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Role of cloned virulence factors (mannose-resistant haemagglutination, mannose-resistant adhesins) from uropathogenic *Escherichia coli* strains in the release of inflammatory mediators from neutrophils and mast cells

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SUMMARY

Genetically cloned *E. coli* strains expressing cloned virulence factors were studied with regard to their capability to induce inflammatory mediator release from various target cells. Among the strains were *E. coli* strains with mannose-resistant haemagglutination (MRH$^+$) and mannose-resistant adhesins, e.g. *E. coli* 536/21 pANN 801/4, *E. coli* 536/21 pANN 921 and *E. coli* 536/21 pANN 801-1. In comparison, *E. coli* 536/21, *E. coli* 536/21 pGB 30 int and *E. coli* K12, without and with mannose-sensitive haemagglutination (MSH$^\pm$), and adhesins were studied. The properties of the various strains for human PMN with regard to adherence and phagocytosis, chemiluminescence, 5-lipoxygenase activation of arachidonic acid, leukotriene formation, granular enzyme release and release of histamine from rat mast cells were analysed. It is evident that the various biochemical processes of cell activation are dissociated events. The highest chemiluminescence response is obtained with strains expressing MSH$^+$, P-MRH$^+$ or S-MRH$^+$; the presence of S-adhesins suppressed the response. Highest leukotriene formation is obtained with *E. coli* 536/21 pANN 801-4, while *E. coli* with MSH was inactive. The concomitant presence of haemolysin secretion enhanced mediator release significantly. Our data suggest a potent role for mannose-resistant haemagglutination (MRH), adhesins and haemolysin as virulence factors in inducing the release of inflammatory mediators.

INTRODUCTION

Escherichia coli causes, in more than 80%, urinary tract infections (UTI). Several characteristics of *E. coli* contribute to urinary tract virulence, including their ability to adhere to urinary tract epithelial cells, their serum resistance and haemolysin production (Kallenius *et al.*, 1980; Marré *et al.*, 1986; Vaisänen *et al.*, 1981, 1984; Waalwijk, MacLaren & de Graaf, 1983). In addition, the expression of specific haemagglutination, and O- and K-antigens may contribute to the virulence of *E. coli* strains (Evans *et al.*, 1981; Kusecek *et al.*, 1984). In a variety of experimental models the interaction of bacteria with erythrocytes, epithelial cells and granulocytes was demonstrated (Björksten & Kaijser, 1978; Björksten & Wadström, 1982; Blumenstock & Jann, 1982; Mangan & Snyder, 1979; Perry, Ofek & Silverblad, 1983; Salit & Cotschlich, 1977; Svanborg-Eden *et al.*, 1984). *E. coli* strains that cause urinary tract infections bind to target cells via distinct adhesins and receptors. The adherence may or may not be inhibited by α-mannosides and is then termed mannose-sensitive (MSH) or mannose-resistant haemagglutination (MRH). Furthermore, the fimbriae can be subdivided according to their receptor requirements for adhesion. MS-fimbriae recognize manno-oligosaccharide-containing glycoproteins. MR-fimbriae can be subdivided according to their different receptor specificities: the P-fimbriae of urinary tract-infective *E. coli* strains recognize α-galactosyl-1,4-β-galactose as receptor (Parkinen *et al.*, 1983; Vaisänen-Rhen *et al.*, 1984). MRH factors which recognize a sialic acid-containing receptor have been termed S-fimbriae. Strains with this binding specificity occur more frequently in cases of newborn meningitis and sepsis. The mannose-sensitive or mannose-resistant hemagglutinating properties can reside on the outer membrane as well as on the fimbriae. Genetically cloned *E. coli* strains are a

Abbreviations: BHI, brain heart infusion; Fim, fimbriae; 5-HETE, 5-hydroxy eicosatetraenoic acid; Hly, haemolysin; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; LT, leukotrienes; MRH, mannose-resistant haemaglutination; MSH, mannose-sensitive haemagglutination; PBS, phosphate-buffered saline; PMN, polymorphonuclear neutrophils; RIA, radioimmunoassay; UTI, urinary tract infection.

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useful tool to understand the properties of the various adhesins (Hacker *et al.*, 1985). In addition to adhesins, the *E. coli* α-haemolysin, an extracellular protein which lyses erythrocytes of different species, is also isolated from patients with extra-intestinal infections such as urinary tract infections, bacteraemia and septicaemia (Cavalieri, Bohach & Snyder, 1984; Gadeberg, Orskov & Rhodes, 1983; Hacker *et al.*, 1983; Hughes *et al.*, 1982; Linggood & Ingram, 1982; Minishew *et al.*, 1978). Furthermore, we recently provided evidence that haemolysin by itself induces inflammatory mediator release from various cells. Therefore, in order to estimate the potency of the various adhesins as to mediator induction, a haemolysin-producing bacterial strain with defined genetical background was included as a well characterized activator of PMN and mast cells.

It is well established that granulocytes upon stimulation answer with the production of O_2 metabolites, the release of enzymes and arachidonic acid metabolites like 5-HETE, LTB_4 and LTC_4 (Bremm *et al.*, 1983; 1984a, b). Activation of mast cells is accompanied by the release of the preformed mediator histamine.

It was the purpose of our study to analyse genetically cloned *E. coli* strains expressing either mannose-sensitive haemagglutination and adhesins or mannose-resistant haemagglutination and adhesins with regard to mediator induction from human polymorphonuclear neutrophils and rat mast cells.

MATERIAL AND METHODS

Buffers

Unless stated otherwise, the medium used for washing the cells and for mediator release was phosphate-buffered saline (PBS) and contained 120 mM of NaCl/10 mM of Na_2PO_4 2 × H_2O/3 mM of KH_2PO_4 (pH 7·4). Bacterial growth was carried out in brain-heart-infusion broth (BHI, Oxoid, Wesel).

Preparation of cells

Human leucocytes were obtained from heparinized blood of healthy donors and separated on a Ficoll-metrizoate gradient followed by dextran sedimentation (Böyum, 1968). This method leads to >97% pure PMN.

Rat peritoneal cells were obtained from Wistar rats. The cell suspension contained 3–5% mast cells. The remainder cells are macrophages. The integrity of the cells after purification and mediator release in the presence of haemolysin was assessed by enzymatic (LDH) release as well as microscopic evaluation (König & Ishizaka, 1976).

Bacterial strains

Cloning and functional characterization of the plasmid-encoded determinants was performed at the Institut für Genetik und Mikrobiologie, Universität Würzburg. All mutants and transformants are derived from the uropathogenic *E. coli* strain 536 isolated from a patient with urinary tract infection (Berger *et al.*, 1982). Strain 536 exhibits a mannose-resistant, neuraminidase-sensitive, S-specific haemagglutination (MRH^+) and production of protein fimbriae (Fim^+). The strain is also serum resistant and produces haemolysin. Strain 536/21 is a spontaneous mutant of strain 536 that has lost the ability to produce haemolysin and the MRH phenotype. Table 1 summarizes the strains under study. Their molecular characteristics have been described elsewhere (Hacker *et al.*, 1985).

Table 1. Summary of strains under study

E. coli 536/21	Hly^- MSH^- MS-Fim^- MRH^- MR-Fim^-
E. coli 536/21 pGB30int	Hly^- MSH^+ MS-Fim^+ MRH^- MR-Fim^-
E. coli 536/21 pANN801-1	Hly^- MSH^- MS-Fim^- S-MRH^+ S-Fim^-
E. coli 536/21 pANN801-4	Hly^- MSH^- MS-Fim^- S-MRH^+ S-Fim^+
E. coli 536/21 pANN921	Hly^- MSH^- MS-Fim^- P-MRH^+ P-Fim^+
E. coli 536/21 pANN5311	Hly^+ MSH^- MS-Fim^- MRH^- MR-Fim^-

A haemolysin-producing strain (*E. coli* 536/21 pANN5311) without adhesins (MSH^-, MRH^-, Fim^-) was used throughout the experiments as a well-defined activator of mast cells and granulocytes. The cloning and functional characterization of this strain has been previously described (Berger *et al.*, 1982).

Bacterial growth

Brain–heart–infusion broth (10 ml) was inoculated with 100 μl of an overnight culture; bacterial growth proceeded for 3·5 hr at 37° on a shaker (150 r.p.m.) (Scheffer *et al.*, 1985).

Analysis of adherence and phagocytosis

This was performed as previously described (Scheffer *et al.*, 1985). In brief, [^{3}H]thymidine-labelled bacteria (50 μl) were added to 500 μl of PMN (1×10^7) and incubated at 37°. Adherent bacteria were removed from the granulocytes by incubating them on ice with Tris buffer (pH 7·35, 25 mM) containing NaCl (120 mM), KCl (4 mM) EDTA (40 mM and lysozyme (100 μg/ml).

Haemolysin assay

The production of haemolysin was analysed on sheep blood agar plates. A quantitative haemolysin assay was performed as described previously (König *et al.*, 1986).

Chemiluminescence

Chemiluminescence was measured at 37° in a Lumacounter M 2080 (Lumac, The Netherlands). Samples for chemiluminescence were obtained by adding a PMN suspension (50 μl, 106 cells) to polypropylene tubes containing PBS (300 μl) and luminol (20 μl, 0·25 mM) (Scheffer *et al.*, 1985).

Leukotriene release from human PMN

Human PMN (2×10^7) were suspended in 1 ml of PBS buffer. For stimulation, bacterial cell suspension (100 μl; 1×10^9 bacterial) was added to the cells and incubated as described in the Results. The supernatant of stimulated cells (1·000 μl) was analysed for leukotrienes by high-pressure liquid chromatography (HPLC) and radioimmunoassay (RIA).

Analysis of leukotriene release

For analysis of leukotriene release, the supernatants of the stimulated cells were deproteinized, evaporated to dryness and resuspended in 400 μl of methanol/water (30 : 70 v/v) for reverse phase HPLC (Köller *et al.*, 1985).

RIA for LTC_4 and LTB_4

In addition to HPLC analysis, the cell supernatants were studied by RIA for LTB_4 and LTC_4 (Aehringhaus *et al.*, 1982). The minimal quantities detected were approximately 20 pg for LTB_4. For the LTC_4 determination, the cross-reactivity with LTD_4 was

< 35%; for LTB_4 and LTE_4 the cross-reactivity was < 2%. The RIA for LTB_4 was obtained from Wellcome Diagnostics, Dartfort, Kent, U.K. The antiserum reacted with the isolated LTB_4 isomer. The correlation of the results obtained by HPLC analysis was determined to be $r = 0{\cdot}92 \pm 0{\cdot}1$ ($n = 6$) for LTC_4 and $r = 0{\cdot}85 \pm 0{\cdot}08$ ($n = 6$) for the LTB_4. The standard variation of the RIA was 7–11%.

Analysis of 5-lipoxygenase activity

The reaction mixture contained PMN (1×10^7) in PBS (500 μl), 26 mM $CaCl_2$ (50 μl), 3·7 kBq ^{14}C-arachidonic acid (50 μl of the stimulus (Stüning, Raulf & König, 1985). Radioactivity was detected with an Isomess Radio-Dünnschicht-analysator IM 3.000 (Isotopenmeßgeräte GmbH, Straubenhardt).

Determination of marker enzymes and protein

Lysozyme (EC 3.2.1.17), β-glucuronidase (EC 3.2.1.31) and lactate dehydrogenase (LDH) (EC 1.1.1.27) were determined as previously described (Stüning *et al.* 1985). Enzyme release was quantified as percentage of total activities from sonicated cells.

Histamine release

Rat peritoneal cells (5×10^6–1×10^7 total cells with 5–10% mast cells) were suspended in PBS buffer (500 μl) and 100 μl of bacterial suspension were added. Incubation proceeded for 60 min at 37°. Histamine content was analysed by the fluorophotometric analyser technique (König & Ishizaka, 1976). Cells in the presence of buffer and the bacterial supernatant at the appropriate dilutions served as controls. Viability of the cells before and after histamine release was assessed by LDH release and microscopic evaluation with toluidine blue. Standard variation of the histamine assay ranged between 3% and 5%.

Statistics

All the data were calculated as means ± SD and the significance was evaluated with Student's *t*-test for independent means.

RESULTS

Adherence and phagocytosis

The rate of adherence as well as the degree of phagocytosis was analysed. For this purpose, human PMN were incubated for 10 min with the radiolabelled *E. coli* strains listed in Table 1 (Fig. 1). As became apparent, *E. coli* 536/21 pANN 801-4 showed the highest degree of phagocytosis (16·0 ± 3·2%) and an adherence rate of 7·9 ± 1·9%, *E. coli* 536/21 pANN 801-1 demonstrated a phagocytosis rate of 15·4 ± 1·6% and an adherence rate of 0·3 ± 0·1%. In contrast *E. coli* 536/21 pANN 921(P-MRH$^+$, P-Fim$^+$) as well as *E. coli* 536/21 (MSH$^-$, MRH$^-$, Fim$^-$) showed significantly lower rates of phagocytosis (≈5%) and adherence (0·5 ± 1·4%) ($P < 0{\cdot}01$). A comparison of *E. coli* strains expressing MSH$^+$ and MS$^+$ adhesins, such as *E. coli* K12 and the genetically cloned *E. coli* 536/21 pGB 30 int, led to the following results: both strains showed a remarkably high phagocytosis (≈13%) and adherence rate (≈4%). These results suggest that, beside MSH$^+$, the S-MRH favours the induction of phagocytosis; the concomitant presence of S-adhesins also supports the adhesion to the cells ($P < 0{\cdot}01$).

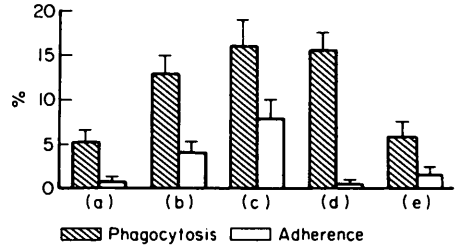

Figure 1. Adherence and phagocytosis of various genetically cloned *E. coli* strains. The experiment was carried out three times in triplicates. (a) *E. coli* 536/21; (b) *E. coli* 536/21 pGB 30 int; (c) *E. coli* 536/21 pANN 801-4; (d) *E. coli* 536/21 PANN801-1; (e) *E. coli* 536/21 pANN921.

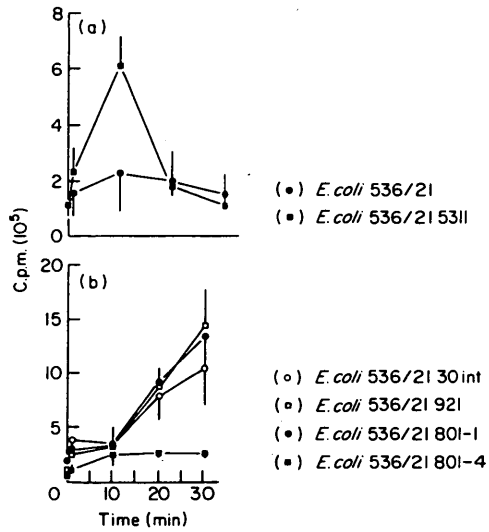

Figure 2. Kinetics of the chemiluminescence response by (a) mannose-sensitive and (b) mannose-resistant *E. coli* strains. Background level of the control, i.e. granulocytes in the absence of bacteria, was 1·02 × 105 c.p.m.

The haemolysin-producing strain (*E. coli* 536/21 pANN5311 40% haemolysis) showed a two- to three-fold higher adherence rate as compared to the isogenic haemolysin-negative strain (data not shown; $P < 0{\cdot}01$).

Studies on chemiluminescence

Experiments were carried out to study the induction of a chemiluminescence response by various *E. coli* strains. The incubation proceeded for 30 min. *E. coli* 536/21 (MRH$^-$, MSH$^-$, Fim$^-$) and *E. coli* 536/21 pANN 801-4 (S-MRH$^+$, S-Fim$^+$) induced a low to intermediate chemiluminescence response, while *E. coli* 536/21 pGB 30 int (MSH$^+$, MS-Fim$^+$), *E. coli* 536/21 pANN 801-1 (S-MRH$^+$, S-Fim$^-$) as well as *E. coli* 536/21 pANN 921 (P-MRH$^+$, P-Fim$^+$) were potent inducers of chemiluminescence ($P < 0{\cdot}01$) (Fig. 2). A steady increase was obtained over the time (30 min) analysed. The *E. coli* strain expressing S-MRH and S-adhesins was always low in the CL response, suggesting an inhibitory role for S-adhesins ($P < 0{\cdot}01$).

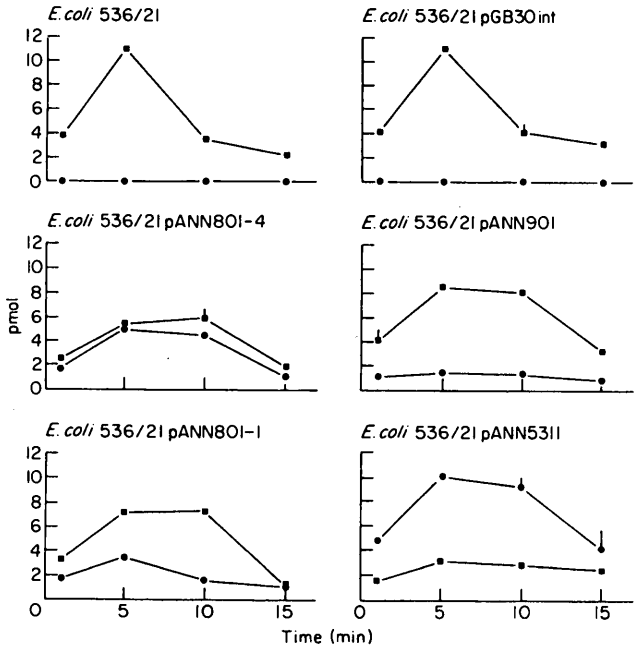

Figure 3. Kinetics of [^{14}C] arachidonic acid metabolism. Generation of 5-HETE ■ and LTB_4 ● by bacteria. Each value represents the mean of three independent experiments.

The experiment was performed with five different donor cells. Although the absolute height differed to a certain extent (±25%) the pattern was similar. The presented results show the values obtained from an individual donor cell.

The concomitant expression of haemolysin led to the following results: *E. coli* 536/21 pANN5311 (≈40% haemolysis) induced a chemiluminescence response which significantly exceeded that of haemolysin negative bacteria ($P < 0{\cdot}01$).

Release of mediators of inflammation

5-HETE and leukotrienes from human PMN. Human PMN (1×10^7) labelled with ^{14}C-arachidonic acid were incubated for various times with washed bacteria ($2{\cdot}5 \times 10^8$). The generation of 5-HETE and LTB_4 from exogenously added arachidonic acid was analysed. It is apparent that *E. coli* 536/21 (MSH^-, MRH^-, Fim^-) as well as *E. coli* 536/21 pGB 30 int (MSH^+, $MS-Fim^+$) only induced 5-HETE formation. A maximum was obtained after 5 min of incubation, which showed a decline after 10 and 15 min (Fig. 3). *E. coli* 536/21 pANN 921 ($P\text{-}MRH^+$, $P\text{-}Fim^+$) as well as *E. coli* 536/21 pANN 801-1 ($S\text{-}MRH^+$, $S\text{-}Fim^-$) leads, in addition to 5-HETE formation, to a significant generation of LTB_4 ($P < 0{\cdot}01$), which was less than the 5-HETE concentrations. In comparison, *E. coli* 536/21 pANN 801-4 ($S\text{-}MRH^+$, $S\text{-}Fim^+$), which also expressed mannose-resistant S-adhesins, released 5-HETE and LTB_4 to a similar degree, with a maximum after 5 and 10 min of incubation.

The haemolysin-producing strain *E. coli* 536/21 pANN5311 induced low amounts of 5-HETE and high amounts of LTB_4. LTB_4 formation after 5 min of stimulation exceeded by threefold the amounts obtained with haemolysin-negative bacteria ($P < 0{\cdot}01$). Further experiments were carried out to analyse the endogenous leukotriene generation. For this purpose human PMN (2×10^7/ml) were incubated with the washed bacterial

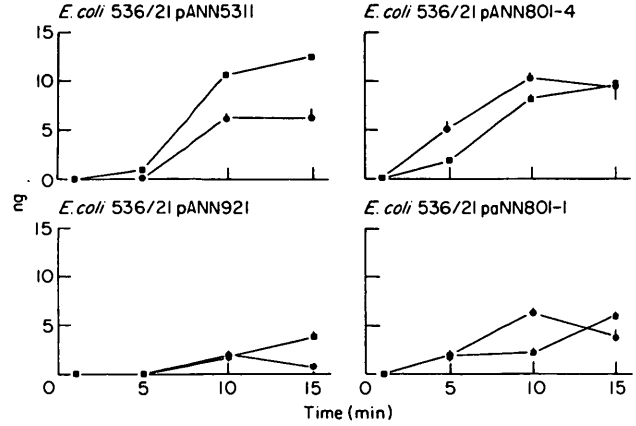

Figure 4. Kinetics of leukotriene release (LTB_4, ●, LTC_4, ■) from human PMN by adherent strains. The experiment was repeated four times in duplicates.

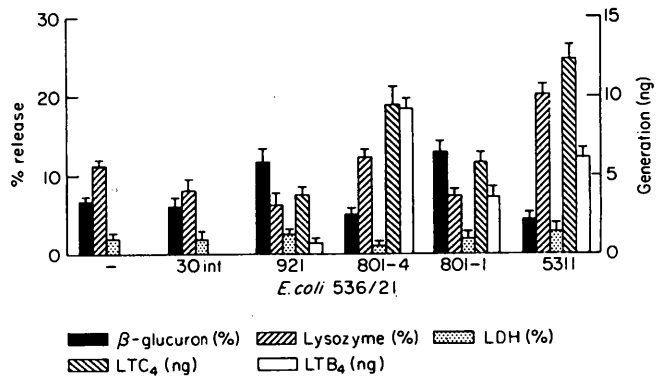

Figure 5. Enzyme and leukotriene release from human PMN by adherent *E. coli* strains. Leukotriene analysis was carried out by HPLC and by RIA (if values were lower than 2 ng). The experiments were carried out four times in duplicates.

strains. After various times (1, 5, 10, 15 min) the supernatant of the stimulated cells was analysed by HPLC or RIA if the values were lower than 2 ng. As is shown in Fig. 4, *E. coli* expressing S-mannose-resistant haemagglutination were significantly more active compared to *E. coli* expressing P-MRH ($P < 0{\cdot}01$). *E. coli* 536/21 pANN 801-4 ($S\text{-}MRh^+$, $S\text{-}Fim^+$) induced LTB_4 and LTC_4 release, which showed a maximum after 10 min with a negligible decline after 15 min. In comparison lesser amounts of LTB_4 and LTC_4 were induced by *E. coli* 536/21 pANN 801-1 ($S\text{-}MRH^+$, $S\text{-}Fim^-$) ($P < 0{\cdot}01$). *E. coli* 536/21 (MRH^-, MSH^-, Fim^-) and *E. coli* 536/21 pGB 30 int (MSH^+, $MS\text{-}Fim^+$) revealed neither LTC_4 nor LTB_4 release from endogenous arachidonic acid.

In contrast the haemolysin-producing strain *E. coli* 536/21 pANN5311 is a potent inducer for LTB_4 and LTC_4 ($P < 0{\cdot}01$). After 10–15 min of incubation with human PMN the amounts for LTB_4 ranged up to 10·5–12·4 ng and for LTC_4 up to 6·2 ng. Comparing the potency of the leukotriene-generating stimuli, it has been shown previously that the calcium ionophore A23187, at its optimal concentration ($7{\cdot}3 \times 10^{-6}$ M), induces LTB_4 and

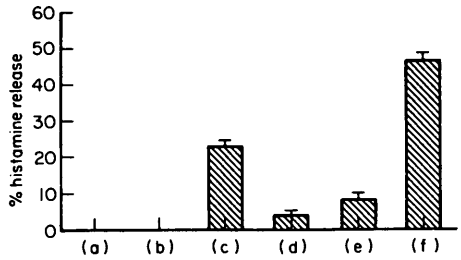

Figure 6. Histamine release from rat peritoneal mast cells by adherent *E. coli* strains. Histamine was quantified as the percentage of total activity. Histamine (100%) ranged between 495 and 520 ng/assay. (a) *E. coli* 536/21; (b) *E. coli* 536/21 pGB 30 unit; (c) *E. coli* 536/21 pANN801-4; (d) *E. coli* 536/21 pANN921; (e) *E. coli* 536/21 pANN801-1; (f) *E. coli* 536/21 pANN5311.

LTC_4 release up to 190 ± 26 and 53 ± 11 ng, opsonized zymosan (2 mg) for LTB_4 and LTC_4 up to $22{\cdot}1 \pm 0{\cdot}6$ and $2{\cdot}5 \pm 1{\cdot}0$ ng, FMLP ($1{\cdot}9 \times 10^{-5}$ M) for LTB_4 and LTC_4 up to $10{\cdot}2 \pm 5{\cdot}2$ and $1{\cdot}3 \pm 0{\cdot}4$, the α-haemolysin-containing bacterial supernatant for LTB_4 and LTC_4 up to $35{\cdot}2 \pm 5{\cdot}5$ and $6{\cdot}6 \pm 2{\cdot}6$, respectively. In this regard α-haemolysin-containing bacterial supernatants appear to be even more active as opsonized zymosan.

Release of granular constituents from human PMN and rat mast cells. Human PMN (2×10^7/ml) were incubated with the various washed *E. coli* strains (100 μl, 5×10^9/ml) for 15 min at 37°. It is evident that the bacterial strains induced lysozyme release from human PMN (Fig. 5). No direct evidence for adhesin induced cell triggering was observed, however. As far as histamine release is concerned, remarkable differences became apparent. Rat peritoneal cells (5–10% mast cells) were incubated with the various washed bacterial strains (5×10^8) for 60 min at 37°. It is apparent from Fig. 6 that *E. coli* 536/21 pGB 30 int (MSH$^+$, MS-Fim$^+$), *E. coli* 536/21 pANN 921 (P-MRH$^+$, P-Fim$^+$), *E. coli* 536/21 pANN 801-1 (S-MRH$^+$, S-Fim$^-$), and *E. coli* 536/21 pANN 801-4 (S-MRH$^+$, S-Fim$^+$) trigger cells for lysozyme secretion. *E. coli* 536/21 pANN 801–4 is a potent inducer of histamine (22·2%) from rat mast cells, unlike the other strains which have no or negligible effects ($P < 0{\cdot}01$). Beta-glucuronidase, a granular and cytosolic enzyme, was released in low amounts (12–13%) by *E. coli* 536/21 pANN 921 and *E. coli* 536/21 pANN 801–1; the other strains showed similar release rates as were obtained for the buffer control. As has been previously stated, *E. coli* 536/21 pANN 801-4 (S-MRH$^+$, S-Fim$^+$) was the most potent stimulus for release of granular constituents.

The haemolysin-producing strain *E. coli* 536/21 pANN5311 (MSH$^-$, MRH$^-$, Fim$^-$, Hly$^+$), unlike the haemolysin-negative strain *E. coli* 536/21, even released less β-glucoronidase from human granulocytes. No release of lactate dehydrogenase occurred from either mast cells or granulocytes.

DISCUSSION

The contribution of adhesin-carrying strains for various diseases has been described elsewhere (Björksten & Kaijser, 1978; Evans *et al.*, 1981; Kallenius *et al.*, 1980). It has been shown that 81% of the pyelonephritis-associated *E. coli* strains possess the P-specific recognition mechanism. The glycolipids which serve as receptors for P-specific fimbriae are present not only on erythrocytes but on many cell types, including the epithelium of the urinary tract (Machert & Kloch, 1980). In contrast, P-receptors are not present on rat tissue cells (Marre *et al.*, 1986). *E. coli* strains with S-MRH have also been preferentially suggested to induce newborn meningitis and sepsis in humans. But up to now the isolated interaction between different adhesins and cells has not been investigated.

Our data clearly demonstrate that *E. coli* strains with mannose-sensitive adhesins (MS-Fim) and haemagglutination properties (MSH), S-mannose-resistant adhesins (S-Fim) and S-haemagglutination properties, P-mannose-resistant adhesins (P-Fim) and P-mannose-resistant haemagglutination (P-MRH) differ in the induction of cellular responses.

It has been clearly established that granulocytes bear on their surface receptors for type I fimbriae, which explains the high phagocytosis and adherence rate of *E. coli* expressing MS-adhesins and MS-haemagglutination (*E. coli* 536/21 pGB 30 int). These pili are involved in enteroadherence. The strain showing mannose-resistant S-adhesin and S-haemagglutination revealed the highest phagocytosis and adherence rate. The deletion in S-adhesins reduced the adherence rate, which could be due to a quantitatively diminished adhesin receptor interaction. Thus, the distribution and amount of receptors appear to be decisive for microbial cell interaction.

Our results did not show a correlation of the chemiluminescence response with the binding of bacteria to PMN. The highest chemiluminescence response was obtained with the strains expressing MS-adhesins, MSH or S-MRH; the presence of mannose-resistant S-adhesins suppressed the response. Similarly, as with the above strains, *E. coli* with P-adhesins and P-MRH induced a high chemiluminescence response but slightly adhered to the surface of the PMN-membrane. One may suggest that in addition to adhesins the surface properties, such as hydrophobicity and charge of the bacterial outer membrane, may trigger granulocytes to an extent that a chemiluminescence response occurs (Robinson *et al.*, 1984). Differences in the distribution of receptor structures might also account for differences in inflammatory mediator release. No leukotrienes were generated from granulocytes after interaction with strains without any adhesins, with MS-adhesin or MSH. The strain expressing P-adhesin and P-MRH induced only slight amounts of leukotrienes. The isogenic strain with S-adhesins and S-MRH was the most potent stimulus for leukotriene formation. The deletion in S-adhesins reduced the capacity for inflammatory mediator release. The potency to release histamine from rat mast cells correlated well with the induction of leukotriene formation from PMN. Again, *E. coli* with S-adhesins and S-MRH was the most potent stimulus. The bacterial strains which were not able to induce leukotriene formation triggered the cells to a high 5-lipoxygenase activation. The effect of the various strains on 5-HETE and leukotriene formation may explain the differences as to the inflammatory response. 5-HETE is far less chemotactically active compared with LTB_4. These differences are not due to a differential arachidonic acid uptake in the presence of the different transformants.

Our results suggest that the chemiluminescence response, 5-lipoxygenase activation, leukotriene formation, histamine and lysosomal enzyme release are dissociated events which are controlled by defined or less defined receptor interactions; after cellular activation it appears that different and independent membrane biochemical mechanisms are initiated. The fact that the strain with S-adhesins and S-MRH is the most potent stimulus in leukotriene release could suggest that these strains are prone to induce a proinflammatory response which may be

beneficial or, if excessive, deleterious, such as in sepsis and meningitis.

The introduction of the haemolysin as a virulence factor into an adhesin-negative strain revealed that haemolysin by itself induced an increase in adherence, chemiluminescence and led to a high leukotriene formation. Thus, these results support the notion that the haemolysin is not merely a cellular toxin but rather an activator of various cells (König *et al.*, 1986; Scheffer *et al.*, 1985, 1988; Scheffer, Vosbeck & König, 1986). The fact that adhesins by themselves are able to trigger an inflammatory response may explain the pathophysiological prerequisites whereby bacteria may initiate defined disease processes such as pyelonephritis or meningitis. The concomitant release of haemolysin may then even potentiate the inflammatory response once the interaction of bacterial adhesin with tissue receptors has occurred.

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