INFECTION AND IMMUNITY, Nov. 1979, p. 520-527
0019-9567/79/11-0520/08$02.00/00

Vol. 26, No. 2

Mannose-Sensitive Interaction of *Escherichia coli* with Human Peripheral Leukocytes In Vitro

DENNIS F. MANGAN† AND IRVIN S. SNYDER*

Department of Microbiology, West Virginia University Medical Center, Morgantown, West Virginia 26506

Received for publication 8 August 1979

The ability of *Escherichia coli* which possess or lack mannose-sensitive adherence factors (adhesins) to associate with human peripheral leukocytes in vitro in the absence of serum was studied. *E. coli* 19+, which have mannose-sensitive adhesins, were derived from *E. coli* strain 19 by culturing in static Trypticase soy broth at 37°C. *E. coli* 19−, which lack mannose-sensitive adhesins, were derived from *E. coli* 19 by culturing in agitated Trypticase soy broth at 30°C. *E. coli* 19+ attached to leukocytes and stimulated the release of lysozyme but not β-glucuronidase or lactate dehydrogenase. In contrast, *E. coli* 19− showed poor attachment to the leukocytes and failed to stimulate lysosomal enzyme release. During a 60-min incubation with the leukocytes, the number of viable 19+ organisms decreased, whereas the number of viable 19− remained constant. Purified type 1 pili from *E. coli* 19+ agglutinated the leukocytes but did not stimulate lysosomal enzyme release. Pretreatment of leukocytes with type 1 pili failed to prevent the adherence of *E. coli* 19+. The association of 19+ with leukocytes and subsequent release of lysozyme could be blocked by α-methyl-D-mannoside but not by equivalent concentrations of dextrose and sucrose. These results show that mannose-sensitive adhesins on *E. coli* mediate association of the organisms with leukocytes in the absence of serum components. The identity of the adhesins involved in leukocyte association has yet to be determined.

The ability of leukocytes (WBCs) to recognize and ingest bacteria is a remarkable but poorly understood phenomenon. It is well known that serum components (complement and antibody) coat particles and facilitate engulfment of bacteria by WBCs. However, WBCs engulf some bacteria even in the absence of serum, indicating that recognition can be mediated by factors other than serum. Van Oss (36) has stressed the importance of bacterial surface characteristics in WBC recognition. Bacteria with hydrophobic exteriors (37) or surface projections, e.g., pili (29, 30), are rapidly ingested by WBCs, whereas organisms with hydrophilic capsules or without projections resist ingestion.

Escherichia coli, like many other gram-negative bacteria, exhibit different surface characteristics depending on the culture conditions. When grown in broth at temperatures of 37°C or above, *E. coli* produce mannose-sensitive (MS) adherence factors (adhesins) which mediate attachment of the organisms to many types of eucaryotic cells, including human WBCs (1, 29). One such MS adhesin, type 1 pili (fimbriae), received extensive examination in recent years (3, 4, 27, 28). Another *E. coli* MS adhesin, the MS lectin (12), has recently been identified but has not been well characterized.

The purpose of this research was to investigate the role of MS adhesins in *E. coli*-WBC association in vitro in the absence of serum, and to determine the effect this association has on both the WBCs and the bacteria. The results show that *E. coli* with MS adhesins associate with WBCs to a much greater extent than do *E. coli* which lack MS adhesins. Furthermore, this association causes release of WBC lysosomal enzymes as well as a decrease in the number of viable *E. coli*. The addition of α-methyl-D-mannoside (αMM) to the test suspensions prevents association of the *E. coli* with the phagocytes and the subsequent killing of the *E. coli*. These results emphasize the significance of MS adhesins in *E. coli*-WBC interactions.

MATERIALS AND METHODS

E. coli. All strains used in this study, with the exception of strain Bam, were clinical isolates from the West Virginia University Hospital. *Escherichia coli* Bam, the prototype strain for type 1 pili, was kindly donated by Charles C. Brinton, Jr., University of Pittsburgh, Pittsburgh, Pa. Most studies used *E. coli* strain 19, which was isolated from a urinary tract

† Present address: Department of Oral Biology, Dental Research Institute, University of Michigan, Ann Arbor, MI 48109.

infection. Isolates were identified as *E. coli* by Gram stain and by biochemical characteristics (11).

Preparation of *E. coli* cultures for WBC studies. Strains of *E. coli* were grown under conditions which stimulate or depress type 1 pili production (P) and MS hemagglutination (HA) of guinea pig erythrocytes (RBCs) (19). P+, HA+ cultures were obtained by repeated subculture of the *E. coli* strain in Trypticase soy broth (TSB) with dextrose (BBL Microbiology Systems, Cockeysville, Md.), incubated at 37°C without agitation. P−, HA− cultures were obtained by repeated subculture of the *E. coli* strain in TSB at 30°C with agitation. The final subcultures were stored at 5°C and used as a stock culture.

A 0.1-ml amount of the stock culture was inoculated into 10 ml of TSB 18 to 20 h before use and incubated at 37°C without agitation or at 30°C with agitation. The organisms were then washed twice in 0.01 M phosphate-buffered 0.15 M NaCl, pH 7.4 (PBS), and suspended in PBS to the desired concentration. Organisms from the HA+ and HA− cultures of a single strain of *E. coli* gave identical reactions with 17 different biochemical tests (11). However, negative-stain and shadow-cast electron microscope preparations of the washed cells demonstrated many heavily piliated bacteria in the HA+ suspensions (>70% piliated), whereas few if any piliated cells were found in the HA− suspensions (<2%). HA reactions of the cultures were unaffected by washing in PBS.

In this paper, HA+ cultures will refer to TSB cultures grown at 37°C which gave MS HA reactions with guinea pig RBCs. PBS-washed suspensions of these cultures will be designated as "plus" (e.g., 19+, Bam+). HA− cultures will refer to TSB cultures grown at 30°C which do not agglutinate guinea pig RBCs. These *E. coli* will be designated as "minus" (e.g., 19−, Bam−).

Heat and formaldehyde pretreatment of *E. coli*. *E. coli* were washed, suspended in PBS to an optical density at 625 nm of 0.5 to 0.7 (1.2×10^9 to 1.8×10^9 *E. coli* per ml) and then heat-treated by incubation for 60 min in a water bath adjusted to 56 or 75°C or in boiling water (100°C) for 15 min. Formaldehyde-killed cells were prepared by adding an equal volume of 2% formaldehyde (final concentration, 1%) to an *E. coli* suspension and incubating for 60 min at room temperature. A portion of an untreated cell suspension was kept at 4°C and used as a control preparation.

The pretreated and control *E. coli* were centrifuged, washed twice in PBS, and suspended in PBS to the desired optical density at 625 nm. Viability was assessed by plating a loopful of the pretreated suspensions on Trypticase soy agar and incubating for 48 h at 37°C.

HA assays. HA was determined by slide agglutination, using 2.5% guinea pig RBCs in 0.4% bovine serum albumin (BSA)-PBS. *E. coli* cultures, which were HA+ in the presence of 50 mM dextrose or sucrose but HA− in the presence of 50 mM αMM, were labeled MS.

Purification of type 1 pili. *E. coli* 19+ were grown in minimal glucose media (22) with rotation (120 rpm) at 37°C for 24 to 36 h. Pili were detached from the cell bodies by blending and purified by isoelectric and $(NH_4)_2 SO_4$ precipitations and by isopycnic centrifugation in CsCl as described by Salit and Gotschlich (27). The purified pili were dialyzed against PBS before being added to the test suspensions. The protein concentration was estimated by the method of Lowry et al. (21), using crystalline BSA as the standard. When viewed under the electron microscope, purified pili appeared free of contaminating substances and identical to the appendages isolated by other investigators (3, 27). Purified pili, at concentrations greater than 2 μg/ml, gave complete HA of guinea pig RBCs. This HA activity was inhibited by 10 mM αMM, but not by 100 mM dextrose or sucrose, which is characteristic of type 1 pili (27).

WBC preparation. Human blood was obtained from healthy volunteer donors. For a given experiment, all tests were performed with WBCs obtained from a single blood donor. All glassware, except pipettes, was siliconized.

Peripheral venous blood was collected in 10-ml, heparinized (143 U/10-ml tube) Vacutainer tubes (Becton-Dickinson & Co., Rutherford, N.J.). WBCs were separated from RBCs by dextran sedimentation (2) and washed in 0.1% BSA-PBS. Residual RBCs were removed by hypotonic saline lysis (6). After an additional wash with 0.1% BSA-PBS, the WBCs were suspended and adjusted to the desired concentration in 0.4% BSA-PBS. Suspensions typically contained 65 to 80% polymorphonuclear neutrophils.

***E. coli*-WBC association on cover slips.** WBCs were attached to cover slips as described by Swanson et al. (33). One drop (0.05 ml) of a suspension of leukocytes (1×10^6 WBCs/ml) was pipetted onto glass cover slips (22 by 22 mm). The cover slips were incubated in a chamber with high humidity at 37°C for 30 min. Nonadherent WBCs were then removed by dipping the cover slips in PBS.

Duplicate slips were placed in plastic culture dishes (15 by 60 mm) containing 1.0 ml of *E. coli* suspension and 9.0 ml of 0.04% BSA-PBS. For inhibition tests, the test saccharide at 50 mM was added to the suspension. The dishes were incubated at 37°C with a slow rotation (60 rpm) for 20 min, after which each cover slip was rinsed in cold PBS, dipped in 0.1% BSA-PBS, and allowed to air dry.

The dried cover slips were Wright stained, washed with water, air dried, and mounted on glass slides with a drop of Permount (Fisher Scientific Co.). They were examined under oil immersion (1,000×), and each WBC was scored as positive if two or more *E. coli* could be seen either within or attached to the cytoplasmic periphery.

In some experiments, WBCs were pretreated with purified pili (1,750 μg/ml) in PBS at room temperature. Control WBCs were treated with PBS. After 15 min, the cover slips were washed in PBS and then added to the bacterial suspensions.

***E. coli* survival assay.** The survival of *E. coli* during incubation with WBCs in suspension was assayed in sterile, siliconized, glass scintillation vials. Each assay contained the following: 3.5 ml of PBS, 1.0 ml of *E. coli* suspension (1.8×10^7 colony-forming units per ml), and 1.0 ml of WBC suspension (7.5×10^6/ml). Vials containing 1.0 ml of 0.4% BSA-PBS in place of the WBCs were included to determine the

amount of bacterial growth during incubation. The vials were incubated at 37°C with rotation (200 rpm) for 60 min.

Portions (0.1 ml each) of the test suspensions were removed from each vial immediately after the WBCs were added and at intervals of 30 and 60 min. Each portion was diluted in 9.9-ml water blanks, and the tubes were vigorously shaken for 1 min to disrupt the WBCs. The number of colony-forming units in the water blanks was determined by duplicate pour plate methods.

Lysosomal enzyme release. A 0.75-ml portion of an *E. coli* suspension or purified pili was mixed with an equal volume of WBCs (7.5×10^6/ml) in duplicate, sterile, siliconized, glass scintillation vials. The vials were then incubated at 37°C for 60 min in a rotating water bath at 200 rpm. Control vials consisted of WBCs incubated with PBS under identical conditions. At the end of the incubation period, vials were placed in crushed ice for 5 min and then centrifuged at 500 × *g* for 5 min at room temperature. The WBC pellet was discarded, and the supernatant was recentrifuged at 3,500 × *g* for 20 min at room temperature to pellet the bacteria.

Because of their lability, β-glucuronidase and lactate dehydrogenase (41) were assayed within 2 h after collection of the sample supernatant fluids. Samples for lysozyme assay were frozen at −20°C and assayed on the following day. Total concentrations of these enzymes varied from one experiment to another; therefore, test results are calculated as a percentage of total activity released in vials containing 0.75 ml of WBCs and 0.75 ml of 0.4% Triton X-100 (final concentration, 0.2%).

Lysozyme was assayed by measuring the decrease in absorbancy at 450 nm of a suspension of *Micrococcus lysodeikticus* (0.15 mg/ml; Sigma Chemical Co.) in 0.1 M phosphate buffer, pH 6.2 (26, 42). Enzyme activities (micrograms per milliliter) were determined by comparison with a standard curve prepared with egg white lysozyme (Sigma). Lactate dehydrogenase was assayed by the method of Wacker et al. (38). One unit of activity was defined as an increase in absorbancy at 340 nm of 0.001/min per 0.02 ml of sample at 25°C.

β-glucuronidase was measured by the methods described by Brittinger et al. (5) and Hoffstein et al. (14), using phenolphthalein-glucuronic acid (Sigma) as the substrate. The amount of free phenolphthalein was measured at 550 nm, using a standard curve prepared with phenolphthalein at pH 10.4.

To test the possibility that *E. coli* was adsorbing and inactivating released β-glucuronidase, the following experiment was done. WBC β-glucuronidase was prepared in crude form by suspending PBS-washed WBCs in distilled water to a concentration of 7.2×10^6/ml and agitating the mixture to disrupt the cells. The cell fragments and the remaining intact WBCs were pelleted by centrifugation at 1,000 × *g* for 15 min at 5°C, and the supernatant fluid was used as the enzyme preparation. Several concentrations of *E. coli* 19+ were added to the enzyme preparation. A control suspension contained an equal amount of the enzyme preparation incubated with PBS. After incubation at 37°C for 60 min, the suspensions were centrifuged at 3,500 × *g* for 20 to 25 min, and the supernatant fluid was assayed for β-glucuronidase activity.

RESULTS

Association of *E. coli* 19. *E. coli* 19+ had much higher WBC association percentages than did 19− (Table 1). The addition of 50 mM αMM to the 19+ suspensions reduced the amount of WBC association to nearly that found for 19−. Glucose and sucrose at the same concentrations as αMM did not inhibit 19+ association. Glucose even appeared to enhance the association of 19+ cells with the WBCs.

Pretreatment of *E. coli* 19+. To partially characterize the *E. coli*-WBC interaction, the bacteria were pretreated with 1% formaldehyde or with heat before being assayed for WBC association (Table 2). Formaldehyde decreased *E. coli* association by about 20% as compared

TABLE 1. *Association of E. coli 19 with WBCs: effect of saccharides*[a]

E. coli	Addition	*E. coli*-WBC association (%)[b]	% Control
19+	PBS (control)	84.0 (78–90)	100
	αMM (50 mM)	7.0 (7–7)	8
	Dextrose (50 mM)	97.0 (95–99)	115
	Sucrose (50 mM)	79.5 (79–80)	95
19−	PBS	7.5 (5–10)	9

[a] WBCs on cover slips were added to suspensions of *E. coli* 19 (8×10^7 CFU/ml) in 0.04% BSA-PBS with and without added saccharides. Suspensions were incubated at 37°C with rotation for 15 min.

[b] Results are expressed as the percentage of 200 WBCs examined having two or more bacteria attached to or within the cytoplasmic periphery. Values in parentheses indicate range.

TABLE 2. *Association of E. coli 19 with WBCs: pretreatment of E. coli 19+ with formaldehyde and heat*[a]

Pretreatment[b]	*E. coli*-WBC association (%)[c]	Control (%)
None (control)	85.5 (78–93)	100
Formaldehyde	69.0 (56–82)	80.7
56°C, 60 min	64.5 (57–72)	75.4
75°C, 60 min	14.0 (7–21)	16.4
100°C, 15 min	1.5 (1–2)	1.8

[a] WBCs attached to cover slips were added to suspensions of pretreated *E. coli* 19+ (8×10^7/ml) in 0.04% BSA-PBS and incubated at 37°C with rotation for 15 min.

[b] *E. coli* 19+ were pretreated as described in the text.

[c] Results are expressed as the percentage of 200 WBCs examined having two or more bacteria attached to or within the cytoplasmic periphery. Data are from a single experiment tested in duplicate. Values in parentheses indicate range.

with untreated (control) 19+. Heating the bacteria at 56°C for 60 min decreased the association by about 25%, whereas heating at 75°C for 60 min or 100°C for 15 min decreased association by 83.6 and 98.2%, respectively. Viable *E. coli* were recovered after heating at 56°C, but no viable bacteria were found after pretreating with formaldehyde or heating at 75 or 100°C.

Pretreatment of WBCs with purified pili. Preliminary studies showed that pili in concentrations greater than 2 μg/ml caused complete agglutination of a suspension of WBCs (1×10^7/ml). The addition of αMM inhibited this agglutination, showing that the attachment of pili to the human WBCs is an MS reaction.

In an attempt to specifically block *E. coli* 19+ association, the WBCs on cover slips were pretreated with purified pili (1,750 μg) from 19+ cultures. The association percentages for 19+ were similar for WBCs pretreated with either pili (97.7%) or PBS (99.6%).

Ingestion of *E. coli* 19+. Because the association experiment did not show whether the bacteria were ingested or attached to the surface of the WBCs, the following experiment was done. WBCs on cover slips were incubated with suspensions of *E. coli* 19+ at 37°C, a temperature at which phagocytosis occurs readily, and at 4°C, a temperature at which phagocytosis is sluggish. After incubation, the cover slips were washed in PBS to remove nonadherent bacteria or in PBS containing 50 mM αMM to remove nonadherent bacteria as well as bacteria attached to the external surfaces of the WBCs (24). Thus, after washing in αMM-PBS, only bacteria which were internalized would be seen on the stained preparations.

The results of this experiment are presented in Table 3. *E. coli* 19+ associated with the WBCs slightly better at 37°C (94.0%) than at 4°C (80.7%). If the WBCs incubated with 19+ at 37°C were then washed in PBS with αMM, there was only a small decrease in the bacterial association percentage. However, if the WBCs incubated with 19+ at 4°C were washed in PBS with αMM, there was a larger decrease in the association percentage of 19+ (about 20%). The results show that after incubation at 4°C, more *E. coli* were detached from the surface of the WBCs as compared with *E. coli* incubated at 37°C. After incubation at 37°C, it appears the bacteria were ingested and were insensitive to the αMM displacement.

***E. coli* survival.** Knowing that 19+ associate with WBCs both in suspension and on glass cover slips, we determined the survival of the bacteria during incubation with WBCs. Bacterial survival was assayed by removing portions of the *E. coli*-WBC mixture at different times and adding these portions to water blank tubes. The tubes were shaken vigorously to disrupt the WBCs and release ingested bacteria. Thus, in these assays, both the viable extracellular and intracellular bacteria were measured.

Figure 1 shows the results of a survival assay

TABLE 3. *Association of E. coli 19+ with WBCs: incubation at 4°C and 37°C*[a]

Incubation temp (°C)	Post-incubation wash[b]	*E. coli*-WBC association (%)[c]	Total WBC counted
37	PBS	94.0 (94.0–94.1)	219
	αMM	90.6 (88.4–92.7)	262
4	PBS	80.7 (80.0–81.3)	228
	αMM	59.6 (52.0–67.3)	204

[a] WBCs on cover slips added to suspensions of *E. coli* 19+ (1.5×10^8 CFU/ml) in 0.04% BSA-PBS and incubated at the desired temperature for 15 min.

[b] After incubation, cover slips were washed with either cold PBS or cold PBS containing 50 mM αMM.

[c] Results are expressed as the percentage of WBCs examined having two or more bacteria attached to or within the cytoplasmic periphery. Data were obtained from a single experiment tested in duplicate. Values in parentheses indicate range.

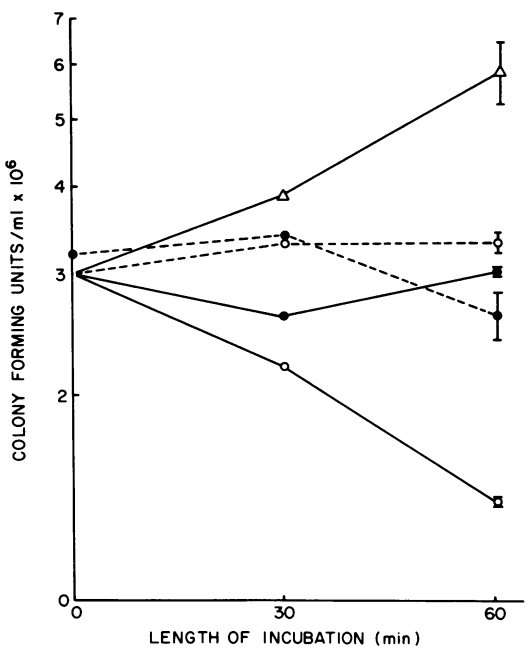

FIG. 1. *Survival of E. coli 19 during incubation with suspensions of WBCs. E. coli 19 (1.8×10^7 CFU) were added to vials with (——) and without (- - -) WBCs (7.5×10^6). Each point represents the average total colony-forming units (both intra- and extracellular) in duplicate test vials. The range of colony-forming units in the vials at 60 min is indicated. Symbols: 19+ (○); 19– (●); 19+ with 10 mM αMM (△).*

using *E. coli* 19+ and 19− as test organisms. Similar results were obtained from other *E. coli* strains (Bam+, 30+, 38−, 47−; data not shown). The number of viable 19+ as well as Bam+ and 30+ decreased approximately 50% over the 60-min period, whereas counts of viable 19− (38−, 47−) remained nearly the same as controls containing 19+ or 19− without WBCs. The addition of 10 mM αMM to the suspensions of 19+ abolished the killing of these bacteria. The number of viable 19+ cells in control suspensions containing 10 mM αMM without WBC increased from 3.0×10^6 CFU/ml to 3.3×10^6 CFU/ml during the 60-min incubation (data not shown). The data show that 19+ cells associated with and were killed by WBCs, and that this interaction between bacteria and WBCs was prevented by αMM.

Lysosomal enzyme release. Lysozyme and β-glucuronidase were used as markers of WBC lysosomal enzyme release. Lactate dehydrogenase, a cytoplasmic enzyme, was used to assess WBC damage.

Figure 2 shows the release of lysozyme from WBCs incubated with *E. coli* 19+ and 19−. At a bacteria-to-WBC ratio of 200:1, 19+ caused release of 38.9% of the total enzyme. An equal number of 19− released 12.4%, which was slightly above the amount of lysozyme released by nonstimulated (PBS) control WBCs (7.2%). Lysozyme release by 19+ was reduced by the addition of 25 mM αMM but not by 25 mM dextrose. The addition of 50 mM αMM reduced the release of lysozyme from 19+-exposed WBCs to approximately the same amount released by live 19+.

The release of enzymes from WBCs incubated with various numbers of 19+ is illustrated in Fig. 3. At each bacteria-to-WBC ratio, the amount of lysozyme (percent total) released was greater than the amount of lactate dehydrogenase or β-glucuronidase released. A ratio of 10 19+ cells per WBC released more enzyme (23%) than did 200 19− cells per WBC (19.5%), thus demonstrating the enhanced ability of 19+ cells to release lysozyme compared to 19− cells. Adding

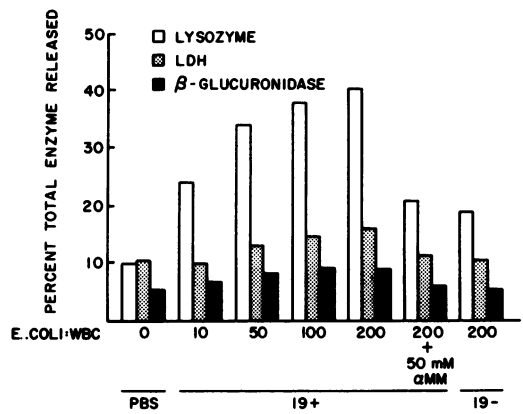

FIG. 3. *Extracellular release of WBC enzymes after incubation with various numbers of E. coli 19 per WBC. Vials were incubated at 37°C for 60 min. Total enzyme released by 0.2% Triton X-100: lysozyme = 13.2 to 22.3 μg of egg white lysozyme equivalents per 5 × 10⁶ WBCs; β-glucuronidase = 270 to 324 μg of phenolphthalein released per 18 h per 5 × 10⁶ WBCs; lactate dehydrogenase = 548 to 590 U/5 × 10⁶ WBCs. Results are reported as the mean value of two experiments, with each test performed in duplicate.*

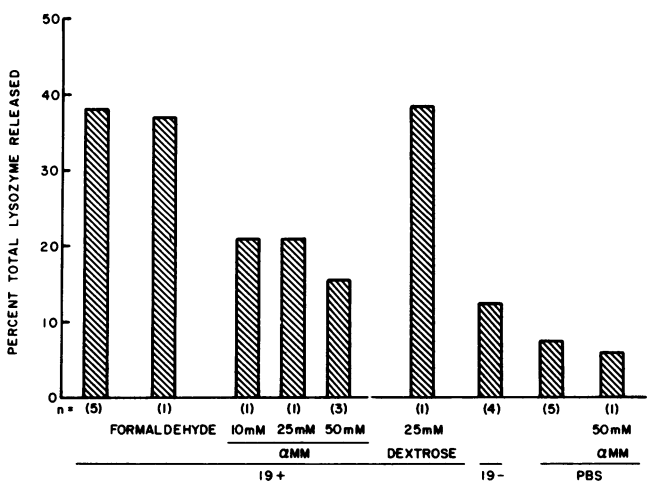

FIG. 2. *Extracellular release of lysozyme from WBCs after incubation with E. coli 19. Vials were incubated at 37°C for 60 min. Bacteria-to-WBC ratio = 200:1. Total lysozyme released by 0.2% Triton X-100 = 16.5 to 27.7 μg of egg white lysozyme equivalents per 5 × 10⁶ WBCs. Results reported as the mean value of n experiments, with each test performed in duplicate.*

increased numbers of 19+ per WBC resulted in only a small increase in released lactate dehydrogenase and β-glucuronidase. The addition of 50 mM αMM to the test suspension containing 200 19+ cells per WBC reduced the release of enzymes to approximately the same amounts released by 19−.

To test the possibility that 19+ was adsorbing or inactivating β-glucuronidase, *E. coli* 19+ were added to a cell-free preparation of WBC enzymes and incubated for 60 min at 37°C. After incubation, the bacteria were pelleted, and the supernatant was assayed for β-glucuronidase activity. At a concentration of *E. coli* 19+ equivalent to 250 bacteria per WBC, β-glucuronidase activity decreased by 16% compared with the PBS control. At ratios of 50 and 25 *E. coli* per WBC, there were decreases of 3 and 0%, respectively, in β-glucuronidase activity.

In an attempt to determine the role of pili in WBC lysosomal enzyme release, purified pili from *E. coli* 19+ were added to suspensions of WBCs and incubated for 60 min at 37°C. Pili, in concentrations of up to 133 μg/ml, failed to induce the release of lysozyme, lactate dehydrogenase, or β-glucuronidase.

DISCUSSION

The results of these experiments show that in the absence of serum opsonins, *E. coli*, which hemagglutinate guinea pig RBCs and possess type 1 pili (P), associated with human WBCs to a greater extent than *E. coli* cells from the same strain which lack HA activity and pili. Furthermore, the HA+, P+ *E. coli* are killed during the interaction with the WBCs, probably as a result of ingestion by the WBCs. This report supports and extends the work of Silverblatt et al. (29), which shows that piliated *E. coli* are more susceptible to phagocytosis than the same *E. coli* after removal of their pili by ultraviolet irradiation. In our experiments, as in those of Silverblatt, D-mannose (or its derivative, αMM) inhibited bacterial adherence to the WBCs, suggesting an MS association. *E. coli* are reported to possess two MS adherence factors: type 1 pili (3, 4, 27, 28) and the recently isolated MS lectin (12). Though type 1 pili and the MS lectin may actually be the same (23), differences in the amino acid composition and molecular weight of the subunits suggest that they are different (12). Thus, the identity of the *E. coli* MS adhesin which mediates adherence to WBC has yet to be determined.

We have obtained some evidence which suggests that type 1 pili do not mediate attachment of the *E. coli* to WBCs. Our attempts to block *E. coli* 19+ association with WBC by pretreating the WBC with purified pili from 19+ were unsuccessful. Similar pretreatment of epithelial cells with purified pili inhibited the attachment of homologously piliated *E. coli* to these cells (18).

Bacteria with two adhesins, one which mediates attachment to epithelial cells and another which mediates association with WBCs, have been reported in the literature. *Neisseria gonorrhoeae* possess pili (8) which are involved in the adherence of *N. gonorrhoeae* to epithelial cells. In addition, *N. gonorrhoeae* have a leukocyte association (LA) factor which is involved in the attachment of *N. gonorrhoeae* to WBCs (32). A similar factor may exist on *E. coli* 19+ cells (the MS lectin?), but identification will have to await the isolation of a strain of *E. coli* lacking type 1 pili (*pil*) but retaining the ability to associate with WBCs (LA+).

The microbicidal activity against *E. coli* 19+ appears to be the result of the interaction of 19+ with the WBCs. The possibility exists, however, that *E. coli* 19− failed to associate with the WBCs and be killed because of a capsule (K antigen) or another antiphagocytic substance (16, 17). However, since K antigen formation is favored at 37°C (13), it would be expected that *E. coli* 19+, which was cultured at 37°C, would possess the largest capsule and therefore be resistant to phagocytosis. Since 19+ readily associated with the WBCs, it is doubtful that the differences in WBC association between 19+ and 19− cells are due to capsule formation.

In the present study, we found that HA+, P+ *E. coli* were able to trigger lysosomal enzyme release from the WBCs in the absence of serum. The magnitude of enzyme released was directly related to the ratio of bacteria to WBCs. The appearance of the enzymes was not a result of bacteria-induced WBC damage, since lactate dehydrogenase was not released. In addition, bacterial viability was not required for the discharge of lysosomal enzymes from WBCs; formaldehyde-killed *E. coli* 19+ stimulated the release of equal amounts of lysozyme as did viable *E. coli* 19+.

Our study shows that *E. coli* 19+ stimulated a large release of WBC lysozyme, an enzyme found in both primary and secondary granules (31), but only a small release of β-glucuronidase, a primary granule enzyme (31). This suggests that mainly secondary granule enzymes are released during WBC association with 19+. This was a surprising result, since enzymes from both primary and secondary granules are typically released into the extracellular media during interaction of phagocytes with bacteria (7, 34, 35, 41). The release of enzymes from only secondary (specific) granules has been reported, but only for noningestible substances, referred to as in-

complete stimuli (39), such as human leukocytic pyrogen (19), phorbol myristate acetate (40), the calcium ionophore A23187 (40), and concanavalin A (ConA) (15).

The inability of 19+ to stimulate β-glucuronidase release may, nevertheless, be more apparent than real. *E. coli* 19+, mixed with a crude preparation of β-glucuronidase obtained from WBCs by hypotonic lysis, caused a decrease in the activity of this enzyme after a 60-min incubation at 37°C. Thus, the low amount of β-glucuronidase in the cell-free supernatant may result from an adsorption or inactivation of released enzyme by 19+.

Type 1 pili and ConA, a plant lectin, agglutinate the same species of cells and the cell agglutination (20, 25, 27) is inhibited by D-mannose. This suggests that receptors for type 1 pili and ConA are, at least in part, related. Evidence for the sharing of a common receptor is also provided by the fact that ConA inhibits agglutination of guinea pig RBCs by type 1 pili (27) and phagocytosis of type 1 piliated *E. coli* by human WBCs (29). Therefore, since ConA is known to cause a release of specific granule enzymes from human WBCs (15), it was expected that purified pili would also cause a release of these enzymes. Isolated pili, however, were unable to stimulate release of lysosomal enzymes from the WBCs. The reason for this is unclear.

Many of the biological activities induced by ConA are associated with the ability of this multivalent lectin to cross-link receptors on the WBC cell surface (8, 10). Similarly, pili on *E. coli* cells may simultaneously bind to several receptors in one small area of the WBC surface and form a receptor patch. Isolated pili may, however, fail to adhere to the WBC in a manner which forms the necessary clustering of receptors needed to stimulate lysosomal enzyme release.

In conclusion, the studies reported in this paper were designed to provide information regarding the interaction of *E. coli* with human phagocytes in a simple medium (PBS) and in the absence of serum. The role of the MS adhesins in the pathogenicity of *E. coli* in vivo is only speculative at this time and must await further studies. For now, however, the *E. coli*-WBC interaction system, because of its reversible nature with αMM, provides investigators with a useful tool with which to probe the complex cascade of events that takes place during phagocytosis.

ACKNOWLEDGMENTS

This work was supported in part by Biomedical Research Support grant SO7 RR 05433 17 and by the West Virginia University Medical Corporation.

We thank Robert Burrell for reviewing this manuscript.

LITERATURE CITED

1. **Bar-Shavit, Z., I. Ofek, R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. Biochem. Biophys. Res. Commun. **78**:455–460.
2. **Böyum, A.** 1968. Isolation of leukocytes from human blood: further observations. Methylcellulose, dextran, and ficoll as erythrocyte-aggregating agents. Scand. J. Clin. Lab Invest. **21**(Suppl. 97):31–50.
3. **Brinton, C. C., Jr.** 1965. The structure, function, synthesis, and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans. N.Y. Acad. Sci. **27**:1003–1054.
4. **Brinton, C. C., Jr.** 1967. Contributions of pili to the specificity of the bacterial surface, and a unitary hypothesis of conjugal infectious heredity, p. 37–70. *In* B. D. Davis and L. Warren (ed.), The specificity of cell surfaces. Prentice-Hall, Inc., Englewood Cliffs, N.J.
5. **Brittinger, G., R. Hirschhorn, S. D. Douglas, and G. Weissmann.** 1968. Studies on lysosomes. XI. Characterization of a hydrolase-rich fraction from human lymphocytes. J. Cell Biol. **37**:394–411.
6. **Clark, R. A., and H. R. Kimball.** 1971. Defective granulocyte chemotaxis in the Chediak-Higashi syndrome. J. Clin. Invest. **50**:2645–2652.
7. **Crowder, J. G., R. R. Martin, and A. White.** 1969. Release of histamine and lysosomal enzymes by human leukocytes during phagocytosis of staphylococci. J. Lab. Clin. Med. **74**:436–444.
8. **Cunningham, B. A., B. A. Sela, I. Yahara, and G. M. Edelman.** 1976. Structure and activities of lymphocyte mitogens, p. 13–30. *In* J. J. Openheim and D. L. Rosenstreich (ed.), Mitogens in immunobiology. Academic Press Inc., New York.
9. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. **74**:397–411.
10. **Edelman, G. M., B. A. Cunningham, G. N. Reeke, Jr., J. W. Becker, M. J. Waxdal, and J. L. Wang.** 1972. The covalent and three-dimensional structure of concanavalin A. Proc. Natl. Acad. Sci. U.S.A. **69**:2580–2584.
11. **Edwards, P. R., and W. H. Ewing.** 1972. Differentiation of Enterobacteriaceae by biochemical reactions, p. 21–47. *In* Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
12. **Eshdat, Y., I. Ofek, Y. Yashour-Gan, N. Sharon, and D. Mirelman.** 1978. Isolation of a mannose-specific lectin from *Escherichia coli* and its role in the adherence of the bacteria to epithelial cells. Biochem. Biophys. Res. Commun. **85**:1551–1559.
13. **Glynn, A. A., and C. J. Howard.** 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. Immunology **18**:331–346.
14. **Hoffstein, S., I. M. Goldstein, and G. Weissmann.** 1977. Role of microtubule assembly in lysosomal enzyme secretion from human polymorphonuclear leukocytes. A reevaluation. J. Cell Biol. **73**:242–256.
15. **Hoffstein, S., R. Soberman, I. Goldstein, and G. Weissmann.** 1976. Concanavalin A induces microtubule assembly and specific granule discharge in human polymorphonuclear leukocytes. J. Cell Biol. **68**:781–787.
16. **Howard, C. J., and A. A. Glynn.** 1971. Some physical properties of K antigens of *Escherichia coli* related to their biological activity. Infect. Immun. **4**:6–11.
17. **Howard, C. J., and A. A. Glynn.** 1971. The virulence for mice of strains of *Escherichia coli* related to the effects of K antigens on their resistance to phagocytosis and killing by complement. Immunology **20**:767–777.
18. **Isaacson, R. E., P. C. Fusco, C. C. Brinton, and H. W. Moon.** 1978. In vitro adhesion of *Escherichia coli* to

porcine small intestinal epithelial cells: pili as adhesive factors. Infect. Immun. **21**:392–397.
19. **Klempner, M. S., C. A. Dinarello, and J. I. Gallin.** 1978. Human leukocytic pyrogen induces release of specific granule contents from human neutrophils. J. Clin. Invest. **61**:1330–1336.
20. **Lis, H., and N. Sharon.** 1973. The biochemistry of plant lectins (phytohemagglutinins). Annu. Rev. Biochem. **42**:541–574.
21. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
22. **Novotny, C., W. S. Knight, and C. C. Brinton, Jr.** 1968. Inhibition of bacterial conjugation by ribonucleic acid and deoxyribonucleic acid male-specific bacteriophages. J. Bacteriol. **95**:314–326.
23. **Ofek, I., E. H. Beachey, and N. Sharon.** 1978. Surface sugars of animal cells as determinants of recognition in bacterial adherence. Trends Biochem. Sci. **3**:159–160.
24. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
25. **Old, D. C.** 1972. Inhibition of the interaction between fimbrial hemagglutinins and erythrocytes by D-mannose and other carbohydrates. J. Gen. Microbiol. **71**: 149–157.
26. **Parry, R. M., Jr., R. C. Chandan, and K. M. Shahan.** 1965. A rapid and sensitive assay of muramidase. Proc. Soc. Exp. Biol. Med. **119**:384–386.
27. **Salit, I. E., and E. C. Gotschlich.** 1977. Hemagglutination by purified type 1 *Escherichia coli* pili. J. Exp. Med. **146**:1169–1181.
28. **Salit, I. E., and E. C. Gotschlich.** 1977. Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. **146**:1182–1194.
29. **Silverblatt, F. J., J. S. Dreyer, and S. Schauer.** 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. Infect. Immun. **24**:218–223.
30. **Silverblatt, F. J., and I. Ofek.** 1978. Influence of pili on the virulence of *Proteus mirabilis* in experimental pyelonephritis, p. 49–59. *In* E. Kass and W. Brumfitt (ed.), Infection of the urinary tract. Proceedings of the Third International Symposium on Pyelonephritis, London. Chicago University Press, Chicago.
31. **Spitznagel, J. K., F. G. Dalldorf, M. S. Leffell, J. D. Folds, I. R. H. Welsh, M. H. Cooney, and L. E. Martin.** 1974. Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab. Invest. **30**:774–785.
32. **Swanson, J., E. Sparks, D. Young, and G. King.** 1975. Studies on gonococcus infection. X. Pili and leukocyte association factor as mediators of interactions between gonococci and eukaryotic cells in vitro. Infect. Immun. **11**:1352–1361.
33. **Swanson, J., E. Sparks, B. Zeligs, M. A. Siam, and C. Parrott.** 1974. Studies on gonococcus infection. V. Observations on *in vitro* interaction of gonococci and human neutrophils. Infect. Immun. **10**:633–644.
34. **Taichman, N. S., and W. P. McArthur.** 1976. Interaction of inflammatory cells and oral bacteria: release of lysosomal hydrolases from rabbit polymorphonuclear leukocytes exposed to gram-positive plaque bacteria. Arch. Oral Biol. **21**:257–263.
35. **Tew, J. G., W. M. Hess, and D. M. Donaldson.** 1969. Lysozyme and β-lysin release stimulated by antigen-antibody complexes and bacteria. J. Immunol. **102**:743–750.
36. **Van Oss, C. J.** 1978. Phagocytosis as a surface phenomenon. Annu. Rev. Microbiol. **32**:19–39.
37. **Van Oss, C. J., and C. F. Gillman.** 1972. Phagocytosis as a surface phenomenon. I. Contact angles and phagocytosis of non-opsonized bacteria. RES J. Reticuloendothel. Soc. **12**:283–292.
38. **Wacker, W. E. C., D. D. Ulmer, and B. L. Vallee.** 1956. Metalloenzymes and myocardial infarction. II. Malic and lactic dehydrogenase activities and zinc concentrations in serum. N. Engl. J. Med. **255**:449–456.
39. **Weissmann, G.** 1978. Leukocytes as secretory organs of inflammation. Hosp. Pract. **13**:53–62.
40. **Wright, D. G., D. A. Bralove, and J. I. Gallen.** 1977. The differential mobilization of human neutrophil granules. Am. J. Pathol. **87**:273–284.
41. **Wright, D. G., and S. E. Malawista.** 1972. The mobilization and extracellular release of granular enzymes from human leukocytes during phagocytosis. J. Cell Biol. **53**:788–797.
42. **Worthington Biochemical Corp.** 1972. Lysozyme, p. 100–101. *In* Worthington enzyme manual. Worthington Biochemical Corp., Freehold, N.J.