Role of Monocytes and Bacteria in Staphylococcus epidermidis Endocarditis

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The endocardial vegetation which is formed in the course of bacterial endocarditis (BE) contains tissue factor (TF)-dependent procoagulant activity. Earlier studies showed that monocytes are the main source of TF in the vegetations. The TF activity (TFA) of vegetations isolated from Streptococcus sanguis-infected rabbits depended on the numbers of bacteria as well as monocytes in the vegetation. In this study, we investigated whether for Staphylococcus epidermidis, a frequent pathogen in BE, an effect similar to that found for S. sanguis could be shown. In vitro, S. epidermidis was found to stimulate TFA of fibrin adherent monocytes significantly. This stimulation was maximal at a bacterium-to-monocyte ratio of 7. In vivo, TFA was found to be significantly higher in S. epidermidis-infected than in sterile catheter-induced vegetations. Reduction of vegetational bacterial numbers by teicoplanin treatment lead to a small but significant decrease of TFA. Reduction of monocyte numbers by etoposide did not affect vegetational TFA. Comparison of data for S. epidermidis and S. sanguis revealed that at equivalent bacterial numbers, vegetational TFAs were approximately the same for both microorganisms. Combining the results of the present study with those of a previous study using S. sanguis, we conclude that the main factor determining monocyte-dependent vegetational TFA is the number of vegetation-associated bacteria. The lower TFA found for S. epidermidis-infected than for S. sanguis-infected vegetations can be explained by the significantly lower bacterial numbers in the infected vegetations and consequently a lower stimulation of vegetation-associated monocytes.

Bacterial endocarditis (BE) is an inflammatory process on heart valves, in which activation of the coagulation system plays a major role (7). A fibrin clot containing monocytes, granulocytes, thrombocytes, matrix proteins, and infecting microorganisms, called an endocardial vegetation (11), is formed on the heart valve. Activation of the coagulation system occurs via the extrinsic pathway (7). A key protein in this process is the cell-associated tissue factor (TF). In an in vitro model of BE, we demonstrated that the expression of monocyte TF activity (TFA) depends not only on an interaction with bacteria but also on the adherence of these cells to a fibrin surface (3). In earlier studies in the rabbit model of BE, we have shown that monocytes do account for the TFA of endocardial vegetations (4, 15). After streptococci, staphylococci are the most frequent causative microorganisms in BE. Staphylococcus epidermidis is frequently isolated in prosthetic valve endocarditis (1, 6). In the rabbit model of BE, the effects of warfarin treatment on the induction and course of the infection of catheter-induced vegetations were studied for S. epidermidis and Streptococcus sanguis (12, 13). Results indicated that with S. epidermidis, warfarin-treated rabbits needed larger bacterial inocula to induce infection, with a lower degree of infection of the vegetations (13), whereas with S. sanguis, warfarin treatment had no effect on the induction or course of the infection (12). The results of these two studies suggest that species-specific effects occur in the pathogenesis of BE. Data from recent studies, both in an in vivo and in an in vitro model for BE, suggest that with S. sanguis, the numbers of monocytes as well as of bacteria are positively correlated with the TFA of endocardial vegetations (3, 4). The main objective of the present study was to investigate whether for S. epidermidis a similar effect on monocytes in the activation of the coagulation system could be found, in vitro as well as in vivo. To achieve this goal, we studied in vitro the ability of S. epidermidis to induce TF expression on fibrin-adherent monocytes. In vivo, the effects of monocytopenia and antibiotic treatment were assessed in the rabbit model of BE.

MATERIALS AND METHODS

Microorganism: S. epidermidis (ATCC 14990) was cultured overnight at 37°C in Todd-Hewitt broth (Oxoid, London, England). Cultures were washed twice with phosphate-buffered saline and diluted to appropriate concentrations. For the in vivo experiments, S. epidermidis suspensions were diluted to approximately 109 CFU/ml.

Adherence of S. epidermidis to a fibrin surface. The adherence of S. epidermidis to a fibrin surface was assessed as described for S. sanguis (3, 4).

Effect of S. epidermidis on TF of fibrin-adherent monocytes. The effect of S. epidermidis on TF of fibrin-adherent monocytes was determined as described for S. sanguis (4, 16).

Rabbit model of BE. BE was induced in male New Zealand White rabbits as described elsewhere (2, 4, 5, 8).

Cytostatic drug. The cytostatic drug etoposide (Vepesid; kindly donated by Bristol-Meyers Squibb BV, Woerden, The Netherlands) was used as described previously (4) to induce a selective monocytopenia.

Antibiotic treatment. On 2 consecutive days, one daily dose of teicoplanin (30 mg/kg of body weight; Gist-Brocades, Delft, The Netherlands) was injected subcutaneously. The first dose was given 20 h after injection of staphylococci. Rabbits were sacrificed 24 h after the last injection of teicoplanin (72 h after infection). The blood concentration of teicoplanin was determined with the Innufluor reagent set for the quantitative determination of teicoplanin (International Bioclinical, Inc., Portland, Oreg.). The MIC and MBC of teicoplanin were determined as described previously (4). The MIC of teicoplanin was 8 μg/ml; the MBC was 16 μg/ml.

Quantitation of blood monocytes. Monocyte numbers in 1-ml blood samples were determined as described before (4).

Blood cultures. Immediately before rabbits were sacrificed, 1 ml of blood was drawn from a marginal ear vein and collected in vials containing 10 mg of EDTA. Two hundred μl of blood was plated on blood agar plates. After overnight incubation at 37°C, the CFU/milliliter of blood was determined.
Handling of vegetations. The isolated vegetations were weighed and homogenized in 2 ml of PBS. Part of the homogenate was used to determine the log CFU/gram of vegetation, while another part was used for measurement of the TFA as described elsewhere (4). The procedure for measuring TFA on the surface of fibrin-adherent monocytes in the presence of S. epidermidis was the same as described previously (3) for S. sanguis.

Statistical analysis. For determination of significance of differences between the vegetational TFAs, weights, and infections of control rabbits, etoposide-treated rabbits, and teicoplanin-treated rabbits, multifactorial analysis of variance was used with Newman-Keuls correction. The significance level α was 5%.

RESULTS

S. epidermidis-induced monocyte TFA in vitro. Overnight staphylococcal cultures were washed and layered on the fibrin plates in concentrations ranging from $1.5 \times 10^4$ to $1.5 \times 10^{10}$ CFU/ml. The percentage adherence of S. epidermidis was not affected by dilution of the cultures, being $\pm 7\%$ of the inoculum. Next, TFA of fibrin-adherent monocytes was assessed after a 4-h incubation of the cells at 37°C and 5% CO$_2$ in the absence or presence of staphylococci. As shown before (3), the adherence of the monocytes to the fibrin plate induced a TFA of $55 \pm 13$ pmol of factor Xa (FXa)/min/10$^6$ monocytes. The presence of S. epidermidis led to an inoculum-dependent increase of monocyte TFA (Fig. 1). A bacterium-to-monocyte ratio of 7 was needed to give a maximal TFA (Fig. 1). At this maximum, TFA was increased twofold by the addition of staphylococci. These results were comparable to those with S. sanguis, where monocyte TFA reached a maximum with a twofold increase at a bacterium-to-monocyte ratio of 4.5 (3).

Effect of etoposide on the number of peripheral blood monocytes and granulocytes. On 6 consecutive days, one daily dose of 12.5 mg of etoposide was injected in a marginal ear vein. At the time of catheterization (day 4 of etoposide treatment), the numbers of peripheral blood monocytes had dropped to 5 to 10% of initial values, and they remained at this level during the rest of the experiment, even after infection of the vegetation. As shown before, the number of blood granulocytes ($\pm 9.9 \times 10^5$ cells/mm$^3$) did not significantly change during the experiment (4).

Effects of monocytopenia on vegetations. After 48 h of infection, all vegetations of S. epidermidis-injected rabbits were infected. The degree of infection was $7.01 \pm 1.16$ log CFU/g of vegetation. Monocytopenia did not cause a difference in vegetational infection ($7.53 \pm 0.92$). All control rabbits had sterile vegetations. Blood cultures of staphylococcus-challenged monocytopenic rabbits were S. epidermidis positive, while all blood cultures of nonmonocytopenic rabbits were sterile. Also, after 48 h of infection, vegetational weights of S. epidermidis-infected rabbits were higher than those of noninfected rabbits, being $16.11 \pm 9$ and $11.19 \pm 5.07$ mg, respectively ($P < 0.008$). Etoposide treatment slightly increased this difference, the weights of S. epidermidis-infected and noninfected vegetations being $19.54 \pm 6.36$ and $11.40 \pm 4.53$ mg, respectively ($P < 0.005$). There were no differences in the vegetational weights of noninfected control and noninfected etoposide-treated rabbits. However, contrary to expectation, vegetational weights of etoposide-treated S. epidermidis-infected rabbits were slightly but not significantly higher than those of non-etoposide-treated S. epidermidis-infected rabbits, being $19.54 \pm 6.36$ and $16.11 \pm 9.00$ mg, respectively. At day 2 of infection, the TFA of infected vegetations was significantly higher than that of sterile vegetations, being $162 \pm 8$ versus $116 \pm 23$ pmol of FXa/g of vegetation/min for the nonmonocytopenic rabbits ($P < 0.05$) and $158 \pm 32$ versus $99 \pm 21$ pmol of FXa/g of vegetation/min for the monocytopenic rabbits ($P < 0.006$). The TFA of sterile vegetations of monocytopenic rabbits was found to be fractionally lower than that of nonmonocytopenic rabbits, but this difference was not significant ($116 \pm 23$ versus $99 \pm 21$ pmol of FXa/g of vegetation/min). Etoposide treatment did not affect the TFA of staphylococcus-infected vegetations ($162 \pm 8$ versus $158 \pm 32$ pmol of FXa/g of vegetation/min for infected and etoposide-treated infected vegetations).

Effect of antibiotic treatment on vegetations. Although serum levels of teicoplanin did not permanently reach the MBC, at 72 h of infection, bacterial numbers of the vegetations dropped below the detection level ($<3.8$ log CFU/g), whereas in non-teicoplanin-treated rabbits, the infection level was $7.18 \pm 1.75$ log CFU/g. Vegetational weights did not differ between teicoplanin-treated and control rabbits, being $18.24 \pm 11.9$ and $18.53 \pm 8.69$ mg, respectively. At day 3 of infection, all blood cultures were sterile. TFA of vegetations from S. epidermidis-infected rabbits was significantly higher than that of S. epidermidis-infected teicoplanin-treated rabbits ($100 \pm 19$ versus $80 \pm 9$ pmol of FXa/g of vegetation/min; $P < 0.01$).

DISCUSSION

From the results of this study, we conclude that in vitro, S. epidermidis can adhere to fibrin and stimulate monocytes to express TFA. In vivo, we found that infection of the vegetation with S. epidermidis resulted in an increase of the vegetational weight and TFA compared to that of noninfected vegetations. Treatment of the rabbits with etoposide led to a 95% reduction of peripheral blood monocytes. Blood cultures from etoposide-treated infected rabbits were positive, whereas those of control infected rabbits remained sterile. Therefore, the slight though not significantly higher weights of vegetations in the former group might be due to reseeding of the vegetation from the circulation. Further, no significant changes in TFA or infection of the vegetations in etoposide-treated rabbits were observed. Reduction of the vegetational infection with teicoplanin led to a significant decrease of the vegetational infection and also to a small but significant decrease of the TFA of the vegetations but did not affect the vegetational weights. In vitro, S. epidermidis and S. sanguis have the same effect on the TFA of fibrin-adherent monocytes. They induce the same maximal twofold increase in TFA at comparable bacterium-to-monocyte ratios (7 for S. epidermidis and 4.5 for S. sanguis [3]).
vegetation after penicillin treatment is approximately $10^7$, midis etations. Interestingly, at equivalent bacterial numbers, TFAs teicoplanin treatment. Apparently TFA in the TFA was found after etoposide treatment and only a small but bers by penicillin treatment resulted in a significantly lower treatment as well as reduction of vegetational bacterial num-

S. sanguis bacteria as well as a lower TFA for S. epidermidis-infected rabbits with teicoplanin reduces bacterial these groups are comparable (Table 1). Treatment of S. epidermidis-infected rabbits with teicoplanin reduces bacterial numbers by etoposide treatment had no effect and reduction of bacterial numbers by teicoplanin had only a very small though significant effect.

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**REFERENCES**


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