Staphylococcal infections impair the mesothelial fibrinolytic system:
The role of cell death and cytokine release

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Summary
Bacterial peritonitis is a serious complication of peritoneal dialysis patients and of patients after abdominal surgery. Especially episodes due to Staphylococcus aureus can harm the peritoneum severely, resulting in peritoneal fibrosis. Human peritoneal mesothelial cells play a critical role in maintaining the integrity of the peritoneum, as they release components of the fibrinolytic system and regulate the influx of immune cells by expressing chemokines and adhesion molecules. Using cultured human peritoneal mesothelial cells (HMCs) and blood mononuclear cells, we analyzed the effect of different staphylococcal strains on mesothelial fibrinolysis and on inflammatory reactions and show that only S. aureus strains with an invasive and hemolytic phenotype decrease the production of fibrinolytic system components, most likely via cell death induction. Furthermore, HMCs react to invading staphylococci by enhanced expression of chemokines and adhesion molecules. Mononuclear cells were activated by all staphylococcal strains tested, and their culture supernatants impaired mesothelial fibrinolysis. Simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, efficiently enhanced the mesothelial fibrinolytic capacity under these inflammatory conditions, but did not protect HMCs against S. aureus-induced cell death. We conclude that only selected S. aureus strains decrease the release of fibrinolytic system components and provoke a mesothelial inflammatory response. These factors most likely contribute to peritoneal fibrosis and might account for the severe clinical presentation of S. aureus peritonitis.

Keywords
Bacterial peritonitis, peritoneal fibrosis, mesothelial fibrinolytic system, statins

Introduction
Staphylococci, particularly S. aureus, can cause a wide spectrum of infections from superficial skin infections to serious conditions as sepsis (1, 2). A dangerous and life-threatening form of infection is bacterial peritonitis, which remains a frequent complication in peritoneal dialysis and abdominal surgery (3–5). Especially in peritoneal dialysis, the use of a permanent indwelling catheter and numerous bag exchanges facilitate the entrance of microorganisms to the peritoneal cavity causing peritonitis (5). Repeated episodes often induce peritoneal fibrosis, which can result in serious complications, as technical failure of peritoneal dialysis, intestinal obstruction, chronic pelvic pain and infertility in women (6, 7).

Human peritoneal mesothelial cells (HMCs), which line the whole peritoneal surface, play an important role in maintaining the integrity of the peritoneum, as they express the pro-fibrinolytic enzyme tissue-type plasminogen activator (t-PA) and its specific inhibitor, plasminogen activator inhibitor-1 (PAI-1) (8). During bacterial peritonitis the fibrinolytic capacity of the peritoneum is severely impaired (9, 10), which can lead to delayed removal and subsequent organization of fibrin, including fibroblast ingrowth, collagen deposition and vessel formation (11). These mechanisms contribute to peritoneal fibrosis which is often found after bacterial peritonitis (6, 12).

Furthermore, mesothelial cells provide the first line of antimicrobial defense and play a major role in the recruitment and activation of phagocytes (13–15). They produce high levels of interleukin-8 (IL-8), a neutrophil chemoattractant, and mono-
Table 1: S. epidermidis and S. aureus strains used in this study. All S. epidermidis and S. aureus strains were tested for invasiveness in HMCs and for hemolysis. Invasion was determined by a flow cytometric invasion assay and is expressed relative to that of Cowan I. Results represent means ± SEM of five independent experiments performed in duplicates. Hemolytic activity due to toxin expression was determined on sheep blood agar plates after 24 h and is listed semi-quantitatively in four categories: -, no hemolysis; +/-, borderline; +, ++ effective hemolysis.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Invasiveness (mean ± SEM)</th>
<th>Hemo-lysis</th>
<th>Properties</th>
<th>Reference or source</th>
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<tbody>
<tr>
<td>S. epidermidis 19</td>
<td>1.1 ± 0.1</td>
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<td>Biofilm-forming strain</td>
<td></td>
</tr>
<tr>
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<td>+/-</td>
<td>NCTC8530 (isolated from septic arthritis)</td>
<td>ATCC 12598</td>
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<tr>
<td>S. aureus 8325–4</td>
<td>28.6 ± 7.0</td>
<td>++</td>
<td>NCTC8325 cured of prophages</td>
<td>(48)</td>
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<tr>
<td>S. aureus Wood 46</td>
<td>5.4 ± 1.7</td>
<td>++</td>
<td>SpA-deficient; NCTC 7121</td>
<td>ATCC 10832</td>
</tr>
<tr>
<td>S. aureus 6850</td>
<td>101.4 ± 8.3</td>
<td>++</td>
<td>Wild-type isolate</td>
<td>(30)</td>
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<tr>
<td>S. aureus ST 239, 635/93</td>
<td>87.8 ± 4.3</td>
<td>++</td>
<td>Wild-type isolate, MRSA</td>
<td>(31)</td>
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</table>

Our study was designed to investigate the effect of staphylococcal infections on the mesothelial fibrinolytic system in a cell culture model of bacterial peritonitis. Using various defined staphylococcal strains we evaluated the inflammatory reactions of cultured HMCs and freshly isolated blood mononuclear cells (MNCs) after bacterial infection and their impact on the mesothelial fibrinolytic system.

Material and methods

Media and reagents
M199 medium and RPMI 1640 were obtained from Gibco (Karlsruhe, Germany); tissue culture plastics were from Costar (Cambridge, MA, USA). Human serum was from PAA (Pasching, Austria) and stored at −20°C. Newborn calf serum was purchased from Gibco and was heat-inactivated (30 minutes [min] at 56°C) before use. Collagenase type II was from Worthington (Freehold, NY, USA). Lysostaphin was from WAK Chemie Medical GmbH (Bad Soden, Germany). Tumor necrosis factor-α (TNF-α) was from Calbiochem (Bad Soden, Germany). Endothelial cell growth supplement (ECS) was from PromoCell (Heidelberg, Germany). Fibronectin was purchased from Roche (Mannheim, Germany).

Cell culture of HMCs and MNCs
HMCs were isolated from the omental tissue of consenting patients undergoing elective surgery and characterized as described previously (8, 29). The experimental protocol was approved by local ethics committee. Briefly, HMCs were grown in fibronectin-coated dishes in M199 supplemented with 20 mM HEPES (pH 7.4), 2 mM glutamine, 10% (v/v) human serum, 5% (v/v) newborn calf serum, ECSG (150 µg/ml), 5 IU/ml heparin, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in a 5% CO₂/95% air atmosphere (cell culture medium). The medium was replaced every 2–3 days. Cells were subcultured with mild trypsinization (1–2 min), using trypsin/ethylene diamine tetraacetic acid (EDTA) (Gibco, Karlsruhe, Germany), after which the cells were replated with a split ratio of 1:3. For the experiments, confluent cultures were used at the second or third passage. The day before the experiment the cells were supplemented with incubation medium, M199 with 20 mM HEPES (pH 7.4), 10% (v/v) human serum, 2 mM glutamine and antibiotics (penicillin 100 IU/ml and streptomycin 100 µg/ml). Under these conditions, HMCs remained in a non-proliferative, viable state.

MNCs were freshly isolated from heparin-treated (10 IU/ml) blood of healthy donors. Ficoll-Paque (Amersham Pharmacia, Freiburg, Germany) and Leucosep-tubes (Greiner, Frickenhausen, Germany) were used according to the manufacturers’ instructions. MNCs were collected and cultured in RPMI 1640 supplemented with 2% heat-inactivated human serum and antibiotics as above. For the measurement of cytokine production 2.5x10⁶ cells/ml were cultured at 37°C in 5% CO₂ atmosphere in 24-well plates.

Bacterial strains and cultures
The laboratory staphylococcal strains used in this study are listed in Table 1. S. aureus strain 6850 and ST 239, 635/93 were kindly provided by R. Proctor, Madison, WI, USA (30) and by M. C. Enright (Bath, United Kingdom) (31), respectively. The hemo-

cyte chemoattractant protein-1 (MCP-1), which contributes to the influx of mononuclear cells (16). Moreover, mesothelial cells express adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (17), which allow leukocytes to bind to the mesothelial cell surface and facilitate their movement into the peritoneal cavity (14, 18, 19). Hence, bacterial peritonitis provokes the release of diverse proinflammatory mediators from resident as well as infiltrating cells.

Bacteria frequently found during peritonitis are S. epidermidis or S. aureus (20). Several studies have shown that the clinical presentation and the outcome of peritonitis are dependent on the organisms involved. Peritonitis episodes with S. aureus have usually poorer outcomes than episodes due to coagulase-negative staphylococci (21, 22). S. aureus expresses a variety of specific surface and secreted proteins which interact in complex and diverse ways with host cells (1, 2). A major toxin of S. aureus is α-hemolysin (α-toxin), which is a pore-forming toxin that can cause cytolysis at higher concentrations, whereas sublytic doses lead to cell activation and induce the production of proinflammatory factors (23–25). Furthermore, S. aureus can invade host cells, persist and even replicate intracellularly, whereas S. epidermidis adheres less effectively to mesothelial cell monolayers and is barely invasive (26–28).
lytic activity was determined visually in a semi-quantitative manner on Columbia sheep blood agar plates. Bacterial supernatants were prepared as described (23). Briefly, bacteria were grown in 5 ml of brain-heart-infusion broth (Merck, Germany) in a rotatory shaker (200 rpm) at 37°C for 12–14 hours (h) and pelleted for 5 min at 3,350 g. Supernatants were sterile-filtered through a Millex-GP filter unit (0.22 µm; Millipore, Bedford, MA, USA) and stored at -20°C. Formaldehyde-fixed bacteria were prepared by incubation of staphylococci in 1% formaldehyde for 2 h. Live bacteria were grown in 5 ml MH-medium overnight. Staphylococci were sonicated gently, washed with PBS and were used with PBS and 1% HSA for the experiments.

**Stimulation of the cells with test compounds**

Confluent HMC monolayers were incubated with TNFα, bacterial supernatants (10% v/v) or fixed bacteria (multiplicity of infection; MOI of 50) at the indicated concentrations. To determine the effect of live *S. aureus* (bacterial invasion) on mesothelial cells, confluent HMCs were washed with M199 and then invasion medium (1 ml of 1% HSA and 10 mM HEPES, pH 7.4, in M199) with cocci was added to the cells. Cells were preincubated for 30 min at room temperature to allow sedimentation of cocci and were then shifted to 37°C for 3 h for invasion. Subsequently, cells were washed with PBS and lysostaphin (20 µg/ml) was added for 30 min to lyse extracellular or adherent staphylococci. Cells were then washed and supplemented with fresh incubation medium. After 24 h or 48 h the cell culture supernatants were collected to measure cytokines, chemokines and compounds of the fibrinolytic system. Conditioned media were centrifuged to remove cells and cellular debris, and samples were frozen at -20°C until use. Remaining cells were used to measure the expression of adhesion molecules on the cell surface by flow
Table 2: Effect of live staphylococci, formalin-fixed staphylococci or bacterial supernatants on HMC membrane damage. Confluent HMCs were stimulated with live staphylococci, formalin-fixed staphylococci (MOI 50), bacterial supernatants or with TNFα (500 U/ml) for 48 h as described in Figure 1. After trypsinization, the percentage of trypan blue-positive cells was determined. Incubation with 200 µM hydrogen peroxide was used as a positive control and resulted in 100% cell death. Data are shown as means ± SD of one representative experiment out of three performed in triplicate.

<table>
<thead>
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<th>Trypan blue-positive cells (%)</th>
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<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Cells treated with live staphylococci and lysostaphin</td>
<td>9.1 ± 2.7</td>
</tr>
<tr>
<td>Cells treated with fixed staphylococci without lysostaphin</td>
<td>13.9 ± 2.9</td>
</tr>
<tr>
<td>Cells treated with staphylococcal supernatants (10% vol/vol)</td>
<td>6.6 ± 0.7</td>
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Flow cytometric invasion assay
The flow cytometric invasion assay was performed as described previously with minor modifications (32). Briefly, HMCs were plated at a density of 2 x 10⁵ cells in 12-well plates. Cells were washed with M199, and then 0.5 ml of 1% HSA, 10 mM HEPES (pH 7.4) in M199 and 50 µl of FITC-labeled bacterial suspension (OD₅₄₀ 1) were added to the cells. Culture dishes were preincubated for 30 min at room temperature to allow sedimentation of bacteria and were then shifted to 37°C for 3 h. Cells were analyzed by flow cytometry as described (28, 32, 33). The invasiveness of the laboratory strain Cowan I was set as 100%.

Assays for fibrinolytic components and cytokines
The levels of t-PA antigens and PAI-1 antigens in the cell culture supernatants of HMCs were measured with enzyme-linked immunosorbent assays Coazilas for t-PA and PAI-1 (Haemochrom Diagnostica, Essen, Germany). The results are given as absolute values (ng/10⁵ cells) and are expressed as percentage values to the corresponding control. The chemokines MCP-1 and IL-8 in the cell culture supernatants of HMCs were determined by immunoassays from Biosource (Solingen, Germany). The cytokines TNFα and IL-1β were measured in the cell culture supernatants of MNCs by immunoassays from Biosource.

Flow cytometric analysis of cell surface expression of adhesion molecules
Analysis of cell surface expression of ICAM-1 and VCAM-1 was performed as recently described (34) with minor modifications. Briefly, after infection with live staphylococci for 3 h, the cells were treated with lysostaphin as described above and washed with PBS. Then the cells were incubated for 20 h. The cells were collected for flow cytometry by trypsinization and washing in PBS. Then, 5% goat serum in PBS was added as blocking agent for 1 h followed by centrifugation and washing. Cells were incubated with primary monoclonal antibodies to ICAM-1 (mouse IgG1κ; BD Biosciences, USA), VCAM-1 (mouse IgG1κ; BD Biosciences, Franklin Lakes, NJ, USA) or with mouse IgGκ, clone MOPC-21 antibodies (BD Biosciences) as an isotype control. After washing, cells were incubated with allophycocyanin (APC)-conjugated goat-anti-mouse Ig (BD Biosciences) for 45 min in dark. The cells were washed twice and resuspended in PBS for analysis on a FACS Calibur (Becton Dickinson).

Statistics
Statistical analysis was performed using the Wilcoxon matched-pairs signed rank test for non-parametric data; a p-value less than 0.05 was considered to indicate statistically significant differences.

Results
S. aureus strains with an invasive and hemolytic phenotype reduce mesothelial release of t-PA and PAI-1 by inducing cell death
To investigate the direct effect of bacteria and bacterial products on the mesothelial fibrinolytic system (release of t-PA and PAI-1), we incubated HMC monolayers with live staphylococci, formalin-fixed staphylococci or culture supernatants of various S. epidermidis and S. aureus strains. The different strains were characterized for their invasiveness in HMCs and for their hemolytic activity (α-toxin production), as shown in Table 1. After 48 h, only live S. aureus strains 6850 and ST 239, both with an invasive and hemolytic phenotype (Table 1), resulted in decreased levels of t-PA and PAI-1 antigens in the cell supernatants (Fig. 1). Recently we have demonstrated that these two S. aureus strains efficiently induce cell damage and cell death (27), which could be an explanation for the decreased release of fibrinolytic system components. As shown in Table 2, live bacteria of the S. aureus strains 6850 and ST 239 markedly increased the number of damaged cells (30.9% ± 8.4% for S. aureus 6850 and 59.7% ± 4.7% for S. aureus ST 239 vs. 9.1% ± 2.7% in control cells), whereas other S. aureus and S. epidermidis strains, formalin-fixed staphy-
loccoci, bacterial supernatants or the proinflammatory cytokine TNFα did not affect cell integrity. Light microscopy revealed that S. aureus 6850 and ST 239 clearly destroyed the mesothelial cell monolayer, characterized by shrinkage of the cells and detachment from the culture plates, whereas TNFα caused changes in cell shape from a cobblestone to a spindle shape appearance (Fig. 2) without provoking membrane damage (Table 2). These findings suggest that S. aureus 6850 and ST 239 decrease levels of t-PA and PAI-1 most likely via inducing cell damage and cell death rather than by regulating their expression. The results obtained with staphylococci are clearly different from the effects of proinflammatory cytokines on mesothelial cells (35). TNFα, which is a potent inflammatory mediator, decreased the release of t-PA (Fig. 1A) and increased the expression of PAI-1 (Fig. 1B) suggesting a suppressed fibrinolytic capacity.

**Staphylococci induce the release of proinflammatory cytokines in mesothelial cells and blood mononuclear cells**

The mesothelial fibrinolytic system is known to be regulated by different cytokines (35). Important sources of cytokines in the peritoneal cavity are infiltrating blood cells. Mononuclear cells predominate during resolution of peritonitis (36). Mesothelial cells contribute to the influx of mononuclear cells by expressing chemokines, particularly IL-8 and MCP-1 (16) and the adhesion molecules ICAM-1 and VCAM-1 (14, 17). In our experiments, we tested the effect of live bacteria of different S. aureus and S. epidermidis strains on the expression of both chemokines. As shown in Figure 3 only S. aureus with an invasive phenotype (Cowan I, 6850 and ST 239) significantly enhanced the levels of MCP-1 and IL-8 in the cell supernatants of mesothelial cells, with S. aureus strain ST 239 inducing the strongest increase. These effects were similar to the proinflammatory action of TNFα. Analogous results were obtained by analyzing the expression of adhesion molecules on the mesothelial cell membrane via flow cytometry. After 24 h of incubation the S. aureus strains Cowan I, 6850 and particularly ST 239 induced cell surface expression of ICAM-1 and VCAM-1, whereas the other strains tested were similar to the unstimulated control (Fig. 4).

A time-course (Fig. 5) revealed that the cytokines IL-8 and MCP-1 were upregulated rapidly in response to S. aureus and TNFα being already significantly elevated after 24 h (Fig. 3 and 5B, C). Cytokine levels declined from the 48-h timepoint on, which is most likely due to the increased rate of cell death in response to S. aureus (Fig. 5A). The levels of the fibrinolytic system components t-PA and PAI-1 were diminished after a 48-h incubation period with S. aureus 6850 and ST239, which is reflected by the enhanced rate of cell death. In contrast to S. aureus, the cytokine TNFα clearly decreased levels of t-PA and increased PAI-1 (Fig. 5D, E).

Infiltrating blood cells are known to express a large number of different inflammatory mediators after activation. Among these, TNFα and interleukin-1 (IL-1) were found to impair the mesothelial fibrinolytic system (Fig. 1) (35). Hence, we measured the release of TNFα and IL-1β in mononuclear blood cells following stimulation with live staphylococci. All S. epidermidis and S. aureus strains tested considerably enhanced TNFα and IL-1β levels in the cell supernatants (Fig. 6).
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the release of t-PA and increase the release of PAI-1. Recently, we have shown that simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is an effective stimulator of the mesothelial fibrinolytic activity (37, 38). Here, we demonstrate that simvastatin also increased t-PA and decreased PAI-1 levels in our in-vitro cell culture model of bacterial peritonitis. However, simvastatin did not protect mesothelial cells from *S. aureus* (strains 6850 and ST 239)-induced cell damage or cell death (Table 3).

Supernatants of activated mononuclear cells impair the fibrinolytic system, but this effect is reversed by simvastatin

To mimic bacterial peritonitis in an in-vitro cell culture model, we stimulated mesothelial cells with supernatants of bacteria-activated mononuclear cells. For this, freshly isolated MNCs were incubated with different staphylococci (as described in Fig. 6), and after 24 h cell supernatants were collected and used for stimulation of mesothelial cells. Confluent HMC monolayers were incubated with the obtained supernatants, and after 24 h the release of t-PA and PAI-1 was determined by ELISA. Figure 7 shows that all MNC-supernatants tested were able to decrease the release of t-PA and increase the release of PAI-1. Recently, we have shown that simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is an effective stimulator of the mesothelial fibrinolytic activity (37, 38). Here, we demonstrate that simvastatin also increased t-PA and decreased PAI-1 levels in our in-vitro cell culture model of bacterial peritonitis. However, simvastatin did not protect mesothelial cells from *S. aureus* (strains 6850 and ST 239)-induced cell damage or cell death (Table 3).

![Figure 3](image1.png)  
**Figure 3:** Mesothelial release of IL-8 and MCP-1 after incubation with different staphylococcal strains. Confluent HMCs were stimulated with live *S. aureus* or *S. epidermidis* strains (MOI 50) for 24 h as described in Figure 1. TNFα (500 U/ml) was used as a positive control. The conditioned media were analyzed for IL-8 (A) and MCP-1 (B) antigens. Results shown are means ± SEM of at least five experiments. *Significant increase (p<0.05) with corresponding control values.

![Figure 4](image2.png)  
**Figure 4:** Mesothelial expression of ICAM-1 and VCAM-1 after incubation with different staphylococcal strains. Confluent HMCs were stimulated with live *S. aureus* or *S. epidermidis* strains (MOI 50) for 24 h as described in Figure 1. TNFα (500 U/ml) was used as a positive control. Cells were analyzed for ICAM-1 (A) and VCAM-1 (B) expression on the cell surface by flow cytometry. In every experiment unspecific binding was tested as described, which was not elevated by any stimuli used (data not shown). Results are expressed as geometrical mean fluorescence intensity and data are means ± SD of three independent experiments.
Figure 5: Time-course of the effects of *S. aureus* strains and TNFα on cell damage and release of IL-8, MCP-1, t-PA and PAI-1 in MNCs. Confluent HMCs were stimulated with live bacteria of *S. aureus* strains Cowan I, 6850 and ST 239 (MOI 50) or with TNFα (500 U/ml) and after different time points (0, 6, 24, 48, 72 h) cells were used for the trypan blue exclusion assay and culture supernatants were collected for measurements of IL-8, MCP-1, t-PA and PAI-1. Data are shown as means ± SD of one representative experiment out of three performed in triplicate.
Discussion

Bacterial peritonitis remains a frequent complication of peritoneal dialysis and abdominal surgery and is associated with a high rate of mortality (3–5, 20). During bacterial peritonitis resident as well as infiltrating cells from the blood contribute to combat an infection. Mesothelial cells constitute the main cell population of the inner peritoneal lining. They exert many functions to preserve the integrity of the peritoneal membrane, e.g. they express growth factors, metalloproteinases and components of the fibrinolytic system (t-PA and PAI-1) (8, 39, 40). Directly after injury, t-PA and PAI-1 regulate fibrin removal, which is the first critical step in fibrosis development, and there is increasing evidence that mesothelial cells mainly account for the fibrinolytic capacity within the peritoneal cavity (41, 42).

In this study we demonstrate that only selected S. aureus strains with both, a hemolytic and invasive phenotype (S. aureus strains 6850 and ST 239), decrease the levels of t-PA and PAI-1 in the supernatants of cultured mesothelial cells. This effect is most likely due to mesothelial cell damage and an increased rate of cell death. Previously, we demonstrated that live S. aureus strains 6850 and ST 239 efficiently induce mesothelial cell death, characterized by apoptotic and necrotic features (27). By contrast, non-invasive and non-hemolytic S. aureus strains, as well as S. epidermidis strains, did not affect mesothelial cell viability and the release of t-PA and PAI-1. Furthermore, the effects of live S. aureus 6850 and ST 239 are clearly different from the actions of inflammatory cytokines. It is known that the mesothelial fibrinolytic system is strongly regulated by inflammatory mediators (35, 38). Proinflammatory cytokines as well as bacterial lipopolysaccharides (LPS) decrease t-PA expression and increase PAI-1 release, which most likely contribute to the insufficient fibrinolytic capacity during infections (9, 10, 35, 43). However, in previous work we found that heat-killed bacteria were also able to increase the expression of PAI-1 in HMCs (44). This effect could be elicited by various bacterial cell wall-associated or -released proteins at high doses. By contrast in our present study we used live staphylococci in a much lower concentration (MOI of 50 vs. MOI of 1,000 [44]). As S. aureus strains 6850 and ST239 very efficiently induce mesothelial cell death at low doses, it is reasonable to speculate that S. aureus can cause more peritoneal damage through killing of mesothelial cells than by directly regulating the mesothelial fibrinolytic system. In this respect, peritonitis-models and clinical observations indicate that a loss of mesothelial cells is associated with an impaired fibrinolytic capacity in the abdominal cavity and peritoneal fibrosis (45, 46).

After bacterial infection mesothelial cells were able to react to invading organisms by expressing chemokines (IL-8, MCP-1) and adhesion molecules (ICAM-1, VCAM-1). These factors contribute to attract immune cells and facilitate their movement into the peritoneal cavity, respectively (14, 16). As most of the clinical S. aureus isolates exhibit an invasive and hemolytic phenotype, whereas S. epidermidis is generally non-hemolytic and barely invasive in HMCs (27, 32), it is likely that S. aureus-infection...
tions cause more inflammatory reactions and cell damage than S. epidermidis-peritonitis. This could be an explanation for the severe inflammation and worse outcome of peritonitis caused by S. aureus compared to coagulase-negative staphylococci (21, 22).

During the resolution phase of bacterial peritonitis leukocyte influx into the peritoneal cavity is predominated by mononuclear cells (36). In our experiments with mononuclear cells all S. aureus and S. epidermidis strains enhanced the secretion of the cytokines TNFα and IL-1β. The extent of cytokine expression varied among several staphylococci, but all tested strains induced a significant increase. As expected, supernatants from the bacteria-stimulated mononuclear cells, which contained most likely a multitude of cytokines besides TNFα and IL-1β, increased the expression of PAI-1 and decreased t-PA release in HMCs. In any circumstance, these effects could be prevented by preincubation of mesothelial cells with simvastatin. Recently, we described simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, as an effective stimulator of local peritoneal fibrinolytic activity, as it increased t-PA and decreased PAI-1 production in unstimulated as well as cytokine-activated HMCs (37, 38). Here we demonstrate that simvastatin is also active in our cell culture model of bacterial peritonitis, suggesting that it might be a useful drug to increase the mesothelial fibrinolytic capacity during an inflammatory state. However, simvasta-
tin failed to protect mesothelial cells from *S. aureus*-induced cell death, which could limit its therapeutic potential in *S. aureus*-peritonitis.

We conclude that only live *S. aureus* strains with an invasive and hemolytic phenotype efficiently induce mesothelial cell death, which could contribute to denudation of the peritoneum, loss of the local fibrinolytic capacity and development of fibrosis after infection. Furthermore, invading staphylococci cause enhanced expression of chemokines and adhesion molecules in mesothelial cells, which attract immune cells and aggravate the inflammatory situation. As host cell invasion and hemolysis are typical features of *S. aureus* strains, these factors might account for the worse outcome of peritonitis caused by *S. aureus* than by coagulase-negative staphylococci.

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### References