Whole blood-mediated endothelial permeability and adhesion molecule expression: a model study into the effects of bacteria and antibiotics

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Aim: To investigate whether the inflammatory response of cultured endothelial cells, as induced by conditioned plasma, depends on the bacterial species or type of antibiotic used for incubation with whole blood.

Materials and methods: Blood from healthy volunteers was stimulated ex vivo with different microorganisms, and with bacteria killed with different antibiotics. The resultant plasmas were incubated on monolayers of cultured human endothelial cells, followed by measurement of their permeability to albumin and expression of E-selectin and intercellular adhesion molecule-1.

Results: Incubation of Escherichia coli in blood yielded plasmas that induced a marked increase in endothelial permeability and E-selectin expression. The response to Bacteroides fragilis or Enterococcus faecalis was generally weaker. Similar effects were observed after incubation of whole blood with lipopolysaccharide (LPS). Much of the permeability and adhesion molecule response to E. coli remained after removal of intact microorganisms from the culture. Whereas antibiotic treatment of E. coli with imipenem or cefuroxime resulted in a divergent production of tumour necrosis factor-α (TNF-α) in blood, no significant differences between these treatments were observed with respect to the plasma-induced endothelial response.

Conclusion: Bacteria differ in their capacity to generate a whole blood-mediated increase of endothelial permeability and adhesion molecule expression; this response depends, at least in part, on soluble bacterial components, such as LPS. Whereas treatment with various antibiotics may generate varying amounts of TNF-α, these differences are not translated into differences in endothelial permeability or adhesion molecule expression.

Keywords: lipopolysaccharide, cytokines, sepsis, β-lactams, HUVEC

Introduction

Systemic activation and damage of the endothelium is a key event in the pathogenesis of the sepsis syndrome.1,2 Whereas massive destruction of the endothelial barrier results in severe loss of fluid from the circulation into the surrounding tissue,3,4 systemic activation of the endothelium is characterized by the overexpression of cell adhesion molecules, such as E-selectin (CD62e) and intercellular adhesion molecule (ICAM)-1 (CD54), and the subsequent recruitment of leukocytes at non-infectious locations. Together, these events may induce the destruction of healthy tissue and represent a crucial step in the development of shock and multi-organ dysfunction.5

The endothelial response to sepsis is believed to be mediated, at least in part, by the generation of host-derived pro-inflammatory mediators, such as the cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), by monocytes and other circulating immune cells. The effects of these individual mediators on endothelial cells have been studied extensively in vitro. For example, we have previously demonstrated that IL-1β and TNF-α cause a dose-dependent increase in the permeability of human umbilical vein endothelial cells (HUVEC) cultured on semipermeable membranes.6

The generation of TNF-α, IL-1β and other cytokines is induced after exposure to various Gram-positive and Gram-negative bacteria. In ex vivo stimulated whole blood, Gram-negative...
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bacteria have been shown to be more potent inducers of IL-1β and IL-6 than Gram-positive micro-organisms. In this respect, *Escherichia coli* was among the most potent microorganisms, whereas *Bacteroides fragilis* and *Enterococcus faecalis*, which are also frequently isolated in abdominal sepsis, induced a lower cytokine response. These bacteria also induced the expression of E-selectin on endothelial cells in vitro. However, despite the fact that increased plasma cytokine, e.g. IL-6, concentrations correlate positively with disease severity and mortality in septic patients, the relationship between the bacterial capacity to generate cytokines in vitro and the clinical course is less obvious. Possibly, such a correlation may exist on the level of the endothelial response to bacteria.

Bacterial cell wall components, or endotoxins, have been considered key determinants of the inflammatory host response during sepsis. They include lipopolysaccharide (LPS) from Gram-negative bacteria, and to a lesser extent, their Gram-positive equivalents, lipoteichoic acid and peptidoglycan. Endotoxins such as LPS, which are released upon phagocytotic or antibiotic-induced bacteriolysis, are not primarily cytotoxic but exert their effect by abusing physiological target cell functions to elicit an inappropriate host response. Administration of LPS induces many of the characteristic features observed in sepsis.

LPS also directly acts on endothelial cells through the plasma-mediated binding and transfer to LPS-binding protein, and sCD14, respectively, which is followed by the association of the complex with Toll-like receptors (TLRs) present on endothelial cells. Activation of TLRs may ultimately result in transcriptional induction of pro-inflammatory genes, including those for E-selectin and ICAM-1. However, indirect but more powerful activation of the endothelium is achieved by the LPS-induced production of IL-1β and TNF-α, that result from binding of LPS to the CD14 receptor present on monocytes and macrophages.

LPS may also be generated as a result of antibiotic therapy. Despite their undisputed beneficial effect on the treatment of sepsis, antimicrobial agents that liberate significant amounts of LPS from killed bacteria may induce or intensify the inflammatory host response. Numerous studies, predominantly performed in vitro, have demonstrated that various antibiotics may act differently in this respect. For example, treatment of *E. coli* with cefuroxime resulted in marked activity in the *Limulus* amoebocyte lysate (LAL) assay and significantly increased production of IL-1β and IL-6 in blood, when compared with that induced by imipenem. A similar phenomenon has been reported for IL-6 production by cultured endothelial cells, but as yet its relevance for further endothelial functions has not been investigated. Despite these distinct in vitro effects, the clinical significance of antibiotic-induced endotoxin release remains to be clarified. Clinical studies, where administration of combinations containing—among others—imipenem and cefuroxime, were compared in patients with severe acute pancreatitis or intra-abdominal infection, did not reveal any difference with respect to mortality.

The potential effects of various bacteria, LPS and individual cytokines on endothelial functions have been investigated in vitro. However, almost invariably the important and regulatory role of circulatory cells and mediators, which comprise the normal environment of the endothelium, has not been considered. In previous studies, we have investigated whole blood-mediated effects of LPS on endothelial adhesion molecule expression and permeability. In the present study, we used the same model to compare the effect of Gram-negative and Gram-positive bacteria, as well as the effect of antibiotic treatment.

Materials and methods

Materials

Culture medium M199 (containing 25 mM HEPES, Earle’s salts, and l-glutamate), heat-inactivated newborn calf serum, penicillin–streptomycin, and trypsin/EDTA were obtained from Life Technologies (Paisley, Scotland, UK). Heat-inactivated normal human serum was purchased from ICN (Costa Mesa, CA, USA). Culture flasks and multi-well tissue culture plates were obtained from Costar (Cambridge, MA, USA); collagenase type 1 (from *Clostridium histolyticum*), pig skin gelatin (type A) was purchased from Sigma Chemical (St Louis, MO, USA). A crude fraction of endothelial cell growth factors (ECGF) was extracted from bovine brain and kindly provided by the Department of Paediatrics, University Medical Center, Nijmegen, The Netherlands. Heparin was obtained from Leo Pharmaceutical Products (Weesp, The Netherlands). Monoclonal antibodies against human E-selectin (clone ENA1) and ICAM-1 (HM2) were a generous gift of Wim Buurman, Department of Surgery, University Medical Center, Maastricht, The Netherlands. Imipenem/cilastatin (Tienam) was obtained from Merck Sharp & Dohme, Haarlem, The Netherlands. Cefuroxime (Zinacef) was obtained from GlaxoSmithKline, Zeist, The Netherlands. Polymyxin B was obtained from Pfizer, Cappelle a/d IJssel, The Netherlands.

Culture of microorganisms

*E. coli* (ATCC 25922), *B. fragilis* (ATCC 10584) or *E. faecalis* (ATCC 29212) were cultured in brain–heart infusion broth, and the number of bacteria was adjusted to the required turbidity by reading the optical density. Diluted cultures (5 × 10⁶ cfu/mL) were heat-killed (0.5 h at 56°C) prior to the addition of the bacteria to human whole blood (see below).

Whole blood incubation with bacteria or LPS

A modification of a previously described method was used. Briefly, blood from healthy volunteers was drawn in a sterile vacuum tube (Sherwood Medical, Ballemoney, N. Ireland, UK) containing 50 IU/mL of pyrogen-free heparin as anticoagulant. Blood was then divided in 2 mL aliquots in endotoxin-free polypropylene tubes, and 10 μL aliquots of bacterial suspension (10⁶ –10⁷ cfu/mL) or control (culture medium without bacteria) was added per mL of blood. Subsequently, the blood samples were incubated at 37°C for 18 h, and plasma was collected by centrifugation and stored at −20°C until use. In separate experiments, LPS (from *E. coli* 055:B5; Sigma Chemical, St Louis, MO, USA) or control (0.1% BSA) was added to whole blood in a final concentration of 10–100 pg/mL and processed likewise.

Antibiotic treatment

In experiments where the effect of antibiotic treatment was investigated, *E. coli* was grown in TSB medium, diluted in broth to a concentration of 5 × 10⁶ cfu/mL in the presence of either cefuroxime (80 mg/L), imipenem/cilastatin (50 mg/L) or polymyxin B (15 mg/L) for 24 h at room temperature. The final concentrations of the antibiotics corresponded to serum peak levels that occur after
the administration of therapeutic doses in humans. Heat-killed E. coli that showed morphologically intact bacteria on Gram stain was used as control. The antibiotic-treated E. coli cultures were diluted to a final concentration of 10^5 bacteria/mL and incubated in whole blood as mentioned earlier.

**Isolation and culture of HUVEC**

Endothelial cells were isolated and cultured as described previously, with minor modification.30 Fresh umbilical cords were obtained after delivery and consent from healthy women at the Department of Obstetrics and Gynaecology at our hospital. Briefly, after harvesting the cells from the umbilical vein, the isolates were seeded on gelatinized culture flasks and cultured in medium M199 containing 10% newborn calf serum, 10% normal human serum, 50 U/mL penicillin, 50 μg/mL streptomycin, 150 μg/mL crude ECGF and 5 U/mL heparin. All experiments were performed on cells that had been subcultured by trypsinization three times. The endothelial origin of the cultures was confirmed by phase contrast microscopy. This revealed confluent monolayers displaying typical cobblestone morphology; meanwhile immunocytochemical analysis showed positive staining for von Willebrand Factor and CD31 [platelet endothelial cell adhesion molecule (PECAM)-1].

**Incubation of HUVEC with plasma**

Plasma obtained from whole blood incubations was diluted to 20% v/v in medium M199 without serum components, and is further referred to as ‘plasma mix’. For the induction of permeability, quadruple samples of the plasma mix were added to confluent HUVEC grown on Transwell-COL (0.4 μm pore diameter size) membranes, and incubated for 18 h. For the induction and detection of E-selectin and ICAM-1, quadruple samples of the plasma mix were incubated for 4–6 h and 24 h, respectively, on HUVEC grown on gelatinized 96-well dishes.

**Permeability studies**

The permeability of endothelial cultures was measured as previously described.3 Briefly, for detection of macromolecular passage across the endothelial monolayers, a tracer solution containing FITC-BSA (250 μg/mL) was prepared in complete medium. At the upper compartment of the Transwell insert, the plasma mix was replaced with 0.5 mL of the tracer solution, whereas the volume in the lower compartment was replaced with 1.5 mL fresh medium. After 1 and 2 h of equilibration of the tracer solution, 250 μL samples were drawn from the lower compartment and the amount of FITC-BSA in the samples was measured on a Hitachi F-3000 fluorescence spectrophotometer (Tokyo, Japan), at emission/ excitation wavelengths of 495/520 nm. In order to allow quantitative comparison between multiple experiments, data are expressed as the relative increase in the amount of FITC-BSA (when compared to the concentration of FITC-BSA measured after a control stimulus run in each individual experiment).

**ELISA detection of E-selectin or ICAM-1**

After incubation with plasma mix, the expression of E-selectin and ICAM-1 was determined by a modified ELISA.31 Briefly, the HUVEC cultures were washed with M199 to remove serum components, and fixed with a 0.025% glutaraldehyde solution in phosphate buffered saline. The tissue culture plates were then pre-incubated for 1 h at 37°C with murine monoclonal anti-human E-selectin (clone ENA1, 1:1000) or anti-ICAM-1 (clone HM2, 1:1000) in ELISA buffer (0.5% BSA in PBS). After four washing steps, specific binding was detected by incubation with horseradish peroxidase-conjugated goat-anti-mouse (1:1000; DAKO, Glostrup, Denmark,) for 1 h at 37°C; enzyme activity was analysed by using orthophenyl diamine substrate and measuring the absorbance at 490 nm on a Bio-Rad 550 plate reader.

**Detection of TNF-α**

The amount of TNF-α in the plasma samples used for endothelial stimulation was measured by radio-immunoassay, as described previously.32 The detection limit was 0.04 ng/mL.

**Statistical analysis**

Comparison of different treatments and differences between stimulated and control cultures were analysed by one-way ANOVA followed by Tukey’s test and Dunnett’s Multiple Comparisons test, respectively, using GraphPad InStat v3.05 software. Differences were considered statistically significant at P < 0.05.

**Results**

**Effects of bacteria and bacterial products**

Incubation of increasing concentrations of heat-killed E. coli (10^4 – 10^6 cfu/mL) in blood produced plasmas that dose dependently increased the amount of FITC-BSA that passed across the endothelial monolayer up to a concentration of 7.0 ± 0.5 μg/mL in 2 h, or 183%, when compared with the amount of FITC-BSA measured after incubating the monolayers with control plasma (plasma obtained from blood without bacteria; 3.8 ± 0.6 μg/mL; Figure 1). In contrast, only the highest concentrations (10^6 cfu/mL) of B. fragilis or E. faecalis induced a significant increase in the amount of FITC-BSA (when compared to the concentration of FITC-BSA measured after a control stimulus run in each individual experiment).

**Figure 1.** Permeability response of HUVEC monolayers to plasma obtained from blood after incubation with bacteria. Dilutions of heat-killed E. coli (black bars), B. fragilis (hatched bars), or E. faecalis (open bars) were added to whole blood at final concentrations in the range 10^4 – 10^6 cfu/mL. Cultures containing 10^6 cfu/mL were also filter sterilized (FS) prior to the incubation in blood. Bars represent mean ± S.E.M. (n=4) relative increase of the permeability to FITC-BSA, as compared with that induced by control plasma (plasma obtained from whole blood was treated with culture medium without bacteria). Asterisks depict significant differences (P < 0.05) with control plasma.
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permeability increase to 210% and 155%, respectively. In order to gain an impression of the source that induced the plasma-mediated permeability response, different bacterial cultures were filter sterilized through a 0.2 μm filter to remove intact microorganisms. Incubation of the filtrates in whole blood produced plasmas that elicited a permeability response that was not significantly different from that induced by unfiltered cultures.

The plasmas produced by incubating E. coli, B. fragilis or E. faecalis in whole blood dose-dependently induced the expression of E-selectin (Figure 2a) on endothelial cells, whereas incubation with control plasma did not induce significant adhesion molecule expression (not shown). The magnitude of the E-selectin response increased from E. faecalis to B. fragilis to E. coli. Whereas E. faecalis also induced the weakest ICAM-1 response, all concentrations of E. coli and B. fragilis induced a marked and almost similar level of ICAM-1 expression (Figure 2b). Removal of intact bacteria by filter sterilization prior to the incubation in blood completely abrogated the adhesion molecule response to E. faecalis and reduced the ICAM-1 response to B. fragilis, whereas the response to E. coli remained unaffected.

These bacterial strains also differed in their capacity to induce TNF-α production in whole blood: E. coli (10⁴–10⁶ cfu/mL) induced a dose-dependent production of TNF-α in whole blood up to 10.6 ng/mL, whereas only the highest concentration of both B. fragilis and E. faecalis induced measurable amounts of TNF-α, of 11.1 ng/mL and 1.0 ng/mL, respectively. Furthermore, the TNF-α response to E. coli was hardly affected by filter sterilization of the culture prior to the incubation in whole blood (data not shown).

Plasma obtained from whole blood exposed to heat-killed E. coli thus significantly increased the permeability of HUVEC monolayers and endothelial adhesion molecule expression, whereas these responses were weaker after stimulation with E. faecalis and B. fragilis. Furthermore, the response to E. coli was maintained when intact microorganisms were removed from the culture, suggesting that soluble components, such as LPS, are instrumental for these phenomena.

Effects of LPS

In separate experiments, the effect of incubation of low doses of LPS in blood on endothelial permeability and adhesion molecule expression was examined. As depicted in Figure 3(a), blood that had been treated with as little as 25 pg/mL of LPS produced plasma that induced a significant (P<0.05) permeability increase, when compared with that induced by control plasma, and the response was dose dependent over a range up to

![Figure 2](https://academic.oup.com/jac/article-abstract/55/2/150/856779)

**Figure 2.** Effect of different bacteria on whole blood-induced E-selectin and ICAM-1 expression. For further explanations, see legends to Figure 1. Bars represent mean ± S.E.M. (n = 4) E-selectin (a) and ICAM-1 (b) expression by HUVEC.

![Figure 3](https://academic.oup.com/jac/article-abstract/55/2/150/856779)

**Figure 3.** Effect of LPS on whole blood-induced endothelial permeability and adhesion molecule expression. Blood of four donors was incubated separately in four-fold. Data are represented as the mean (n = 16) ± S.E.M. (a) relative increase in permeability as compared with the response to control plasma (unconditioned plasma obtained from the same donor), (b) expression of E-selectin (hatched bars) and ICAM-1 (black bars). Asterisks denote significant (P<0.05) differences with control plasma.
100 pg/mL LPS. On the level of adhesion molecule expression, doses as low as 10 pg/mL of LPS in blood were sufficient to induce significant (P < 0.05) expression of both E-selectin and ICAM-1 (Figure 3b).

Effects of antibiotics on cytokine production

In order to investigate if antibiotic killing of bacteria would lead to increased cytokine production, *E. coli* (10⁵ cfu/mL) were incubated for 24 h with either polymyxin B (15 mg/L), imipenem (50 mg/L) or cefuroxime (80 mg/L) prior to addition to whole blood. All antibiotics adequately killed the *E. coli*, which was confirmed by the absence of viable bacteria in subcultures. In whole blood, bacteria treated with antibiotics, as well as heat-killed bacteria, induced measurable amounts of TNF-α (Figure 4). However, large differences were present in the cytokine levels produced. When compared with heat-killed bacteria, which induced the production of 4.7 ± 1.6 ng/mL of TNF-α, both imipenem and cefuroxime treatment further increased the production, to 6.9 ± 2.0 ng/mL (NS) and 10.4 ± 1.7 ng/mL (P < 0.05), respectively. In contrast, polymyxin B treatment resulted in a four-fold reduction of the TNF-α response, when compared with that induced by heat-killed bacteria. The inhibitory effect of polymyxin B was also present when this antibiotic was added to heat-killed bacteria, whereas the addition of imipenem or cefuroxime to heat-killed bacteria did not alter the TNF-α response. Similar, but non-significant effects were observed for the production of IL-1β (data not shown).

Effects of antibiotics on endothelial function

In order to examine whether the antibiotic-induced differences in whole blood TNF-α production would be translated into altered endothelial function, HUVEC monolayers were exposed to the resultant plasmas. When compared with the effect of untreated control plasma, incubation of polymyxin B-, imipenem- or cefuroxime-treated *E. coli* in blood all produced plasmas that significantly increased the permeability (Figure 5a), whereas addition of antibiotics without bacteria to whole blood or to endothelial monolayers had no effect on the permeability (data not shown). The permeability response to imipenem- or cefuroxime-treated bacteria was not significantly different from that induced by heat-killed bacteria. On the other hand, the response to polymyxin B-treated *E. coli* was ~50% of that induced by heat-killed bacteria. This blunted response was also observed when polymyxin B was added to heat-killed cultures prior to the incubation in whole blood. The addition of imipenem or cefuroxime to heat-killed bacteria had no influence on the permeability response, when compared with that induced by heat-killed bacteria without antibiotics.

![Figure 4](https://academic.oup.com/jac/article-abstract/55/2/150/856779)

**Figure 4.** The effect of antibiotic killing on TNF-α production. Viable *E. coli* (10⁵ cfu/mL, open bars) were killed by polymyxin B (PolyB, 15 mg/L), imipenem (IPM, 50 mg/L), or cefuroxime (CXM, 80 mg/L) and incubated in whole blood. Incubation of blood with heat-killed (HK, black bar) bacteria, or with heat-killed bacteria with subsequent antibiotic treatment (hatched bars), as well as untreated blood (C, grey bar) was used for comparison. Data are presented as the mean ± S.E.M. of four repeat experiments. Asterisks denote a significant (P < 0.05) difference with heat-killed bacteria.

![Figure 5](https://academic.oup.com/jac/article-abstract/55/2/150/856779)

**Figure 5.** Effect of antibiotic killing on endothelial responses. Permeability (a): viable (open bars), and heat-killed (HK, hatched bars), *E. coli* were treated with polymyxin B (PolyB), imipenem (IPM), or cefuroxime (CXM) prior to the addition to whole blood (final concentration: 10⁵ cfu/mL). The effect of the resultant plasmas on endothelial permeability was compared with that induced by plasma obtained from whole blood incubated with heat-killed bacteria without antibiotic treatment (black bar) and untreated control plasma (C, grey bar) and are represented as the mean ± S.E.M. (*n* = 16) relative increase of the permeability to FITC-BSA. Data were collected from four repeat experiments. (b) E-selectin (hatched bars) and ICAM-1 (black bars) expression: data were collected from three repeat experiments and are represented as the mean ± S.E.M. (*n* = 12). Asterisks mark significant differences (P < 0.05) from the response elicited by heat-killed bacteria.
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The effects observed on endothelial permeability were also present for endothelial adhesion molecule expression (Figure 5b). Antibiotic or heat-killed E. coli produced plasmas that strongly induced the expression of E-selectin and ICAM-1 on HUVEC. However, again no significant differences were present between heat-killed, imipenem- and cefuroxime-treated bacteria, whereas a significant reduction of the E-selectin response was induced by polymyxin-treated E. coli. On the level of ICAM-1 expression, incubation of plasmas obtained from the different whole blood treatments resulted in small and non-significant differences, when compared with the effect induced by heat-killed bacteria.

Discussion

The incubation of different microorganisms with whole blood results in the generation of plasmas that differ in their capacity to induce endothelial permeability, E-selectin, and ICAM-1 expression. Furthermore, this endothelial response to bacteria roughly correlates with the levels of TNF-α produced in whole blood and to the number of bacteria added. Incubation of HUVEC monolayers with plasma obtained from E. coli-treated whole blood generally induced a stronger permeability and adhesion molecule response than that induced by B. fragilis, whereas weak effects were measured after incubation with E. faecalis. These different responses were also reflected in the production of TNF-α (this study) and various other cytokines, including IL-1β and IL-6, in whole blood. These observations in a relevant ex vivo model suggest that Gram-negative infections with high cytokine induction may lead to a more acute and more severe clinical picture than infection with Gram-positive bacteria. However, Friel et al. showed that this relation is not obvious. It remains to be determined if microorganisms that induce few cytokines but a serious clinical picture, such as Capnocytophaga canimorsus, are capable of inducing a strong endothelial permeability and adhesion molecule response.

The observation that removal of intact microorganisms from E. coli cultures, prior to incubation with whole blood, did not alter the permeability and adhesion molecule response, additionally proves that the inflammatory response to bacteria largely depends on the availability of soluble bacterial components, such as LPS. Incubation of bacteria in blood yielded plasma that increased adhesion molecule expression, sometimes even in the absence of measurable levels of plasma TNF-α, as was the case for lower concentrations of B. fragilis and E. faecalis. This finding indicates that in addition to TNF-α at least one other mediator must be responsible for regulating the endothelial responses. Despite the possible involvement of other bacterial components and host mediators, we propose that IL-1β is the other major molecule involved in this phenomenon. In previous studies, it was demonstrated that, next to TNF-α, plasma IL-1β is instrumental in the induction of endothelial permeability, E-selectin and ICAM-1 by LPS-stimulated whole blood.

It has been demonstrated that different antibiotics can liberate widely ranging concentrations of LPS from bacterial cultures. These variations in LPS-liberating potential may result in different production of TNF-α by monocytes or in whole blood. The clinical significance of this phenomenon remains to be established.

In a murine sepsis model, antibiotics significantly differed in their capacity to reduce mortality, but without changing the kinetic appearance of pro-inflammatory cytokines and LPS in plasma. The notion that the type of antibiotic, and thus its LPS (and TNF-α) generating capacity, may determine the inflammatory response to bacteria, and that this phenomenon may also be present on the level of endothelial cells, was supported by two results from the present study. Firstly, soluble components in E. coli cultures seemed to be responsible for the induction of endothelial permeability and adhesion molecule expression, as was demonstrated by the effects of filter-sterilized microbial cultures. Secondly, small differences in physiologically relevant concentrations of purified LPS in blood (of the same E. coli strain) yielded plasmas that induced different endothelial responses. In order to address the question whether antibiotics differ in their capacity ultimately to induce endothelial permeability and adhesion molecule expression, we examined the effects of E. coli killed by three antibiotics that are supposed to have different LPS-generating capacity. Although differences between cefuroxime-, imipenem- and polymyxin B-treated E. coli were present on the level of TNF-α production in whole blood, imipenem- and cefuroxime-treated E. coli induced a similar plasma-mediated permeability increase and adhesion molecule expression. In fact, the endothelial responses to imipenem- and cefuroxime-killed E. coli were not significantly different from that induced by heat-killed bacteria. On the other hand, polymyxin B treatment largely prevented the E. coli-induced cytokine and endothelial responses, confirming the important role of LPS and the LPS-neutralizing capacity of polymyxin B.

In this and previous studies, we have demonstrated that cefuroxime-killed E. coli induced more TNF-α, IL-1β and IL-6 in blood than imipenem-killed E. coli. Cefuroxime also induced increased activity of the culture supernatant in the LAL assay, which indicated increased production of bacterial fragments by this antibiotic. We have also previously demonstrated that TNF-α and IL-1β mediated the permeability and adhesion molecule response to LPS-treated whole blood. On the basis of these findings one might expect a difference between (classes of) antibiotics in their capacity to induce endothelial responses. However, the present results indicate that the initial antibiotic-dependent differences that occur on the level of cytokine (e.g., TNF-α) production in blood do not translate into endothelial permeability and adhesion molecule expression. One might still argue that the amount of TNF-α and other mediators that are produced in blood upon bacterial challenge would exceed levels required for maximum stimulation of endothelial cells. However, this seems unlikely since, in the present experiments, ~ two-fold permeability increases were observed over control values; previously we demonstrated the occurrence of ~ 10-fold increases, using high concentrations of LPS in whole blood.

In conclusion, our data do not support the opinion that the choice of antibiotic for the treatment of sepsis should mainly depend on its LPS-liberating properties. It remains to be clarified whether the plasma-mediated endothelial responses to antibiotic-killed bacteria are also prevented by neutralization of IL-1β and TNF-α. It is entirely possible that factors other than LPS that are released by E. coli can explain the lack of a difference that is observed after cefuroxime and imipenem treatment.
References


