Escherichia coli-Induced Activation of Neutrophil NADPH-Oxidase: Lipopolysaccharide and Formylated Peptides Act Synergistically To Induce Release of Reactive Oxygen Metabolites

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The prevailing view of neutrophil NADPH-oxidase activation during interaction with bacteria is that the production of toxic oxygen metabolites should be directed into the phagosome containing the engulfed prey. However, in this report we show that a common *Escherichia coli* strain, HB101, may induce a release of neutrophil oxygen metabolites to the extracellular milieu. This phenomenon is dependent on three factors: (i) the mobilization (upregulation) of neutrophil secretory vesicles prior to interaction with the bacteria, (ii) soluble bacterial factors binding to the formylmethionyl-leucyl-phenylalanine receptor and tentatively identified as formylated peptides, and (iii) a bacterium-associated priming factor identified as lipopolysaccharide.

Phagocytic cells (neutrophil granulocytes and monocytes/macrophages) form the front line in our defense against infection, playing an important role in eliminating invading bacteria (15). To fulfill this task, the phagocytes depend on their capacity to engulf the bacteria, to secrete inflammatory mediators, and to produce large amounts of reactive oxygen species following ingestion of the bacteria (19).

The tissue damage resulting from many bacterial infections is caused not only by bacterial virulence factors but also by the constituents of various host defense systems. The neutrophil bactericidal systems (i.e., lysosomal enzyme release and production of oxygen metabolites) that are activated upon challenge with a stimulus are toxic not only to bacteria but also to the surrounding host tissue (21). Under circumstances ideal for the host, the interaction between neutrophils and invading bacteria should result in bacterial killing with minimal damage to the host tissue. This is achieved by the phagocytic process whereby the bacterium is engulfed in an intracellular vacuole into which the lysosomal proteins and oxygen metabolites can be released. However, in cases not so favorable for the host, toxic oxygen metabolites may leak out to the surrounding tissue (25, 29).

Not only bacteria but also primary and secondary metabolites that are produced during bacterial growth can interact with the phagocytes (8, 17, 27). In contrast to neutrophil activation by particulate stimuli resulting in phagocytosis and intraphagosomal production of oxygen metabolites, the NADPH-oxidase activation induced by the bacterium-derived peptide and chemoattractant formylmethionyl-leucyl-phenylalanine (fMLP) is associated with a rapid and extensive extracellular release of reactive oxygen species (13). However, because neutrophil fMLP receptors are hidden, stored in the easily mobilized secretory vesicles (30), cells isolated from healthy individuals are hyporesponsive to fMLP (1, 11). In order for the peptide to induce a signal transduction cascade that activates the super-

oxide-generating NADPH-oxidase, the neutrophil secretory vesicles must first be mobilized to the cell surface.

In this study, we have investigated the consequences of the interaction between neutrophils and *Escherichia coli* cells with respect to the capacity of the bacteria and bacterial growth products to activate the neutrophil NADPH-oxidase and to induce an extracellular release of the oxygen metabolites produced.

MATERIALS AND METHODS

Isolation of phagocytic cells. Neutrophils were isolated from buffy coats obtained from apparently healthy adults. After dextran sedimentation at $1 \times g$, hypotonic lysis of the remaining erythrocytes, and centrifugation in a Ficoll-Paque gradient (6), the neutrophils were washed twice and resuspended in Krebs-Ringer phosphate buffer containing glucose (10 mM), Ca^{2+} (1 mM), and Mg^{2+} (1.5 mM) (KRG; pH 7.3). The cells were either stored on a melting ice bath (nonmobilized cells with respect to the secretory vesicles, hereafter referred to as control cells) or first incubated at 22°C for 60 min to mobilize their secretory vesicles to the cell surface (hereafter referred to as upregulated cells [1]). The cells were used within 120 min of preparation, and the viability was $\geq 95\%$.

Bacterial strains. The model bacterial strain used in this study, *E. coli* HB101, has been characterized in earlier studies (5). In addition, six clinical isolates of *E. coli* were used. These strains were obtained from patients suffering from urinary tract infections and were not further characterized.

Cultivation. The bacterial strains were grown on Luria agar plates for 16 to 18 h. The bacteria and bacterial growth products were harvested by addition of 3 ml phosphate-buffered saline (PBS; pH 7.3) to the plates followed by resuspension of the colonies and adjustment of the number of bacteria to 10^9 /ml. The bacteria were either used directly or washed once in PBS prior to adjustment of the cell number. To separate bacterial particles from soluble metabolites, the bacterial suspension was diluted to 10^9 /ml and centrifuged ($15,900 \times g$, 5 min). The supernatant was collected, and the bacteria were washed once and resuspended to 10^9 /ml.

Immunostaining and flow cytometry. The expression of complement receptor 3 (CR3) was measured by adding 10 μl of phycoerythrin-conjugated mouse monoclonal anti-CR3 antibody of immunoglobulin G2 class (Becton Dickinson, Mountain View, Calif.) to paraformaldehyde-fixed (4% in PBS; 5 min at 4°C) neutrophil pellets (around 10^6 cells). The cells were incubated at 4°C for 30 min, washed twice, and resuspended (1 ml) in PBS-EDTA (0.1 mM EDTA, 0.02% sodium azide) (26). The immunostained cells were examined in a flow cytometer (FACScan; Becton Dickinson) to obtain values for the absolute number of cells and the mean fluorescence intensity, complexity, and mean cell size of the cell population. The two cell populations had equally low autofluorescence, and there was no binding of a nonspecific phycoerythrin-conjugated antibody (data not shown).

Neutrophil respiratory burst activity. The NADPH-oxidase activity was measured by using a luminol-enhanced chemiluminescence (CL) system (12). The

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FIG. 1. Room temperature treatment upregulates CR3. The graph shows the expression of CR3 on the surface of control (left curve) and upregulated (right curve) neutrophils measured by immunostaining for CR3 and analyzed by flow cytometry. A representative experiment is shown. The means of the fluorescence values were 240 ± 158 ($n = 4$) and 458 ± 191 ($n = 4$) ($P < 0.001$; Student's *t* test) for the control and upregulated cell populations, respectively.

CL activity was measured in a six-channel Biolumat LB 9505 (Berthold Co., Wildbad, Germany), using disposable 4-ml polypropylene tubes. Samples (0.1 ml) were withdrawn from the neutrophil suspensions and added to the reaction mixture (0.8 ml; see below), and the tubes were placed in the Biolumat to equilibrate for 5 min at 37°C. The stimulus (0.1 ml) was added, and the light emission was recorded continuously. To quantify intracellularly and extracellularly generated reactive oxygen species, two different reaction mixtures were used. Tubes used for measurement of extracellular release of the reactive oxygen species contained KRG, neutrophils, azide (a low-molecular-weight myeloperoxidase inhibitor that gains access to intracellular compartments; 1 mM), horseradish peroxidase (HRP; an azide insensitive peroxidase; 4 U), and luminol (2×10^{-5} M). Azide alone totally inhibits the CL response, but because HRP is azide insensitive and a large molecule that has no access to intracellular sites, the CL activity is regenerated in the presence of HRP, provided that the metabolites produced are released from the cells. Tubes used for measurement of intracellular generation of reactive oxygen species contained KRG, neutrophils, superoxide dismutase (SOD; a membrane-impermeable scavenger of O_2^- ; 200 U), catalase (a membrane-impermeable scavenger of H_2O_2 ; 2,000 U), and luminol (2×10^{-5} M). In the presence of SOD and catalase, the extracellularly released O_2^- and H_2O_2 will be removed, thus providing a measurement of the remaining intracellular response (for a review, see reference 12).

Specific desensitization of fMLP-induced NADPH-oxidase activity. Upregulated neutrophils were preincubated at 15°C for 5 min, after which fMLP (10^{-7} M, final concentration) was added and the incubation was continued for another 10 min to allow fMLP to bind to the fMLP receptor. The cells were then transferred to a heated water bath (37°C) and incubated for 5 min to allow for the fMLP receptor-ligand complexes to be internalized. These desensitized cells with no exposed fMLP receptors were then used in the CL assay.

Coating of latex particles with LPS. Latex particles (0.81 μ m, 10^9 /ml in PBS) were incubated with lipopolysaccharide (LPS; 40 μ g/ml in PBS) at room temperature overnight. The particles were washed twice and resuspended to the original concentration in PBS. The LPS concentration on the latex particles was 1.2 μ g/ 10^9 particles.

Statistical analysis. Significance was defined by a two-sample *t* test (unpaired). *P* values exceeding 0.05 were considered not significant.

Reagents. fMLP, butoxy-carbonyl-methionyl-leucyl-phenylalanine (Boc-MLP), phorbol myristate acetate, polymyxin B, LPS (Sigma L2530), and luminol were obtained from Sigma Chemical Co. (St. Louis, Mo.). SOD, catalase, and HRP were purchased from Boehringer (Mannheim, Germany), and the cell separation chemicals (dextran and Ficoll-Paque) were obtained from Pharmacia

(Uppsala, Sweden). The latex particles were from Difco Laboratories (Detroit, Mich.).

RESULTS

The neutrophil secretory vesicles are mobilized to the cell surface during room temperature storage. The secretory vesicles that contain a pool of hidden receptors such as the fMLP receptor and CR3 (1, 30, 33) are by far the most mobilizable of the different neutrophil granule populations (32). To demonstrate the extreme sensitivity by which the neutrophils respond to environmental changes, the expression of CR3 on the surface of control and upregulated neutrophils was measured by immunostaining with anti-CR3-antibodies and examination by flow cytometry (Fig. 1). The expression of CR3 was pronounced on upregulated cells but was almost lacking on control cells, indicating that the upregulated neutrophils had indeed mobilized their secretory vesicles to the cell surface.

***E. coli* induces intracellular production of reactive oxygen metabolites in control neutrophils.** The metabolic responses induced in control neutrophils (kept at 4°C prior to stimulation to avoid receptor upregulation [4, 14, 34]) upon interaction with *E. coli* HB101 were monitored by a CL technique. A distinct metabolic response was obtained and was found to be of intracellular origin since it remained of the same magnitude upon extracellular addition of the scavengers SOD and catalase (Fig. 2). The response varied somewhat between different experiments, but in general the peak value was reached after 50 min, and the CL value in the peak was repeatedly found to be around 10^7 cpm.

Production of extracellular oxygen metabolites in response to *E. coli* requires the mobilization of neutrophil secretory vesicles. Two cell populations, control cells and upregulated cells (incubated at 22°C for 60 min), were allowed to interact with *E. coli* HB101, and the production of reactive oxygen metabolites was monitored. The two cell populations produced

FIG. 2. Activation of control neutrophils in response to *E. coli* HB101. The graph shows the time courses of the neutrophil CL responses induced by *E. coli* HB101. The increased CL is registered following stimulation with bacteria in the absence (solid line) and presence (dashed line) of SOD (50 U) and catalase (2,000 U). The bacteria were harvested in PBS directly added to a Luria agar plate and suspended to 10^9 /ml by addition of PBS. Control neutrophils (10^6 cells) were transferred to the Biolumat and were allowed to interact with the bacteria (10^8 bacteria).

FIG. 3. Activation of control and upregulated neutrophils in response to *E. coli* HB101. The graph shows the time courses of the neutrophil CL responses induced by *E. coli* HB101 (10^8 bacteria prepared as described in the legend to Fig. 1) in control (resting) and upregulated neutrophils (10^6 cells). The intracellular responses (a) were measured in the presence of the scavengers SOD (50 U) and catalase (2,000 U), while the extracellular responses (b) were measured in the presence of HRP (4 U) and azide (1 mM).

roughly the same amount of reactive oxygen species when the intracellular activity was measured (Fig. 3a). However, the upregulated neutrophils induced a strong extracellular release of reactive oxygen species, while the control cells released virtually no reactive oxygen metabolites (Fig. 3b). When phorbol myristate acetate (a potent inducer of extracellular release of reactive oxygen metabolites) or serum-opsonized zymosan (a potent inducer of an intracellular production of reactive oxygen species) was used as a control stimulus, no increased extracellular activity in primed neutrophils was observed (data not shown). These results show that the neutrophil receptors mediating the release of reactive oxygen species are localized in a subcellular compartment (probably the secretory vesicles) that is mobilized to the cell surface upon incubation of the neutrophils at room temperature.

Six different clinical *E. coli* isolates from patients suffering from urinary tract infections were investigated with respect to the ability to induce an extracellular release of reactive oxygen species similar to that induced by strain HB101. Two of the clinical isolates induced a neutrophil release of oxygen metabolites of the same magnitude as that induced by HB101, whereas no extracellular release was induced by the other four strains (Table 1).

The fMLP receptor mediates the neutrophil release of reactive oxygen species in response to *E. coli*. The chemotactic peptide fMLP induces a very rapid neutrophil response, similar in time course to the extracellular phase of the response induced by the *E. coli* HB101. Since the bacteria could be expected to produce formylated peptides, and since the neutrophil fMLP receptor is stored in the secretory vesicles, we decided to investigate if the fMLP receptor could be involved in the response induced by the bacteria. The activation of neutrophils by fMLP occurs through a receptor-operated guanine nucleotide-binding protein (G protein). Binding of the receptor-ligand complex to the cell cytoskeleton laterally segregates the receptor from the G protein, resulting in an inactivation of the occupied receptors and a termination of the fMLP-induced oxidative response (22, 23). Such cytoskeletal binding resulting in a specific desensitization is, for instance, induced when neutrophils are allowed to interact with fMLP at 15°C. Desensitization at 15°C occurs without any oxidative activation of the neutrophils. The neutrophil NADPH-oxidase response to PMA was not altered by this procedure (data not shown), indicating that the desensitizing process is specific for fMLP and not due to any direct effects on the NADPH-oxidase.

Upregulated neutrophils were downregulated with respect to their fMLP receptors as described above, and excess fMLP was removed by washing. Downregulation was visualized when these cells were once again exposed to fMLP (at 37°C), and the NADPH-oxidase activity measured was very low; i.e., the cells were desensitized to fMLP (Fig. 4, inset). When these desensitized cells were allowed to interact with bacteria (HB101), no release of reactive oxygen metabolites was detected (Fig. 4), indicating that the fMLP receptor is involved in the HB101-induced response. This conclusion is further supported by the fact that the bacterium-induced release of reactive oxygen species is inhibited by Boc-MLP (Fig. 5), a structural analog to fMLP which binds to the fMLP receptor and functions as a competitive antagonist (18).

A soluble factor(s) is required for the *E. coli* HB101-induced neutrophil release of reactive oxygen species. A number of different bacteria have been shown to produce N-formylated peptides (9, 10, 27, 28), and a release of formylated molecules from *E. coli* HB101 could be a plausible explanation of the neutrophil activation. In agreement, no activity was obtained with bacteria alone. When HB101 cells were pelleted by centrifugation, washed once, and then allowed to interact with the

TABLE 1. Interaction between neutrophils and six clinical *E. coli* isolates

Strain	Induction of release of oxygen metabolites ^a	fMLP desensitization ^b	Priming capacity ^c
838	—	NR ^d	+
916	+	Yes	+
970	+	Yes	+
977	—	NR	+
983	—	NR	+
986	—	NR	+

^a Ability of crude bacterial suspensions to induce release of reactive oxygen species in upregulated neutrophils. Bacteria were harvested in PBS from an agar plate and diluted in PBS.

^b Effect of desensitization with fMLP on the release of reactive oxygen metabolites. Neutrophils were desensitized to fMLP prior to stimulation with bacteria.

^c Capacity of washed bacteria to prime the response of upregulated neutrophils to fMLP.

^d NR, not relevant.

FIG. 4. Downregulation of the fMLP receptor inhibits the neutrophil response to the bacteria. The graphs show the time courses of the extracellular CL responses induced in upregulated neutrophils (10^6 cells) by *E. coli* HB101 (10^8 bacteria prepared as described in the legend to Fig. 1) or fMLP (10^{-7} M; inset). The neutrophils were incubated for 10 min at 15°C in the absence (solid line) or presence (dashed line) of fMLP (10^{-7} M), prewarmed for 5 min at 37°C , and then stimulated with bacteria or fMLP. The responses were measured in the presence of HRP (4 U) and azide (1 mM).

upregulated neutrophils, no release of oxygen metabolites was obtained. However, to our surprise, the bacterium-free supernatant could not stimulate the upregulated neutrophils either (Fig. 6). The stimulus was not destroyed by the centrifugation procedure, since the activity was restored when the washed bacteria were resuspended in the original supernatant (Fig. 6). Consequently, both the bacteria and some soluble bacterial factor(s) are required for the extracellular release of reactive oxygen species. The finding that the supernatant could be diluted 1/3 in a reconstitution assay with only a minor loss in activity suggests that the soluble factor(s) is present in excess (Fig. 7).

Substimulating doses of fMLP can replace the soluble factor(s). In upregulated neutrophils, no respiratory burst activity is induced by fMLP at a concentration of 10^{-9} M (or less). This concentration of fMLP could, however, replace the bacterial supernatant in a reconstitution experiment in which washed bacteria together with the substimulating dose of fMLP induced a release of reactive oxygen species (Fig. 8); i.e., the bacterial particle primes the neutrophil response to fMLP.

Clinical *E. coli* isolates could replace strain HB101 as the priming factor. Combining substimulating doses of fMLP with washed bacteria induced a neutrophil release of reactive oxygen species with all six clinical isolates studied, independent of the stimulating capacity of the original bacterial suspension (Table 1).

The priming effect is inhibited by polymyxin B. To investigate if the priming factor is bacterium-bound LPS, we tried to inhibit the bacterial priming of the fMLP response with polymyxin B. Polymyxin B inhibits biological effects of LPS by forming complexes with LPS (24). Bacteria were pretreated with polymyxin B (100 $\mu\text{g}/\text{ml}$) 30 min prior to addition of the bacteria together with fMLP (10^{-9} M). Polymyxin B readily inhibited the priming effect of the bacterial particles (Fig. 9), indicating that LPS is responsible for this effect.

When we replaced the bacterial particles with LPS-coated

latex beads, we could not demonstrate any priming capacity; i.e., substimulating doses of fMLP combined with LPS-coated latex beads gave no extracellular release of oxygen metabolites from upregulated neutrophils. This discrepancy may be due to symmetrical differences in LPS arrangement on the bacteria and the latex particle.

DISCUSSION

We interpret the results of this study to indicate that the ability of enterobacteria to induce an extracellular release of reactive oxygen species from human neutrophils is influenced by (i) the mobilization state of the neutrophil with respect to the secretory vesicles, (ii) bacterial production and release of formylated peptides, and (iii) a priming factor associated with the bacterium suggested to be LPS.

The existence of a very easily mobilized vesicle that could account for upregulation of different surface receptors has been suggested (1), but it was only recently that the alkaline phosphatase-containing granule subset (36) was identified as that organelle (3, 35). The presence in this organelle, the secretory vesicle, of receptor molecules (i.e., CR3 and fMLP receptor) and the extreme sensitivity with which the neutrophil responds to stimulus by mobilizing the secretory vesicles suggest that this organelle plays a primary role in regulating adhesion of neutrophils to endothelial cells during diapedesis. Indirect evidence obtained with exudated neutrophils supports this hypothesis (7, 16, 31). Since neutrophil secretory vesicles can be expected to be mobilized when phagocytes are attracted to sites of infection, our experimental system, in which phagocytes were induced to mobilize their vesicles before interaction with the bacteria, mimics an *in vivo* situation. We found that when we compared control neutrophils with neutrophils that had mobilized their secretory vesicles (i.e., upregulated neutrophils), there was a marked difference in response to the bacteria. Control neutrophils showed an exclusively intracellular response, while the neutrophils with mobilized secretory

FIG. 5. Boc-MLP inhibits the neutrophil response to the bacteria. The graphs show the time courses of the extracellular CL responses induced in upregulated neutrophils (10^6 cells) by *E. coli* HB101 (10^8 bacteria prepared as described in the legend to Fig. 1) or fMLP (10^{-7} M; inset) in the absence or presence of Boc-MLP (10^{-5} M). The responses were measured in the presence of HRP (4 U) and azide (1 mM).

FIG. 6. Separation (through centrifugation) of bacteria from a cell-free supernatant resulted in loss of stimulating capacity. The graphs show the time courses of the extracellular CL responses induced in upregulated neutrophils (10^6 cells) by *E. coli* HB101 (10^8 bacteria prepared as described in the legend to Fig. 1) before separation (top), by the bacteria and supernatant after separation (middle), and by a reconstituted system of bacteria and supernatant (bottom). The responses were measured in the presence of HRP (4 U) and azide (1 mM).

vesicles, in addition to the intracellular response, also exhibited a pronounced extracellular response corresponding to a release of reactive oxygen metabolites.

In order for an interaction between *E. coli* HB101 and human neutrophils to result in a release of reactive oxygen metabolites from the phagocyte, soluble bacterial factors were required. These were tentatively identified as formylated peptides on the basis of the findings: (i) the time course of the extracellular response induced by strain HB101 is very similar to that induced by fMLP; (ii) the fMLP receptors are stored in the secretory vesicles and are upregulated to the cell surface upon incubation at room temperature, which results in an enhanced extracellular oxidative response to fMLP, an effect also seen upon stimulation with HB101; (iii) desensitization of the fMLP receptor abolishes the HB101-induced activation; (iv) Boc-MLP, a competitive fMLP antagonist, inhibits the neutrophil response to HB101; and (v) fMLP can replace the soluble bacterial factor(s) in a reconstitution assay.

An interesting observation is that substimulating concentrations of fMLP (and the soluble bacterial factors) are turned into cell-activating concentrations only when the binding of fMLP to its neutrophil receptor occurs concomitant with a neutrophil-bacterium interaction. This finding suggests that the bacterial particle functions as a priming agent. From the results obtained with the clinical isolates, we conclude that the priming capacity of the bacterial particle is a general (or commonly occurring) character of *E. coli*. Priming has frequently been observed in many in vivo situations, and a number of different substances, including bacterial cell wall LPS, have been shown to induce in vitro priming in neutrophils (2, 16, 20). *E. coli* can be expected to produce (and expose) substantial amounts of LPS. When the bacterial particles were treated with polymyxin B, an inhibitor of the biological activities of

LPS, the priming capacity of the bacterial particles was lost. These results indicate that LPS is indeed responsible for priming the neutrophil response to fMLP. However, it should be mentioned that an alternative (although not very likely) expla-

FIG. 7. Time courses of the extracellular CL responses induced in upregulated neutrophils (10^6 cells) by *E. coli* HB101 (10^8 bacteria) separated (through centrifugation) from a cell-free supernatant and reconstituted in PBS (0%) or the supernatant undiluted (100%) or diluted in PBS to 50, 30, and 10%, as indicated. The responses were measured in the presence of HRP (4 U) and azide (1 mM).

FIG. 8. Subinhibitory concentrations of the chemotactic peptide fMLP can replace the soluble factor(s) in a reconstitution experiment. The graph shows the extracellular CL responses of upregulated neutrophils (10^6 cells) induced by *E. coli* HB101 alone (10^8 bacteria, washed and resuspended in PBS), by fMLP (10^{-9} M) alone, and by *E. coli* HB101 (10^8 bacteria) together with fMLP (10^{-9} M). The responses were measured in the presence of HRP (4 U) and azide (1 mM).

nation of the inhibition achieved with polymyxin B is that being a large molecule, polymyxin B may hide other bacterial surface structures with priming capacity. The immediate priming effect achieved with the bacterium-bound LPS differs considerably from that of soluble LPS, which requires an interaction time of around 30 min in order to induce a primed state in neutrophils

FIG. 9. Polymyxin B inhibits the priming effect of the bacterial particle. The graph shows the extracellular CL responses of upregulated neutrophils (10^6 cells) induced by *E. coli* HB101 (10^8 bacteria, washed and resuspended in PBS) together with fMLP (10^{-9} M). Prior to neutrophil activation, the bacteria were incubated in the presence (dashed line) or absence (solid line) of polymyxin B (PMB; 100 μ g/ml) for 30 min. The responses were measured in the presence of HRP (4 U) and azide (1 mM). Polymyxin B was without effect on the response induced by a stimulating concentration (10^{-7} M) of fMLP.

(20). Hence, there may be arrangement requirements for the LPS to function as priming agent. The fact that latex particles coated with LPS (presumably exposed in a nonorderly fashion) could not replace the bacterial particle somewhat supports this theory. It should be stressed that no phagocytosis occurred during the time period of the extracellular oxidative response, implying that the priming effect is independent of the phagocytic process.

In accordance with the belief that phagocyte responsiveness is of importance for tissue-destructive activities during infection, we have previously described differences between clinical bacterial isolates with respect to the ability to induce neutrophil release of reactive oxygen species (29). Since released oxygen species presumably may be toxic to the host, we suggested that this characteristic could be of importance in *E. coli* pathogenesis. However, the tissue-damaging capacity of the clinical *E. coli* isolates included in this study, with only the priming ability of the bacterial particle, compared with that of isolates with both this ability and the capacity to generate and release soluble factor(s) may only be speculated upon. However, future studies of host-parasite interactions, trying to correlate neutrophil activity with clinically relevant parameters, have to take into account not only direct activity induced by the bacteria but also production and release of soluble substances as well as priming activities and the responsiveness of the phagocytes.

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