for 120 min at 4°C, and after the incubation period, each well was rinsed four times with 1 ml of ice-cold HBSS, pH 7.4. Monolayers were subsequently treated with 0.5 ml of Triton X-100 (Sigma) in sterile water for 10 min. Middlebrook 7H9 broth (0.5 ml) was then added in each sample, and the bacteria were harvested. Adherent bacteria were quantitated by plating for CFU onto Middlebrook 7H10 agar.

To measure internalization of adherent bacteria, bacteria were allowed to adhere as described above; after removal of nonadherent bacteria, 1 ml of prewarmed tissue culture medium was added to each assay well, and internalization was allowed to proceed for 120 min at 37°C in a 5% CO_2 atmosphere. Intracellular *M. avium* was quantitated after treatment of monolayers with amikacin as described below.

Invasion assay. The invasion assay was a modification of the assay described by Isberg and Falkow (14). Prior to the assay, the culture medium of the monolayers was removed and replaced with prewarmed culture medium. Bacterial samples (10^6 bacteria per well) were added as described above. Invasion assays were incubated at 37°C in a 5% CO_2 incubator for 120 min. Assays were terminated by replacing the overlying medium with 1 ml of tissue culture medium supplemented with 100 μg of amikacin per ml. Amikacin, an aminoglycoside antibiotic, at the concentration used kills extracellular *M. avium*, while intracellular bacteria remain viable (Fig. 1). After the incubation with amikacin for 120 min at 37°C in a 5% CO_2 atmosphere, the monolayers were washed twice with HBSS, and the viable intracellular bacteria were released by incubation of the monolayers with 0.5 ml of 0.1% Triton X-100 in sterile water for 10 min. Controls were run in parallel to ensure viability of the bacteria. Samples were vigorously mixed with 0.5 ml of Middlebrook 7H9 broth and harvested. Viable bacteria were quantitated by plating for CFU onto Middlebrook 7H10 agar.

Effect of temperature. To examine the effect of temperature on the ability of *M. avium* to invade mucosal cells, bacteria were grown for 5 days in Middlebrook 7H9 broth and then separated in three groups: one to grow at 40°C, another to grow at 37°C, and one to grow at 30°C. Bacteria were maintained at these temperatures for 24 h. After the incubation, bacteria were washed with HBSS and used in the assay.

Treatment of cells with cytochalasin B. Cytochalasin B (Sigma) was prepared as a 1.0-mg/ml stock solution in dimethyl sulfoxide (Sigma) and diluted in RPMI 1640 to concentrations of 0.05 to 5 μg/ml. Cytochalasin B was applied to the culture monolayers for 2 h prior to the infection. Monolayers were subsequently washed three times with HBSS, and bacterial inoculum was then added.

Signal transduction inhibitors. All inhibitors were dissolved in dimethyl sulfoxide, and stock solutions were divided into aliquots and stored at −20°C. The stock concentrations were as follows: staurosporine (Boehringer Mannheim), 1 mM; genistein (ICN), 100 mM; 1-(5-isoquinolinesulfonyl) 2-methylpiperazine (H7; Sigma), 300 mM; and H8 (Sigma), 300 mM.

Effect of signal transduction inhibitors on bacterial viability. Bacterial cultures (10^5 bacteria in 100 μl of HBSS) were incubated with 1 ml of RPMI 1640 supplemented with 5% fetal bovine serum with or without an inhibitor. The cultures were subsequently placed at 37°C for various times, and the numbers of CFU for treated and untreated bacteria were compared.

RESULTS

Efficacy of the washing procedure. Incubation of *M. avium* with HT-29 and HEp-2 cell lines was followed by washing of the monolayers in order to remove the extracellular bacteria and enable us to quantitate the bacteria associated with the cells. To ensure the efficacy of the process, we performed a number of preliminary experiments in which 10^6 organisms were added to wells lacking mammalian cells. The wells were then treated as described above for the binding and invasion assays for 2 h and subsequently washed with HBSS or incubated with 100 μg of amikacin per ml for 2 h before washing. This concentration of amikacin was shown to kill all extracellular bacteria in 2 h (Fig. 1).

Percentages of the initial inoculum of bacteria (10^6) recovered in the monolayers after washing were as follows: 4°C for 2 h, <0.001; 4°C for 6 h, <0.001; 37°C for 2 h, <0.003; and 37°C for 2 h plus amikacin (100 μg/ml for 2 h), <0.001. These results indicated that the washing process was efficient when monolayers were incubated at 4°C and that treatment with amikacin improved the efficiency of the washing after incubation at 37°C.

Effect of growth conditions on binding and invasion. Mycobacteria are known to be able to survive within mammalian cells both in a latent state and in the logarithmic phase of growth (28). To verify that binding and invasion properties of the bacteria were linked to the phase of growth in vitro, we incubated bacteria in the logarithmic phase of growth (5 days in Middlebrook 7H9 broth under agitation) and the stationary phase of growth (25 days in Middlebrook 7H9 broth without agitation) with HEp-2 and HT-29 epithelial cells and determined their ability to adhere and invade in a 2-h assay. Figure 2 shows log-phase *M. avium* ingested by HEp-2 cells. As shown in Table 1, bacteria in the logarithmic phase of growth are significantly more efficient than bacteria in the stationary phase

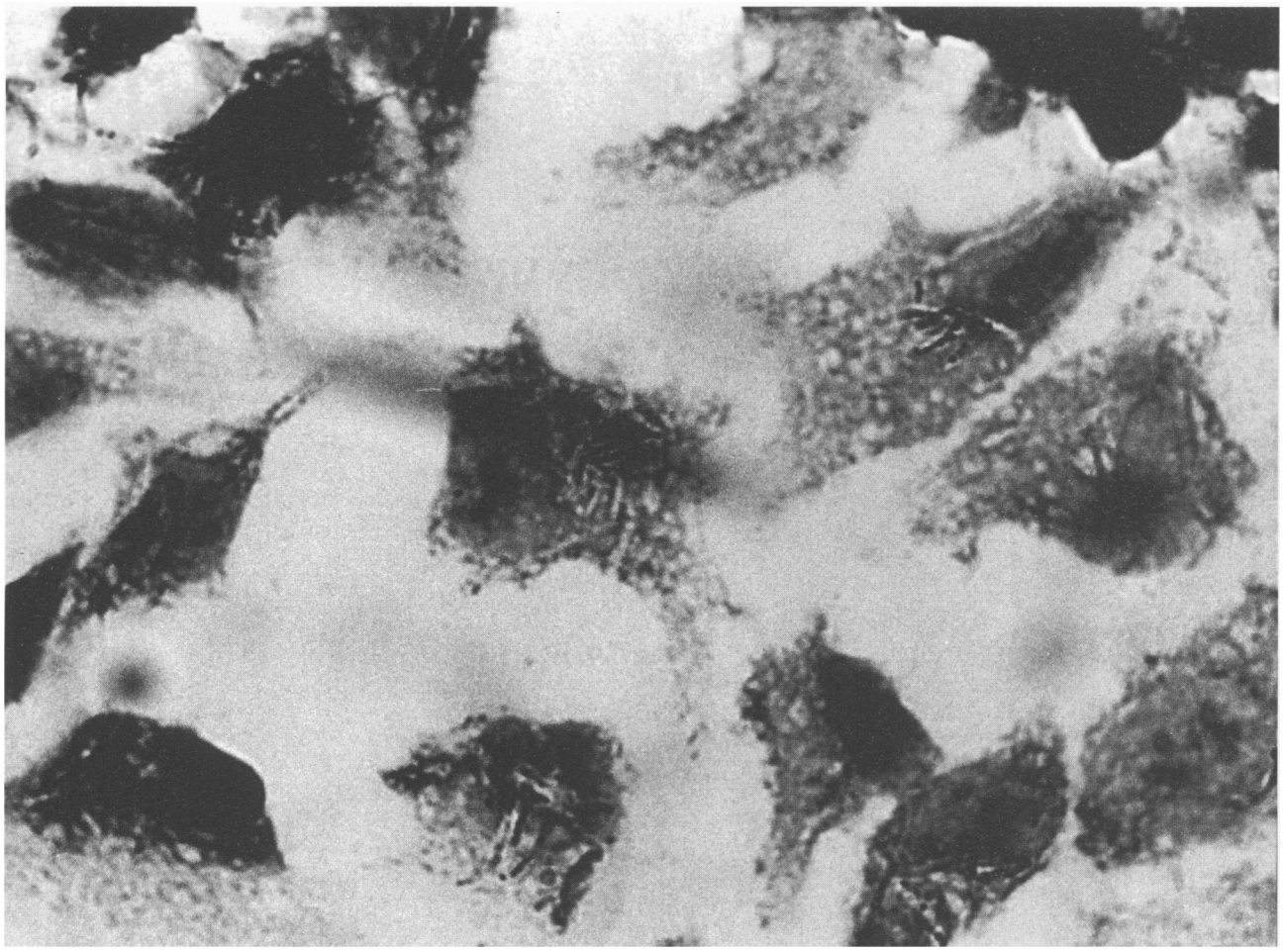

FIG. 2. HEp-2 cells monolayer exposed to *M. avium* 101 for 2 h. A number of bacteria can be seen within cells.

of growth in binding and invading HEp-2 laryngeal and HT-29 intestinal cell lines, although only marginally more efficient in binding to the cells.

Effect of temperature in the ability of *M. avium* to bind and invade epithelial cells. To determine whether the temperature at which the bacteria were cultured would have any effect on the ability to bind and invade epithelial cells, *M. avium* 100, 101, 109, and CDC 86-2486 (serovars 8, 1, 4, and 16, respectively) were grown 24 h at 30, 37, and 40°C and subsequently used in the assays. As shown in Table 2, all four strains were capable of binding and invading with more efficiency when grown at 37 or 40°C than 30°C. It was observed that an increase in the temperature by 3°C (from 37 to 40°C) resulted in a small increase in the efficiency of invasion of strains 100, 101, and 109 but not strain CDC 86-2486.

Effect of time in the binding and invasion of *M. avium*. One of the difficulties that a bacterium must overcome to bind and invade intestinal cells is peristalsis (continuous intestinal movement). Therefore, the ability to bind and invade cells rapidly is probably very important in the pathogenesis of infection.

M. avium's ability to bind to HT-29 cells was measured during the course of 6 h. As shown in Fig. 3, *M. avium* binding to HT-29 cells increased up to 4 h of incubation. Binding at 6 h of incubation was not statistically significantly greater than binding after 4 h of incubation.

Effect of cytochalasin B on *M. avium* invasiveness. Cytochalasin B is an inhibitor of the cell cytoskeleton. Treatment of the culture monolayer with cytochalasin B prior to incubation with bacteria at concentrations of ≥ 0.5 μg/ml significantly inhibit invasion of *M. avium* in both HEp-2 and HT-29 cell lines. Higher concentrations of cytochalasin B virtually eliminated uptake (Table 3).

Effect of signal transduction inhibitors on *M. avium* internalization. In eukaryotic cells, protein phosphorylation is often involved in the transduction of extracellular signals. If protein kinase activity is necessary for transducing *M. avium* uptake signals, inhibitors of these enzymes should inhibit bacterial invasion. To test this hypothesis, we used staurosporin and H7,

TABLE 1. Binding and subsequent invasion of *M. avium* 101 by HEp-2 and HT-29 cell lines[a]

Bacterial culture	Mean % of initial inoculum of 10^6 bacteria ± SD (n = 3)			
	HEp-2		HT-29	
	Binding	Invasion	Binding	Invasion
Stationary phase	4.2 ± 1.6	1.4 ± 0.6	3.5 ± 0.7	0.9 ± 0.04
Logarithmic phase	7.2 ± 2.1	5.9 ± 0.8	6.7 ± 1.2	5.4 ± 1.1

[a] The binding assay was performed at 4°C for 2 h; the invasion assay was performed at 37°C for 2 h.

TABLE 2. Effect of temperature on *M. avium* binding and invasion of HT-29 epithelial cells

M. avium complex strain	Mean % of initial inoculum of 10^6 bacteria ± SD (n = 3)					
	Binding			Invasion		
	30°C	37°C	40°C	30°C	37°C	40°C
100	2.7 ± 0.7	6.9 ± 2.0	7.3 ± .9	1.6 ± 0.4	6.7 ± 2.1	7.5 ± 1.7
101	2.9 ± 0.9	7.2 ± 2.1	7.5 ± 1.6	1.9 ± 0.3	5.7 ± 1.4	6.6 ± 2.6
109	2.5 ± 0.6	6.7 ± 1.8	7.1 ± 1.5	1.2 ± 0.4	5.9 ± 1.7	6.5 ± 2.1
86-2486	1.6 ± 0.6	3.1 ± 0.7	3.3 ± 1.0	0.5 ± 0.1	2.6 ± 0.8	2.9 ± 1.1

two protein kinase inhibitors (protein kinase C and cyclic AMP-dependent protein kinase). Our results show that staurosporin and H7 exhibited dose-dependent inhibition of invasion of HT-29 cells by *M. avium* (Fig. 4A and B). Incubation of *M. avium* with staurosporin (1 μM) for 2 h had no effect on the viability of the bacterium (extracellular and intracellular).

Since staurosporin also inhibits some tyrosine kinase activities (31, 32), we examined the effect of a tyrosine kinase inhibitor on the uptake of *M. avium* by HT-29 cells. Genistein inhibits tyrosine kinase by inhibiting the binding of ATP to the enzyme (31), and it is a highly specific tyrosine kinase inhibitor (31). Genistein strongly inhibited the uptake of *M. avium* by HT-29 cells (Fig. 4C). Genistein at 300 μM also did not affect *M. avium* viability (intracellular or extracellular). H8, a protein kinase A inhibitor, at 0.05, 0.5, 1, 2, or 4 μM had no effect on the ability of *M. avium* to invade HT-29 and HEp-2 cells (data not shown).

DISCUSSION

Infections caused by organisms of the *M. avium* complex are common in patients with AIDS (4, 10, 33). Current evidence suggests that a large majority of patients with AIDS acquire *M. avium* infection following colonization of the gastrointestinal tract. Histopathologic studies show that large numbers of organisms are found in the intestinal mucosa and submucosa of patients with *M. avium* infection, and a few studies suggest that intestinal colonization precedes the onset of bacteremia (11, 19).

To infect the intestinal mucosa, *M. avium* must be able to bind and invade the intestinal epithelial cells; however, little is known about how this is accomplished. In an attempt to better understand the interaction between *M. avium* and intestinal mucosal cells, we have adapted the *Yersinia*-based experimental systems described by Isberg (12, 13), Isberg and Falkow (14), and Miller and Falkow (22) to mycobacteria.

Our results show that *M. avium* can bind to and invade two nonphagocytic cell lines such as the HT-29 and HEp-2 epithelial cell lines. Such results suggest that *M. avium* contains mechanisms that allow the bacterium to interact and quickly penetrate epithelial cells before translocating into the lamina propria. This interaction was more efficient when the bacterium was grown at 37 or 40°C than 30°C, indicating that moieties associated with binding and penetration of mucosal cells must have their expression up-regulated when the bacterium is within the susceptible host. This phenomenon has also been described for *Y. enterocolitica* and *Y. pseudotuberculosis* (15). Yersiniae harbor a thermoinducible protein, invasin, which is associated with invasion of epithelial cells.

The ability of *M. avium* to penetrate epithelial cells is dependent on the phase of growth of the organism. Organisms in the logarithmic phase of growth are significantly more efficient in penetrating epithelial cells than organisms in the stationary phase of growth. However, ability to adhere apparently is not significantly different between bacteria grown to logarithmic and stationary phases of growth, which suggests different mechanisms of binding and invasion.

Previous studies by Shepard have shown that *M. tuberculosis* as well as *M. smegmatis*, *M. fortuitum*, and *M. phlei* can infect HeLa epithelial cells (30). Furthermore, Shepard reported a relationship between the ability of *M. tuberculosis* to invade HeLa cells and the ability to cause disease in humans and experimental animals (29).

Studies carried out with *Yersinia* sp. and *Salmonella typhimurium* have demonstrated the presence of multiple pathways for cellular penetration (7, 11, 12, 22, 23), but current evidence suggests that the mammalian cell plays an active role in taking up the bound microorganisms (7, 23). Similar findings were obtained for *M. avium* and supported by the observation that cytochalasin B, a microfilament inhibitor, inhibits entry of *M. avium* into mammalian epithelial cells. Our results indicate that the mammalian cell receptor(s) recognized by the bacte-

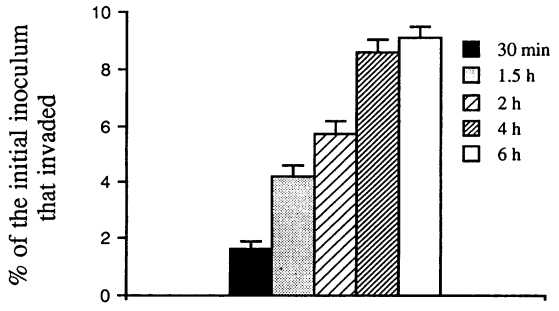

FIG. 3. Kinetics of binding of *M. avium* 101 to HT-29 intestinal cells.

TABLE 3. Effect of cytochalasin B on *M. avium* invasiveness

Cytochalasin B concn (mM)	Mean % invasion ± SD[a]	
	HEp-2	HT-29
0	100 ± 5	100 ± 8
0.05	79.2 ± 6	76 ± 6
0.1	73.4 ± 5	70 ± 4
0.5	49 ± 8[b]	42 ± 6[b]
1.0	37.6 ± 4[b]	36 ± 3[b]
2.0	21 ± 6[b]	22.5 ± 5[b]
5.0	9 ± 2[b]	8.2 ± 3[b]

[a] Ability of bacteria to invade HEp-2 and HT-29 cells after 2 h of incubation. The results were normalized such that the invasiveness of bacteria in the absence of cytochalasin B equals 100%. The actual percentages of the inoculum that entered HEp-2 cells and HT-29 cells were 6.2 and 6.8%, respectively.

[b] Statistically significant ($P < 0.05$) compared with control.

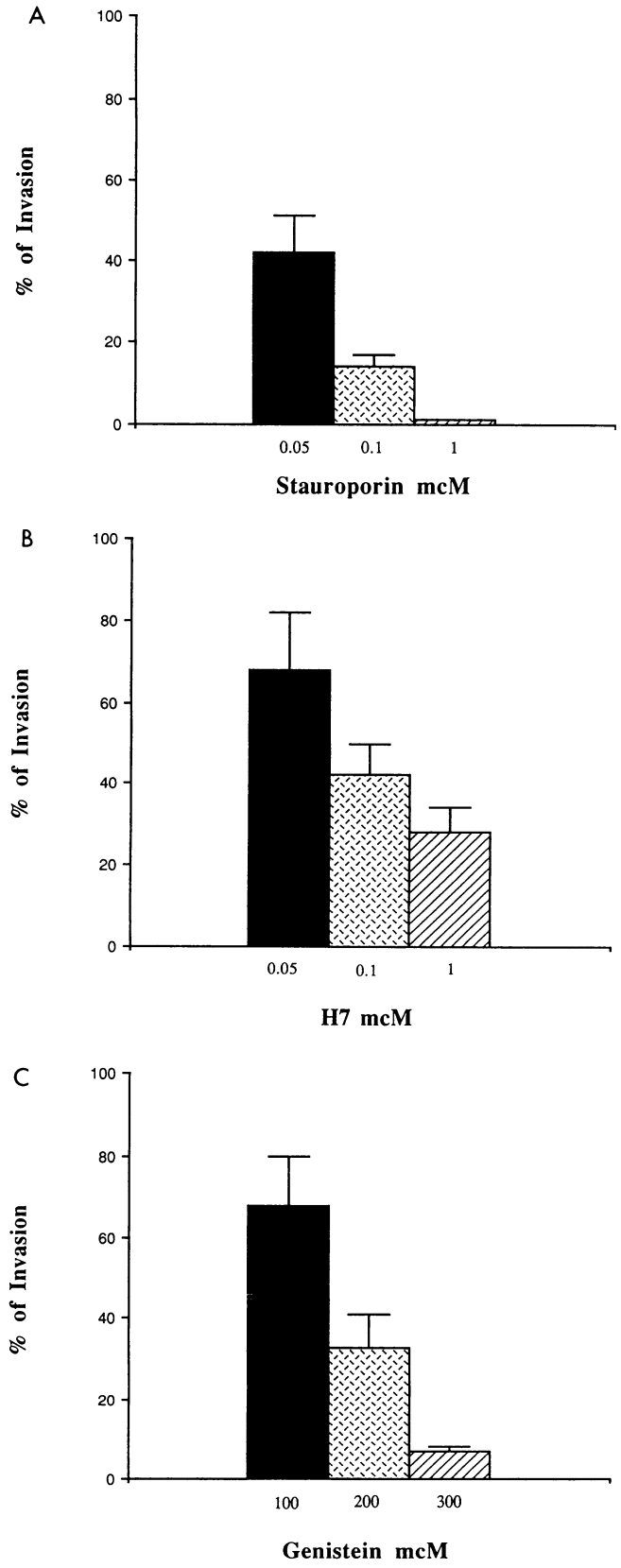

FIG. 4. Effects of staurosporin (A), HT (B), and genistein (C) treatment on invasion of HT-29 cells by *M. avium*. Values are means of three experiments ± standard deviations.

rium must somehow be linked to the cell cytoskeleton. This linkage may be the critical difference between pathogens that simply bind to the surface of mammalian cells and those that invade. In fact, the ability of cytochalasin B to inhibit entry of *M. avium* has been demonstrated previously with serovar 2 of *M. avium* and Henle 407 epithelial cells (21).

Recently, an adhesin for fibronectin has been characterized in a number of species of mycobacteria (1, 25) and has been associated with the ability of *M. bovis* to bind to bladder cells (26).

Our results also suggest a role for tyrosine phosphorylation in *M. avium* entry as well. Protein kinase inhibitors and a tyrosine kinase inhibitor were able to block *M. avium* entry into cultured HT-29 cells. Inhibition in the presence of H7 was not complete; however, in contrast with staurosporin, H7 has been found to be a weak inhibitor of protein kinase (27). Similar findings have been reported for invasin-mediated entry of yersiniae into cultured cells. In fact, integrins such as $\alpha_4\beta_1$ and $\alpha_5\beta_1$ on the surface of mammalian cells are membrane receptors for fibronectin, and tyrosine phosphorylation has been shown to play a role in signal transduction through integrins (18). Furthermore, the β_1 integrins were recently shown to be associated with *M. leprae* binding to nasal epithelial cells (3).

In conclusion, *M. avium* is capable of interacting with mammalian epithelial cells. We observed a difference between the ability of *M. avium* strains 100, 101, and 109 and one strain of *M. intracellulare* (CDC 86-2486) to penetrate cells. Further studies are necessary to establish the bacterial structure(s) responsible for the invasion of host cells.

ACKNOWLEDGMENTS

We thank Clark Inderlied for helpful discussion during the course of this work and Monica F. Mapa for preparing the manuscript.

This work was supported by grant AI-25769 from the National Institutes of Health.

REFERENCES

1. **Abou-Zeid, C., T. Garbe, and R. Lathingra.** 1991. Genetic and immunological analysis of *Mycobacterium tuberculosis* fibronectin-binding proteins. Infect. Immun. **59:**2712–2718.
2. **Bermudez, L. E., M. Petrofsky, P. Kolonoski, and L. S. Young.** 1992. An animal model of *Mycobacterium avium* complex disseminated infection after colonization of the intestinal tract. J. Infect. Dis. **165:**75–79.
3. **Byrd, S. R., R. Gelber, and L. E. Bermudez.** 1993. Roles of soluble fibronectin and β1 integrin receptors in the binding of *Mycobacterium leprae* to nasal epithelial cells. Clin. Immunol. Immunopathol. **69:**266–272.
4. **Ellner, J. J., M. J. Goldberger, and D. M. Parenti.** 1991. *Mycobacterium avium* infection and AIDS: a therapeutic dilemma in rapid evolution. J. Infect. Dis. **163:**1326–1335.
5. **Falkow, S.** 1991. Bacterial entry into eukaryotic cells. Cell **65:**1099.
6. **Finlay, B. B., and S. Falkow.** 1988. Comparison of the invasion strategies used by *Salmonella choleraesuis*, *Shigella flexneri*, and *Yersinia entercolitica* to enter cultivated animal cells: endosome acidification is not required for bacterial invasion or intracellular replication. Biochimie **70:**1089–1099.
7. **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.
8. **Gray, J. R., and L. Rabeneck.** 1989. Atypical mycobacterial infection of the gastrointestinal tract in AIDS patients. Am. J. Gastroenterol. **84:**1521–1525.
9. **Holinka, R., C. Davie, J. Choong, and P. Wyrick.** 1988. Ultrastructural study of endocytosis of *Chlamydia trachomatis*. Infect. Immun. **56:**1456–1463.
10. **Horsburgh, C. R., Jr.** 1991. *Mycobacterium avium* complex in the

acquired immunodeficiency syndrome (AIDS). N. Engl. J. Med. **324:**1332–1338.
11. **Horsburgh, Jr., C. R., B. G. Metchock, J. McGowan, and S. E. Thompson.** 1991. Progression to disseminated infection in HIV-infected persons colonized with mycobacteria other than tuberculosis. Am. Rev. Respir. Dis. **143:**A279.
12. **Isberg, R. R.** 1990. Pathways for the penetration of enteroinvasive *Yersinia* into mammalian cells. Mol. Biol. Med. **7:**73–82.
13. **Isberg, R. R.** 1991. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. Science **252:**934.
14. **Isberg, R. R., and S. Falkow.** 1985. A single genetic locus encoded by *Yersinia pseudotuberculosis* permits invasion of cultured animal cells by *Escherichia coli* K-12. Nature (London) **317:**262–264.
15. **Isberg, R. R., A. Swain, and S. Falkow.** 1988. Analysis of expression and thermoregulation of the *Yersinia pseudotuberculosis inv* gene with hybrid proteins. Infect. Immun. **56:**2133–2138.
16. **Isberg, R. R., D. L. Voorhis, and S. Falkow.** 1987. Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells. Cell **50:**769–778.
17. **Jacobson, M. A., P. C. Hopewell, D. M. Yajko, W. K. Hadley, E. Lazarus, P. K. Mohanty, G. W. Modin, D. W. Feigal, P. S. Cusick, and M. A. Sande.** 1991. Natural history of disseminated *Mycobacterium avium* complex infection in AIDS. J. Infect. Dis. **164:**994–998.
18. **Juliana, R. L., and S. Haskill.** 1993. Signal transduction from the extracellular matrix. J. Cell Biol. **120:**577–585.
19. **Klatt, E. C., D. F. Jensen, and P. R. Meyer.** 1987. Pathology of *Mycobacterium avium-intracellulare* infection in acquired immunodeficiency syndrome. Hum. Pathol. **18:**709–714.
20. **Lee, C. A., and S. Falkow.** 1990. The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. Proc. Natl. Acad. Sci. USA **87:**4304–4308.
21. **Mapother, M. E., and J. G. Sanger.** 1984. In vitro interaction of *Mycobacterium avium* with intestinal epithelial cells. Infect. Immun. **45:**67–73.
22. **Miller, V. L., and S. Falkow.** 1988. Evidence for two genetic loci in *Yersinia entercolitica* that can promote invasion of epithelial cells. Infect. Immun. **56:**1242–1248.
23. **Miller, V. L., B. B. Finlay, and S. Falkow.** 1988. Factors essential for penetration of mammalian cells by *Yersinia*. Curr. Top. Microbiol. Immunol. **138:**15–39.
24. **Orme, I. M., S. K. Furney, and A. D. Roberts.** 1992. Dissemination of enteric *Mycobacterium avium* infections in mice rendered immunodeficient by thymectomy and CD4 depletion or by prior infection with murine AIDS retrovirus. Infect. Immun. **60:**4747–4753.
25. **Ratliff, T. L., R. McCarthy, W. B. Telle, and E. J. Brown.** 1993. Purification of a mycobacterial adhesin for fibronectin. Infect. Immun. **61:**1889–1894.
26. **Ratliff, T. L., J. O. Palmer, and J. A. McGarr.** 1987. Intravesical bacillus Calmette-Guerin therapy for murine bladder tumors. Initiation of the response by fibronectin-mediated attachment of bacillus Calmette-Guerin. Cancer Res. **47:**1762–1766.
27. **Rosenshine, I., V. Duronimo, and B. B. Finlay.** 1992. Tyrosine protein kinase inhibitors block invasin-promoted bacterial uptake by epithelial cells. Infect. Immun. **60:**2211–2217.
28. **Scordamaglila, A., M. Bagnasco, and G. W. Canonsca.** 1988. Immune response to mycobacteria: characterization of immunocompetent cells in tuberculosis lesions of humans, p. 81–97. *In* M. Berdinelli and H. Friedman (ed.), Mycobacterium tuberculosis. Plenum Press, New York.
29. **Shepard, C. C.** 1957. Growth characteristics in HeLa cells of the rapidly growing acid fast bacteria, *Mycobacterium fortuitum*, *Mycobacterium phlei*, and *Mycobacterium smegmatis*. J. Bacteriol. **73:**722–726.
30. **Shepard, C. C.** 1957. Growth characteristics of tubercle bacilli and certain other mycobacteria in HeLa cells. J. Exp. Med. **105:**39–48.
31. **Tamaoki, T., and H. Nakano.** 1990. Potent and specific inhibition of protein kinase C of microbial organisms. Bio/Technology **8:**732–735.
32. **Watson, S. P., J. McNally, L. J. Shipman, and P. P. Godfrey.** 1988. The action of the protein kinase C inhibitor, stavrosporin, on human platelets. Evicence against a regulatory role for protein kinase C in the formation of inositol triphosphate by thombine. Biol. Chem. J. **249:**345–350.
33. **Young, L. S., C. B. Inderlied, O. G. Berlin, and M. S. Gottlieb.** 1986. Mycobacterial Infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev. Infect. Dis. **8:**1024–1033.