INFECTION AND IMMUNITY, June 1994, p. 2229–2235
0019-9567/94/$04.00+0

Vol. 62, No. 6

Entrance and Survival of *Salmonella typhimurium* and *Yersinia enterocolitica* within Human B- and T-Cell Lines

GEORGES M. G. M. VERJANS,[1]* JEFFREY H. RINGROSE,[1] LOEK VAN ALPHEN,[2] T. E. W. FELTKAMP,[1] AND JOHANNES G. KUSTERS[3]

Department of Ophthalmo-Immunology, The Netherlands Ophthalmic Research Institute,[1] and Department of Medical Microbiology, University of Amsterdam,[2] Amsterdam, and Department of Bacteriology, Institute of Infectious Diseases and Immunology, School of Veterinary Medicine, University of Utrecht, Utrecht,[3] The Netherlands

Received 12 October 1993/Returned for modification 10 December 1993/Accepted 11 March 1994

Lymphocytes, located within the Peyer's patches, might be involved in the dissemination of enteropathogenic *Salmonella typhimurium* and *Yersinia enterocolitica* bacteria. To test this hypothesis, we have investigated the susceptibility of human B- and T-cell lines to bacterial adhesion and invasion. The two *S. typhimurium* strains analyzed were highly invasive, while the two *Y. enterocolitica* (O:8) strains adhered to the B- and T-cell lines but did not enter the cell lines in significant amounts. We hypothesize that the incapability of the *Y. enterocolitica* (O:8) strains to enter the human B- and T-cell lines is most probably due to the bacterial inability to induce the internalization process upon adhesion to both cell lines. Although immortalized B- and T-cell lines were used in this study, the results presented suggest the possibility that both cell types could play a role in the dissemination of intracellularly residing *S. typhimurium* in vivo.

The genera *Salmonella* and *Yersinia* include members that are severe pathogens for both humans and animals. Enteropathogenic *Salmonella* and *Yersinia* strains are capable of causing disease types ranging from mild diarrhea to systemic infections like typhoid fever (14, 15, 36). Extraintestinal manifestations like reactive arthritis have been associated with infections of both bacterial species (30). *Yersinia* and *Salmonella* spp. are transmitted by the fecal-oral route. When ingested, they proceed to the small bowel, where they penetrate the intestinal epithelium. After penetration of the intestinal epithelial cell barrier, these bacteria are capable of entering the underlying lymphoid tissue of the Peyer's patches and disseminate to the regional lymph nodes, spleen, and liver (14).

Both bacterial species are classified as facultative intracellular pathogens. Although both bacterial species replicate extracellularly, in vivo, it has been suggested that professional phagocytes, i.e., macrophages, may act as carriers transporting intracellularly residing bacteria throughout the host (6, 12, 23, 36). The persistence of *Salmonella typhimurium* in macrophages is generally accepted, and there is substantial evidence that this bacterium inhabits an intracellular niche in vivo (10).

In addition to macrophages, migrating B and T cells located within the Peyer's patches may function as a "Trojan horse" to deliver *S. typhimurium* and *Yersinia enterocolitica* throughout the host. Therefore, we investigated whether *S. typhimurium* and *Y. enterocolitica* bacteria are able to enter and survive within well-defined immortalized human B- and T-cell lines.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. typhimurium* SR11 (17) and SL1344 (22) were obtained from R. Curtiss III (Department of Biology, Washington University, St. Louis, Mo.). *Y. enterocolitica* O:8 strains WA (19) and 8081 (41) were obtained from J. Heesemann (Institute of Hygiene and Microbiology, Würzburg University, Würzburg, Germany). Both *Y. enterocolitica* strains carry the 70-kb virulence plasmid. For control experiments, virulence plasmid-cured derivatives of both strains were used. The nonadhesive, noninvasive *Escherichia coli* K-12 strain HB101 was used as a negative control (3). Bacterial stocks were stored at −70°C in Luria-Bertani (LB) broth containing 20% glycerol. For the cell culture infection experiments, fresh overnight cultures of bacteria were prepared by incubating LB broth inoculated with bacteria from frozen (−70°C) glycerol stocks. All bacteria were grown with vigorous shaking, *S. typhimurium* and *E. coli* at 37°C and *Y. enterocolitica* at 30°C. *S. typhimurium* and *E. coli* bacteria were plated onto LB agar plates and incubated overnight at 37°C. *Y. enterocolitica* bacteria were plated onto blood agar plates and incubated overnight at 37°C under 5% CO_2 and 100% humidity (CO_2 incubator).

Cell cultures. All tissue culture reagents were obtained from GIBCO Laboratories (Grand Island, N.Y.). Cells were grown in culture medium which consisted of RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS) supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml) at 37°C in a CO_2 incubator. The human leukemic T-cell lines H9 (ATCC HTB 176) and Jurkat (ATCC TIB 152) and the human larynx epithelium cell line HEp-2 (ATCC CCL 23) were obtained from the American Type Culture Collection (Rockville, Md.). The Epstein-Barr virus (EBV)-transformed B-cell line JY has been described by Peters et al. (37). The EBV-transformed B-cell line JP was kindly donated by F. G. C. M. UytdeHaag (Department of Immunobiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) (42). The B- and T-cell lines were routinely grown in 175-cm^2 culture flasks and subcultured twice a week. HEp-2 cells were trypsinized and split at a 1:10 ratio approximately twice a week. For invasion assays, 2×10^5 HEp-2 cells were seeded in six-well dishes and incubated in a CO_2 incubator to semiconfluent monolayers (approximately 5×10^5 cells per well). Prior to the bacterial invasion assays, the cell lines were incubated overnight in culture medium without antibiotics.

* Corresponding author. Mailing address: Department of Ophthalmo-Immunology, The Netherlands Ophthalmic Research Institute, P.O. Box 12141, NL-1100 AC Amsterdam, The Netherlands. Phone: 31 20 5664535. Fax: 31 20 6916521.

Invasion and intracellular replication assay. Exponentially growing cultures of *S. typhimurium*, *E. coli*, and *Y. enterocolitica* were prepared by diluting overnight cultures 1:100 in prewarmed RPMI 1640 medium and then incubating them for 3.5 h with shaking. *S. typhimurium* and *E. coli* were incubated at 37°C, and *Y. enterocolitica* was incubated at 30°C. Prior to infection, tissue culture plates (5×10^5 HEp-2 cells per well) or tubes with nonadherent cells (15×10^5 B- and T-cell lines) were washed twice with RPMI 1640 medium. Cells were infected with 1 ml of a diluted bacterial suspension, resulting in a nominal multiplicity of infection of 10. Tissue culture plates or tubes, containing the cell-bacterium suspension, were centrifuged ($800 \times g$, 10 min, 25°C) to promote intimate contact between the bacteria and cells. Adhesion and subsequent invasion of the bacteria were allowed to take place for 30 min at 37°C in a CO_2 incubator. Cells were washed three times with phosphate-buffered saline (PBS) plus 5% FCS (washing buffer) to remove free bacteria and incubated in RPMI 1640 medium supplemented with 10% FCS and 200 μg of gentamicin per ml for 1 h at 37°C in a CO_2 incubator. Extracellular bacteria are killed by this antibiotic treatment, while the viability of intracellular bacteria is not affected (43). Cells were washed twice with washing buffer and maintained in RPMI 1640 medium supplemented with 10% FCS and 20 μg of gentamicin per ml to prevent reinfection of the cells. At various times (1, 4, and 24 h postinfection), the amount of intracellular bacteria was determined in separate wells or tubes in triplicate. The cells were washed twice with washing buffer to remove the gentamicin. Subsequently, the cells were lysed by incubation with PBS plus 1% (wt/vol) saponin (1 ml per sample) for 5 min at room temperature to release the intracellular bacteria. This procedure has been described for *Neisseria gonorrhoeae* (33), and it did not influence the viability of the bacterial strains used in this study (data not shown). Appropriate dilutions in PBS of each sample were plated onto LB or blood agar plates to determine the number of intracellular bacteria (CFU) by colony counting. In some experiments, cytochalasin D (5 μg/ml) was added to the cells 1 h before the bacteria were added and was also included throughout the infection period (13).

The total number of cell-associated bacteria was determined in the same way as the number of intracellular bacteria was determined, but with the following modification. After incubation of the bacteria with the cells for 30 min, the cells were washed thoroughly and lysed immediately with PBS plus 1% (wt/vol) saponin as described above. The percentage of cell-associated bacteria was calculated as follows: [(number of cell-associated bacteria)/(total number of bacteria added)] × 100. Experiments were repeated at least three times.

Flow cytometry analysis of integrin expression on the B- and T-cell lines. The following monoclonal antibodies (MAb), kindly provided by A. Sonnenberg (The Netherlands Cancer Institute, Amsterdam, The Netherlands), were used: anti-VLA-3 α-chain MAb J143 (20), anti-VLA-4 α-chain MAb HP2/1 (40), anti-VLA-5 α-chain MAb SAM-1 (29), anti-VLA-6 α-chain MAb J8H (21), and anti-VLA-β1 subunit MAb K20 (1).

To examine the expression of various VLA antigens on the B-cell line (JY) and the T-cell line (H9), the cells were washed twice with washing buffer (PBS plus 0.2% bovine serum albumin) and resuspended in 0.5 ml of washing buffer. Subsequently, 50 μl of cell suspension ($\sim 5 \times 10^5$ cells) was mixed with 50 μl of an appropriate dilution of MAb and incubated for 30 min at 4°C. Labeled cells were washed twice, resuspended in 50 μl of washing buffer, and incubated with 50 μl of an appropriate dilution of fluorescein isothiocyanate-conjugated polyclonal goat anti-mouse antiserum for 30 min at 4°C. The cells were washed twice, resuspended in 150 μl of washing buffer, and analyzed by using a fluorescence-activated cell sorter (FACScan; Becton Dickinson, Mountain View, Calif.). Cells solely incubated with the secondary antibody were used as a negative control.

RESULTS

Invasion and survival of *S. typhimurium* and *Y. enterocolitica* in human B- and T-cell lines. Cells of the EBV B-cell line (JY) and T-cell leukemic cell line (H9) were infected with the *S. typhimurium* strains SR11 and SL1344 and the *Y. enterocolitica* O:8 strains WA and 8081 to analyze whether these bacteria are able to enter and survive within human B and T cells. The results of a representative experiment are shown in Fig. 1.

The numbers of bacteria that entered the cell lines differed significantly among the bacterial species analyzed. The B-cell line (Fig. 1A) contained higher numbers of intracellular bacteria than the T-cell line (Fig. 1B). Both *S. typhimurium* strains entered the B- and T-cell lines very efficiently. Infection of the B-cell line with the *Y. enterocolitica* strains resulted in an invasion rate identical to that of the noninvasive *E. coli* strain HB101. The amount of *Y. enterocolitica* internalized into the T-cell line was 10-fold higher than that of *E. coli* HB101. By using cytochalasin D, which is known to block phagocytosis of bacteria by inhibiting microfilament formation of the host cell (13), we observed a nearly complete inhibition of the internalization of all three bacterial species in both the B- and T-cell lines (data not shown). Thus, it can be concluded that the bacteria are indeed internalized and that *Y. enterocolitica*, when compared with *E. coli* HB101, is not able to enter the B- and T-cell lines in significant amounts. Upon internalization, *S. typhimurium* survived intracellularly but did not replicate extensively (Fig. 1). Experiments performed with an additional EBV B-cell line (JP) and T-cell line (Jurkat) gave essentially the same results as those obtained with the JY and H9 cell lines (data not shown).

Invasion and survival of *S. typhimurium* and *Y. enterocolitica* in HEp-2 cells. To demonstrate whether the observed lack of invasion of the *Y. enterocolitica* strains is due to a (genetic) defect of the strains itself, we infected a *Y. enterocolitica* permissive cell line, HEp-2, with the *Y. enterocolitica* and *S. typhimurium* strains.

The results of a representative experiment are shown in Fig. 2. Both bacterial species were able to enter HEp-2 cells efficiently, and *Y. enterocolitica* was about 10-fold more invasive than *S. typhimurium*. The number of *Y. enterocolitica* bacteria within HEp-2 cells did not increase significantly over time, while *S. typhimurium* replicated about 10-fold within 24 h.

Analysis of integrin expression by the B- and T-cell lines. Studies of the facultative intracellular bacterium *Yersinia pseudotuberculosis* have demonstrated that the bacterial outer membrane protein invasin mediates the attachment and internalization within nonphagocytic cells by binding to four members of the β1-chain integrin family, namely, VLA-3, VLA-4, VLA-5, and VLA-6 (27). Since binding of *Y. enterocolitica* to HEp-2 cells is inhibited by anti-β1 integrin serum, it has been suggested that the homologous invasin proteins of *Y. pseudotuberculosis* and *Y. enterocolitica* bind to the same host cell receptors (44).

The inability of *Y. enterocolitica* to enter the B- and T-cell lines could be due to the absence of the putative *Y. enterocolitica* invasin receptors present on the surface of both cell lines. Therefore, the expression of the α subunits of VLA-3, VLA-4,

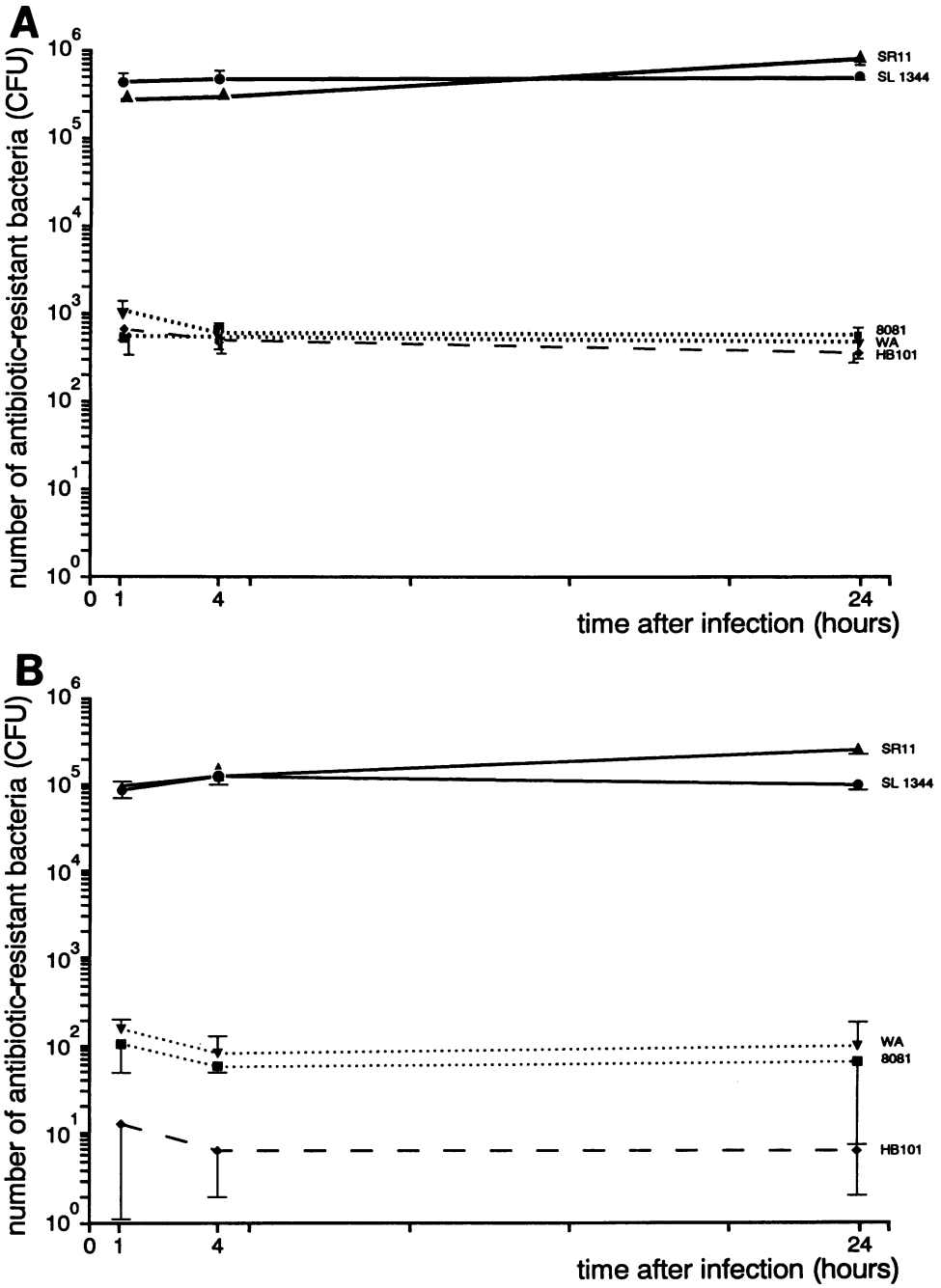

FIG. 1. Bacterial growth kinetics within the human EBV B-cell line (JY) (A) and T-cell line (H9) (B). Triplicate cell samples (5×10^5) were incubated with bacteria at a multiplicity of infection of 10. Symbols: ■, *Y. enterocolitica* 8081; ▼, *Y. enterocolitica* WA; ▲, *S. typhimurium* SR11; ●, *S. typhimurium* SL1344; ◆, *E. coli* HB101. Data are expressed as the average number of gentamicin-resistant bacteria (CFU) of three cell samples plus standard error.

VLA-5, and VLA-6 and the β1 subunit by both cell lines was determined by fluorescence-activated cell sorter analysis (Fig. 3). The β1 subunit was significantly expressed by the T-cell line (H9), whereas the β1 subunit expression by the B-cell line (JY) was negligible. The H9 cell line expressed high amounts of VLA-3 and VLA-4 and moderate amounts of VLA-5 and VLA-6. The B-cell line (JY) expressed moderate amounts VLA-4, negligible amounts of VLA-5, and no VLA-3 and VLA-6. The higher level of VLA-4 present, compared with β1, on the surface of the B-cell line (JY) is most likely due to the expression of the integrin β7 subunit in association with α4 (9).

Adhesion of *S. typhimurium* and *Y. enterocolitica* to the HEp-2-, B-, and T-cell lines. Adhesion of bacterial pathogens to the host cell is essential for internalization (26). To examine whether the inability of *Y. enterocolitica* to enter the B- and T-cell lines is due to a lack of bacterial adherence, we analyzed the interaction of *Y. enterocolitica* and *S. typhimurium* strains with cells of the HEp-2 and B- (JY) and T- (H9) cell lines.

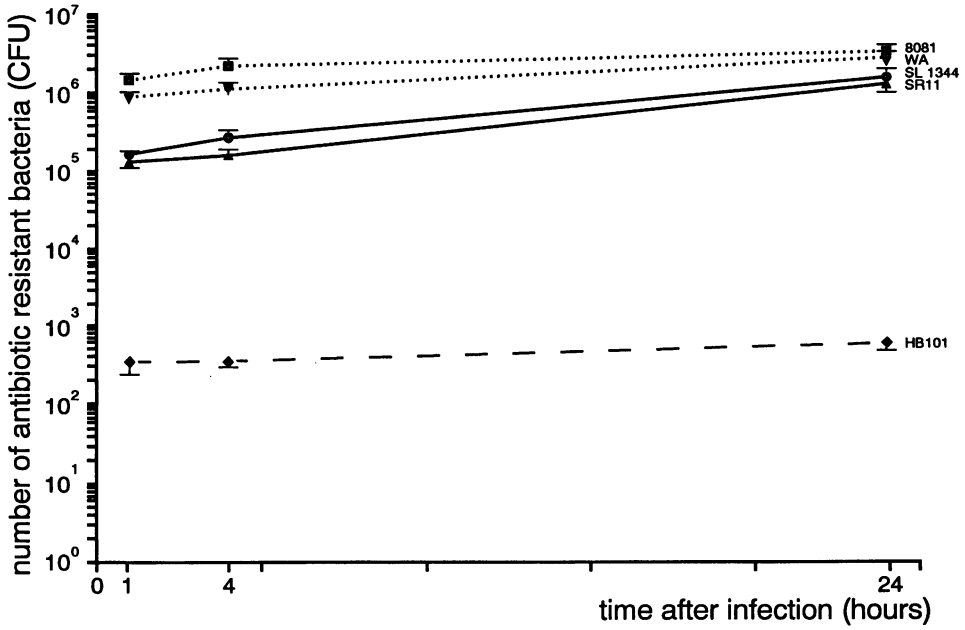

FIG. 2. Bacterial growth kinetics within the HEp-2 cell line. Triplicate cell samples (5×10^5) were incubated with bacteria at a multiplicity of infection of 10. Symbols: ■, *Y. enterocolitica* 8081; ▼, *Y. enterocolitica* WA; ▲, *S. typhimurium* SR11; ●, *S. typhimurium* SL1344; ◆, *E. coli* HB101. Data are expressed as the average number of gentamicin-resistant bacteria (CFU) of three cell samples plus standard error.

The number of cell-associated bacteria, both attached and intracellular, was determined after 30 min of incubation of the cells with the bacteria (Table 1). The numbers of cell-associated bacteria differed significantly between the bacterial species. Although *Y. enterocolitica* did not invade the B- and T-cell lines, the bacteria adhered very efficiently to these cell lines. Furthermore, the number of *Y. enterocolitica* bacteria that adhered to the B- and T-cell lines was significantly higher than the number of *S. typhimurium* bacteria. The data on the adherence of both bacterial species to the HEp-2 cells were similar as described previously (31, 34). Similar results were obtained with the EBV B-cell (JP) and T-cell (Jurkat) lines (data not shown).

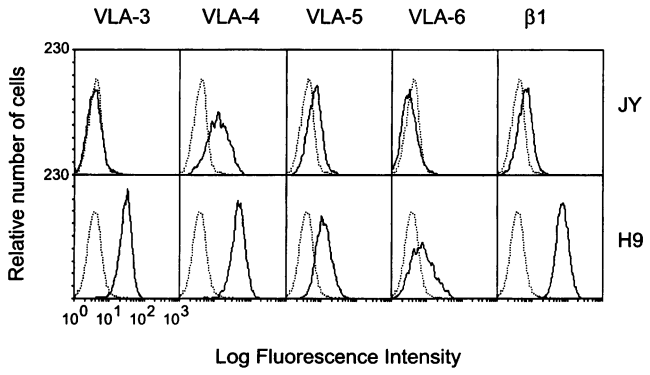

FIG. 3. Analysis of cell surface expression of VLA proteins on the human EBV B-cell line (JY) and T-cell line (H9) by flow cytometry. Cells were indirectly stained with mouse MAb against the α chains of VLA-3 (J143), VLA-4 (HP2/1), VLA-5 (SAM-1), and VLA-6 (J8H) and against the β1 chain (K20) by using fluorescein isothiocyanate-conjugated polyclonal goat anti-mouse antiserum. The fluorescence intensity is expressed on a log scale. Cells incubated only with the secondary antibody were used as a negative control (dotted line).

DISCUSSION

Marked differences were observed in the efficiencies of invasion of *S. typhimurium* and *Y. enterocolitica* (O:8) into the human B- and T-cell lines analyzed. *Y. enterocolitica* was not able to enter the B- and T-cell lines significantly, while *S. typhimurium* was internalized very effectively. *S. typhimurium* did not replicate extensively within the B- and T-cell lines (Fig. 1). Although unable to enter the B- and T-cell lines, *Y. enterocolitica* was highly invasive for the HEp-2-cell line (Fig. 2). The results obtained with HEp-2 cells are in concordance with a previous report showing that *Y. enterocolitica* is highly invasive for HEp-2 cells and that these bacteria fail to grow significantly within the host cell (13, 43). As expected, *S. typhimurium* replicated extensively within the HEp-2 cell line (Fig. 2) (13). Although *Y. enterocolitica* did not invade the B- and T-cell lines, this bacterium bound to the B and T cells even better than *S. typhimurium* (Table 1).

Bacterial adherence to and invasion of eukaryotic cells are thought to constitute a receptor-mediated process. This process depends both on (several) bacterial factors and the host cell receptor(s) to which the bacterial ligand(s) binds (5, 26). Enteropathogenic *Yersinia* spp. possess three independent pathways for the attachment and internalization into mammalian cells (12). Two of these determinants (invasin and *ail*) are encoded by the bacterial chromosome, while the third one (YadA), known to be less efficient than that promoted by invasin (4, 24), is encoded by a gene located on the 70-kb virulence plasmid that is present in all pathogenic *Yersinia* strains (25). In addition to YadA, the *Yersinia* virulence plasmid encodes operons involved in the expression of virulence proteins known as *Yersinia* outer membrane proteins (Yops) (5). YopH and YopE secretion, induced upon bacterial

TABLE 1. Numbers and percentages of cell-associated bacteria for JY, H9, and HEp-2 cells

Cell line	Bacterial strain[a]	No. of bacteria added to cells	No. of cell-associated bacteria[b]	% Cell-associated bacteria[c]
JY	HB101	4.8×10^6	$(2.3 \pm 0.3) \times 10^5$	4.7
	SR11	5.8×10^6	$(12.0 \pm 2.8) \times 10^5$	20.8
	SL1344	5.4×10^6	$(12.8 \pm 1.6) \times 10^5$	23.9
	8081	5.2×10^6	$(19.7 \pm 3.1) \times 10^5$	37.8
	WA	5.2×10^6	$(18.7 \pm 3.3) \times 10^5$	35.1
H9	HB101	6.1×10^6	$(1.9 \pm 0.2) \times 10^5$	3.1
	SR11	5.8×10^6	$(7.8 \pm 2.8) \times 10^5$	14.1
	SL1344	5.4×10^6	$(8.8 \pm 1.6) \times 10^5$	13.5
	8081	5.8×10^6	$(26.3 \pm 6.4) \times 10^5$	45.1
	WA	4.9×10^6	$(23.1 \pm 3.1) \times 10^5$	46.5
HEp-2	HB101	5.3×10^6	$(1.1 \pm 0.2) \times 10^5$	2
	SR11	5.8×10^6	$(7.9 \pm 1.4) \times 10^5$	13.7
	SL1344	6.5×10^6	$(7.1 \pm 0.9) \times 10^5$	10.1
	8081	5.0×10^6	$(30.1 \pm 4.5) \times 10^5$	60.1
	WA	5.7×10^6	$(36.5 \pm 5.1) \times 10^5$	63.7

[a] SR11 and SL1344 are *S. typhimurium* strains, WA and 8081 are *Y. enterocolitica* (O:8) strains harboring the 70-kb virulence plasmid, and HB101 is an *E. coli* K-12 strain.

[b] Values represent the averages of triplicate samples plus standard errors of a representative experiment.

[c] Percentage of cell-associated bacteria with respect to the initial inoculum.

attachment to the host cell and maximally expressed at 37°C, has been demonstrated to inhibit bacterial internalization (4, 32). Furthermore, the pathways of *Yersinia* internalization are thermoregulated. Expression of invasin is maximal at ambient temperatures, while the *ail* and YadA proteins are maximally expressed at 37°C (12, 24, 38).

In the case of *Y. pseudotuberculosis*, several members of the β1-chain integrin family of cell adhesion molecules (VLA-3, VLA-4, VLA-5, and VLA-6) have been shown to be the extracellular receptors for invasin (27). Binding of *Y. pseudotuberculosis* to these β1-chain integrins is sufficient to promote internalization (26). Analogous to *Y. pseudotuberculosis*, invasin-mediated attachment of *Y. enterocolitica* to HEp-2 cells can be inhibited by antiserum to the β1 integrin subunit. This observation suggests that the homologous invasin proteins of both *Y. enterocolitica* and *Y. pseudotuberculosis* probably bind to the same host cell receptors (44). By performing fluorescence-activated cell sorter analysis, we have demonstrated that the putative invasin receptors of *Y. enterocolitica* are significantly expressed by the T-cell line (H9), while the B-cell line (JY) expressed almost no detectable β1 integrins (Fig. 3). Despite the lack of significant amounts of invasin receptors on the B-cell line (JY), the levels of adherence of *Y. enterocolitica* to the JY and H9 cell lines were almost similar (Table 1). Since β1 integrins have also been demonstrated to play a possible role in the YadA-mediated attachment to and invasion of HEp-2 cells by *Y. pseudotuberculosis* (4), we hypothesize that attachment of the *Y. enterocolitica* strains to the JY cells is most probably not invasin or YadA mediated. The observed attachment to this cell line could therefore be mediated by the bacterial *ail* protein, while attachment to the H9 cells is more likely to be a combined effect of invasin-, *ail*-, and possibly YadA-mediated adhesion (11). The similar numbers of attached *Y. enterocolitica* bacteria observed for both the JY and H9 cell lines could be due to cell type-specific levels of bacterial adhesion sites (i.e., receptors) for either the *ail*- or invasin- and YadA-mediated attachment (26). Control experiments, using plasmid-cured derivatives of both *Y. enterocolitica* strains, did not alter significantly the observed invasiveness or attachment to the B- and T-cell lines (data not shown). It can therefore be concluded that the lack of internalization of the *Y. enterocolitica* strains into the B- and T-cell lines is most likely not due to the inhibitory effect of YopH and YopE. Furthermore, growth of the *Y. enterocolitica* strains (harboring the virulence plasmid) at 37°C prior to the invasion assay gave amounts of internalized bacteria in the B- and T-cell lines similar to those observed during preincubation at 30°C (data not shown). It can therefore be concluded that even under conditions in which invasin (at 30°C) or *ail* and YadA (at 37°C) are maximally expressed, these proteins do not significantly contribute to the internalization of both strains into the B- and T-cell lines.

The pronounced differences in invasion of the *Y. enterocolitica* strains analyzed in HEp-2 cells versus the B- and T-cell lines, despite significant adherence to the B- and T-cell lines, suggest that the incapability of these strains to enter the B- and T-cell lines is not the result of a (genetic) defect of the bacterial strains used or the invasion procedure followed. We therefore hypothesize that, as suggested for the cell type specificity of *ail*-mediated invasion of *Y. enterocolitica* (34), the *Y. enterocolitica* strains used in this study are unable to induce the internalization process upon attachment to the B- and T-cell lines.

In contrast to our results, Asai et al. (2) have shown that a *Y. enterocolitica* (O:3) strain was able to penetrate human EBV-transformed B-cell lines. Consistent with their report, and by using a clinical isolate of *Y. enterocolitica* (O:3), we have observed that this particular strain entered both B- and T-cell lines in significant amounts (data not shown). The discrepancy in internalization efficiency between the *Y. enterocolitica* serotypes O:8 and O:3 may be a serotype-specific phenomenon (44). Alternatively, the discrepancy could be due to *Y. enterocolitica* strain-specific genetic differences which influence the internalization process, e.g., polymorphism of the *inv* gene of *Y. enterocolitica* (35, 39).

Several chromosomal loci required for *Salmonella* invasion have been identified (5). Upon infection of eukaryotic cells, invasive *Salmonella* bacteria elicit membranous ultrastructures called ruffles. Ruffle formation, induced at the site of bacterium-host cell interaction, is most probably a prerequisite for *Salmonella* entry (16). The mechanism of ruffle induction by *Salmonella* spp. is not understood. Although the epidermal growth factor receptor expressed by the target cell is stimulated during *S. typhimurium* infection (18), it has recently been shown that it is not required for *Salmonella* entry (16). Therefore, epidermal growth factor receptor stimulation is thought to be more likely a secondary phenomenon caused by *Salmonella*-induced membrane ruffling (16).

S. typhimurium bacteria are known to reside and replicate within host cell-derived vacuoles, i.e., phagosomes (15). *S. typhimurium* is capable of inhibiting the fusion of phagosomes with lysosomes (7, 28). Inhibition of phagosome-lysosome fusion is associated with enhanced intracellular survival and possibly replication (7). The pronounced replication of *S. typhimurium* within HEp-2 cells, compared with that in B and T cells, could be due to the cell type-specific intracellular environment, i.e., antimicrobial mechanisms (6).

In conclusion, the differential abilities of *S. typhimurium* and *Y. enterocolitica* (O:8) to enter human B and T cells clearly reflect the unique internalization pathways utilized by both bacterial species (5). Although the ability of *S. typhimurium* to enter and survive within B and T cells has now been demon-

strated by using immortalized lymphocytic cell lines, the results presented here indicate that both cell types, located within the Peyer's patches (8), could play a role in the trafficking of intracellularly residing *S. typhimurium* in vivo. The likelihood of this hypothesis will have to be addressed in future experiments by using freshly isolated human B and T cells. Although it has been demonstrated that *Y. enterocolitica* O:3 strains, in contrast to the *Y. enterocolitica* O:8 strains WA and 8081, were able to enter human B-cell lines, it is not likely that the B and T cells play a major role in the dissemination of intracellularly residing *Y. enterocolitica*. Additional experiments are needed to elucidate the possibly serotype-specific, differential invasiveness of separate *Y. enterocolitica* strains and to analyze the adhesion and invasion of the *Y. enterocolitica* strains by using freshly isolated human B and T cells.

ACKNOWLEDGMENTS

Anemiek de Melker (Department of Cell Biology, The Netherlands Cancer Institute) is acknowledged for determining the VLA-3 to VLA-6 expression by fluorescence-activated cell sorter analysis. We thank C. E. M. van Doornik for technical assistance.

This work was supported by Het Nationaal Reumafonds of The Netherlands. J.G.K. was supported by a fellowship from the Royal Netherlands Academy of Arts and Sciences.

REFERENCES

1. **Amiot, M., A. Bernard, H. C. Tran, G. Leca, J. M. Kanellopoulos, and B. Boumsell.** 1986. The human cell surface glycoprotein complex (gp 120,200) recognized by monoclonal antibody K 20 is a component binding to phytohaemagglutinin on T cells. Scand. J. Immunol. **23:**109–118.
2. **Asai, S., I. Namikawa, and Y. Ito.** 1983. Invasion of Burkitt's lymphoma cell lines by *Yersinia enterocolitica*. Proc. Soc. Exp. Biol. Med. **172:**243–249.
3. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:**525–557.
4. **Bliska, J. B., M. C. Copass, and S. Falkow.** 1993. The *Yersinia pseudotuberculosis* adhesin YadA mediates intimate bacterial attachment to and entry into HEp-2 cells. Infect. Immun. **61:**3914–3921.
5. **Bliska, J. B., J. E. Galan, and S. Falkow.** 1993. Signal transduction in the mammalian cell during bacterial attachment and entry. Cell **73:**903–920.
6. **Buchmeier, N. A., and F. Heffron.** 1989. Intracellular survival of wild-type *Salmonella typhimurium* and macrophage-sensitive mutants in diverse populations of macrophages. Infect. Immun. **57:**1–7.
7. **Buchmeier, N. A., and F. Heffron.** 1991. Inhibition of macrophage phagosome-lysosome fusion by *Salmonella typhimurium*. Infect. Immun. **59:**2232–2238.
8. **Bye, W. A., C. H. Allan, and J. S. Trier.** 1984. Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. Gastroenterology **86:**789–801.
9. **Chan, B. M. C., J. Elices, E. Murphy, and M. E. Hemmler.** 1992. Adhesion to vascular cell adhesion molecule 1 and fibronectin: comparison of α4β1 (VLA-4) and α4β7 on the human B cell line JY. J. Biol. Chem. **267:**8366–8370.
10. **Dunlap, N. E., W. H. Benjamin, Jr., R. D. McCall, Jr., A. B. Tilden, and D. E. Briles.** 1991. A "safe-site" for *Salmonella typhimurium* is within splenic cells during the early phase of infection in mice. Microb. Pathog. **10:**297–310.
11. **Ennis, E., R. R. Isberg, and Y. Shimizu.** 1993. Very late antigen 4-dependent adhesion and costimulation of resting human T cells by the bacterial integrin ligand invasin. J. Exp. Med. **177:**207–212.
12. **Falkow, S., R. R. Isberg, and D. A. Portnoy.** 1992. The interaction of bacteria with mammalian cells. Annu. Rev. Cell. Biol. **8:**333–363.
13. **Finlay, B. B., and S. Falkow.** 1988. Comparison of the invasion strategies used by *Salmonella choleraesuis*, *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.
14. **Finlay, B. B., and S. Falkow.** 1989. Common themes in microbial pathogenicity. Microbiol. Rev. **53:**210–230.
15. **Finlay, B. B., and S. Falkow.** 1989. *Salmonella* as an intracellular parasite. Mol. Microbiol. **3:**1833–1841.
16. **Francis, C. L., T. A. Ryan, B. D. Jones, S. J. Smith, and S. Falkow.** 1993. Ruffles induced by *Salmonella* and other stimuli direct macropinocytosis of bacteria. Nature (London) **364:**639–642.
17. **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.
18. **Galan, J. E., J. Pace, and M. J. Hayman.** 1992. Involvement of the epidermal growth factor receptor in the invasion of cultured mammalian cells by *Salmonella typhimurium*. Nature (London) **357:**588–589.
19. **Heesemann, J., and R. Laufs.** 1983. Construction of a mobilizable *Yersinia enterocolitica* virulence plasmid. J. Bacteriol. **155:**761–767.
20. **Hemler, M. E., C. Huang, and L. Schwarz.** 1987. The VLA protein family. J. Biol. Chem. **262:**3300–3309.
21. **Hogervorst, F., I. Kuikman, E. Noteboom, and A. Sonnenberg.** 1993. The role of phosphorylation in activation of the α6Aβ1 laminin receptor. J. Biochem. **268:**18427–18430.
22. **Hoiseth, S. K., and B. A. Stocker.** 1981. Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. Nature (London) **291:**238–239.
23. **Hsu, H. S.** 1989. Pathogenesis and immunity in murine salmonellosis. Microbiol. Rev. **53:**390–409.
24. **Isberg, R. R.** 1989. Determinants for thermoinducible cell binding and plasmid-encoded penetration detected in the absence of the *Yersinia pseudotuberculosis* invasin protein cell binding and penetration detected in the absence of invasin. Infect. Immun. **57:**1998–2005.
25. **Isberg, R. R.** 1990. Pathways for the penetration of enteroinvasive *Yersinia* into mammalian cells. Mol. Biol. Med. **7:**73–82.
26. **Isberg, R. R.** 1991. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. Science **252:**934–938.
27. **Isberg, R. R., and J. M. Leong.** 1990. Multiple β1 chain integrins are receptor for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell **60:**861–871.
28. **Ishibashi, Y., and T. Arai.** 1990. Specific inhibition of phagosome-lysosome fusion in murine macrophages mediated by *Salmonella typhimurium* infection. FEMS Microbiol. Immunol. **64:**35–44.
29. **Keizer, G. D., A. A. te Velde, R. Schwarting, C. G. Figdor, and J. E. de Vries.** 1987. Role of p150,95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. Eur. J. Immunol. **17:**1317–1322.
30. **Kingsley, G., and J. Sieper.** 1993. Current perspectives in reactive arthritis. Immunol. Today **14:**387–391.
31. **Kusters, J. G., G. A. W. Mulders-Kremers, C. E. M. van Doornik, and B. A. M. van der Zeijst.** 1993. Effects of multiplicity of infection, bacterial protein synthesis, and growth phase on adhesion to and invasion of human cell lines by *Salmonella typhimurium*. Infect. Immun. **61:**5013–5020.
32. **Lian, C. J., W. S. Hwang, and C. H. Pai.** 1987. Plasmid-mediated resistance to phagocytosis in *Yersinia enterocolitica*. Infect. Immun. **55:**1176–1183.
33. **Makino, S., J. P. M. van Putten, and T. F. Meyer.** 1991. Phase variation of the opacity outer membrane protein controls invasion by *Neisseria gonorrhoeae* into human epithelial cells. EMBO J. **10:**1307–1315.
34. **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.
35. **Miller, V. L., J. J. Farmer III, W. E. Hill, and S. Falkow.** 1989. The *ail* locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. Infect. Immun. **57:**121–131.
36. **Miller, V. L., B. B. Finlay, and S. Falkow.** 1988. Factors essential for the penetration of mammalian cells by *Yersinia*. Curr. Top. Microbiol. Immunol. **138:**15–39.
37. **Peters, P. J., J. J. Neefjes, V. Oorschot, H. D. Ploegh, and H. J. Geuze.** 1991. Segregation of MHC II molecules from MHC class I

molecules in the Golgi complex for transport to lysosomal compartments. Nature (London) **349:**669–675.

38. **Pierson, D. E., and S. Falkow.** 1990. Nonpathogenic isolates of *Yersinia enterocolitica* do not contain functional *inv*-homologous sequences. Infect. Immun. **58:**1059–1064.
39. **Portnoy, D. A., S. L. Mosely, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31:**775–782.
40. **Pulido, R., M. J. Elices, M. R. Campanero, L. Osborn, S. Schiffer, A. Garcia-Pardo, R. Lobb, M. E. Hemler, and F. Sanchez-Madrid.** 1991. Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. Correlation with distinct α4 epitopes. J. Biol. Chem. **266:**10241–10249.
41. **Small, P. L. C., R. R. Isberg, and S. Falkow.** 1987. Comparison of the ability of enteroinvasive *Escherichia coli*, *Salmonella typhimurium*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica* to enter and replicate within HEp-2 cells. Infect. Immun. **55:**1674–1679.
42. **van Binnendijk, R. S., C. A. van Baalen, M. C. M. Poelen, P. de Vries, J. Boes, V. Cerundolo, A. D. M. E. Osterhaus, and F. G. C. M. UytdeHaag.** 1992. Measles virus transmembrane fusion protein synthesized de novo or presented in immunostimulating complexes is endogenously processed for HLA class I- and class II-restricted cytotoxic T cell recognition. J. Exp. Med. **176:**119–128.
43. **Vaudaux, P., and W. A. Waldvogel.** 1979. Gentamycin antibacterial activity in the presence of human polymorphonuclear leukocytes. Antimicrob. Chemother. **16:**743–749.
44. **Young, V. B., S. Falkow, and G. K. Schoolnik.** 1992. The invasin protein of *Yersinia enterocolitica*: internalization of invasin-bearing bacteria by eukaryotic cells is associated with reorganization of the cytoskeleton. J. Cell Biol. **116:**197–207.